Genetic, Epigenetic and Phenotypic analyses along a Continuum from ROHHAD to Monogenic Obesity Disorders linked to Hypothalamic dysregulation

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HARVENGT Julie

Promotor: Prof. Vincent BOURS

Thesis submitted to fulfil the requirements for the degree of

Doctor of Philosophy in Medical Sciences

Academic year 2025-2026

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Jury

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Abstract

Rapid-onset obesity with hypothalamic dysfunction, hypoventilation, autonomic dysregulation and neural crest tumor (ROHHHAD[NET] is a rare pediatric disorder characterized by rapid-onset obesity and hypothalamic dysfunction. Despite its acronym suggesting a defined clinical entity, no formal diagnostic criteria have been validated, and recent findings support a broader ROHHAD spectrum, including adult-onset cases and presentations lacking rapid weight gain. Genetic, autoimmune, and paraneoplastic hypotheses have been explored without conclusive results. However, epigenetic mechanisms remain non investigated, despite observations such as discordant monozygotic twins, suggesting a role for epigenetic alterations. ROHHAD syndrome exemplify the complexity of rare forms of early-onset obesity, among which monogenic causes are few and often underdiagnosed, suggesting that other mechanisms, such as hypothalamic inflammation, environmental disturbances, and epigenetic alterations, may be relevant for these forms of early-onset obesity. The diagnostic yield for monogenic forms remains low as demonstrated by our study on real world evidence data in a cohort of 223 patients. Expanding genomic approaches may improve diagnostic yield and help delineate patients who could benefit from targeted therapies. This thesis aims to explore the genetic, epigenetic, and phenotypic continuum between ROHHAD and monogenic hypothalamic obesities, contributing to a better understanding of the ROHHAD condition and the early-onset obesities.

Résumé

Analyse génétique, épigénétique et phénotypique d'un continuum entre le syndrome ROHHAD et les obésités monogéniques liées à une dérégulation hypothalamique

L'obésité d'apparition rapide avec dysfonctionnement hypothalamique, hypoventilation, dérégulation du système nerveux autonome et tumeur de la crête neurale (ROHHHAD[NET]) est une maladie pédiatrique rare caractérisée par une obésité à apparition rapide et un dysfonctionnement hypothalamique. Bien que son acronyme suggère une entité clinique définie, aucun critère diagnostique formel n'a été validé, et des découvertes récentes soutiennent le concept d'un spectre ROHHAD plus large, incluant des cas apparaissant à l'âge adulte et des présentations sans prise de poids rapide. Des hypothèses génétiques, auto-immunes et paranéoplasiques ont été explorées sans résultats concluants. Cependant, les mécanismes épigénétiques restent inexplorés, malgré des observations telles que des jumeaux monozygotes discordants, suggérant un rôle des altérations épigénétiques. Le syndrome de ROHHAD illustre par ailleurs la complexité des formes rares d'obésité précoce, parmi lesquelles les causes monogéniques sont en faible proportion et souvent sous-diagnostiquées, ce qui amène à suggérer que d'autres mécanismes, tels que l'inflammation hypothalamique, les perturbations environnementales et les altérations épigénétiques, pourraient être responsables de ces obésités précoces. Le rendement diagnostique des formes monogéniques reste faible, comme le démontre notre étude sur des données réelles issues d'une cohorte de 223 patients. L'élargissement des approches génomiques pourrait améliorer le rendement diagnostique et aider à identifier les patients qui pourraient bénéficier de thérapies ciblées. Cette thèse vise à explorer le continuum génétique, épigénétique et phénotypique entre le ROHHAD et les obésités hypothalamiques monogéniques, contribuant ainsi à une meilleure compréhension du syndrome de ROHHAD et des obésités précoces.

Acknowledgments

Having been immersed in the fascinating field of pediatric obesity throughout my pediatric endocrinology training, the meeting and diagnosis of a patient with ROHHAD syndrome became a turning point that inspired me, in 2020, to embark on this PhD project. It was personally meaningful to engage in research that would allow for an in-depth exploration of a rare disorder while also keeping a connection with a more common clinical challenge, thus contributing—however modestly—to advancing knowledge in the field of pediatric obesity.

I would like to begin by warmly thanking the patients and their families. Their collaboration made it possible to explore a new and previously unstudied aspect of this syndrome—epigenetics. I am deeply moved by the trust they have placed in our team, and I remain mindful of the hopes and expectations they carry. It is with sincere gratitude that I dedicate these words to them today.

I am also very grateful to the many colleagues and collaborators from different countries who reached out, shared their experiences, and actively contributed to this project. Their openness and willingness to exchange ideas have strengthened our collective understanding and have been an invaluable source of support throughout this journey.

This thesis also explores genetic diagnosis in the context of early-onset obesity. I am deeply grateful for the invaluable collaboration with Muriel Hannon, the scientific lead for the gene panel analysis discussed in this work. Thank you, Muriel, for your support, your attentive listening, your thoughtful and precise feedback, and your energy, which will allow us to continue, beyond this thesis, to pursue ever more stimulating projects together.

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Although this thesis formally began in 2020, it was built on years of prior experience—clinical practice, research project submissions, and stimulating discussions about the role of early inflammation in obesity. I sincerely thank Professor Lebrethon for my training, which, before this thesis even began, enabled me to develop a critical and curious mindset within the vast field of pediatric obesity. All of this was, of course, enriched by the interactions with Professor AS Parent and the pediatric endocrinology team, with whom I had the privilege of working before joining the Genetics Department.

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A project like this reminds us how important the support of friends is. Thank you to all of you.

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List of publications

First author

HARVENGT, J., GERNAY, C., Mastouri, M., FARHAT, N., LEBRETHON, M.-C., Seghaye, M.-C., & Bours, V. (2020). ROHHAD(NET) Syndrome: Systematic review of the clinical timeline and recommendations for diagnosis and prognosis. Journal of Clinical Endocrinology and Metabolism. doi:10.1210/clinem/dgaa247

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List of abbreviations

AA: amino acid

ACMG: American College of Medical Genetics and Genomics

ADH: antidiuretic hormone

ACTH: adrenocorticotropic hormone

AHA: anti-hypothalamus antibodies

AKT: protein kinase B involved in the PI3K/AKT/mTOR pathway

AL: anterior lobe of the ante hypophysis

αMSH: α-melanocyte stimulating hormone

AP: adenohypophysis

APA: anti-hypophysis antibodies

ARC: arcuate nucleus

AgRP: agouti related peptide

BBB: Blood brain barrier

BDNF: (brain-derived neurotrophic factor

BF: basal forebrain

BP: breakpoints

CCHS: Congenital Central Hypoventilation Syndrome

CNS: Central Nervous System

CSF: Spinocerebellar fluid

CX3CL1: Fractalkine

CpGs: cytosine-phosphate-guanine sites

DI: intellectual disability

DMH: dorsomedial hypothalamic nucleus

DMRs: differentially methylated regions

DNA: deoxyribonucleic acid

DPSCs: dental pulp stem cells

DR: dorsal raphe nucleus

EMA: European Medicines Agency

ELISA: Enzyme-Linked Immunosorbent Assay

FDA: Food and Drug Administration

FSH: follicle-stimulating hormone

FTA: frontal temporal area

GABA: gamma-aminobutyric acid

GH: growth hormone

HFD: high-fat diet

HLA-DQ: major histocompatibility complex

HPA: hypothalamic–pituitary–adrenal

IL-1β: interleukin 1 beta

IL-6: interleukin 6

JAK: januse kinase

LC: locus coeruleus

LEPR: leptin receptor

LH: luteinizing hormone

lncRNAs: long non-coding RNAs

LPT: lateral paragigantocellular nucleus

LH: Lateral hypothalamus

MC3R: melanocortin 3 receptor

MCR: melanin-concentrating hormone receptor

miRNAs: microRNAs

mtDNA: mitochondrial DNA

MRAP2: Melanocortin 2 receptor accessory protein 2

MRI: magnetic resonance imagery

MRS: methylation risk score

MSH: melanocyte-stimulating hormone

ncRNAs: non-coding RNAs

NET: neural crest tumors

NICU: Neonatal Intensive Care Unit

NIV: Non-invasive ventilation

NO: Nitric oxide

NPY: neuropeptide Y

OMS: opsoclonus myoclonus syndrome

OX2Rs: OX2-receptors

PAG: phenylacetylglycine

PCSK1: proprotein convertase subtilisin/kexin type 1

PHIP-Seq: Phage ImmunoPrecipitation Sequencing

PI3K: Phosphatidylinositol 3-kinase

piRNAs: PIWI-interacting RNAs

POMC: proopiomelanocortin

PP: neurohypophysis

PRL: prolactin

PRRs: pattern recognition receptors

PRS: Polygenic risk score

PVN: paraventricular nucleus

PWS: Prader Willi Syndrome

PNX: Phoenixin

RLBA: Radioligand Binding Assay

RNA: ribonucleic acid

RNA-seq: RNA sequencing

ROHHAD: Rapid-onset Obesity with Hypothalamic Dysfunction, Hypoventilation, and Autonomic Dysregulation

ROHHADNET: ROHHAD with Neural crest tumor

RRBS: reduced representation bisulfite sequencing

SCN: suprachiasmatic nucleus

SFAs: saturated fatty acids

SH2B1: SH2B adaptor protein 1

siRNAs: small interfering RNAs

SLD: sublaterodorsal nucleus

SST: somatostatin

STAT3: Signal transducer and activator of transcription 3

SCN: Suprachiasmatic Nucleus

TFs: transcription factors

TGF-β: tumor necrosis factor beta

TNF-α: tumor necrosis factor alpha

TMN: tuberomammillary nucleus

TSH: thyroid-stimulating hormone

UPD: uniparental heterodisomy

UPF: ultra-processed food

UTF: ultra-transformed food

VEGFα: vascular endothelial growth factor A

VLPO: ventrolateral preoptic nucleus

VTA: ventral tegmental area

VPN: Ventrolateral Preoptic nucleus

WES: whole-exome sequencing

WGBS: whole-genome bisulfite sequencing

WGS: whole genome sequencing

WHO: World Health Organization

ZI: zona incerta

293 T CBA: homemade cell-based assay using HEK293 cells

1. Introduction

1.1. General considerations in the field of pediatric obesities

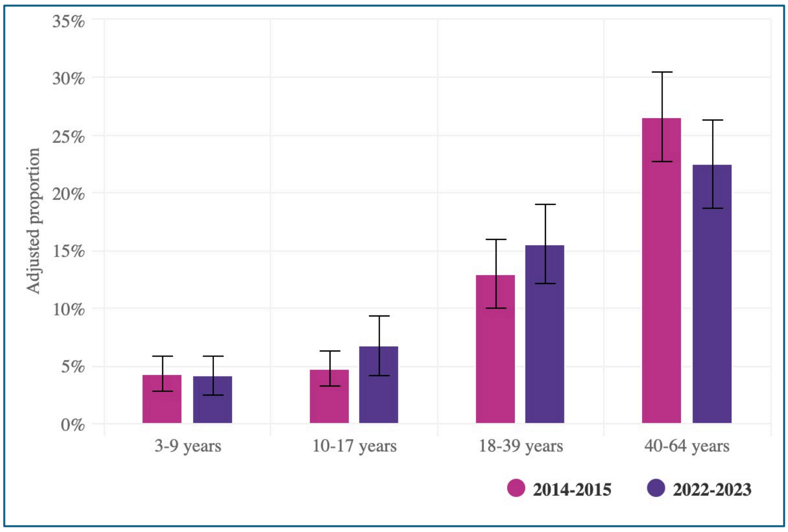
1.1.1. Epidemiology

Childhood obesity has been recognized as one of the most serious public health problems of the 21st century and is defined by the World Health Organization as an abnormal or excessive accumulation of body fat, sufficient to cause adverse health effects. The body mass index (BMI, calculated by dividing weight [kilograms] by the height squared [meters]) is the clinical current and easy standard measure of overweight and obesity. The BMI provides an estimation of adiposity in children and adults and indicates the level of severity of the obesity (Da Fonseca ACP *et al.,* 2017). A severe obesity is classically defined as a BMI of 120% or more of the 95th percentile of BMI for age and sex (based on CDC2000 growth charts), a definition that can be used in both clinical practice and research (Jebeile H. *et al.,* 2022). A strong link between a severe obesity in children and subsequent comorbidities in adulthood is currently well established. A severe obesity in children persists into adulthood in 70-80% of cases and cardiovascular risk in adulthood is associated with obesity in childhood and/or adolescence.

In 2019, the World Obesity Federation estimated there would be 206 million children and adolescents aged 5–19 years living with obesity in 2025, and 254 million in 2030. The prevalence of severe obesity in the pediatric population has grown in many high-income countries, even though overall prevalence of obesity has been stable (GBD 2021 Adolescent BMI Collaborators,2025). Indeed, a survey of European countries has shown that, approximately a quarter of children with obesity were classified with severe obesity, a finding that has implications for the management of obesity clinical services, because such children will need more specialized and intensive therapy (Spinelli A. *et al.*, 2019).

In Belgium, recent epidemiological studies reveal that nearly half (49%) of the adult population aged more than 18 years is overweight (BMI ≥ 25) and 16% is obese in Flanders and 25% in Wallonia (BMI ≥ 30) (Sciensano, 2024). Considering pediatric statistics, 19% of children and 21% of adolescents have overweight (including obesity), while 4% of children and 7% of adolescents have obesity. Among the most educated, 11% have obesity compared to 23% among the least educated. The levels of overweight and obesity have remained unchanged in 2022-2023 compared to 2014-2015, especially for the younger group of 3-9 years (Figure 1). However, the subcategorization with the proportion of Belgian pediatric severe obesity is not available.

Figure 1 - Proportion of obesity in the Belgian population aged 3 to 64 years, by years and age, Belgium, 2022-2023.



The proportion has remained unchanged between 2014-2015 and 2022-2023, across all age groups. Adjusted = results weighted for season, age, sex, and socioeconomic status, and adjusted according to age and sex based on linear regression model (using the Belgian population of 2022-2023 as reference).

In adults, obesity is defined by the World Health Organization (WHO) as having a BMI above or equal to 30.0 kg/m². In children and adolescents, this classification is age and sex specific as defined by Cole & Lobstein (2012). (Figure from Sciensano, Food Consumption Survey 2022-2023, June 2024)

During the last decades, international and national laws and regulations have helped to promote health in children and adolescents through the provision of healthy and nutritious food, safe activities and opportunities for physical activity and sports participation. This is presumed to be a major reason for the observation of stable levels for obesity in children during the last 10 years. Nevertheless, 4% of the younger part of the population present an obesity for whom there is a need to identify the shared risk factors through studies that investigate the complex mechanisms involved in the early-onset obesity. These investigations are motivated by a common aim in supporting the development of relevant interventions and accurate therapeutic approach for the broad spectrum of early-onset obesity, since the current epidemiological results show a clear failure of lifestyle interventions on the pediatric population. Early-onset obesity must be investigated considering the complex interactions of psycho-social, environmental and genetic factors (Güngör N.K., 2014). Parental employment status, social anxiety, parents' nutrition knowledge, pressure to eat, family function and perception of child weight have been pointed as contributor elements in the genesis or the persistence of the weight gain in children (Zhu H. *et al.*, 2024). Nevertheless, genetic and epigenetic factors should be considered crucial triggers in cases of severe early-onset obesity in children under the age of 10, as this subgroup may still be relatively unaffected by a range of societal and nutrition-related behaviors.

1.1.2. Etiologies

The causes of pediatric obesity can be classified into six categories (Figure 2):

(1) extremely rare monogenic obesities (such as leptin deficiency),

(2) rare syndromic conditions such as Prader-Willi syndrome and an estimated number of 78 other syndromes,

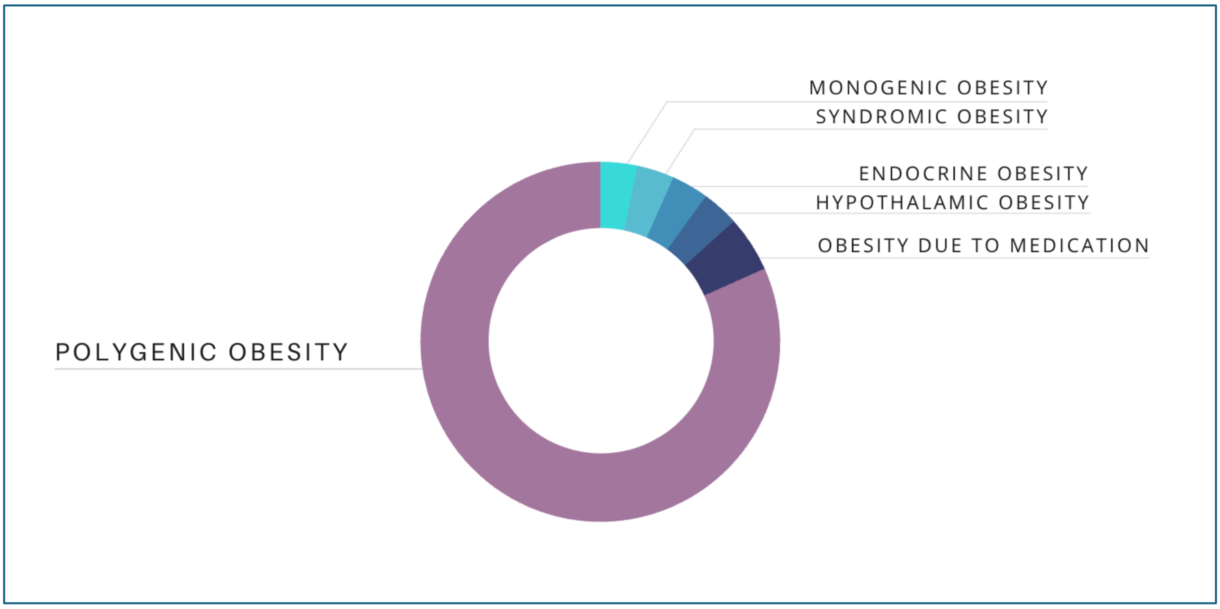
(3) endocrine obesities include Cushing's syndrome, hypothyroidism,

(4) acquired (CNS injury) and non-acquired hypothalamic dysregulation (e.g. ROHHAD),

(5) obesities due to medication interacting with the satiety regulation are also considered in a separate category (Sivakumar *et al*., 2024; Kaur et al,.2017),

(4) the so-called "polygenic obesity" or "common obesity" or "multifactorial obesity" or "pediatric obesity" represent the most common category.

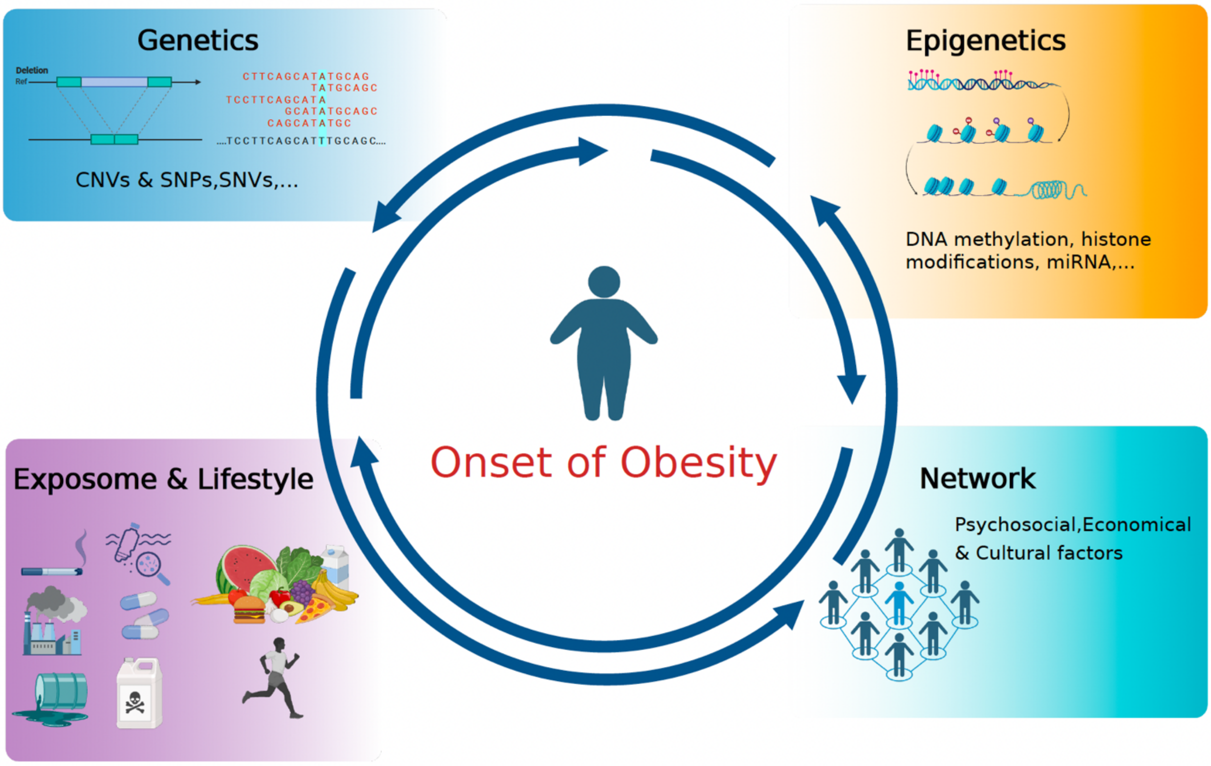
Figure 2 – Illustration of the repartition of the six categories for etiologies in early-onset obesities.



The proportions of each category are derived from various sources in the literature. Polygenic obesity represents the largest group, while the other five categories—monogenic, syndromic, endocrine forms, hypothalamic and obesity due to medications altering the satiety—are considered (very) rare. Due to limited and heterogeneous data, it remains challenging to provide accurate prevalence estimation for each of these rare forms (with the courtesy of Pr MC Lebrethon for the adaptation of the original slide).

This last category of polygenic obesity is a broad entity in terms of phenotype, which may explain why the overall management of these patients remains disappointing across the various published series. Pediatric obesity has its basis in genetic susceptibilities influenced by a permissive environment starting in utero and extending through childhood and adolescence (Styne D.M. *et al*., 2017) leading to epigenetics susceptibilities. Furthermore, in recent years, it has become evident that our chronic exposure to a multitude of pollutants and stress factors represents a major area of investigation that must be integrated into the global framework for understanding the onset of obesity.

Figure 3 - Interrelations of genetics, epigenetics, and environmental factors in the development of obesity at an individual level.



The onset of obesity results from a complex interaction between genetics, epigenetics, and external factors including daily behavior (lifestyle and diet), exposition to a large number of pollutants (exposome) and components from the social and cultural environment. Single nucleotide polymorphisms (SNPs) and variants (SNVs), short insertions and deletions (indels), as well as copy number variations (CNVs) are genetic variations contributing to obesity susceptibility. Modifications such as DNA methylation, histone modifications, and microRNA (miRNA) regulation influence gene expression without altering the DNA sequence, playing a crucial role in obesity (adapted from Keller V. *et al*., 2025; Catalan V. *et al*.,2022) (Created with BioRender.com).

Further investigations are needed to refine phenotypic characterization of pediatric obesity considering subgroups such as the (very)-early-onset childhood obesity group, which is the group of interest for this thesis. The improvement of the phenotypic description will aim to increase the yield of genetic diagnoses in this population and subsequently optimize clinical management strategies in the respect of the new era of precision medicine (Cuda S.E. & Censani M., 2022). Furthermore, the exploration of polygenic risk factors (PRS) - and more recently of the methylation risk score (MRS) - in obesity is ongoing through different worldwide studies, with the goal of improving the risk stratification for each patient. Those PRS and MRS will be certainly a promising tool for our understanding of this complex population of pediatric patients with obesity (Fenwick P. *et al*., 2019; Littleton S.H. *et al*., 2020).

Overall, among the children presenting a severe obesity in non-consanguineous population, it is currently assumed that around 5% (2-10% depending on the series) present chromosomal abnormalities and/or highly penetrant genetic variants that contribute to their obesity (Marenne G. *et al*., 2020).

The prevalence of other underlying medical causes of early-onset obesity, particularly those classified under endocrine-related obesity, has been insufficiently studied which contributes to the difficulty in providing accurate prevalence estimates for this subgroup. A recent study by Kleinendorst *et al*. involving a cohort of 282 patients assessed in a tertiary obesity center and systematically evaluated according to the pediatric-obesity 2017 endocrine society clinical guidelines, reported a diagnostic yield of 13% for monogenic obesity, 2.8% for cerebral lesions, 3.2% for medication-induced obesity, and 0% for specific endocrinopathies (Kleinendorst L. *et al*., 2020; Styne D.M. *et al*., 2017) (Figure 4).

Figure 4 – Box illustration of genetic and endocrine obesity conditions and their respective clinical main features. Examples of main diagnoses are listed for both etiologies.

GENETIC OBESITY	
Monogenic conditions	**Syndromic conditions**
Obesity starting in early childhood (<4-6yrs)	Young age at onset
Young adults (18-25yrs) with BMI ≥ $40 kg/m^2$	Dysmorphic features
Familial pedigree suggestive of inheritance **OR** major weight discordance between family members	Intellectual disability/ Developmental delay/Autism
Hyperphagia	Short stature
Hypopigmentation	Polydactyly
Red hair	Renal abnormalities/Nephropathy
	Severe myopia or retinopathy
	Congenital deafness
EXAMPLES	
LEP or LEPR deficiency MC4R deficiency POMC deficiency PCSK1 deficiency	Prader-Willi Syndrom* Bardet-Biedl Syndrom Asltröm Syndrom 16q11.2 deletion

ENDOCRINE OBESITY	
Early-onset obesity + Main additional feature: Alteration of growth velocity	
Primary hormonal dysregulation	**Hypothalamic dysregulation**
EXAMPLES	
Cushing Syndrom	**Acquired** SNC involvment (tumor, injury) Medication interfering with satiety regulation
Hypothyroidsism	**Non-acquired** ROHHAD

1.2. Hypothalamic Inflammation in the pathogenesis of early-onset obesity

Preclinical studies have consistently shown that hypothalamic inflammation and gliosis contribute to diet-induced obesity in rodents. Recent human data support these findings, highlighting the role of central neuroinflammatory processes in obesity pathogenesis.

In 2005, the first publications emerged demonstrating the presence of inflammatory changes in the hypothalamus of rodents subjected to a high-fat diet (HFD): the expression of proinflammatory cytokines IL-1β, TNFα, and IL-6 were increased in these animals after 16 weeks of HFD (De Souza C.T. *et al.*, 2005). Early hypothalamic inflammation in rodents exposed to a lipid-rich diet was also demonstrated in a 2012 study, which identified hypothalamic gliosis lesions on histological sections (Thaler J.P. *et al.*, 2012). These lesions notably affect proopiomelanocortin (POMC) neurons, which normally exert anorexigenic effects through the release of α-melanocyte-stimulating hormone (α-MSH).

If exposure to a HFD is prolonged, central lesions may extend beyond the hypothalamus. Neuronal dysfunction in mesolimbic and dopaminergic circuits, impairing reward signaling pathways, contributes to hyperphagia secondary to reward system dysregulation (Berthoud H.R., 2011). Disruption of the central circadian clock, along with disturbances in circadian rhythm and sleep regulation, appears also linked to central inflammatory damage (Tsang A.H. *et al.,* 2014). Animal studies suggest that these central damages occur early after HFD exposure.

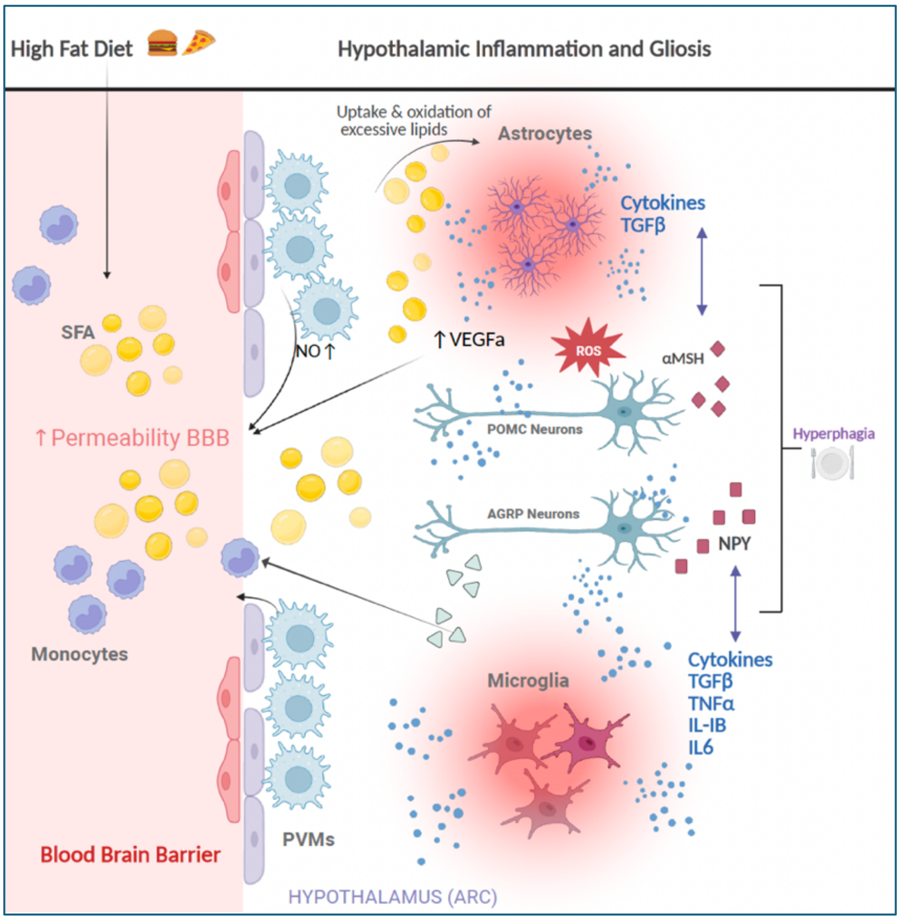
In parallel to these animal studies, Thaler *et al.* identified signs of hypothalamic gliosis on brain MRI scans performed in patients with obesity (Thaler J.P. *et al.*, 2010; Thaler J.P. *et al.*, 2012). Between 2012 and 2023, central hypothalamic damage has been explored through brain imaging or through post-mortem histopathological observations in a total of 24 publications (Sewaybricker L.E. *et al.*, 2023). Only a few publications refer to studies conducted in young adults or adolescents, showing anatomical changes or emergence of gliosis-type lesions (Thaler J.P. *et al.*, 2012). In the study by Yau *et al.*, brain MRIs performed in adolescents with diagnosed metabolic syndrome revealed smaller hippocampal volumes and reduced microstructural integrity in white matter (Yau P.L. *et al.*, 2012).

Most of the other results implicate the medio basal hypothalamus region (where is located the arcuate nucleus) which has predominantly been the main study area of mechanistic preclinical studies. However, the hypothalamus remains challenging to evaluate by MRI because of its small size, a lack of readily discernible anatomic margins and the proximity to the third ventricle and sinuses leading to a high risk of artefacts. These changes should also be interpreted cautiously for the results of the adolescents considering the physiological brain modifications occurring during puberty. Computerized segmentation of the hypothalamus and identification of its subnuclei by MRI have been developed based on adult brain references but still pose a challenge to apply to the pediatric population, especially infants and neonates (Sewaybricker L.E. *et al*., 2023). Brain imaging remains nevertheless a vast promising field of investigation, with current and future technical capabilities potentially enabling targeted studies of neurons located in the hypothalamic arcuate nucleus. A current research question is whether hypothalamic damage occurs early or late during weight gain, as current MRI studies do not allow for a temporal correlation between the onset of gliosis and the initiation of weight gain in individuals. Future research should also assess whether these lesions are potentially reversible in humans (Thaler J.P., 2013).

Central inflammation in obesity differs from peripheral inflammation in that it precedes the onset of obesity and is rapidly triggered by the consumption of an HFD. Within 24 hours of HFD initiation, as mentioned before, proinflammatory cytokine gene expression is induced in the hypothalamus of rodents, highlighting the immediacy of the central response to dietary fat (Tzounakou A.M. *et al*., 2024). The activation of hypothalamic microglia in obese mice appears to be driven more by dietary composition and fat- and gut-derived hormones than by obesity or adiposity itself. The cytokines secreted in response to HFD consumption rapidly activate a complex network of cells. Figure 5 provides a summary of the cascade of events involved, illustrating the complex regulatory mechanisms leading to the central gliosis.

HFD consumption leads to elevated levels of saturated fatty acids (SFAs) in the bloodstream, which subsequently cross the blood-brain barrier (BBB). SFAs trigger both reactive astrocytosis and microgliosis in the hypothalamus, with astrocytosis occurring as early as 24 hours post-HFD intake. Hypothalamic astrocytes produce various inflammatory mediators, notably transforming growth factor beta (TGF-β), which induces oxidative RNA stress and results in atypical NF-κB activation (Yan J., 2014). While astrocytes play a physiological role in lipid uptake and oxidation, excessive SFAs levels exacerbate oxidative reactions. This inflammatory activation also promotes increased BBB permeability through the release of vascular endothelial growth factor A (VEGF-α) (Tzounakou A.M. *et al*., 2024). In parallel, elevated central SFAs levels enhance nitric oxide (NO) production by perivascular macrophages, further compromising BBB integrity (Lee C.H. *et al.,* 2020).

Figure 5 - Schematic representation of hypothalamic gliosis induced by high-fat diet (HFD).



Following HFD consumption, elevated levels of SFAs in the bloodstream cross the BBB, triggering early activation of astrocytes and microglia in the hypothalamus. Astrocytes respond by releasing inflammatory mediators such as TGF-β, contributing to oxidative RNA stress and atypical NF-κB activation (not shown in the figure). This astrocytic activation also promotes increased BBB permeability via VEGF-α secretion. In parallel, perivascular macrophages produce NO, further compromising BBB integrity. Microglia initiate the release of a broad spectrum of cytokines (including TGF-β, TNF-α, IL-1β, IL-6), which are also modulated bi-directionally (purple arrows) by neuropeptides such as α-MSH and NPY. These cytokines interfere with leptin and insulin signaling pathways (JAK–STAT3 and PI3K–AKT, not illustrated), leading to dysregulation of POMC and AgRP expression in their respective neurons and contributing to central leptin and insulin resistance but also to a disbalance between NPY and αMSH appetite regulation resulting *in fine* to hyperphagia. Additionally, microglial inflammation induces CX3CL1 (fractalkine, represented by grey triangles) expression in neurons, facilitating peripheral monocyte recruitment and perpetuating the inflammatory process.

The figure illustrates a part of the cellular interactions and molecular pathways involved in the early and sustained inflammatory response to HFD, tanycytes and oligodendrocytes cells are not represented to maintain clarity and comprehensibility.

SFAs = saturated fatty acids; BBB= blood-brain barrier; NO= nitric oxide △ CX3CL1 Cytokines

(Created with BioRender.com)

Microglia initiate the release of a broad spectrum of cytokines, among which TGF-β, TNF-α, IL-1β, and IL-6. Neuropeptides such as α-MSH and NPY modulate also microglial cytokine production through complex and bidirectional interactions (Delgado R., 2011; Ferreira R., 2011). Subsequent inflammation-induced cytokines disrupt neuropeptide synthesis by inducing leptin and insulin resistance. This involves interference with key hypothalamic signaling pathways, including the leptin receptor–JAK–STAT3 pathway and the insulin receptor–PI3K–AKT pathway, leading to dysregulation of POMC and AgRP gene expression. Additionally, activation of the Toll-like receptor–IKK–NFκB pathway further promotes cytokine production (Alexander Jais & Jens C. Brüning, 2017). These disruptions contribute to energy imbalance and hyperphagic behavior observed in response to HFD exposure.

Microglial inflammation also rapidly induces the expression of the chemokine CX3CL1 (fractalkine) in hypothalamic neurons, as demonstrated in a mouse study by Morari J. *et al*. (Morari J. *et al*., 2014). CX3CL1 plays a pivotal role in the inflammatory process by recruiting peripheral monocytes, thereby amplifying the inflammatory response.

More recently, it has been shown that mice fed with HFD also exhibit increased hypothalamic expression of the *PNX* gene. Phoenixin (PNX), a neuropeptide with two active isoforms—PNX-14 and PNX-20—plays a role in reproduction, food intake, memory, anxiety, neuronal and microglial activity, and inflammation regulation (Liang H., 2022). Emerging evidence suggests that HFD may stimulate PNX release, which acts on GnRH neurons and may affect puberty timing and fertility in individuals with obesity. Although PNX appears to exert protective anti-inflammatory effects in the hypothalamus, its role in central gliosis remains unclear. Its upregulation may influence both the gonadotropic axis and feeding behavior (Valsamakis G. *et al.,* 2021).

Hypothalamic inflammation involves a complex cellular network, including not only glial cells such as microglia and astrocytes, but also oligodendrocytes and tanycytes (Dali R. *et al*.,2015). Tanycytes are specialized glial cells essential for leptin transport and signaling, while NG2 glial cells are required to maintain leptin responsiveness in neurons (Alexander Jais & Jens C. Brüning, 2017). These cell types form a complex regulatory network that maintains metabolic homeostasis in healthy state. However, chronic exposure to hypercaloric diet stimuli create eventually permanent glial scars that disrupts the normal homeostatic signaling in this complex neuronal and glial network, promotes further weight gain through the chronic hyperphagia, and may also induce a resistance to weight loss (Sewaybricker L.E. *et al*., 2023).

In this context, understanding interactions between neuronal and non-neuronal cells in hypothalamic inflammation remains a vast field of investigations across common, genetic, and syndromic obesities—including ROHHAD—where hyperphagia and altered food intake reflect complex interaction between genetic and inflammatory mechanisms.

1.3. Monogenic obesity

The current challenge in clinical practice is to detect the genetic forms of obesity among the large cohort of young patients with severe obesity (children and adolescents). Until now, the common practice was to start genetic investigations in cases of inappropriate weight gain in comparison to diet and lifestyle. As a major point, sustained severe hyperphagia (sometimes with nocturnal symptoms) from early childhood is a feature of the genetic obesity syndromes (Farooqi IS; 2021). Some of these syndromic conditions are associated with learning difficulties, behavioral troubles, and developmental delay (e.g., Prader–Willi syndrome) and other evident clinical problems (e.g., visual loss/renal abnormalities encountered in Bardet–Biedl syndrome) (Forsythe E. *et al.*, 2013). When this kind of association of symptoms is present, children are generally investigated at a young age by pediatricians (Farooqi IS, 2021).

Over the last 20 years, a group of genetic disorders characterized by severe obesity as the only presenting feature has been increasingly described. However, it is recently—starting in 2025—that specific clinical criteria have begun to emerge to help distinguish the nature of weight gain in these monogenic forms. A European retrospective study conducted in 2025 identified a BMI cutoff of ≥24 kg/m² at 2 years of age as a significant indicator with good diagnostic performance for biallelic forms of monogenic obesity (Zorn S. et al., 2025). These disorders are driven by molecular alterations in pathways involved in appetite and weight regulation. The growing understanding of these mechanisms has provided new insights into the identity and function of central neuronal circuits, particularly within the hypothalamus.

1.3.1. A low diagnostic yield

For now, current estimations from different studies dedicated to monogenic etiologies suggest that 5% of the patients with severe early onset obesity are linked to a monogenic condition related to the melanocortin pathway. From one point of view, a mean of 5% for the efficiency rate for the targeted panels may be discussed as underestimated due to limited access to genetic investigations for a wide range of patients. Medical compliance and socioeconomic status should be cited as two factors of under evaluation for this category of patients. On the other hand, the 5% diagnostic yield should also be interpreted with caution, as it might be overestimated. A thorough analysis of previously published series reveals an inflated rate of positive results due to inconsistencies between studies and differing criteria for variant classification not systematically referring to ACMG criteria, which could lead to the misclassification of variants of uncertain significance (VUS) as positive results (Kleinendorst

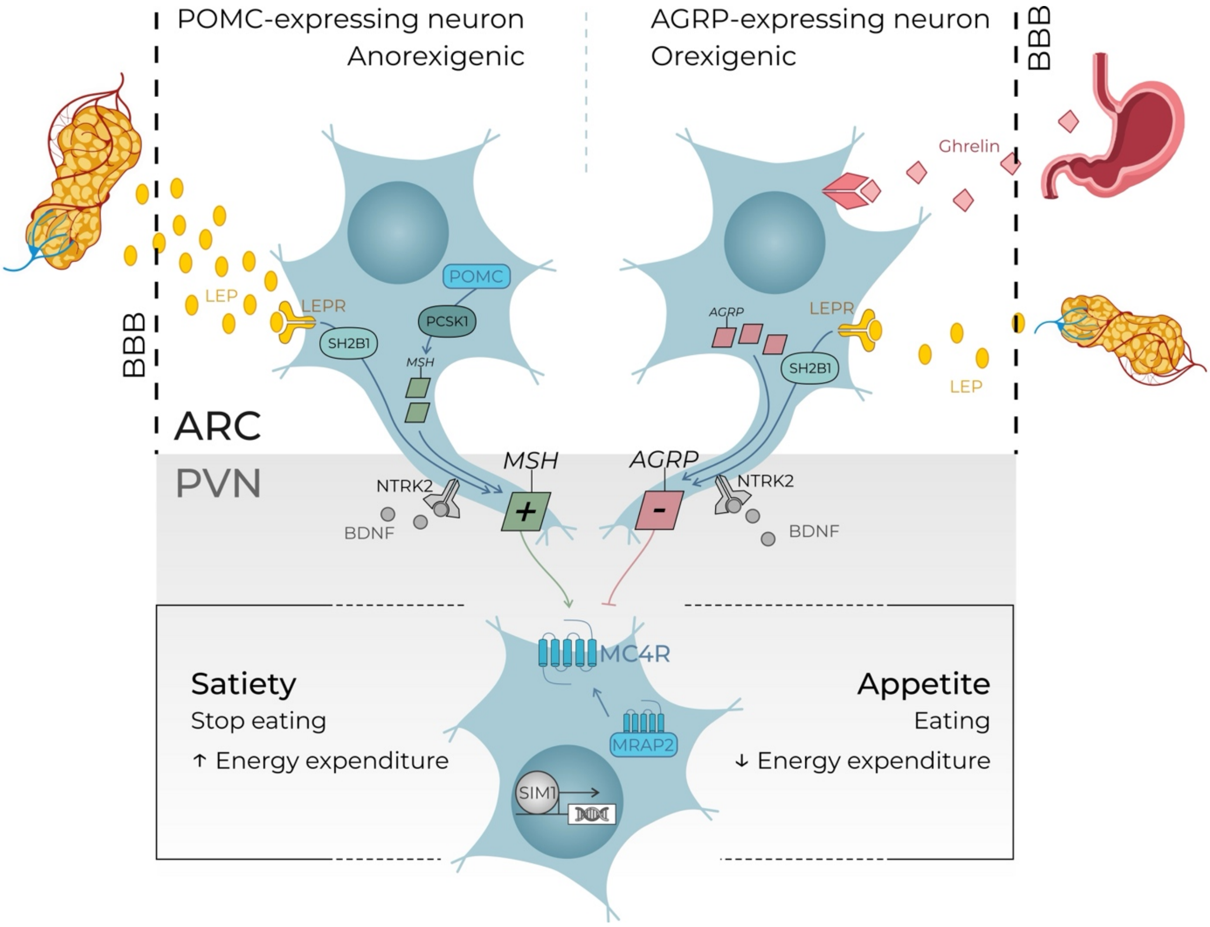
L. *et al*., 2018; Mohammed I. *et al*., 2023; Bonetti G. *et al*., 2022).

1.3.2. The MC4R hypothalamic pathway- impact on the appetite regulation

The central disruption of appetite regulation is primarily due to the impairment of the hypothalamic leptin–melanocortin pathway which is the main mechanism implicated in monogenic non-syndromic forms of obesity. Figure 6 illustrates this pathway, showing the balance between orexigenic (AgRP-expressing) and anorexigenic (POMC-expressing) neurons. In non-obese individuals, this system maintains a well-balanced caloric intake relative to energy expenditure. In contrast, patients with obesity exhibit a dysregulation of this balance, characterized by enhanced appetite and diminished satiety signaling.

To expand the understanding of the molecular components involved in the leptin–melanocortin pathway, Table 1 provides a summary of clinical and biological characteristics for each gene, along with a summary of its specific role in the pathway. The following section offers a detailed overview of the implication of each gene in obesity based on current literature.

Figure 6 - An overview of the leptin–melanocortin pathway in the hypothalamus.



Leptin is secreted in the white adipose tissue. Leptin levels depend on the "fed status": leptin levels increase in case of refeeding after food starvation and leptin levels decrease in case of food deprivation. Leptin hormone peptides act on the hypothalamus where POMC-expressing neurons and agouti-related protein (AGRP)-expressing neurons are located, more precisely in the arcuate nucleus. These neurons send a signal to the MC4R-expressing neurons in the paraventricular nucleus of the hypothalamus (PVN) which controls through their central neural projections *in fine* the level of appetite or satiety.

BDNF (brain-derived neurotrophic factor) is thought to be an actor in this pathway, through its binding to NTRK2 (neurotrophic receptor tyrosine kinase 2) leading to a regulation in the synaptic plasticity of neurons, including those present in the ARC and PVN. The transcription factor SIM1 is also essential for the correct development of the PVN.

+, agonist; −, antagonist; LEPR, leptin receptor; MRAP2, melanocortin receptor accessory protein 2; MSH, melanocyte-stimulating hormone; SH2B1, SH2B adaptor protein 1.

ARC, arcuate nucleus; AgRP, agouti related peptide; NPY, neuropeptide Y; POMC, propiomelanocortin; αMSH, α-melanocyte stimulating hormone; MCR, melanin-concentrating hormone receptor; SST, somatostatin.

(Adapted from: Farooqi IS, 2021; Rohde K. *et al*., 2019; Da Fonseca ACP. *et al*., 2017; Loos RJF & Yeo GSH, 2022; Baldini G & Phelan KD, 2019; Kumar U & Singh S,2020)

Gene ID	GeneName/OMIM number	Chromosomal location	Inheritance	Clinical Features	Biological (or additional) features	Role in the leptin/melanocortin pathway
LEP	LEPTIN #164160	7q32.1	AR	Normal birth weight Early-onset severe obesity Hyperphagia Hypogonadism Hypothyroidism Immune disorders (T-Lymphopenia)	Recurrent ear infections. Frequent upper respiratory and pulmonary infections.	Circulates in proportion to fat mass. ↑ hunger when circulating levels decline below a minimum threshold.
LEPR	LEPTIN RECEPTOR #601007	1p31.3	AR	Severe obesity, onset from birth Hyperphagia Hypogonadism (not systematic) Hypothyroidism Immune disorders (T-Lymphopenia)	Frequent upper respiratory tract infections Normal linear growth but short stature in final adult height.	Receptor for leptin, transmitting its neuroendocrine functions through the activation of the JAK/STAT3 pathway. Mediating binding and endocytosis of leptin at the human blood brain barrier.
PCSK1	PROPROTEIN CONVERTASE #162150	5q15	AR	Severe obesity and Hyperphagia Intestinal malabsorption - diarrhea (AR) Hypogonadotropic hypogonadism	Postprandial hypoglycemia (AR) Glucocorticoid deficiency (AR)	Encodes one of the prohormone convertases critical for processing POMC
POMC	PROOPIOMELANOCORTIN #176830	2p23.3	AR	Severe obesity starting in infancy Hyperphagia Ginger and red hair Pale skin	Adrenal insufficiency (ACTH deficiency). Neonatal cholestasis. Gonadotropin, growth hormone, and TSH deficiency (may become apparent in the teenage years)	Complex pro-polypeptide that is processed into ACTH, melanocortin peptides that signal to MC4R in the brain, and into ß endorphin that signal to the opioid receptors.
MC4R	MELANOCORTIN 4 RECEPTOR #155541	18q21.32	AD,AR	Severe obesity (AR) or light, mild or severe obesity (AD) Hyperphagia Tall Stature	Hyperinsulinemia (at a rate disproportionate to the degree of obesity) Increased risk of T2D Relatively low blood pressure for degree of obesity	Receptor that binds the melanocortin peptides and AGRP to regulate appetitive behavior and autonomic functions
SH2B1	SH2B ADAPTOR PROTEIN 1 #608937	16p11.2	De novo microdeletion/ AD in rare cases	Severe obesity Hyperphagia Short Stature Behavioral disturbances		A signaling molecule just downstream of the leptin receptor. Crucial role in signal transduction through different pathways (mainly JAK2/STA3-PI3K/AKT-MAPK)
			AD	Severe early-onset obesity Disproportionate insulin resistance	/	
MRAP2	MELANOCORTIN 2 RECEPTOR ACCESSORY PROTEIN 2 #615410	6q14.2	AD	(Mild) obesity or excessive weight gain.	Hyperglycemia Arterial hypertension	A protein trafficking MC4R to the cell surface
SIM1	SIM bHLH TRANSCRIPTION FACTOR 1 #603128	6q16.3-q21	*	Severe obesity Hyperphagia Prader-Willi-Like	Autonomic Dysfunction Neurobehavioral disturbances	A transcription factor crucial for the development of the PVN and consequently regulating expression of MC4R (among other genes)
BDNF	BRAIN-DERIVED NEUROTROPHIC FACTOR #113505	11p14.1	*	Severe obesity Hyperphagia Developmental Delay Nociception alteration	Salivary BDNF concentration currently under evaluation as a potential biomarker of predicting obesity related complications	A regulator of the neuronal synaptic plasticity in the CNS including the hippocampal area

Table 1- Description of the main clinical, genetic and mechanistic features for the most prevalent monogenic obesities.

Abbreviations: T2D: type 2 diabetes/PVN: Para-ventricular nucleus/CNS: central nervous system/ AD: autosomal dominant/ AR: autosomal recessive

(Ref: Selvaraju V. *et al*., 2022; Golden P.L. *et al*., 1997;Liu *et al*., 2022;Farooqi IS, 2021; Da Fonseca ACP *et al*., 2017; Loos RJF & Yeo GSH, 2022; Singh RK *et al*., 2017; Baron M. *et al*., 2019; Valk E.S. *et al*., 2019)

1.3.2.1. Leptin and Leptin Receptor

Some genetic obesity conditions can result from pathogenic variants that disrupt the production or the action of leptin on its targets (Clément K. *et al*., 1998; Montague C.T. *et al*., 1997). *LEP* and *LEPR* pathogenic variants are found in around 1% of patients with severe obesity and are principally observed in consanguineous families (Littleton S.H. *et al*., 2020; Farooqi I.S. *et al*., 2007). Clinical characteristics of leptin deficiency include severe obesity, short stature, hyperphagia, emotional lability and social disability (Littleton S.H. *et al*., 2020). In case of *LEPR* bi-allelic pathogenic variants, 100% had early-onset obesity (< 6 years of age) and 96% had hyperphagia. Anthropometrics studies showed that children have generally a BMI > 27 kg/m2 at two years of age and > 33 kg/m2 at five years of age (equivalent to a BMI Z-score > +4 SDS) (Dubern B. *et al*., 2021). Mild hypothyroidism, hypogonadism, and impaired T-cell-mediated immunity are also described among patients carrying *LEPR* variants. Interestingly, systematic reviews revealed that only one third of the patients showed one or more pituitary deficiency (Kleinendorst L. *et al*., 2020). In a clinical point of view, patients with *LEP* or *LEPR* variants do not exhibit risk factors for cardiovascular disease such as hypertension, lipid dysregulation, or hyperglycemia. Treatment with exogenous administration of a recombinant human leptin can relieve the symptoms including hyperphagia, weight gain as well as immunologic abnormalities and hypogonadism (Rohde K. *et al*., 2019). In the natural history of the disease, immune and endocrine function may improve spontaneously in some adult patients (Ozata M. *et al*., 1999).

1.3.2.2. POMC and MC4R

At a molecular level, leptin stimulates specifically neurons expressing POMC (proopiomelanocortin), whose gene product promotes production of alpha-melanocyte-stimulating hormone (α-MSH) binding to the melanocortin 4 receptor (MC4R) (Figure 1) (Rohde K. *et al*., 2019). This pathway is part of the anorexigenic signaling in response to a ‘fed state’ in humans. Pathogenic variants in *POMC* and *MC4R* are consequently highly impacting the appetite and weight regulation.

1.3.2.2.1. POMC

POMC (proopiomelanocortin) is an appetite inhibitory molecule that produces α-, β- and γ-MSH (melanocyte-stimulating hormone), and works mainly through the MC4R and MC3R to inhibit appetite (Singh R.K., 2017). A deficiency of the POMC protein causes an absence of ACTH and α-MSH, which are normally cleaved from the POMC protein (Mahmoud R. *et al*., 2022). Clinically, a deficiency of POMC (bi-allelic variants) leads to hyperphagia, lower resting metabolic rate, and severe obesity with cutaneous pigmentation abnormalities (red hair and pale skin). The impact of heterozygous *POMC* variants on obesity is still unclear. A recent publication of 2023 concludes that heterozygous pathogenic *POMC* variants do not contribute to monogenic obesity, but that they slightly increase body mass index (Le Collen L. *et al*., 2023). Further data will be probably available in the next few years considering the subsequent question of a treatment such as Setmelanotide (a MC4R agonist) in this indication.

1.3.2.2.2. MC4R

MC4R is the first cause of monogenic obesity with a prevalence estimated at 5% in the obese pediatric cohorts and 2% in the obese adult cohorts. It was first discovered to be related to body weight in 1998, and multiple studies have investigated its mechanism and the molecular alterations in case of pathogenic variants (Mahmoud R. *et al*., 2022). MC4R is one of the most important melanocortin receptor (MCR) in the melanocortin pathway. MC4R is also a member of the protein G-receptor family and is expressed in hypothalamus, brain, muscle, adipocytes, astrocytes. It is involved not only in energy homeostasis and food intake but also in anti-inflammatory regulation, drug tolerance, and sexual behavior (Singh R.K. *et al*., 2017; Semple E. & Hill J.W., 2017). Patients with homozygous variants are very rare in Europe, few consanguineous families are described from specific ethnicities (e.g. in Pakistan). Homozygous patients present a highly severe phenotype with a very early-onset of the hyperphagia. In contrast, heterozygous patients present a variable degree in the severity of the obesity ranging from severe to mild forms without other distinctive symptoms.

1.3.2.3. PCSK1

Patients with homozygous and compound heterozygous pathogenic variants in *PCSK1* (proprotein convertase, subtilisin/kexin-type, 1) present an alteration in the POMC processing. Patients manifest obesity accompanied by a glucocorticoid deficiency, hypogonadotropic hypogonadism, and postprandial hypoglycemia with high phenotypic variability (Rohde K. *et al*., 2019).

The involvement of heterozygous *PCSK1* variants in monogenic obesity is rarely reported, as first-degree carrier relatives of patients with *PCSK1* deficiency are mostly not described to be obese (Van Dijck E. *et al*., 2022).

1.3.2.4. BDNF

In addition to activating the classical leptin/MC4R signaling pathway, leptin rapidly induces modifications in synaptic connectivity between neurons—a form of structural plasticity that is essential for its downstream effects. The brain-derived neurotrophic factor (BDNF) is among the key mediators of this hypothalamic neuronal plasticity and is widely expressed throughout the central nervous system, including the hippocampus. Loss-of-function variants of BDNF that impair hippocampal synaptogenesis have been associated with neurobehavioral disorders (Sonoyama T. *et al.*, 2020). Furthermore, sporadic disruptions in BDNF expression have been linked to phenotypes characterized by both metabolic and neurological manifestations, such as severe obesity, hyperphagia, cognitive deficits, and hyperactivity (Da Fonseca ACP *et al.*, 2021; Harcourt B.E. *et al.*, 2018).

Despite these findings, further clinical evidence is required to clarify the role of pathogenic BDNF variants in obesity. To date, no definitive genotype-phenotype correlations have been established in the literature. It remains particularly important to differentiate the effects of isolated single nucleotide polymorphisms from those of broader chromosomal deletions encompassing BDNF, which may contribute to complex phenotypes involving multiple genes. Notably, salivary BDNF concentration is currently being investigated as a non-invasive biomarker for predicting obesity-related complications in pediatric populations. Recently, BDNF has been classified within a novel category of molecules termed "metabokines," reflecting its regulatory functions in energy homeostasis and food intake (Selvaraju V. *et al.*, 2022).

1.3.2.5. MRAP2

Loss-of-function *MRAP2* (Melanocortin 2 receptor accessory protein 2) pathogenic variants are related to monogenic hyperphagic obesity associated with hyperglycemia and hypertension. This contrasts with the other monogenic forms of obesity characterized by excessive hunger, including MC4R deficiency, that present generally with low blood pressure and normal glucose tolerance. The pleiotropic metabolic effect of pathogenic variants in *MRAP2* might be due to the functional defect of different protein-G receptors regulated by MRAP2 in various tissues including pancreatic islets (Baron M. *et al.*, 2019). A recent publication of 2023 highlights that MRAP2 is required for the localization of MC4R to the primary cilia and the function of MC4R neurons. This emerging knowledge provides new insights in recent theories linking energy homeostasis and primary cilia. In this perspective, further studies searching for candidate genes for human obesity should be also oriented to genes controlling localization of MC4R to primary cilia (Bernard A. *et al.*, 2020). As deficiency in MRAP2 partly impacts the MC4R pathway, the sub-sequent energy homeostasis dysregulation and obesity in MRAP2-deficient subjects might be theoretically treated by the MC4R agonist Setmelanotide (Baron M *et al.*, 2019). Further studies are needed to improve our knowledge on this field.

1.3.2.6. SIM1

SIM1 has been found as responsible of monogenic obesities in 2013 with the report of 13 *SIM1* variants in 28 unrelated patients with severe obesity. Nine of the first 13 variants identified have been shown to significatively reduce the activity of SIM1 and to co-segregate with obesity in extended family studies with nevertheless a variable penetrance. Since 2013, few reports have been dedicated specifically to SIM1 obesity. Consequently, no OMIM phenotype have been validated for now (Bonnefond A. *et al*., 2013).

Interestingly, animal models have investigated mice lacking specifically CREB-Regulated transcription coactivators, CRTC1 and CRTC2, in Sim1 cells (in the PVH) and demonstrated that CRTCs in Sim1 cells have a physiological role of gene expression regulation and suppress excessive fat intake, especially in female mice (Tanaka J. *et al*., 2021).

1.3.3. Therapeutic perspectives

More than an added value, the confirmation of monogenic obesity should imply therapeutic perspectives. For example, the bariatric surgery effectiveness in patients with monogenic obesity remains debated. Genetic alterations, such as MC4R dysfunction, disrupt appetite regulation, potentially explaining the observation of differences in the long-term outcome in percentage of weight loss in comparison to patients with a non-genetic obesity (Bonetti G. *et al.,* 2022). The new genetic knowledges have also opened the field for current and future therapies, as seen initially with the treatment of congenital leptin deficiency by recombinant leptin (Faccioli N. *et al*., 2023). *POMC, PCSK1, LEPR* deficiencies and Bardet-Biedl syndrome (*BBS*) can be counter-regulated by the MC4R-agonist Setmelanotide (Clément K. *et al*., 2020; Faccioli N. *et al*., 2023).

Setmelanotide was approved by the EMA in July 2021 and is currently available in some European country (IMCIVREE®) for adult patients and children older than 6 years of age. The current specific indications are either a genetically confirmed Bardet Biedl syndrome (BBS) or a deficiency in pro-opiomelanocortin (POMC), in proprotein convertase subtilisin/kexin type 1 (PCSK1) or in leptin receptor (LEPR). Clinical trials are still ongoing to extend the genetic indications to other genes involved in the MC4R pathway.

One remaining question is to determine the impact of MC4R agonists in patients with heterozygous *POMC* variants, identifying a targeted group of responders based on variant types. These variants affect protein cleavage pathways (ß-endorphins or α-MSH), paving the way for more specific targeted therapy research.

Therefore, improving genetic diagnosis for early-onset obesity will probably offer appropriate, preventive and dedicated care management according to the genetic etiology and its associated risks (e.g. ophthalmopathy, renal failure, metabolic disturbances…).

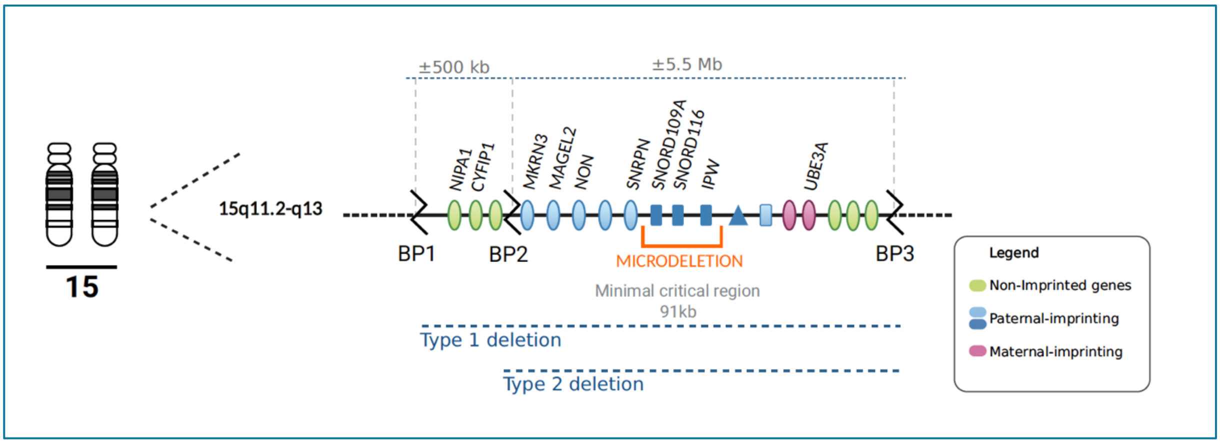
1.4. Syndromic early-onset obesity conditions and hypothalamic involvement

1.4.1. Prader Willi Syndrome versus ROHHAD

The similarity in disrupted systems in both ROHHAD and PWS, including the hypothalamus and autonomic nervous system (ANS), makes it plausible that the rapid-onset obesity observed in these two syndromes may derive from similar mechanisms, albeit resulting from distinct etiologies.

Prader-Willi syndrome (PWS) is a rare genetic neurodevelopmental disorder characterized by hyperphagic obesity, central hypogonadism, and growth hormone deficiency. It is caused by the loss of paternally expressed genes in an imprinted region of chromosome 15q (Figure 7). This loss of gene expression results either from deletions in the paternally inherited 15q11–q13 chromosomal region or from maternal uniparental heterodisomy (UPD). The 15q11.2–q13 region is subdivided by three common deletion breakpoints (BP). The proximal non-imprinted region between BP1 and BP2 contains four biparentally expressed genes: NIPA1, NIPA2, CYFIP1, and GCP5. The "PWS paternal-only expressed region" between BP2 and BP3 includes five protein-coding genes (*MKRN3, MAGEL2, NECDIN* (*NDN*), *SNURF-SNRPN*, and *C15orf2*), a cluster of C/D box small nucleolar RNA genes (snoRNAs), and several antisense transcripts, including the antisense transcript to *UBE3A* (Correa-da-Silva F. *et al*., 2021; Butler M.G., 2023).

Figure 7 – Summary of the genetic map of the 15q11.2-q13 region.



PWS is caused by loss of expression of paternally inherited genes located in chromosome 15. The delineation of the deletion establishes relation for the severity of the phenotype knowing that patients with a lack of expression of genes in the non-imprinted region are reported to present more serious neurodevelopmental symptoms. The map order was determined by the latest human genome assembly (UCSC Genome Browser,GRCh38/hg38) (Adapted from Burnett L.C. et al., 2017; F Correa-da-Silva. 2021; Butler M.G. 2023 and Driscoll D.J., 1993-2024).

These breakpoints define two main classes of deletions: type 1 (long deletion, BP1–BP3) and type 2 (short deletion, BP2–BP3). To delineate the critical region sufficient to cause the major physical and neuroendocrine phenotypes of PWS, five shorter microdeletions have been identified. Their overlap defines a critical 91 kb region containing three noncoding RNA genes: *SNORD109A*, *SNORD116*, and *IPW* (Burnett L.C. *et al*., 2017).

ROHHAD and PWS are clinically distinct conditions, although they share overlapping features, as summarized in Table 2. Both syndromes encompass the core signs defined by the ROHHAD acronym, but the pattern of symptom presentation differs. For instance, rapid weight gain occurs at different ages, and hypoventilation in ROHHAD is due to central dysregulation, whereas in PWS it is primarily associated with obstructive sleep apnea. PWS is a genetic condition consistently associated with intellectual disability, which is not a feature of ROHHAD.

Table 2 - Clinical comparison of ROHHAD and PWS phenotypes.

Clinical features	ROHHAD	PWS
Obesity	Yes *Over 3–12 months / Starting classically between 2-7yrs*	Yes *A more gradual trajectory. Age of onset 1-3 yrs.*
Hypoventilation	Yes *Central hypoventilation*	Sometimes *Obstructive and/or sleep apneas*
Hypothalamic dysfunction	Yes	Yes
Hyperprolactinemia	Yes	No
Hypothyroidism	Sometimes	Sometimes
Central adrenal insufficiency	Sometimes	Sometimes
Growth hormone insufficiency	Sometimes	Yes *GHD is responsible of the short stature feature of PWS patients.*
Altered pubertal onset	Sometimes *precocious*	Sometimes *delayed*
Autonomic dysfunction	Yes	Yes
Bradycardia	Sometimes	No
Gastrointestinal dysfunction	Yes	Sometimes
Thermal dysregulation	Yes *most typically as hypothermia*	Yes *hyper- or hypothermic easily occur*
Ophthalmologic manifestations	Yes *Strabismus*	Yes *Strabismus*
Altered pain perception	Yes	Yes
Neural crest tumors	Yes *50% of occurrence*	No
Seizures	Sometimes *Seizures generally in a context of hypoxemia*	Sometimes *Generalized seizure in a subset of PWS patients*
Neonatal hypotonia	No	Yes
Intellectual disabilities	No	Yes
Autism spectrum disorder	No	Yes
Behavioral disorder	Sometimes *Possibly linked to inadequate ventilation management*	Yes *Temper tantrums, stubbornness, rigidity, compulsiveness, controlling or manipulative, psychosis (5-10%)*
Sleep abnormalities	No	Yes

Both syndromes involve hypothalamic and autonomic dysfunctions. For each clinical feature, brief annotations highlight differences (*in italics*) in presentation between ROHHAD and PWS. PWS also includes characteristic neurodevelopmental signs, which may be absent or differ significantly in ROHHAD patients. Table adapted from Barclay S.F. *et al*.,2018; Burnett L.C. *et al*., 2017; Victor K. *et al*., 2023.

Despite these differences, the impaired development and function of the hypothalamus in both conditions have prompted comparative genetic studies. Extensive whole-exome sequencing (WES) analyses of ROHHAD patients have confirmed that the genetic etiology of ROHHAD does not overlap with that of PWS, as all major genes within the PWS region have been thoroughly investigated and excluded (Barclay S.F. *et al*., 2018).

In a recent study, dental pulp stem cells (DPSCs) derived from neurotypical controls, from ROHHAD, and from CCHS patients were differentiated into neuronal cultures and subjected to RNA sequencing (RNA-seq). The resulting transcriptomic profiles were compared with previously published data from PWS. This comparative analysis identified six genes significantly dysregulated in both ROHHAD and PWS compared to controls. Among these, three genes—*ID1, OAZ3*, and *CNN3*—were uniquely shared between ROHHAD and PWS, but not CCHS (Victor K. *et al*., 2023). ID1 is a transcriptional regulator, OAZ3 is involved in cell proliferation and maintenance, and CNN3 contributes to cytoskeletal organization through actin-binding. Although these genes were excluded from direct involvement in obesity pathophysiology due to their limited relevance to metabolic regulation, the shared transcriptomic signature between ROHHAD and PWS—both characterized by rapid-onset obesity and autonomic dysfunction with distinct evolutionary patterns— may suggest that research should be pursued to explore common molecular mechanisms underlying hypothalamic dysfunction in both conditions.

Furthermore, a recent publication analyzing the differentially methylated regions in PWS emphasize the need for continued investigation into epigenetic regulation and hypothalamic involvement in PWS (Salles J. *et al*., 2021). The pathophysiology of PWS remains not completely understood and may potentially overlap with the concept of a broader “ROHHAD spectrum,” warranting consideration in future comparative studies and perspectives of this research project.

1.5. ROHHAD

1.5.1. Definition of ROHHAD

ROHHAD Syndrome is a rare endocrine syndromic entity characterized by early onset hyperphagia and obesity, alveolar hypoventilation, dysautonomia, hypothalamic dysfunction and behavioral disorders. Central hypothyroidism, endocrine abnormalities, ionic disorders and respiratory failure may be associated with the syndrome. Less than 200 patients have been described in the literature to date. The major criterion is an excessive weight gain (approximately 9 to 13 kg over a period of 3 to 12 months) associated with alveolar hypoventilation appearing between the ages of 1.5 and 7 years in a previously healthy child. This rapid onset obesity is the first sign of hypothalamic dysfunction. At least one other clinical sign in favor of hypothalamic dysfunction is required for diagnosis, such as hyperprolactinemia, central hypothyroidism, diabetes insipidus, growth hormone deficiency, central corticotropic insufficiency, or pubertal developmental disorder. Dysregulation of the autonomic nervous system is mainly marked by central hypoventilation and may also manifest itself later in thermal dysregulation, excessive sweating, cardiovascular manifestations (arrhythmias, blood pressure regulation disorders), strabismus, abnormal papillary reaction to light or gastrointestinal or sensory disorders. Behavioral disorders (such as aggressivity) or mood disorders may be present and appear to be related to suboptimal ventilation. The risk of presenting neuroendocrine tumors in this syndrome is estimated to be about 50%; they are mainly ganglioneuromas or ganglioneuroblastomas with mainly intra-abdominal localization. Patients with an associated neuroendocrine tumor are referred to as ROHHADNET (J Harvengt, Orphanet, www.orpha.net/fr/disease/detail/293987).

1.5.2. Epidemiologic considerations in ROHHAD

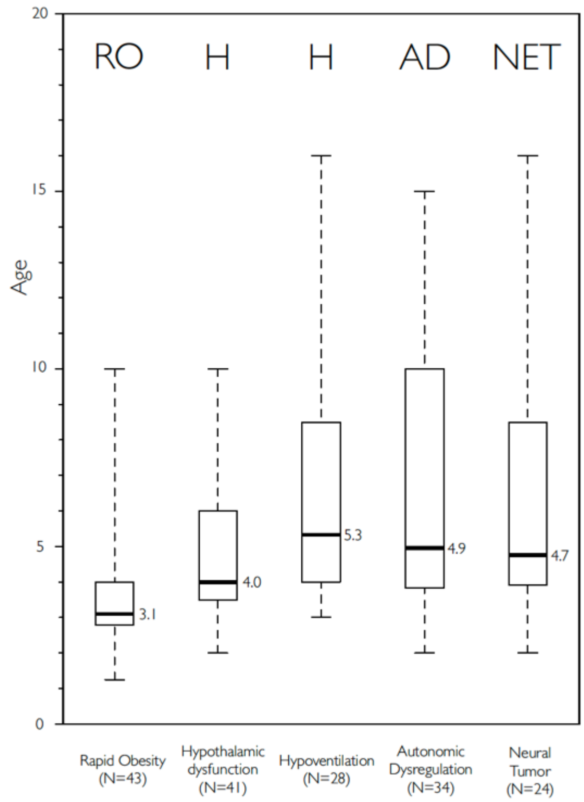
ROHHAD is now recognized as an ultra-rare disorder, with fewer than 100 cases described in the literature before 2020. Our systematic review, published in 2020, compiled data from 33 publications, from which 43 individual patient data were included in accordance with PRISMA guidelines. Although it is likely that the number of ROHHAD patients worldwide now exceeds 100, the precise prevalence remains difficult to determine. Our updated literature review, covering publications from 2020 through June 2025, identified 41 additional ROHHAD cases for which the individual data are provided. A summary and detailed description of this supplementary cohort is proposed below, in the section 4.2. The reported cases are globally distributed, with no apparent geographic clustering or predisposition ruling out a strict association with specific environmental factors.

An international ROHHAD consortium was established in December 2020 and continues to operate, holding biannual meetings. One of the consortium primary objectives is to develop a consensus statement on the clinical presentation and symptomatology of ROHHAD. This step is essential to refine diagnostic criteria and to define key elements for future registry-based follow-up. In this view, American and European registries try to collect data that may help to appreciate the global burden of ROHHAD disease, still unknown.

1.5.3. Clinical timeline of ROHHAD

ROHHAD syndrome is characterized by a sequence of clinical features emerging with advancing age, commonly accepted as appearing in the order of the acronym: rapid-onset obesity with hypothalamic dysfunction, hypoventilation followed by autonomic dysregulation syndrome (Barclay S.F. *et al*., 2018; Ize Ludlow D. *et al*., 2007). There is however no evidence for a systematic sequence of symptoms. ROHHAD syndrome has also been associated with a series of comorbidities or associated symptoms such as neural crest tumors (NET) (Bougnères P. *et al*., 2008; Calvo C. *et al*., 2019). Before 2020, the only large clinical description of a ROHHAD cohort described 15 patients in 2007, when the group of Ize-Ludlow *et al.* defined for the first time the term of ROHHAD disease (Ize Ludlow D. *et al*., 2007). In 2020, it appeared that no specific recommendation for the management or timing of the investigations for ROHHAD patients was available. Considering the aforementioned elements, we published a review in JCEM because although knowledge of the disease has improved since 2007, a comprehensive overview of the clinical timeline of the disease was still missing (Harvengt J *et al*., 2020; Barclay S.F. *et al*., 2015; Ize Ludlow *et al*., 2007). All the articles that meet the definition of ROHHAD(NET) and provide chronological clinical data were reviewed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis individual patient data guidelines (PRISMA guidelines). The data were grouped into 7 categories: hypothalamic dysfunction, autonomic dysregulation, hypoventilation, NET, psychiatric symptoms, other clinical manifestations, and outcome. We proposed for the first time a summary of the symptoms timeline that has been observed in all the published clinical cases (before 2020) (Figure 8).

Figure 8 - Rapid-onset obesity with hypoventilation, hypothalamic dysfunction, and autonomic dysregulation syndrome (neural crest tumor) (ROHHAD[NET]) general timeline.



Age of outcome for each specific term of the ROHHAD(NET) acronym. Box plots show the median values and the first and third quartiles for each group. T-bars represent the rest of the data. Outlier data were not removed. (adapted from Harvengt J. *et al*., 2020)

The initial event is systematically a rapid weight gain followed by a second hypothalamic dysfunction (Figure 8). Our description shows that hypoventilation appears at a median of 2.2 years (0-3.75 years) after the first sign of obesity. Because patients were not systematically evaluated with a polysomnography at the beginning of the pathology, knowing precisely when hypoventilation starts is highly difficult. We would recommend vigilance with such young patients with a weight curve showing a rapid weight gain without any other clear etiology. If the child also presents with an endocrinology disorder or a dysautonomic disturbance, a polysomnography seems to be a safe preventive exam to confirm ROHHAD syndrome. In case of high suspicion, the exam must be repeated annually for a minimum of 5 years. In our review, central hypoventilation was diagnosed for 83% of the patients during the first 5 years after the beginning of the obesity.

Hypothalamic dysfunctions were reported rapidly after the onset of obesity, with a median interval timing of less than 1 year (0.9 years). The principal hormonal dysregulation reported in the literature was hyperprolactinemia with values ranging from slightly elevated (44.7 ng/mL) to significantly increased (380 ng/ mL). Central hypothyroidism and GH deficiency were frequently reported, with GH levels almost undetectable after GH stimulation test (Abaci A. *et al*., 2013; Cemeroglu A.P. *et al*., 2016; Ibáñez-Micó S. *et al*., 2017; Kocaay P. *et al*., 2014; Kot K. *et al*., 2016; Lucas-Herald A.K. *et al*., 2012). Natremia disorders were a frequent observation with different types of water imbalance description. A series of patients were initially managed in the pediatric intensive care unit (N = 8). In this context, hypernatremia, hyponatremia, and syndrome of inappropriate antidiuretic hormone or diabetes insipidus were described (Barclay S.F. *et al*., 2015; Aljabban L. *et al*., 2016; Ibáñez-Micó S. *et al*., 2017; Jacobson L.A. *et al*., 2016; Jalal Eldin A.W. *et al*., 2019; Patwari P.P. *et al*., 2011; Sethi K. *et al*., 2014; Sumanasena S.P. *et al*., 2012). Some patients recovered but not all. Other patients presented initially with polyuria-polydipsia syndrome (N = 5), some of them requiring undergoing treatment with desmopressin (Graziani A. *et al*., 2016; Grudnikoff E. *et al*., 2013; Patwari P.P. *et al*., 2011).

Hypoventilation is a key symptom of the disease. Sleep apnea or a pattern of mixed apnea can be described initially. All the patients were treated initially with a nocturnal NIV. Tracheostomy was reported during acute decompensation (N = 5/12), and for some patients it was necessary to optimize the chronic ventilation (maximum 24 hours/day) (N = 7/12). One patient was mentioned as having a pacemaker diaphragm without a more precise specific description of his ventilation (Grudnikoff E. *et al.,* 2013). The management of ventilation is an essential point to avoid respiratory failure and the negative impact of hypercapnia, and to help control body mass index. Altered ventilation can be the cause of behavioral disorders or neurocognitive impairment (Ibáñez-Micó S. *et al*., 2017). Our data suggest such a link: the occurrence of aggressiveness or mood disorders was described mostly at a median of 1.5 years before the diagnosis and management of the hypoventilation.

Dysautonomia is a major clinical point in the syndrome. Impaired sweating and thermal dysregulation were principally reported. Hypothermia or hyperthermia can be objectivized, as mentioned in 15 cases in our cohort. Therefore, temperature instability reported in a young patient with obesity must lead to considering the possibility of ROHHAD syndrome. Notably, cold hands and feet were visible signs observed in 6 patients in the series.

Altered perception -mostly a decreased sensation - of pain is an essential point for which the clinician must be attentive. All the reported dysautonomia signs were independent of each other. Among them, strabismus and sweating alteration are the first 2 clinical signs encountered after initial weight gain.

NETs are classically diagnosed in approximately 50% of ROHHAD patients (Bougnères P. *et al.,* 2008). Our review consolidates this statistic with 56% of patients encountered with NET. Most of the tumors were GN located mainly at an adrenal location. All the patients were asymptomatic, except for one with a retro-pancreatic mass and a metastatic process causing weight loss and jaundice. The clinical presentation and characterization of neuroblastoma seems to be specific in ROHHAD patients compared to the general population with more indolent presentation and diagnosis at an older age (Shohet J. & Foster J, 2017; Swift C.C. *et al*., 2018). Nevertheless, only one ROHHAD patient was observed with a marker of pejorative prognostic factor and correlated with aggressive features at diagnosis.

1.5.4. ROHHAD in adult patients

At our knowledge, there are only 2 recent publications with adult data of ROHHAD patients diagnosed during childhood. The first mentioned particularities for airway management and obstructive apnea concurrent with central hypoventilation linked to severe obesity (Graziani A. *et al*., 2016). The second focused on the natural history of one 27-year-old ROHHAD patient who presented with fatty liver disease over time and a hepatocarcinoma at age 26 years (Jalal Eldin A.W. *et al*., 2019). In the total cohort from our review of 43 patients in 2020, no other case was described with hepatic disturbances during childhood. The patient described by Jalal-Eldin *et al.* was also the only one with reported diabetes diagnosed at age 14 years. Lipid metabolic disturbances were noted for 2 patients (hypertriglyceridemia for one and hypercholesterolemia for the other). With regards to improving the management of metabolic disturbances, further descriptions from young ROHHAD adult patients would be useful.

In the January 2024 issue of ERJ Open Research, the group of Á. Ortega-González *et al.* published the world's first clinical description of a ROHHAD syndrome case with an adult onset in a patient aged of 57 years at diagnosis. The clinical description fulfills the criteria for ROHHAD disease except for the age of onset. This female adult patient started a major weight gain at 57 years with pathologic hyperphagia (+30kg in 3 months, with initial BMI at evaluation at 29.7 kg/m^2). The obesity was rapidly followed by asthenia, hypersomnia, syncopal episodes, muscle weakness, gait instability and strabismus. The medical work-up confirmed a severe OSA requiring NIV. The ventilation management kept challenging, and a central hypoventilation was confirmed a few months later. The endocrinological assessment found central hypothyroidism, central adrenal insufficiency, hyperprolactinemia and hypogonadotropic hypogonadism. A progressive basal bradycardia was reported as the only one cardiological alteration. The behavior was described as modified with a neurocognitive impairment with a lack of impulse control that was never described before for her. Importantly, the patient had no previous medical history except a follow up in a context of chronic autoimmune hepatitis, recent menopause and inactive multinodular goiter (Ortega-Gonzalez Á. *et al*., 2024).

Figure 9 – Description of the clinical evolution of the first ROHHAD patient described with an adult onset.

Initial Symptoms
Hyperphagia
Rapid weight gain (30 kg in 3 months)
Hypersomnia
Gain instability
Strabismus

Medical History
Autoimmune pancreatitis
Menopause transition

First clinical evaluation
Mild motor impairment
Shoulder girdle muscles
Transitory left oculomotor nerve palsy

First Hospitalization
•MRI (brain-spinal cord) and Thoraco-abdominal CT: No relevant findings
•Electroencephalogram: No specific diffuses lowing activity, bitemporal irritative activity during sleep.
•Biology: tumor markers, βHCG, Ig ,ACE and serologies (HIV,Hepatitis-A,B,C-,EBV,CMV,HSV),treponemal tests: normal or negative. VZVIgM/IgG:positive.
•Autoinmune panel: positive ANA-HEp-2(1:640, positive Anti-dsDNA, hypocomplementemia(C3/C4): In relation to autoimmune hepatitis.
Onco-neural, cell neuronal surface antigens (serum/CSFincluding mGluR1/R5,DPPX,Neurexin3,IgLON5)and AChR/anti MuSK antibodies and porphyrins: All negative.
•Cerebrospinal fluid pattern: unspecific with negative ganglioside antibodies, cultures and routine serologies.

Endocrinological assessment
Central hypothyroidism ,central adrenalin sufficiency , hyperprolactinemia and hypogonadotropic hypogonadism.
R/ Hydrocortisone and Levothyroxine
Psychiatric evaluation
Neurocognitive impairment with lack of impulse control.
Control ultra-high-field brain MRI and abdominal CT
Showed no changes.
Genetics
PHOX2B : No pathogenic variant.

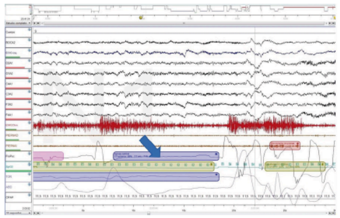
♀57 years
ONSET OF SYMPTOMS

Syncope - Second hospitalization
Severe Hypercapnic acidosis
Intubation

SUDDEN DEATH
32 months

6 months
3 months
START NIV
23 months

Outpatient follow-up
Hyperphagia and increasing obesity persisted together with episodic daytime sleepiness lasting from seconds to several minutes.
•Polysomnography: AHI:54/hour,CT90: 11.4% and Titration Polysomnography (CPAP9.5cmH2O): AHI:13.4/hour,CAI:2.1/hour,Sleeplatency:1min,REM latency:264min



Central event in polysomnography of the patient

•Narcolepsy evaluation :Discarded by MSLT,HLA-DQB1*06:02/DQA1*01:02 absence and no response to Modafinil course.
•Electromyogram: Mild proximal myopathic changes.
•Phrenic nerve conduction: normal.

°The patient requires NIV with 50% of daily hours support
°Persisting recurrent episodes of day time sleepiness with extreme desaturation and episodic bradycardia

Therapeutic option
Gamma globulin infusion (4gr/kg/day(twice): Negligible response

Patient asleep during day time with oxygen desaturation

Result obtained in Postmortem : ZSCAN1 positive

Clinical timeline since the onset of the symptoms in a 57 year-old female and her sudden death 32 months after the initial symptoms management. Pictures and clinical data reproduced with the permission and collaboration of Dr Angel Ortega-Gonzalez (Based on the poster presented by Dr Angel Ortega-Gonzalez at the ATS 2022, San Francisco).

The detection of ZSCAN1 antibodies was positive in her CSF establishing the world's first ever detection of these antibodies in an adult-onset ROHHAD syndrome case. This observation strengthens the hypothesis of an immune-mediated pathogenesis of the syndrome.

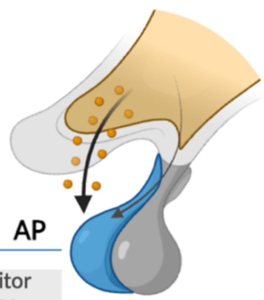
Together with the clinical description of this case, these findings may encourage to define new diagnostic criteria including the ZSCAN1 biomarker and the expansion of the definition of ROHHAD to adult patients. This new finding strongly highlights the need of searching for ROHHAD among obese adult populations with severe hypoventilation and central features of unknown origin.

1.5.5. The Hypothalamus: central disruption in the pathophysiology of ROHHAD

1.5.5.1. Comprehensive Overview of Hypothalamic Anatomy and Neuroendocrine Regulation

The hypothalamus is recognized as the central coordinator of the endocrine system. It integrates a wide array of signals originating from higher cortical regions, autonomic centers, and environmental stimuli such as light, temperature, and food intake, as well as feedback from peripheral endocrine organs. In response, the hypothalamus transmits highly specific signals to the pituitary gland, which subsequently releases hormones that regulate nearly all endocrine systems.

Figure 10 – Pituitary gland: schematic overview.



hormone-secreting endocrine cells	Hormone	Progenitor lineage
Lactotrophs	PRL	POU1F1
Somatotrophs	GH	
Thyrothrophs	TSH	
Corticotrophs	ACTH	TBX19
Melanotrophs	MSH	
Gonadotrophs	LH & FSH	NR5A1

(Original figure based on Willis T.L. *et al.*,2022; Tadross J.A. *et al.*, 2025)

Created with BioRender.com

The pituitary gland is anatomically divided into two main components: the posterior pituitary (PP, neurohypophysis) and the anterior pituitary (AP, adenohypophysis) (Figure 10). The PP comprises axon terminals that originate in the hypothalamus and extend through the pituitary stalk. Its main functions are to store and secrete antidiuretic hormone (ADH) and oxytocin, both synthesized by hypothalamic neurons. Additionally, the PP relays stimulatory signals from the hypothalamus to the AP, facilitating hormone release (Willis T.L. *et al*., 2022).

The AP contains the majority of hormone-secreting endocrine cells including: lactotrophs (secreting prolactin, PRL); somatotrophs (secreting growth hormone, GH); thyrotrophs (secreting thyroid-stimulating hormone, TSH); gonadotrophs (secreting luteinizing hormone, LH, and follicle-stimulating hormone, FSH); corticotrophs (secreting adrenocorticotropic hormone, ACTH). In mice, melanotrophs are located in the intermediate lobe (IL) and secrete melanocyte-stimulating hormone (MSH), whereas all other differentiated endocrine cells reside within the anterior lobe (AL) of the AP. These hormone-producing cell types originate from three distinct progenitor lineages, each defined by specific transcription factors: POU1F1 (previously PIT1)(somatotrophs, lactotrophs, and thyrotrophs); TBX19 (previously TPIT) (corticotrophs and melanotrophs); NR5A1 (previously SF1) (gonadotrophs) (Willis T.L. *et al*., 2022).

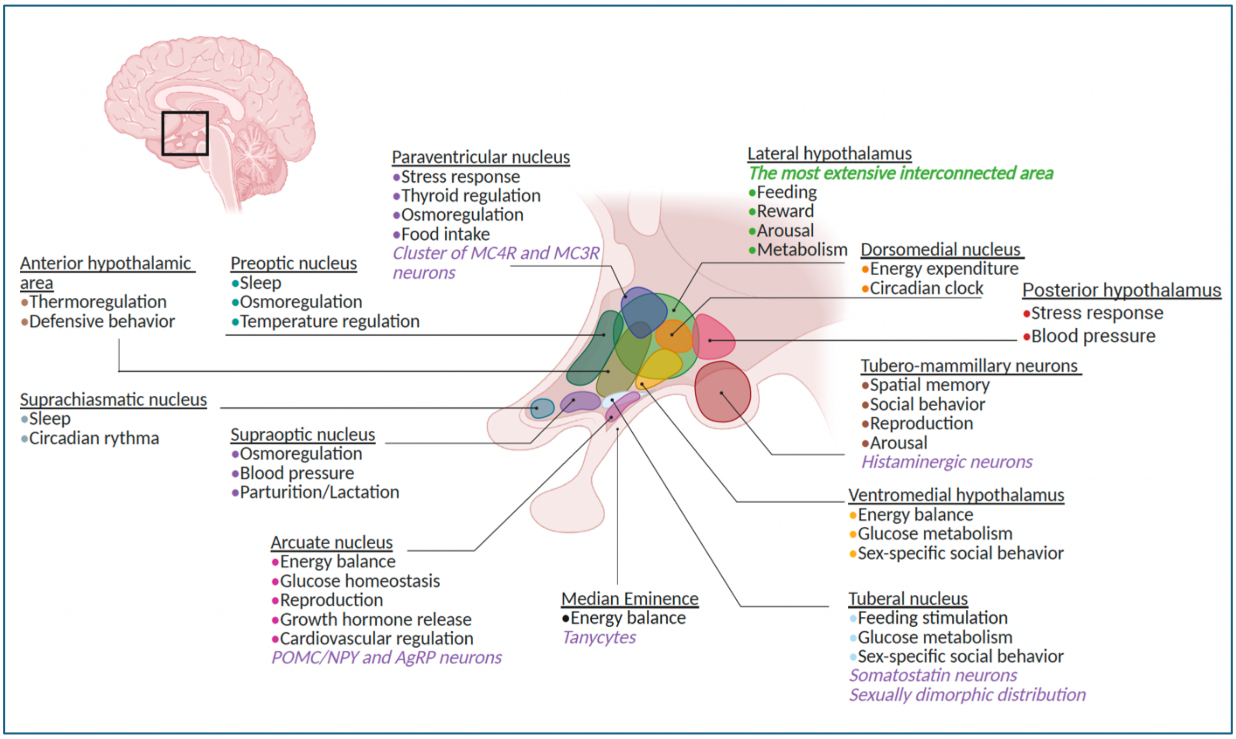
From an anatomical perspective, the hypothalamus is situated at the base of the brain, beneath the third ventricle and directly above the optic chiasm and pituitary gland (Figure 11). Afferent projections to hypothalamic nuclei originate from the brainstem, thalamus, basal ganglia, cerebral cortex, and olfactory regions. Main efferent pathways include the dorsal longitudinal fasciculus, connecting the hypothalamus to brainstem reticular centers, projections to the autonomic nervous system and thalamus and a hypothalamo-neurohypophysial tract, linking the paraventricular and supraoptic nuclei (which produce ADH and oxytocin) to nerve terminals in the median eminence and the posterior pituitary (Nakamura. 2022).

Since recently (Tadross J.A. *et al*., 2025), HYPOMAP provides a detailed atlas of the human hypothalamus in a spatial context. Out of the 452 hypothalamic known cell types, 291 neuronal clusters are showed to be significantly enriched for expression of body mass index (BMI) genome-wide association study genes. This enrichment is specifically driven by 426 'effector' genes. Rare deleterious variants in six of these genes (*MC4R, PCSK1, POMC, CALCR, BSN* and *CORO1A*) associate with BMI at population level, but *CORO1A* has not been linked previously to BMI. Importantly, conservation of neuronal cell types between humans and mice, as based on transcriptomic identity, is generally high.

But notable exceptions have been highlighted through this new human map research. Specifically, significant disparities exist in the identity of POMC neurons and in the expression levels of G-protein-coupled receptors between the two species, human and mice. These disparities carry direct implications for current pharmaceutical studies involving the MC4R and POMC pathway based on mouse models.

The internal structure of the hypothalamus is classically described based on the division on twelve nuclei distributed across the four anatomical regions of the hypothalamus: preoptic, anterior (supraoptic), tuberal (middle), and mammillary (posterior) (Figure 11).

Figure 11 - Schematic anatomic representation of the hypothalamic nuclei, their specific functions and main neuronal populations.



The lateral hypothalamus (LH) is represented as the most extensive interconnected area explaining its predominant role as hypothalamic nuclei. Details for LH role and orexin neurons are developed in the Figure 12. The arcuate nucleus is the specific area for POMC/NPY and AgRP neurons, details are provided in Figure 6. Importantly, cluster of MC4R and MC3R neurons are identified in the paraventricular nucleus but they have also a wide repartition in the global hypothalamic area.

Figure adapted from Tadross J.A. *et al*., 2025; Burdakov D & Karnani M.M. 2020; Kaitlyn H. *et al*., 2022; Krieger, 1980; Bolborea M. *et al*.,2013, Placzek M. *et al*.,2025. Figure Created with BioRender.com.

Among these twelve nuclei, the ARC is a critical region for energy homeostasis and reproduction. ARC contains neuronal cells, but also glial cells as discussed above in our section dedicated on hypothalamic inflammation and behavioral food disturbances. It has notably been shown that selective Ca2+ activation of glia in the mouse arcuate nucleus (ARC) reversibly induces increased food intake while disruption of Ca2+ signaling pathway in ARC glia reduces food intake (Chen N., 2016). These findings highlighted the role of glial cells and the multiple cellular networks involved in the appetite regulation. These findings also suggest a possible causal link between increased ARC glial Ca2+ during astrogliosis and hyperphagia during ARC inflammation (details in the section: hypothalamic inflammation in the pathogenesis of early onset obesity) (Kanemaru *et al*., 2013; De Souza et *al*., 2005).

The paraventricular nucleus (PVN) relays information from the hypothalamus to the body. PVN^{Trh} neurons release thyrotropin-releasing hormone to the pituitary to control the hypothalamic–pituitarythyroid axis. PVN^{Crh}-expressing neurons are the central regulators of the hypothalamic–pituitary–adrenal (HPA) axis being part of the critical stress response regulation through the modulation of the cortisol levels. PVN^{MC4R} neurons receive inputs from hypothalamic nuclei such as ARC and promote feeding (Hajdarovic K.H. *et al*., 2022).

The tuberal nucleus anatomically encircles the third ventricle of the brain. Feeding behavior is regulated within this nucleus through the direct action of ghrelin, as well as via mechanisms driven by peripheral hunger signals. Whether this regulation is sexually influenced remains an open question, especially considering recent findings that the tuberal nucleus exhibits a sexually dimorphic distribution, which may underlie sex-specific behavioral patterns. Notably, leptin and a high-fat diet have been shown to modulate various aspects of tuberal cell specification, including neurogenesis, axon guidance, and synaptic connectivity (Placzek M. *et al*., 2025). These findings underscore the broader impact of inflammation across multiple hypothalamic regions.

Median eminence plays also a key role in the appetite regulation due to the presence of the tanycytes, glial-like cells that line the third ventricle. Tanycytes are glucosensitive and are able to respond to transmitters associated with arousal and the drive to feed. At least some tanycytes are stem cells and, in the median eminence, may be stimulated by diet to generate new neurons (Bolborea M. *et al.*, 2013).

The lateral hypothalamic area (LH) is a vital controller of arousal, feeding, and metabolism. Burdakov and Karnani showed that there is near-zero local connectivity in the LH, suggesting that incoming synaptic input is integrated primarily within individual neurons (Burdakov D. & Karnani M.M., 2020). These results suggest that input from other brain structures is decisive for selecting active populations in the LH. Consequently, coordination of activity in upstream networks is required for the rapid and coordinated activity of LH neurons during behavior control, reward process and feeding regulations. The lateral hypothalamus (LH) is also the most extensively interconnected area of the hypothalamus, allowing it to control and convey a variety of essential autonomic and somatomotor functions (Burt J. *et al*., 2011; Fukushi I. 2025). Such extensive connectivity is thought to represent the anatomical support that drives sleep-wake regulation, energy homeostasis, as well as cognitive, reward-related, and emotion-related functions. The function of the LH can be mainly attributed to orexin neurons that synthesize orexin A and B (also called hypocretin-1 and -2), respectively 33 and 28 amino acid peptides, cleaved from the precursor protein prepro-orexin (Burt J. *et al*., 2011).

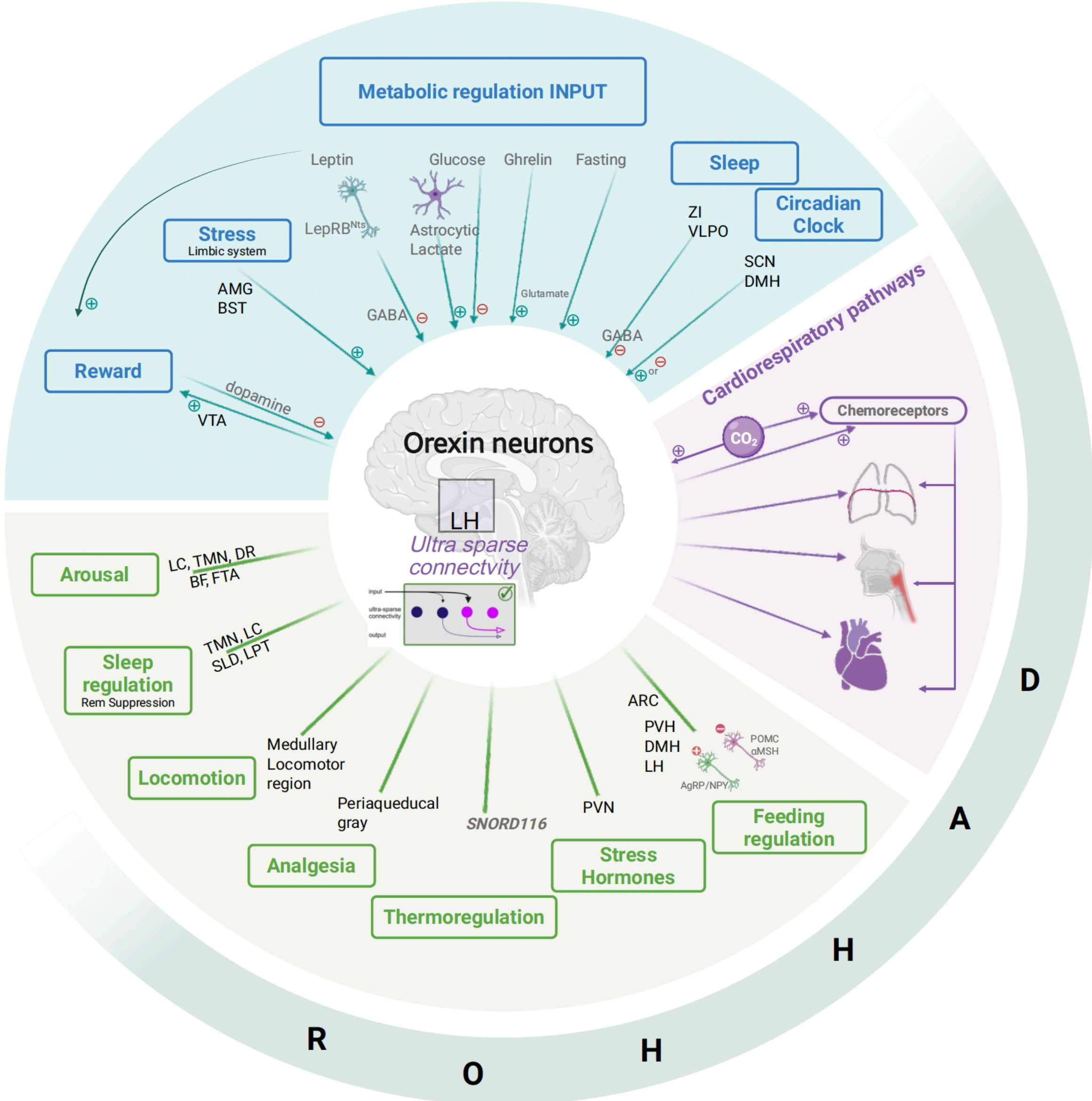
1.5.5.2. Orexins neurons and hypothalamic network (face to ROHHAD symptoms)

1.5.5.2.1. Orexin neurons

Orexin (hypocretin) neurons, through their pivotal roles in energy balance, cardiorespiratory regulation, sleep–wake cycles, neuroendocrine and autonomic outflow, stress responses, analgesia, and behavioral responses to food reward and addictive drugs, exemplify the intricate architecture of hypothalamic networks. A comprehensive overview of the orexin system provides critical insights into the neurobiological mechanisms potentially underlying the clinical manifestations of ROHHAD and offers theoretical hypotheses to guide future investigations (Figure 12).

The orexin neurons represent a relatively small population of neurons; however, they can coordinate diverse physiological functions. Indeed, despite their small population, they have extensive projections throughout the brain and spinal cord, allowing them to influence multiple systems simultaneously and amplifying their impact. Furthermore, the local network of orexin neurons allows them to stay at an active state (intrinsically in a depolarized state) and/or to recruit a larger number of orexin neurons, using excitatory neurotransmitters, such as orexins and glutamate. Orexin A or B activate OX2-receptors (OX2Rs), which in turn open nonselective cation channels to depolarize orexin neurons (Burt J. *et al*., 2011).

Figure 12 - Orexin Neurons – A Schematic Overview of Broad Anatomical Connections and Functional Regulation



This figure provides a detailed schematic representation of the orexin neuron system, illustrating its principal inputs and outputs, and its role in various physiological processes. Orexin neurons are located in the lateral hypothalamus (LH), where they exist in small numbers but exhibit high responsiveness to a wide range of inputs, enabling activation of diverse efferent pathways. A distinctive feature of the LH is its ultra-sparse neuronal connectivity, which contributes to the specificity of orexin signaling.

Orexin neurons operate at the intersection of sleep regulation, reward processing, and energy homeostasis. They also serve as a critical link to cardiorespiratory and autonomic regulatory systems.

The pink-shaded area of the figure summarizes the cardiorespiratory homeostatic reflex pathway, emphasizing the role of CO_2 chemoreceptors. Central chemoreceptors, which are distributed across various regions of the hindbrain—including the LH—receive input from orexin neurons and directly regulate CO_2-sensitive neurons (Nattie E. & Li A., 2012). This regulation influences diaphragmatic movement frequency, modulates pharyngeal dilator activity, and governs vascular tone and heart rate (Kuwaki T., 2015).

The primary inputs to orexin neurons are depicted in the blue-shaded area. The limbic system activates orexin neurons in response to perceived risks, initiating a stress response. The reward system interacts with orexin neurons via direct dopaminergic regulation. During sleep, orexin neurons are typically silent, likely due to inhibition by both local and long-range GABAergic inputs. Metabolic inputs originate from multiple sources. Leptin plays a central role in orexin-mediated appetite regulation by acting on LEPRBNts-specific neurons, which inhibit orexin neurons through GABAergic signaling (Leinninger G.M. *et al.,* 2019). In states of negative energy balance, orexin neurons are activated to promote arousal and food-seeking behavior. They are stimulated by fasting and directly by ghrelin, which acts through complex amplitude-sensitive mechanisms, while glucose and leptin inhibit their activity (Arrigoni E. *et al*., 2019). The importance of leptin signaling via LepRb Nts neurons in body weight regulation has been demonstrated in studies using Nts-LepRbKO mice, which fail to reduce body weight in response to acute leptin administration. However, glucose exerts a dual effect: rapid increases in extracellular glucose inhibit orexin neurons, while astrocytes locally convert glucose into lactate, which subsequently excites orexin neurons.

The main outputs of orexin neurons are illustrated in the green-shaded area, showing their extensive connections to many physiological systems. These outputs support stress responses and modulate thermoregulation, pain sensitivity, and feeding behavior. Orexin peptides directly excite arcuate NPY neurons and indirectly inhibit arcuate POMC neurons by increasing GABAergic afferent input and reducing glutamatergic input (Arrigonia *et al.,* 2019). The regulatory pathways governing orexin neuron activity may be relevant to clinical manifestations observed in ROHHAD syndrome.

LH – Lateral hypothalamus; LC – Locus Coeruleus; DR – Dorsal Raphe nucleus; BF – Basal Forebrain; FTA – Frontal Temporal Area; SLD – SubLaterodorsal nucleus; LPT – Lateral Paragigantocellular nucleus; PVN – Paraventricular Nucleus; ARC – Arcuate Nucleus; DMH – Dorsomedial Hypothalamic nucleus; SCN – Suprachiasmatic Nucleus; ZI – Zona Incerta; VLPO – Ventrolateral Preoptic nucleus; VTA – Ventral Tegmental Area – TMN - tuberomammillary nucleus;

(Based on Tsujino N., 2009; Arrigoni E. *et al*., 2019; Kuwaki T., 2015; Nattie E. & Li A., 2012; Leinninger G.M.*et al.,* 2019; Flores A. ,2013; Kuwaki T., 2021; Giardino W.J. ,2014)

(Created with BioRender.com and Corel Draw)

1.5.5.2.2. Stem cells and emerging research areas in hypothalamic studies

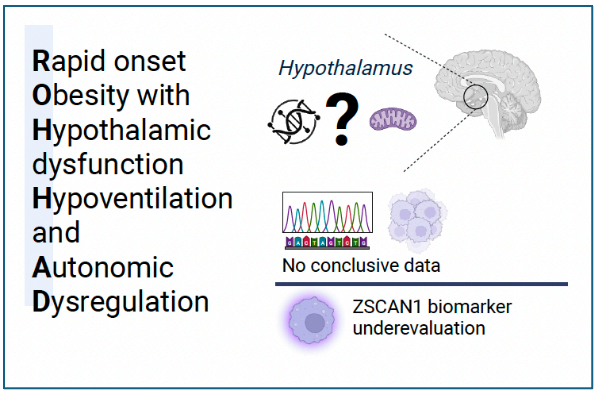
To give a complete overview of the different cell type involved in the hypothalamic regulatory network, stem cells appear also to be one of the important signaling actor within the anterior pituitary (Willis T.L. *et al*., 2022). Russel *et al.* demonstrated that progenitor multiplication depends on the paracrine WNT secretion from SOX2+ primary stem cells. Their results indicate that stem cells can hold additional roles in tissue expansion and homeostasis, acting as paracrine signaling centers to coordinate the proliferation of neighboring cells (Russel J.P. *et al*., 2021). Cellular networks and communication between stem cells represent a vast field of investigation that should be more easily assessable in the near future thanks to the optimization of the in-situ sequencing and snRNAseq technologies coupled with accurate functional validation tests (Hajdarovic K.H. *et al*., 2022).

Moreover, a new classification of transcription factors (TFs) has emerged whereby they can be termed 'pioneer' if able to modulate local condensed chromatin structure to gain access to otherwise inaccessible genomic regions. Pioneer TFs, either alone or together with co-factors, unwind heterochromatin revealing underlying specific binding motifs and might be involved in etiological process for still unresolved condition, such as ROHHAD (Willis T.L. *et al*., 2022).

1.5.6. ROHHAD: Unknown etiology

The etiology of ROHHAD Syndrome is currently not determined. Hypotheses have been put forward with the possibility of genetic mechanisms, paraneoplastic and/or autoimmune processes or epigenetic alterations.

Figure 13 –ROHHAD unknown etiology: illustrative box.



The **genetic hypothesis** has been investigated by several teams since 2007 without finding a specific candidate gene. Genes involved in respiratory control, autonomic control and neuronal plasticity (*NECDIN, ASCL1, PHOX2B, TRKB, BDNF* genes) had been first investigated, with no significant results identified (De Pontual L. *et al.*, 2008; Barclay S.F. *et al.*,2015). In 2011, CM Rand *et al.* published a study based on the analysis of candidate genes (Rand C.M. *et al.*, 2011). The choice was made to analyze 3 genes: the *HTR1a* gene (OMIM#109760), serotonin receptor (role in the regulation of appetite, energy and cardiorespiratory control), *OTP* (OMIM#604529; role in cell specification during the development of the hypothalamus) and the *PACAP* gene (OMIM#102980) expressed at the hypothalamus level (role in the management of cardiorespiratory regulation and energy metabolism control). No significant difference between the variants identified in ROHHAD patients and control patients could be demonstrated for these three genes. After that, SF Barclay *et* directed their genetic analyses in a cohort of 16 patients towards the study of the *HCRT, HCRTR1* and *HCRTR2* genes involved in the hypocretin pathway *al* (Barclay S.F. *et al.*, 2015). The question of a mechanism related to narcolepsy-cataplexy arose following the observation of a patient with a ROHHAD syndrome associated with narcolepsy confirmed by a hypocretin deficiency in cerebrospinal fluid (Dhondt K. *et al.*, 2013). No pathogenic variant could be identified in *HCRT, HCRTR1* and *HCRTR2*, only common polymorphisms in the general population were observed. In 2015, a team from Chicago published data on exome analysis carried out on a homogeneous cohort of 35 ROHHAD patients (Barclay S.F. *et al.*, 2015). They looked for de novo mutations in the coding sequences based on an initial analysis of 7 patients and their parents (trio analyses). They added the analysis of 4 tumor samples from these 7 patients and the analysis of the exome of an unaffected monozygotic twin. From their analyzed data, they identified 13 genes of interest and searched in the entire cohort for potentially pathogenic variants in these genes. No gene of interest was retained at the conclusion of this study, apart from the *c17orf53* gene but with limitations in the molecular interpretation since the variants were identified only in patients with no additional data to determine the inherited status or not of these variants. Somatic comparisons were also performed in this study revealing no coding differences between any blood and tumor samples or between the two discordant monozygotic twins included in their cohort. More recently, an exome sequencing study involving 33 individuals (33 families including 27 trios, 1 duo and 5 singletons) with ROHHAD looking at variants under monogenic inheritance did not find variant of interest. (Iannello G. *et al.,* 2021). Another high-depth whole-genome sequencing study involving 40 individuals with ROHHAD looked also at candidate variants under monogenic inheritance models. The WGS enhances the ability compared to WES to capture non-coding genetic variation and improved capacity to detect structural and copy number variants. Considering these technological advantages, an Italian team performed also trio WGS on two ROHHAD families (Grossi A. *et al*., 2023). Nevertheless, none of these WES and WGS studies was able to identify a valuable candidate gene (Khaytin V. *et al*., 2023). In addition, several recent published case reports (after 2020) have also reported performing WES in individuals diagnosed with ROHHAD without identifying any clinically pathogenic variants (Roby P. *et al*., 2023; Marpuri I. *et al.,* 2021; Priyadarshini S. *et al*., 2024; Desse B. *et al*., 2022).

The evidence arguing for a **paraneoplastic and/or autoimmune hypothesis** for ROHHAD includes the presence of neural crest tumors, spinal fluid inflammation markers, brain inflammation at autopsy, concurrent autoimmunity, immunotherapy response, and, most recently, identification of a putative antibody biomarker, ZSCAN1.

The presence of neural crest tumors is a feature also encountered in opsoclonus myoclonus syndrome (OMS), a well-established paraneoplastic syndrome in which an immune response, presumed targeting the neural crest tumor, is believed to cross-react with the cerebellum leading to damage in pathways that regulate oculomotor function and coordination. Currently, anti-neuronal antibodies are inconsistently detected in OMS and a common single antigen has not yet been identified (Khaytin V. *et al*., 2023). Knowing that, the physiopathology of ROHHAD and OMS might be considered as similar despite different phenotypes. Both diseases are considered to result from the involvement of different CNS regions, with the targeting of the brainstem and hypothalamus in ROHHAD (Gorman M.P., 2010). Patients with OMS overlapped with ROHHAD in term of findings common to paraneoplastic syndromes including young age of onset in previously healthy children, the presence of CNS inflammatory markers reported for a series of cases, associated low-grade neural crest tumors in around half of the patients, and a description of variable response to immunosuppression (Mitchell W.G. *et al*., 2015; Sartori S. *et al*., 2014; Lee J.M. *et al*., 2018). Also, the ROHHAD phenotype, like OMS in most cases, continues to unfold even after surgical resection of the tumor or continue with a variable evolution after receiving immunomodulatory therapy.

Autoimmune associations have been initially retained based on observations, particularly the detection of oligoclonal bands in the cerebrospinal fluid of four patients (2 cases in the cohort published in 2020, and 2 patients published after 2020) (Sartori S. *et al*., 2014; Gharial J. *et al*., 2020; Aldirawi M. *et al*., 2023) and a case described with post-mortem lymphocyte infiltration in the hypothalamus (Sethi K. *et al*., 2014). Some patients are also described with a favorable initial response to immunosuppressive treatment such as Endoxan° or Rituximab° (Paz-Priel I. *et al*., 2011; Giacomozzi C. *et al*., 2019; Hawton K. *et al*., 2022). There are currently no published patients with long-term follow-up after immunosuppressive treatment, all the cases described the initial course of the disease and the evolution of the patients few months after therapy. Interestingly, for the case described in 2022, elevated interleukin-6 levels were detected by cytokine serology, and the level normalized after rituximab treatment (Hawton K. *et al*., 2022). Furthermore, after rituximab treatment, the patient weight decreased significantly in 12 months. The therapy is generally administrated in the acute phase, during the first devastating symptoms (severe alteration of ventilation parameters or major ionic disturbances requiring NICU management) and there is no report of chronic immunosuppressive therapy.

Another study shows a positivity of anti-hypothalamus (AHA) and anti-hypophysis (APA) antibodies on serum and CSF (Giacomozzi C. *et al*., 2019). This study was carried out on post-mortem samples from a 3-year-old ROHHAD patient with a severe clinical picture described as a ROHHAD syndrome associated with narcolepsy-cataplexy. These results should be considered with caution given the difficulty of interpretation for AHA and APA antibody measurements, their poor reproducibility and low sensitivity (Cocco C. *et al*., 2017). There are also reports of the identification of anti-Nax and anti-SFO antibodies in patients diagnosed with ROHHAD syndrome as it has been found in patients with autoimmune hypernatremia, justifying a discussion of a possible common autoimmune mechanism for the two conditions (Nakamura-Utsunomiya A., 2022).

Additionally, concurrent autoimmunity has been reported in ROHHAD for 1 patient, occurring 5 years after the initial rapid weight gain (Cemeroglu A.P. *et al*., 2015). A few reports have described onset of ROHHAD following primary infectious disease leading to consider a possible infectious trigger in the physiopathology of ROHHAD (Gharial J. *et al*., 2021; Mandel-Brehm C. *et al*., 2022). Autoimmunity-predisposing alleles have also been found within the HLA-DQ complex in five out of nine

ROHHAD patients investigated in a single study (De Pontual L *et al*., 2008). Since 2009, no supplemental data have been published to enhance statistical conclusions related to HLA-complex locus of susceptibility. In this view, HLA-DQ complex analysis is not being part of the initial evaluation of ROHHAD patients.

Investigations by high-resolution MRI or functional MRI focused on the hypothalamic-pituitary region may give information on the presence of localized inflammatory lesions. This examination requires access to high-tech equipment and examination conditions that must be acceptable for pediatric patients. Such data have not yet been published. Only one case report described a case with mild brain inflammation suspected by single photon emission computed tomography in a 12-year-old patient (Hasuike S. *et al*., 2024). But postmortem analyses for the same patient (died at 21 years) revealed no obvious inflammation of the pituitary gland or hypothalamus. A total of 5 autopsy descriptions has been identified in ROHHAD dedicated publications. These autopsies revealed hypothalamic lesions for two out of the five descriptions. The case of Gharial *et al*. presented specifically hypothalamic infiltrates in multiple regions, including the anterior, paraventricular, arcuate, and ventromedial nuclei. Overall, perivascular lymphocytic infiltration distributed in the brain are a common feature of ROHHAD autopsies reports and have notably also been described in patients with OMS (Bougnères P. *et al*., 2008; Sethi K. *et al*., 2014; Gharial J. *et al*., 2021; Hasuike S. *et al*., 2024).

The recent discovery of a potentially reliable antibody biomarker, ZSCAN1 (*see section*: ZSCAN1 as a new biomarker) detectable in both cerebrospinal fluid and serum of ROHHAD patients, provides further support for the autoimmune hypothesis.

The hypothesis of an **epigenetic mechanism** was essentially and firstly suggested by the observation of a patient with an unaffected monozygotic twin (Patwari P.P. *et al*., 2011). For monozygotic twins, the accumulation of variations in their epigenome throughout their lives is the factor that mainly influences their phenotype difference. However, this phenotype difference might also be due to a very early-onset mosaicism during the first steps of the embryogenesis. Nevertheless, no coding differences have been currently identified between the two discordant twins (Barclay S.F. *et al*., 2018).

Epigenetic mechanisms have a well-established role in the genesis of cancers and might be involved in the genesis of the neural crest tumors of ROHHAD patients. Such studies were not considered until now. Epigenetics is also increasingly studied for different pediatric chronic pathologies (Thürmann L. *et al*., 2023) or genetic conditions revealing epigenetics patterns that may be helpful in diagnostic setting. For example, the Prader Willi Syndrome (PWS) is a well-known condition in which genes are subject to parental imprinting. A study has carried out the sequencing of the genes included in the PWS region, without identifying any pathogenic variants in patients with ROHHAD syndrome

(Barclay S.F. *et al.*, 2018). However, this study did not analyze the expression of these genes in ROHHAD patients, which can be altered by epigenetic mechanisms.

Finally, the onset of ROHHAD condition a few years after birth may indicate a potential environmental impact that would influence gene expression through methylation processes occurring in the early life (Sivakumar S. *et al.*, 2024).

Considering the whole current knowledge, the paraneoplastic/autoimmune mechanism should be retained as a potential initiator of the pathology in ROHHAD patients. A genetic or an epigenetic susceptibility to these autoimmune phenomena is an additional possible hypothesis to consider. And the trigger who would lead to the disease in these rare patients would also be to determine.

1.5.7. ZSCAN1 as a new biomarker

ROHHAD remains a particularly challenging diagnosis due to the lack of reliable biomarkers. Several research groups have explored the hypothesis that an autoimmune paraneoplastic mechanism may contribute to the pathogenesis of ROHHAD. In 2022, preliminary findings revealed the presence of ZSCAN1 autoantibodies in 7 out of 9 ROHHAD patients with an associated neural crest tumor. These autoantibodies were notably absent in control groups, which included individuals with non-inflammatory paraneoplastic syndromes and pediatric patients diagnosed with opsoclonus-myoclonus syndrome (OMS). In 2024, a Japanese research team corroborated these findings by detecting ZSCAN1 autoantibodies in ROHHAD patients, even in the absence of any detectable tumor. The antibodies were identified using an in-house ELISA method, suggesting the feasibility of developing a diagnostic assay. A third study published in 2024 further reported the presence of ZSCAN1 autoantibodies in ROHHAD patients without evidence of a paraneoplastic syndrome, including one adult case.

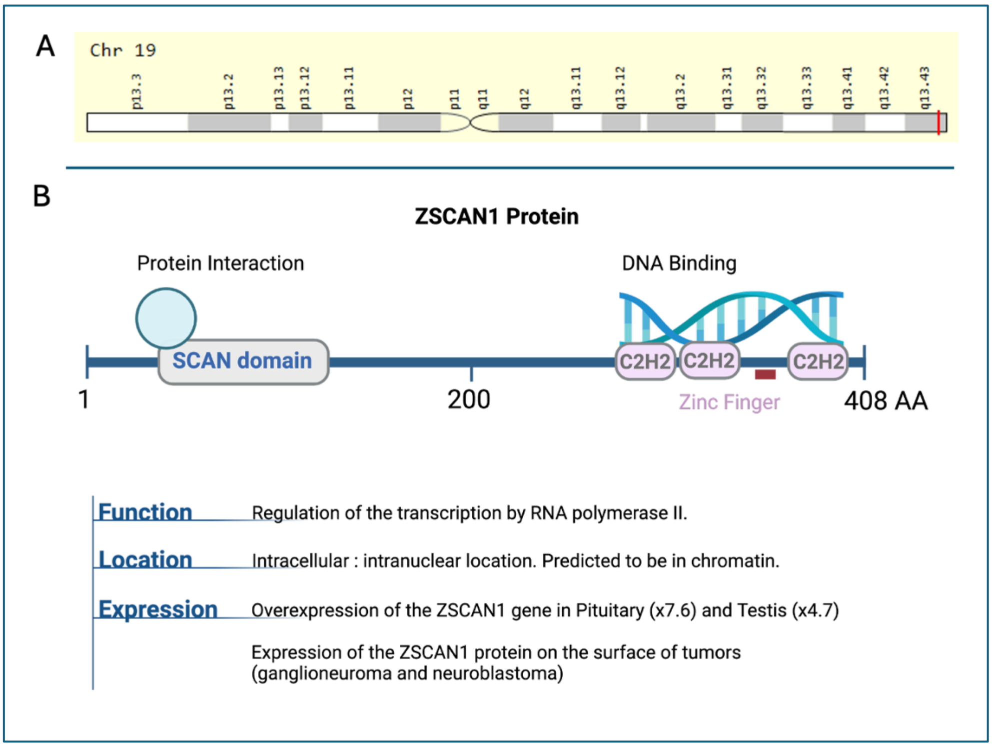
All these recent publications (

Table 3) show that consistent and reliable detection of anti-ZSCAN1 autoantibodies remains technically demanding. The need for standardized, validated assays and the establishment of reference values is critical to ensure accurate clinical interpretation. Current ongoing international collaborations aim to extend testing to larger ROHHAD cohorts, with the dual objective of evaluating the diagnostic performance of the biomarker and investigating potential correlations between anti-ZSCAN1 levels, disease expressivity, and stage.

Although anti-ZSCAN1 appears to be a promising biomarker, its utility in clarifying the pathophysiological mechanisms of ROHHAD remains uncertain. Given that ZSCAN1 is an intracellular antigen (Figure 14), the antibodies are unlikely to be pathogenic. As observed in major subtypes of paraneoplastic neurological syndromes (PNS), antibodies targeting intracellular onconeural antigens typically serve as biomarkers rather than predictors of response to immunosuppressive therapy or tumor resection.

Furthermore, it is important to note that the ZSCAN1 gene is absent in rodents. Based on prior analyses, the putative epitope within ZSCAN1 is likely to be primate specific. This highlights the critical importance of human-based approaches in future research protocols investigating the role of ZSCAN1 in ROHHAD.

Figure 14 - ZSCAN 1- schematic identity card.



A/ **The genetic location** of the ZSCAN1 gene.

Cytogenetic band:19q13.43 by HGNC 19q13.43 by NCBI Gene 19q13.43 by Ensembl

B/ **ZSCAN1 Protein schematic illustration** - The blue bar represents the 408 amino acid ZSCAN1 protein. The ZSCAN family of transcription factors shares a similar DNA binding domain consisting of three or more zinc fingers (Pink) corresponding to the C2H2 domains. Especially, the ZSCAN1 contains three zinc fingers. At the N-terminus, the SCAN domain (grey) functions as a protein interaction domain.

The peptide enrichment data from individual ROHHAD patients from the study of Mandel-Brehm *et al.*, 2022 has revealed a span of antigenic area from which an 11 AA region of overlap in 100% (7/7) patients within the C-terminal domain has been identified (represented here by the dark red color bar).

Figure adapted from M Huang *et al.*, 2019; Mandel-Brehm C. *et al.*, 2022 and http://genecards.org/cgi-bin/carddisp.pl?gene=ZSCAN1. Created with BioRender.com.

	Publication 1 Mandel-Brehm C. *et al.*, 2022	Publication 2 Nakamura-Utsunomiya A. *et al.*, 2024		Publication 3 Serafim A.B. et al., 2024	Publication 4 Tocan V. *et al.* , 2024
STUDY COHORT					
ROHHAD + NET	8	1	/	/	1 (serum sample)
ROHHAD (~~NET~~)	1	4	14	13 6 of them : paired CSF and sera samples 7 of them : only sera samples	/
CONTROLS	41 non-inflammatory individuals with PNS 25 pediatric controls with OMS	/	15 normal subjects 5 subjects with autoimmune disorders	90 patients with inflammatory diseases Serum samples and/or CSF 43 (39CSF) opsoclonus-myoclonus 32 (32 CSF) NMDAR Encephalitis 15 (11 CSF) seronegative autoimmune encephalitis 59 (55 CSF) without inflammatory disease (9 psychiatric disorders +50 Alzheimer disease) 50 Adult healthy blood donors	/
METHODS	1° PhIP-Seq 2° validation by RLBA and 293 T CBA	HuPEXTM human protein array	ELISA + if dosage > 40	293 T CBA	ELISA + if dosage > 40
RESULTS (+ AntiZSCAN1)					
ROHHADNET	7/8 (sera samples)	0/1	/	/	Hight titers : 350 Max
ROHHAD	0/1	4/4	12/14 (sera samples)	0/7 sera samples 4/6 only in CSF and 1/6 CSF+sera	/
CONTROLS	0/66	/	0/20	0/199	/

Table 3 – Review of the publications dedicated to the detection of anti-ZSCAN1

Description of the study cohort (number of patients and nature of the samples (CSF- Spinocerebellar fluid- or Sera)), the methods used for the detection of the autoantibodies and the results obtained in ROHHAD, ROHHADNET and controls Among methods : PHIP-Seq = Phage ImmunoPrecipitation Sequencing/ 293 T CBA = homemade cell based assay using HEK293 cells/ RLBA = Radioligand Binding Assay/ Enzyme-Linked Immunosorbent Assay

1.5.8. Therapeutic management in ROHHAD

ROHHAD syndrome remains a condition of unknown etiology. As a result, current therapeutic strategies are primarily based on symptom-specific management, whether preventive or curative, and are adapted to the clinical progression of each individual patient. Importantly, ROHHAD(NET) is recognized as a life-threatening disorder, with reported mortality occurring around the age of 10 years (Aljabban L. *et al*., 2016). In our 2020 review, the median age at death was estimated at 4.6 years (SD: 3–6), based on data available for three of the six deceased patients. At the time of follow-up, 26 patients were still alive. Notably, two of the six deaths were attributed to sudden cardiac arrest.

The early age at death may reflect a subset of patients presenting with more severe initial clinical manifestations. Additionally, delayed diagnoses may contribute to poorer outcomes by compromising initial prompt therapeutic management, which is essential for better life support.

Figure 15 summarizes the recommended management and follow-up protocols for patients with ROHHADNET syndrome (Harvengt J. *et al.,* 2020). To date, no international consensus or dedicated clinical guidelines have been established for the care of individuals affected by ROHHAD. Management considerations for each item of the ROHHAD acronym are detailed below.

1.5.8.1. RO - Rapid Obesity

The rapid obesity must be countered with appropriate caloric restriction diet and physical to avoid the weight increasing. However, this management remains insufficient. Patients were mainly reported to be treated by oral anti diabetic drugs such as metformin with no major effects. New therapeutic approaches in severe obesity involve GLP-1 agonists such as Semaglutide that enhance satiety by slowing gastric motility and suppressing appetite through activation of GLP-1 receptors in the hypothalamus (Kelly A.S. *et al*., 2013). Frequent side effects include gastrointestinal symptoms and headache. Semaglutide efficacy was reported in few ROHHAD patients but with no long-term data follow-up and the report of one patient with severe adverse effects. Semaglutide has the EMA and FDA authorization for patients aged to more than 12 years, resulting in a limitation of the therapeutic options for younger patients.

Dextro-amphetamines have also been sporadically used in specific cases with specifical difficulties in the management of hypothalamic obesity (Van Engelen N. *et al*., 2025; Marpuri I.*et al*., 2021). Dextro-amphetamines are not recommended for ROHHAD patients but are a new approach that can be discussed in severe hypothalamic obesities, keeping in mind the potential secondary effects (dry mouth, hypertension, tachycardia, and gastrointestinal symptoms) and the need to prescribe this treatment in collaboration with pediatric psychiatrist (Van Schaik J. *et al*., 2022).

Figure 15- Guidance for management and follow-up in rapid-onset obesity with hypoventilation, hypothalamic dysfunction, and autonomic dysregulation syndrome (neural crest tumor) (ROHHAD[NET]). A multidisciplinary approach is highlighted. All caregivers should be supported by a Reference Centre for Rare Disease. (Harvengt J. et al., 2020)

Guidelines for management and follow-up in ROHHAD(NET)

	Investigations/Screening	Therapeutic options
Ro/	**Rapid obesity**	
	- Initial clinical and biological general evaluation - Cerebral MRI to exclude central tumor - Complete endocrine work up * to exclude other differential diagnosis of precocious obesity * evaluation of metabolic disturbances: dyslipidaemia, insulin resistance min 1x/year	- BMI stabilisation * strict calorie intake control * regular physical activities (endurance training) - Oral Antidiabetic drugs - Anti lipid treatment
H/	**Hypothalamic dysfunction**	
	- Hormonal investigations : 1-2x/year. * Hypothyroidism? * Hyperprolactinemia? * GH deficiency? * Puberty delay? * Adrenal insufficiency? * ...?	Specific hormonal substitution (according to biological results)
H/	**Hypoventilation**	
	- Polysomnography + nocturnal gaz exchange: Nocturnal hypoventilation? * If negative: control 1x/year during 5 years * After 5 years: control according to the symptoms - Prevention of respiratory infections	- Artificial ventilation: * VNI (during sleeping time or 24h/day) * Tracheostomy * Diaphragmatic pacemaker - Influenza vaccination (winter) 1x/year (according to local recommendations) - Eviction from school at first respiratory symptoms - Consider antibiotics treatment - Airway support
Ad/	**Autonomic dysregulation**	
	- ECG- Cardiac ultrasound: 1x/year 72h Holter 1x/year + repeat in case of syncopes (risk of severe bradycardia)	- Cardiac pacemaker
	- Control blood pressure * 1x/3months, and at each medical visit * Monitoring blood pressure during VNI monitoring (at hospital)	- Anti-hypertensive drugs
	- Gastro-enterologic screening 1x/year * Celiac disease: transglutaminases autoantibodies analysis 1x/year * Screening for food intolerance according to symptoms * Transit dysregulation: constipation/diarrhea	- Gluten free diet - Lactose free diet - Drugs for transit control
	- Ophthalmologic evaluation: 1x/year * Strabismus? * Delayed pupil response to light?	
NET/	**Neural tumor**	
	- Screening program to detect NET * Chest and Abdominal MRI 1x/year * MIBG I^{123} scintigraphy 1x/year (Abdominal ultrasound in case of MIBG scintigraphy not available). > Resulting in an exam every 6 months during 2 years. - After 2 years of follow up : * Chest and abdominal MRI 1x/year (or MIBG I^{123} scintigraphy depending of patient and hospital conditions)	- In case of NET: Staging of the tumor and recommended treatment * low risk: surgical option * high risk: multimodal and aggressive treatment
	Neurologic Impact	
	- EEG in case of seizures - Behavioral disturbances: hallucinations, aggressiveness, flat affect …	- Anti-epileptic drugs - Anti psychotic drugs or specific treatment if symptoms are not controlled
	Genetic considerations	
	- Exclude a PHOX2B mutation - Exclude Genetic Obesity (consider Prader Willi Syndrome) - Exome sequencing: to exclude other genetic cause	

Only one case report has described the consideration of a surgical intervention following the sequential use of three pharmacological agents—topiramate (for its GABAergic effects and inhibition of neuropeptide Y), dextroamphetamine, and semaglutide (Marpuri I. *et al.*, 2021). In this case, sleeve gastrectomy was planned for an 11-year-old female patient at the time of publication. No subsequent data have been published regarding her postoperative outcome.

Despite the presence of severe obesity, ROHHAD patients appear to exhibit a relatively low prevalence of major metabolic comorbidities. Among the 44 cases described in our review, lipid metabolism disturbances were reported in only two patients—one with hypertriglyceridemia and another with hypercholesterolemia (Harvengt J. *et al.*, 2020). Type 2 diabetes mellitus is rarely documented in this population. However, long-term follow-up data remain very limited and will be essential to better characterize the metabolic profile of young adult ROHHAD patients and to inform future preventive and therapeutic strategies. In this context, regular monitoring of metabolic parameters is strongly recommended throughout the course of follow-up.

1.5.8.2. H - Hypothalamic Dysfunction

Specific hormonal substitution is required according to the hormonal deficiencies presented by each patient. Hypothalamic dysfunctions were reported rapidly after the onset of obesity, with a median interval timing of less than 1 year (0.9 years) arguing for a regular follow up of the endocrine parameters. Central hypothyroidism and GH deficiency were frequently reported, with GH levels almost undetectable after GH stimulation test (Abaci A. *et al.*, 2013; Cemeroglu A.P. *et al.*, 2016; Ibáñez-Micó S. *et al.*, 2017; Kocaay P. *et al.*, 2014; Kot K. *et al.*, 2016; Lucas-Herald A.K. *et al.*, 2012). Natremia disorders are a frequent observation with different types of water imbalance description: hypernatremia, hyponatremia and syndrome of inappropriate antidiuretic hormone (Barclay S.F. *et al.*, 2015; Aljabban L. *et al.*, 2016; Ibáñez-Micó S. *et al.*, 2017; Jacobson L.A. *et al.*, 2016; Jalal Eldin A.W. *et al.*, 2019; Patwari P.P. *et al.*, 2011; Sethi K. *et al.*, 2014; Sumanasena S.P. *et al.*, 2012). Some patients recovered in case of transitory water imbalance but not all and a part of them required long-term treatment with desmopressin (Graziani A. *et al.*, 2016; Grudnikoff E. *et al.*, 2013; Patwari P.P. *et al.*, 2011).

1.5.8.3. H - Hypoventilation

Artificial ventilation should be initiated promptly upon the identification of pathological respiratory patterns on polysomnography. Patients with ROHHAD syndrome typically present with central hypoventilation, which may occur with or without concomitant sleep apnea syndromes.

The choice of ventilatory support is determined by the severity of respiratory impairment. Non-invasive ventilation (NIV) is commonly utilized, while tracheostomy may be required during acute decompensations or in cases necessitating continuous (24-hour) ventilatory assistance. Diaphragmatic pacing constitutes an alternative approach; however, its use remains rare. To date, only one pediatric case involving diaphragmatic pacing has been reported in the 2020 literature review, with insufficient data to evaluate its effectiveness (Grudnikoff E. *et al.*, 2013). In the adult ROHHAD case described in 2024, diaphragmatic pacing was not pursued due to anatomical limitations, despite normal phrenic nerve conduction studies.

In addition to ventilatory strategies, ROHHAD patients must be protected from severe respiratory infections to prevent further decompensation. Preventive measures such as vaccination and the timely administration of appropriate antibiotic therapies should be considered for all individuals affected by ROHHAD syndrome.

1.5.8.4. Ad - Autonomic dysregulation

Dysautonomia represents a key clinical feature of ROHHAD syndrome. The most frequently reported manifestations include impaired sweating and thermoregulatory dysfunction, which generally do not require specific therapeutic intervention. Cardiac dysautonomia may result in altered central regulation of blood pressure, necessitating periodic monitoring as part of routine follow-up. In more severe cases, cardiac dysregulation can lead to arrhythmias or profound bradycardia, potentially requiring the implantation of a cardiac defibrillator at an early age. In our cohort described in 2020, three out of 44 patients underwent defibrillator implantation, with a median age of 7.5 years.

Gastrointestinal dysmotility is another systemic manifestation of dysautonomia in ROHHAD, presenting as either severe constipation or diarrhea. In such cases, the use of transit-regulating agents is recommended. Given the hypothesized autoimmune component of the syndrome, annual screening for biological markers of celiac disease may be warranted. Additionally, some patients follow restrictive diets, such as gluten-free or dairy-free regimens, primarily based on personal well-being rather than medical rationale. Only one confirmed case of celiac disease was identified in the 2020 review, with no additional cases reported in the literature between 2020 and 2025.

1.5.8.5. NET - Neural tumor

Although we have no formal evidence that tumor resection improves symptoms in patients with ROHHADNET, this intervention is routinely recommended because of the possible compressive local effect of the tumor and its potential for malignant transformation (Calvo C. *et al.*, 2019; Khaytin V. *et al.*, 2023).

1.5.8.6. Immunomodulation treatment

In our systematic review, immunomodulatory treatments were reported in six patients, including the use of glucocorticoids, intravenous immunoglobulins, and targeted immunosuppressive agents such as cyclophosphamide and rituximab. However, these data were not subjected to detailed analysis due to insufficient information regarding treatment timing and dosage variability. The scope of our review was extended through July 2025, yet no systematic investigations have specifically addressed this therapeutic approach. Robust data on the efficacy and reliability of these treatments remain lacking, particularly in the context of controlled or placebo-based studies and standardized dosing protocols.

Notably, the first documented case of an adult patient with ROHHAD syndrome in 2024 described a therapeutic regimen comprising hydrocortisone (40 mg/day), levothyroxine (1 µg/kg/day), and intravenous immunoglobulin (4 g/kg/day), administered twice at distinct disease stages. This intervention yielded minimal clinical response (Ortega-Gonzalez A. *et al*., 2024). Collectively, these pediatrics and adult case reports suggest a potential role for immunomodulatory therapies and underscore the need for accurate designed clinical trials to evaluate their efficacy in long term evolution of ROHHAD syndrome.

2. Purpose of the study

Rapid-onset obesity with hypothalamic dysfunction, hypoventilation, autonomic dysregulation and neural crest tumor (ROHHAD[NET]) is a rare pediatric disorder characterized by sudden-onset obesity and hypothalamic dysfunction. Despite its acronym suggesting a well-defined clinical entity, no validated diagnostic criteria or biomarkers were available as of 2020. To date, no definitive genetic, autoimmune, or paraneoplastic cause has been identified, although these hypotheses have been explored. The epigenetic hypothesis, however, remains largely unexplored, despite reports of discordant monozygotic twins, suggesting a potential role for epigenetic alterations.

Moreover, studying ROHHAD highlights the need to better understand the etiological landscape of early-onset obesity. Despite its growing prevalence, monogenic forms remain underdiagnosed and poorly characterized in current literature. In this view, our study is structured into two interconnected research workflows (Figure 16):

1/ The objectives of the ROHHAD EPIGEN project are:

-To generate the first epigenetic dataset from ROHHAD patients by:

* Collecting biological samples from patients worldwide.
* Collecting detailed clinical data to match the descriptive criteria outlined in our review of 2020.

-To compare DNA methylation profiles between ROHHAD patients and healthy first-degree relatives using whole-genome bisulfite sequencing (WGBS).

-To investigate genetic hypotheses and exclude differential diagnoses through whole-exome sequencing (WES) and long-read sequencing.

-To integrate WGBS and long-read data using Oxford Nanopore Technology, enabling direct detection of epigenetic modifications.

-To describe ROHHAD-like phenotypes, contributing to the delineation of the ROHHAD spectrum and identifying potential phenocopies.

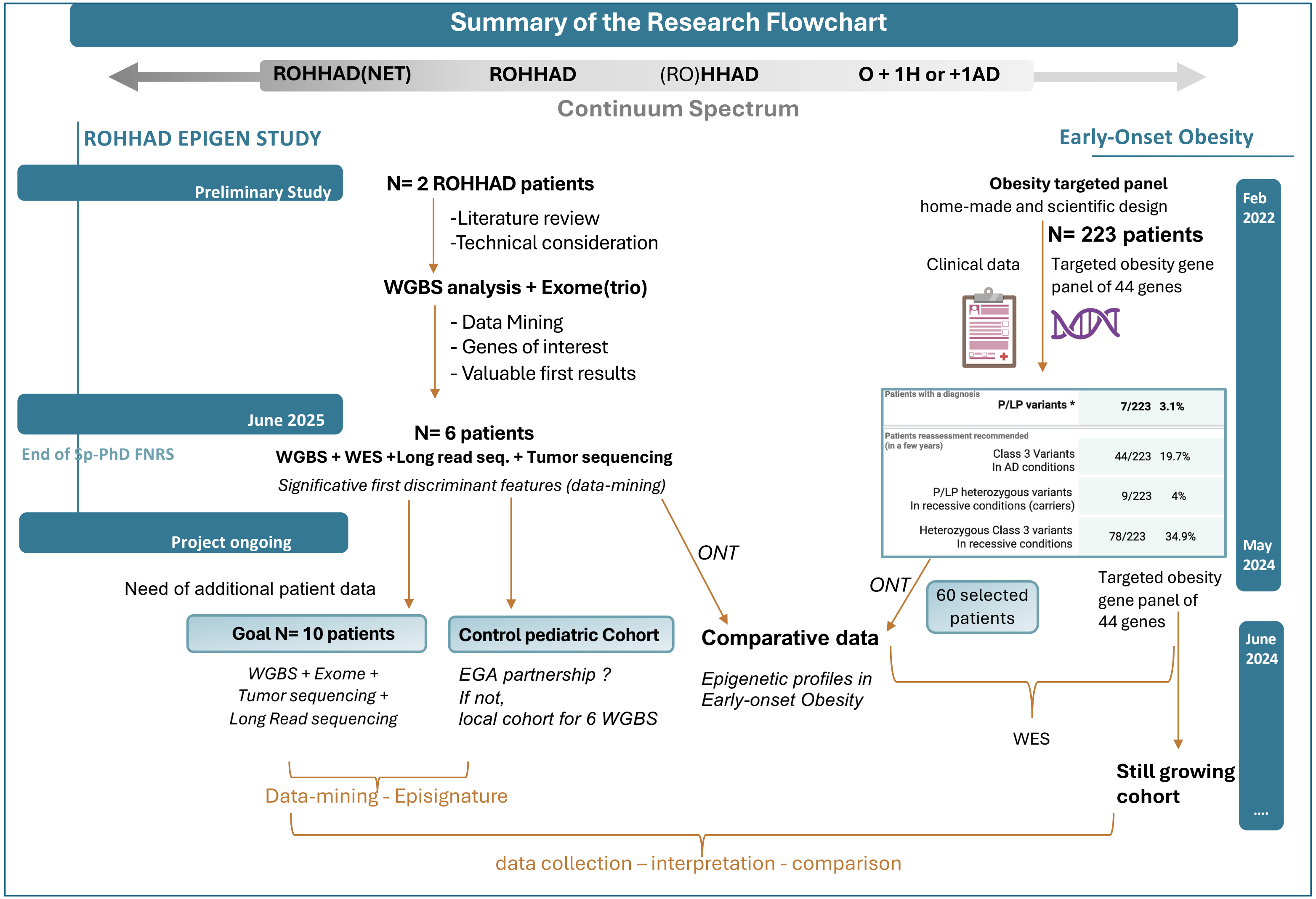
2/ The objectives of the Early-Onset Obesity Cohort project:

-To analyze results from a targeted gene panel in children with early-onset obesity.

-To assess diagnostic yield and compare findings with previous studies.

-To provide a comprehensive interpretation of identified variants and their functional consequences, with potential implications for therapeutic management.

Figure 16 – Summary of the research flow chart representing two study arms: the EPIGEN Project and the Early Onset Obesity project.



The two arms have been investigated between 2020 and 2025, and all the published and unpublished results are part of this thesis.

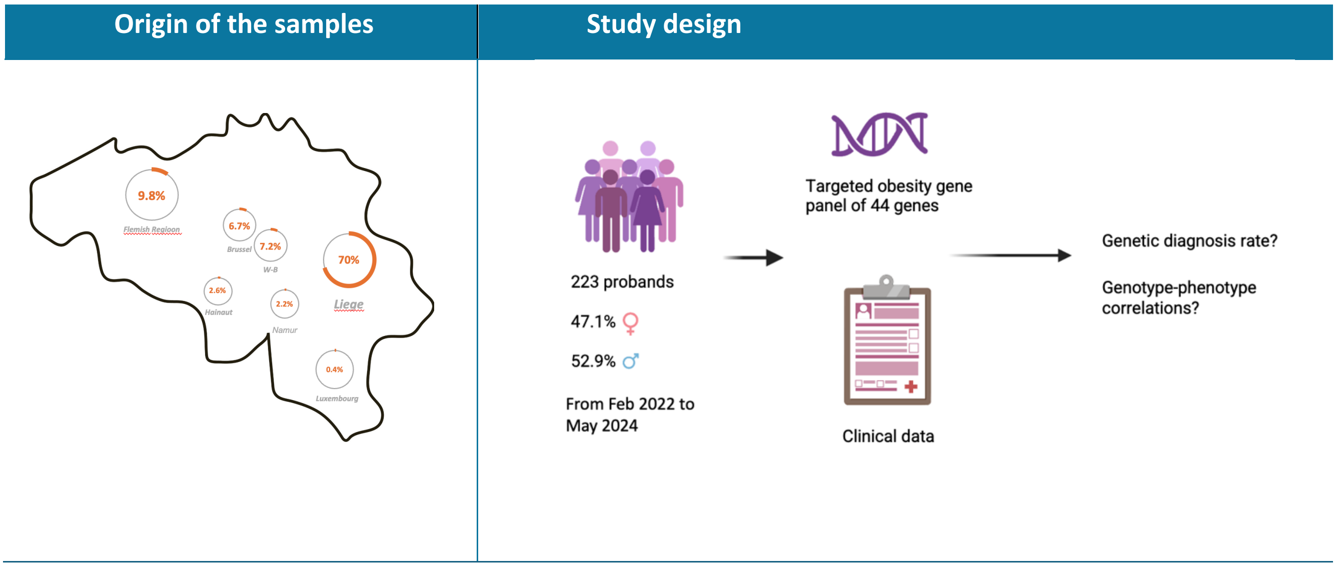
The two projects are still ongoing and details on the current workflows are also mentioned on this schematic study plan.

3. Monogenic etiologies in a cohort of early onset obesity: a real-world experience from Belgium

Julie Harvengt, Muriel Hannon, Leonor Palmeira, Marie-Christine Lebrethon, Vinciane Dideberg & Vincent Bours. (**2025**)

Frontiers in Endocrinology

3.1. Graphical Study design



Created partially with BioRender.com

3.2. Results summary

Our real-world evidence data published in August 2025 provided the analysis of 223 patients evaluated through a targeted obesity panel. The diagnostic yield of our targeted panel is 3.1% (7 of 223). However, additional positive results were found in the cohort: array CGH revealed positive results for three patients, but we know that one of these three patients was initially identified with a 16p11.2 deletion by the obesity-targeted panel. A pathologic variant in *DDX3X* was found through an intellectual disabilities WES panel. Finally, six patients from the cohort were investigated by a WES, with one positive result identified for one out of the 6 patients: a young girl with HIDEA Syndrome previously published in 2023 (Harvengt J. *et al*., 2023).

A carrier status for recessive diseases was found for nine patients (9/223 = 4%). Regarding the percentage of VUS found in our gene panel, 44 patients (19.7%) present at least one VUS in genes associated with conditions with an autosomal dominant inheritance, and 78 patients (34.9%) of the cohort present at least one VUS associated with a recessive condition.

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Monogenic etiologies in a cohort of early onset obesity: a real-world experience from Belgium

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Introduction: Obesity is a major global health issue with multifactorial etiologies. Among them, recent advances in the comprehension of eating and energy regulation showed that around 60 genes involved in the hypothalamic leptin/melanocortin pathway contribute to the development of rare monogenic or syndromic forms of obesity.

Objective: To better delineate the genetic diagnostic rate and the phenotype in a cohort of early onset obesity and to integrate our results in guidance for genetic testing.

Methods: In a diagnostic setting, 223 patients with early onset obesity were screened through a targeted panel including 44 genes for severe early onset obesity. Genetic results and clinical descriptions were reviewed for the entire cohort.

Results: A diagnostic yield of 3.1% was established. Likely pathogenic or pathogenic variants were found in *MRAP2*, *MC4R*, *BBS2*, and *BBS4*, and a 16p11.2 deletion was confirmed. Clinically, 23% of the cohort had early onset obesity at <1 year, 47% at 1–4 years, and 30% at >4 years. No discriminative clinical feature appears to enhance the diagnostic yield. Thirty-six percent of the cohort presented additional neurological complaints that led to more extensive genetic investigations with a diagnosis rate of 1.8% in this subgroup.

Conclusion: Our work found a diagnostic yield of 3.1%. Additionally, 19.7% of heterozygous variants of unknown significance were found in genes related to autosomal conditions and 34.9% in genes related to recessive conditions. These results highlight the need for accurate genotype-phenotype correlations. Genetic laboratory expertise in obesity is highly recommended, especially in the context of the availability of new targeted anti-obesity therapies that open the field for current and future perspectives of these targeted genetic investigations.

KEYWORDS
early-onset obesity, monogenic obesity, MC4R, Bardet–Biedl syndrome, hypothalamic obesity

Introduction

Childhood obesity has been recognized as one of the most serious public health problems of the 21st century. In Belgium, recent epidemiological studies (1) reveal that nearly half (49%–55%) of the adult population aged more than 18 years is overweight (BMI ≥ 25) and 16% is obese (BMI ≥ 30). In the group of children and adolescents (2–17 years), 19% present an excess weight (85th percentile ≤ BMI < 95th percentile), and 5.8% are obese (BMI ≥ 95th percentile). A standard pediatric categorization defines obesity in three classes by using the 95th percentile for age and sex as the reference threshold and categorizing 100%–120% of the 95th percentile as class I obesity, 120%–140% as class II obesity, and more than 140% as class III obesity (2, 3). Among the children presenting severe obesity (i.e., classes II and III obesity), at least 5%–10% present chromosomal abnormalities and/or highly penetrant genetic mutations that contribute to their obesity (4).Individually, these monogenic disorders are considered (very) rare. But at a population level, the impact of these diagnoses may be significant on public health care. Moreover, the current medical practices tend to be more oriented to a better precision medicine that might be started at younger ages for children accurately diagnosed.

Clinicians face the challenge of identifying the rare genetic forms of obesity among the large population group of severely obese young children and adolescents. Until now, one major criterion has been to start genetic investigations in cases of inappropriate weight gain in comparison to diet and lifestyle. As a main symptom, sustained severe hyperphagia (moreover with nocturnal eating) from early childhood is a feature of the genetic obesity syndromes (5, 6). Learning and behavioral troubles, developmental delay (e.g., Prader–Willi syndrome), ophthalmological issues and/or kidney failure (e.g., visual loss/renal abnormalities encountered in Bardet–Biedl syndrome (7)) are classically encountered in these syndromes, leading generally to an exhaustive genetic work-up at a very young age. However, over the last 20 years, a group of genetic disorders with severe obesity as the only presenting feature has emerged. These monogenic non-syndromic obesity disorders are mainly driven by molecular alterations in hypothalamic pathways involved in appetite regulation and weight regulation through the leptin-melanocortin pathway.

In this context, we have performed since 2022 a custom NGS targeted panel of 44 genes dedicated to monogenic and syndromic forms of severe and early onset obesity. The aim of this study is to assess the diagnostic yield of our approach in a diagnostic setting and to propose a clinical description of the whole cohort studied and a short description of individual cases to illustrate, secondarily, the perspectives and the need for guidance for genetic testing.

Materials and methods

Patients: inclusion criteria and data collection

Patients investigated through our targeted genetic obesity panel must present a severe early onset obesity starting ≤ 4 years of age as an isolated symptom or an obesity starting at 4 years or a few years later (in a pre-puberty stage) as a non-isolated symptom. Symptoms are assessed by the specialists who prescribe the analysis: (pediatric) endocrinologists but also other experts in the field of obesity management. Important, but not mandatory, criteria for genetic testing are the hyperphagia (defined by pathologic, insatiable hunger accompanied by abnormal food-seeking behaviors, including sometimes nocturnal eating) (8) or the lack of satiety. At that time, no mandatory dedicated and validated hyperphagia questionnaire was in use in Belgium, leaving this clinical criterion to the evaluation of the specialist. A family history is not mandatory, knowing that some genetic conditions are *de novo*.

A cohort of a total of 223 probands was tested between February 2022 and May 2024. Our cohort included only those patients for whom we received a well-filled clinical form that included specific clinical criteria and familial data (Table 1). For each patient, all the items have been recorded in an anonymized Excel database in accordance with the PGDR legacy and our internal hospital ethical legacy.

Patients were included to perform solo, duo (proband and one parent), or trio (proband and both parents) analyses depending on the availability or not of the blood samples from the parents.

All the patients agreed to this study through the signatures of a consent form.

TABLE 1 Cohort description.

Cohort Description			
Total of index patients = 223		47.1% ♀	52.9% ♂
Obesity targeted panel **First tier test= 71% patients**	TRIO	n= 110	49.3%
	DUO	n=33	14,7%
	SOLO	n=69	30.9%

Number of index patients and genre repartition. Percentages of obesity targeted panels performed in trio, duo or solo.

Abbreviations: BMI, Body mass index; LEP, Leptin; LEPR, Leptin receptor; POMC, Proopiomelanocortin; MC4R, Melanocortin 4 receptor; PCSK1, Proprotein convertase, subtilisin/kexin-type, 1; BDNF, Brain-derived neurotrophic factor; MRAP2, Melanocortin 2 receptor accessory protein 2; EMA, European Medicines Agency; VUS, Pathologic variant (Class 5 variant in ACMG classification); LPV, Likely Pathologic variant (Class 4 variant in ACMG classification); PV, Variant of uncertain significancy (Class 3 variant in ACMG classification).

Sequencing and bioinformatics methods

Our genetic test is an "in-house LDT test" developed from commercial kits. The targeted obesity gene panel contains 44 obesity-related genes: *ADCY3*, *ALMS1*, *BBS1*, *BBS2*, *BBS3 (ARL6)*, *BBS4*, *BBS5*, *BBS6 (MKKS)*, *BBS7*, *BBS8 (TTC8)*, *BBS9*, *BBS10*, *BBS11 (TRIM32)*, *BBS12*, *BBS13 (MKS1)*, *BBS14 (CEP290)*, *BBS15 (WDPCP)*, *BBS16 (SDCCAG8)*, *BBS17 (LZTFL1)*, *BBS18 (BBIP1)*, *BBS19 (IFT27)*, *BDNF*, *CREBBP*, *EP300*, *DYRK1B*, *GNAS*, *INPP5E*, *LEP*, *LEPR*, *MAGEL2*, *MC3R*, *MC4R*, *MRAP2*, *MYT1L*, *NTRK2*, *PCSK1*, *PHF6*, *POMC*, *RAB23*, *SETD2*, *SH2B1*, *SIM1*, *TBX3*, and *TUB*.

The list of the 44 genes was established based on an extensive literature review and a comparative review of the previously described worldwide obesity panel. Technically, each gene included in the panel had sufficient coverage by NGS sequencing. All the materials and methods used for the design of our custom panel were documented and accredited according to local regulations.

Genomic DNA was extracted from peripheral blood mononuclear cells in EDTA tubes using the Nucleomag Blood 200 µl kit (Macherey Nagel, Germany) on a MICROLAB STARlet (Hamilton, Reno, USA). DNA was quantified using NanodropOne (Thermo Fischer Scientific, MA, USA).

NGS sample preparation and enrichment was performed on 50 ng of DNA using the TWIST technology according to the manufacturer's recommendations (TWIST Biosciences, CA, USA). The custom probes were designed to capture the exonic regions ± 14 bp of our targeted genes (captured region of about 134 kb). Samples were pooled by 16 before cluster generation and paired-end sequencing on a MiSeq using the MiSeq Reagent Kit V3 150 cycles (Illumina, San Diego, USA).

Raw data demultiplexing and generation of the FASTQ files were performed internally using "bcl2fastq" (Illumina). Sequencing reads were then analyzed via our internal bioinformatics pipeline (Humanomics, https://doi.org/10.5281/zenodo.13739359), which maps and prepares raw reads before inferring SNPs and INDELs following the GATK Best Practices. QC parameters are monitored following our internal diagnostics procedure and presented for interpretation using MultiQC.

Variant interpretation

The analysis and interpretation of variants reaching a minimal 30X coverage were performed using Alissa Interpret Software 5.3 (Agilent Technologies, CA, USA) and according to ACMG interpretation variant guidelines (9) and their updates by the Sequence Variant Interpretation Working Group published on the ClinGen website.

Results

Our total cohort includes 223 patients. (Table 1) For 110 patients, we had trio samples, and for 33 patients, we collected duo samples. Thirty percent of samples came from national external centers, and 70% came from the geographical area linked to our university location.

The clinical dataset from all the cohorts of the 223 recruited patients reveals that 45% (N = 102/223) are described as hyperphagic. Regarding the other patients (121 of 223), the eating behavior was not clearly mentioned to allow an appropriate interpretation.

To evaluate epidemiologically the type of early onset obesity tested, patients were divided into three groups: weight gain started ≤ 1 year of age, between 1 and 4 years of age, or after 4 years of age. Data are missing for seven of the 223 patients. The first group represents 23% of the cohort, the second 47%, and the third 30%. (Table 2) Associated symptoms or features are detailed in Table 2. Specifically, among the 2.3% of patients reported with red hair, no *POMC* variants have been detected. Only two patients presented with retinitis pigmentosa. One of them has a confirmed diagnosis of BBS. The second had all the criteria for BBS without molecular confirmation at that time. Overgrowth was reported for 10% of the cohort (22 of 220), and no positive cases were found among these 10%. A subset of 3.7% of this last group of patients was found to be investigated also by an overgrowth-targeted panel. More generally, 36% of the cohort was reported with neurological concerns, including motor delay (11%), language delay (19%), and intellectual disabilities (16%). Neurological features were found in the same ratio between the patients with positive or negative results. Additional genetic investigations for neurodevelopmental troubles have been performed using an array CGH in 37% (79 of 214), an intellectual disability panel in 7.4% (16 of 216), and a dedicated test for *FMR1* in 12.5% (Table 3).

Parents themselves are reported with the general criteria of being affected by obesity (BMI > 30 with no precision) in 59.5% (112 of 188) of the mothers and 48.8% (82 of 168) of the fathers. The exact BMI (normal or in the criteria of obesity) was specifically recorded for 82 fathers and 92 mothers, of whom 51 and 65, respectively, had a BMI ≧ 30 kg/m^2. The median BMI for the fathers affected by obesity is 35.3 kg/m^2 (M = 36.8; SD = 6.64), and the median BMI for the mothers affected by obesity is 38.05 kg/m^2 (M = 39.1; SD = 7.03). Only one father has been reported to have died (unexplained cause).

As an indicator of familial severe obesity, parental history of bypass surgery was reported in 13% (n = 21/164) of fathers and 25% of mothers (n = 47/187).

The diagnostic yield of our targeted panel is 3.1% (7 of 223) (Table 4A). The seven positive results identified in six families are listed in Table 4B. Our panel was performed as a first-tier analysis in 71.5% of the cases (156 of 218). The genetic test for Prader-Willi was additionally requested for 9.1% (19 of 218) of the patients and showed no positive results.

However, additional positive results were found in the cohort: array CGH revealed positive results for three patients (Table 4A), but we know that one of these three patients was initially identified with a 16p11.2 deletion by the obesity-targeted panel. For this specific case, array CGH was completed to investigate additional chromosomal abnormalities.

A pathologic variant in *DDX3X* was found through an intellectual disabilities WES panel. Finally, six patients from the

TABLE 2 Auxological and clinical descriptive features of the total cohort.

	Mothers		Fathers	
Obesity (BMI>30)	59.5%	112/188	48.8%	82/168
Mean BMI kg/m² (SD)	39.1 (±7.0)	65/112	36.8 (±6.6)	50/82
Bypass history	25%	47/187	13%	21/164

Birth Parameters

GA (wks)		BW		BH	
n=142		*n=130*		*n =116*	
38.6 (±2.2SD)		3.2 kg (±0.7) Min;Max 0.74;4.88		49.6 cm (±2.8)	
≤ 35 GA	**8.4%**	% ≥4kg	**15%**	% ≥52 cm	**12%**

BMI at last visit

	Adult >18yrs	≤18yrs	Early onset ≤4 yrs
BMI kg/m² mean (SD)	*n=19/188* 45 (±10.4)	*n=162/188* 29.6 (±8.0)	*n =36/188* 23.1 (±2.9)
BMI Z score mean (±SD)	/	*n= 96/188* 4.75 (±2.1)	*n =19/188* 4.9 (±1.9)

Age of onset of obesity

< 1 year	1-4 years	≥ 4years
23%	47%	30%

Associated clinical features

	Number of positive reported cases n	%
Hyperphagia	102/223	45%
Dysmorphism	28/199	14%
Overgrowth	22/220	10%
Short stature	6/220	2.7%
Intellectual Disabilities	35/220	16%
Motor delay	26/220	11%
Langage delay	42/220	19%
Red hair	5/218	2.3%
Retinitis pigmentosa	2/223	0.8%

Auxological data. For each patient, age of onset of obesity, birth parameters, BMI at last visit and associates' symptoms were collected.
To assess the timing of early onset obesity, patients were divided into three groups: weight gain started ≤1 year of age (23%), between 1 and 4 years of age (47%), or after 4 years of age (30%).
Birth parameters show that 8.4% of the patients were born prematurely (<35GA). To estimate the number of patients in the criteria of macrosomia/overgrowth at birth, we calculated that 15% presented a BW >4kg and 12% a BH >52cm.
For each patient, available data from the parents included BMI >30, exact BMI at the time of sample collection and bypass history. Each item was calculated in percentage or mean BMI for both groups of mothers and fathers.
Clinical descriptive features. For each patient, associated symptoms were described by the prescribers and/or reviewed by our team. The list of features is described with the number (*n*) and the percentage (%) of positive patients for each item and for each group (total cohort, patients with a positive genetic result and patients with a negative genetic result).

cohort were investigated by a WES, with one positive result identified for one of the 6 patients: a young girl with HIDEA Syndrome previously published in 2023 (10).

Out of our cohort, seven patients presented an LPV or PV in the genes *MC4R*, *MRAP2*, and *BBS2*, and a 16p11.2 deletion for one patient. (Table 4B) The median BMI for these positive cases is 35.2 kg/m^2 (SD = 10.4, n = 6/7), and the median age at the time of genetic investigations is 11.66 years (SD = 6.6). Five out of the seven positive patients presented an early onset of obesity starting ≤ 4 years; more specifically, two of them started ≤ 1 year.

A carrier status was found for nine patients (9/223 = 4%) (Table 4A). They present either heterozygous LPV or PV in genes related to recessive OMIM conditions. The genes encountered in this subgroup are *LZTFL1*, *BBS7*, *BBS1*, *CEP290*, *POMC*, *LEP*, and *PCSK1*.

Regarding the percentage of VUS found in our gene panel, 44 patients (19.7%) present at least one VUS in genes associated with conditions with an autosomal dominant inheritance, and 78 patients (34.9%) of the cohort present at least one VUS associated with a recessive condition. (Table 4A) Lack of segregation analyses or lack of literature consensus are the main reasons why the pathogenicity of these variants remains uncertain until now. Reevaluation of these 122 (54.7%) patients [considering patients with a VUS for both AD (19.7%) and AR (34.9%) conditions] might be proposed in the next few years to perform a reevaluation of the genotype-phenotype correlations and expand the genetic testing thanks to the new genomic technologies that should be available in a diagnostic setting.

Regarding specifically the BBS variants, 38 patients (17%) encountered at least one BBS VUS, and seven LPV/PV were found in seven additional patients. These seven patients are heterozygous, and no second variant in BBS genes was identified. For three patients, the clinical suspicion remains highly significant and investigations encompassing intronic region analyses are still ongoing to try to identify a second variant for one patient. Segregation analyses are still lacking for two patients.

TABLE 3 Number and type of additional genetic investigations performed in the cohort.

Number and type of additional genetic investigations		
	%	n
Array CGH	37%	79/214
ID gene panel	7.4%	16/216
Overgrowth gene panel	3.7%	8/215
FMR1 gene analysis	12.5%	27/216
Prader-Willi Syndrome	9.1%	19/208
Whole Exome Sequencing	2.8%	6/212
Angelman		3
BBS targeted gene panel		3
BWS		3
Temple syndrome		6
MODY panel		3
Metabolic work-up		4

Discussion

The diagnosis of monogenic obesity in children remains a current challenge. In that view, since 2022, a targeted panel of 44 genes specifically dedicated to the genetic forms of severe and early onset obesity has been implemented in the CHU of Liège, Belgium. 223 index probands were tested. Seven patients were found with LPV or PV, which represents a diagnostic yield of 3.1%. Nevertheless, this descriptive series also highlights a significant number of heterozygous class 3 variants (19.7% related to autosomal dominant conditions and 46.6% to recessive conditions) and 4% of heterozygous carriers of an LPV or PV in a gene encountered in recessive conditions.

The seven positive cases diagnosed in our cohort present variants in *MC4R*, *MRAP2*, and *BBS* genes and a 16p11.2 deletion. All these molecular alterations lead to a dysregulation of the appetite control, mainly through a disturbance of the hypothalamic leptin-melanocortin pathway, the main cause of monogenic non-syndromic obesities (Figure 1). Clinically, it is well known that a deficiency of *POMC* (bi-allelic variants) leads to hyperphagia, lower resting metabolic rate, and severe obesity with cutaneous pigmentation abnormalities (red hair and pale skin) (16). However, the impact of heterozygous *POMC* variants on obesity is still unclear. A recent publication of 2023– (17) concludes that heterozygous pathogenic *POMC* variants do not contribute to monogenic obesity but that they slightly increase the BMI (17). Further data will probably be more accurate in the next few years considering the subsequent question of the relevance (or not) of using new treatments such as setmelanotide in this indication. In our cohort, we found three patients with *POMC* VUS for whom the interpretation should improve with better segregation data, but familial DNA samples are not available. One of them presents, nevertheless, a highly questioning VUS that raises the question of the impact of specific variants located on cleavage sites of the POMC protein. (Figure 2) POMC is cleaved by pro-hormone convertases at dibasic sites, which are generally well conserved between species (18). The expression of the *POMC* gene is based on complex mechanisms that regulate the release of POMC-derived peptides such as MSH, ACTH, and ß-endorphins (Figure 2). Our patient presents the variant *POMC* c.706C>G, p.(Arg236Gly),

TABLE 4A Results summary showing the number of variants (P/LP/VUS) identified in the cohort through the different genetic tests.

Results summary

	Number of patients with variants (P/LP/VUS3) identified in the cohort			
	Obesity Targeted Panel	Array CGH	WES	Other targeted panel
Patients with a diagnosis **P/LP variants ***	**7/223 3.1%**	3/79 3.8%	1/223 0.4%	1/223 0,4%
Patients reassessment recommended (in a few years) Class 3 Variants In AD conditions	44/223 19.7%	19/79 24%	1/223 0,4%	/
P/LP heterozygous variants In recessive conditions (carriers)	9/223 4%			
Heterozygous Class 3 variants In recessive conditions	78/223 34.9%			

The diagnosis yield for the obesity panel is 3.1%. The total diagnosis yields all tests included for the whole cohort is 4.9.%. The results discriminate furthermore the number of patients who are carriers of a heterozygous LP/P in a gene associated with a recessive condition and the number of patients for whom the genetic test found at least one VUS in respectively autosomal conditions and recessive conditions.

*The P/PL variants reported for the patients with a confirmed diagnosis are in heterozygous state for autosomal conditions or in a homozygous or composite heterozygous state for recessive conditions.

TABLE 4B Molecular results identified for each patient (total of 11 positive case) and listed according to the genetic test performed.

Patient ID	Results from the targeted panel	
1	Class 4 variant c.68G>C, p.(Arg23Pro) homozygous in the *BBS2* gene	NM_031885.4 (BBS2)
	And a classs 3 variant c.884G>A, p.(Arg295Gln) in the *BBS4* gene	NM_033028.5 (BBS4)
2	Class 4 variant c.535delG, p.(Val179Phefs*39) in the *MC4R* gene (NM_005912.3) at a heterozygous status. Maternally inherited.	NM_005912.3
3	Class 4 variant c.181G>T, p.Glu61* in the MC4R gene at a heterozygous status. Parental analyses not performed.	NM_005912.3 NM
4	Class 4 variant c.240C>A, p.(Tyr80*) in the MC4R gene at a heterozygous status	NM_005912.3
5	Class 4 variant c.91_92delinsTA, p.(Gly31*) in the *MRAP2* gene at a heterozygous status. Maternally inherited.	NM_138409.4
6	Class 4 variant c.91_92delinsTA, p.(Gly31*) in the *MRAP2* gene at a heterozygous status. Maternally inherited.	NM_138409.4
7	Deletion of the exon 1 of the *SEZ6L2* gene at a heterozygous status (region of the deletion 16p11.2)	NM_012410
Array CGh results		
7	arr[GRCh37] 7q36.3(158269643_158599209)x3 pat,16p11.2(29592783_30190568)x1 pat	
8	arr[GRCh37] 16p12.2(21837492_22407931)x1 dn	
9	arr[GRCh37] 9p21.1(28219132_28377937)x1 including *LINGO2*.	
ID panel		
10	Class 4 variant c.[1021T>G];[=],p.[Cys341Gly];[=] in the DDX3X gene at a heterozygous status. De novo.	NM_001193416.3
WES		
11	Class 5 variant c.1207_1216delinsCACTGTGACA; p. Lys406ThrfsTer3 homozygous in the *P4HTM* gene.	NM_177939.3

which is located on the cleavage site involved in the generation of ß-endorphins. ß-endorphins are known to play a role in the regulation of analgesia but also in the regulation of food intake through their specific activation of the µ-opioid receptors and not the MC4R. A study of mice with deletion of ß-endorphins reveals that male mice were obese and hyperphagic (19). In addition to this anorexigenic role, ß-endorphins are also involved in the positive regulation of the appetite through the reward behavior system. Processing of POMC is therefore a complex and subtle pathway that might need more detailed knowledge to better appreciate the functional consequences of each specific variant. Notably, in the case of a variant leading to a specific ß-endorphin deficiency, a treatment such as MC4R-agonist would not be indicated.

Interestingly, *MC4R* remains the first cause of monogenic obesity, with an estimated prevalence of 5% and 2% in the obese pediatric and adult cohorts, respectively (16). MC4R is expressed in the hypothalamus, brain, muscle, adipocytes, and astrocytes and is involved not only in energy homeostasis and food intake but also in anti-inflammatory regulation, drug tolerance, and sexual behavior (20, 21). Patients with homozygous variants are extremely rare in Europe; a few consanguineous families are described showing a highly severe phenotype with a very early onset of hyperphagia. In contrast, heterozygous patients present a wide phenotypical spectrum ranging from very early onset hyperphagia to minor excess weight in adulthood. Three patients with an LPV or PV in *MC4R* were detected in our cohort (3/223 = 1.35%): two *MC4R* pathogenic variants previously published (22, 23) and one *MC4R* LP variant c.535delG (p.(Val179Phefs*39) never reported until now. (Patient 2, Figure 3). Our diagnosis yield is probably lowered because, in case of suspicion of MC4R, Belgian clinicians have the possibility to prescribe *MC4R*-targeted tests, leading to a statistical estimation of the incidence of MC4R-positive patients that is difficult without a specific registry.

Another key component in the leptin-melanocortin pathway is the melanocortin 2 receptor accessory protein 2 (MRAP2). (Figure 1) Since 2023, MRAP2 is known to be required for the localization of MC4R to the primary cilia and the function of MC4R neurons (24), an emerging knowledge providing new insights in recent theories linking energy homeostasis and primary cilia. In this perspective, research for new candidate genes should also be oriented to genes controlling the localization of MC4R to the primary cilia (24). Loss-of-function pathogenic variants in *MRAP2* are related to monogenic hyperphagic obesity associated with hyperglycemia and hypertension, contrasting with the other monogenic forms of obesity that present generally with low blood pressure and normal glucose tolerance. As deficiency in MRAP2 partly affects the MC4R pathway, the subsequent energy homeostasis dysregulation and obesity in *MRAP2*-deficient subjects might be theoretically improved by an MC4R-agonist treatment (25). However, further studies are needed to better delineate mechanisms and efficacy of this therapeutic option in *MRAP2*-related obese patients. In our cohort, a new MRAP2 variant was found in two related probands: *MRAP2* loss-of-function LPV c.91_92delinsTA, p.(Gly31*) (Figure 3).

Early onset obesity causes are evidently broader, encompassing environmental, hormonal, and oligogenic factors. Among these oligogenic predispositions, the MC4R pathway seems to play a key role, and the future would be to consider both genetic studies, monogenic and oligogenic, of our patients in

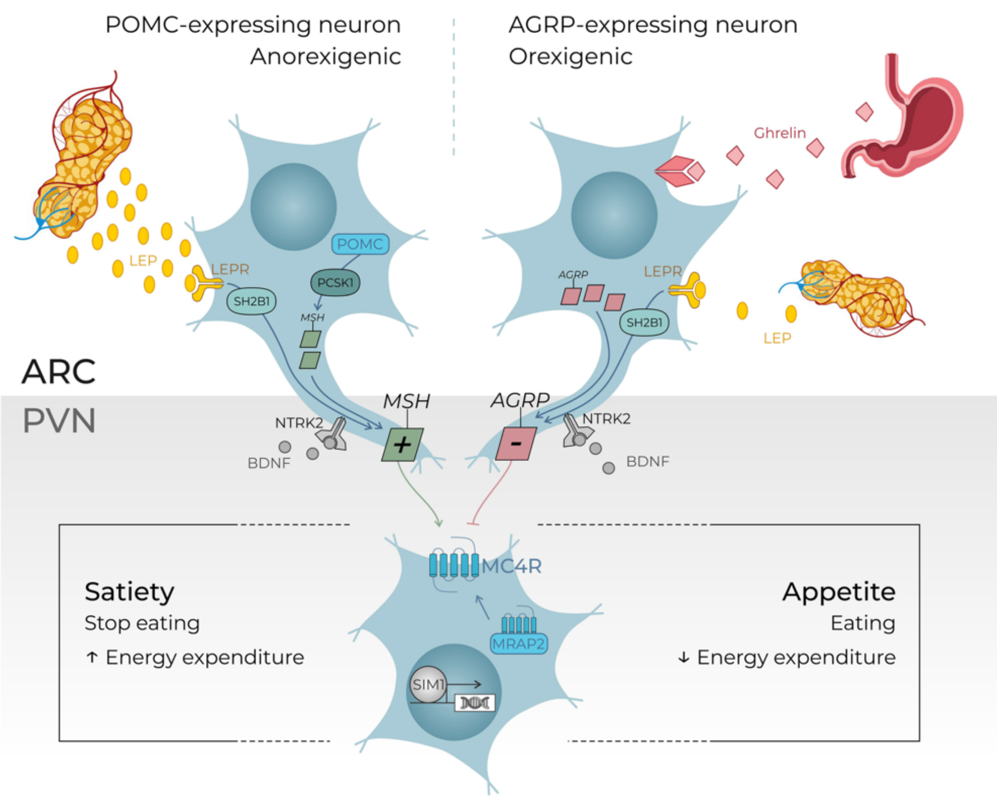

FIGURE 1
An overview of the leptin–melanocortin pathway in the hypothalamus. Leptin is secreted in the white adipose tissue. Leptin levels depend on the "fed status": leptin levels increase in case of refeeding after food starvation and leptin levels decrease in case of food deprivation. Leptin hormone peptides act on the hypothalamus where POMC-expressing neurons and agouti-related protein (AGRP)–expressing neurons are located, more precisely in the arcuate nucleus. These neurons send a signal to the MC4R-expressing neurons in the paraventricular nucleus of the hypothalamus (PVN) which controls through their central neural projections *in fine* the level of appetite or satiety. BDNF (brain-derived neurotrophic factor) is thought to be an actor in this pathway, through its binding to NTRK2 (neurotrophic receptor tyrosine kinase 2) leading to a regulation in the synaptic plasticity of neurons, including those present in the ARC and PVN. The transcription factor SIM1 is also essential for the correct development of the PVN. This overview gives comprehensive examples of regulator genes investigating through our targeted gene panel. (5, 11–15). +, agonist; −, antagonist; LEPR, leptin receptor; MRAP2, melanocortin receptor accessory protein 2; MSH, melanocyte-stimulating hormone; SH2B1, SH2B adaptor protein 1. ARC, arcuate nucleus; AgRP, agouti related peptide; NPY, neuropeptide Y; POMC, propiomelanocortin; αMSH, α-melanocyte stimulating hormone; MCR, melanin-concentrating hormone receptor; SST, somatostatin.

diagnostic settings. For now, current estimations from different studies dedicated to monogenic etiologies suggest that 5% of the patients with severe early onset obesity are linked to a monogenic condition related to the melanocortin pathway. From one point of view, a mean of 5% for the efficiency rate for the targeted panels may be discussed as underestimated due to limited access to genetic investigations for a wide range of patients. Medical compliance and socioeconomic status should be cited as two factors of under evaluation for this category of patients. On the other hand, the 5% diagnostic yield should also be interpreted with caution, as it might be overestimated. A thorough analysis of previously published series reveals an inflated rate of positive results due to inconsistencies between studies and differing criteria for variant classification, which could lead to the misclassification of variants of uncertain significance (VUS) as positive results (26–28). In our cohort, the diagnostic yield for the targeted panel itself was 3.1%. However, a total of 4.9% of diagnoses in our cohort were confirmed through additional genetic investigations performed on highly suspicious cases. (Table 4B) Moreover, our results found that 54.7% of the patients had at least one heterozygous VUS; specifically, 19.7% of VUS were related to autosomal conditions and 46.6% to recessive conditions. For a limited number of the patients of the cohort, stronger genotype-phenotype correlations or additional genetic investigations in a research setting are still ongoing due to a high suspicion of variant pathogenicity. Nevertheless, a systematic re-evaluation might be recommended for all the patients presenting at least one VUS (54.7%) in a few years to expand the potential for new positive results. Notably, we hypothesize that the future availability in a diagnostic setting of genomic testing technologies (WGS) or long-read sequencing should be key next steps to improve the detection of second variants in bi-allelic conditions.

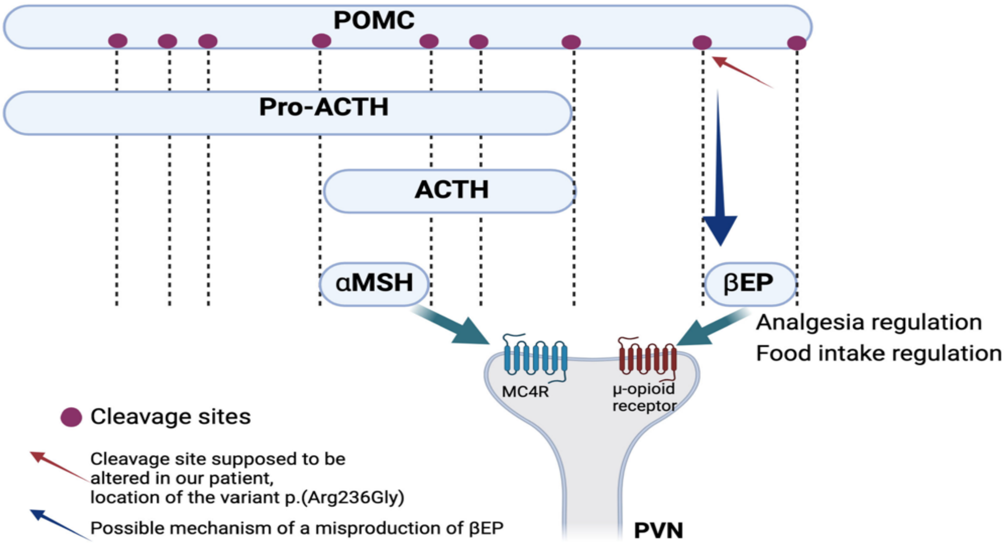

FIGURE 2
Simplified schema of the processing of POMC leading to the generation of functional peptides including αMSH and ß-endorphins. These two peptides act through the binding to two different types of receptors, respectively MC4R and μ-opioid receptor. Red arrow shows the cleavage site that is thought to be altered in our patient hypothesizing an alteration of the leptin melanocortin pathway through the alteration of the ß-endorphins action which should probably lead to food intake dysregulation but also to analgesia and reward process dysregulation (17–19).

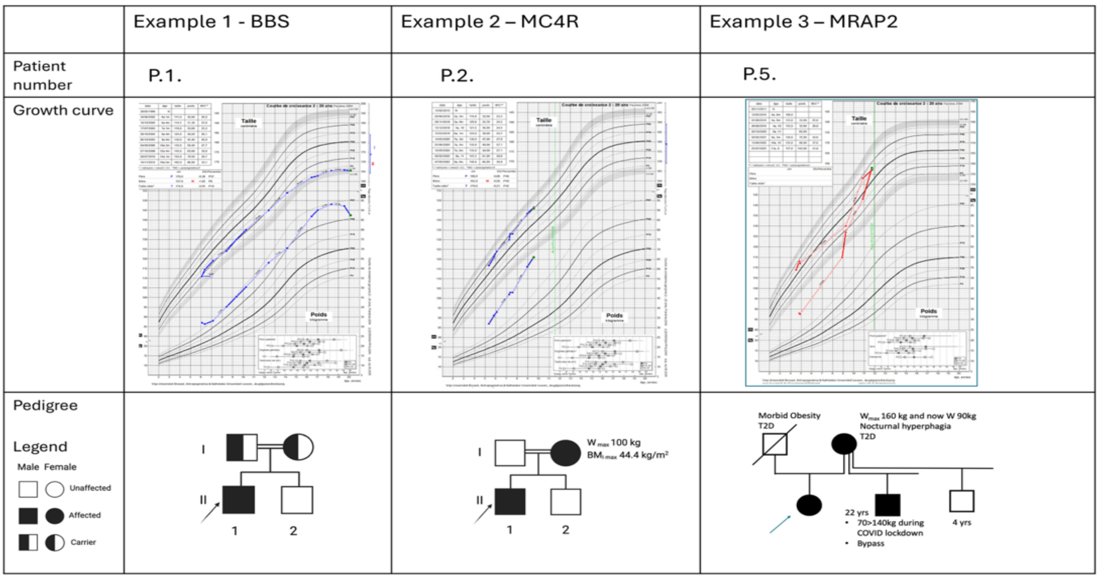

FIGURE 3
Description of three illustrative positive cases. For each patient, the familial pedigree shows the inheritance pattern. The growth curves reveal for the three cases a severe obesity with early onset and a weight relatively stabilized at adult age for the case P1. At last evaluation, they have all a significant pathologic BMI. P.1 presents associated features typically in accordance with a BBS syndrome. The two other cases present no discriminative clinical pattern that may potentially orient the diagnosis hypotheses.

In the field of monogenic obesity, depending on laboratory resources, a targeted panel provides advantages of relatively short turnaround times, avoidance of incidental findings, and higher sequencing quality (by improving the coverage) (29). For these reasons, investigations through a targeted panel as a first-tier analysis remain currently considered to be a more effective screening method than WES in the population of patients with severe early onset obesity.

In pediatrics, it is currently well established that obesity starting ≤ 4 years must be investigated with a genetic test (30, 31). Our diagnosis rate calculated for our cohort subgroup of obesity starting ≤ 4 years is 3.3%, which is not significantly discriminant compared to obesity starting > 4 years. This cutoff age of 4 years for the onset of obesity might not be systematically used as a stringent criterion in clinical practice for requesting genetic testing. Among our seven positive cases, three are presented (Figure 3) to illustrate the types of growth curves observed in childhood genetic obesity and the challenges in establishing a cutoff based on clinical data and growth parameters for genetic testing. Furthermore, our findings do not reveal any distinguishing clinical features between the seven positive cases and the rest of the cohort (Table 2). Similarly, the statistical comparisons between the group of patients presenting a VUS and the rest of the cohort did not reveal any significant differences. Larger sample sizes should be recommended to enhance the statistical performance test. Nonetheless, the variability and minimal clinical differences among monogenic disorders reinforce the notion that clinical criteria alone are insufficient to restrict access to genetic testing.

Regarding the young adult patients, no consensual recommendations have yet been published on this topic. From our perspective, genetic investigations should be implemented more widely for all the young adults (especially between 18 and 25 years) with an extremely severe BMI (BMI ≥ 40 kg/m^2) and a medical history of prepubertal childhood obesity with no evident explanation (no adverse drug effect, no diet imbalance).

More than an added value, the confirmation of monogenic obesity should imply therapeutic perspectives. For example, the bariatric surgery effectiveness in patients with monogenic obesity remains debated. Genetic alterations, such as MC4R dysfunction, disrupt appetite regulation, potentially explaining the observation of differences in the long-term outcome in percentage of weight loss in comparison to patients with a non-genetic obesity (28). The new genetics knowledge has also opened the field for current and future therapies, as seen initially with the treatment of congenital leptin deficiency by recombinant leptin (32). *POMC*, *PCSK1*, and *LEPR* deficiencies and Bardet-Biedl syndrome (*BBS*) can be counter-regulated by the MC4R agonist setmelanotide (33, 34). One remaining question is to determine the impact of MC4R agonists in patients with heterozygous *POMC* variants, identifying a targeted group of responders based on variant types. These variants affect protein cleavage pathways (ß-endorphins or α-MSH), paving the way for more specific targeted therapy research.

However, improving genetic diagnosis for early onset obesity is the way to offer appropriate, preventive, and dedicated care management according to the genetic etiology and its associated risks (e.g., ophthalmopathy, renal failure, metabolic disturbances…).

Conclusion

Monogenic causes of early onset obesity are still challenging to diagnose due to a lack of clinical discriminant criteria. Current guidance for clinicians proposes to identify candidates for genetic investigations among those patients with early onset obesity and hyperphagia. In our experience, based on that guidance, the diagnostic yield for a genetic diagnosis is 3.1% for the total cohort, increasing to 4.9% with additional molecular investigations. However, 19.7% and 46.6% of variants, respectively, associated with autosomal or recessive conditions, remain of unknown significance, highlighting the need to reevaluate systematically our patients in a few years in a diagnostic setting and to offer further research testing in selected cases. Our literature review underlines the discrepancies between the previous reported series and the non-uniformization for the reporting of the positive results. In the era of precision medicine, strengthening expertise in genetic obesity is essential for accurate diagnoses and to orient our patients through effective targeted (future) therapies.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the study involving human samples in accordance with the local legislation and institutional requirements because [reason ethics approval was not required]. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

JH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MH: Conceptualization, Investigation, Methodology, Resources, Validation, Writing – review & editing. LP: Methodology, Writing – review & editing. M-CL: Writing – review & editing. VD: Supervision, Writing – review & editing. VB: Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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4. ROHHAD(NET) Syndrome: Systematic Review of the Clinical Timeline and Recommendations for Diagnosis and Prognosis

<u>Julie Harvengt</u>, Caroline Gernay, Meriem Mastouri, Nesrine Farhat, Marie-Christine Lebrethon, Marie-Christine Seghaye & Vincent Bours. (**2020**)

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4.1. Original publication

ROHHAD(NET) Syndrome: Systematic Review of the Clinical Timeline and Recommendations for Diagnosis and Prognosis

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Context: Rapid-onset obesity with hypothalamic dysfunction, hypoventilation, autonomic dysregulation and neural crest tumor (ROHHHAD[NET]) is a rare and potentially fatal disease. No specific diagnostic biomarker is currently available, making prompt diagnosis challenging. Since its first definition in 2007, a complete clinical analysis leading to specific diagnosis and follow-up recommendations is still missing.

Objective: The purpose of this work is to describe the clinical timeline of symptoms of ROHHAD(NET) and propose recommendations for diagnosis and follow-up.

Design: We conducted a systematic review of all ROHHAD(NET) case studies and report a new ROHHAD patient with early diagnosis and multidisciplinary care.

Methods: All the articles that meet the definition of ROHHAD(NET) and provide chronological clinical data were reviewed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis individual patient data guidelines. The data were grouped into 7 categories: hypothalamic dysfunction, autonomic dysregulation, hypoventilation, NET, psychiatric symptoms, other clinical manifestations, and outcome.

Results: Forty-three individual patient data descriptions were analyzed. The timeline of the disease shows rapid-onset obesity followed shortly by hypothalamic dysfunction. Dysautonomia was reported at a median age of 4.95 years and hypoventilation at 5.33 years, or 2.2 years after the initial obesity. A NET was reported in 56% of the patients, and 70% of these tumors were diagnosed within 2 years after initial weight gain.

Conclusion: Because early diagnosis improves the clinical management and the prognosis in ROHHAD(NET), this diagnosis should be considered for any child with rapid and early obesity. We propose guidance for systematic follow-up and advise multidisciplinary management with the aim of improving prognosis and life expectancy. (***J Clin Endocrinol Metab* 105: 2119–2131, 2020)**

Freeform/Key Words: ROHHAD, precocious obesity, central hypoventilation, dysautonomia, sinusal bradycardia, neural crest tumor

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Abbreviations: GH, growth hormone; GN, ganglioneuroma; MRI, magnetic resonance imaging; NET, neural crest tumor; NIV, noninvasive ventilation; ROHHAD, rapid-onset obesity with hypoventilation, hypothalamic dysfunction, and autonomic dysregulation syndrome; WES, whole-exome sequencing; (ROHHHAD[NET]), Rapid-onset obesity with hypothalamic dysfunction, hypoventilation, autonomic dysregulation and neural crest tumor.

Rapid-onset obesity with hypoventilation, hypothalamic dysfunction, and autonomic dysregulation syndrome (ROHHAD) is a very rare autonomic and respiratory pediatric disorder associated with a high risk of mortality (1). The first case report appeared in 1965 (1), and in 2000 this specific clinical disorder was named *late-onset central hypoventilation with hypothalamic dysfunction (LO-CHS/HD)* and described in a series of 11 patients (2). In 2007, the acronym ROHHAD was proposed to improve patient identification (3). At that time, it was possible to distinguish it from congenital central hypoventilation syndrome with genetic testing: Congenital central hypoventilation syndrome is associated with the *PHOX2B* mutation, whereas ROHHAD is not. The acronym of the disease was amended in 2008 to ROHHAD neural crest tumor (NET) to include the risk of ganglioneuroma (GN) or ganglioneuroblastoma, observed in about 40% of ROHHAD patients (4). The etiology of the disease remains unclear and there is still no significant genetic result. An autoimmune process or epigenetic disorders are currently considered as possible etiological hypotheses (5, 6). Previous studies have not yet established whether ROHHAD and ROHHAD(NET) syndromes share the same etiology.

The definition of ROHHAD syndrome is currently based on clinical criteria, defined first by Ize-Ludlow et al in 2007 (3). The major criterion is dramatic weight gain associated with central hypoventilation appearing between age 1.5 and 7 years in a previously healthy child.

This rapid-onset obesity is considered to be the first sign of hypothalamic dysfunction. At least one more piece of evidence of hypothalamic dysfunction is necessary for the diagnosis, such as hyperprolactinemia, central hypothyroidism, disordered water balance, growth hormone (GH) abnormal response, adrenocortical insufficiency, or puberty disorders.

Central hypoventilation is caused by a dysfunction of the autonomic nervous system with an abnormal response to hypoxia and hypercapnia. This autonomic dysfunction may also manifest later with thermal dysregulation, excessive sweating, cardiovascular manifestations (arrhythmias or blood pressure dysregulation), strabismus, abnormal pupillary reaction to light, or gastrointestinal or sensitive disturbances.

Because precocious obesity has become a frequent reason to consult a pediatrician, the challenge is to keep this rare condition in mind and to perform appropriate investigations in a child with suspected ROHHAD. We report the case of a ROHHAD patient that highlights the importance of a prompt diagnosis for better management regarding the high risk of sudden death due to cardiorespiratory arrest. In parallel, we conducted a literature review to propose a comprehensive clinical approach for diagnosis and follow-up. This review demonstrates that knowledge of the disease has improved since 2007 (3, 6) but a comprehensive overview of the clinical timeline of the disease is currently missing. The present review intends to analyze all reported cases published since 2007 to better describe the clinical events in ROHHAD(NET) and to propose recommendations for appropriate multidisciplinary management.

Case Presentation

Our patient is a Caucasian boy who was born to nonconsanguineous parents with a normal birth weight at full term and normal delivery. There is no relevant family history.

He showed severe hyperphagia and rapid weight gain. His weight increased from 15 kg (0 SD) at age 3 years and 6 months to 22 kg (+2 SD) at age 3 years and 9 months (the initial evaluation), and reached 26 kg at age 4 years. (Fig. 1) During the first 3 months of symptoms he was very hungry, including nocturnal eating. After that time, his calorie intake was strictly controlled by the parents with a permanent rigorous diet.

The parents initially reported some sleep alteration with jerky breathing and grinding of teeth.

During the first year of symptoms the child developed behavioral disturbances including frustration tantrums and aggression. He has excellent cognitive skills, particularly high for memory activities.

At first examination, he was not dysmorphic. There were no stretch marks and no nigricans acanthosis. Somatic examination was normal except for generalized obesity. The first investigations, including cerebral magnetic resonance imaging (MRI), abdominal ultrasound, cortisolemia (including an overnight dexamethasone test and three 24-hour urinary free cortisol), and thyroid hormone levels, were normal.

Polysomnography demonstrated a pattern of severe central hypoventilation with hypercapnia (mean partial pressure of transcutaneous carbon dioxide, $PtcCO_2$: 59 mm Hg; maximal $PtcCO_2$: 61 mm Hg; time spent with $PtcCO_2$ > 50 mm Hg: 100%). Hypocretin concentration in cerebrospinal fluid was normal (333 ng/L; normal value 224-653 ng/L), excluding narcolepsy. Treatment with nocturnal noninvasive ventilation (NIV) with facial mask was started at age 4 years. The diagnosis of ROHHAD syndrome was then confirmed and complementary investigations highlighted a central hypothyroidism, a normal insulin-like growth factor 1 level, a moderate polyuria-polydipsia without diabetes insipidus, and an arterial hypertension.

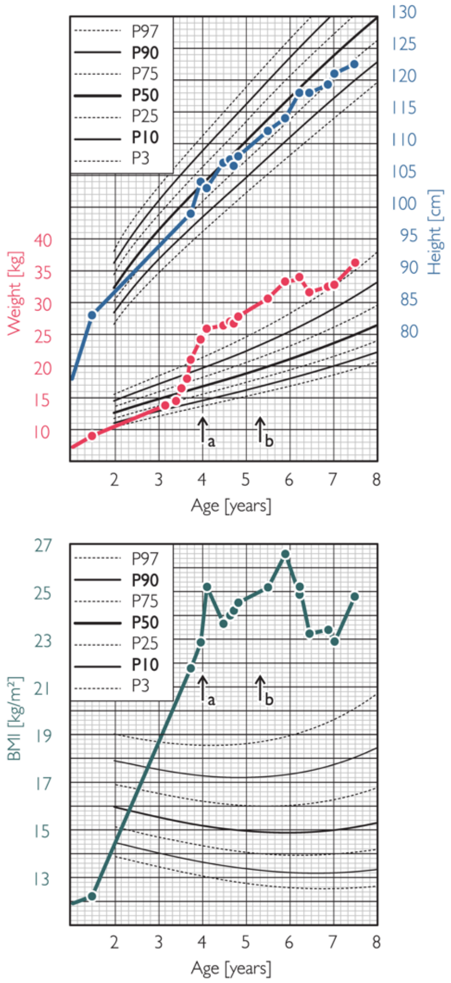

Figure 1. Weight, height, and body mass index (BMI) curve of our patient (Growth Chart Flanders, 2004). Rapid weight gain since age 3 years, with progressive stabilization of BMI, due to strict control of calorie intake and daily physical activities. Arrow a, Time of rapid-onset obesity with hypoventilation, hypothalamic dysfunction, and autonomic dysregulation syndrome (ROHHAD) diagnosis and start of noninvasive ventilation during sleep time. Arrow b, Pacemaker implantation.

After 15 months of follow-up, the patient presented with repeated syncope, occurring generally after intense laughing or severe anger and followed by general muscular hypotonia and postcritical state. The parents had to resuscitate with noninvasive ventilation by mask. A 21-day Holter-electrocardiogram recording demonstrated an episode of sinus pause of 15 seconds that was concomitant with a syncope. A cardiac pacemaker with a ventricular stimulation lead, ensuring a minimal heart rate of 60 beats per minute, was therefore implanted at age 5 years and 4 months. Since this intervention, the patient has been syncope free.

Today, at age 7.5 years, the patient continues to engage in a lot of physical activities, essentially based on endurance. He wears a connected device (digital watch) to track his physical activity. Management of his nocturnal respiratory assistance is demanding but well controlled with the NIV. Screening for NET is still negative.

Genetic investigations revealed no mutation in *PHOX2B*. A comparative genomic hybridization array was normal. Prader-Willi syndrome was excluded. Whole-exome sequencing (WES) did not identify any pathogenic variant that could explain the phenotype.

Consent from the patient and his parents was obtained for the present case report.

Methods

Search methods

We conducted a systematic analysis of the medical literature to identify all published clinical cases of ROHHAD and/or ROHHAD(NET) using the online database PubMed, until September 30, 2019. Language was restricted to English. The search query was limited with the terms *ROHHAD* and *ROHHAD(NET)*. All publications identified were included and analyzed. These were supplemented with the incorporation of all secondary references found in each article. The research was limited to articles published since 2007.

We reviewed each article adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis individual patient data guidelines (7) (Fig. 2).

Eligibility criteria

We collected clinical case reports written in English. The clinical description had to include the timing of the onset of the different symptoms. The chronology had to be mentioned, with a minimum of 2 references to the age of the patient.

The clinical cases had to match the definition of the ROHHAD syndrome as published by Ize-Ludlow and colleagues in 2007 (3): [1] onset of rapid and extreme weight gain after age 1.5 years in a previously healthy child, [2] evidence of hypothalamic dysfunction, [3] central hypoventilation, and [4] features of autonomic dysregulation. Cases were eligible if they present with criteria [1] + [3] + one clinical sign of hypothalamic dysfunction [2]. In the absence of criteria [2] or [3], if a neuroendocrine tumor is present, we collected the data because of the possibility of further evolution in these patients with later onset of criteria [2] and [3] (in particular in case reports of young children).

To collect more individual data, abstracts presenting a complete overview of the clinical evolution of patients were also collected for the present review.

Exclusion criteria

Original articles such as review articles that do not contain individual data were not included. Letters to editors, commentary, and general publications about ROHHAD(NET) syndrome were not included if they did not contain any individual data.

Clinical cases that did not match the ROHHAD definition were excluded.

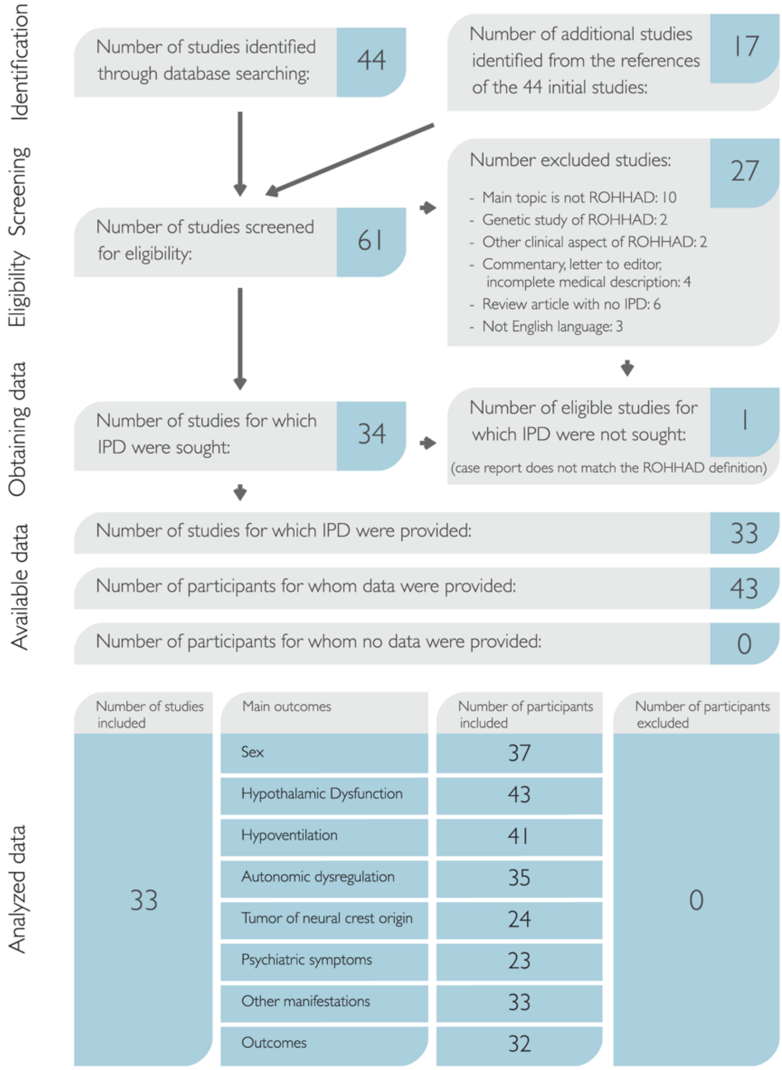

Figure 2. Preferred Reporting Items for Systematic Reviews and Meta-Analysis individual patient data (IPD) flow diagram (based on Stewart et al [7]).

Data extraction

Data were extracted from all the included case reports. The demographic information included age, sex, and ethnicity. Clinical manifestations were divided into 7 categories (1): hypothalamic dysfunction (2), autonomic dysregulation (3), hypoventilation (4), NET (5), psychiatric symptoms (6), other clinical manifestations, and (7) outcome. For each category, data were collected regarding clinical description, apparition of each symptom, laboratory findings, and management strategy.

Data analysis

Data were collected to calculate the mean age at which each symptom appeared. Mean age was calculated for each item for all patients for whom these data had been reported. Mean, median, SD, minimum value, maximum value, 25th percentile, and 75th percentile were automatically generated for each item with a conventional spread sheet. Data are expressed as medians (25th-75th percentiles) or percentages in the text. R software was used to generate Box-Plots graphs (R Core Team, 2013).

Results

Sixty-one specific articles dedicated to ROHHAD or ROHHAD(NET) syndrome were considered, and 33 articles were selected for extraction of individual patient data (4, 5, 8-38). Forty-three patient descriptions were extracted from these case reports. Descriptions of approximately 70 supplementary ROHHAD patients were not included because of the possibility of duplication of patients and a lack of precise clinical description in these case series.

General description

Case reports of 43 patients were included, 29 female and 8 male (N = 37; 6 missing data), with a female to male ratio of 3:6. All cases were described at pediatric age except for 2 young adults (diagnosis made during infancy, and follow-up until maximum age of 27 years).

Fig. 3 summarizes the results concerning the timing of onset of the different major clinical signs encountered in ROHHAD patients.

Hypothalamic dysfunction. The main criterion of the disease is the onset of rapid weight gain that appeared at median age 3.1 years (2.8-4 years) (N = 43) with a description of hyperphagia that started at median age 3 years (2-3.6 years) (N = 23).

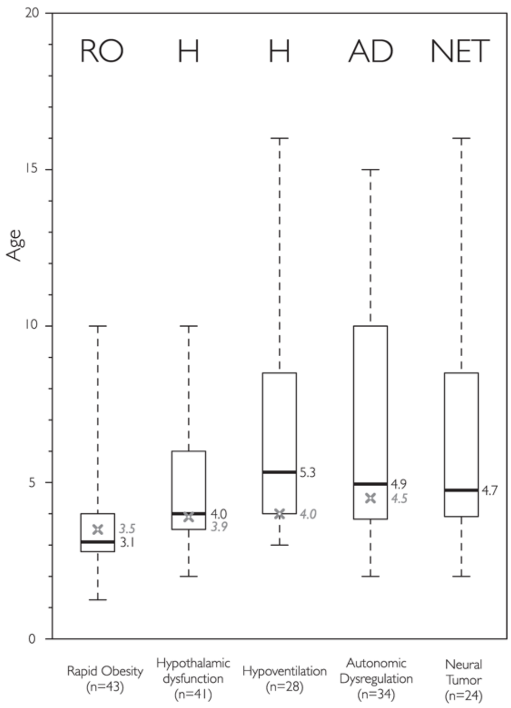

Figure 3. Rapid-onset obesity with hypoventilation, hypothalamic dysfunction, and autonomic dysregulation syndrome (neural crest tumor) (ROHHAD[NET]) general timeline. Age of outcome for each specific term of the ROHHAD(NET) acronym. Box plots show the median values and the first and third quartiles for each group. T-bars represent the rest of the data. Outlier data were not removed. The gray cross shows the time of outcome of each symptom for our patient. No NET was detected at that time, after 3 years and 6 months of follow-up.

Fig. 4A illustrates the timing of onset of different hypothalamic dysfunction encountered in ROHHAD syndrome, and Fig. 4B shows the number of symptoms presented by each patient, with a median of 2 different symptoms reported per patient.

Hypoventilation. Hypoventilation was mentioned for 41 patients (Fig. 4C). Two cases are not described in terms of hypoventilation; they are young patients (age 2.5 and 3.8 years) with NET and obesity. Central hypoventilation occurred at a median age of 5.3 years (4-8.5 years) (N = 28) and was diagnosed for 83% of the patients in the 5 first years after the beginning of obesity.

Obstructive apneas were also described for 15 children at a median age of 4 years (3.2-5.25 years). For 5 patients, an exclusive central hypoventilation was explicitly reported without obstructive apnea. Cyanotic episodes were observed in 7 patients at a median age of 4.7 years (2.9-5 years).

Tracheostomy was needed for 12 patients at a median age of 4.8 years (4.1-5.1 years), secondary to the management of acute respiratory distress (N = 5/12) or secondary to the need to optimize ventilator chronic management (N = 7/12).

NIV was started at a median age of 6.25 years (4-7.9 years) for the 10 cases that specified this timing. Two other cases described NIV treatment without reporting a specific timing.

Eleven patients presented with acute respiratory failure and required intubation during ROHHAD disease (N = 3) or at the beginning of the diagnosis process (N = 8) at a median age of 5 years (3.3-9.5 years).

Autonomic dysregulation. Autonomic disturbances were described for 35 patients. There were no available data for 8 patients. Table 1 lists autonomic dysfunctions. They are encountered at various ages (Fig. 4D) and stages of the disease. Concerning thermal dysregulation, 4 patients were reported with hypothermia, 6 with hyperthermia, and 5 with fluctuating hypothermia or hyperthermia (N = 15).

Neural crest tumor. Twenty-four patients were reported to have NETs, representing 56% of the cohort. The median age of occurrence was 4.75 years (4-8.45 years). The type of tumor was a ganglioneuroblastoma for 2 patients, a neuroblastoma for 2 patients, and a GN for 18 patients. One child was described with an aggressive neuroblastoma presenting as metastasis.

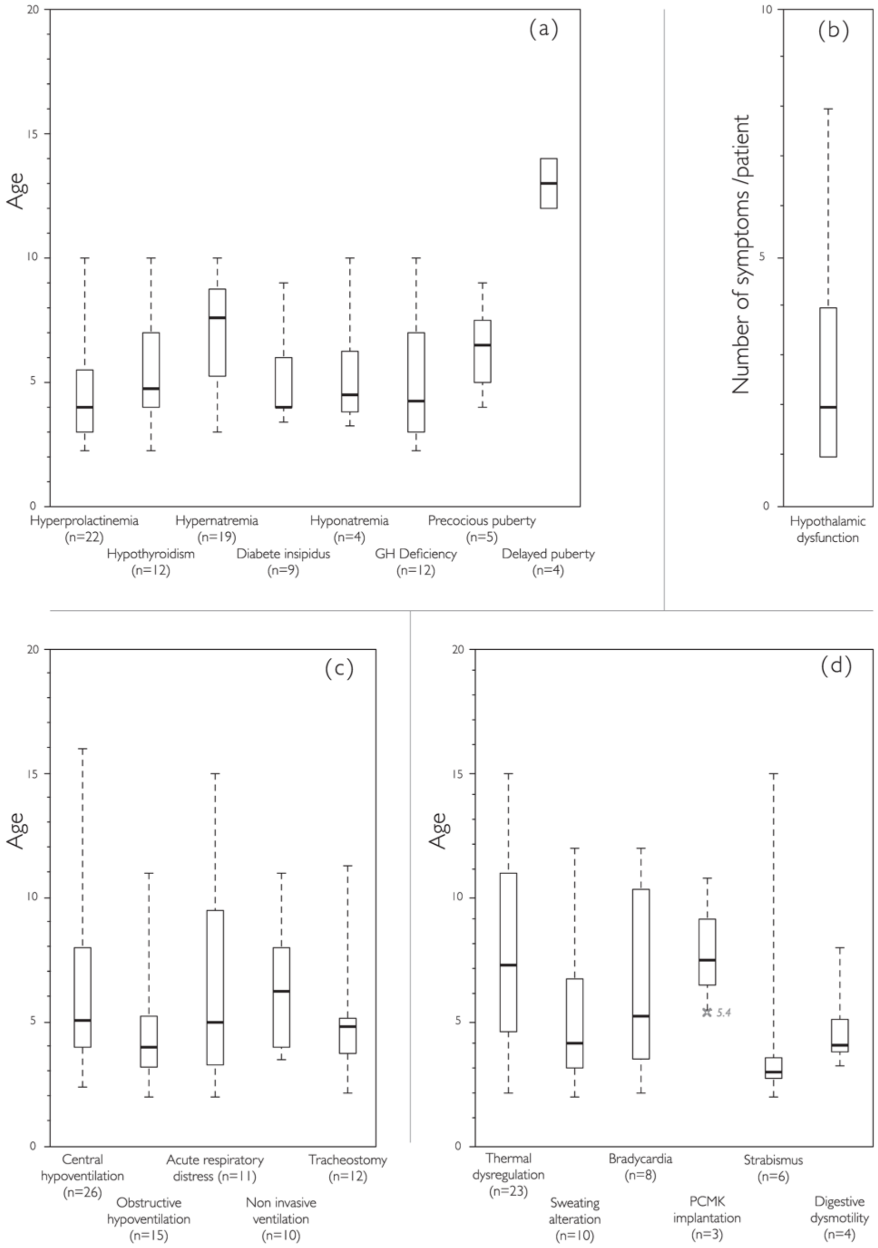

Figure 4. A, Hypothalamic dysfunction. Age of outcome for the main hypothalamic disorders reported in rapid-onset obesity with hypoventilation, hypothalamic dysfunction, and autonomic dysregulation syndrome (neural crest tumor) (ROHHAD[NET]). All symptoms appeared before age 10 years, except of course for delayed puberty. Box plots show the median values and the first and third quartiles for each group. T-bars represent the rest of the data. B, Hypothalamic dysfunction. Number of symptoms encountered by patients during the course of the disease. C, Hypoventilation. Age of outcome of different type of respiratory events reported in the cohort. Central and obstructive hypoventilation were reported, acute respiratory distress was mentioned for 11 patients, and therapeutic management was reported with noninvasive ventilation or tracheostomy. D, Autonomic dysregulation. Age of outcome of the different types of autonomic dysregulation and therapeutic management. Gray cross shows the time of pacemaker implantation for our patient, at the younger age currently described.

Table 1. Description of different types of autonomic dysregulations reported in ROHHAD[NET] and median age of outcome

	No.	Mean age, y	Median	SD
Digestive				
Gastrointestinal dysmotility	4	4.9	4.1	2.1
Constipation	2	6.3	6.3	2.4
Ophthalmic				
Strabismus	6	4.7	3.0	4.6
Other ophthalmic manifestations	5	4.7	4.5	1.1
Bilateral ptosis	1	3.4	3.4	/
Thermic				
Cold hands and feet	6	4.8	4.2	2.7
Thermal dysregulation	23	7.8	7.3	3.9
Hypothermia/ hyperthermia[a]	15	–	–	–
Altered sweating	10	5.4	4.2	3.4
Cardiac				
Bradycardia	8	6.6	5.3	3.8
Syncopes	1	3.0	3.0	
PCMK implantation	3	7.9	7.5	2.7
Pain				
Altered perception of pain	7	3.9	3.8	1.7
Others				
Dysarthria	2	11.0	11.0	5.7
Pulmonary hypertension	1	9.0	9.0	–

Abbreviations: No., number of patients reported with symptom; PCMK, pacemaker.
[a]Hypothermia N = 4; hyperthermia N = 6; episodes of hypothermia and hyperthermia N = 5.

The different neural tumor locations reported included adrenal N = 9, thoracic N = 5, paravertebral cervical N = 1, paravertebral abdominal N = 1, and retropancreatic N = 1. One of the reported tumors was a benign hamartoma with proliferating neural elements.

In addition to these neural tumors, one other case presented with a hepatocarcinoma at age 26 years in the context of hepatic fibrosis due to initial nonalcoholic fatty liver disease.

The period between rapid weight gain and the diagnosis of NET was recorded for 24 patients. According to these data, 50% of the patients (28% of the total cohort) presented with such a tumor 0.75 years after weight gain, and 70% of the patients (40% of the total cohort) were affected by a neural tumor within 2 years after the initial onset of obesity.

Psychiatric/behavioral disorders. Psychiatric manifestations or behavioral problems are reported in ROHHAD(NET) patients.

Hallucinations were reported in 3 patients (median age of 10 years [7.5-11 years]). Major anxiety was reported in these 3 patients and in 1 additional case at a median age of 4.5 years (4.25-6.6 years).

Psychosis was also reported for 2 patients at ages 4.75 and 5 years, and flat affect for 4 other patients (median age of 3 years [2.9-4.25 years]).

Behavioral problems were described for 13 patients at age 3.8 years (3-4.3 years): aggressiveness for 7 patients at age 3.8 years (3.25-4.2 years), hyperactivity for 2 of these 7 patients at age 3 and 3.8 years, and irritability for 3 other patients at age 4 years (3.6-4.75 years).

Other clinical manifestations. Cerebral MRI results were reported for 23 patients and were normal except for 2 patients for whom particularities were noted: One case presented with an absence of the posterior bright spot, and one presented with a normal pituitary axis but with a general cerebral atrophy.

A developmental disorder was encountered in 9 cases with a developmental regression described for 4 patients at age 2.8 years (2.6-3.2 years).

Pneumonia can be a trigger of respiratory decompensation and was encountered in 3 cases.

Seizures were described for 3 patients at a median age of 4 years (3.5-7.5 years).

Among the 43 patients described, there were 2 with narcolepsy confirmed with decreased hypocretin levels. Hypersomnolence was reported in 6 other cases, without more clinical information about the eventual diagnosis of narcolepsy. Symptoms were often described as sleep attacks.

Hypercholesterolemia was mentioned for 2 patients with major hypertriglyceridemia (1062 mg/dL) for one of them. Metabolic syndrome was reported for 2 patients and 1 of them developed severe insulin resistance evolving toward clinical diabetes at age 14 years.

Rectal prolapses were reported for 2 patients, as a secondary sign of dysautonomia and dysregulation of digestive function.

Celiac disease was reported for 1 patient, occurring 5 years after the initial rapid weight gain.

Scoliosis was reported for 1 patient at age 9 years.

Therapeutic management: Three patients were reported to be treated with intravenous immunoglobulin, 6 patients with cyclophosphamide, 5 patients with rituximab, and 3 with corticoids.

Outcome. The outcome was reported for 32 patients of the cohort. Six of the 32 patients died. The age of death was available for only 3 of these patients, allowing the calculation of a median age of 4.6 years (4.5-6.3 years). Among the reported causes of death, 2 were consecutive to a sudden cardiorespiratory arrest, 1 was secondary to a blocked tube during ventilation, and another 1 was caused by septic shock with multiple organ failure. An autopsy was conducted only for this last case and revealed hypothalamic encephalitis.

Twenty-six patients were clearly described to be still alive in their respective publications, with a median age of 8 years (5.2-12.8 years) (N = 14) at the end of the described follow-up; among them, 70% were younger than 12 years.

Discussion

Diagnosing ROHHAD syndrome is challenging because of the lack of any specific marker. Clinical criteria offer clues to diagnosis but they do not all appear concurrently.

In our patient, the ROHHAD diagnosis was made rapidly thanks to the initial explorations. The prompt diagnosis was the first step in medical care and was essential to anticipate many of the symptoms such as the necessity for early implantation of a cardiac pacemaker.

In addition to the vital management in ROHHAD syndrome, a lot of comorbidities and associated symptoms such as the risk of NET require rigorous follow-up. To better understand the clinical evolution and timeline of the disease, we analyzed, for the first time, the detailed evolution of 43 patients described to date in the literature.

ROHHAD syndrome is characterized by a sequence of clinical features emerging with advancing age, commonly accepted as appearing in the order of the acronym: rapid-onset obesity with hypothalamic dysfunction, hypoventilation followed by autonomic dysregulation syndrome (3, 39). There is however no evidence for a systematic sequence of symptoms. ROHHAD syndrome has also been associated with a series of comorbidities or associated symptoms such as NET (4, 14). Currently, there is no specific recommendation for the management or timing of the investigations. The last large clinical description of a ROHHAD cohort of 15 patients was published by Ize-Ludlow et al in 2007, when her group defined for the first time the term *ROHHAD disease* (3).

The initial event is always rapid weight gain accompanied rapidly by a second hypothalamic dysfunction. Hypoventilation has been found more or less rapidly after the onset of obesity (Fig. 3). Our description shows that hypoventilation appears at a median of 2.2 years (0-3.75 years) after the first sign of rapid obesity. Because patients were not systematically evaluated with a polysomnography at the beginning of the pathology, knowing precisely when hypoventilation starts is very difficult. We would recommend vigilance with such young patients with a weight curve showing a rapid weight gain without any other clear etiology. If the child also presents with an endocrinology disorder or a dysautonomic disturbance, a polysomnography seems to be a safe preventive exam to confirm ROHHAD syndrome. In case of high suspicion, the exam has to be repeated annually for a minimum of 5 years. In our review, central hypoventilation was diagnosed for 83% of patients during the first 5 years after the beginning of the obesity.

Hypothalamic dysfunctions were reported rapidly after the onset of obesity, with a median interval timing of less than 1 year (0.9 years). The principal hormonal dysregulation reported in the literature was hyperprolactinemia with values ranging from slightly elevated (44.7 ng/mL) to significantly increased (380 ng/mL). Central hypothyroidism and GH deficiency were frequently reported, with GH levels almost undetectable after GH stimulation test (9, 15, 23, 26-28).

Natremia disorders were a frequent observation with different types of water imbalance description. A series of patients were initially managed in the pediatric intensive care unit (N = 8). In this context, hypernatremia, hyponatremia, and syndrome of inappropriate antidiuretic hormone or diabetes insipidus were described (6, 8, 23, 24, 26, 30, 34, 36). Some patients recovered but not all. Other patients presented initially with polyuria-polydipsia syndrome (N = 5), requiring some of them to undergo treatment with desmopressin (21, 22, 30, 37).

Hypoventilation is a key symptom of the disease. In our cohort, central hypoventilation occurred at a median age of 5.33 years, 2.2 years after the onset of obesity. Sleep apnea or a pattern of mixed apnea can be described initially. All the patients were treated initially with a nocturnal NIV. Tracheostomy was reported during acute decompensation (N = 5/12), and for some patients it was necessary to optimize the chronic ventilation (maximum 24 hours/day) (N = 7/12). One patient was mentioned as having a pacemaker diaphragm without a more precise specific description of his ventilation (22).

The management of ventilation is an essential point to avoid respiratory failure and the negative impact of hypercapnia, and to help control body mass index. Altered ventilation can be the cause of behavioral disorders or neurocognitive impairment (23). Our data suggest such a link: The occurrence of aggressiveness or mood disorders was described mostly at a median of 1.5 years before the diagnosis and management of the hypoventilation. In our case, the patient presented with severe aggressive anger in the beginning of the disease. With perfect management of the NIV at home and strict regularity of sleeping hours, the patient has improved very well with normal behavior for his age.

Dysautonomia is a major clinical point in the syndrome. Impaired sweating and thermal dysregulation were principally reported. Hypothermia or hyperthermia can be objectivized, as mentioned in 15 cases in our cohort. Therefore, temperature instability reported in a young obese patient must lead to considering the possibility of ROHHAD syndrome. Cold hands and feet were visible signs observed in 6 patients in the series. Altered perception of pain is an essential point for which the clinician has to be attentive. All the reported dysautonomia signs were independent of each other. Strabismus and sweating alteration are the first 2 clinical signs encountered after initial weight gain.

NETs are classically diagnosed in approximately 50% of ROHHAD patients (4). Our review consolidates this statistic with 56% of patients encountered with NET. The majority of the tumors were GN located mainly at an adrenal location. All the patients were asymptomatic, except for one with a retropancreatic mass and a metastatic process causing weight loss and jaundice. The clinical presentation and characterization of neuroblastoma seems to be specific in ROHHAD patients compared to the general population (Table 2) (40, 41) with more indolent presentation and diagnosis at an older age. Nevertheless, only one ROHHAD patient was observed with *MYCN* amplification, a marker of pejorative prognostic factor and correlated with aggressive features at diagnosis.

No practical recommendations are currently available to define NET screening. In some publications, ROHHAD clinical follow-up included urinary catecholamine dosages, neuron-specific enolase (a biomarker of tumor proliferation in case of neuroblastoma) screening, ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy, or chest and abdominal MRI. In our patient, the strategy is to screen with ^{123}I-MIBG scintigraphy every year because the use of MRI is strictly restricted because of the cardiac pacemaker. An annual tumor screening seems to be the most usual clinical practice, but our survey supports the need for more frequent screenings during the 2 first years after the ROHHAD diagnosis. Our data show that 70% of the NETs were detected in the first 2 years after the initial weight gain and 50% at a maximum of 0.75 years. A chest and abdominal MRI should be performed every year, alternating with annual ^{123}I-MIBG scintigraphy, allowing surveillance every 6 months. In case of contraindications or unavailability for one of these exams, abdominal ultrasound is recommended as an alternative option. None of the ROHHAD patients tested positive for catecholamines to establish follow-up recommendations on this basis. On the contrary, this measure is falsely reassuring for such patients. After the first 2 years of follow-up, annual screening should be performed based on chest and abdominal MRI or ^{123}I-MIBG scintigraphy, depending on the patient's medical conditions and the local hospital conditions (Fig. 5). Because of some tardive occurrence of NET and the isolated case of an aggressive tumor 11 years after the initial rapid weight gain, screening must be continued. Decreasing the frequency of imaging to every 2 years (3) could be discussed for adult follow-up. Data from young ROHHAD(NET) adult patients are needed to improve these clinical recommendations.

Currently, there are only 2 recent publications with adult data. The first (21) mentioned particularities for airway management and obstructive apnea concurrent with central hypoventilation linked to severe obesity. The second (25) focused on the natural history of one 27-year-old ROHHAD patient who presented with fatty liver disease over time and a hepatocarcinoma at age 26 years. In the total cohort of 43 patients, no other case was described with hepatic disturbances during childhood. The patient described by Jalal-Eldin and colleagues was also the only one with reported diabetes diagnosed at age 14 years (25). Lipid metabolic disturbances were noted for 2 patients (hypertriglyceridemia for one and hypercholesterolemia for the other). With regards to improving the management of metabolic disturbances, further descriptions and young adult data would be useful. Close monitoring of metabolic parameters is nevertheless recommended during the entire follow-up.

WES of the patient and his parents did not identify any genetic variant that could explain the phenotype, which confirms previous reports (6, 30, 42-44). Nevertheless, our guidance (Fig. 5) recommends WES to rule out other differential diagnoses. For example, Thaker et al (45) revealed a patient with a clinically similar ROHHAD evolution with an *RAI1* mutation corresponding to a diagnosis of Smith-Magenis syndrome.

Three main hypotheses for the cause of ROHHAD are currently being discussed, though research remains inconclusive. First, genetic studies have investigated candidate genes in neuronal development (among which especially *BDNF* and *TRKB*) or in the hypothalamic and autonomic dysfunction pathway (among which *HTR1a*, *OTP*, *PACAP*, *HCRT*, *HCRTR1*, and *HCRTR2)* but did not identify any significant genetic variant (6, 30, 39, 40, 42-44, 46). A 2018 genetic study investigated the 11 genes of the Prader-Willi syndrome region, including *MAGEL2* (39). No mutations were found; however, the expression levels of these genes have not been studied in ROHHAD patients and could be altered by epigenetic

Table 2. Comparison between neural crest tumor (NET) in the general population and in ROHHAD(NET) patients

	General population (40, 41)	ROHHAD(NET) patients (N = 24)
Epidemiology	Incidence 1/10 000 live-born	56% in the cohort (n = 24/43)
Median age of diagnosis	19 mo	4.75 y
Localization	1/Adrenal (46%) 2/Extra-adrenal abdominal location (18%) 3/Posterior mediastinum or thorax (14%)	1/Adrenal (N = 9) 2/Thoracic (N = 5) 3/Paravertebral cervical (N = 1)
Catecholamines metabolizing	Positive in 75% of patients (high levels of vanillylmandelic acid and homovanillic acid)	Never reported as pathologic
Symptomatology	Abdominal pain or distension Hypertension Scoliosis Neurologic signs (medullar compression)	No relevant symptoms Diagnosis made during screening
Metastasis at diagnosis	50% of cases	1 case (N = 1/24)
Staging	Low risk Intermediate risk High risk 50%	1 metastatic case (N = 1/24) 1 high risk reported (N = 1/24)
MYCN amplification (pejorative factor)	20% of the primitive tumors	1 case reported

Neuroblastoma is commonly used to describe a spectrum of neuroblastic tumors including neuroblastomas (the most common type), ganglioneuroblastomas, and ganglioneuromas (40).
The *MYCN* gene is a cellular proto-oncogene: Its amplification in the tumor is a pejorative factor.

or (post)transcriptional mechanisms. A study of a pair of monozygotic twins with different phenotypes (6, 30) highlights this last notion and is consistent with the second etiological hypothesis, epigenetic disturbance. For the monozygotic twins the accumulation of variants in their epigenome is indeed the main factor influencing their phenotype differences.

Finally, some literature suggests an autoimmune origin for ROHHAD syndrome. Immunosuppressive treatment with high-dose cyclophosphamide was reported to have positive effects, in particular on body mass index stability and neuropsychological function, but with limited follow-up data (a maximum of 18 months backward) (24, 31). Two patients were found to have an intrathecal synthesis of oligoclonal bands at the time of diagnosis (33), but no other cases reported a measurement of oligoclonal bands in our cohort; in addition our patient tested negative. An immune process was also supported by the unique autopsy of a 5-year-old girl with ROHHAD with evidence of hypothalamic encephalitis with a perivascular and mild parenchymal chronic inflammatory infiltrate of CD3 T cells without vascular necrosis (34). Giacomozzi and colleagues in 2019 appeared to go further with the identification for the first time of antipituitary and antihypothalamus autoantibodies on serum and cerebrospinal fluid in a case of a ROHHAD girl who died at age 3 years (5).

In our review, immunomodulating treatments were described for 6 patients, with the use of glucocorticoids, or intravenous gamma globulin or specific immunosuppressive treatments (cyclophosphamide, rituximab). These data were not specifically analyzed because of the lack of precise information (time of administration, different dosages).

ROHHAD(NET) syndrome is considered a life-threatening condition with death occurring around age 10 years (8). In this review, the median age of death was estimated at 4.6 (3-6) years (age of death reported for only 3 patients), but 26 patients were still alive at the end of the follow-up. Two of the six patients died as a result of sudden cardiac arrest. The young age at death could suggest that some cases may occur with more severe initial clinical presentation. Early death can also be linked to a delayed diagnosis affecting the initial management, which is essential for better life support.

Conclusions

We report a ROHHAD case with early diagnosis and a multidisciplinary care program. This illustrates the need for clear diagnosis criteria and standardized monitoring. Based on a literature review, we propose several recommendations for the diagnosis and follow-up of these patients in the absence of reliable biomarkers and a clear etiology. Early diagnosis and appropriate follow-up probably improve to a great extent prognosis and life expectancy.

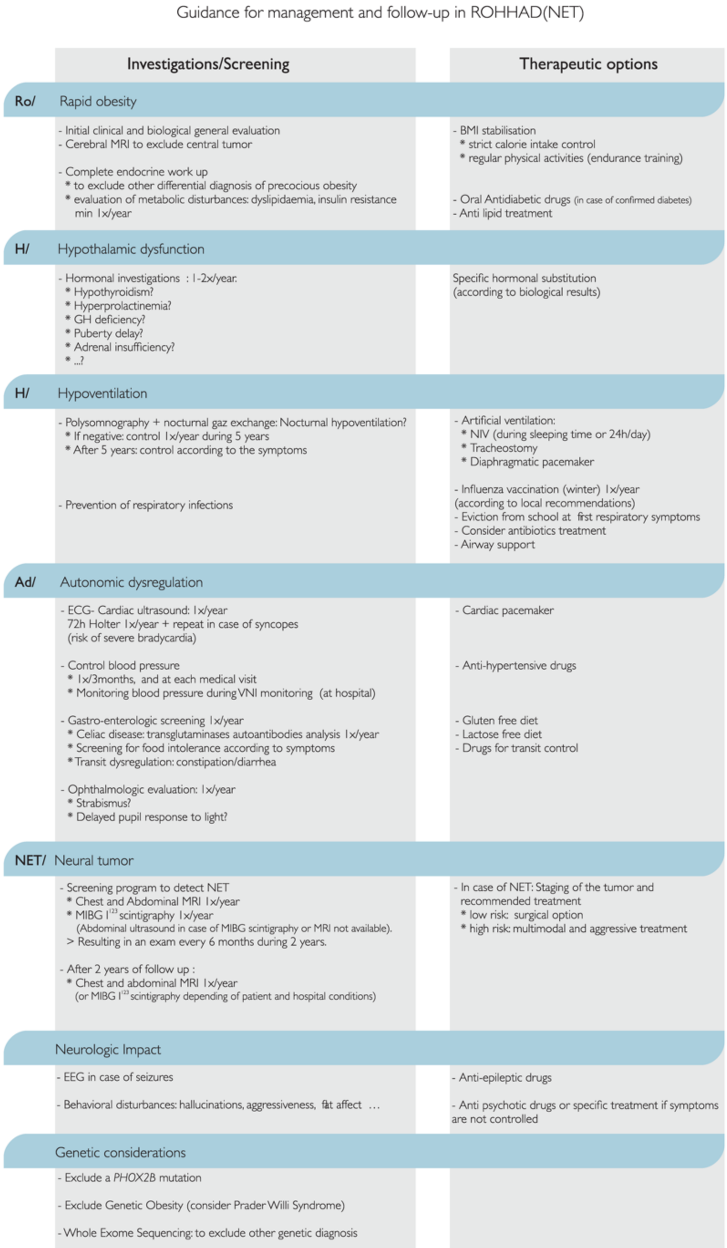

Figure 5. Guidance for management and follow-up in rapid-onset obesity with hypoventilation, hypothalamic dysfunction, and autonomic dysregulation syndrome (neural crest tumor) (ROHHAD[NET]). A multidisciplinary approach is highlighted. All caregivers should be supported by a Reference Centre for Rare Disease.

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Disclosure Summary: The authors have nothing to disclose.

Data Availability: The data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

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4.2. Systematic Review: Updated Findings

Our review of 2020 intended to analyze all the reported cases published since 2007 to better describe the clinical events in ROHHAD(NET) and to propose recommendations for appropriate multidisciplinary management. Since 2020, no consensual recommendations have not yet been published but a series of additional case reports and review have been documented.

Our objective is to update the current data of 2020 to refine the understanding of the full clinical spectrum of ROHHAD, especially by exploring the emergence (or not) of new or divergent features in its evolution.

4.2.1. Research methods

We conducted a systematic analysis of the medical literature to identify all new published clinical cases of ROHHAD and/or ROHHAD(NET) using the online database PubMed, between the 1st of May 2019 and the 1st of August 2025. Language was restricted to English. The search query was limited with the terms ROHHAD and ROHHAD(NET).

4.2.2. Eligibility criteria

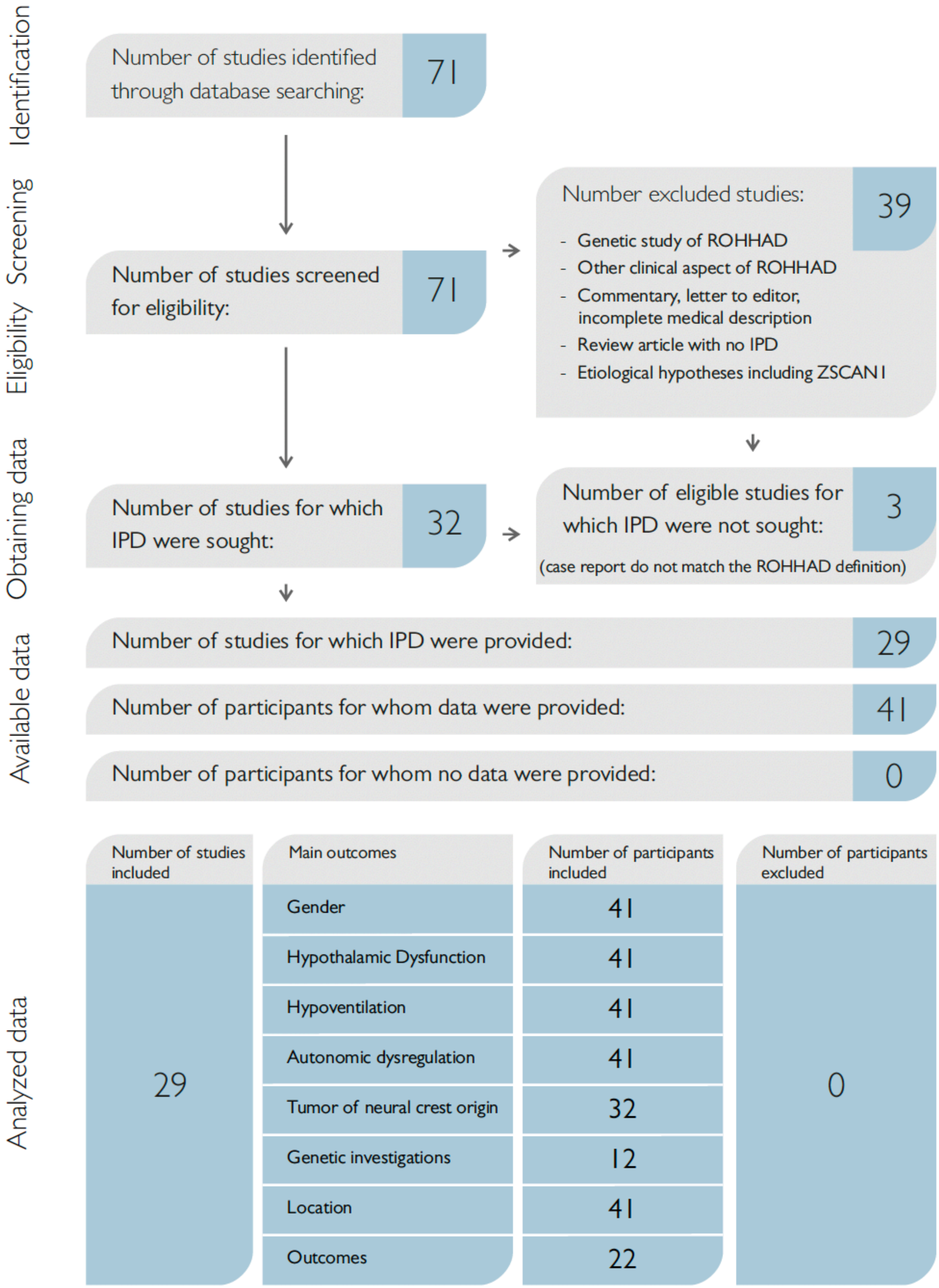
We collected clinical case reports written in English. The clinical description had to include the timing of the onset of the different symptoms. The chronology had to be mentioned, with a minimum of 2 references to the age of the patient.

The clinical cases had to match the definition of the ROHHAD syndrome as published by Ize-Ludlow and colleagues in 2007 (Ize-Ludlow D. *et al*., 2007): [1] onset of rapid and extreme weight gain after age 1.5 years in a previously healthy child, [2] evidence of hypothalamic dysfunction, [3] central hypoventilation, and [4] features of autonomic dysregulation. Cases were eligible if they present with criteria [1] + [3] + one clinical sign of hypothalamic dysfunction [2]. In the absence of criteria [2] or [3], if a neuroendocrine tumor is present, we collected the data because of the possibility of further evolution in these patients with later onset of criteria [2] and [3] (in case reports of young children).

4.2.3. Data extraction

Data were extracted from all the included case reports. The demographic information included age, sex, and ethnicity. Clinical manifestations were divided into 7 categories (1): hypothalamic dysfunction (2), autonomic dysregulation (3), hypoventilation (4), NET (5), psychiatric symptoms (6), other clinical manifestations, and (7) outcome. For each category, data were collected regarding clinical description, apparition of each symptom, laboratory findings, and management strategy.

Figure 17 – PRISMA flow diagram for the updated review.



Preferred Reporting Items for Systematic Reviews and Meta-Analysis individual patient data (IPD) flow diagram (based on Stewart *et al*., 2015) presenting the methodology for the conduction of a review of the recent published data on ROHHAD patients. Our review includes all the published cases after May 2019 to propose a completion of the literature between our publication in May 2020 and the current situation (August 2025).

4.2.4. Results

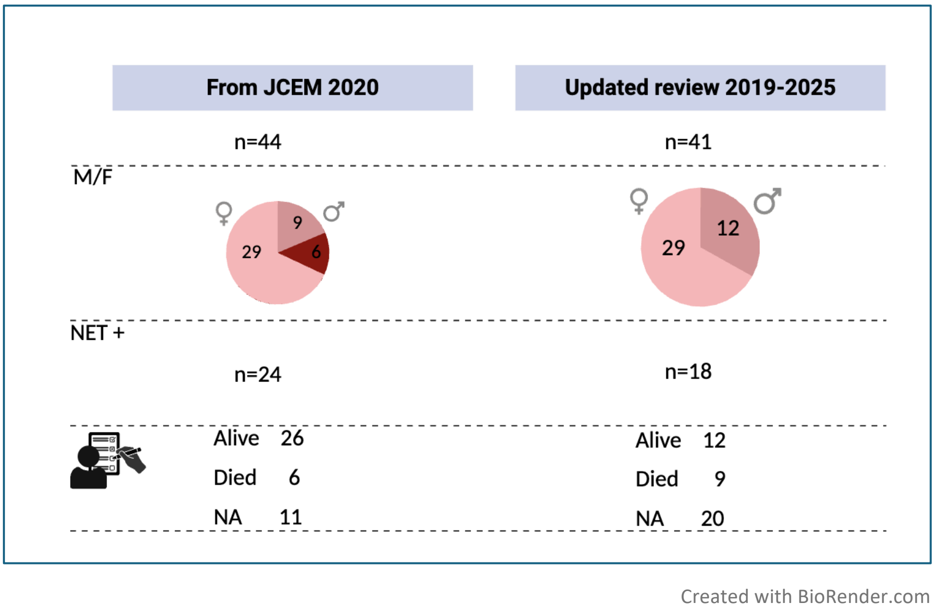
Based on our updated review, the ROHHAD condition appears to follow the same timeline of progression as previously reported in our 2020 publication. The proportion of patients presenting with a neuroendocrine tumor (NET) remains stable, with 24 out of 43 cases reported before 2020 and 18 out of 41 in the updated dataset. This results in an overall tumor prevalence of 49,4% (42/85) among ROHHAD patients.

Regarding tumor types, only neural crest-derived tumors were identified. The updated data did not reveal any additional histological types, and notably, no highly malignant forms were observed—contrasting with the initial review, which included one hepatocarcinoma and one aggressive neuroblastoma. For all ROHHAD patients described between 2019 and 2025, tumors were predominantly located in the adrenal region or in thoracic/abdominal paravertebral areas.

Concerning patient outcomes, the 2020 data reported six deceased patients at the time of publication and 26 explicitly alive. In the updated dataset, nine patients were reported as deceased, 12 still alive, and for 20 patients, the outcome was not clearly specified. The age at death was available for only three individuals: one died at age 11, another at 21, and the third at 57. The latter case corresponds to a particular adult-onset ROHHAD patient, whose death occurred 32 months after symptom onset.

Importantly, this updated cohort includes, for the first time, an adult patient fulfilling all ROHHAD diagnostic criteria—except for the pediatric age requirement. This case provides a significant clinical observation that should be considered in future discussions aimed at refining the definition and scope of ROHHAD, and at establishing more precise clinical criteria.

Figure 18 – Comparison of the results from the 2 phases of reviewing knowledges on ROHHAD patients



5. The ROHHAD spectrum: Clinical and Phenotypic Insights

5.1. ROHHAD syndrome without rapid-onset obesity: A diagnosis challenge.

Desse B., Tran A., Butori M., Marchal S., Afanetti M., Barthélemy S., Bérard E., Baechler E., Debelleix S., Lampin M.-E., Macey J., Massenavette B., *Harvengt J.,* Trang H., & Giovannini-Chami L. (**2022**)

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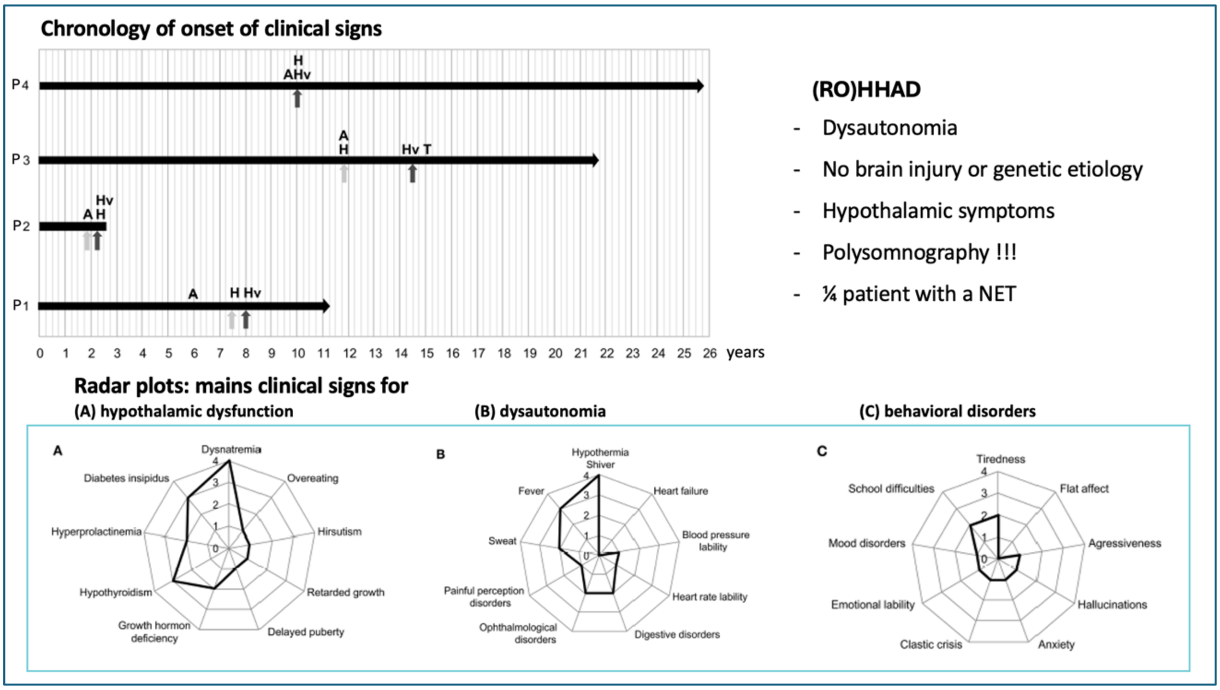
Figure 19 - Graphical Highlights: Chronological onset and distribution of symptoms for the four patients.



5.1.1. Summary and highlights

As part of our work on ROHHAD phenotype delineation, collaborations with other teams have emerged and conducted to a contribution to a new concept of ROHHAD syndrome without rapid-onset obesity{(RO)HHAD)}. This diagnosis challenge has been described by Desse *et al*. in a study published in 2022. Our team has been involved in the discussion and genetic investigations for two of the four patients described in the cohort. Our raw data from our review of 2020 have been the support to the elaboration of statistical comparative analysis for symptom onset comparisons between ROHHAD and (RO)HHAD.

By this study, Desse B. *et al*. provide a retrospective, observational, multicenter study including all cases of (RO)HHAD diagnosed in France from 2000 to 2020.

Four patients were identified. Median age at diagnosis was 8 years 10 months. Median body mass index was 17.4 kg/m². Signs of autonomic dysfunction presented first, followed by hypothalamic disorders. All four patients had sleep apnea syndrome. Hypoventilation led to the diagnosis. Three of the four children received ventilatory support, all four received hormone replacement therapy, and two received psychotropic treatment. One child in the cohort died at 2 years 10 months old after PICU management. For the three surviving patients, median duration of follow-up was 7.4 years.

(RO)HHAD is described here for the first time, as a particular entity, appearing later than ROHHAD. This entity should be considered in the presence of dysautonomia disorders without brain damage. Likewise, the occurrence of a hypothalamic syndrome with no identified etiology requires a sleep study to search for apnea and hypoventilation. Identifying (RO)HHAD remains a clinical challenge with significant implications for patient prognosis. Further research is needed to better define the ROHHAD spectrum, including the prevalence and natural progression of (RO)HHAD.

Finally, this initial clinical observation of (RO)HHAD raises new research questions regarding the etiology of ROHHAD. It suggests that the pathophysiological mechanisms of (RO)HHAD may not involve MC4R-expressing hypothalamic neurons, or that an alternative central hypothalamic counterregulatory pathway may preserve feeding regulation.

5.1.1. Original publication

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ROHHAD syndrome without rapid-onset obesity: A diagnosis challenge

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Background: ROHHAD syndrome (Rapid-onset Obesity with Hypothalamic dysfunction, Hypoventilation and Autonomic Dysregulation) is rare. Rapid-onset morbid obesity is usually the first recognizable sign of this syndrome, however a subset of patients develop ROHHAD syndrome without obesity. The prevalence of this entity is currently unknown. Alteration of respiratory control as well as dysautonomic disorders often have a fatal outcome, thus early recognition of this syndrome is essential.

Material and methods: A retrospective, observational, multicenter study including all cases of ROHHAD without rapid-onset obesity diagnosed in France from 2000 to 2020.

Results: Four patients were identified. Median age at diagnosis was 8 years 10 months. Median body mass index was 17.4 kg/m². Signs of autonomic dysfunction presented first, followed by hypothalamic disorders. All four patients had sleep apnea syndrome. Hypoventilation led to the diagnosis. Three of the four children received ventilatory support, all four received hormone replacement therapy, and two received psychotropic treatment. One child in our cohort died at 2 years 10 months old. For the three surviving patients, median duration of follow-up was 7.4 years.

Conclusion: ROHHAD syndrome without rapid-onset obesity is a particular entity, appearing later than ROHHAD with obesity. This entity should be considered in the presence of dysautonomia disorders without brain damage. Likewise, the occurrence of a hypothalamic syndrome with no identified etiology requires a sleep study to search for apnea and hypoventilation. The identification of ROHHAD syndrome without rapid-onset obesity is a clinical challenge, with major implications for patient prognosis.

KEYWORDS

ROHHAD syndrome, hypothalamic syndrome, child, central hypoventilation, dysautonomia

Introduction

ROHHAD syndrome (Rapid-onset Obesity with Hypothalamic dysfunction, Hypoventilation and Autonomic Dysregulation) is a rare disease that was initially described in 1965 under the name "Late-Onset Central Hypoventilation Syndrome with Hypothalamic Dysfunction (LO-CHS/HD)" (1). Subsequently, just over 150 cases have been reported in the literature (2). Recognition of this sydrome constitutes a challenge for clinicians. In 2007, the Ize-Ludlow team (3) suggested diagnostic criteria for ROHHAD combining: (i) rapidly evolving morbid obesity in the first years of life, (ii) signs of hypothalamic dysfunction, (iii) central hypoventilation occurring in childhood, (iv) signs of dysautonomia.

Due to the association with neural crest tumors in 15–50% of cases, the hypothesis of a paraneoplastic etiology has been suggested, but not confirmed (2, 4). The ROHHANET syndrome acronym (ROHHAD and NEural Tumors) have been suggested to describe the entity but ROHHAD remains the preferred term because it accounts for those with and without tumors. No candidate gene has been identified *via* exome sequencing (3–6) and the hypothesis of a monogenic etiology has been challenged by the description of a case in only one of a pair of monozygotic twins (7). The dysimmunity hypothesis seems probable, in particular with the presence of oligoclonal bands in the cerebrospinal fluid of two patients (8) and of antihypothalamic and antipituitary autoantibodies in the serum and cerebrospinal fluid of another patient (9).

Morbid obesity with rapid onset driven by hypothalamic dysfunction is the first recognizable sign of this syndromic sequence in 80–100% of ROHHAD cases. This occurs early in life (median 3 years old) and has been described by Ize-Ludlow as one of the mainstays of the diagnosis (3, 10). A better prognosis of pathology in ROHHAD is associated with an early diagnosis (11).

The main objective of this work was to describe the characteristics of ROHHAD syndrome without rapid-onset morbid obesity (RO) at diagnosis, in cases retrieved from the French cohort of Disorders of Ventilatory Control, registered in the RespiRare® (French Reference Center for Rare Lung Diseases) database. The secondary objective was to compare this cohort with a recent systematic review of cases of ROHHAD with RO (10).

Methods

Population

All cases of ROHHAD without RO who were <18 years old at the time of diagnosis, and registered in the "RespiRare®" French national pediatric database for rare lung diseases, were included through a multicenter national, retrospective study. The RespiRare® database was established in 2006, and includes 33 French "competence centers." Each patient and/or their legal representative gave informed consent prior to their details being entered in the database. For cases that were not listed in the database, data were retrieved by contacting all the French competence centers for rare lung diseases asking for cases followed between 2000 and 2020. The database was approved by the French National Data Protection Authorities and the study was approved by the Institutional Review Board of the "Société de Pneumologie de Langue Française" (France).

The inclusion criteria were:

- hypoventilation confirmed on a polysomnography recording, without pulmonary or cardiac etiology, nor brainstem lesion
- at least one of the following signs of hypothalamic dysfunction: growth hormone deficiency, diabetes insipidus, puberty advance or delay, hypogonadism, hyperprolactinemia, central hypothyroidism, corticotropic insufficiency
- at least one symptom of dysautonomia (hypo/hyperthermia, cold hands and feets, sweating dysregulation, bradycardia, syncopes, orthostatic hypotension, bladder dysmotility, digestive dysmotility (alternating between constipation, diarrhea), strabismus, ptosis, altered perception of pain, ...)
- absence of rapid-onset obesity defined by a body mass index greater than or equal to IOTF-30 when diagnosing ROHHAD. IOTF-30 (International Obesity Task Force) has been defined as child centile curve of BMI corresponding to an adult BMI cut-off points of 30 kg/m2 (12).

Data collection

Data regarding clinical features at the time of diagnosis, investigations, treatment, and outcome were collected using a standardized questionnaire sent to each coordinator physician at the 33 French centers that had declared a patient.

The following patient data, as at diagnosis, were collected and analyzed further: *sociodemographic* (age, sex, weight, height, body mass index, siblings, personal and family history); *diagnostic* (clinical signs, date of onset of specific signs); *hypothalamic involvement* (type of involvement, laboratory parameters, pituitary MRI results); *hypoventilation* (CO_2 response test, results of polysomnography at diagnosis, results of functional respiratory examinations, results of the walking test or stress test, results of the blood gas); *autonomic nervous system damage* (results of ECG and blood pressure holters, cardiac ultrasound data); *behavioral disorders*, if present; *neurological damage*, if present (cerebrospinal fluid analysis, brain MRI); *neuroendocrine tumor*, if present (date of diagnosis, nature and location of the tumor, type of treatment and effect on symptoms, data from thoracic and abdominal imaging); *therapeutic measures* (respiratory treatment: type of ventilatory support, duration, need for a tracheostomy; immunomodulatory treatment: administration of intravenous immunoglobulins, immunosuppressive treatment, type of molecule used, dose and start date; hormonal treatment: control of water intake, molecules used; surgical treatment: adenoidectomy, tonsillectomy, tumor excisional surgery, others; psychiatric treatment); *outcome* [reasons and date of hospitalization (excluding follow-up)]; *education and social support*; *date of the latest evaluation*; *date and cause of death*, if applicable.

ROHHAD with RO cohort

Data from the systematic review of cases of ROHHAD with RO published in the literature (13) were extracted. Additional data not presented in the publication were kindly provided to us by the first author.

Statistics

Descriptive analysis of the sample and figures were performed with R software (www.r-project.org). Data are expressed as median and interquartile range for quantitative variables. The comparison of the quantitative variables was carried out with the Wilcoxon-Mann-Whitney test. The results were considered statistically significant when the p-value was < 0.05.

Results

Ten cases were identified from the initial screening of the French database. Of these ten cases, five were excluded because of obesity at the time of diagnosis, after checking BMI on IOTF-30 curves. Of the five remaining eligible cases, one was excluded due to a clinical and paraclinical presentation closer to encephalitis.

Demographic data

Our study population thus included four children, all male. Consanguinity was not present in two of patients, with data not available for the other two.

Three of the four children had a significant family history. Two parents had dysthyroidism and one parent had been treated for Hodgkin's disease at the age of 9 years. The sibling of one patient was followed by ENT for recurrent infections, while the sibling of another patient presented with Prader-Willi syndrome (PWS).

Diagnosis

The median age at diagnosis of ROHHAD syndrome was 8 years 10 months [2 years 9 months—12 years 7 months]. The first signs of the disease, defined retrospectively, were different depending on the child: hypersomnia; association of fever and excessive sweating; panhypopituitarism; seizure in the context of hypothermia. Hospitalization following onset of the first symptoms occurred for three of four patients, median 3 months [3–3.1] prior to diagnosis. These three children were, respectively, hospitalized for hypernatremic coma, hypoxemic pneumonia, and prolonged unexplained fever with constant strabismus. These hospitalizations did not lead to the diagnosis of ROHHAD. The chronology of onset of clinical signs is summarized in Figure 1. The median body mass index was 17.4 kg/m^2 [16.5–19.35] (Figures 2A–C).

The median time to diagnosis of ROHHAD syndrome was 4.4 months [2.8 months—12.6 months]. The maximum time to diagnosis was 35.4 months. Two diagnoses were evoked by a pediatric pneumopediatrician, the other two, respectively, by a pediatric intensivist and a pediatric endocrinologist. All four patients were screened for any PHOX2B pathogenic variant involved in central hypoventilation syndrome (14). No patient had a pathogenic variant in this gene. An exome sequencing was performed in one patient of the cohort and no pathogenic variant was identified.

Hypothalamic dysfunction

All four children showed hypothalamic dysfunction, with a median age at onset of 8 years 8 months [6 years 2 months—10 years 3 months]. These signs appeared a median of 2 months before the diagnosis of ROHHAD [1–12 months]. The median number of manifestations of hypothalamic dysfunction per child was 5 [3.75–6.5] (Figure 3A).

Hypoventilation

All four children showed hypoventilation, with a median age at onset of 8 years 9 months [6.4–11]. Hypoventilation led to the ROHHAD diagnosis. One child had no respiratory complaints. Two of the four patients presented with sleep apnea. Two of the four children presented episodes of respiratory distress in an infectious context. None of the four patients presented with an attack of cyanosis. All children underwent polysomnography. For 2 patients, the clinical signs of hypoventilation prompted polysomnography. For 2 patients, hypothalamic dysfunction

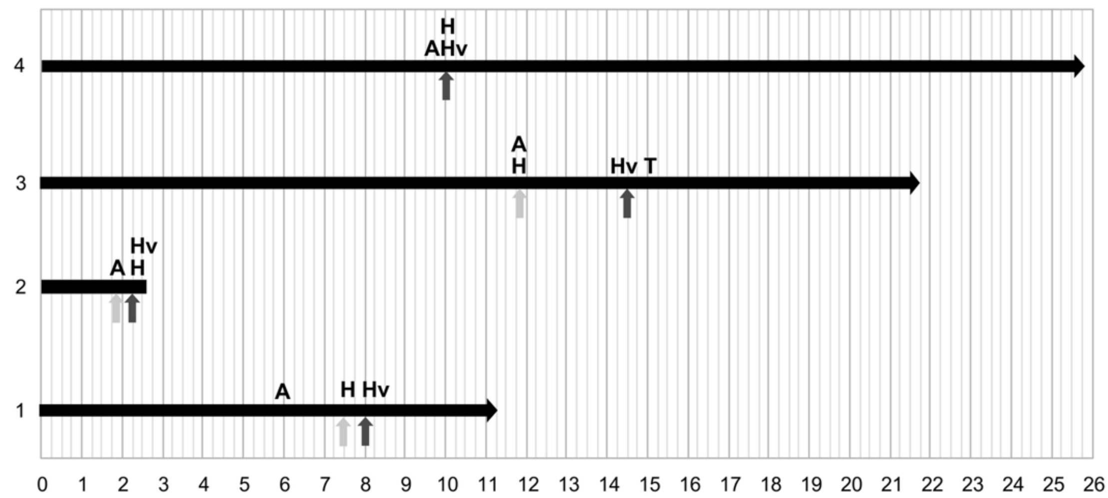

FIGURE 1
Chronology of onset of clinical signs. 1–4 correspond to each patient of the cohort and axial axis to the age at onset of symptoms in years. The end of the black horizontal arrow corresponds to the last follow-up of the patient, or patient death. H represents hypothalamic involvement, A dysautonomia, Hv hypoventilation, T the occurrence of a tumor. The light-gray arrows indicate the time of the first hospital referral and the dark-gray arrows the time of diagnosis.

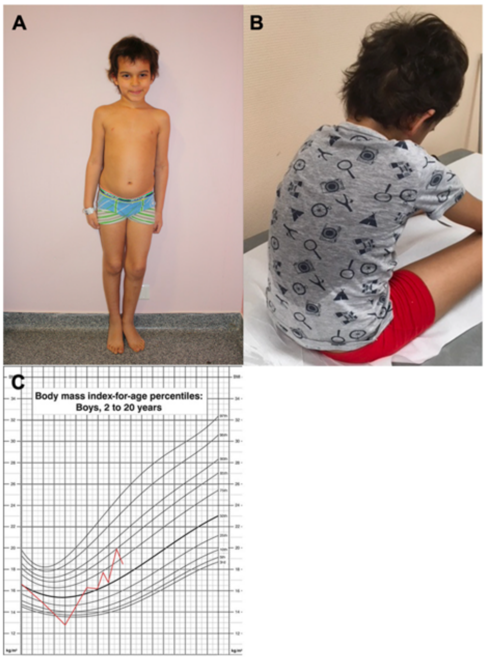

FIGURE 2
Pictures of a patient of the cohort. Panel showing **(A)** normal BMI at diagnosis **(B)** excessive sweating **(C)** follow-up of BMI.

was the main prompter of polysomnography. This was performed at a mean age of 9.3 years [6.5–11.9], 4.5 months after first respiratory symptoms leading to diagnosis [0.4–9]. The results of the initial polysomnography are summarized in Table 1. This initial polysomnography showed hypoventilation in the four cases. Isolated OSA before of onset hypoventilation was not diagnosed in our cohort. Only one patient underwent a test of ventilatory response to carbon dioxide, which was abnormal. Respiratory functional explorations were performed in three children in our cohort, showing a restrictive syndrome for one and being normal for the two others. Only two children in our cohort underwent a stress test, one was normal and the other sub-maximal, nevertheless showing limited aerobic capacity for exercise. Venous blood gas results were available for three of the four patients showing a median capnia of 60 mmHg [56.5–85], median pH of 7.30 [7.27–7.34], median alkaline reserve of 40 mmol/L [33.7–43]. Nasofibroscopy could be performed in two of our patients and was normal.

Dysautonomia

All four children showed dysautonomia, with a median age at onset of the first signs at 7 years 10 months [4.8–10.3]. These signs appeared 15 months before diagnosis of ROHHAD [3.8–27.6]. The median number of clinical signs indicating

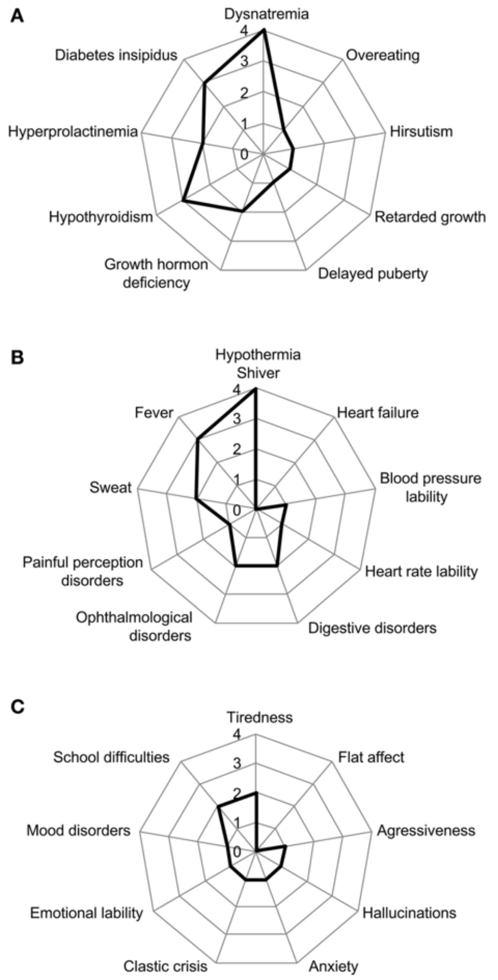

FIGURE 3
Distribution of main clinical signs. Radar plots showing mains clinical signs encountered concerning the fields of **(A)** hypothalamic dysfunction, **(B)** dysautonomia, **(C)** behavioral disorders.

dysautonomia was 3.5 per patient [1.75–5.75] (Figure 3B). No patient presented with cardiac rhythm disorders and no patient presented pulmonary hypertension.

Behavioral disorders

All four children showed behavioral disorders, with a median age at onset of 6 years [4.2–10.1]; 2.9 months [1.3–13.9] before ROHHAD diagnosis (Figure 3C).

Neurological involvement

Three out of the four children presented with neurological involvement, at a median age of 9 years 10 months; 0.26 months [0.13–1.7] after ROHHAD diagnosis. Two of these three patients presented with initial disturbances of consciousness. One patient presented with febrile convulsions at the time of diagnosis. Two children presented with trouble sleeping and one patient presented with attacks of ataxia with catatonia. The cerebrospinal fluid of two children was analyzed, showing no hyperproteinorachia, but seven extra oligoclonal bands in one child. Of two children who underwent electroencephalographic recordings, one was normal, while one patient showed slow bilateral activity. Brain MRI results, performed on average 1.7 months after diagnosis, were available for three patients and did not show any abnormalities, notably of hypothalamic-pituitary structures.

Tumor involvement

Only one child developed a tumoral lesion, in the psoas area, during the course of his disease. This lesion has never been biopsied and is monitored regularly without any sign of progression to date.

Therapeutic care

Three of the four children received ventilatory support, which was noninvasive and nocturnal-only in two cases, and invasive *via* tracheostomy for one. A single patient received intravenous corticosteroid pulses, intravenous immunoglobulins at a dose of 2 g/kg four times, rituximab at a dose of 375 mg/m²/week five times, three sessions of plasmapheresis, ten sessions of immunoadsorption, and four injections of cyclophosphamide. This was the patient with a severe form of ROHHAD with a rapidly unfavorable course, and for whom analysis of the cerebrospinal fluid had identified the oligoclonal bands. Two of the four patients received psychotropic treatment (risperidone, clonidine, lorazepam). The fluid intake of three of the four patients was monitored. All patients received opotherapy (L-thyroxine 3/4, desmopressin ¾, Growth hormone 2/4, hydrocortisone hemisuccinate and testosterone 1/4). The median number of molecules was 2 [2–2.5].

Follow-up

Two children were readmitted to hospital for decompensation subsequent to the hospitalization leading to the ROHHAD diagnosis; one patient for accidental drug

TABLE 1 Results of the initial polysomnography.

	Patient 1	Patient 2	Patient 3	Patient 4
Recording	Night	Nap	Night with ventilatory support	Night
Age (years)	7.8	2.4	15.2	10.8
Paradoxical sleep (%)	20	na	22.7	na
Apnea-hypopnea index (/hour)	8	3.5	2.1	4
Average SpO_2 (%)	94.2	90.8	na	94
Minimal SpO_2 (%)	na	68	83	90
Average $TcPCO_2$ (mmHg)	48	na	na	50
Maximal $TcPCO_2$ (mmHg)	55	60	50	52
% time with $TcPCO_2$ >50 mmHg	38	na	0	10

intoxication with risperdone, while the other case was rehospitalized four times because of episodes of paralysis of the lower limbs, either associated, or not, with hypothermia.

One child in our cohort died at age 2 years 10 months, 5 months after being diagnosed with ROHHAD. The death occurred as a result of limited care in a context of respiratory decompensation. For the three surviving patients, the median duration of follow-up was 7.4 years [5.3–11.6]. The schooling was arranged for two children.

None of these three patients developed obesity during this follow-up period.

Comparison with data from the literature of ROHHAD with RO

We compared the age of onset of the different symptom groups constituting ROHHAD syndrome with data from the systematic review of the literature (10). These data are represented on the Kaplan-Meier curves in Supplementary Figure S1. The disorders seem to occur later in our population, in particular for hypothalamic disorders. However, this difference was not statistically significant due to our limited number of patients. We moreover compared the frequency of each symptom in the two cohorts (Supplementary Table 1). Hypernatremia and diabetes insipidus were significantly more frequent in our cohort and tumor of neural crest significantly lest frequent. Unusual symptoms were described in our cohort such as ataxia, paralysis of the lower limbs and a begnin tumor of the psoas. Our cohort was moreover exclusively constituted of boys.

Discussion

In order to make the diagnosis easier, in 2007 (3) the acronym ROHHAD was proposed to describe the clinical criteria for syndromic diagnosis based on the chronology of symptom onset. Rapidly progressive obesity was described as being the first warning sign of this pathology. We now describe the first cohort of children who were not obese when diagnosed with ROHHAD and who did not develop obesity during follow-up.

In a systematic review of recent literature focusing on the chronology of symptom onset (10), the median age of onset of this obesity was 3.1 years (2.8–4), sometimes preceded by a phase of hyperphagia appearing at the mean age of 3 years (2–3.6). This review (which retains obesity as necessary for the diagnosis) collected 43 cases from the literature for which chronological data were available. The review reported that rapid-onset obesity was always the first sign in this cohort. No study in the literature has, to date, specifically described the entity of ROHHAD without obesity at diagnosis. Its prevalence is therefore very difficult to assess.

Of the fifteen children in the Ize-Ludlow study population (3), obesity was not the inaugural symptom in two children (13.3%). However, both of these children progressed to major obesity within 1 to 5 years. The alveolar hypoventilation in these two cases followed obesity, and one could imagine that the diagnosis was not made on the initial symptoms of dysautonomia, the procession of other symptoms having arisen around obesity.

Likewise, in the cohort of De Pontual et al. (15), rapid-onset obesity was not the first sign of the pathological sequence in three of the thirteen patients included (23%). Only one patient in the cohort did not progress to obesity. However, looking closely at the data presented in this paper, this 2-year-old child was already obese at diagnosis (BMI 20.5), even if not as massively obese as the other patients.

Descriptions in the literature often describe onset of obesity preceded by a period of overeating. In our sample, periods of transient overeating were described in one patient, without significant weight gain. A recent and rapid weight gain was described for the youngest patient in our cohort, but without mention of obesity. The outcome for this patient was unfavorable over a few months and it is likely that weight gain was limited by the clinical condition. In our cohort, the median age at ROHHAD diagnosis was 8 years 10 months, later than the median age described in the literature (4.75 years) (9). The prolonged follow-up of our surviving patients (median of 7.4 years) suggests that they have a specific form of ROHHAD syndrome that does not progress to obesity.

The average time to ROHHAD diagnosis in our population was 4.4 months. The lack of obesity led to diagnostic uncertainty. Several articles in the literature indicate consideration of ROHHAD syndrome when obesity occurs, especially when it is isolated, early, or severe (16, 17), making this manifestation an essential sign for a ROHHAD diagnosis. However, even when early-onset obesity occurs, there can be a significant delay from presentation to diagnosis of ROHHAD syndrome, notably in countries with high prevalence of morbid obesity like the United States. A diagnostic delay of more than 10 years in a young 19-year-old woman was recently reported. This patient had been suffering from severe morbid obesity since the age of 3 years, she presented obstructive sleep apnea at 3 years old, hypoventilation at 5 years old and hypothalamic deficit at 7 years old. The diagnosis of ROHHAD was made at the age of 19 years when severe dysautonomic signs appeared after three visits to the intensive care unit (18). The story is similar for the patient described by Jalal Eldin et al. (19) in 2019. Apart from the absence of obesity, patients in our study population met the diagnostic criteria for ROHHAD syndrome.

The earliest signs found retrospectively in our population were signs of autonomic dysfunction. These were described around a mean age of 6 years and were mostly disorders of thermal regulation. These data are consistent with data from the 2020 literature review (13). No patients of our cohort presented with tumor of the neural crest.Dysautonomia is rare in pediatrics and is often difficult to identify due to the large polymorphism and non-specific nature of clinical signs. Brain damage is the main underlying cause of dysfunction (20). In the three of our patients who underwent brain imaging, findings were normal and it is likely that an infectious etiology was initially suggested to explain the thermal regulation disorders. ROHHAD syndrome should be mentioned when signs of dysautonomia appear in the absence of a history suggesting brain damage or in the event of normal brain imaging.

In our population, on average 21 months after the signs of dysautonomia, the signs of hypothalamic dysfunction appeared, at a median age of 8 years 8 months. In a recent review of the literature, signs of hypothalamic dysfunction were present in 41 of 43 patients with a median age of onset of 4 years (10). Hyperprolactinemia and dysnatremia were the earliest signs. Hypernatremia and diabetes insipidus were significantly more frequent in our cohort compared to Harvengt series. Central hypothyroidism is present in 30% of ROHHAD patients and growth hormone deficiency in approximately half of patients (21, 22). In a study published by Bougnères et al. (17) in 2008, describing endocrine abnormalities in patients diagnosed with ROHHAD, all of the patients presented blood serum abnormalities, hyperprolactinemia and dysthyroidism. The endocrine profile of our patients is similar, apart from early and rapidly developing obesity, but dyshypothalamic disorders appear to occur later.

Psychiatric and behavorial problems were very similar to those encountered in ROHHAD with RO cohorts. Neurological symptoms such as ataxia and paralysis of the lower limbs in a context of dysautonomia and mild rhabdomyolysis have never been described to our knowledge in previously published case reports.

Hypoventilation is the cardinal symptom of the non-obese ROHHAD entity. In fact, the clinical signs of hypoventilation or the results of polysomnography made it possible to make the diagnosis in all of our patients faced with a combination of hypoventilation - dysautonomia - hypothalamic disorders. In our population, hypoventilation occurred at a median age of 8.7 years [6.4–11]. A retrospective study published in 2016 (4) showed that ROHHAD patients initially presented with obstructive sleep apnea and that hypoventilation only appeared secondarily, associated with respiratory irregularity during wakefulness. In this study, the median age of onset of hypoventilation was 7.2 years. In the study by Harvengt et al. (13), there was also a lag between the age of observation of obstructive sleep apnea (4 years) and the age at diagnosis of hypoventilation (5.3 years). Obstructive sleep apnea is not a diagnostic criterion for ROHHAD, but it seems to occur before hypoventilation and could indicate this diagnosis more quickly. Isolated OSA before of onset hypoventilation was not diagnosed on the initial polysomnography in our cohort in contrast to prior studies in ROHHAD. The respiratory clinical signs were mainly a symptomatology of sleep apnea or acute decompensations in an infectious context. However, diagnosis was only made by a pediatric pulmonologist in two of the four cases. The diagnosis can be suggested by intensivists during acute episodes of decompensation (23) or during severe dysautonomia, by pediatric pulmonologists during diagnostic exploration for hypoventilation or by pediatric endocrinologists in the event of hypothalamic involvement. It is clear that these signs must be interpreted together in order to reach the diagnosis, provided that this syndromic association is known by these different specialists.

To date, no curative treatment for ROHHAD exists. As for diagnosis, the management of these children must be multidisciplinary. Ventilatory impairment and dysautonomia impact negatively on prognosis for these children. The installation of adapted ventilatory support makes it possible to limit morbidity and to prevent the occurrence of cardiorespiratory arrests. The association with neural crest tumors in more than half of the life-threatening cases (13) indicates oncological screening in these children early in the treatment and on a regular basis. Efficacy of the immunomodulatory treatments varies according to the studies (24–27). The correction of endocrine disorders and monitoring of water and sodium intake if necessary, as well as dietary management, are essential. Patients with ROHHAD syndrome may also present behavioral disorders of multifactorial origin (28, 29), for which management and care should not be forgotten. Psychological and social support for the family must be organized. As ROHHAD syndrome is little known, emergency departments must be informed in advance of the risk of cardiopulmonary arrest in these children and of the actions to be taken.

The prognosis for this pathology can be severe. One of our four patients died. In the literature, the mortality rate varies from 6% (3) to 30% (17) depending on the study. The prognosis and quality of life of surviving children has not been established. There are a few articles reporting ROHHAD cases that have become adults. One patient presented with severe metabolic syndrome and hepatocellular carcinoma at the age of 26 years, with fatty liver disease (19). A 22-year-old patient presented with acute respiratory failure on non-invasive ventilation (30).

Our study is the first to describe the clinical and evolutionary characteristics of patients with genuine ROHHAD syndromes without RO (Rapid-onset Obesity). Persistent absence of obesity during follow-up constitutes a newly described phenotype of the disease, even if endotype remains unknown. An understanding of the pathogenesis of this entity would be noteworthy in order to classify this entity as a sub-type of ROHHAD or as a really new entity. Knowledge of this particular entity will allow early recognition of affected patients and avoid diagnostic errancy that could be life-threatening. Management of the various conditions and early detection of associated tumors will limit the associated morbidity and increase the duration and quality of life of these patients.

The main limitation of our study is the small size of our population, probably linked to the lack of knowledge of this entity, non-consensual diagnostic criteria, retrospective design of the study and therefore the non-recognition of affected patients. Moreover, obstructive and central apnea-hypopnea index were not available as some centers reported only a global hypopnea index and polysomnography reports did not determine if hypoventilation was worse in non-REM sleep or REM sleep. Larger studies are needed to more accurately describe the typical profile of patients with ROHHAD without RO. These studies may also make it possible to progress with knowledge of the pathophysiology of this syndrome.

Conclusion

ROHHAD syndrome without RO (Rapid-onset Obesity) appears to be a separate, later onset entity. Its identification is difficult due to the absence of the most easily identifiable and earliest sign in the sequence of classic ROHHAD syndrome. It is likely that without being described in the literature, this syndrome is underdiagnosed. Given its severity, it seems important to discuss this diagnosis in the context of cases where signs of dysautonomia appear in the absence of a history suggesting brain injury or the appearance of several signs of pituitary dysfunction. In the absence of a diagnostic or genetic test, performing repeated polysomnography in these situations could better identify these patients and therefore improve their prognosis. International studies are needed to better describe the clinical and progressive features of this syndrome.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by société de Pneumologie de Langue Française IRB. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

LG-C and HT designed the study, critcally revised the first draft, and approved the final draft. BD collected the data and wrote the first draft and approved final draft. AT, JH, MB, EBe, EBa, SM, MA, SB, SD, M-EL, JM, BM, HT, and LG-C provided data, critically revised the initial draft, and approved the final version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.910099/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Kaplan Meier curves of clinical signs age of onset concerning **(A)** hypoventilation, **(B)** hypothalamic dysfunction, **(C)** autosomic dysregulation. In black, data from our population of ROHHAD without RO; in dotted lines, data from the study by Harvengt et al. (13) of ROHHAD with RO.

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5.2. HIDEA syndrome: A new case report highlighting similarities with ROHHAD syndrome.

Harvengt J., Lumaka A., Fasquelle C., Caberg J.-H., Mastouri M., Janssen A., Palmeira L. & Bours V.

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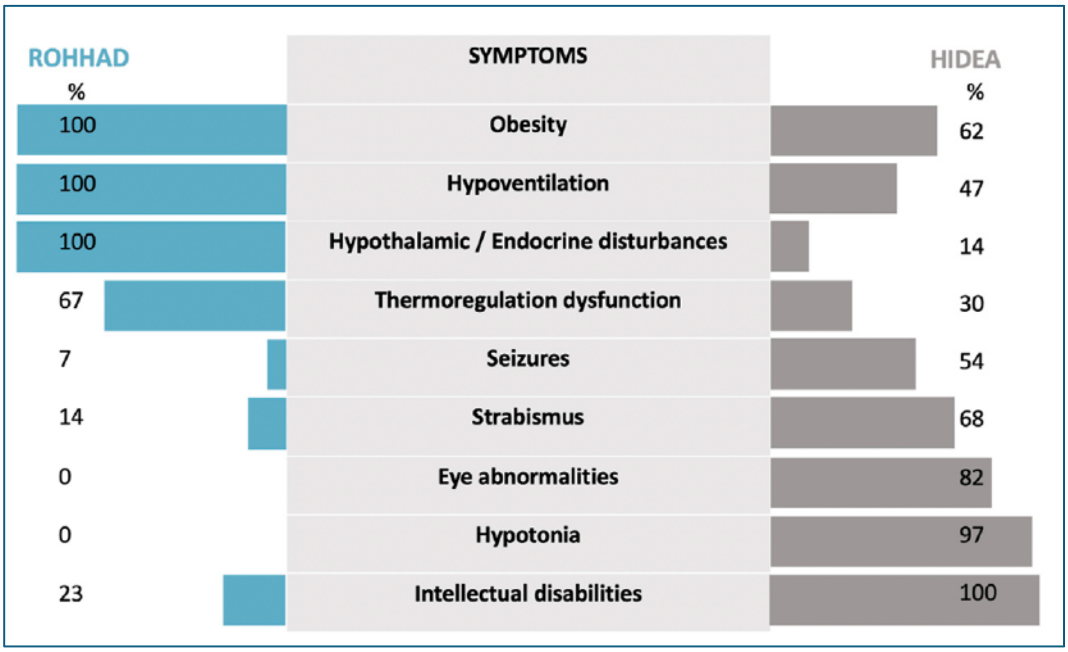
Figure 20 -Comparison of ROHHAD and HIDEA features



The phenotypic features of HIDEA patients demonstrate an overlap with ROHHAD characteristics.

5.2.1. Summary and highlights

In the context of our research setting on ROHHAD, our expertise has been required to evaluate patients encompassing a ROHHAD-like phenotype. These patients do not fulfill the criteria of ROHHAD as defined by Ize-Ludlow *et al*., motivating a whole genetic investigation.

The patient presented here was a 21-month-old girl at inaugural presentation. She had a history of severe respiratory infections requiring intensive care but also hypotonia, abnormal eye movements, and rapid weight gain. Polysomnography identified a severe central hypoventilation suggesting ROHHAD as a possible diagnosis. During her follow-up, a significant psychomotor delay and the absence of language were gradually observed. The prolactin levels were initially increased. Hypothermia was reported at 4 years. Because of the abnormal neurodevelopmental evolution, exome sequencing has been performed and reevaluated twice. The second interpretation ultimately identified a novel homozygous truncating *P4HTM* variant establishing the diagnosis of HIDEA syndrome; *P4HTM* acting as a regulator of calcium dynamics and gliotransmission.

HIDEA – acronym for hypotonia, hypoventilation, impaired intellectual development, dysautonomia, epilepsy, and eye abnormalities - present similarities with ROHHAD, including hypoventilation, obesity, and dysautonomia. To date, only 14% of endocrinological disturbances have been reported in HIDEA patients, considering the data from 30 patients described in Kraatari-Tiri *et al*. (Kraatari-Tiri M. *et al*., 2022). However, this point of comparison must be interpreted cautiously knowing that hormonal monitoring of these patients has not been described or carried out systematically.

Practically, our clinical observation suggests to systematically analyzed P4HTM in addition to the PHOX2B testing in ROHHAD patients, in case of clinical evidence of ROHHAD in a child with abnormal neurological development or eye abnormalities.

A better delineation of the natural history of HIDEA is required to allow further comparisons between features of HIDEA and ROHHAD. The clinical similarities could potentially orient some molecular hypotheses in the field of ROHHAD research.

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HIDEA syndrome: A new case report highlighting similarities with ROHHAD syndrome

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Context: ROHHAD syndrome presents a significant resemblance to HIDEA syndrome. The latter is caused by biallelic loss-of-function variants in the *P4HTM* gene and encompasses hypotonia, intellectual disabilities, eye abnormalities, hypoventilation, and dysautonomia. We report the first patient identified with HIDEA syndrome from our ROHHAD cohort.

Clinical case: Our patient was a 21-month-old girl who had a history of severe respiratory infections requiring intensive care, hypotonia, abnormal eye movements, and rapid weight gain. Polysomnography identified severe central hypoventilation. During her follow-up, a significant psychomotor delay and the absence of language were gradually observed. The prolactin levels were initially increased. Hypothermia was reported at 4 years. Exome sequencing identified a new homozygous truncating *P4HTM* variant.

Discussion: Our patient met the diagnosis criteria for ROHHAD, which included rapid weight gain, central hypoventilation appearing after 1.5 years of age, hyperprolactinemia suggesting hypothalamic dysfunction, and autonomic dysfunction manifesting as strabismus and hypothermia. However, she also presented with severe neurodevelopmental delay, which is not a classic feature of ROHHAD syndrome. HIDEA syndrome presents similarities with ROHHAD, including hypoventilation, obesity, and dysautonomia. To date, only 14% of endocrinological disturbances have been reported in HIDEA patients. Better delineation of both syndromes is required to investigate the eventual involvement of *P4HTM*, a regulator of calcium dynamics and gliotransmission, in ROHHAD patients.

Conclusion: In the case of clinical evidence of ROHHAD in a child with abnormal neurological development or eye abnormalities, we suggest that the *P4HTM* gene be systematically interrogated in addition to the analysis of the *PHOX2B* gene. A better delineation of the natural history of HIDEA is required to allow further comparisons between features of HIDEA and ROHHAD. The clinical similarities could potentially orient some molecular hypotheses in the field of ROHHAD research.

Abbreviations: ROHHAD, rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysregulation; ROHHAD (NET), rapid-onset obesity with hypothalamic dysfunction, hypoventilation, autonomic dysregulation, and neural crest tumor; HIDEA, hypotonia, hypoventilation, impaired intellectual development, dysautonomia, epilepsy, and eye abnormalities.

KEYWORDS

ROHHAD, rapid-onset obesity with hypoventilation, hypothalamic dysfunction and autonomic dysregulation, central hypoventilation, HIDEA syndrome, *P4HTM*, childhood obesity

Introduction

Rapid-onset obesity with hypothalamic dysfunction, hypoventilation, autonomic dysregulation, and neural crest tumor [ROHHAD (NET)] is a rare and potentially fatal disease for which a specific diagnostic biomarker is currently unavailable. The definition of ROHHAD syndrome is currently based on clinical criteria, defined first by Ize-Ludlow et al. (2007). The major criterion is dramatic weight gain associated with central hypoventilation appearing between 1.5 and 7 years of age. This rapid-onset obesity is considered to be the first sign of hypothalamic dysfunction. At least one other sign of hypothalamic dysfunction is necessary for its diagnosis, such as hyperprolactinemia, central hypothyroidism, disordered water balance, abnormal growth hormone (GH) response, adrenocortical insufficiency, or puberty disorders. Central hypoventilation can occur rapidly after the initial weight gain or can appear during the following years (83% were reported to have been diagnosed with hypoventilation after a maximum of 5 years of follow-up). This autonomic dysfunction may also manifest later with other dysregulations such as thermal dysregulation, excessive sweating, cardiovascular manifestations (arrhythmias or blood pressure dysregulation), strabismus, abnormal pupillary reaction to light, or gastrointestinal or sensory disturbances.

In cases of patients presenting with rapid weight gain, hypoventilation, and hypothalamic dysfunction, an early diagnosis of ROHHAD has to be considered to improve management and prognosis. Nevertheless, at the same time, etiological investigations need to be extended to explore rare differential diagnosis (Harvengt et al., 2020). The current recommendations include the systematic screening of ROHHAD patients for *PHOX2B* gene disorder to rule out congenital central hypoventilation syndrome. However, the wider availability of whole exome sequencing (WES) analyses tends to show new results in patients who have been initially investigated for clinical signs that may partly overlap with those of ROHHAD syndrome.

We report here, for the first time, the case of a patient encompassing some ROHHAD criteria in whom HIDEA syndrome has been identified. HIDEA (OMIM #618493), first described in 2019, is an acronym for hypotonia, hypoventilation, impaired intellectual development, dysautonomia, epilepsy, and eye abnormalities.

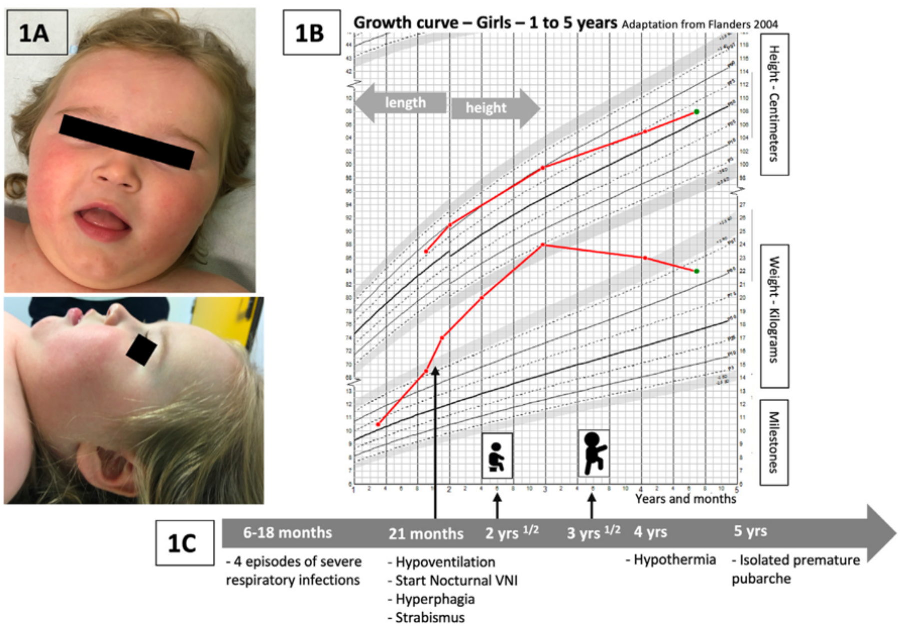

FIGURE 1
(A) Pictures of the patient: face and profile. No specific dysmorphism is discriminated, but similarities with previous publications are encountered: global facial hypotonia, tented upper lip vermilion, and a low nasal bridge (Kraatari-Tiri et al., 2022). **(B)** Weight and height curves of our patient (growth chart—Flanders, 2004, for girls, 1–5 years). Rapid weight gain starting just before 2 years, significant increase between 2 and 3 years of age, and progressive improvement and stabilization of weight related to height at around 4 years of age. **(C)** Timeline with the main relevant clinical data of the follow-up of our patient.

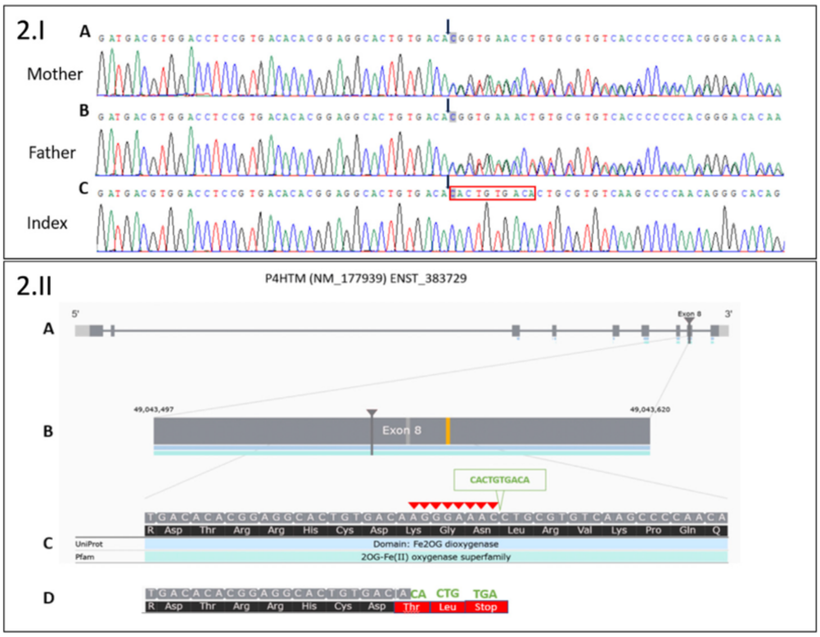

FIGURE 2
2.I Sanger sequencing electropherograms of a novel deletion 8 bp/insertion 10 bp in exon 8 of the *P4HTM* gene: **(A)** in the heterozygous state in the healthy mother, **(B)** in the heterozygous state in the healthy father, and **(C)** in the homozygous state in the index. Black arrows indicate the start of 8 bp deletion (AGGGAAAC)/10 bp insertion (CACTGTGACA). The red box indicates the 10 bp insertion in Index. 2.II. Moon visualization of the *P4HTM* gene. **(A)** Transcript version NM_177939 of *P4HTM* gene with nine coding exons. **(B)** The deletion (8 bp)/insertion (10 bp) mutation is located in exon 8 of the transcript. **(C)** Red arrowheads indicate 8 bp deletion (AGGGAAAC). The green arrowhead indicates 10 bp insertion (CACTGTGACA). **(D)** The substitution creates a premature stop codon (three codons downstream): NM_177939.3: c.1217_1224delinsCACTGTGACA; NP_808808.1:p.(Lys406Thrfs*3).

Clinical case

Our patient (Figure 1A) is a girl who was initially hospitalized in the context of persistent hypercapnia at 21 months of age. Her neonatal history revealed a prematurity of 35 weeks of gestation, BW 2.8 kg (0.13 SD) and BL 48 cm (2.2 SD) but otherwise the pregnancy, delivery and neonatal period had been uneventful. Her parents were healthy and unrelated with no particular family history. Between the ages of 6 and 18 months, she presented with four severe viral respiratory infections [bronchiolitis, one episode due to an RSV (respiratory syncytial virus)] requiring intensive care and invasive ventilation for three of these episodes. The fifth infectious episode occurred at 21 months of age, and persistent hypercapnia was observed with confirmation of central hypoventilation on the polysomnography record. Nocturnal non-invasive ventilation was started and well tolerated. At the same time, she presented with rapid weight gain (Figure 1B). Her parents reported a total lack of satiety. Hormonal workup showed initially increased prolactin levels and no hypothyroidism. IGF1 dosages were slightly increased (340 ng/mL). The initial evaluation was complemented by an ophthalmologic assessment because of abnormal eye movements. Severe strabismus was confirmed but with no other retinal abnormalities. Cardiac ultrasound and electrocardiogram were normal. Global hypotonia was observed during hospitalizations, more severely at 21 months of age, with poor postural control. Cerebral MRI was normal.

During her follow-up, severe psychomotor delay was progressively noted. She sat at 2.5 years and walked at 3.5 years (Figure 1C). She developed no language before the age of 4 years and only a few short words at 6 years. The swallowing function was also delayed; however, she could eat solid food and drink liquids with assistance at the age of 6 years. At 4 years of age, hypothermia was observed with a temperature measurement of 34°C without particular symptoms (the temperature measurement was taken daily during the COVID-19 pandemic in her school institution, which led to the incidental detection of the hypothermia episodes).

Currently, she has no signs of epilepsy. The weight gain is being progressively controlled with a healthy diet and the stimulation of limited physical activities (she walks with assistance). The respiratory function is relatively well controlled with nocturnal non-invasive ventilation, with only one recent acute respiratory decompensation at 6 years. Notably, she presented with an isolated premature pubarche starting at the age of 5.5 years.

Molecular investigations first encompassed *PHOX2B* gene analysis, molecular karyotyping, and Prader–Willi syndrome analysis. All of these were normal. A WES-filtered panel dedicated to intellectual disabilities initially identified no pathogenic variant. Because of the patient's clinical evolution, at 5 years of age, the whole exome analysis was requested, and a new homozygous pathogenic variant in the *P4HTM* gene was identified, providing the diagnosis of HIDEA syndrome (Figure 2).

Methods

Molecular investigations

Trio-based exome (in the proband and her unaffected parents) was performed at the Human Genetics Laboratory, CHU of Liège. Genomic DNA was extracted from whole blood with the NucleoMag® Blood 200 µL kit (Macherey-Nagel) automated on a STARlet platform (Hamilton) following the manufacturer's instructions. DNA concentrations and quality were measured using the Qubit® DNA Assay Kit in a Qubit® 2.0 Fluorometer (Invitrogen) and agarose gel electrophoresis. A total of 1.0 µg genomic DNA per sample was used as the input material for the exome library preparation. The exome was captured using the Agilent SureSelect Human All Exon V6 following the manufacturer's recommendations. Sequencing was performed using the Illumina NovaSeq 6000 system in paired-end (2 × 150 cycles). The average depths of coverage obtained were 24× for the proband, 21× for the healthy father, and 35× for the healthy mother.

The raw results were aligned to the reference genome (GRCh37/hg19) using a homemade pipeline (Humanomics v2.0) that utilizes published algorithms in a sequential manner according to the GATK v3.8 toolkit: BWA-MEM v0.7.17 for mapping the reads, Picard v2.20 for marking the end and removing optical and PCR duplicates, HaplotypeCaller for detecting variants, and GenotypeGVCFs for producing VCF files. VCF files are used for the interpretation of variants using the Moon software from Invitae. Genome Reference Consortium Human Build 38 (GRCh38/hg38) was used as the genome assembly reference.

Several variants were detected in exon 8 of the *P4HTM* gene, suggesting an indel in this region. PCR was performed on genomic DNA to amplify exon 8 of the *P4HTM* gene with the following primers: 5′CTCACCTCTCCCCACAAGTT3′ and 5′GGGCAG ATGGAGTCAGTACA3′ (PCR product size, 1,906 bp). Sanger sequencing was performed on the PCR product with the same primers. The electropherogram analysis revealed deletion (8 bp)/insertion (10 bp) in the heterozygous state for the healthy parents and in the homozygous state for the patient. The deletion (in-frame deletion) of 8 bp (AGGGAAAC) was compensated by the substitution of 10 bp (CACTGTGACA). The substitution created three stop codons downstream: NM_177939.3: c.1217_1224delinsCACTGTGACA; NP_808808.1:p.(Lys406Thrfs*3). According to the current ACMG classification guidelines, this result is considered a class 4 variant (PVS1 and PM2 positive criteria).

Discussion

This report highlights for the first time the clinical similarities between HIDEA and ROHHAD syndromes. Few data regarding HIDEA are currently available in the literature. A recent cohort description has been published describing 24 previously reported patients and 6 new patients (Rahikkala et al., 2019; Maddirevula et al., 2020; Hay et al., 2021; Kraatari-Tiri et al., 2022). A comparison with a ROHHAD descriptive cohort of 44 patients (Harvengt et al., 2020) was made (Figure 3). The phenotypic features of the HIDEA patients demonstrated an overlap with the ROHHAD characteristics.

First, obesity and central hypoventilation are the initial symptoms of both syndromes. A ROHHAD diagnosis is based on clinical criteria, and the lack of a biological marker makes it difficult for the clinician to rule out the diagnosis in a young patient with central hypoventilation and rapid weight gain. The symptoms in ROHHAD typically (but not categorically) begin in a child with no previous specific medical history (Harvengt et al., 2020). One major factor in discriminating the diagnoses of both ROHHAD and HIDEA is carefully following the clinical evolution of the patient during the few months following the initial symptoms. This is especially illustrated by patients such as ours for whom during the initial diagnosis investigation phase, no evident argument could distinguish between the two hypotheses of ROHHAD and HIDEA. Regarding hypoventilation, successive hospitalizations for severe bronchiolitis were probably due to the decompensation of untreated chronic hypoventilation. The other clinical signs presented by our patient could also be clear arguments for the HIDEA diagnosis but only on retrospective analysis. Our patient presented progressive hypotonia and motor delay. It was not clear at the time whether the hypotonia was related to a syndromic disorder or to poor socio-educational conditions. Molecular analysis was, therefore, considered to be the most appropriate way in this case to investigate the differential diagnoses of ROHHAD. A panel of genes dedicated to intellectual disabilities was initially negative. The analysis was extended to the WES to finally establish the diagnosis of HIDEA syndrome.

Dysautonomia is a second similar feature. It is an essential clinical sign encountered in ROHHAD patients that has been reported in 30% of HIDEA patients through thermal dysregulation. Consequently, it is not clear whether cardiac rhythm alterations, sweating alterations, or digestive dysmotility could be part of this syndrome. Strabismus has been reported in 68% of HIDEA patients, and we do not know whether strabismus is an independent symptom that is related to neurological dysfunction or whether it could be a part of the dysautonomia process. In ROHHAD patients, strabismus is only described as a part of the dysautonomia symptoms in a total of 6/44 patients but has not been excluded as a symptom that could be more frequent in ROHHAD, considering frequent incomplete case reports (Harvengt et al., 2020).

As the last point of comparison, hypothalamic or endocrine disturbances have been reported for only 14% of HIDEA patients. The 14% includes a total of three patients: one with precocious adrenarche (Rahikkala et al., 2019), one with hypothyroidism (Maddirevula et al., 2020), and one (the subject of our case report) with premature pubarche. A better delineation and description of HIDEA syndrome is required to determine whether hormonal dysfunction may be more significant than expected.

As another issue, in the case of ROHHAD, the risk of developing a neural crest tumor is well established and has been reported for 56% of ROHHAD patients (Bougnères et al., 2008; Harvengt et al., 2020). No data about tumor development in HIDEA patients has

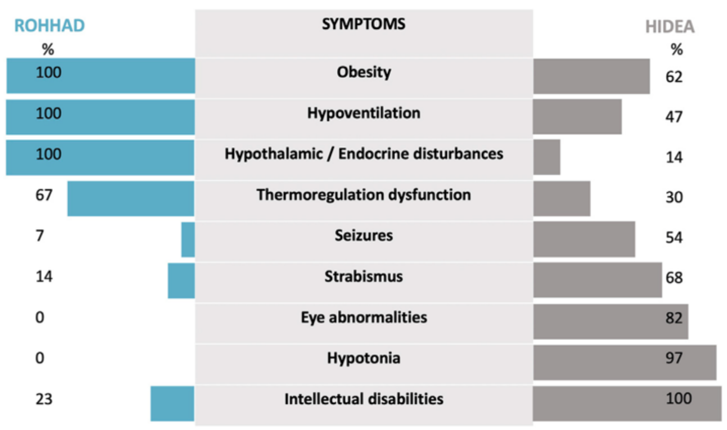

FIGURE 3
The phenotypic features of HIDEA patients demonstrate an overlap with ROHHAD characteristics. For HIDEA: percentages based on the data of 31 patients—our patient and 30 patients—described in the review of Kraatari-Tiri et al. (2022). [which included 24 previously reported patients (Kaasinen et al., 2014; Rahikkala et al., 2019; Maddirevula et al., 2020; Hay et al., 2021; Järvelä et al., 2021; Lim et al., 2022) and six new patients]. For ROHHAD: percentages are based on the data from Harvengt et al. (2020).

been published until now. Currently, this outcome is possibly lacking because some of the HIDEA patients are still young.

The first complete phenotypic description of patients with biallelic loss-of-function *P4HTM* gene variants was recently detailed in 2019 and extended in 2022 (Kaasinen et al., 2014; Rahikkala et al., 2019; Kraatari-Tiri et al., 2022). *P4HTM* was previously not integrated in the panels of the core genes list of intellectual disabilities. New versions of dedicated panels and updated algorithms for exome interpretations can currently highlight pathogenic variants in *P4HTM*. Therefore, it might be suggested to clinicians to ask for exome reinterpretations for patients with phenotypes similar to those with HIDEA or for patients with atypical ROHHAD presentation. Thanks to these technological improvements, more HIDEA patients will probably be diagnosed in the coming years, facilitating a better HIDEA phenotypic description.

In the same way, regarding the ROHHAD phenotypic description, a recent publication highlights the spectrum of ROHHAD patients without RO (Desse et al., 2022). An accurate phenotypic description of ROHHAD and ROHHAD-like patients is important to guide further research toward a better understanding of ROHHAD disease. The involvement of hypothalamic mechanisms seems to be the key point in this spectrum. In this respect, previous genetic and clinical comparisons have been described, for example, between ROHHAD and Prader–Willi syndrome (Barclay et al., 2018). It is currently unknown how to link Prader–Willi syndrome, central congenital hypoventilation syndrome (CCHS), or HIDEA to the spectrum of ROHHAD, but all these comparisons must be continuously studied in future clinical or molecular research studies.

At the molecular level, HIDEA is caused by biallelic pathogenic variants in the *P4HTM* gene. P4HTM is an endoplasmic reticulum transmembrane prolyl-4-hydroxylase (P4H) whose function is currently not exactly known (Rahikkala et al., 2019). P4Hs are enzymes that are not only involved in collagen synthesis but also in the regulation of the cellular response to hypoxia (Williams et al., 2007). A dysfunction in this last role seems to be a possible mechanism pathway interfering with mitochondrial function. Currently, intracellular mechanisms of the hypothalamic neurons are not completely understood, especially the possible role of the mitochondria in the regulation of metabolism and energy homeostasis (Jin and Diano, 2018). Apart from its role in the mitochondria, P4HTM has also been recently identified as a novel regulator of calcium signaling in astrocytes with potential disturbance to gliotransmission (Byts et al., 2021). Further knowledge on the role of P4HTM in central neurotransmission and hypothalamic neuronal connections is required to explore the possible mechanism of linking ROHHAD and HIDEA at the molecular level and investigate novel therapeutic research approaches in the future.

Conclusion

Both ROHHAD and HIDEA are challenging diagnoses. In the case of clinical evidence of ROHHAD in a child with abnormal neurological development or eye abnormalities, the *P4HTM* gene should be systematically interrogated in addition to the *PHOX2B* analysis. In the future, a better delineation of the natural history of HIDEA is required to allow further comparisons between HIDEA and ROHHAD features, especially for dysautonomia and hypothalamic disturbances. The clinical similarities between the two syndromes should be better detailed to orient some molecular hypotheses. The investigation of P4HTM, an actor in neurotransmission, could bring new insight into the mechanism, possibly linking ROHHAD and HIDEA syndrome.

Data availability statement

The data sets for this article are not publicly available due to concerns regarding participant/patient anonymity. Requests to access the data sets should be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

HJ collected data from her personal follow-up of the patient, drafted the initial manuscript, and revised the manuscript. MM was involved in performing the diagnosis of the patient. MM and JA managed the respiratory follow-up of the patient. They agree with the manuscript. LA, FC, and CJ performed and interpreted the exome analyses. They drafted the Methods section of the manuscript and revised the manuscript. PL revised the manuscript. BV reviewed and revised the manuscript. All authors have approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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5.2.2. Poster

Harvengt J., Lumaka Zola A., Fasquelle C. & Bours V. HIDEA Syndrome: A new case report highlighting similarities with ROHHAD Syndrome.

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ROHHAD International Consortium Virtual Symposium.

HIDEA Syndrome : A new case report highlighting similarities with ROHHAD Syndrome.

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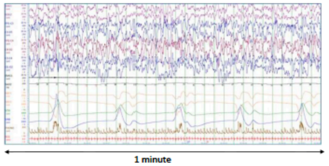
Patients with ROHHAD syndrome present significant resemblance to HIDEA syndrome. This entity is caused by bi-allelic loss of function variants in *P4HTM* gene and encompasses Hypotonia, Intellectual Disabilities and Eye Abnormalities, Hypoventilation and Dysautonomia. We report the first HIDEA syndrome patient identified from our ROHHAD cohort.

Case report

Girl
Birth at 35 6/7 WG
BH = 48 cm BW = 2.8 kg

6-18 months
- 5 hospitalizations in PICU for viral severe respiratory infections
- In the 5th episode: persistent hypercapnia (difficult conditions for the extubation)
- Global hypotonia

21 months
- Polysomnography: Central Hypoventilation (AHI 300/h, desaturation index 48/h, 69% of the time with PtCO2>50mmHG)
- Rapid weight gain (P50>P90)
- Hyperphagia
- Strabismus
- Psychomotor delay
- Global Hypotonia
- No language



-Hormonal work up: Prolactin 653 mUI/l. TSH at subnormal range. IGF1 340 ng/ml (↑)
-Cerebral MRI normal
-Cardiac US: normal

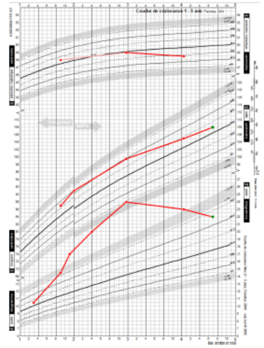
PHOX2B negative
Array CGH : (1-22,X)x2

21 months-5years
- START BPAP during sleep
- Hypothermia (34°C measured on several times at 4 years, without symptoms)
- Sitting position at 2 ½ years
- First walking at 3 ½ years

Prader Willi: Negative
ID Panel: No pathogenic variant

5 ½ years
- Nocturnal BPAP
- Controlled gain weight
- No language (limited to few words and sounds)
- No seizures
- Walking with assistance
- Isolated early pubarche at 5 years

Exome (WES) re-analysis:
Homozygous VUS Class 5 NM_177939.3(P4HTM):
c.1217_1224delinsCACTGTGACA;p.Lys406ThrfsTer3



Discussion

HIDEA vs ROHHAD — Clinical Data

The phenotypic features of HIDEA patients demonstrate an overlap with the ROHHAD characteristics.

Feature	%
Hypotonia	100%
Intellectual Disability	100%
Strabismus	93%
Eye abnormalities	60%
Seizures	45%
Obesity (ROHHAD Symptoms)	50%
Hypoventilation (ROHHAD Symptoms)	50%
Thermoregulation Dysfunction (ROHHAD Symptoms)	30%
Hypothalamic / Endocrine disturbance (ROHHAD Symptoms)	4%

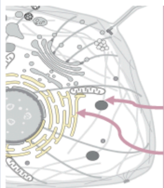
Percentage based on the data of 22 patients : 13 patients reported by Rahikkala E. *et al.*(2019), 7 patients by Maddirevula S. *et al.*(2020), 1 patient by Hay E. *et al.*(2021) and our case report.

Molecular level

State of knowledge on the potential molecular disruption in case of P4HTM deficiency.

P4HTM loss of function — ROHHAD Causes ?

- P4HTM: An Endoplasmic Reticulum transmembrane Prolyl-4-Hydroxylase (P4H)



Proline hydroxylation in Hypoxia Inducible Factors (HIFs): role in proteasomal degradation and polyubiquitination

Possible targets :
*RNA Polymerase II: aberrant levels of hundreds proteins needed for mitochondrial function
*ATF4: role in mitochondrial stress

ER membrane location: role trough the mechanism of Mitochondria associated membranes (MAMs)

Mitochondrial dysfunction?

- P4HTM in astrocytes: the inactivation of P4HTM disturb calcium signaling
Gliotransmission abnormalities?

Diagnosis Workup

Two possible diagnosis modalities for HIDEA: importance of a multidisciplinary approach.

Neuropediatric Workup

Intellectual Disability in children

In case of unexplained rapid weight gain:
- Screen for Polysomnography
- WES analysis: be careful to P4HTM (2/16 reported families: P4HTM identified after the exome re-analysis)

ROHHAD evaluation

Rapid Obesity and Hypoventilation
- Indication of systematic WES analysis in case of extended neurological phenotype

Both ROHHAD and HIDEA syndromes are challenging diagnoses. Multidisciplinary work helps to improve the diagnosis process. In case of clinical evidence of ROHHAD in a child with abnormal neurological development or eye abnormalities, *P4HTM* gene should be systematically interrogated in addition to the *PHOX2B* analysis. A better delineation of the natural history of HIDEA is required to allow further comparison between HIDEA and ROHHAD features. The clinical similarities could orient some molecular hypotheses in the field of ROHHAD research.

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6. Epigenetic landscape of ROHHAD Syndrome: early observations from a patient cohort

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6.1. Introduction

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6.2. Material and Method

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6.3. Results

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6.4. Discussion

6.5. Conclusion

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7. Discussion and perspectives

As part of our work, a first exploration into the epigenetic hypotheses of ROHHAD has been proposed. This initial approach of epigenetic patterns in ROHHAD remains a preliminary step in a vast field of research that will certainly be in interconnection with a wide range of conditions, all related to feeding and central hypothalamic regulations. Given the societal and medical impact of these questions, there is an urgent need to pursue such studies with a holistic approach encompassing clinical, biological and molecular perspectives, and integrating emerging concepts as the potential role of the mitochondria in the regulation of hypothalamic neurons homeostasis (Jin & Diano, 2018).

In this context, the following sections present new evidence and additional considerations that contribute to a more in-depth reflection, with the aim of guiding current and future research projects.

7.1. Monogenic obesities

7.1.1. Perspectives from our cohort study

Considering our publication presenting the first Belgian real-world evidence of monogenic obesity testing, an updated version of the cohort is presented below highlighting the maintenance of our diagnostic yield and the continued growth of the cohort.

A total of 108 samples, including 64 index cases, were processed between May 2024 and the end of July 2025. Among these 64 patients, we identified one likely pathogenic variant in *MC4R*, four highly suspicious class 3 variants and a total of 64 class 3 variants distributed across 33 patients. These findings illustrate consistency in diagnostic yield. Although 1.5% of positive results have already been confirmed, 6% remain under evaluation. Current evidence from two of these cases supports the hypothesis that their variants may be reclassified as likely pathogenic considering further clinical assessment giving additional supporting criteria for the ACMG reclassification. This would place our total diagnostic yield between 3.5% and 4.2% (10 to 12 positive results/287 patients).

Table 4 – Results summary of the P/LP/VUS3 identified in the cohort of early-onset patients analyzed through the obesity targeted panel from February 2022 until July 2025.

Results summary - Obesity Targeted Panel

Number of patients with variants (P/LP/VUS3) identified in the cohort

	Feb 2022 - May 2024	May 2024 - July 2025	Total cohort
Patients with a diagnosis			
P/LP variants *	7/223 3.1%	1/64 1.5%	**8/287 2.8%**
Patients reassessment recommended (in a few years)			
Class 3 Variants In AD conditions	44/223 19.7%	11/64 17 %	55/287 19%
Heterozygous Class 3 variants In recessive conditions	78/223 34.9%	25/64 39 %	103/287 36%
P/LP heterozygous variants In recessive conditions (carriers)	9/223 4%	0/64	9/287 3%
Variants under evaluation			
Highly suspicious Class 3 Variants In AD conditions	1/223 0.4%	4/64 6%	**5/287 1.7%**

The diagnosis yield for the obesity panel is 3.1% between February 2022 and May 2024 and is estimated at 1.5% during the period of May 2024 and July 2025. This last result will be subject to modification knowing that four results during the same time period are under evaluation and potentially candidates to be reevaluated as likely pathogenic variants.

The results discriminate furthermore the number of patients who are carriers of a heterozygous LP/P in a gene associated with a recessive condition and the number of patients for whom the genetic test found at least one VUS in respectively autosomal conditions and recessive conditions. Highly suspicious class 3 variants mean that one ACMG criteria is lacking to consider the class 4 categorization.

*The P/PL variants reported for the patients with a confirmed diagnosis are in heterozygous state for autosomal conditions or in a homozygous or composite heterozygous state for recessive conditions.

Continued data collection is essential to expand the cohort and enable future reinterpretation of results, considering both updated variant classifications and evolving clinical data. Our project aims to reconnect with all prescribers five years after the initial testing to request updated clinical information, particularly in cases of persistent obesity, and to reassess the relevance of previously identified variants by increasing the value of phenotype-genotype correlations. Our goal is to offer a genetic test that remains dynamic over time. Even in the absence of initial positive findings, it is crucial to maintain vigilance and consider re-evaluation, especially considering emerging therapeutic targeted options.

Beyond reassessment, the technological limitations inherent to targeted panels and short-read sequencing may be overcome by implementing whole-exome (WES), whole-genome (WGS), and long-read sequencing (e.g., Oxford Nanopore Technologies) in selected patients with strong clinical suspicion. In other areas of genetic investigation, such as intellectual disability, it is well established that the diagnostic yield of WES increases upon reanalysis after approximately 18 months. Long-read sequencing further improves diagnostic yield by detecting structural variants and variants located in regions poorly covered by WES—commonly referred to as "dead zones"—with an additional diagnostic rate ranging from 7% to 17%, depending on the cohort (Del Gobbo GF & Boycott KM, 2025). However, the data remain limited and less robust in the context of monogenic obesity and it is thus likely that these technological advances will lead to better diagnostic yields in the coming years.

Finally, our findings will be shared in the European Registry to facilitate collaboration for patients requiring further investigations and to contribute to the advancement of knowledge through broader collaborative databases.

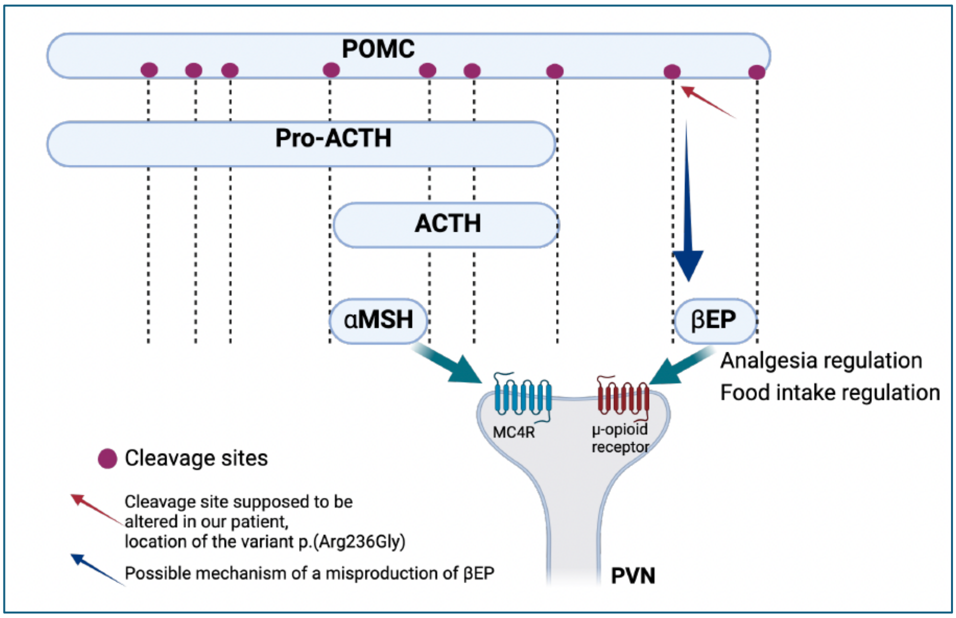
7.1.2. The impact of variant interpretation on therapeutic perspectives

7.1.2.1. POMC

To provide an illustration of the complexity of the POMC variant interpretation, in our cohort published in 2025, we found 3 patients with *POMC* VUS for whom the interpretation should be improved with better segregation data. But one of them presents interestingly a highly questioning VUS that raises the question of the impact of specific variants located on cleavage sites of the POMC protein (Figure 21). POMC is cleaved by pro-hormone convertases at dibasic sites which are generally well conserved between species (Harno E. *et al*., 2018).

The expression of *POMC* gene is based on complex mechanisms that regulate the release of POMC derived peptides: αMSH, ACTH and ß-endorphins (Figure 21). Our patient presents the variant *POMC* c.706C>G, p.(Arg236Gly) which is located on the cleavage site involved in the generation of ß-endorphins. ß-endorphins are known to play a role in the regulation of analgesia but also in the regulation of food intake through their specific activation of the µ-opioid receptors and not the MC4R. A study of mice with deletion of ß-endorphins reveals that male mice were obese and hyperphagic (Appleyard S.M. *et al*., 2003). In addition to this anorexigenic role, ß-endorphins are also involved in the positive regulation of the appetite through the reward behavior system. Processing of POMC is therefore a complex and subtle pathway that might need more detailed knowledge to better appreciate the functional consequences of each specific variant. Notably, in case of a variant leading to a specific ß-endorphins deficiency, a treatment with MC4R-agonist would not be indicated.

Figure 21- Simplified schema of the processing of POMC



Processing of POMC leads to the generation of functional peptides including αMSH and ß-endorphins. These two peptides act through the binding to two different types of receptors, respectively MC4R and μ -opioid receptor. Red arrow shows the cleavage site that is thought to be altered in our patient hypothesizing an alteration of the leptin melanocortin pathway through the alteration of the ß-endorphins action which should probably lead to food intake dysregulation but also to analgesia and reward process dysregulation (Le Collen L. *et al*., 2023; Harno E. *et al*., 2018; Appleyard S.M. *et al*., 2003) (Created with BioRender.com).

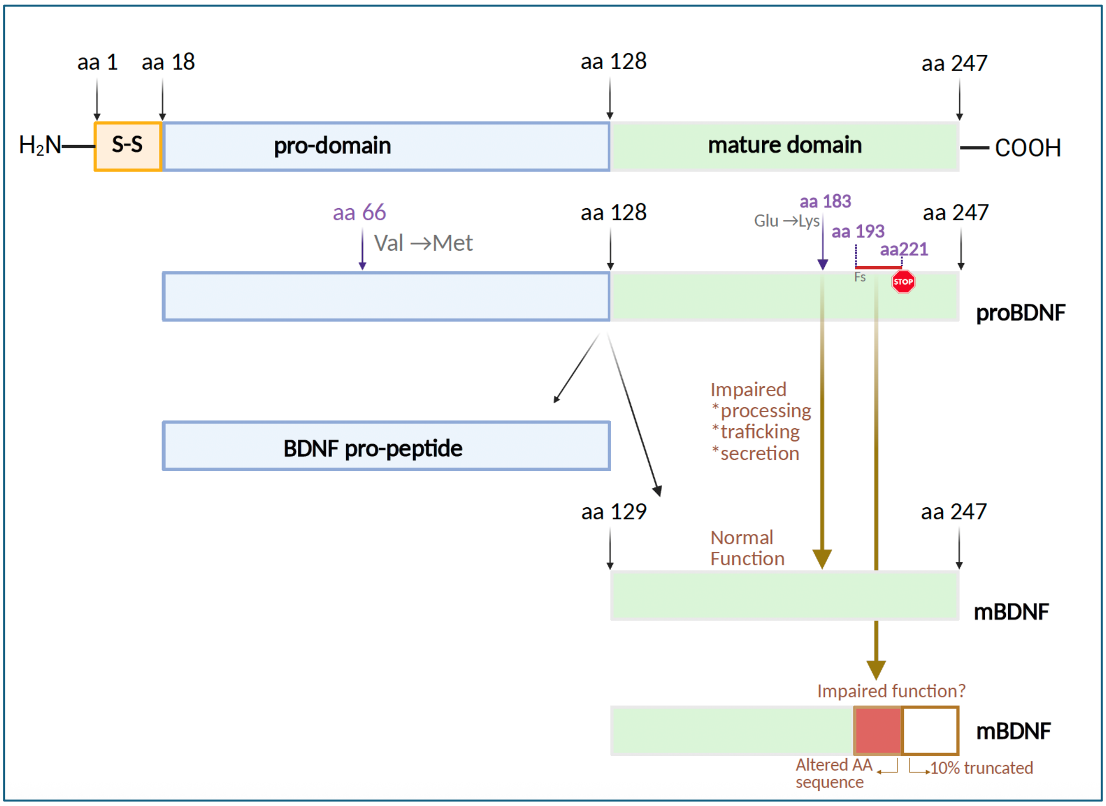
7.1.2.2. BDNF

BDNF (brain-derived neurotrophic factor) is a well-characterized protein involved in central processes such as memory, learning, and neuroplasticity. Beyond its neurological functions, BDNF also plays a key role in the regulation of food intake. Reflecting this dual involvement, BDNF has recently been classified as a "metabokine," a term that highlights its regulatory influence on energy homeostasis and appetite control (Selvaraju V. *et al.,* 2022).

Emerging evidence has linked sporadic disruptions in BDNF expression to phenotypes that combine metabolic and neurological features, including severe obesity, hyperphagia, cognitive impairment, and hyperactivity (Da Fonseca ACP *et al*., 2021; Harcourt B.E. *et al*., 2018). Despite these associations, clear BDNF genotype–phenotype correlations remain elusive, largely due to the limited number of well-characterized rare coding variants whereas several common variants in BDNF have been characterized (especially for one well-studied variant p.Val66Met presenting a variant allele frequency of 19%). Previous reports of broader chromosomal deletions encompassing BDNF are also available but not sufficiently discriminant to isolate the specific effect of BDNF, due to the complexity of multigenic phenotypes.

In this context, our updated cohort analysis identified a novel class 3 *BDNF* variant: c.578_582del p.(Lys193Argfs*28). This frameshift variant introduces a premature stop codon, resulting in the truncation of approximately 10% of the protein. To date, no prior data have been reported for this specific variant, making it a potentially valuable addition to the spectrum of BDNF-related alterations. At present, it can only be hypothesized that the structural alteration leads to impaired protein function, although functional studies are required to confirm this. Our hypothesis gains further relevance when considering previous work by Sonoyama *et al.* who investigated a nearby missense variant, p.Glu183Lys, not previously described at that time. Using functional assays and murine models, they demonstrated that this variant significantly impairs secretion, trafficking, and processing of proBDNF, while leaving the mature peptide functional (Sonoyama T. *et al*., 2020). Their results emphasize the need for comprehensive functional characterization of variants, as reduced protein activity may result either from decreased protein levels or from impaired protein function—two distinct mechanisms that imply different therapeutic development strategies.

Figure 22 -Schematic representation of the BDNF protein structure.



The preproBDNF contains three specific sequences: signal sequence represented by (s.s), pro-domain, and mature domain. Both intra- and extracellular cleavage of preproBDNF generates two functionally active isoforms: BDNF pro-peptide and mature BDNF (mBDNF), each of which exhibits a characteristic affinity to a specific type of receptor. Black arrows indicate known cleavage sites involved in the processing of mature BDNF. Purples arrows represent: (1) the position of the frequent single nucleotide polymorphism (rs6265, Val66Met), (2) the position of the p.Glu183Lys variant identified by Sonoyama *et al.*, (3) our new variant positioned at the amino acid 193 and leading to a frameshift with a premature stop codon and a predicted truncation of around 10% of the protein (adapted from Colucci-D'Amato L. *et al.,* 2020 ; Sonoyama T. *et al*., 2020) (Created with BioRender.com).

Additional complexity arises from the dynamic regulation of BDNF expression, which is influenced by environmental factors such as stress, sedentary behavior, and diet, particularly high-fat diets (HFD). These factors have been implicated in BDNF downregulation in neurodegenerative processes (Marcos-Pasero H. *et al*., 2021). Regarding the diet impact, Urbonaite *et al.* demonstrated that HFD exposure in female mice leads to aberrant methylation of the *BDNF* promoter in maternal germline ovarian cells, supporting a model of transgenerational epigenetic BDNF downregulation (Urbonaite G. *et al.,* 2022).

Taken together, these observations highlight the necessity of an integrative approach that considers both genetic and epigenetic contributions to early-onset obesity.

7.2. DNA Methylation

This section on DNA methylation presents recent new studies of epigenetic mechanisms in early-onset obesity. The emphasis on new models linking environmental exposures to epigenomic alterations that may influence early programming of eating behavior opens up new perspectives that should be incorporated into the future delineation of ROHHAD research and etiological hypotheses.

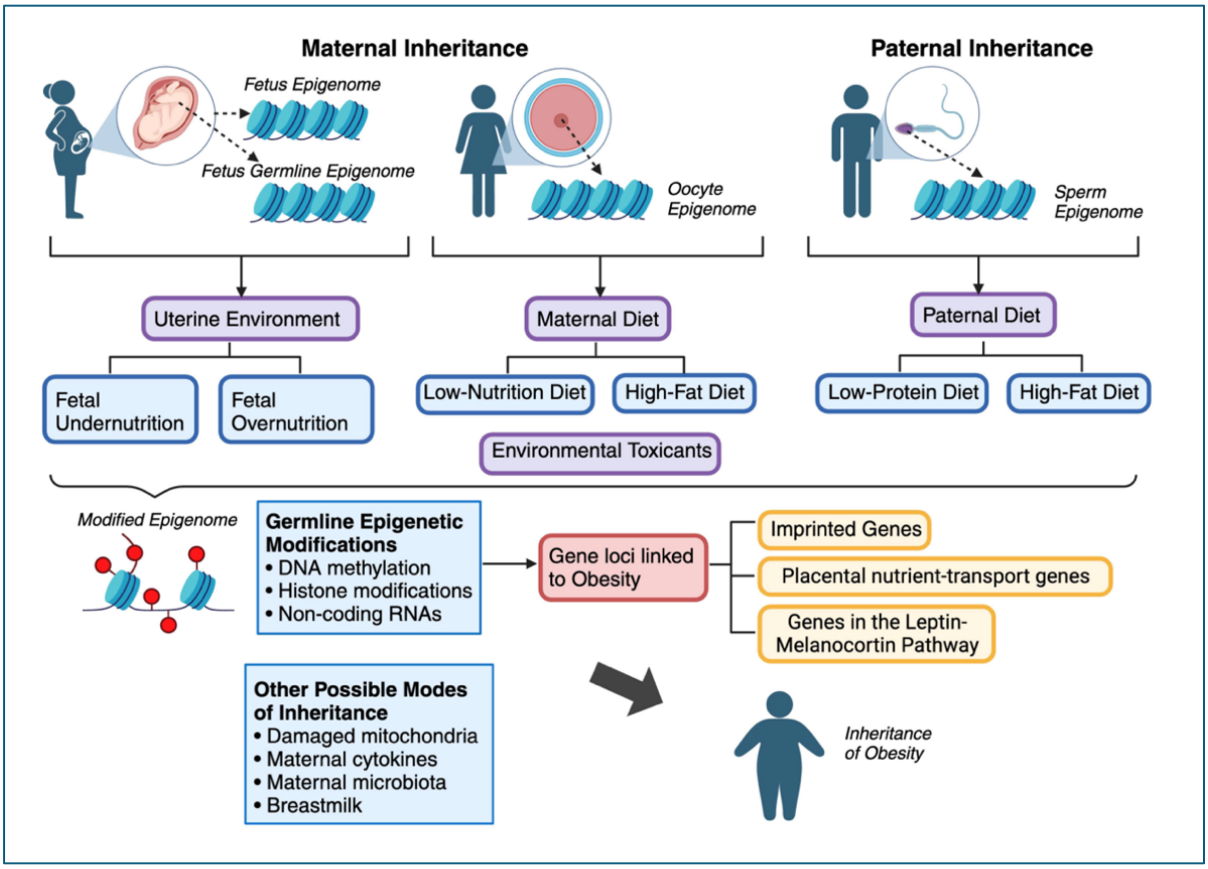
In parallel, our study investigating the epigenetic hypothesis in ROHHAD using WGBS establish first insights that pave the way for future research directions, such as the study of potential episignatures, an emerging concept discussed below.

7.2.1. Epigenomic modifications and environment

Current research unequivocally demonstrates the intergenerational and transgenerational transmission of obesity. This transmission involves various epigenetic modifications, including DNA methylation at CpG sites, histone modifications that alter chromatin structure (either opening or closing it), and non-coding RNAs—both short-chain (such as siRNAs, miRNAs, and piRNAs) and long non-coding RNAs (lncRNAs).

For years, it has been widely accepted that the epigenome is highly sensitive to environmental influences such as lifestyle, dietary habits, gut microbiota, and other external factors (Alegría-Torres J.A. *et al*., 2011; Sharma M. *et al*., 2019). Exposure to these factors early in life or in utero can alter metabolic outcomes through developmental epigenetic reprogramming. Notably, studies in both mice and humans have clearly shown that undernutrition and overnutrition contribute to metabolic disorders in offspring, highlighting the influence of parental nutritional status and diet on both first and second generations.

Figure 23 - Summary of the mode of inheritance of obesity through different mechanisms evolving epigenomic modifications and other mechanisms influenced by environmental factors.



In addition to nutritional factors, various environmental toxicants may interfere with the parental or fetal epigenome, either synergistically or independently. Notable examples include plastics such as BPA, phthalates (DEHP and DBP), bisphenol S, the herbicide glyphosate, insecticides (DDT, methoxychlor), and the biocide tributyltin (TBT). More recent data have identified that maternal consumption of low-calorie sweeteners (e.g., aspartame and rebaudioside A) has long term effects on offspring metabolism and neural development. These effects are likely mediated through the gut microbial co-metabolite phenylacetylglycine (PAG), establishing a link between nutritional chemicals, microbiota alterations, and disruption of hypothalamic regulation. Indeed, these first in vivo explorations revealed that administration of PAG decreases the density of POMC circuits while it increases the density of AgRP neural pathways (Bouret S.G. *et al*., 2022; Park *et al*., 2023). Emulsifiers—components of ultra-processed foods (UPFs)—have also been shown to alter the regulation of POMC and MSH expression in mouse models (Milà-Guasch M. *et al*., 2023; Franssen D. & Parent A.S., 2023).

These examples illustrate the vast field that still need to be explored to better understand the impact of environmental and nutritional trigger on the epigenome itself or on factors regulating the hypothalamic network.

The number of potential disruptors continues to grow, not only due to newly identified chemicals but also because of re-evaluations of previously considered safe substances. To stay alert on these topics might be useful to elaborate new hypotheses linking environment and early onset obesities including ROHHAD.

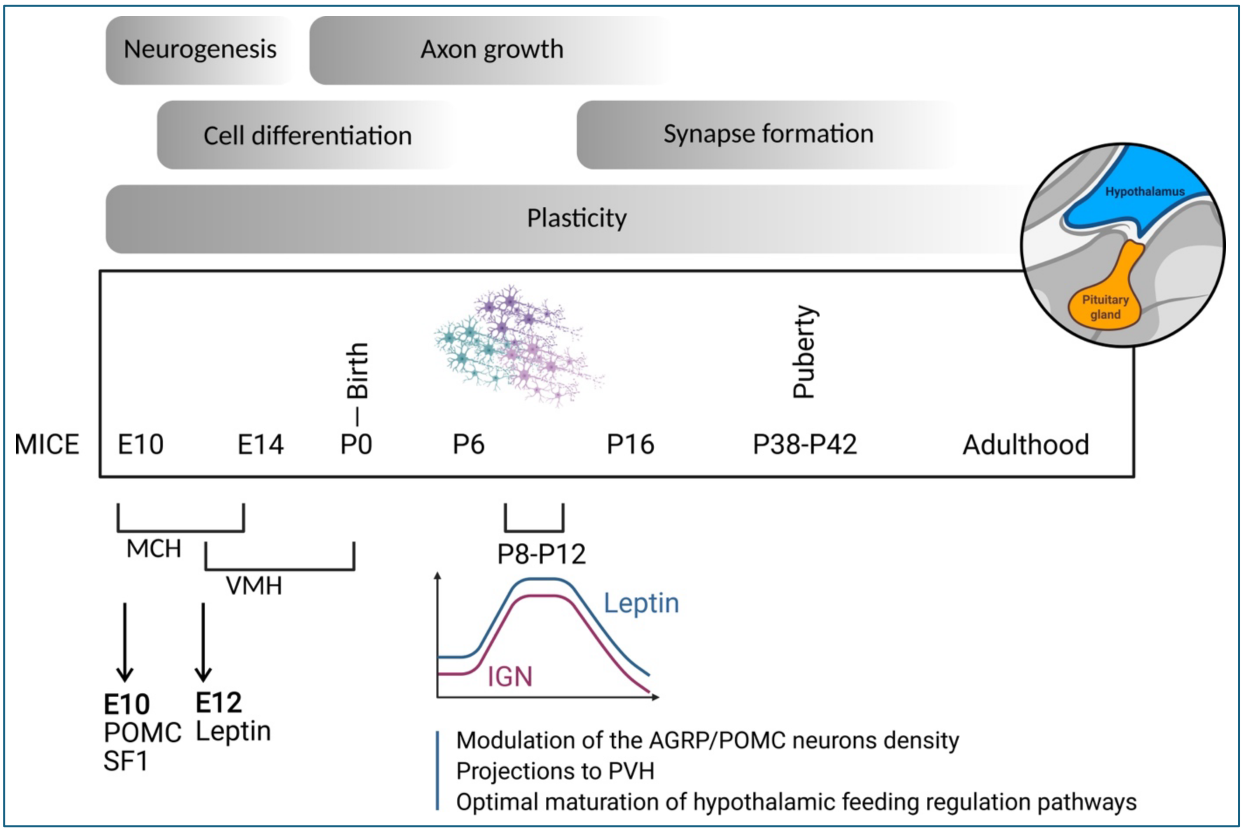
Moreover, maternal cytokines may also play a key role in interfering with the regulation of epigenetic markers, emphasizing the importance of maternal inflammatory status. This raises further questions about the link between chronic inflammation—a medical concept increasingly associated with modern lifestyles and dietary patterns—and metabolic disturbances in children, ranging from insulin resistance to severe early-onset obesity and how this chronic inflammation also alters epigenetics regulation in genes involved in placental nutrient function.

7.2.2. Programming the hypothalamus: Early-Life Determinants of feeding regulatory neurons

The hypothalamus follows a distinct growth trajectory that begins early in fetal gestation and continues throughout the postnatal period. Mouse models have established developmental windows characterized by multiple processes that determine the formation of hypothalamic nuclei, including those involved in the regulation of energy balance.

The neurogenesis, the axon growth and the synaptogenesis successively occur and represent three stages of vulnerability during which alterations in the prenatal and early postnatal period by environmental toxic factors may lead to long-term and potentially irreversible consequences on hypothalamic development and function as observed in mouse models (Figure 24).

Figure 24 – Schematic timeline of the hypothalamic neuronal genesis and maturation steps according to age in mice.



Neuronal genesis and maturation steps according to age (pre and postnatally) with a schematic representation of the relevant major events in the formation of the leptin melanocortin pathway and the representation of the leptin and IGN surges in mice.

IGN = Intestinal neoglucogenesis

Notably, leptin exhibits a specific surge during the first two weeks of life in mice neonates. This increase in leptin levels plays a key role in the development of leptin-sensitive neurons and in enhancing axonal density within the ARC of the hypothalamus. Leptin does not have an anorexigenic role during this critical period but a specific role on the establishment of hypothalamic neuronal circuits. In parallel, intestinal neoglucogenesis has been shown to complement leptin action during this same period, modulating the density of AgRP/POMC projections to the paraventricular nucleus. The detailed mechanistic pathways modulating the regulation of AgRP/POMC neurons by both leptin and neoglucogenesis surges are not completely elucidated and may encompass determinants involved in epigenetics regulation.

In mice, leptin exhibits a marked surge during the first two weeks of life, a phenomenon not observed in humans, where circulating leptin levels typically decline during the first weeks after birth. However, cord blood leptin concentrations are generally high and correlate with maternal nutritional status. The impact of these early leptin levels in humans remains unclear, despite hypotheses suggesting a potential role in modulating hypothalamic neurogenesis, as observed in mouse models.

Similarly, evidence from mouse studies linking intestinal neoglucogenesis to breast milk composition—whether natural or formula—underscores the importance of maternal diet in shaping breast milk composition and, by extrapolation to humans, the possible influence of common dietary components (including artificial sweeteners) on epigenetic programming, hypothalamic neuronal maturation, and long-term metabolic outcomes in the child (Helland I. *et al*., 1998 ;Chatmethakul T. *et al*., 2022).

7.2.3. Episignature as an emerging concept

An episignature may be defined as a recurring epigenetic pattern associated with a genetic or environmental etiology in a particular patient population. In other words, episignatures represent clusters of differentially methylated cytosines (DMCs) that serve as molecular landmarks for specific diseases. In diagnostic setting, episignatures have proven to be useful for diagnosing certain neurodevelopmental disorders with more than 50 episignatures reported so far for such syndromes (Aref-Eshghi E. *et al*., 2020; Husson T. *et al*., 2024). Nevertheless, independent studies are only starting to be published and rise question regarding the reproducibility of the epigenetics results (Husson T. *et al*., 2024). Knowing that —and with all the necessary caution given the high complexity of the field—promising tools and approaches may help to identify such episignature patterns in ROHHAD patients. Working on the identification of methylation patterns could support the diagnostic process and potentially contribute to a better understanding of the disease etiology. In this perspective, our WGBS dataset from ROHHAD patients and controls has been analyzed using machine learning and data mining models. However, the preliminary results are not yet sufficiently robust. To improve robustness, a first approach is to expand our patient cohort to collect additional WGBS data and refine data mining strategies.

However, it is particularly difficult in case of very rare disease, such as ROHHAD, to enroll enough patients. Classical methods to identify differentially methylated regions and episignatures are usually based on intergroup comparisons, requiring many samples in each group to reach robust and statistically significant results (Jaffe A.E. *et al*., 2012). In the context of rare diseases, those methods present limits due to either the cohort size or the intra-group heterogeneity. Single patient-based analyses might be used to address those issues and support the personalization of diagnosis in future perspectives. Grolaux *et al*. developed this concept in their recent publication, explaining two current statistical methods and building a new protocol of single patient-based method to detect differentially methylated regions (Grolaux R. *et al*., 2022). Notably, the authors highlight the need to integrate covariates such as smoking status, ethnicity, and BMI. In their approach, they compile a list of CpGs known to be correlated with these different covariates and removed all DMRs containing them from their analyses. Also, a careful match should always be made between the patient and the control group. That point is critical in the field of ROHHAD studies due to the current difficulty to find these control groups, because of limited availability of pediatric (public or shared) methylation data.

Outside the specific context of ROHHAD, the association between DNA methylation in obesity-related genes and body mass index (BMI) percentile in adolescents has been relatively understudied. One study by He *et al.* which analyzed reduced representation bisulfite sequencing (RRBS) data from 263 adolescents, revealed that obesity-related genes were significantly enriched among 103,466 intragenic sites (He *et al*., 2018). Notably, increased methylation at a specific site within *SIM1* was significantly associated with a higher BMI percentile. Although their findings require external validation, the study underlines the potential interconnections between emerging epigenetic knowledge and childhood obesity and provides insights for building future study design plans for our early-onset obesity cohort.

7.2.4. Biological pathway analysis approach

Rather than focusing on individual genes or isolated regions with differential methylation profiles, an alternative strategy is to assess the cumulative impact of DMRs or DMCs on a given biological pathway. Functional annotation-based gene sets regroup genes according to their involvement in specific molecular functions, biological processes, and curated annotations. The Kyoto Encyclopedia of Genes and Genomes (KEGG) provides comprehensive insights into how gene groups orchestrate specific biomolecular activities through networks of interactions and reactions (Kanehisa & Goto, 2000). From the KEGG database, a selection of pathways involving molecular signaling mechanisms that may be associated with ROHHAD-related disturbances has been investigated: oxytocin signaling pathway, serotonergic synapse, dopaminergic synapse, circadian entrainment and prolactin signaling pathway. No formal result emerged out of our cohort analysis except a trend of hypermethylation in the oxytocin pathway for ROHHAD patients. Interestingly, the oxytocin pathway was previously investigated in RRBS datasets from patients with Prader-Willi Syndrome (PWS); oxytocin is a well-established neuropeptide implicated in PWS, known to modulate social behavior, hyperphagia, and autism-like traits (Tauber M. *et al*., 2017). These preliminary observations, despite the need of more relevance, deserve further explorations including an integration of covariate in the statistical interpretation.

A second approach to identify relevant gene sets involves using data from omics studies or functional analyses specific to the condition under investigation. Following this rationale, methylation level differences between ROHHAD patients and controls have been compared across two targeted gene lists. The first list is derived from the study by Salles J. *et al.,* which identified 18 genes linking DMRs associated with Prader-Willi Syndrome to pathways related to addiction and obesity (Salles J. *et* *al.*, 2021). These 18 genes were included in our pathway analysis. However, no significant methylation differences were observed between ROHHAD and control samples suggesting that no major discrepancy warrants further investigation in this specific gene set. The second gene set is based on Victor K. *et al*., who conducted RNA-seq analysis on differentiated patient-derived dental pulp stem cells, identifying 58 genes with differential expression in ROHHAD and CCHS (Congenital Central Hypoventilation Syndrome [OMIM # 209880]) neurons compared to controls (Victor K *et al*., 2023). Our current methylation analysis revealed significant differences in 5 out of the 58 genes, which need to be reassessed in an extended cohort before considering detailed discussion.

In this first approach of the pathway analysis for our cohort, the ZSCAN1 gene has been added in the pathway lists in an attempt to detect potential methylation differences that might be worth investigating. However, no significant differences were identified in any of these pathway analyses.

A remark must be made to better apprehend the gene annotated sets strategy. Approximately 70% of human genes currently lack functional annotations and are therefore excluded from databases such as KEGG. Moreover, within annotated gene sets, the biological relevance of individual genes may vary, with some playing central roles and others peripheral ones. It may thus be more informative to weight gene contributions accordingly when assessing methylation impacts, especially given the hypothesis that ROHHAD may involve dysfunction in genes with central roles within key pathways (Silberstein M. *et al*., 2022).

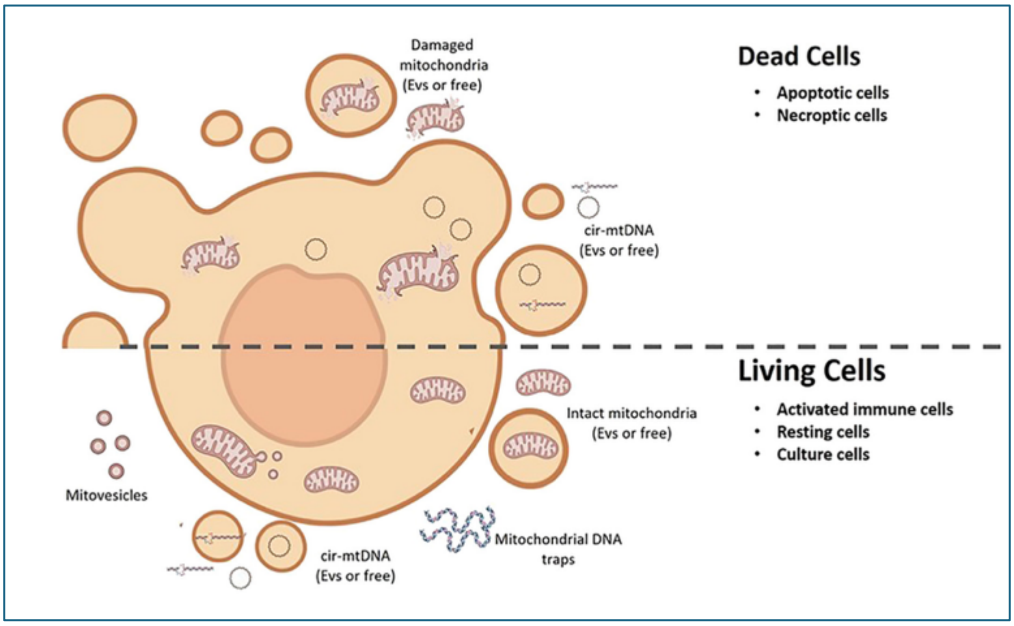
In the perspectives, future development of AI-driven algorithms capable of integrating relative gene weight based in intra- and inter-pathway roles could provide a more nuanced understanding of our future results in the field of ROHHAD and biological consequences of altered methylation patterns.

7.3. The mitochondrial hypothesis

Until recently, mitochondrial functions were regarded as being restricted to the parental cell. However, several recent studies have pointed out that these organelles and their components can be released outside the cell into the extracellular milieu, under both physiological and pathological conditions. Different components may be found in the extracellular milieu in a variety of forms and structures, including intact free, fragmented, and vesicle encapsulated mitochondria, as well as free components (Figure 25) (Al Amir Dache Z. & Thierry A.R., 2023).

Outside the cells, these structures may participate in a series of functions, ranging from the maintenance of homeostasis to the modulation of immune responses and to intercellular communication. Knowledge relative to all these functions is still growing and not yet fully elucidated.

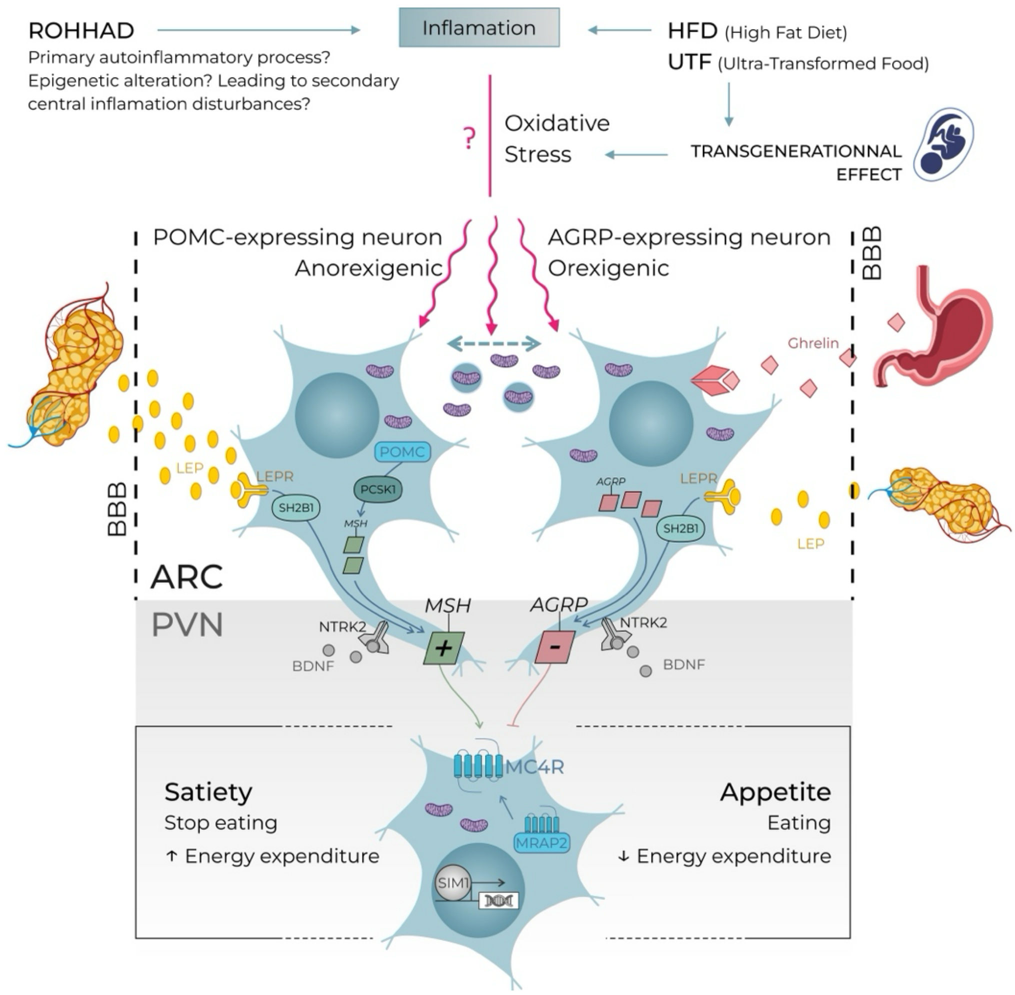
Figure 25 – Representation of the mitochondria-derived components.



The mitochondria-derived components are released outside of the cell during cell death or by active secretion. These organelles can in fact be present within the circulation as free whole mitochondria, as fragmented components, or encapsulated within vesicles.

Because of their structural similarity to their bacterial ancestor, extracellular mitochondria and their components may be recognized by the immune system (activated through the recognition of pattern recognition receptors - PRRs) and stimulate inflammatory cascade events. As explained in the section dedicated to hypothalamic inflammation, inflammation is the earliest mechanism activated in the hypothalamus after the introduction of a high-fat diet and may play a role in the development of mitochondrial abnormalities in diet-induced obesity. In this specific context of hypothalamic inflammation, the findings of Albornoz *et al.* support the hypothesis that the immunoproteasome (a specialized proteasomal complex implicated in inflammation and cellular homeostasis) may play a crucial role in the hypothalamic neuroinflammation caused by HFD and obesity. Especially, their study using a specific immunoproteasome inhibitor (ONX-0914) shows a preservation of mitochondrial membrane potential and an attenuation of mitochondrial ROS (reactive oxygen species) production in the presence of palmityl acid in HFD fed mice. Moreover, they found a reduction in mitophagy in the presence of palmityl acid. They establish by this study the role of the immunoproteasome in controlling mitochondrial homeostasis, and the link between HFD, immunoproteasome defect and mitochondrial alterations. But it remains necessary to investigate how this mitochondrial dysfunction impacts the function of the hypothalamic neurons and to determine how the mitochondria disrupt *in fine* the impact on the neuronal regulation of body energy homeostasis (Albornoz N. *et al*., 2024).

Figure 26- Schematic and simplified overview of the leptin-melanocortin hypothalamic pathway integrating the action of hypothalamic central inflammation and mitochondrial potential dysfunction.



*Hypothalamic central inflammation is proved to be induced by external factors such as the consumption of ultra transformed food and is responsible of a dysregulation of the appetite control. In this view, inflammatory process is also responsible of mitochondria damage due to the negative impact from the oxidative stress. New concepts have recently emerged on the mitochondrial role in neural communication in the hypothalamus through intra- and extra-cellular modulation, especially in the microglia. The mitochondrial communication seems therefore to be altered in inflammatory states. Moreover, the inflammatory state may impact the development of hypothalamic neuronal circuits during fetal development.

*Our diagram illustrates the hypothesis of a potential central dysregulation in ROHHAD, similar that observed in cases of inflammatory damage.

(Ref: Jin S. *et al*., 2018; Albornoz N. *et al*., 2024; Carraro R.S. *et al*., 2018; Al Amir Dache Z. & Thierry A.R., 2023)

Regarding the intercellular communication, since the key study of Rustom *et al.*, it is established that mitochondria and their components can be horizontally transferred via a nanotubular network between mammalian cells, resulting in changes of mitochondrial genes, bioenergetics profiles, and other functional characteristics of recipient cells (Rustom A. *et al*., 2004). Extensive research subsequently revealed that, through this transfer, damaged cells can be rescued by the incorporation of exogenous mitochondria into their mitochondrial network (Al Amir Dache Z and Thierry AR, 2023).

Inside the cells, mitochondrial morphological changes due to fusion and fission that control mitochondrial shape, size, and number defined the mitochondrial dynamics which is a mechanism in support of the energetic needs of the cell. However, these mitochondrial dynamics have been recently involved in the hypothalamic neuronal regulation. For example, recent studies have shown that mitochondrial dynamics and mitochondria-endoplasmic reticulum interaction in hypothalamic neurons play a role of modulator in nutrition sensors and systemic metabolic regulation (Slapnik B. *et al*., 2024; Heymsfield S.B. *et al*., 2025; Desai M. *et al*., 2022). In particular, the deletion of DRP1 in POMC neurons improve sensitivity to glucose and leptin, DRP1 being a protein regulating the mitochondrial fission. The precise mechanisms underlying the mitochondrial dynamics and subsequent neural adaptations remain unclear, but it is well proved that mitochondrial dynamics in most hypothalamic neurons is involved in regulating energy metabolism and glucose homeostasis.

Moreover, the recent emerging field of mitochondrial epigenetics reveals that mitochondrial DNA (mtDNA) itself is subject to epigenetic modifications, which may modulate the effects of environmental stressors on metabolic programming (Kumar A. *et al*., 2024).

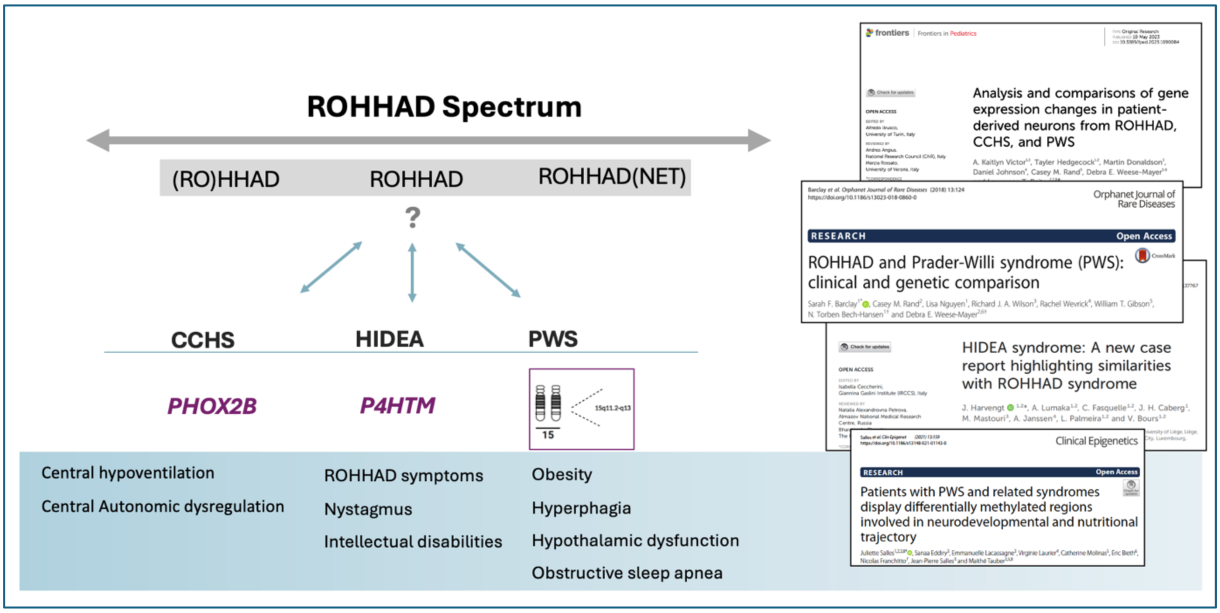
Finally, recent data have also revealed that mitochondrial biogenesis may be subtly regulated at the hypothalamic level through a complex network of interactions. One study postulated that phoenixin 20 (PNX-20) plays a role in promoting neuronal mitochondrial biogenesis by modulating the CREB–PGC1α signaling pathway (Muzammil A.N. *et al*., 2024). The ongoing emergence of these new findings, coupled with increasingly precise data, is expected to significantly improve our understanding of mtDNA, mitochondrial dynamics and regulation of hypothalamic neurons in the next few years.

These developments are likely to position this domain as a new field of investigation within the context of ROHHAD research.

7.4. ROHHAD Spectrum

Regarding the phenotypic characterization of ROHHAD, our previous work—along with the recent publication by Ortega *et al*.—highlights the need to define a broader concept of the "ROHHAD spectrum." These studies describe a continuum ranging from patients with ROHHAD without rapid-onset obesity (RO), to ROHHAD phenocopies such as HIDEA syndrome, and even to the first reported case of adult-onset ROHHAD (Desse B. *et al*., 2022; Harvengt J. *et al*., 2022; Ortega-Gonzalez A. *et al*., 2024)(Figure 27).

Figure 27 – Delineation of the concept of ROHHAD spectrum.



Central to this spectrum, the involvement of hypothalamic mechanisms appears to be a research direction, as developed in the introduction of this thesis. In that view, previous clinical and genetic comparisons—such as those between ROHHAD and Prader–Willi syndrome—have already been proposed (Barclay S.F. *et al*., 2018). Although the exact relationship between ROHHAD, Prader–Willi syndrome, but also central congenital hypoventilation syndrome (CCHS), and HIDEA remains unclear, these comparisons warrant continued investigations. Future clinical and molecular studies should aim to build meaningful connections across these conditions by integrating diverse datasets—particularly clinical, genetic, epigenetic, and proteomic data—to uncover potentially significant relationships.

8. Conclusion

Table 5 – Results overview supporting the conclusion

	ROHHAD EPIGEN PROJECT	EARLY-ONSET OBESITY GENETIC ETIOLOGIES
Recruitment period	From 2020 to currently active	1/ Dataset from Feb. 2022 to July 2025 2/ Currently active
Cohort description	9 ROHHAD patients 10 controls (parents or siblings) International recruitment	287 probands Belgian recruitment
Clinical Data	ROHHAD clinical form	Clinical forms for each patients
Tests	WGBS Analyses Long read sequencing / WES	Targeted Obesity Panel (NGS) Other tests : Array CGH/WES/Other gene panels
Results	837 DMRs No valuable clustering	2.8 to 4.5 % of (likely) pathogenic variants
Publications	*HARVENGT, J. et al(2020). ROHHAD(NET) Syndrome: Systematic review of the clinical timeline and recommendations for diagnosis and prognosis. JCEM . doi:10.1210/clinem/dgaa247 *Desse, B., Tran, A., Butori, M., Marchal, S., Afanetti, M., Barthélemy, S., Bérard, E., Baechler, E., Debelleix, S.,Lampin, M.-E., Macey, J., Massenavette, B., Harvengt, J., Trang, H., & Giovannini-Chami, L. (2022). ROHHAD syndrome without rapid-onset obesity: A diagnosis challenge. Frontiers in Pediatrics, 10. doi:10.3389/fped.2022.910099 *HARVENGT J, et al. (2023). HIDEA syndrome: A new case report highlighting similarities with ROHHAD syndrome. Frontiers in Genetics, 14, 1137767. doi:10.3389/fgene.2023.1137767	*HARVENGT, J., Hannon, M., Palmeira, L., Lebrethon, M.-C., Dideberg, V., & Bours, V. (01 August 2025). Monogenic etiologies in a cohort of early onset obesity: a real-world experience from Belgium. Frontiers in Endocrinology, 16. doi:10.3389/fendo.2025.1608398

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Undeniably, studying ROHHAD significantly contributes to understanding the etiological landscape of early-onset obesity. A whole expertise in the continuum of ROHHAD and early-onset obesity encompassing monogenic obesity disorders linked to hypothalamic dysregulation is essential to navigate through different hypotheses.

Our results from the early-onset obesity cohort reveal a relatively low diagnostic yield for monogenic obesity, around 3%. This finding supports the need to investigate additional causes within this patient population. Although most comprehensive genetic studies will probably improve this diagnostic rate, it is most probable that the majority of childhood obesities are not monogenic disorders. The identification of oligogenic effects, as well as polygenic risk scores, environmental triggers and epigenetic factors will provide further information on this very heterogeneous condition. Treatment options will have to be based on a precision medicine approach and thus on a better definition of the whole early obesity spectrum. As discussed in our work, hypothalamic neurons are involved in the regulation of food intake. Their cellular and molecular pathways will be scrutinized to develop novel therapeutic options.

In our first epigenetic investigation of ROHHAD, WGBS analyses comparing ROHHAD patients with controls underscore the need to enlarge the study cohort and include additional subgroups, notably pediatric patients and those with early-onset obesity. Establishing links with related conditions — such as monogenic obesity syndromes or Prader–Willi syndrome — through specific cohorts could improve our understanding of ROHHAD and childhood obesity. The complexity of these datasets will benefit from ongoing advances in multilayer covariate analysis using robust algorithms, as well as from improved access to growing biomedical databases. Moreover, collaborative and synergistic efforts are crucial to generate the evidence needed to identify potential biomarkers — including epigenetic ones — that could advance the definition and clinical delineation of the ROHHAD spectrum.

To conclude, our genetic and epigenetic investigations are part of a broader effort to map the etiological landscape of early-onset obesity, a major public health concern. Their purpose is to raise awareness on rare obesity syndromes and to support further research aimed at improving diagnosis and guiding personalized therapeutic decisions.

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10. List of publications and communications

10.1. Articles accepted in reviewed journal

Harvengt, J., HANNON, M., PALMEIRA, L., Lebrethon, M.-C., Dideberg, V., & Bours, V. (01 August 2025).
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DOCAMPO MARTINEZ, E., MARTIN, M., GANGOLF, M., HARVENGT, J., BULK, S., SEGERS, K., LEROI, N., LETE, C., PALMARICCIOTTI, V., Freire Chadrina, M., LAMBERT, F., & Bours, V. (2021). Hérédité et cancer. *Revue Médicale de Liège, 76* (5-6), 327-336. https://hdl.handle.net/2268/264946

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10.2. Articles accepted in conference proceedings

VALDES SOCIN, H. G., LIBIOULLE, C., HARVENGT, J., Pintiaux, A., JONAS, C., PARENT, A.-S., GEENEN, V., CORMAN, V., Debray, F.-G., DIDEBERG, V., T'Sjoen, G., De Leerner, A., Beckers, D., Destree, A., Roland, D., Lederer, D., Boscolo, M., BOURS, V., Maiter, D., & BECKERS, A. (2018). Brain imaging and genetics in patients with congenital hypogonadotropic hypogonadism: a multicenter Belgian study. In J. O. Jorgensen, *NENEG Abstract Book Communications* (pp. 64). Aarhus, Denmark: Pfizer. https://hdl.handle.net/2268/223610

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10.3. Posters presentations in conferences with scientific selection committee

Wechsung, K., Al-Halak M, Avdjieva-Tzavella, D., Bechtold-Dalla Pozza S, Beger, C., Gausche, R., Grasemann, C., & Harvengt, J. (16 November 2024). *Real World Effectiveness of Vosoritide in 217 Children with Achondroplasia - Data from a Multicenter European Registry* [Poster presentation]. ESPE 2024 LIVERPOOL, LIVERPOOL, United Kingdom. https://hdl.handle.net/2268/325411

Harvengt, J., Docampo Martínez, E., Jacquinet, A., Alkan, S., Martin, M., Debray, F.-G., BULK, S., & Bours, V. (12 April 2024). *New paradigms in clinical geneticist practice in the era of emerging therapies: examples of two pediatric syndromes* [Poster presentation]. BESHG Annual Symposium 2024. https://hdl.handle.net/2268/316514

Harvengt, J., Dimitrov, B., Docquier, P.-L., Boudewyns, A., De Vloo, P., Laumen, A., Plasschaert, F., De Rademaeker, M., Schrouff, I., DeWaele, K., & Mortier, G. (12 April 2023). *Management of achondroplasia in Belgium: Overview of the current practice based on a multicentric survey* [Poster presentation]. BESHG Annual Symposium 2024. https://hdl.handle.net/2268/316512

KEMPENEERS, C., Bricmont, N., Bonhiver, R., GUISSARD, F., HOUGRAND, O., DELVENNE, P., JACQUINET, A., HARVENGT, J., DOCAMPO MARTINEZ, E., BOURS, V., Benchimol, L., POIRRIER, A.-L., LEFEBVRE, P., CALMES, D., LOUIS, R., & SEGHAYE, M.-C. (17 March 2022). *Ciliary videomicroscopy at room temperature lacks sensitivity for PCD diagnosis* [Poster presentation]. Congress of the BVK-SBP, Brussels, Belgium. https://hdl.handle.net/2268/291276

HARVENGT, J., Lumaka Zola, A., FASQUELLE, C., & BOURS, V. (26 February 2022). *HIDEA Syndrome : A new case report highlighting similarities with ROHHAD Syndrome* [Poster presentation]. ROHHAD International Consortium Virtual Symposium. https://hdl.handle.net/2268/289103

Bibliometric synthesis

28 references of which 28 peer reviewed (22 in scholarly journals)
Total downloads: 11,166 (118 ULiège)
Total views: 5,712 (288 ULiège)
Total Scopus citations: 287 (245 without self citations) -- H-index (Scopus): 8 -- H-index (Scopus without self citations): 8
Total OpenCitations: 85 -- H-index (OpenCitations): 4
Total OpenAlex: 328 -- H-index (OpenAlex): 9