A new *NOTCH2* Variant in Hajdu-Cheney Syndrome: A case report and literature update

DIU de Dysmorphologie et Anomalies du Développement

2024 / 2025

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1. Abstract

Introduction: Hajdu-Cheney syndrome (HCS) is a very rare (< 1/1,000,000 live births) autosomal dominant connective tissue disorder. HCS results from pathogenic variants in exon 34 of the Notch homolog protein 2 (*NOTCH2*) gene. The Notch signalling pathway is implicated in bone development and homeostasis by controlling cell proliferation and differentiation. In HCS, truncated Notch2 protein accumulation leads to a continuous excessive signal responsible for developmental skeletal disorders such as acro-osteolysis and osteoporosis, as well as short stature, craniofacial features and systemic abnormalities. We describe a woman and her sister who initially presented with finger deformities, and we subsequently identified a novel heterozygous c.6187delG (p.Asp2063Metfs*5) class IV variant in exon 34 of the *NOTCH2* gene.

Method: Our patients' features were compared to a literature review of 40 cases published after July 2020, as Cortés-Martín *et al.* had previously conducted a systematic review covering data up to that time.

Results: Our two patients presented typical clinical and radiological features. However, they did not have osteoporosis or short stature. Their diagnosis was delayed and only confirmed around the age of 40 through genetic testing, based on deformities in the fingers and toes, associated pain, and a positive family history.

Conclusion: A novel *NOTCH2* variant was identified in our patients, exhibiting variable expressivity intrafamilially and in comparison to previously reported cases. Due to the rarity of HCS, a delayed diagnosis appeared to be common in this condition. Our review provides new descriptive data improving the latest retrospective descriptive cohort published in 2020 by Cortés-Martin *et al*. Specific treatment guidelines for HCS are not currently established and need accurate genotype-phenotype correlations. Current therapeutic objectives for HCS are to minimise complications and to reduce pain and osteoporosis. Antiresorptive (bisphosphonates, denosumab) and anabolic (teriparatide) agents have been used, without clear evidence of efficacy.

2. Introduction

Hajdu-Cheney syndrome (HCS) (OMIM 102500) is a very rare (< 1/1,000,000) and multi-systemic genetic disorder of the connective tissue classified among the osteolysis syndromes. Initially, Hajdu and Kauntze reported the disease in 1948 with a 37-year-old man who presented with severe osteoporosis, acro-osteolysis and neurological complications; he died 12 years later. This syndrome was more extensively described by Cheney in 1965 in a family of Michigan, providing evidence for autosomal dominant inheritance. A comprehensive review of the literature was later published in 2020. HCS is a heterogeneous condition with progressive bone phenotypic manifestations characterised by focal bone destruction with severe generalised osteoporosis leading to fractures and vertebral deformities, as well as acro-osteolysis of distal phalanges. Craniofacial dysmorphisms such as coarse features, long philtrum, high arched palate and retro-/micrognathia are commonly observed. Other systemic clinical features as congenital heart or vessel defects, recurrent respiratory infections and polycystic kidneys have also been reported. Otological, ocular, neurological and dental disorders have been described in few patients.

HCS is caused by heterozygous pathogenic variants in exon 34 of the Notch homolog protein 2 (*NOTCH2*) gene. The inheritance mode of HCS is autosomal dominant. Most cases are familial but sporadic cases also occur (1,2,3). To date, mosaicism has not been reported in HCS, although mosaicism has been identified in several other skeletal disorders (4). The Notch signalling network is a highly conserved intracellular communication pathway determining cell fate decisions and differentiation, as well as function of cells of multiple lineages during embryonic development and cell regeneration (e.g., organ formation, tissue function and repair, skeletal tissue remodeling). The *NOTCH2* gene encodes the NOTCH2 transmembrane receptor protein implicated in skeletal development and bone remodeling by influencing both osteoclast and osteoblast lineage cells. The *NOTCH2* gene also contributes to the development of cardiovascular, renal, and hepatic systems. In HCS, nonsense pathogenic variants, intragenic duplications or deletions resulting in a frameshift variant lead to the production of a truncated NOTCH2 protein that lacks a conserved region of its intracellular domain. This protein truncation increases the stability of the NOTCH2 protein, and in the NOTCH signalling pathway is excessively activated, resulting in a gain-of-function effect with uncontrolled bone resorption (5-8).

We present the case of a 43-year-old woman and her sister who initially consulted for the evaluation of progressive distal fingers deformities.

3. Patients & Methods

Informed consent was obtained from both sisters for this scientific report, including photographs.

The proband was evaluated at the age of 43 during a genetic consultation at the University Hospital of Liège (Belgium), with the aim of establishing appropriate medical follow-up and staying informed about therapeutic advancements.

DNA extracted from peripheral blood leukocytes was obtained from both sisters. Due to clinical suspicion in the sister, molecular analysis involving sequencing of exon 34 of the *NOTCH2* gene was previously performed at the Rheumatology Laboratory of the Cliniques Universitaires Saint-Luc in Brussels. The variant identified in the sister was subsequently investigated in our proband by Sanger sequencing, which confirmed the diagnosis.

A literature review of HCS was conducted by searching in the Pubmed and Google Scholar databases, complemented by a manual review of references from all the selected articles. The research terms used were « Hajdu-Cheney Syndrome », « Acro-osteolysis », « Osteoporosis », « NOTCH2 gene», « Notch2 receptor », « Notch pathway », « Serpentine fibula-polycystic kidney syndrome » with the logical operators "AND" and "OR" to optimise and refine the research. We documented all the cases published after July 2020, without applying specific inclusion criteria, but excluding duplicate cases.

4. Case Report

Appendices 1 *and* ***2*** *describe clinical and radiological features of our two patients.*

The case report section is presented in a chronological order to facilitate understanding of the clinical description, while respecting the fact that patient 1 is the patient initially admitted to another centre.

The sister of the proband (Patient 1)

At the age of 39-year-old, the sister of the proband visited the rheumatology clinic initially to investigate finger deformities. She presented finger deformities progressing over the past eight years, which first appeared in the fourth finger of the right hand, initially suspected to be traumatic. Subsequently, she noticed deformities affecting the third finger of the left hand and the fifth finger of the right hand. The distal phalanges were predominantly involved with clubbing and ridged nails on several fingers. She suffered from joint pain affecting her hands and feet, with no signs of Raynaud's phenomenon, no skin thickening or sicca syndrome documented. The patient sustained multiple

fractures including a distal metaphyseal-epiphyseal fracture of the second phalanx of the right fourth finger, a fracture involving multiple segments of the third digit of the left hand, a fracture of the base of the middle phalanx of the right third ray, a spiral fracture of the distal right fibula, a fracture of the lateral base of the right fifth metatarsal, and a posterior rim fracture of the lateral tibial plateau in the left knee. Her medical history revealed asthma with atopy, recurrent bronchitis and otitis, ossiculoplasty and tympanoplasty procedures for hearing impairment, as well as surgical correction of dental malocclusion due to prognathism and a narrow palate. She reported that her sister (patient 2) had also two deformed fingers and that their father had a short stature (adult height of approximately 1.65 meters), finger deformities, and facial features similar to her own. Her brother presented ridged nails. Nevertheless, the patient's family history is unavalaible because she had no contact with other members of her family apart from her sister.

On clinical examination, she had a height of 1.64 meters (-0.43 SD) and a weight of 57 kilograms (-0.35 SD). She presented with accordion-like deformities of the distal part of the left third ray, and of the right fourth and fifth rays with clubbing. Generalised hypermobility was noted.

Laboratory blood tests, including complete blood count, renal and hepatic function, electrolytes (notably calcium and phosphate levels), C-reactiv protein as well as autoimmune screening, were all within normal limits. Alkaline phosphatase and vitamin D levels were normal. X-rays confirmed acro-osteolysis of the left third ray, as well as the right fourth and fifth rays of the hands. Skull and cervical radiographies revealed an irregular appearance and lack of fusion of the sutures. There was an absence of frontal sinus pneumatization, hypoplasia of the maxillary sinuses, and a block vertebra involving C2 and C3.

According to these observations, HCS was firstly suspected, leading to the request for genetic analyses for both sisters. Consequently, echocardiography and renal ultrasound were performed in patient 1, revealing leaflet billowing and no polycystic kidneys.

Proband (Patient 2)

Our patient consulted in the rheumatology clinic at the age of 37 (two years after her sister's visit) due to severe pain in her hands and feet. In the past, she had multiple non traumatic long bones fractures such as the left third and fourth metacarpals, the proximal phalanx of the fourth ray of the right hand, and of the left second and third metatarsals. Surgical intervention was required for left hallux valgus. She also had generalised hypermobility with recurrent shoulder dislocations. Major orthodontic treatment was performed during adolescence to correct a narrow palate. She developed hearing loss at age 36, followed by ossiculoplasty and tympanoplasty six months later.

On clinical examination, her height was 1.68 meters (+0.25 SD) and her weight was 55 kilograms (-0.61 SD). Deformities of the second and third phalanges were observed in the second and third rays of the left hand, and in the second, third and fifth rays of the right hand, associated with digital clubbing affecting several fingers. X-rays confirmed acro-osteolytic changes affecting the distal phalanges of the left second and third digits, right second, third, and fifth digits, and right hallux. Skull X-rays demonstrated irregularities and lack of fusion of the sutures, with absence of aeration in the frontal sinuses.

5. Results

5.1. Analysis of the *NOTCH2* gene variant

The c.6187delG (p.Asp2063Metfs*5) variant in exon 34 of the *NOTCH2* gene (NM_024408) was identified in heterozygous state in both sisters, with suspected paternal inheritance (father was clinically affected but untested).

The variant is not reported in the literature or in the LOVD database. It is not present in the population control database (gnomAD) and not found in ClinVar.

The c.6187delG (p.Asp2063Metfs*5) variant induces a frameshift at the amino acid position 2063/2472, resulting in a premature stop codon, five positions downstream.

Based on these data, this variant was classified as a class IV (likely pathogenic) variant according to ACMG criteria.

5.2. Comparison with HCS cases in the literature

The clinical and radiological characteristics of our patients were compared to 40 patients (1 prenatal and 39 postnatal cases) described in the literature between 2020 to 2025. *The prenatal case was analysed separately. Only the clinical and radiological features, the systemic abnormalities and other findings reported in the papers were included* (7-40) (*cf.* ***Appendix 3***).

To improve our comparative analysis, we considered the systematic review performed by Cortés-Martin *et al.* in 2020 encompassing literature data prior to 2020. The authors selected 76 articles out of 193 excluding systematic reviews, duplicate articles, and those that were exclusively case reports. Their results were reported in the form of four thematic subjects including disease genetics,

description of the disease and phenotypic evolution, diagnosis and differential diagnosis, and treatments but without providing quantitative details (2). Consequently, their data could not be compared in a statistically relevant approach to our cases. A manual review of their 76 articles was not performed because it would also be statistically limited due to restricted access to most of the older papers.

5.2.1. Clinical features

Although sometimes dysmorphic features are present at birth, they are initially non-specific for HCS. The clinical symptoms emerge early in life and gradually progress, with the pathognomonic features of HCS becoming increasingly recognisable over time. Several recurring dysmorphic features were observed. Similarly to our family, craniofacial characteristics contain coarse hair (*n*=7/39), coarse face (*n*=7/39) and thick bushy eyebrows (*n*=12/39). Prominent forehead (*n*=5/39) is present in our proband but not in her sister. Although hypertelorism is frequently reported in the literature (*n*=11/39), it was not observed in our patients. Downslanted palpebral fissures have also been described (*n*=3/39), as our sisters. Midface hypoplasia (*n*=7/39) with flat and broad nasal bridge (*n*=9/39), long philtrum (*n*=11/39) similar to our patient 1, high-arched palate (*n*=10/39) as seen in our proband, and micrognathia (*n*=14/39) are also very common. Patients may manifest pain and brachydactyly of the hands and feet, along with clubbing and nail hypoplasia (*n*=17/39). Finger deformities, accompanied by inflammation, pain, swelling, and occasionally paresthesia, led to impaired fine motor skills and hand weakness in both sisters, with significant impact on their dayly working lives. In most of the cases, the hands tend to be more severely affected. These symptoms initially motivated the first investigations in our family, as mentioned in some previously published cases (8-14). Flat feet (*n*=4/39) were also present.

5.2.2. Systemic abnormalities

Other manifestations consisted of congenital heart diseases including atrial or ventricular septal defects (*n*=6/39), patent ductus arteriosus (*n*=9/39) and mitral insufficiency (*n*=2/39), recurrent respiratory tract infections (*n*=7/39), and renal cysts (*n*=7/39). Serpentine fibula polycystic kidney syndrome is a rare skeletal dysplasia sharing key features with HCS, including acro-osteolysis, polycystic kidneys, and short stature. This syndrome is now considered part of the HCS spectrum (41).

A cardiac and renal ultrasound, along with spinal radiography, are planned for our proband to complete the medical evaluation.

5.2.3. Radiological features

Typical radiological findings involve the skull, spine and extremities. Cranial abnormalities as persistent wormian bones (*n*=11/39), open skull sutures (*n*=12/39) and hypoplasia of frontal sinus (*n*=4/39) are all radiological features shared by our patients. Dolichocephaly (*n*=8/39) and occipital protuberance are also mentioned in the literature. There may be J-shaped elongated and enlarged sella turcica (*n*=8/39). Acro-osteolysis is one of the main features of HCS (*n*=25/39). Dual-energy X-ray absorptiometry is used to highlight bone mineral density loss. Interestingly, osteoporosis was not found in our patients in adulthood, whereas osteopenia/osteoporosis is frequently reported in the literature (*n*=30/39). Osteoporosis may result in basilar invagination and platybasia (*n*=8/39), biconcave vertebral deformities ("fishbone") (*n*=6/39), as well as compression or fractures (*n*=14/39), and kyphoscoliosis (*n*=12/39). These skeletal changes can lead to neurological manifestations such as intense headaches, hydrocephalus (n=6/39), and respiratory failure. Additional spinal complications, including spondylolisthesis (n=4/39) and syringomyelia (n=3/39), have also been reported and may further compromise neurological function. Furthermore, kyphoscoliosis can contribute to restrictive ventilatory impairment, increasing the risk of both respiratory and cardiac complications (3,38). The patient 1 reported headaches with cervical restricted mobility (vertebral block at C2-C3), osteoarthritis and incorrect posture (kyphotic tendency); spinal osteoarthritis was recently demonstrated in the context of polyalgia. She described hypoesthesia in the left lower limb and paresthesias in both hands and feet. Long bone fractures, also frequently documented (n=13/39), were indeed present in both sisters. Fibulae can be characterised by elongation, widening, and pronounced medial bowing.

5.2.4. Other findings

Short stature is found in many cases (*n*=17/39); however, our two patients have a normal adult height. Joint hypermobility and subluxations were also documented in the literature (*n*=12/39) as in our patients. Dental anomalies as malocclusion, premature loss, underdevelopped or delayed teeth were found in more than half of the patients reported in the literature (*n*=21/39). Our patients have no premature loss of teeth, but one has dental malocclusion and the other delayed eruption of permanent teeth. Regarding otorhinolaryngology, conductive or sensorineural hearing loss (*n*=15/39) (along with deep, hoarse, and low-pitched voice) have been reported as observed in our patients. Finally, mild developmental delay was reported *in* 8/39 patients; no information for neurodevelopmental milestones is avalaible for our patients. They completed secondary education.

5.2.5. Prenatal case

During the prenatal period, ultrasound examination can reveal shortened upper and lower limbs (mesomelic and rhizomelic, respectively), bowed femurs, slender and/or curved ("s-shaped") fibulae, inadequate mineralisation of the calvaria, open fontanelles, and hypoplasia or absence of nasal bones. Moreover, cardiac, pulmonary, urogenital, and renal malformations may be present, as observed in postnatal HCS. Only two foetal cases have been reported in the literature at our knowledge, in 2014 (described below but no included in our cases serie) and in 2023. In 2023, Deb *et al.* described a prenatal case of HCS diagnosed by ultrasound in the second trimester, with growth restriction, particularly short and curved long bones in the limbs, and severe cardiopathy. A medical termination of pregnancy was performed. The fetopathological examination showed supplementary findings as micromelia, open fontanelle, facial dysmorphism (as hypertelorism and microstomia), and histological anomalies of the kidneys (15) (*cf.* ***Appendix 3***). The second foetus with HCS was described by Martin *et al.* (2014) with shortened femora at 19 weeks of pregnancy. Ultrasound reexamination at 23 weeks revealed shortened, bowed long bones with ulnar deviation of both hands. Moreover, fetopsy identified cardiac anomalies and poor bone mineralisation. Histological analysis showed an impaired transition from prehypertrophic to hypertrophic chondrocytes, appearing to be atypically small. This supports the fact that Notch signalling is essential for the differentiation process (16).

To date, no cases of HCS have been reported in the medical literature with specific details regarding genetic counseling or prenatal diagnosis.

6. Discussion

Initially, the diagnosis of HCS relies on distinctive clinical manifestations and imaging results. Brennan and Pauli (2001) proposed a diagnostic tool with inclusion criteria for HCS, based on physiological factors and genetic inheritance patterns from London Dysmorphology Database. This tool incorporated 10 major manifestations of HCS as well as positive family history and took into account the differences between adults and children, considering the evolution of the phenotype over time (*cf.* ***Appendix 4***).

Currently, the gold standard is based on genetic analyses demonstrating a pathogenic variant or likely pathogenic in the terminal exon of NOTCH*2*.

6.1. Physiopathology

The *NOTCH2* gene produces a 2471-amino acid Notch2 protein, a member of the four Notch transmembrane receptor family (Notch1 to 4). *NOTCH1*, *NOTCH2*, *NOTCH3*, and *NOTCH4* genes are respectively located on chromosomes 9, 1, 19, and 6.

NOTCH2 is located on chromosome 1p12. The Notch signalling pathway is an evolutionarily conserved mechanism of intercellular communication between adjacent cells that governs cell fate, differentiation, and tissue patterning. NOTCH2 is widely expressed in various tissues, such as the central nervous system, bone, heart and vascular epithelium, kidneys, lungs, teeth, liver, immune system, skin, and epithelial tissues. Its role varies depending on the cell type and developmental stage (4,21,41).

The four Notch receptors are transmembrane receptors that share structural similarities with three major parts: Notch extracellular domain (NECD), transmembrane domain (TMD) and Notch intracellular domain (NICD). The NECD contains multiple epidermal growth factor (EGF)-like repeats that typically interact with ligands, and a negative regulatory region (NRR). The NRR consists of three cysteine-rich Lin12-NOTCH repeats (LNRs) and a heterodimerization (HD) region required for cleavage. The transmembrane domain (TMD) is located between NECD and NICD. The NICD include an Rbpjk-association module (RAM) domain, multiple ankyrin (ANK) repeats and, nuclear localisation sequences (NLS) around ANK. This NICD is essential for activating the transcription of Notch target genes. The C-terminal extremity of NICD is composed by conserved proline (P)-, glutamic acid (E)-, serine (S)-, threonine (T)-rich (PEST) domain, recognised by ubiquitin ligases (*cf.* ***Appendix 5*** *figure 1*) (42).

In contrast to classical signalling pathways driven by G protein-coupled receptors (GPCRs) and enzyme-linked receptors, which involve multiple intermediates between membrane receptors and nuclear effectors, the canonical Notch pathway functions independently of such intermediates. The receptors are directly translocated into the nucleus after three cleavage events.

In signalling-receiving cells, Notch precursors are produced in the endoplasmic reticulum (ER) after transcription and translation, and glycosylated at the EGF-like repeat domain essential to Notch stability and function. Once glycosylated, Notch precursors are transported to the Golgi and cleaved at the HD (S1 cleavage) resulting in a heterodimer. The mature form of the receptor is transported to the cell membrane. Continuous endocytosis, promoted by ubiquitin ligases, modulates the abundance of Notch receptors at the cell membrane. A significant portion of these receptors is ubiquitinated and subsequently degraded by the proteasome, while the remaining receptors are retained at the membrane to mediate signal transduction. A large proportion of these receptors undergoes

ubiquitination and then a degradation by the proteasome, while the others are preserved at the membrane to facilitate the signal transduction.

In signalling-sending cells, five classic Notch ligands are described: Jagged (Jag1 and Jag2) and Delta-like (Dll1, Dll3 and Dll4). These ligands are transmembrane proteins, and their extracellular domains are also constitued by multiple EGF-like repeats, which enable binding to the complementary Notch receptor. Ligand concentrations and activity are also regulated through ubiquitynation and endocytosis. Ligand binding to the NECD of the Notch receptor initiates endocytosis of the ligand and generates a pulling force for the bound receptor. This phenomenon extends the LNR domain and exposes the S2 site for cleavage by a disintegrin and metalloprotease (ADAM). S2 cleavage generates Notch extracellular truncation (NEXT), which contains TMD and NICD. NEXT can be cleaved by γ-secretase (S3 cleavage) on the cell membrane or, in the endosome after endocytosis, and release NICD. Otherwise, NEXT can be transported to the lysosome for degradation. If NICD is translocated into the nucleus, it acts as a transcription factor by interacting with CBF1, Suppressor of Hairless, Lag1 (CSL) and mastermind-like proteins (MAMLs) that release transcriptional co-repressors (CoR) and recruit co-activators (CoA). This interaction allows expression of Notch target genes (*cf.* ***Appendix 5*** *figure 2*). Phosphorylation of the PEST domain results in the dissociation of NICD-MAML-CSL complex, after which NICD is ubiquitinated and degraded in the lysosomes (6,43).

In HCS, the pathogenic variants mainly include nonsense variants, deletions, duplications and insertions, which cause frameshifts upstream of the PEST domain, leading to the production of truncated Notch2 proteins lacking this PEST domain (5). Because the PEST domain is critical for targeting Notch2 for ubiquitination and degradation, its loss increases protein stability and ultimately results in a gain-of-function phenotype. As the premature stop codon is located in the terminal exon of the *NOTCH2* gene, the nonsense-mediated mRNA decay (NMD) does not operate. As a result, the NOTCH2 altered mRNA is not degraded, and its transcript levels remain unchanged (4,41).

Interestingly, somatic pathogenic variants in NOTCH2 causing loss of the PEST domain lead to increased NOTCH activation and are associated with certain types of B-cell lymphomas. However, no increased incidence of lymphoma has been observed in patients with HCS (42).

6.2. How do pathogenic variants in the *NOTCH2* gene, associated with Hajdu-Cheney syndrome, contribute to bone loss? What mechanisms are currently hypothesised to underlie other systemic manifestations?

Canalis *et al.* investigated the effects of a pathogenic variant in the *NOTCH2* gene in murine (2018) and human (2024) models to assess its influence on osteogenic and osteoclastogenic cell differentiation. Human models using induced pluripotent stem cells (iPSc) were generated. Osteopenia was identified in both models, due to excessive bone resorption by increased number of osteoclasts (42,44) (*cf.* ***Appendix 6***).

In osteogenesis, studies in mutant mouse models showed no changes in osteoblast differentiation or bone formation due to the pathogenic variant. Under conditions of high bone remodeling due to elevated bone resorption, an increase in bone formation would typically be expected as part of the compensatory mechanism. Surprisingly, in this mouse model, bone formation was instead slightly reduced. In human models, a modest increase in osteogenesis was observed in *NOTCH2* mutant cells. Thus, an imbalance between bone resorption and bone formation is observed. Alkaline phosphatases, primarily produced by osteoblasts in bone tissue (marker of osteoblastic activity), are at the lower limit of the normal range in our index case, in accordance with the evidence of physiopathological osteogenesis imbalance showed in these studies.

In osteoclastogenesis, research in mutant mouse models have shown that overactivation of the Notch pathway in osteoblast-lineage cells contributes to increased expression of Receptor Activator of Nuclear Factor κB Ligand (RANKL). In contrast, in human iPSc models harboring *NOTCH2* pathogenic variants, RANKL expression appears reduced, as in our proband results. This reduction appears to be mediated by HES1, which functions as a repressor of *TNFSF11* (the gene encoding RANKL). In HCS, *NOTCH2* pathogenic variants results in a gain-of-function, with a significant elevated expression of canonical Notch target genes, particularly *HES1* - 100 to 1000 times more than other target genes - highlighting its important role in skeletal cells. Decreased RANKL would typically be expected to reduce bone resorption. Instead, osteoclastogenesis appears to be enhanced via a direct pathway, independent of RANKL (44,45). This may be explained by a cell-intrinsic role of HES1 in myeloid lineage cells, promoting the differentiation of osteoclast precursors without the need for RANKL signalling. Other pathways could potentially be involved but remain to be discovered.

We hypothesise that a hypomorphic NOTCH2 variant may explain the absence of osteoporosis and result in a milder or atypical clinical presentation compared to classic gain-of-function variants, or that protective genetic factors may be present to compensate for bone resorption. Although our patients do not have osteoporosis, they have sustained long bone fractures. One hypothesis should simply be

that the fractures observed in our patients may result from trauma, stress fractures, and joint hypermobility due to intense physical activity, rather than reduced bone density. Additionally, the fractures could be partially attributed to alterations in bone structure caused by premature activation of the Notch pathway in both osteoblast progenitors and mature osteoblasts, which has been shown to reduce trabecular bone volume and promote the deposition of disorganised woven bone (1,6).

According to the literature, acro-osteolysis appears to result from a distinct mechanism compared to the generalised bone loss. Indeed, our patients exhibit acro-osteolysis without osteoporosis in adulthood. Furthermore, in some patients reported in the literature, pharmacological treatment led to an improvement in bone density, with no observed effect on acro-osteolysis. Biopsy findings indicated the presence of inflammation, neovascularisation and fibrosis, but physiopathology remains poorly understood (42,4). Vollersen *et al.* (2017) observed that mutant Notch2 mice did not develop acro-osteolysis. This suggests that the condition may result from external factors such as minor injuries or inflammation. It is also possible that the mouse model was not exposed to sufficient mechanical stress, abrasion or pathogen exposure on their distal phalanges to trigger the development of acro-osteolysis (46). The sister of our patient practiced rock climbing for several years and seem to be more affected than our index case.

The short stature observed in fewer than half of the reported cases with HCS could be explained by the strong inhibitory role of Notch signalling in chondrogenesis. No clear evidence has been published to date on this specific point to elucidate the variability in the impact of *NOTCH2* variants on the growth plate. The mechanisms underlying periodontal disease and tooth loss remain unclear (42).

Notch signalling is essential for the development of the renal glomeruli. In mouse models, reduced expression of NOTCH2 leads to renal hypoplasia; however, no cystic formations have been observed (48).

Finally, the *NOTCH2* gene is involved in cardiovascular development and angiogenesis, which could partially explain the congenital heart malformations observed in HCS (1,48).

However, no clear phenotype–genotype correlation exists, as both skeletal severity and other manifestations can vary within affected families and among unrelated individuals carrying the same variant.

6.3. Differential diagnosis

6.3.1. Prenatal period

Due to the limited number of prenatal descriptions of NOTCH2-related disorders, it remains difficult to precisely define the full spectrum of thoracic and pelvic skeletal anomalies associated with HCS, especially in comparison to better-documented prenatal skeletal dysplasias, such as Stüve-Wiedmann syndrome (SWS), Campomelic Dysplasia (CD), Osteogenesis Imperfecta type II (OI II), and achondroplasia. These conditions constitute the main differential diagnoses of HCS in the prenatal period evocated in case of abnormal curvature of the long bones as a first ultrasound sign.

Upper limb abnormalities may indicate either SWS or HCS. However, if oligohydramnios or intrauterine growth retardation is present, with the tibia more severely affected than the femur, SWS could be considered. A small thoracic cavity may suggest CD or OI II. In contrast, the thoracic measurements in HCS typically appear normal and follow standard growth curves. The presence of poor calvarial mineralisation and multiple fractures is suggestive of OI II, whereas CD is characterised by early and pronounced shortening of the long bones, which may be detectable as early as the first trimester, sometimes accompanied by hypoplastic scapulae. Achondroplasia is often associated with macrocephaly, pronounced rhizomelic limb shortening, as well as vertebral anomalies. Skeletal radiographs are a key diagnostic tool in the prenatal period, particularly for demonstrate a tapered proximal femoral end, which is characteristic of achondroplasia condition. Cardiothoracic and visceral anomalies are frequently observed in HCS, whereas they are typically absent in the other conditions (15,16).

6.3.2. Postnatal period

The broad spectrum of skeletal and non-skeletal manifestations makes the identification of HCS complex. Moreover, the phenotype shows significant clinical variability and evolves over time (4). The differential diagnoses listed below are not exhaustive but includes the main causes of acro-osteolysis and syndromic conditions with features overlapping those of HCS. Notably, both Alagille syndrome and HCS involve disruptions in the NOTCH2 signalling pathway, reflecting a shared genetic basis despite their distinct clinical presentations.

6.3.2.1. Phenotypic overlap with HCS

Acro-osteolysis

Based on the etiology, acro-osteolysis can be classified into primary and secondary types. Radiologically, acro-osteolysis appears as transverse (band-like) osteolysis of the distal phalanx shaft or, more commonly, as longitudinal resorption of the terminal tuft. In HCS, Rothmund-Thomson syndrome, and chronic exposure to polyvinyl chloride, transverse acro-osteolysis of the mid-portion of the phalanges is observed. Differentiating HCS from other conditions that lead to acro-osteolysis is essential, as acro-osteolysis is the main feature of HCS. These conditions can be distinguished based on clinical and imaging findings.

Primary acro-osteolysis

Acro-osteolysis can be observed in various genetic disorders such as HCS, pycnodysostosis, Hutchinson-Gilford Progeria and Rothmund-thomson syndromes. Pycnodysostosis and Hutchinson-Gilford Progeria syndrome (HGPS) are described below in more detail because of certain clinical similarities with HCS.

Pycnodysostosis is an extremely rare lysosomal genetic condition with a prevalence of 1 to 9 cases per 1,000,000 individuals. This condition is characterised by short-limbed short stature, brachydactyly, and distinctive craniofacial features such as frontal bossing, persistently open anterior fontanelle, a prominent nose with a convex nasal ridge, midface retrusion due to maxillary and mandibular hypoplasia, and a high-arched palate. Dental anomalies and nail abnormalities are frequently observed. Radiographic findings typically include osteosclerosis, acro-osteolysis of the distal phalanges, non-pneumatized mastoid bones, delayed fusion of cranial sutures, and an increased susceptibility to fractures. Diagnosis is based on characteristic clinical and radiographic findings and/or confirmed by the identification of biallelic pathogenic variants in the *CTSK* gene through molecular genetic testing (53).

Hutchinson-Gilford Progeria Syndrome (HGPS) is an extremely rare disorder (<1/1,000,000), characterised by premature aging that begins postnatally with alopecia and osteolysis. As described in HCS, acro-osteolysis associated with hypoplasia of the nails, short stature, osteopenia and conductive hearing loss are present. Craniofacial features include prominent forehead, narrow nasal ridge and tip, small mouth and micrognathia or retrognathia. In the classical form, a specific point mutation in the *LMNA* gene on chromosome 1q leads to the production of an abnormal protein named progerin, in

addition to normal lamin A. The accumulation of progerin causes nuclear membrane abnormalities and reduces cellular lifespan with accumulation in bones (54).

Secondary acro-osteolysis

The secondary causes include hyperparathyroidism, scleroderma, psoriatic arthritis, sarcoidosis, neuropathic diseases (e.g., diabetes), infections, trauma origins, Raynaud's phenomenon, and toxicity from polyvinyl chloride and ergot (49-52).

Lateral Meningocele Syndrome

Lateral Meningocele (LMS) or Lehman Syndrome (<1/1,000,000) is characterised by craniofacial abnormalities, hypotonia and meningocele, accompanied by related neurological impairment. Skeletal manifestations are varied and include craniofacial developmental defects, short stature, scoliosis and low bone density. The overlap in craniofacial and skeletal manifestations between LMS and HCS can complicate the differential diagnosis. Exome sequencing of individuals with LMS has revealed punctual mutations or small deletions in exon 33 of *NOTCH3*, upstream of the PEST domain. As in HCS, these pathogenic variants result in the production of a truncated, stable protein lacking the PEST domain, likely leading to a gain-of-function of *NOTCH3* (42).

Singleton-Merten Syndrome

Singleton-Merten Syndrome (SMS) (<1/1,000,000) is a type I interferonopathy including aortic calcification, dental dysplasia, osteopenia, acro-osteolysis, foot deformities, anomalies of the metacarpal and phalangeal diaphyses. SMS also involves glaucoma, psoriasis-like eruption, idiopathic fever and muscular weakness. This syndrome is caused by mutations in the *IFIH1* or *DDX58* genes (atypical form) and the transmission is autosomal dominant (55).

6.3.2.2. One gene, two syndromes: contrasting Alagille and HCS

Alagille Syndrome (ALGS) and HCS share molecular physiopathology involving the Notch signalling pathway (with a loss- or gain-of-function, respectively) and can make the differential diagnosis challenging if clinical presentations are atypical or incomplete with overlapping signs. ALGS has an estimated prevalence of approximately 1 in 30,000 based on genetic analyses. It is a multisystemic autosomal dominant disorder caused by dysregulation of the Notch signalling pathway. *JAG1* mutations account for most cases (> 90%) (ALGS1). *NOTCH2* mutations, located in the EGF-like or ANK

repeats, represent the second most frequent cause of ALGS (approximately 2,5% of cases) (ALGS2). These *NOTCH2* variants result in a loss-of-function mechanism. Clinically, ALGS is diagnosed when at least three out of five major criteria are met: hepatic bile duct paucity and cholestasis, cardiovascular defects (as Fallot's syndrome), ocular anomalies (like posterior embryotoxon), abnormal vertebral segmentation (e.g., butterfly vertebrae or hemivertebrae) and distinctive facial features (broad forehead, deep-set eyes, and a pointed chin). Additionally, renal and vascular abnormalities are frequently observed (56,57).

6.4. Therapeutics

The treatment and management of HCS depend on the specific organs affected for each patient. As the main mechanism of bone loss in HCS is osteoclast activation, antiresorptive therapy rather than anabolic treatment seems more appropriate for the treatment of osteoporosis and the prevention of fractures. Therefore, antiresorptive drugs were used in most of the reported cases.

Bisphosphonates are known to attach to hydroxyapatite in bone, preventing osteoclasts from binding and thereby decreasing bone resorption. In certain cases of HCS, bisphosphonate treatment led to an improvement in bone mineral density and bone microarchitecture but showed no effect on acro-osteolysis. However, this treatment appears to have been tried only when bone mineral density values were pathological (osteoporosis), and in some cases, no substantial benefit was observed. According to the literature, there is a short-term benefit that tends to reverse quickly once treatment is stopped, despite the skeletal manifestations of the disease being caused by increased bone resorption. The optimal time to initiate treatment and the required duration to maintain a beneficial effect are not yet established.

If the findings from Canalis *et al.* are applicable to humans suffering from HCS, the efficacy of anti-RANKL treatment (e.g., denosumab) may be limited, likely due to its inhibitory effect on residual RANKL activity and bone resorption. Canalis *et al.* reported a woman with elevated serum levels of RANKL who was treated with denosumab for two years, leading to improved bone mineral density and no fractures; however, acro-osteolysis continued to progress (58).

Teriparatide, a synthetic parathyroid hormone, should be administrated with caution as experimental models of HCS demonstrate a bone resorption phenotype that could potentially worsen under treatment. Moreover, the use of teriparatide requires attention due to potential side effects, such as an increased risk of osteosarcoma. Notch signalling is increased in human osteosarcoma, and long-term activation of Notch in osteoblasts can cause osteosarcoma in animal models. Although

osteosarcoma has not been reported in HCS, it would be safer to avoid treating these patients with teriparatide due to a theoretical risk of developing osteosarcoma.

Supplementation with calcium and vitamin D is recommended, as well as daily physical exercise. Smoking cessation and limiting alcohol consumption are recognised as important measures to reduce risk factors contributing to bone loss (5,42,59,60).

Additional studies are required to develop therapeutic guidelines for HCS, especially for osteoporosis and acro-osteolysis. To date, no effective strategies exist for the prevention or treatment of acro-osteolysis. Furthermore, there are no reports specific to the treatment for growth retardation in HCS.

7. Conclusion

Hajdu-Cheney syndrome (HCS) is an extremely rare cranioskeletal dysplasia associated with gain-of-function pathogenic variants in exon 34 of the *NOTCH2* gene. HCS is typically characterised by acro-osteolysis, severe osteoporosis, and distinctive dysmorphic features. Early diagnosis and treatment are crucial to prevent complications such as severe osteoporosis and fractures. It requires a detailed medical history, careful physical examination, and specific genetic testing, which can also be used for genetic counseling and prenatal diagnosis.

To date fewer than 100 cases have been reported in the literature. Due to its rarity and wide phenotypic spectrum, HCS is often diagnosed late or misdiagnosed. In our cases, the diagnosis was delayed (around 40 years old) and made by a rheumatologist, primarily based on digital deformities and long bones fractures. It could be supposed that a serie of patients may remain undiagnosed, especially when the hallmark feature of acro-osteolysis is absent. The global phenotype associated with the heterozygous c.6187delG variant seems correlated with cases in the literature. The phenotype of HCS varies over time within the same individual, among different family members sharing the same mutation, and compared to published cases. Indeed, the proband's sister appears to be more severely affected, especially regarding acro-osteolysis, whereas the two patients have a normal adult height and their father exhibits a short stature. No osteoporosis was found in both sisters. This phenotypic variability suggests the involvement of additional genetic or environmental modifiers influencing the expression of *NOTCH2* pathogenic variants.

Treatment is symptomatic and requires a multidisciplinary approach, addressing issues such as hearing loss, dental abnormalities, and bone fragility. There is currently no specific therapy for HCS. Management focuses on preventing osteoporotic fractures. Bisphosphonates and vitamin D

supplementation are commonly used, although their effectiveness varies and their efficacy has not been proven.

Our review highlights the phenotypic variability of HCS. Better delineate the physiopathology of HCS is essential. Further disease-specific stem cell models and genetic studies may help clarify the underlying mechanisms. Moreover, HCS remains poorly characterised regarding patient quality of life and long-term prognosis. More comprehensive studies involving larger patient cohorts are needed to refine the phenotypic description and establish clear management guidelines.

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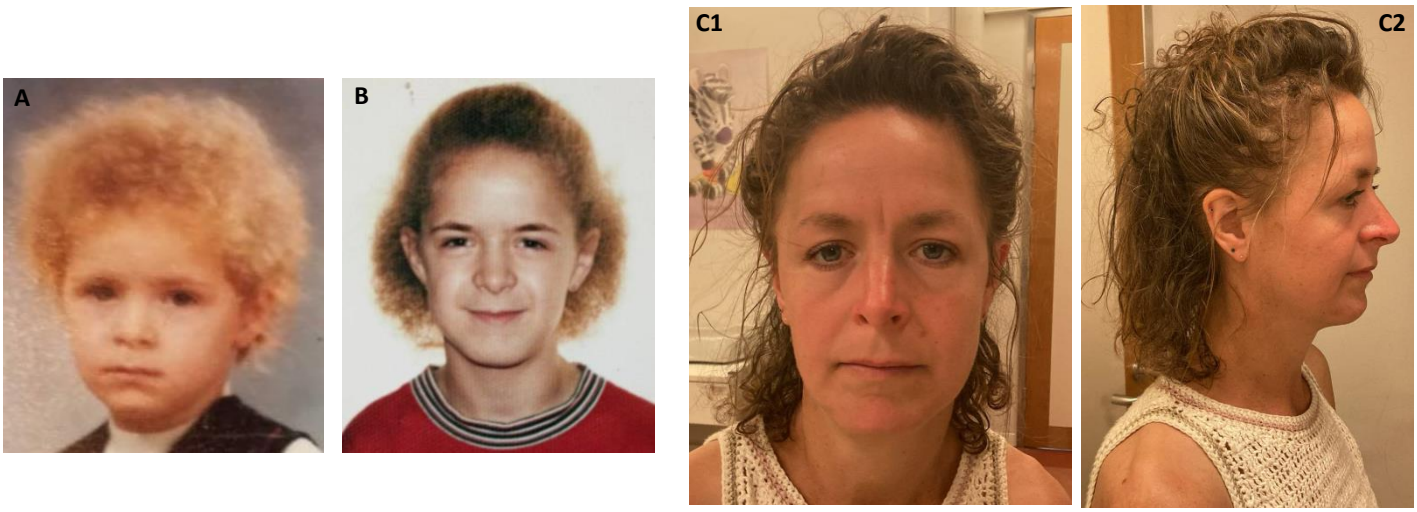
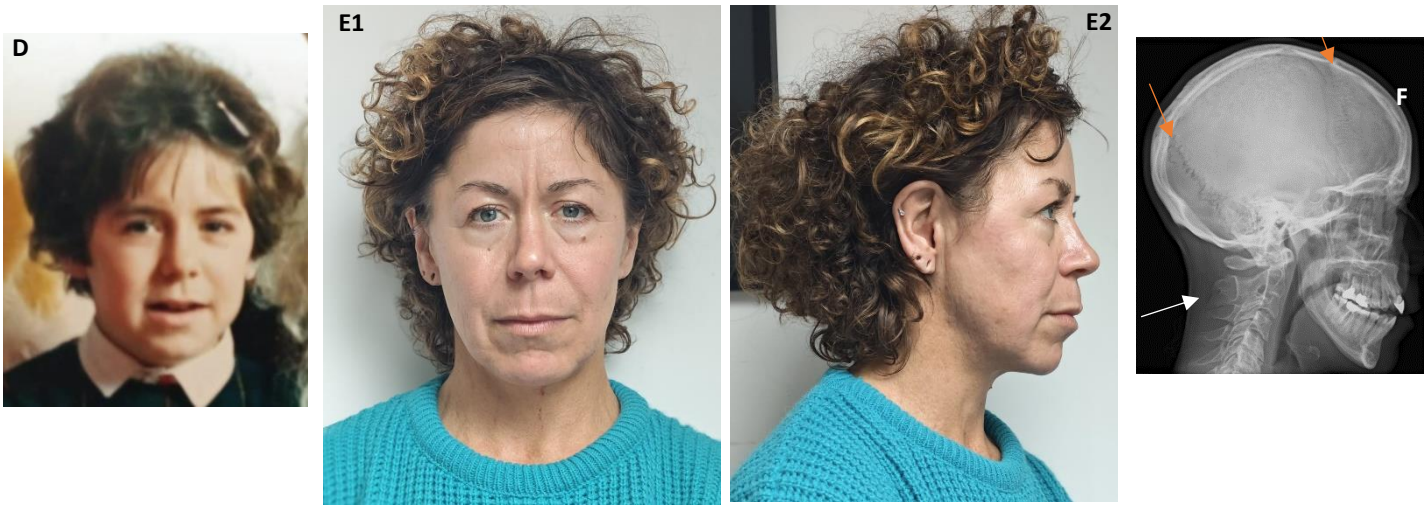
9. Appendices

Appendix 1. Craniofacial features of our patients

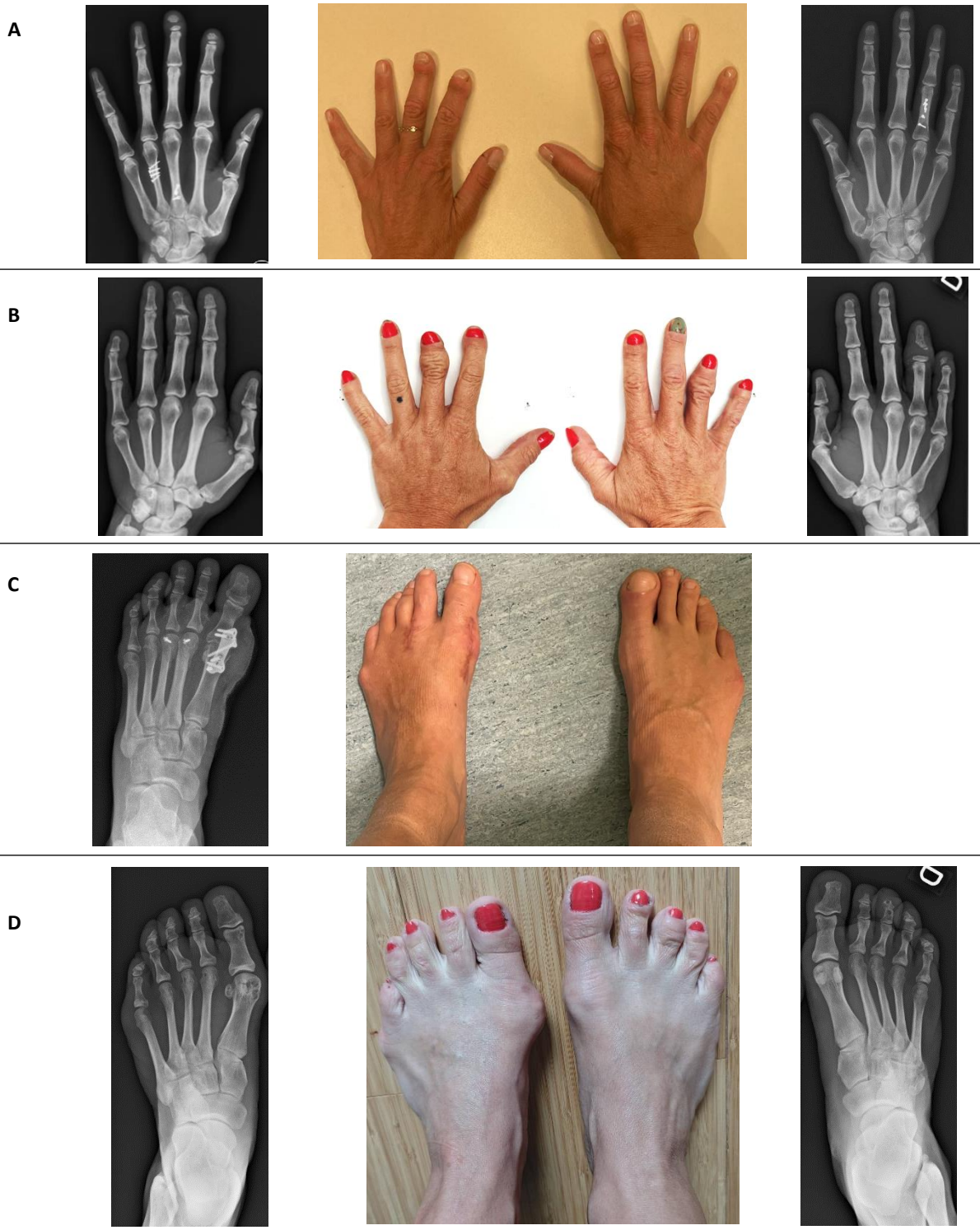


Proband (Patient 2) at early childhood, at 9 and 44 years old.
A, B) Coarse hair predominant in childhood with progressive downslanted palpebral fissures and prominent forehead.
C1, C2) Typical adult features with prominent forehead, bushy eyebrows, downslanted palpebral fissures, and thin upper lip.



Sister of the proband (Patient 1) at 10 and 48 years old.
D) No evident dysmorphic features
E1, E2) Coarse hair, downslanted palpebral fissures with impression of hypotelorism, long philtrum, thin upper lip.
F) Skull X-ray demonstrated irregular appearance and lack of suture fusion (orange arrows), absence of pneumatization in the frontal sinuses, hypoplasia of maxillary sinuses and C2-C3 block vertebra (white arrow).

Appendix 2. Clinical and radiological characteristics of the extremities



A) Proband: deformities of the 2nd and 3rd finger of left hand; 2nd, 3rd and 5th finger on right hand.
B) Sister of the proband: accordion-like deformities of the distal part of the left 3rd finger; of the right 4th and 5th fingers with clubbing.
C) Proband: left metatarsophalangeal joint arthrodesis of the 1st finger; right terminal phalange deformity (X-ray non available).
D) Sister of the proband: bilateral hallux valgus, significant forefoot widening, and deformity of the distal phalanx of the right 3rd ray.
Acro-osteolysis confirmed on X-rays.

Appendix 3. Clinical and radiological characteristics of our patients compared to 40 patients described in the literature between 2020 and 2025.

	Proband	**Sister of the proband**	*Postnatal (39 cases)*	*Prenatal case of Deb et al. (2023)*
Sex	F		21 F / 18 M	M
Age at last evaluation	44 y	48 y	21.4 y (±17.5)	27 + 5WG
Clinical features				
Coarse hair	+	+	7	NA
Coarse face	+	+	7	NA
Prominent forehead	+	-	5	+
Low-set ears	-	-	13	-
Bushy eyebrows	+	+	12	NA
Long eyelashes	-	-	4	NA
Hypertelorism/telecanthus	-	-	11	+
Downslanted palpebral fissures	+	+	3	-
Midface hypoplasia	-	-	7	NA
Flat and broad nasal base	-	-	9	+
Long philtrum	-	+	11	+
Microstomia	-	-	3	+
Cleft palate	-	-	2	-
High-arched palate	+	-	10	NA
Retro-/micrognathia	-	-	14	+
Short neck	-	-	5	NA
Brachydactyly	+	+	18	-
Hypoplasia of the nails/ clubbed fingers	+	+	17	NA
Flat feet	+	+	4	NA
Systemic abnormalities				
Cardiovascular				
Congenital heart defects	ND	-	6	+
Patent ductus arteriosus	ND	-	9	-
Dysplastic cardiac valve	ND	-	2	-
Pulmonary				
Recurrent infections	-	+	7	NA
Chronic lung disease	-	+	4	NA
Lungs malformation	-	-	0	+
Renal				
Polycystic kidneys	ND	-	7	+

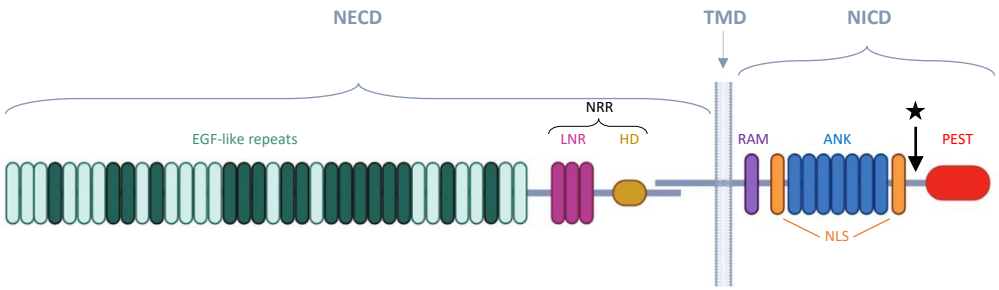
Radiological features				
Wormian bones	+	+	11	NA
Open skull sutures	+	+	12	+
Hypo-/aplasia of frontal sinus	+	+	4	NA
Dolicho-/bathrocephaly	-	-	8	-
Sella abnormalities	ND	ND	8	NA
Acro-osteolysis	+	+	25	NA
Osteopenia/Osteoporosis	-	-	30	-
Platybasia, basilar invagination	-	-	8	NA
Fishbone vertebral deformity	ND	-	6	NA
Vertebral fractures	-	-	14	NA
Kyphosis/Lordosis/Scoliosis	ND	-	12	NA
Hydrocephalus, Chiari malformation	-	-	6	-
Spondylolisthesis	ND	-	4	NA
Syringomyelia	ND	-	3	NA
Long bones fractures	+	+	13	-
Bowed long bones	ND	ND	6	+
Micromelia	-	-	5	+
Nasal bone agenesis	NA	NA	1	+
Other findings				
Oligohydramnios	NA	NA	N/A	+
Short stature	-	-	17	+
Joint hypermobility	+	+	12	NA
Dental abnormalities	+	+	21	NA
Hearing impairment	+	+	15	NA
Developmental delay	-	-	8	NA

Abbreviations : F, female; M, male; NA, non applicable; ND, non descriptible

Appendix 4. Diagnostic criteria of HCS by Brennan and Pauli (2001) (Adapted from Saji et al., 2022)

Diagnostic features of HCS	**Our cases**	**Adults**	*Children*
1. Acro-osteolysis	+	Acro-osteolysis and 3 additional manifestations from 2 to 9 *or* Acro-osteolysis and documented positive family history *or* Documented positive family history and 2 additional manifestations from 2 to 9	*Any 4 manifestations from 1 to 9* *or* *Documented positive family history and any other 2 manifestations*
2. Wormian bones or open sutures	+		
3. Platybasia	-		
4. Premature loss of teeth	-		
5. Micrognathia	-		
6. Coarse facies	+		
7. Coarse hair	+		
8. Midface hypoplasia	-		
9. Short stature (<5th precentile)	-		
10. Documented positive family history	+		

Appendix 5. Notch2 receptor after cleavage at S1 and activation of Notch pathway



NECD : **Notch extracellular domain**
EGF-like repeats : Epidermal growth factor-like repeats
NRR : Negative regulatory region
LNR : Lin12-Notch repeats
HD : Heterodimerization domain

TMD : **Transmembrane domain**

NICD : **Notch intracellular domain**
RAM : Rbpjk-association module
ANK : Ankyrin
NLS : Nuclear localisation sequence
PEST : Proline (P)-, glutamic acid (E)-, serine (S)- and threonine (T)-rich domain
★ **p.(Asp2063Metfs*5) variant position**

Figure 1. Molecular structure of NOTCH2 receptor and variants related to Hajdu-Cheney syndrome (Adapted from Canalis *et al.*, 2019)

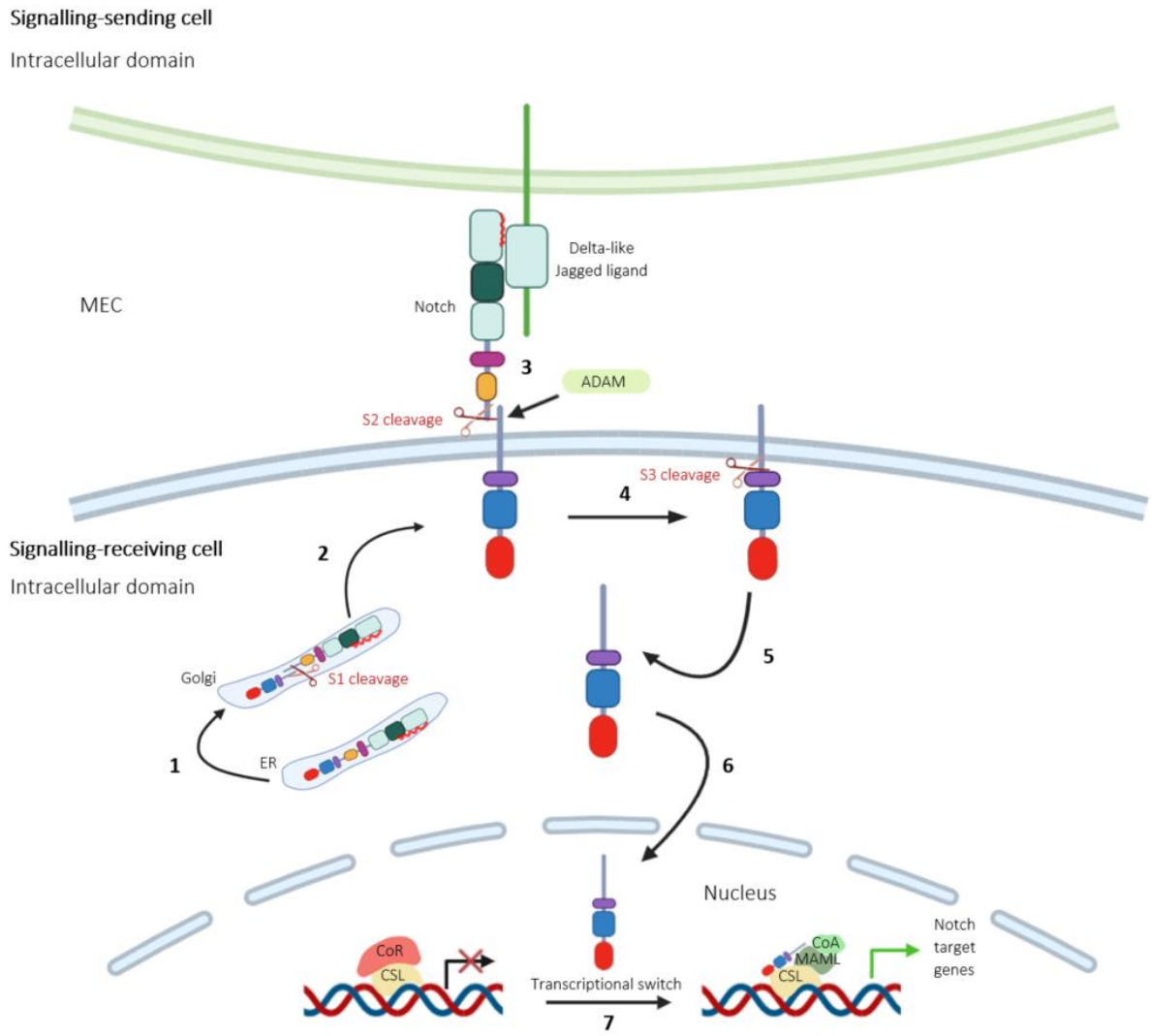
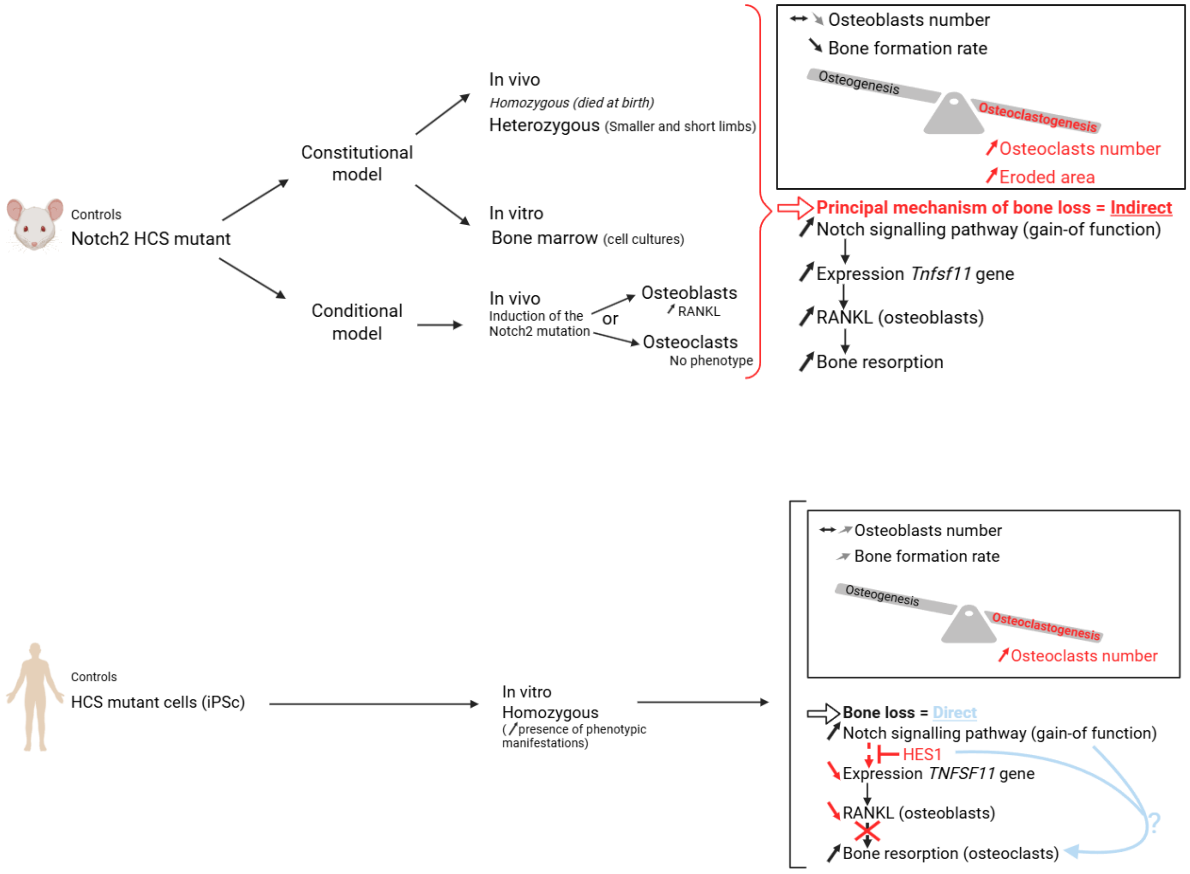


Figure 2. Simplified Notch signalling pathway (Adapted from Zhou *et al.*, 2022)

1) In signal-receiving cell, the Notch receptor is synthesised as a precursor and glycosylated (red curved) in the ER, and undergoes S1 cleavage into heterodimers in the Golgi complex; **2)** Fully functional Notch receptor is transported to the cell membrane; **3)** Notch signal transduction occurs when a ligand from the signal sending-cell (neighboring cell) binds to the Notch receptor. This binding induces conformational changes that expose the S2 cleavage site, which is then cleaved by an ADAM metalloprotease; **4)** This cleavage generates the activated form of Notch receptor or NEXT; **5)** NEXT can be cleaved by intramembrane γ-secretase into NICD (S3 cleavage); **6)** NICD can be translocated into the nucleus and transformed itself into a transcriptional activator; **7)** NICD binds to CSL and recruits MAMLs, leading to the release of corepressors, the recruitment of coactivators, and the activation of transcription of NOTCH target genes.

Abbreviations : ER, endoplasmic reticulum; S, site; ADAM, a desintegrin and metalloproteinase domain-containing protein; MEC, extracellular matrix; CoR, corepressor; CSL, CBF-1/suppressor of hairless/Lag1; MAMLs, mastermind-like proteins; CoA, coactivator.

Appendix 6. Bone loss in HCS: concepts from studies in mouse and human models.



Canalis *et al.* studied both murine and human models of Notch2-related HCS. The mouse model included a constitutional Notch2 HCS mutant and a conditional model activating the mutation specifically in osteoclasts or osteoblasts. For human model, homozygous pathogenic NOTCH2 HCS variant iPS cells (iPSc) were generated.

The Notch2 HCS mutant mouse reproduces key features of human HCS, notably osteopenia resulting from increased bone resorption and osteoclastogenesis, mainly due to elevated RANKL (*Tnfsf11*) expression in osteoblast-lineage cells. The Notch2 mutation had no skeletal impact when induced in osteoclasts (myeloid cells), but caused osteopenia when expressed in osteoblasts via increased RANKL and bone resorption. Notch2 also directly promotes osteoclastogenesis (not shown in this schematic view).

In human iPSc, HCS mutants show decreased RANKL expression. HES1 appears to mediate RANKL regulation, as its inactivation increases expression of the *TNFSF11* gene, showing that HES1 suppresses RANKL expression. Osteoclastogenesis seems also enhanced by a direct, RANKL-independent mechanism (42,44,45).