**"Next-generation sequencing identifies monogenic diabetes in 16% of patients with late adolescence or adult-onset diabetes selected on a clinical basis: a cross-sectional analysis ", by Xavier Donath *et al*.**

**Additional file 2.**

* **Tables S1, S2, S3, S4, S5, S6, S7**
* **Figures S1, S2, S3**

**Table S1.** Cases with class 3/4/5 variants identified in two genes

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **case #** | **Gene** | **Location** | **Nucleotide change** | **Protein effect** | **Variant type** | **Class of pathogenicity** | **References** | **sex** | **Euro-Caucasian** | **n of generations with diabetes** | **age (years)** | **BMI (kg/m²)** | **symptoms** | **HbA1c (%)** | **insulin therapy** |
| 1 | *HNF4A* | Exon 8 | c.1063G>C | p.Gly355Arg | Splice defect | 5 | This report | F | yes | 3 | 17 | 32.6 | yes | na | yes |
| *ABCC8* | Exon 10 | c.1561C>T | p.Arg521Trp | Misense | 3 | This report |
| 2 | *GCK* | Exon 10 | c.1333A>T | p.Ser445Cys | Missense | 4 | [1] | M | yes | 4 | 20 | 23.8 | no | 7.5 | no |
| *KCNJ11* | Exon 1 | c.1040G>A | p.Arg347His | Missense | 3 | [2] |
| 3 | *GCK* | Exon 2 | c.128G>A | p.Arg43His | Missense | 5 | [3] | F | na | 1 | 38 | 20.8 | no | 6.1 | na |
| *HNF1A* | Exon 4 | c.862G>A | p.Gly288Arg | Missense | 3 | This report |
| 4 | *HNF1A* | Exon 4 | c.872dupC | p.Gly292fs | Frameshift | 5 | [4] | M | no | 4 | 37 | 21.1 | no | 6.2 | no |
| *HNF1B* | Exon 3 | c.793G>A | p.Val265Met | Missense | 3 | This report |
| 5 | *GCK* | Exon 5 | c.571C>T | p.Arg191Trp | Missense | 5 | [1] | F | na | 2 | 20 | na | no | na | no |
| *ABCC8* | Exon 33 | c.4058G>C | p.Arg1353Pro | Missense | 4 | [5] |

Sequence variants are numbered with respect to GenBank cDNA sequences. *ABCC8*, NM\_000352.3; *GCK*, NM\_000162.3; *HNF1A*, NM\_000545.6; *HNF1B*, NM\_000458.3; *HNF4A*, NM\_175914.4; *KCNJ11*, NM\_000525.3 and described according to Human Genome Variation Society (HGVS) guidelines (<http://www.hgvs.org/varnomen>). Variants were classified according to ACMG recommendations [see Table S3 for details]. LOF, loss-of-function variants

1. Osbak KK, Colclough K, Saint-Martin C, Beer NL, Bellanné-Chantelot C, Ellard S, Gloyn AL.: **Update on mutations in glucokinase (GCK), which cause maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemic hypoglycemia.** *Hum Mut* 2012, **30**(11): 1512-26.

2. Kapoor RR, Flanagan SE, Arya VB, Shield JP, Ellard S, Hussain K.: **Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism.** *Eur J Endocrinol* 2013, **168**(4): 557-64.

3. Beer NL, Osbak KK, van de Bunt M, Tribble ND, Steele AM, Wensley KJ, Edghill EL, Colcough K, Barrett A, Valentínová L, *et al*: **Insights into the pathogenicity of rare missense GCK variants from the identification and functional characterization of compound heterozygous and double mutations inherited in cis.** *Diabetes Care* 2012, **35**(7): 1482-4.

4. Colclough K, Bellanne-Chantelot C, Saint-Martin C, Flanagan SE, Ellard S.: **Mutations in the genes encoding the transcription factors hepatocyte nuclear factor 1 alpha and 4 alpha in maturity-onset diabetes of the young and hyperinsulinemic hypoglycemia.** *Hum Mut* 2013, **34**(5): 669-85.

5. Magge SN, Shyng SL, MacMullen C, Steinkrauss L, Ganguly A, Katz LE, Stanley CA.: **Familial leucine-sensitive hypoglycemia of infancy due to a dominant mutation of the beta-cell sulfonylurea receptor.** *J Clin Endocrinol Metab* 2004, **89**(9): 4450-6.

**Table S2.** Loss-of-function variants identified in *ABCC8* and *KCNJ11*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Location** | **Nucleotide Change** | **Protein effect** | **Variant type** | **Class of pathogenicity** | **References** |
| *ABCC8* | Exon 2 | c.220C>T | p.Arg74Trp | Missense | 4 | (1) |
| *ABCC8*  *ABCC8* | Exon 6  Exon 10 | c.892C>T  c.1484G>A | p. Arg298Cys  p.Arg495Gln | Misense  Missense | 3  4 | (2)  (3) |
| *ABCC8*  *ABCC8* | Exon 12  Exon 18 | c.1741T>A  c.2294+1G>A | p.Ser581Thr  p.? | Missense  Splice defect | 3  5 | (4)  This report |
| *ABCC8* | Exon 35 | c.4262G>A | p.Arg1421His | Missense | 4 | (5) |
| *KCNJ11*  *KCNJ11* | 5’UTR  Exon 1 | c.-54C>T  c.148C>T | p.0?  p.Arg50Trp | Promoter variant  Missense | 3  4 | (6)  This report\* |
| *KCNJ11* | Exon 1 | c.617G>A | p.Arg206His | Missense | 4 | This report\* |
| *KCNJ11* | Exon 1 | c.934G>T | p.Gly312Cys | Missense | 4 | (7) |

\*variant previously identified in cases diagnosed with recessive form of HHI in the diagnostic’s database of Pitié-Salpêtrière hospital

Sequence variants are numbered with respect to GenBank cDNA sequences. *ABCC8*, NM\_000352.3; *KCNJ11*, NM\_000525.3 and described according to Human Genome Variation Society (HGVS) guidelines (<http://www.hgvs.org/varnomen>). Variants were classified according to ACMG recommendations [see Table S3 for details].

1. Suchi M, MacMullen C, Thornton P, Adzick N, Ganguly A, Ruchelli E, Stanley C.: **Molecular and immunohistochemical analyses of the focal form of congenital hyperinsulinism.** *Mod Pathol* 2006, **19**(1): 122-9.

2. Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, Ganapathy K, Bhatti T, Stanley CA, Ganguly A.: **Genotype and phenotype correlations in 417 children with congenital hyperinsulinism.** *J Clin Endocrinol Metab* 2013, **98**(2): E355-363.

3. Yan F, Lin Y, MacMullen C, Ganguly A, Stanley C, Shyng S: **Congenital hyperinsulinism associated ABCC8 mutations that cause defective trafficking of ATP-sensitive K+ channels: identification and rescue.** *Diabetes* 2007, **56**(9): 2239-48.

4. Salisbury RJ, Han B, Jennings RE, Berry AA, Stevens A, Mohamed Z, Sugden SA, De Krijger R, Cross SE, Johnson PP, *et al*.: **Altered Phenotype of beta-Cells and Other Pancreatic Cell Lineages in Patients With Diffuse Congenital Hyperinsulinism in Infancy Caused by Mutations in the ATP-Sensitive K-Channel.** *Diabetes* 2015, **64**(9): 3182-3188.

5. Saito-Hakoda A, Yorifuji T, Kanno J, Kure S, Fujiwara I.: **Nateglinide is Effective for Diabetes Mellitus with Reactive Hypoglycemia in a Child with a Compound Heterozygous ABCC8 Mutation.** *Clin Pediatr Endocrinol* 2012, **21**(3): 45-52.

6. Huopio H, Jaaskelainen J, Komulainen J, Miettinen R, Karkkainen P, Laakso M, Tapanainen P, Voutilainen R, Otonkoski T: **Acute insulin response tests for the differential diagnosis of congenital hyperinsulinism.** *J Clin Endocrinol Metab* 2002, **87**(10): 4502-4507.

7. Arya VB, Guemes M, Nessa A, Alam S, Shah P, Gilbert C, Senniappan S, Flanagan SE, Ellard S, Hussain K.: **Clinical and histological heterogeneity of congenital hyperinsulinism due to paternally inherited heterozygous ABCC8/KCNJ11 mutations .** *Eur J Endocrinol* 2014, **171**(6): 685-95.

**Table S3.** List of novel pathogenic (Class 5) or likely pathogenic (Class 4) variants

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Criteria for classifying variants according to ACMG guidelines (1) | | | | | | |
| Gene | Location | Nucleotide Change | Protein effect | Variant type | Variant consequence | Functional data | Population data | Segregation data | Computational and predictive evidence | Class of pathogenicity | |
| *ABCC8* | Exon 22 | c.2588A>G | p.His863Arg | Missense |  |  | PM2 | PP1 | PM5 PP3 | 4 | |
| *ABCC8* | Exon 37 | c.4514T>C | p.Met1505Thr | Missense |  | PS3 | PM2 |  | PP3 | 4 | |
| *GCK* | Intron 1 | c.46-1G>T | p.? | Splice defect | PVS1 |  | PM2 |  |  | 5 | |
| *GCK* | Exon 2 | c.208G>C | p.Glu70Gln | Splice defect |  | PS3 | PM2 | PP1 | PM5 PP3 PP2 | 5 | |
| *GCK* | Exon 2-3 | c.46-?\_363+?del | p.? | In-frame exonic deletion | PM4 |  | PM2 |  |  | 4 | |
| *GCK* | Exon 3 | c.236T>C | p.Leu79Pro | Missense |  |  | PM2 | PS2 | PP3 PP2 | 4 | |
| *GCK* | Exon 3 | c.247A>G | p.Asn83Asp | Missense |  |  | PM2 |  | PP3 PP2 | 4 | |
| *GCK* | Exon 3 | c.268A>T | p.Lys90Ter | Nonsense | PVS1 |  | PM2 |  |  | 5 | |
| *GCK* | Exon 4 | c.388A>G | p.Ile130Val | Missense |  |  | PM2 |  | PM5 PP3 PP2 | 4 | |
| *GCK* | Exon 5 | c.485G>C | p.Gly162Ala | Missense |  |  | PM2 |  | PM5 PP3 PP2 | 4 | |
| *GCK* | Exon 5 | c.505A>G | p.Lys169Glu | Missense |  |  | PM2 |  | PM5 PP3 PP2 | 4 | |
| *GCK* | Exon 5 | c.508G>T | p.Gly170Cys | Missense |  |  | PM2 |  | PM5 PP3 PP2 | 4 | |
| *GCK* | Exon 5 | c.513C>G | p.Phe171Leu | Missense | PS1 |  | PM2 |  | PM5 PP3 PP2 | 5 | |
| *GCK* | Exon 6 | c.677T>G | p.Val226Gly | Missense |  |  | PM2 |  | PM1 PP3 PP2 | 4 | |
| *GCK* | Exon 7 | c.686del | p.Gly229fs | Frameshift | PVS1 |  | PM2 |  |  | 5 | |
| *GCK* | Exon 7 | c.770G>A | p.Trp257Ter | Nonsense | PVS1 |  | PM2 |  |  | 5 | |
| *GCK* | Exon 7 | c.781G>C | p.Gly261Arg | Missense | PS1 |  | PM2 | PP1 | PM1 PM5 PP3 PP2 | 5 | |
| *GCK* | Exon 7 | c.821A>C | p.Asp274Ala | Missense |  |  | PM2 |  | PM5 PP3 PP2 | 4 | |
| *GCK* | Exon 7 | c.824\_850del | p.Arg275\_Asn283del | In-frame deletion | PM4 |  | PM2 |  | PP3 | 4 | |
| *GCK* | Exon 7 | c.859C>T | p.Gln287Ter | Nonsense | PVS1 |  | PM2 |  |  | 5 | |
| *GCK* | Exon 8 | c.868G>A | p.Glu290Lys | Missense |  |  | PM2 |  | PM5 PP3 PP2 | 4 | |
| *GCK* | Exon 8 | c.904G>T | p.Val302Leu | Missense | PS1 |  | PM2 |  | PM5 PP3 PP2 | 5 | |
| *GCK* | Exon 8 | c.908G>C | p.Arg303Pro | Missense |  |  | PM2 |  | PM1 PP3 PP2 | 4 | |
| *GCK* | Exon 8 | c.1019G>A | p.Ser340Asn | Splice defect | PVS1 |  | PM2 |  | PM5 PP3 PP2 | 5 | |
| *GCK* | Exon 9 | c.1134\_1151del | p.Ala379\_Ala384del | In-frame deletion | PM4 |  | PM2 |  | PP3 | 4 | |
| *GCK* | Exon 9 | c.1135G>A | p.Ala379Thr | Missense |  |  | PM2 |  | PM5 PP3 PP2 | 4 | |
| *GCK* | Exon 9 | c.1181\_1182delinsCG | p.Arg394Pro | Missense |  | PS3 | PM2 |  | PP3 PP2 | 4 | |
| *GCK* | Exon 9 | c.1238\_1253+14delinsGCCCCCA | p.Tyr413\_Ser418delinsCysProHis | Splice defect | PVS1 |  | PM2 | PP1 | PP3 | 5 | |
| *GCK* | Exon 9 | c.1246C>T | p.His416Tyr | Missense |  |  | PM2 |  | PM5 PP3 PP2 | 4 | |
| *GCK* | Exon 10 | c.1313T>C | p.Phe438Ser | Missense |  |  | PM2 |  | PM5 PP3 PP2 | 4 | |
| *GCK* | Exon 10 | c.1348del | p.Ala450fs | Frameshift | PVS1 |  | PM2 |  |  | 5 | |
| *HNF1A* | Exon 1 | c.26A>G | p.Gln9Arg | Missense |  |  | PM2 |  | PM1 PM5 PP3 | 4 | |
| *HNF1A* | Exon 1 | c.35T>G | p.Leu12Arg | Missense |  |  | PM2 |  | PM5 PP3 | 4 | |
| *HNF1A* | Exon 1 | c.102del | p.Tyr36fs | Frameshift | PVS1 |  | PM2 |  |  | 5 | |
| *HNF1A* | Exon 1 | c.142dup | p.Glu48fs | Frameshift | PVS1 |  | PM2 |  |  | 5 | |
| *HNF1A* | Exon 1 | c.242del | p.Phe81fs | Frameshift | PVS1 |  | PM2 |  |  | 5 | |
| *HNF1A* | Intron 1 | c.326+3\_326+9del | p.? | Splice defect | PVS1 |  | PM2 |  | PP3 | 4 | |
| *HNF1A* | Intron 1 | c.326+4A>G | p.? | Splice defect | PVS1 |  | PM2 |  | PP3 | 4 | |
| *HNF1A* | Exon 2 | c.364T>C | p.Tyr122His | Missense |  |  | PM2 |  | PM1 PP3 | 4 | |
| *HNF1A* | Exon 2 | c.375G>C | p.Gln125His | Missense |  |  | PM2 |  | PM1 PP3 | 4 | |
| *HNF1A* | Exon 2 | c.467C>T | p.Thr156Met | Missense |  |  | PM2 |  | PM1 PP3 | 4 | |
| *HNF1A* | Exon 2 | c.496T>G | p.Tyr166Asp | Missense |  |  | PM2 |  | PM1 PP3 | 4 | |
| *HNF1A* | Exon 2 | c.514G>T | p.Glu172Ter | Nonsense | PVS1 |  | PM2 |  |  | 5 | |
| *HNF1A* | Exon 2 | c.327-?\_526+?del | p.? | Exonic deletion | PVS1 |  | PM2 |  |  | 5 | |
| *HNF1A* | Intron 2 | c.526+1delG | p.? | Splice defect | PVS1 |  | PM2 |  | PP3 | 5 | |
| *HNF1A* | Exon 3 | c.570del | p.Gly191fs | Frameshift | PVS1 |  | PM2 |  |  | 5 | |
| *HNF1A* | Exon 4 | c.775G>A | p.Val259Ile | Missense |  |  | PM2 |  | PM1 PM5 PP3 | 4 | |
| *HNF1A* | Exon 4 | c.825\_827del | p.Glu275\_Ala276delinsAsp | In-frame deletion | PM4 |  | PM2 |  | PM1 PP3 | 4 | |
| *HNF1A* | Exon 8 | c.1623G>A | p. ? | Splice defect | PVS1 |  | PM2 |  | PP3 | 4 | |
| *HNF1A* | Exon 9 | c.1742\_1768+2delinsACAGGG | p.? | Splice defect | PVS1 |  | PM2 |  | PP3 | 4 | |
| *HNF1A* | Exon 9 | c.1697dup | p.His566fs | Frameshift | PVS1 |  | PM2 |  |  | 5 | |
| *HNF1A* | Exon 10 | c.1772\_1773del | p.Ser591fs | Frameshift | PSa |  | PM2 |  |  | 4 | |
| *HNF1B* | Exon 1 | c.34C>T | p.Leu12Phe | Missense |  |  | PM2 |  | PM1 PP3 | 4 | |
| *HNF1B* | Exon 2 | c.377A>G | p.Lys126Arg | Missense |  |  | PM2 |  | PM1 PP3 | 4 | |
| *HNF1B* | Exon 2 | c.473C>A | p.Thr158Asn | Missense |  |  | PM2 |  | PM1 PP3 | 4 | |
| *HNF4A* | Exon 4 | c.325C>T | p.Gln109Ter | Nonsense | PVS1 |  |  |  | PM1 | 5 | |
| *HNF4A* | Exon 4 | c.335G>C | p.Arg112Pro | Missense |  |  | PM2 |  | PM5 PP3 | 4 | |
| *HNF4A* | Exon 5 | c.433\_436del | p.Ser145fs | Frameshift | PVS1 |  | PM2 |  |  | 5 | |
| *HNF4A* | Exon 5 | c.535T>C | p.Trp179Arg | Missense |  |  | PM2 |  | PM1 PP3 | 4 | |
| *HNF4A* | Exon 6 | c.589del | p.Leu197fs | Frameshift | PVS1 |  | PM2 |  |  | 5 | |
| *HNF4A* | Exon 6 | c.625G>A | p.Gly209Arg | Missense |  |  | PM2 | PP1 | PM1 PP3 | 4 | |
| *HNF4A* | Exon 8 | c.851\_852del | p.Gly284fs | Frameshift | PVS1 |  | PM2 | PS2 |  | 5 | |
| *INS* | Exon 2 | c.85C>T | p.His29Tyr | Missense |  |  | PM2 |  | PM5 PP3 | 4 | |
| *INS* | Exon 2 | c.100C>T | p.His34Tyr | Missense |  |  | PM2 |  | PM5 PP3 | 4 | |
| *KCNJ11* | Exon 1 | c.125G>A | p.Cys42Tyr | Missense |  | PS3b | PM2 |  | PM5 PP3 | 4 | |
| *KCNJ11* | Exon 1 | c.952A>G | p.Ile318Val | Missense |  | PS3b |  | PP1 | PP3 | 4 | |

Sequence variants are numbered with respect to GenBank cDNA sequences. *ABCC8*, NM\_000352.3; *GCK*, NM\_000162.3; *HNF1A*, NM\_000545.6; *HNF1B*, NM\_000458.3; *HNF4A*, NM\_175914.4; *INS*, NM\_000207.2; *KCNJ11*, NM\_000525.3 and described according to Human Genome Variation Society (HGVS) guidelines (<http://www.hgvs.org/varnomen>). Variants were classified according to ACMG recommendations [1].

**Variant consequence**: PVS1, Nonsense, frameshift, canonical ±1 or ±2 splice sites, single or multi-exon deletions; PS1, same amino-acid change as a previously established pathogenic variant regardless of nucleotide change; PM4, in-frame deletions in a nonrepeat region. aPS instead of PVS1 as this frameshift is located in the last exon of the *HNF1A* gene

**Functional data**: PS3, well-established *in vitro* functional studies supportive of a damaging effect on the gene product; or bimproved glycaemic response treated with sulfonylureas.

**Population data**: PM2, if variant allele frequency (VAF) < 0.01% in geographically-matched population databases (ExAC [http://exac.broadinstitute.org/] and dbSNP [www.ncbi.nlm.nih.gov/snp])

**Segregation data**: PS2, *de novo* variant; PP1, segregation with diabetes in at least 2 affected relatives in proband’s family;

**Computational evidence**: PM1, located in a mutational hot spot and/or critical and well-established functional domain; PM5, novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before;

**Predictive evidence:** PP3, *in silico* evidence. For missense mutations, use of 4 predictive algorithms of pathogenicity (SIFT, PolyPhen-2, Align-GVGD and CADD); for CADD, we used a cut-off threshold of 20 (>20: considered pathogenic). For intronic variants, PP3 was attributed if both MaxEntScan and Splice site Finder algorithms predicted a splicing defect according to Houdayer et Coll. [2], guidelines (a variant score at least 15% lower than the reference allele for MaxEntScan and at least 5% lower for Splice site Finder); PP2, Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease.

**Patient’s phenotype**: PP4, proband’s and family’s history was suggestive of monogenic diabetes. Criteria for performing MODY genetic testing were systematically checked before NGS analysis, PP4 was attributed to all patients included in this study.

**Class of pathogenicity** determined taking into account all criteria according to rules established by the ACMG [1].

1. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E *et al*: **Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology**. *Genet Med* 2015, **17**(5):405-424.

2. Houdayer C, Caux-Moncoutier V, Krieger S, Barrois M, Bonnet F, Bourdon V, Bronner M, Buisson M, Coulet F, Gaildrat P *et al*: **Guidelines for splicing analysis in molecular diagnosis derived from a set of 327 combined in silico/in vitro studies on BRCA1 and BRCA2 variants**. *Hum Mutat* 2012, **33**(8):1228-1238.

**Table S4.** List of known class 4-5 variants identified in *ABCC8*, *HNF1B*, *KCNJ11* and *INS* genes

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Location** | **Nucleotide Change** | **Protein effect** | **Variant type** | **References** | **Associated phenotype in literature** |
| *ABCC8* | Exon | c.806C>A | p.Ala269Asp | Missense | [1] | NDM |
| *ABCC8* | Exon 28 | c.3547C>T | p.Arg1183Trp | Missense | [2] | TNDM |
| *ABCC8* | Exon 28 | c.3548G>A | p.Arg1183Gln | Missense | [3] | TNDM |
| *ABCC8* | Exon 34 | c.4139G>A | p.Arg1380His (3)a | Missense | [2 ; 4] | TNDM |
| *HNF1B* | Exon 2 | c.529C>T | p.Arg177Ter | Missense | [5] | HNF1B renal disease |
| *HNF1B* | Exon 3 | c.704G>A | p.Arg235Gln | Missense | [7] | HNF1B renal disease |
| *HNF1B* | Exons 1-4 | c.1-?\_1045+?del | p.0? (10)a | Exonic deletion | [6] | HNF1B renal disease |
| *HNF1B* | Exons 1-9 | c.1-?\_1674+?del | p.0? | Gene deletion | [7] | HNF1B renal disease |
| *KCNJ11* | Exon 1 | c.679G>A | p.Glu227Lys | Missense | [8] | TNDM |
| *INS* | Exon 2 | c.16C>T | p.Arg6Cys (2)a | Missense | [9] | MODY |
| *INS* | Exon 2 | c.130G>A | p.Gly44Arg | Missense | [10]; Bellanné-Chantelot, Saint-Martin unpublishedb | MODY |
| *INS* | Exon 3 | c.163C>T | p.Arg55Cys | Missense | [11] | MODY |

Sequence variants are numbered with respect to GenBank cDNA sequences. *ABCC8*, NM\_000352.3; *GCK*, NM\_000162.3; *HNF1A*, NM\_000545.6; *HNF1B*, NM\_000458.3; *HNF4A*, NM\_175914.4; *INS*, NM\_000207.2; *KCNJ11*, NM\_000525.3 and described according to Human Genome Variation Society (HGVS) guidelines (<http://www.hgvs.org/varnomen>).

aNumber of probands into parentheses; b1 additional family diagnosed with TNDM in the diagnostics database of Pitié-Salpêtrière hospital; NDM, neonatal diabetes mellitus; TNDM, transient neonatal diabetes mellitus.

1. Vaxillaire M, Dechaume A, Busiah K, Cave H, Pereira S, Scharfmann R, de Nanclares GP, Castano L, Froguel P, Polak M *et al*: **New ABCC8 mutations in relapsing neonatal diabetes and clinical features**. *Diabetes* 2007, **56**(6):1737-1741.

2. Flanagan SE, Patch AM, Mackay DJ, Edghill EL, Gloyn AL, Robinson D, Shield JP, Temple K, Ellard S, Hattersley AT: **Mutations in ATP-sensitive K+ channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood**. *Diabetes* 2007, **56**(7):1930-1937.

3. Babenko AP, Polak M, Cave H, Busiah K, Czernichow P, Scharfmann R, Bryan J, Aguilar-Bryan L, Vaxillaire M, Froguel P: **Activating mutations in the ABCC8 gene in neonatal diabetes mellitus**. *N Engl J Med* 2006, **355**(5):456-466.

4. Hartemann-Heurtier A, Simon A, Bellanné-Chantelot C, Reynaud R, Cavé H, Polak M, Vaxillaire M, Grimaldi A: **Mutations in the ABCC8 gene can cause autoantibody-negative insulin-dependent diabetes**. *Diabetes Metab* 2009, **35**(3):233-235.

5. Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, Cockburn BN, Lindner T, Yamagata K, Ogata M, Tomonaga O *et al*: **Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY**. *Nat Genet* 1997, **17**(4):384-385.

6. Yorifuji T, Fujimaru R, Hosokawa Y, Tamagawa N, Shiozaki M, Aizu K, Jinno K, Maruo Y, Nagasaka H, Tajima T *et al*: **Comprehensive molecular analysis of Japanese patients with pediatric-onset MODY-type diabetes mellitus**. *Pediatr Diabetes* 2012, **13**(1):26-32.

7. Bellanne-Chantelot C, Clauin S, Chauveau D, Collin P, Daumont M, Douillard C, Dubois-Laforgue D, Dusselier L, Gautier JF, Jadoul M *et al*: **Large genomic rearrangements in the hepatocyte nuclear factor-1beta (TCF2) gene are the most frequent cause of maturity-onset diabetes of the young type 5**. *Diabetes* 2005, **54**(11):3126-3132.

8. Edghill EL, Gloyn AL, Goriely A, Harries LW, Flanagan SE, Rankin J, Hattersley AT, Ellard S: **Origin of de novo KCNJ11 mutations and risk of neonatal diabetes for subsequent siblings**. *J Clin Endocrinol Metab* 2007, **92**(5):1773-1777.

9. Edghill EL, Flanagan SE, Patch AM, Boustred C, Parrish A, Shields B, Shepherd MH, Hussain K, Kapoor RR, Malecki M *et al*: **Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood**. *Diabetes* 2008, **57**(4):1034-1042.

10. Flannick J, Johansson S, Njolstad PR: **Common and rare forms of diabetes mellitus: towards a continuum of diabetes subtypes**. *Nat Rev Endocrinol* 2016, **12**(7):394-406.

11. Molven A, Ringdal M, Nordbo AM, Raeder H, Stoy J, Lipkind GM, Steiner DF, Philipson LH, Bergmann I, Aarskog D *et al*: **Mutations in the insulin gene can cause MODY and autoantibody-negative type 1 diabetes**. *Diabetes* 2008, **57**(4):1131-1135.

**Table S5.** Main characteristics of the 15 patients with HNF1B-MODY.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient #** | **HNF1B**  **Nucléotide change / protein effect** | **Sex F/M** | **Eurocaucasian origin yes/no** | **n of generations with diabetes** | **Age at diagnosis of diabetes (years)** | **BMI at diagnosis of diabetes (kg/m²)** | **Symptoms at diagnosis of diabetesa yes/no** | **HbA1c at diagnosis (%)** | **Insulin therapy at diagnosis yes/no** | **Age at study**  **(years)** | **Diabetes duration**  **(years)** | **Plasma creatinine (µmol/L)** | **Renal morphologyb** |
| 1 | c.34C>T / p.Leu12Phe | M | no | 2 | 32 | 24,4 | yes | 10,3 | yes | 33 | 1 | 56 | na |
| 2 | Whole deletion | F | yes | 2 | 15 | 23,4 | yes | na | yes | 18 | 3 | 53 | cysts |
| 3 | Exons 1-4 deletion | M | na | 2 | 18 | na | no | na | no | 66 | 48 | 212 | one cyst |
| 4 | Whole deletion | M | yes | 3 | 27 | 20,2 | yes | 9,9 | yes | 27 | 0 | 88 | normal |
| 5 | Whole deletion | M | no | 3 | 15 | na | yes | na | yes | 25 | 10 | na | normal |
| 6 | Whole deletion | M | no | 3 | 29 | 19,7 | yes | 14 | yes | 29 | 0 | na | na |
| 7 | Whole deletion | M | yes | 1 | 33 | 21,4 | no | 7,1 | no | 35 | 2 | 79 | normal |
| 8 | Whole deletion | M | no | 3 | 34 | 21,8 | yes | 14,2 | yes | 55 | 21 | 98 | normal |
| 9 | Whole deletion | F | yes | 1 | 22 | 19,1 | no | 5,3 | no | 25 | 3 | 61 | cysts |
| 10 | Whole deletion | M | yes | 2 | 18 | na | yes | na | yes | 25 | 7 | 76 | na |
| 11 | c.377A>G / p.Lys126Arg | M | yes | 2 | 39 | 26,1 | yes | na | yes | 45 | 6 | 78 | normal |
| 12 | Whole deletion | M | no | 3 | 22 | 28,5 | yes | 12,1 | yes | 27 | 5 | 89 | normal |
| 13 | c.473C>A / p.Thr158Asn | M | yes | 3 | 15 | 19 | no | 6,6 | no | 16 | 1 | 65 | normal |
| 14 | Whole deletion | F | no | 1 | 40 | 18,4 | yes | 12 | yes | 49 | 9 | 31 | normal |
| 15 | c.704G>A / p.Arg235Gln | F | yes | 3 | 37 | 16,5 | yes | na | yes | 37 | 0 | 54 | na |

aSymptoms of diabetes: polyuria and/or unexplained body weight loss and/or diabetic ketoacidosis.

bAssessed by ultrasonography and/or computed tomography scan

|  |  |
| --- | --- |
|  |  |

**Table S6.** Main characteristics at the onset of diabetes in patients with monogenic vs. non-monogenic diabetes

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Monogenic** | **Monogenic**  **excluding GCK-cases** | **Non-monogenica** | ***P* ; OR [95% CI]**  **Monogenic vs. non-monogenic** | ***P* ; OR [95% CI]**  **Monogenic excluding GCK**  **vs. non-monogenic** |
| N patients | 254& | 142 | 1241 | 254 vs. 1241 | 142 vs. 1241 |
| Sex: F/M | 168/86 (66%) | 89/53 (63%) | 619/622 (50%) | < 10-4; 1.96 [1.48-2.60] | 0.0045; 1.69 [1.18-2.41] |
| Eurocaucasian/others | 192/37 (84%) | 101/32 (76%) | 600/499 (55%) | < 10-4; 4.32 [2.98-6.26] | < 10-4; 2.63 [1.73-3.98] |
| Age (years) | 24 [18-30] (254) | 24 [18.3-29.8] (142) | 31 [25-39] (1239) | < 10-4 | < 10-4 |
| ≥3 generations with diabetes: yes/no | 144/105 (58%) | 84/55 (60%) | 581/631 (48%) | 0.0053; 1.49 [1.13-1.96] | 0.0055; 1.66 [1.16-2.37] |
| BMI (kg/m²) (n) | 21.8 [20.1-24.2] (221) | 22.8 [20.8-25] (124) | 24.2 [21.6-27.7] (1107) | < 10-4 | < 10-4 |
| BMI: normal/increased (%) | 180/43 (81%) | 92/33 (74%) | 642/475 (57%) | < 10-4; 3.10 [2.18-4.41] | 0.0005; 2.06 [1.36-3.12] |
| Symptoms of diabetesb: yes/no | 37/204 (15%) | 33/102 (24%) | 461/702 (40%) | < 10-4; 0.28 [0.19-0.40] | 0.0005; 0.49 [0.33-0.74] |
| HbA1C (%) | 6.7 [6.3-8.7] (157) | 8.05 [6.6-9.7] (86) | 9.6 [7-12] (588) | < 10-4 | 0.0011 |
| HbA1C (mmol/mol) | 50 [45-72] (157) | 64 [49-83] (86) | 81 [53-108] (588) | < 10-4 | 0.0011 |
| Insulin therapy: yes/no | 38/189 (17%) | 36/94 (28%) | 339/768 (31%) | < 10-4; 0.46 [0.31-0.66] | 0.5455; 0.87 [0.58-1.30] |
| Hypertension: yes/no | 23/133 (15%) | 16/71 (18%) | 214/538 (28%) | 0.0003; 0.43 [0.27-0.70] | 0.0562; 0.57 [0.32-1.00] |
| Dyslipidemia: yes/no | 23/116 (17%) | 16/63 (20%) | 235/461 (34%) | < 10-4; 0.39 [0.24-0.63] | 0.0157; 0.50 [0.28-0.88] |

Values are actual numbers with percentages into parentheses, or median with interquartile range into brackets and numbers of values into parentheses.

aNon-monogenic, no genetic etiology detected by targeted NGS on 7 genes;

bSymptoms of diabetes: polyuria and/or unexplained body weight loss and/or diabetic ketoacidosis;

BMI, body mass index.

**Table S7.** List of variants of uncertain significance (class 3)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Criteria for classifying variants according to ACMG guidelines [1] | | | | | | |
| Gene | Location | Nucleotide Change | Protein effect | Variant type | Variant consequence | Functional data | Population data | Segregation data | Computational and predictive evidence | Class of pathogenicity |
| *ABCC8* | Exon 1 | c.40G>T | p.Ala14Ser | Missense |  |  | PM2 |  | BP4 | 3 |
| *ABCC8* | Exon 3 | c.291G>T | p.? | Splice defect |  |  | PM2 |  |  | 3 |
| *ABCC8* | Exon 3 | c.361G>A | p.Val121Met | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 3 | c.380T>C | p.Ile127Thr | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Intron 4 | c.580-16\_580-14del | p.? | Splice defect |  |  |  |  |  | 3 |
| *ABCC8* | Exon 5 | c.770T>C | p.Met257Thr | Missense |  |  | PM2 |  |  | 3 |
| *ABCC8* | Exon 5 | c.809T>G | p.Phe270Cys | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 6 | c.946G>Aa | p.Gly316Arg | Missense |  |  | PM2 |  | PP3 | 3b |
| *ABCC8* | Exon 9 | c.1337T>C | p.Ile446Thr | Missense |  |  |  |  | PP3 | 3 |
| *ABCC8* | Exon 10 | c.1510C>T | p.Arg504Cys | Missense |  |  |  | BP5 | PP3 | 3 |
| *ABCC8* | Exon 10 | c.1537G>A | p.Ala513Thr | Missense |  |  | PM2 |  | PP3 | 3b |
| *ABCC8* | Exon 15 | c.2104C>T | p.Arg702Cys | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 15 | c.2116+61A>G | p.? | Splice defect |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 21 | c.2491T>A | p.Ser831Thr | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 21 | c.2512A>G | p.Ile838Val | Missense |  |  | PM2 |  |  | 3 |
| *ABCC8* | Exon 21 | c.2546A>C | p.Asn849Thr | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 25 | c.3113C>A | p.Thr1038Asn | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 28 | c.3496G>A | p.Val1166Met | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 31 | c.3778G>A | p.Val1260Met | Missense |  |  | BS1 |  | PP3 | 3 |
| *ABCC8* | Exon 31 | c.3827T>C | p.Leu1276Pro | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 31 | c.3868A>G | p.Met1290Val | Missense |  |  | PM2 |  | BP4 | 3 |
| *ABCC8* | Exon 35 | c.4279C>A | p.Gln1427Lys | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 37 | c.4511T>A | p.Ile1504Asn | Missense |  |  | PM2 |  | PP3 | 3 |
| *ABCC8* | Exon 39 | c.4736G>A | p.Arg1579His | Missense |  |  | PM2 |  | PP3 | 3 |
| *GCK* | Exon 2 | c.142G>A | p.Glu48Lys | Missense |  |  | PM2 |  | PP3 PP2 | 3 |
| *GCK* | Exon 3 | c.325T>C | p.Ser109Pro | Missense |  |  | PM2 |  | PP3 PP2 | 3 |
| *GCK* | Intron 5 | c.580-9T>G† | p.? | Splice defect |  |  | PM2 |  | PP3 | 3 |
| *GCK* | Exon 8 | c.923G>C | p.Arg308Thr | Missense |  |  | PM2 |  | PP3 PP2 | 3 |
| *GCK* | Exon 9 | c.1207C>T | p.Arg403Cys | Missense |  |  | PM2 |  | PP3 PP2 | 3 |
| *GCK* | Exon 10 | c.1373\_1376del† | p.Lys458fs | Frameshift | PM4 |  | PM2 |  |  | 3 |
| *HNF1A* | Promoter | c.-191T>C | p.= | Promoter variant |  |  | PM2 |  |  | 3 |
| *HNF1A* | Exon 1 | c.98C>T | p.Pro33Leu | Missense |  |  | PM2 | PP5 | BP4 | 3 |
| *HNF1A* | Exon 2 | c.490A>G | p.Thr164Ala | Missense |  |  | PM2 | PP5 | PM1 BP4 | 3 |
| *HNF1A* | Exon 2 | c.511C>G | p.Arg171Gly | Missense |  |  | PM2 | PP5 | PM1 BP4 | 3b |
| *HNF1A* | Exon 4 | c.866C>T | p.Pro289Leu | Missense |  |  | PM2 |  | PP3 | 3 |
| *HNF1A* | Exon 7 | c.1380\_1406del | p.Gln460\_Leu468del | In-frame deletion | PM4 |  |  |  |  | 3 |
| *HNF1A* | Exon 8 | c.1522G>A | p.Glu508Lys | Missense |  | PS3 |  |  | PP3 | 3b |
| *HNF1A* | Exon 10 | c.1865T>C | p.Ile622Thr | Missense |  |  | PM2 |  | PP3 | 3 |
| *HNF1B* | Exon 1 | c.118G>A | p.Gly40Arg | Missense |  |  | PM2 |  | PP3 | 3 |
| *HNF1B* | Exon 7 | c.1460T>C | p.Met487Thr | Missense |  |  | PM2 |  | PP3 | 3 |
| *HNF1B* | Exon 7 | c.1484T>A | p.Met495Lys | Missense |  |  | PM2 |  | PP3 | 3 |
| *HNF4A* | Exon 2 | c.203A>G | p.Lys68Arg | Missense |  |  | PM2 |  | PP3 | 3 |
| *HNF4A* | Exon 4 | c.353G>A | p.Arg118Gln | Missense |  |  | PM2 |  | PP3 | 3 |
| *HNF4A* | Exon 5 | c.461T>G | p.Ile154Ser | Missense |  |  | PM2 |  | PP3 | 3 |
| *HNF4A* | Exon 5 | c.478G>A | p.Ala160Thr | Missense |  |  | PM2 |  | PP3 | 3 |
| *HNF4A* | Exon 6 | c.658G>A | p.Val220Met | Missense |  |  | PM2 |  | PP3 | 3b |
| *INS* | Exon 2 | c.11G>A | p.Trp4Ter | Nonsense |  |  | PM2 |  | PP3 | 3c |
| *INS* | Exon 2 | c.155C>T | p.Pro52Leu | Missense |  |  | PM2 |  | PP3 | 3 |
| *INS* | Intron 2 | c.188-15G>A | p.? | Splice defect |  |  | PM2 |  | PP3 | 3b |
| *KCNJ11* | Exon 1 | c.86G>A | p.Arg29His | Missense |  |  | PM2 |  | PP3 | 3 |
| *KCNJ11* | Exon 1 | c.160C>T | p.Arg54Cys | Missense |  |  | PM2 |  | PP3 | 3 |
| *KCNJ11* | Exon 1 | c.341T>C | p.Ile114Thr | Missense |  |  | PM2 |  | PP3 | 3 |
| *KCNJ11* | Exon 1 | c.353C>T | p.Ser118Leu | Missense |  |  | PM2 |  | BP4 | 3b |
| *KCNJ11* | Exon 1 | c.463G>A | p.Val155Met | Missense |  |  | PM2 |  | PP3 | 3 |
| *KCNJ11* | Exon 1 | c.623G>A | p.Ser208Asn | Missense |  |  | PM2 |  | PP3 | 3 |
| *KCNJ11* | Exon 1 | c.662G>A | c.Arg221His | Missense |  |  | PM2 |  | PP3 | 3 |

aVariant identified in two probands; bVariant previously reported in literature; cVariant identified at an heterozygous state and previously reported in literature at homozygous state in type 1-like diabetes [2].

Sequence variants are numbered with respect to GenBank cDNA sequences. *ABCC8*, NM\_000352.3; *GCK*, NM\_000162.3; *HNF1A*, NM\_000545.6; *HNF1B*, NM\_000458.3; *HNF4A*, NM\_175914.4; *INS*, NM\_000207.2; *KCNJ11*, NM\_000525.3 and described according to Human Genome Variation Society (HGVS) guidelines [3].

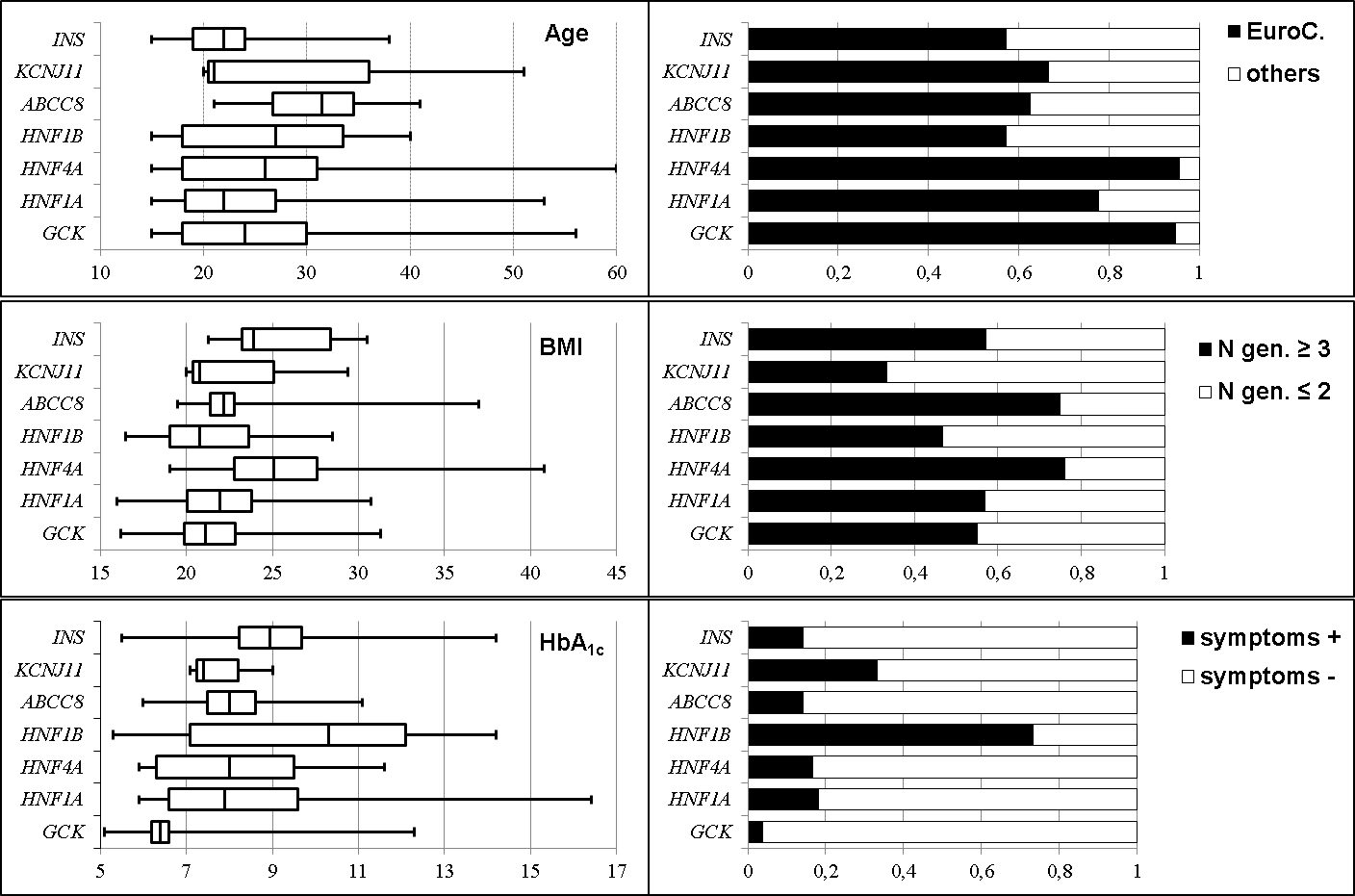
Variant evaluation was conducted as described in **Supplemental Table S2**. One additional criterion was considered for these variants as piece of predictive evidence : BP4, when results of prediction algorithm are contradictory and do not suggest any impact on the gene product.

1. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E *et al*: **Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology**. *Genet Med* 2015, **17**(5):405-424.

2. Di Benedetto M, Richard O, Pélissier P, Darteyre S, Cavé H, Stéphan JL: **[Permanent neonatal diabetes and recessive mutation in the INS gene: a familial history]**. *Arch Pediatr* 2013, **20**(2):199-202.

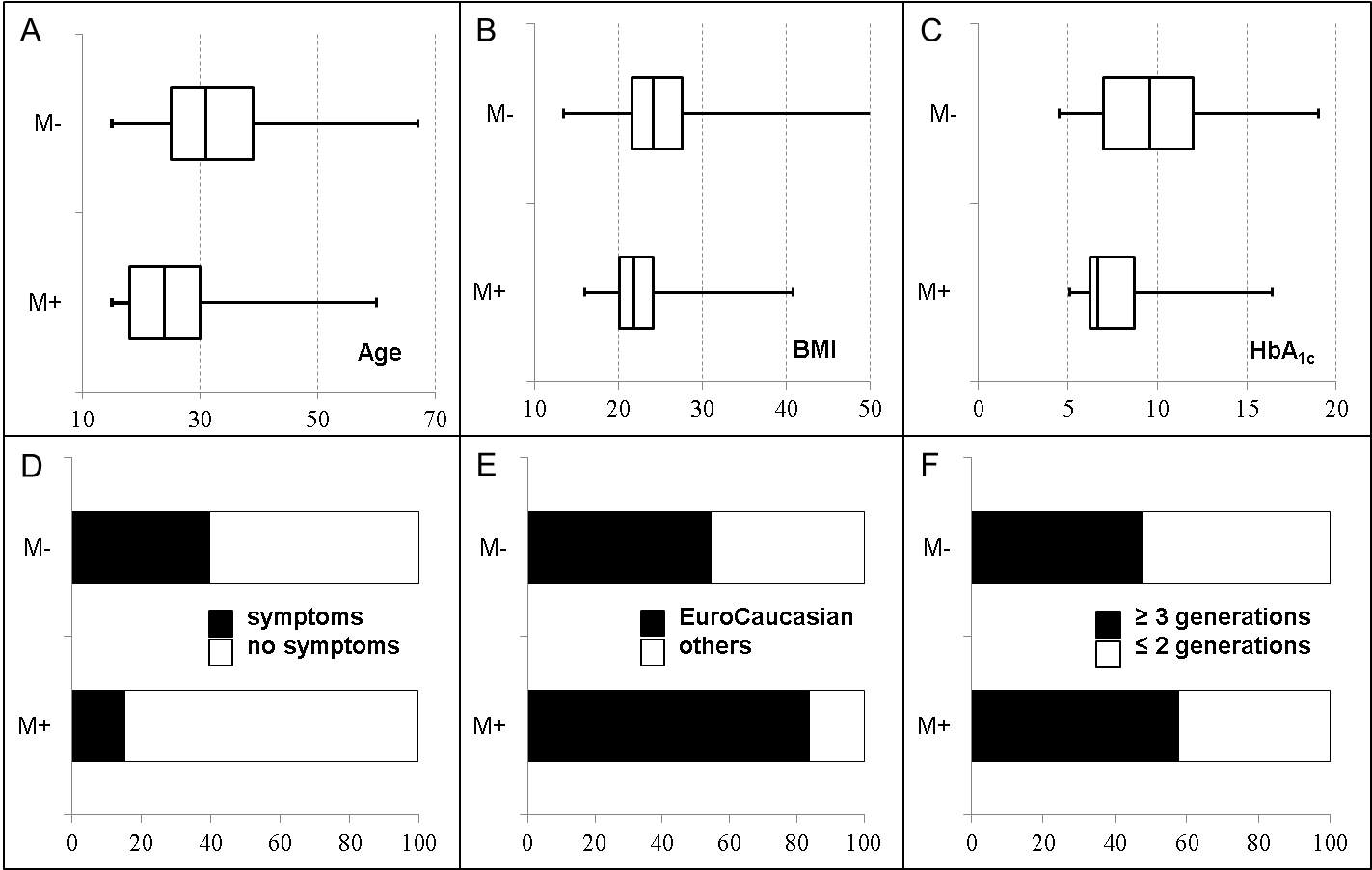
3. den Dunnen JT, Dalgleish R, Maglott DR, Hart RK, Greenblatt MS, McGowan-Jordan J, Roux AF, Smith T, Antonarakis SE, Taschner PE: **HGVS Recommendations for the Description of Sequence Variants: 2016 Update**. *Hum Mutat* 2016, **37**(6):564-569.

**Figure S1.** Main characteristics at diagnosis of diabetes in patients with monogenic diabetes according to the involved gene

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Legend. Box-plot representation of age (years), BMI, body mass index (kg/m²), HbA1c (%), and percentages of geographical origin family history of diabetes in ≥ 3 generations, and presence of diabetes symptoms.

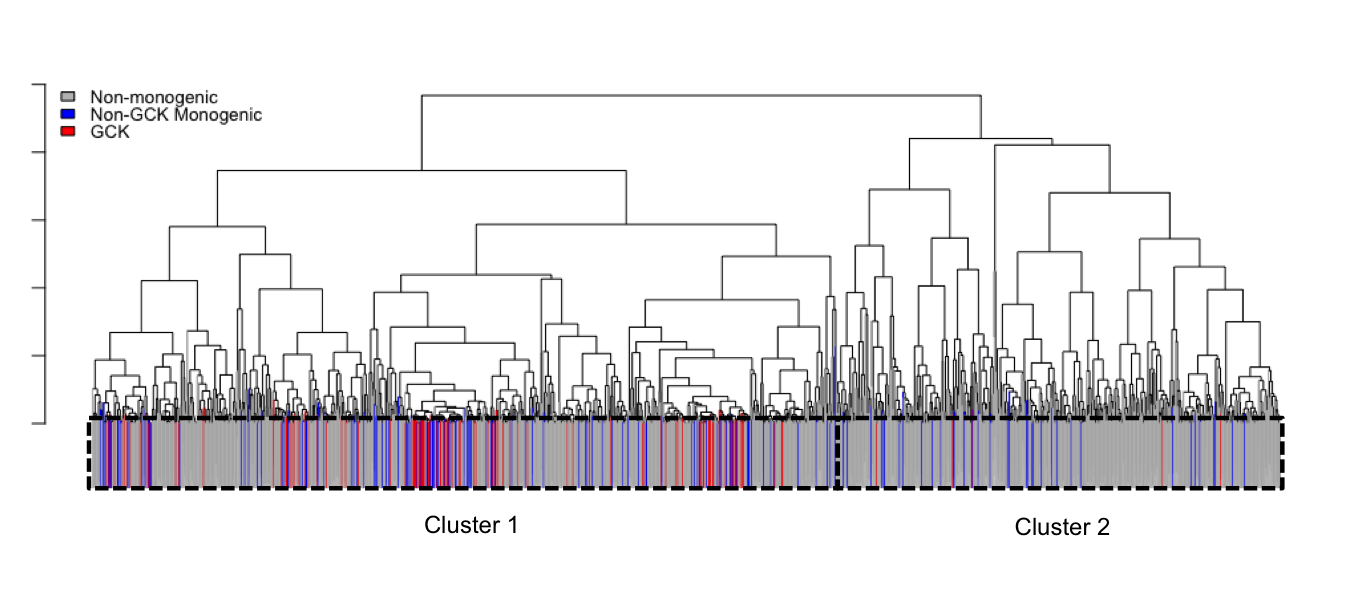
**Figure S2.** Main characteristics at diagnosis of diabetes in patients with (M+) and without (M-) monogenic diabetes

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Legend. Box-plot representation of A, age (years), B, BMI, body mass index (kg/m²), C, HbA1c (%), and percentages of D, presence of

diabetes symptoms, E, geographical origin, and F, family history of diabetes in ≥3 generations.

**Figure S3.** Hierarchical clustering of 1495 patients with a clinical suspicion of monogenic diabetes



Legend. Dendrogram of the non-supervised hierarchical clustering performed in 1495 patients with a clinical suspicion of monogenic diabetes. Patients with loss-of-function variants (n=10) and those with a class 3 variant (n=59) were not included in this analysis.