Maturity-Onset Diabetes of the Young (MODY) in Children with Incidental Hyperglycemia. A Multicenter Italian Study on 172 families

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**Objective:** To investigate MODY prevalence in Italian children with incidental hyperglycemia.

**Research design and methods:** Among 748 subjects with incidental hyperglycemia aged 1-18 years minimal diagnostic criteria for MODY were met by 172 families. Mutational analysis of the glucokinase (GCK) and hepatocyte nuclear factor 1-α (HNF1A) genes was 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 FPG and IFG/IGT frequency after OGTT were higher in GCK patients, who also showed a lower frequency of diabetes mellitus 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.
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., 2 or 3 consecutive generations with hyperglycemia diagnosed before age of 25 years (3,4). Alterations in at least 6 different genes cause MODY (3), with mutations of the GCK and HNF1A genes accounting for up to 85% of MODY in Europe. Defects of other MODY genes are quite rare (3). Aim of the study was the screening of GCK and HNF1A genes in 172 Italian children with incidental hyperglycemia and clinical diagnosis of MODY.

RESEARCH DESIGN AND METHODS

ICA, IAA, IA-2A and GADA were assayed in 748 subjects (480 males, aged 1-18 years) referred to the 35 participating Centers because of incidentally discovered hyperglycemia. Each center provided a report of those subjects with incidental hyperglycemia who satisfied 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, gender and BMI were calculated using standard charts (5,6). Mutation carriers were classified according their fasting plasma glucose (FPG) following the latest recommendations of ADA. When available, OGTT data were analyzed.

Mutation screening: Amplification of GCK and HNF1A genes was accomplished by the polymerase chain reaction (PCR) and various rapid screening methods of PCR products were utilized (e.g., Single Strand Conformational Polymorphism or Denaturing Gradient Gel Electrophoresis) followed by direct DNA sequencing of samples different from reference PCR.

Statistical analysis: Proportions were compared between MODY groups using the Fisher exact test, means and medians using the Student t test and the Mann-Whitney U test, respectively, or the Kruskall Wallis test. Logistic models were fitted to compute the probability of diagnosis of HNF1A (and of GCK), and their 95% confidence interval (95%CI), according to FPG and to response to OGTT. Stata 10 (StataCorp, College Station, TX) was used for computation.

RESULTS

GCK gene screening: 213 subjects from 172 families met MODY diagnostic criteria. A total of 85 different GCK mutations were identified in 109 probands (109/172 = 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 (see Conclusion). (see Table A1 in the online appendix at http://care.diabetesjournals.org). Each mutation was confirmed in the affected parent and available family members with the exception of 3 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., 3 known consecutive generations with diabetes or related conditions.
HNF1A gene screening: We detected 10 different HNF1A mutations (1 novel: p.Arg363Cys), in 12 unrelated patients (12/172= 6.9%) (10). In a single patient mutation p.Pro291fs (c.872duplC) has arose de novo. Ninety percent of HNF1A-MODY families showed 3 consecutive generations with diabetes or related conditions.

MODY with unknown genetic origin: Fifty-one probands out of 172 (or 29.6%) resulted negative to GCK or HNF1A gene screening (MODY “unknown”).

Clinical and metabolic parameters: Age at first evaluation and birth weight were lower in GCK patients than in HNF1A. GCK patients had a lower frequency of normal FPG and higher frequency of IFG than HNF1A. At OGTT, GCK patients showed a higher frequency of IGT and a lower frequency of DM than HNF1A (Table 1).

CONCLUSION
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 can not 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 yet-to be-found locus. We considered pathogenetic two variations of GCK gene at end of exons 1a (c.45G>A) and 4 (c.483G>A) that change a 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 pathogenetic 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 3 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 GCK haplo-insufficiency (11, 12).

In this study, a high prevalence of GCK mutations has been found, similarly 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 below 18 years of age (14). However, true differences in the prevalence of MODY genes between populations can not be excluded at this time, as suggested by 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 candidates for molecular screening of GCK gene.

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REFERENCES

1. Lorini R, Alibrandi A, Vitali L, Klersy C, Martinetti M, Betterle C, d’Annunzio G, Bonifacio E and the Pediatric Italian Study Group of Prediabetes: Risk of type 1 diabetes development in children with incidental hyperglycemia. *Diabetes Care* 24:1210-1216, 2001

2. Lorini R, Vanelli M: Normal values of first-phase insulin response to intravenous glucose in healthy Italian children and adolescents. *Diabetologia* 39:370-371,

3. Vaxillaire M, Froguel Ph. Monogenic diabetes in the young, pharmacogenetics and relevance to multifactorial forms of type 2 diabetes. *Endocr Rev* 29:254-264, 2008

4. Massa O, Meschi F, Cuesta-Muñoz A, Caumo A, Cerutti F, Toni S, Cherubini V, Guazzarotti L, Sulli N, Matschinsky FM, Lorini R, Iafusco D, Barbetti F and the Diabetes Study Group of the Italian Society of Paediatric Endocrinology and Diabetes (SIEDP): High prevalence of glucokinase mutations in Italian children with MODY. Influence on glucose tolerance, first-phase insulin response, insulin sensitivity and BMI. *Diabetologia* 44:898-905, 2001

5. Gairdner D, Pearson J: A growth chart for premature and other infants. *Arch Dis Child* 46, 250:783-787, 1971

6. Rolland-Cachera MF, Cole TJ, Sempé M, Tichet J, Rossignol C, Charraud A. Body Mass Index variations: percentiles from birth to 87 years. *Eur J Clin Nutr* 45:13-21, 1991

7. Guazzini B, Gaffi D, Mainieri D, Multari G, Cordera R, Bertolini S, Pozza G, Meschi F, Barbetti F: Three novel missense mutations in the glucokinase gene (G80S; E221K; G227C) in Italian subjects with maturity-onset diabetes of the young (MODY). *Hum Mutat* 12:136, 1998

8. Gloyn AL: Glucokinase (GCK) mutations in hyper- and hypoglycaemia: maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemia of infancy. *Hum Mutat* 22:353-362, 2003

9. Johansen A, Ek J, Mortenes HB, Pedersen O, Hansen T: Half of clinically defined Maturity-Onset Diabetes of the Young patients in Denmark do not have mutations in HNF-4α, GCK and TCF1. *J Clin Endocr Metab* 90:4607-4614, 2005

10. Ellard S, Colclough K: Mutations in the genes encoding the transcription factors hepatocyte nuclear factor 1 alpha (*HNF1A*) and 4 alpha (*HNF4A*) in maturity-onset diabetes of the young. *Hum Mutat* 27:854-869, 2006

11. Cartegni L, Chew SL, Krainer AR: Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat Rev Genet* 3:285-298, 2002

12. Krawzac M, Reiss J, Cooper DN: The mutational spectrum of single base-pair substitutions in mRNA splice junction of human genes: causes and consequences. *Hum Genet* 90:41-54, 1992

13. Velho G, Blanché H, Vaxillaire M, Bellanné-Chantelot C, Pardini VC, Timsit J, Passa Ph, Deschamps I, Robert J-J, Weber IT, Marotta D, Pilkis SJ, Lipkind GM, Bell GI, Froguel P: Identification of 14 new glucokinase mutations and description of the clinical profile of 42 MODY-2 families. *Diabetologia* 40, 217-224, 1997
14. Bellanné-Chantelot C, Carette C, Riveline JP, Valéro R, Gautier JF, Larger E, Reznik Y, Ducluzeau PH, Sola A, Hartemann-Heurtier A, Lecomte P, Chaillous L, Laloï-Michelin M, Wilhem JM, Cuny P, Duron F, Guerri B, Jeandidier N, Mosnier-Pudar H, Assayag M, Dubois-Laforgue D, Velho G, Timsit J. The type and the position of HNF1A mutation modulate age at diagnosis of diabetes in patients with maturity-onset diabetes of the young (MODY)-3. Diabetes 57:503-508, 2008

15. Gragnoli C, Cockburn BN, Chiaromonte F, Gorini A, Marietti G, Marozzi G, Signorini AM: Early-onset type II diabetes mellitus in Italian families due to mutations in the genes encoding hepatic nuclear factor 1α and glucokinase. Diabetologia 44:1326-1329, 2001

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

|                      | GCK              | HNF1A            | Unknown type | P value |
|----------------------|------------------|------------------|--------------|---------|
| Birth weight (g)*    | 3050 (2790-3370) | 3570 (2975-4205) | 3075 (2950-3520) | 0.100   |
| Age at 1st visit (yrs) | 7.6±3.6*(vs HNF1A, UT) | 13.2±6.2*(vs GCK) | 10.3±3.4*(vs GCK) | <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 & OGTT outcome°  | (vs HNF1A)       | (vs GCK, UT)     | (vs HNF1A)   | <0.001  |
| NFG + 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 mean ± SD unless specified. *median (25th-75th percentiles); ° N (%).

For post-hoc comparisons p<0.017 (after Bonferroni correction) (GCK) vs GCK; (HNF1A) vs HNF1A and (X) vs unknown.