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Letter to the Editor

Identification of novel and recurrent glucokinase mutations in Belgian and Luxembourg maturity onset diabetes of the young patients

To the Editor:

Maturity onset diabetes of the young (MODY) is a monogenic form of diabetes mellitus accounting for approximately 1–2% of non-insulin dependent diabetes. It is characterized by early-onset pancreatic β-cell dysfunction and autosomal dominant inheritance. MODY is a genetic heterogeneous condition for which today seven causal genes have been identified; the hepatocyte nuclear factor 4α (*HNF-4α*) causing MODY1 (1), the glucokinase enzyme (*GCK*) responsible for MODY2 (2), the hepatocyte nuclear factor 1α (*HNF-1α* or *TCF1*) causing MODY3 (3), insulin promotor factor-1 (*IPF1*; MODY4) (4), transcription factor HNF-1β (*TCF2*; MODY5) (5), *Neuro1D* (MODY6) (6) and the carboxyl ester lipase (*CEL*) gene (7). In Europe, GCK-MODY and HNF-1α-MODY are the most prevalent forms (8–11), with their prevalence mainly depending on the way of recruitment.

We have performed molecular screening of the *GCK* gene in 161 patients belonging to 124 families, referred to our centre between 2002 and 2005 from hospitals in Belgium and Luxembourg. All probands fulfilled at least two of the following criteria: early-onset hyperglycaemia (age of onset <40 years), the absence of beta cell autoantibodies and a positive familial history for diabetes, with at least two successive generations affected.

Polymerase chain reaction amplification (primers and conditions available upon request) and sequence analysis of exon 1a and exons 2 to 10 of the *GCK* gene (Genbank XM_041001) resulted in the detection of a mutation in 33 of the 124 probands. Additional mutation analysis performed in available family members of the probands showed cosegregation of the *GCK* mutations, with the MODY phenotype examined in all the families.

19 different mutations were identified (Table 1), including eight previously described missense mutations: p.Arg36Trp (12), p.Cys129Tyr (9), p.Arg191Trp (13), p.Gly223Ser (9), p.Val226Met (14), p.Ala378Thr (15), p.Ser441Trp (9) and p.Arg447Gln (15). Three of the 11 new *GCK* mutations identified during this study are clearly inactivating, as they result in pre-mature termination of translation: c.171delG, c.663-673dupGGTCGGCATGA and c.1261delG cause pre-mature termination codons after respectively 85, 227 and 429 amino acids. Two splice site mutations (680-1 G>A and 680-6 C>A) may also result in aberrant nonfunctional *GCK* transcripts as theoretical splice site prediction (16) shows decrease in splice acceptor consensus value for both 680-1 C>A (from 0.869 to 0.662) and 680-6 C>A (from 0.869 to 0.801) mutations. Unfortunately, no RNA was available to confirm this. Six new missense mutations (p.Phe152Leu, p.Ala188Val, p.Met202Arg, p.Asn231His, p.Leu315Phe and p.Cys434Phe) were detected in diabetic probands and were found to be absent in our control population (100 chromosomes, Belgian origin). All six missense mutations were predicted pathogenic by the theoretical prediction programme SIFT (17), while the Polyphen theoretical prediction programme (18) predicts a damaging effect for four of them, but not for p.Ala188Val and p.Leu315-Phe. However, these two mutations segregate with diabetes in the families tested and are highly conserved in the glucokinases or hexokinases of all the mammalians. Moreover, mutations affecting the alanine188 residue have been reported in diabetic patients before (19, 20). The same is true for the cysteine residue at position 434 (10, 20). The p.Phe152Leu and p.Asn231His missense mutations contribute to the glucose binding site and are believed to disrupt glucose binding.

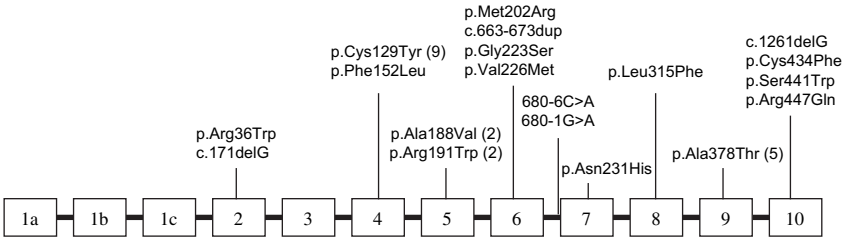

Fig. 1. Mutation distribution of glucokinase enzyme mutations found in Belgium and Luxembourg. If a mutation was found in multiple families the exact number is given in parentheses.

Four mutations were detected in more than one proband; p.Cys129Tyr in nine probands, p.Ala378Thr in five probands and p.Ala188Val and p.Arg191Trp in two probands each. Together, p.Cys129Tyr and p.Ala378Thr represented 42.4% of the *GCK* mutations identified in our GCK-MODY population. All the probands harbouring the p.Ala378Thr mutation originated from the Belgian province of West-Flanders, suggesting a founder mutation with a common ancestor from this region. Additional genealogical research and haplotype analysis, with polymorphic markers flanking the *GCK* gene (D7S2541; 40.5 Mb, D7S691; 41.8 Mb and D7S2506; 47.5 Mb) could confirm this for 3 of the 5 families showing this mutation (families 8, 16 and 23). In five families with p.Cys129Tyr, a possible common haplotype could be found for distal-flanking markers D7S2541 and D7S691, but due to the low number of available affected persons in all the families with this mutation, the overall results were inconclusive. Haplotype analysis showed a common specific haplotype for markers D7S2541, D7S691 and D7S2506 segregating with the p.Ala188Val mutation in the two families with this mutation. Previously, a founder effect for another GCK missense mutation (p.Gly299Arg) has been demonstrated in the Oxford region (UK), but such regional clustering is rather the exception as in most populations a variety of mutations scattered all over the *GCK* gene is identified (9–11, 14, 15, 20).

MODY is defined as an autosomal dominant form of diabetes mellitus, with an early onset, usually before 25 years. However, there are several MODY subtypes with different clinical characteristics. The GCK-MODY phenotype is normally characterized by mild nonprogressive hyperglycaemia present at very young age (21), and GCK-MODY patients rarely develop diabetes-associated complications (14, 21, 22). Also, in our GCK-MODY probands, there was mostly mild, nonprogressive hyperglycaemia (mean of fasting glucose 7.0 $\pm$ 1.2 mmol/l; $n = 30$) and 88% of the probands showed elevated haemoglobin A1c (Table 1). However, we could not detect a clear correlation between nature of the mutation and therapy (insulin/oral hypoglycaemic agents/diet), with different therapies effective for different patients with the same mutation. This may be explained by different attitudes of the treating physicians towards therapy, but may also be caused by additional factors influencing disease expression. The latter is illustrated by proband MODY32 who showed glucose levels up to 50 mmol/l, especially in times of infection, and required insulin therapy combined with diet. Analysis of all coding *GCK* exons showed only a heterozygous p.Arg191Trp mutation and no homozygosity or compound heterozygosity, which would have been compatible with permanent neonatal diabetes mellitus (PNDM). Possibly, this patient has both GCK-MODY and an additional type of diabetes.

Because of the usually mild phenotype GCK-MODY patients are often only detected during routine screenings such those during pregnancy for women. It is therefore not surprising that a significant number of female patients with a *GCK* mutation are classified as having gestational diabetes mellitus (13). Consequently, the age of diagnosis in GCK-MODY patients does not often reflect the real age of onset. Therefore, we preferred also to include in our GCK-MODY screening patients with older age of diagnosis, if they fulfilled the other criteria. In our *GCK* positive probands, the mean age of diagnosis was 18, with 29 of them diagnosed before the age of 40. Four patients were diagnosed between the age of 40 and 60. This indeed illustrates that the cut-off age of diagnosis should not be set too stringent for *GCK* screening.

GCK mutations account for the majority of mutations identified in various European MODY populations (9–11), while in other countries such as the UK, GCK-MODY is reported as the second most prevalent form of MODY after HNF-1α-MODY (8). This difference is mainly explained by the way of recruitment. Indeed, recruitment in adult clinics

Table 1. Clinical and molecular characteristics of MODY2 probands

Family	Ethnic origin	Mutation		Age of diagnosis (years)	Fasting glucose (mmol/l)	HbA1c (%)	normal range	BMI (kg/m^2)	SDS	Actual treatment
		Nucleotide level	Protein level							
MODY2_19	Luxembourg	c.106C>T	p.Arg36Trp	11	6.4	6.6	4–6	23.2	+1.8	Diet
MODY2_04	Belgium	c.171delG	frameshift	5	6.6	6.7	4–6	14.7	−0.8	Diet+OHA
MODY2_03	Belgium	c.386G>A	p.Cys129Tyr	7	9.9	6.4	4–6	14.2	−1.1	Ins
MODY2_13	Belgium	c.386G>A	p.Cys129Tyr	34	7.6	5.5	4–6	25.4	+0.8	OHA
MODY2_14	Belgium	c.386G>A	p.Cys129Tyr	2	na	7	4–6	19.4	+1.0	Ins
MODY2_17	Belgium	c.386G>A	p.Cys129Tyr	28	6.7	6.4	4.5–6.5	23.7	+1.8	Ins
MODY2_18	Belgium	c.386G>A	p.Cys129Tyr	17	6.6	6.5	4–6	21	−0.2	diet
MODY2_21	Belgium	c.386G>A	p.Cys129Tyr	60	7.1	6.9	4–6	23.9	+0.6	diet
MODY2_26	Belgium	c.386G>A	p.Cys129Tyr	11	7.4	6.5	3–6	18.7	+0.6	na
MODY2_27	Belgium	c.386G>A	p.Cys129Tyr	21	7.7	6.8	4–5.5	18.9	−2.0	diet
MODY2_28	Belgium	c.386G>A	p.Cys129Tyr	29	7.7	6.8	4–6	23	+0.2	Ins/OHA
MODY2_11	Philippines	c.454T>C	p.Phe152Leu	13	6.6	6.7	4–6	16	−1.1	None
MODY2_05	Belgium	c.563C>T	p.Ala188Val	5	6.1	6.1	4–6	16.4	+0.64	diet
MODY2_06	Belgium	c.563C>T	p.Ala188Val	6	5.3	6.9	4–6	15.7	+0.14	diet
MODY2_01	Belgium	c.571C>T	p.Arg191Trp	12	6.3	6.5	4.8–6	17.7	−0.4	diet
MODY2_32	Belgium	c.571C>T	p.Arg191Trp	Neonatal	5.3[a]	6.4[b]	4–5.5[c]	15[c]	−2.3	Ins+diet
MODY2_30	Belgium	c.605T>G	p.Met202Arg	39	6.9	5.8	4–5.5	24	+0.3	diet
MODY2_22	Belgium	c.663-673dup	Frameshift	5	8.6	6.3	4–6	17.5	−1.8	Ins
MODY2_29	Belgium	c.667G>A	p.Gly223Ser	10	5.8	6.1	4–6	17	0.0	Diet
MODY2_20	Portugal	c.676G>A	p.Val226Met	5	7.4	6.1	4–6	16.4	+0.7	Diet
MODY2_12	Belgium	680-6C>A	Splice site	42	7.7	5.2	4–6	23.9	+0.3	Diet+OHA
MODY2_10	Belgium	680-1G>A	Splice site	24	7.2	6.8	4–6	21	−0.9	Diet
MODY2_07	Belgium	c.691A>C	p.Asn231His	6	na	na	na	na	na	na
MODY2_09	Turkey	c.943C>T	p.Leu315Phe	9	6.5	7.9	4.3–5.7	14.2	−1.3	Diet
MODY2_08	Belgium	c.1132G>A	p.Ala378Thr	7	6.1	6.3	4–6	14.6	−0.85	OHA
MODY2_15	Belgium	c.1132G>A	p.Ala378Thr	15	7.3	6.4	4–6	21.8	+0.9	Diet
MODY2_16	Belgium	c.1132G>A	p.Ala378Thr	22	6.1	6.5	4–6	22.9	+0.3	Ins
MODY2_23	Belgium	c.1132G>A	p.Ala378Thr	52	7.2	6.4	4–6	22.1	−0.4	Diet
MODY2_25	Belgium	c.1132G>A	p.Ala378Thr	3	11.4	6.2	4–5.5	13	−2.5	Diet
MODY2_02	Belgium	c.1261delG	Frameshift	15	6.4	5.9	< 5.5	18.3	−1.6	Diet
MODY2_33	Belgium	c.1301G>T	p.Cys434Phe	46	6.4	6.3	4–6	25	+0.9	Diet+OHA
MODY2_24	Belgium	c.1322C>G	p.Ser441Trp	12	na	6.9	4–6	na	na	na
MODY2_31	Belgium	c.1340G>A	p.Arg447Gln	7	6.7	6.3	4–6	na	na	na

Ins, insulin; OHA, oral hypoglycaemic agents; na, not available; HbA1c, haemoglobin A1c; BMI, body mass index; SDS, standard deviation score; MODY, maturity onset diabetes of the young.
[a]At age 11.
[b]At age 14.

seems to correlate with higher frequency of HNF-1α-MODY positive patients, while GCK-MODY seems to be the most frequent form in paediatric MODY populations (17, 23–25). This can partially be explained by the observation that GCK-MODY patients often do not continue their medical follow up in adulthood, because often their hyperglycaemia is well under control by diet alone. Our study recruited both in paediatric and adult clinics, and in 26.6% of the 124 probands, a *GCK* mutation was detected. This frequency is comparable with frequencies reported in Germany (22.5%) (11) and Czech republic (31%) (10), but lower than the 41% frequency observed in paediatric populations in Spain (23) and Italy (9). This difference may partially be explained by the less stringent inclusion criteria with regard to age of diagnosis we used.

In conclusion, we show that *GCK* mutations are a frequent cause of MODY presentation in Belgium and Luxembourg, with the p.Cys129Tyr and p.Ala378Thr being most prevalent. Further studies are now needed to determine the prevalence of other MODY types in our population.

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