

Maturity-Onset Diabetes of the Young in Children With Incidental Hyperglycemia:

A multicenter Italian study of 172 families

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cause MODY (3), with mutations of the glucokinase (*GCK*) and hepatocyte nuclear factor 1 α (*HNF1A*) genes accounting for up to 85% of MODY in Europe. Defects of other MODY genes are quite rare (3). The aim of this study was to screen for *GCK* and *HNF1A* genes in 172 Italian children with incidental hyperglycemia and clinical diagnosis of MODY.

OBJECTIVE — To investigate the prevalence of maturity-onset diabetes of the young (MODY) in Italian children with incidental hyperglycemia.

RESEARCH DESIGN AND METHODS — Among 748 subjects age 1–18 years with incidental hyperglycemia, minimal diagnostic criteria for MODY were met by 172 families. Mutational analyses of the glucokinase (*GCK*) and hepatocyte nuclear factor 1 α (*HNF1A*) genes were performed.

RESULTS — We identified 85 *GCK* gene mutations in 109 probands and 10 *HNF1A* mutations in 12 probands. In *GCK* patients, the median neonatal weight and age at the first evaluation were lower than those found in patients with *HNF1A* mutations. Median fasting plasma glucose and impaired fasting glucose/impaired glucose tolerance frequency after oral glucose tolerance testing were higher in *GCK* patients, who also showed a lower frequency of diabetes than *HNF1A* patients.

CONCLUSIONS — *GCK* mutations are the prevailing cause of MODY (63.4%) when the index case is recruited in Italian children with incidental hyperglycemia.

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Between 1992–1999, the Italian Society of Pediatric Endocrinology and Diabetology (ISPED) Study Group on childhood pre-diabetes recruited 748 individuals with incidental hyperglycemia to be screened for markers of type 1 diabetes (1,2). Among autoantibody-

negative subjects, a significant number (~23%) met the criteria for clinical diagnosis of maturity-onset diabetes of the young (MODY), i.e., two or three consecutive generations with hyperglycemia diagnosed before age 25 years (3,4). Alterations in at least six different genes

RESEARCH DESIGN AND METHODS

Islet cell antibodies, insulin autoantibodies, IA-2 antigens, and GAD antibodies were assayed in 748 subjects (480 males, age 1–18 years) referred to the 35 participating centers for incidentally discovered hyperglycemia. Each center provided a report on those subjects with incidental hyperglycemia who satisfied the diagnostic criteria of MODY. Informed consent for genetic analysis was obtained from all families following approval from local ethical committees. The percentile of birth weight after correction for gestational age, sex, and BMI was calculated using standard charts (5,6). Mutation carriers were classified according their fasting plasma glucose (FPG) following the latest recommendations of the American Diabetes Association. When available, oral glucose tolerance test (OGTT) data were analyzed.

Mutation screening

Amplification of *GCK* and *HNF1A* genes was accomplished by PCR, and various rapid screening methods of PCR products were used (e.g., single-strand conformational polymorphism or denaturing gradient gel electrophoresis). This was followed by direct DNA sequencing of samples different from reference PCR.

Statistical analysis

Proportions were compared between MODY groups using the Fisher's exact test, and means and medians were compared using the Student's *t* test and the Mann-Whitney *U* test, respectively, or the Kruskal-Wallis test. Logistic models were fitted to compute the probability of diagnosis of *HNF1A* and *GCK*, and their 95% CIs, according to FPG and OGTT. Stata

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Table 1—Clinical and metabolic analyses in carriers of GCK, HNF1A, and MODY of unknown type (UT) mutations

	GCK	HNF1A	UT	P
Birth weight (g)	3,050 (2,790–3,370)	3,570 (2,975–4,205)	3,075 (2,950–3,520)	0.100
Age at 1st visit (years)	7.6 ± 3.6*	13.2 ± 6.2†	10.3 ± 3.4‡	<0.001
BMI at 1st visit	17 (14–24)	21 (13–25)	17 (16–20)	0.929
FPG (mmol/l)	6.3 (5.8–6.7)	6.1 (5.5–6.6)	6.0 (5.6–6.4)	0.099
OGTT + 120' (mmol/l)	8.37 ± 1.77‡	10.23 ± 4.5§	9.45 ± 2.52‡	0.102
FPG and OGTT outcome				<0.001
NFG and NGT	3 (3)	4 (31)	1 (4)	
IFG or IGT	100 (83)	2 (15)	17 (71)	
DM/DM	17 (14)	7 (54)	6 (25)	
FPIR (pmol/l)	438 (306–624)	300 (240–432)	570 (294–876)	0.084
FPIR percentiles				0.073
<25th	70 (71)	7 (100)	12 (52)	
25th–75th	25 (26)	0	8 (35)	
>75th	3 (3)	0	3 (13)	

Data are median (25th–75th percentile), means ± SD, and n (%). *Vs. HNF1A, UT; †vs. GCK; ‡vs. HNF1A; §vs. GCK, UT. For post hoc comparisons: $P < 0.017$ (after Bonferroni correction) (GCK) vs. GCK; (HNF1A) vs. HNF1A and (X) vs. unknown. DM, diabetes mellitus; FPIR, first-phase insulin response; NFG, normal fasting glucose; NGT, normal glucose tolerance.

10 (StataCorp, College Station, TX) was used for computation.

RESULTS

GCK gene screening

A total of 213 subjects from 172 families met MODY diagnostic criteria, and 85 different GCK mutations were identified in 109 probands (109 of 172 = 63.4%); our group has already reported about 75 of these probands (4,7). In the remaining 34 families, we identified 14 novel and 20 previously described (8,9) mutations. Eleven of the novel mutations were missense mutations and three were point mutations, which, although they do not predict amino acid changes, still could have a pathogenic potential (CONCLUSIONS and online appendix Table A1, available at <http://care.diabetesjournals.org/cgi/content/full/dc08-2018/DC1>). Each mutation was confirmed in the affected parent and available family members with the exception of three subjects in which the mutation arose de novo. All mutations were not found in 200 normal chromosomes. Of note, only 25% of family trees of GCK probands met the stringent criteria for MODY (i.e., three known consecutive generations with diabetes or related conditions).

HNF1A gene screening

We detected 10 different HNF1A mutations (one novel: p.Arg363Cys) in 12 unrelated patients (12 of 172 = 6.9%) (10). In a single patient mutation, p.Pro291fs (c.872duplC) arose de novo. Ninety percent of HNF1A-MODY families showed

three consecutive generations with diabetes or related conditions.

MODY with unknown genetic origin

Of 172 probands, 51 (29.6%) were negative for GCK and HNF1A genes. The genetic defect was therefore unknown in these MODY probands.

Clinical and metabolic parameters

Age at first evaluation and birth weight were lower in GCK patients than in HNF1A patients. GCK patients had a lower frequency of normal FPG and a higher frequency of impaired fasting glucose than HNF1A patients. At OGTT, GCK patients showed a higher frequency of impaired glucose tolerance and a lower frequency of diabetes than HNF1A patients (Table 1).

CONCLUSIONS— We confirmed that in the largest Italian case series of pediatric patients clinically defined as MODY, mutations of GCK are very frequent (63.4%), while HNF1A are relatively rare (6.9%). It is possible, however, that we have slightly underestimated the latter because the methodologies utilized in our investigation cannot detect large deletions. Thus, approximately one-third of our families may carry either a mutation in any of the rare MODY genes (3) or, more likely, in a locus yet to be found. We considered pathogenetically two variations of GCK gene at the end of exons 1a (c.45G→A) and 4 (c.483G→A) that changed guanine to adenine in the third base of the codon (AAG→AAA) (online appendix Table A1). Because both AAG

and AAA encode the amino acid lysine, this variation is usually regarded as “silent.” However, both mutations change the exonic consensus guanine at the 5' exon/intron boundary, a location that in other genes has been demonstrated to determine exon skipping or other defects (11). We also considered pathogenetically an intronic change outside the splice-site consensus sequence (c.1019 + 5G→A) but substituting a highly conserved guanine in the 5' consensus splice site (12). All three mutations were found along three consecutive generations of affected family members and were not detected in 200 normal chromosomes. Though we did not provide in vitro evidence that these mutations have deleterious consequences, it is likely that they cause GK haplo-insufficiency (11,12).

In this study, a high prevalence of GCK mutations has been found, similar to previous investigations conducted in the pediatric setting (3,13). In contrast, HNF1A mutations were rarely detected, probably because of the reduced penetrance of mutations of HNF1A in subjects under 18 years of age (14). However, true differences in the prevalence of MODY genes between populations cannot be excluded at this time, as suggested by the low prevalence of HNF1A mutations (16%) in Italian families with MODY recruited in the adult diabetes clinic (15) (online appendix Table A2). In conclusion, our study indicates that autoantibody-negative children with (stable) incidental hyperglycemia and a parent with the same condition are good candi-

dates for molecular screening of *GCK* gene.

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