Molecular analysis of a large cohort of MODY patients by Next Generation Sequencing

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INTRODUCTION AND OBJECTIVE

Maturity-onset diabetes of the young (MODY) is a mono-genic non-autoimmune form of diabetes mellitus characterized by absence of ketosis, autosomal dominant inheritance, a young age of onset (<25 years) and primary defect in the function of the beta cells of the pancreas. MODY accounts for 2-5% of all cases of diabetes mellitus type 1 and 2 (T1D, T2D), but probably the true prevalence is underestimated as MODY shares clinical features with the more common forms of diabetes T1D and T2D. It is a phenotypically and genetically heterogeneous disorder with at least 14 subtypes.

However, in about 50% of MODY patients, causative mutations in known genes (MODYx) have been described. Recent advances in next-generation sequencing (NGS) technologies make it affordable to search for rare and functional variants for common complex diseases systematically. On the bases of this observation, we decide to analyse 100 MODY patients through NGS approach.

METHODS

Target resequencing in about **100** cases with diagnosis of MODY and/or T2D.

Design array custom for a set of 182 genes

We selected the coding and regulatory regions of genes:
implicated in MODY and different type of diabetes disorders; implicated inT2D; implicated in the pancreatic β cells pathway; causative of diabetes in mice models

RESULTS

SAMPLE	GENE	Nucleotide change	Protein effect	rs OMIM ASSOCIATED
10605	*SLC30A8* *ABCC8*	c.589A>G c.1616A>G	p.Ile197Val p.Tyr539Cys	c.79A>C (p.Ile27Leu) HNF1A rs1169288
A/1257	*GIPR* *IFIH1*	c.983G>C c.2027T>A	p.Arg328Pro p.Leu676His	c.1403C>T (p.Pro446Leu) GCKR rs1260326 IVS4-9G>A WFS1 rs10010131
B/296	*TLE1* *DGKG*	c.1228G>A c.461C>T	p.Val410Met p.Ser154Leu	**c.67G>A (p.Glu23Lys) KCNJ11 rs5219**
B/1422	*ARAP1* *GCK*	c.2965G>C c.448_450delTTC	p.Glu989Gln p.Phe150del	**c.79A>C (p.Ile27Leu) HNF1A rs1169288** **c.484C>G (p.Leu162Val) PPARA rs1800206**
C/171	*PPARA* *ADAMTS9*	c.160T>C c.5303C>T	p.Tyr54His p.Ala1768Val	**c.67G>A (p.Glu23Lys) KCNJ11 rs5219** **c.2911G>A (p.Gly972Arg) IRS1 rs1801278**
B/1401	*SLC2A4* *GLIS3*	c.944A>C c.252A>T	p.Glu315Ala p.Leu84Phe	
A/436	*ABCC8* *RFX6*	c.4500C>A c.1558A>T	p.Ser1500Arg p.Ser520Thr	**c.67G>A (p.Glu23Lys) KCNJ11 rs5219** **c.1403C>T (p.Pro446Leu) GCKR rs1260326**
B/446	*WFS1* *RFX6*	c.2054G>A c.1678G>A	p.Arg685His p.Asp560Asn	
A/261	*INS* *SLC30A8*	c.125T>C c.377C>T	p.Val42Ala p.Ala126Val	
D/823	*RBMS1* *HNF1A*	c.1072G>A c.629C>T	p.Ala358Thr p.Ser210Phe	
D/1114	*HNF1A* *AGMO*	c.1745A>G c.1029T>G	p.His582Arg p.Phe343Leu	**c.67G>A (p.Glu23Lys) KCNJ11 rs5219** **c.484C>G (p.Leu162Val) PPARA rs1800206**
D/1198	*SREBF1* *ARAP1*	c.3062C>T c.344C>T	p.Pro1021Leu p.Pro115Leu	**c.67G>A (p.Glu23Lys) KCNJ11 rs5219**
D/1222	*ARAP1* *ZFAND6*	c.344C>T c.548A>T	p.Pro115Leu p.Tyr183Phe	**c.67G>A (p.Glu23Lys) KCNJ11**
B/1478	*HNF4A* *GLIS3*	c.1360G>T c.422T>C	p.Ala454Ser p.Ile141Thr	**c.67G>A (p.Glu23Lys) KCNJ11 rs5219**
D/1766	*GCK* *ZFP57* *RFX6* *PRC1*	c.52C>T c.1101G>C c.2681C>T c.1795C>T	p.Gln18* p.GLN367His p.Pro894Leu p.Leu599Phe	**c.67G>A (p.Glu23Lys) KCNJ11**
D/2015	*CAMK1D* *THADA*	c.1097T>C c.4610G>A	p.Val366Ala p.Arg1537Gln	
E/13	*ARAP1* *ADAM30*	c.1435G>A c.1923G>A	p.Gly479Ser p.Arg641Gln	c.484C>G (p.Leu162Val) PPARA rs1800206 c.2911G>A (p.Gly972Arg) IRS1 rs1801278
D/1945	*NOTCH2* *IRS1*	c.4647T>G c.2057_2059delGCA	p.Ile1549Met p.Ser686del	c.2911G>A (p.Gly972Arg) IRS1 rs1801278
E/713	*KLF11* *TLE4*	c.145G>A c.2081A>G	p.Glu49Lys p.Lys694Arg	
E/766	*CAPN10* *CDKAL1*	c.1532C>T c.174-9C>A	p.Ala511Val	c.1720A>G (p.Ser574Gly) HNF1A c.67G>A (p.Glu23Lys) KCNJ11 rs5219 c.2911G>A (p.Gly972Arg) IRS1 rs1801278 c.973C>T (p.Arg325Trp) SLC30A8 rs13266634
E/2085	*FAM13A* *TLE4*	c.1261G>A c.1946G>A	p.Gly421Arg p.Arg649His	c.668A>G (p.Gln223Arg) LEPR rs1137101 c.1337T>C (p.Leu446Pro) GCKR rs1260326 c.2836G>A (p.Ala946Thr) IFH1 rs1990760 c.441-26T>C G6PC2 rs560887 IVS4-9G>A WFS1 rs10010131 c.973C>T (p.Arg325Trp) SLC30A8 rs13266634 c.190T>C (p.Trp64Arg) ADRB3 rs4994 c.16010C>T (p.Ser5337Phe) MACF1 rs202106473 c.3905G>A (p.Arg1302His) MAP3K19 rs772898317 c.2024G>A (p.Arg675Gln) MAP3K19 rs141304858 c.2076G>C (p.Lys692Asn) PCSK1 rs570064523 c.409G>A (p.Val137Met) KCNK16 rs147045595 c.184G>A (p.Gly62Arg) PEPD rs748037244

SAMPLE	GENE	Nucleotide change	Protein effect	rs OMIM ASSOCIATED
E/1181	*ADAMTS9* *PAX6* *TRPM5*	c.3274G>T c.500C>G c.3318G>C	p.Asp1092Tyr p.Thr167Ser p.Glu1106Asp	**c.67G>A (p.Glu23Lys) KCNJ11**
B/781	*DGKG* *O3FAR1* *PAX4*	c.2276C>T c.563A>G c.773C>A	p.Thr759Met p.Asp188Gly p.Ala258Glu	**c.79A>C (p.Ile27Leu) HNF1A rs1169288**
E/1081	*RFX6* *GATA2*	c.1875G>A c.1150A>G	p.Met625Ile p.Arg384Gly	**c.67G>A (p.Glu23Lys) KCNJ11 rs5219**
E/1074	*NCR3* *THADA* *THADA*	c.286C>T c.5773G>A c.4018_4020delTCT	p.Arg96Trp p.Glu1925Lys p.Ser1340del	**c.67G>A (p.Glu23Lys) KCNJ11 rs5219**
E/1610	*ADAM30* *MYT1*	c.1807C>A c.1583A>C	p.Leu603Val p.Gln528Pro	c.386T>A(Val129Glu)TMEM154 rs761728172 c.7963G>C (p.Glu2655Gln) LAMA1 rs73390524 c.7736G>A (p.Ser2579Asn) LAMA1 rs73390524 c.1403C>T (p.Pro446Leu) GCKR rs1260326 c.2836G>A (p.Ala946Thr) IFH1 rs1990760 c.441-26T>C G6PC2 rs560887 IVS4-9G>A WFS1 rs10010131
15_0153	*PPARA* *MC4R*	c.259C>T c.508A>G	p.Pro87Ser p.Ile170Val	c.1968G>C (p.Lys656Asn) LEPR rs1805094 c.441-26T>C G6PC2 rs560887 IVS4-9G>A WFS1 rs10010131 c.2069G>C(p.Ser690Thr) PCSK1 rs6235 c.367G>A (p.Val123Ile) CEL rs201336247 c.885-5C>T GLP1R rs201451844
E/2212	*UHRF1BP1* *BLK*	c.4084A>G c.211G>A	p.Ile1362Val p.ALa71Thr	**c.622G>A(p.Val208Ile) SDC3 rs2491132** **c.2836G>A (p.Ala946Thr) IFH1 rs1990760** **c.441-26T>C G6PC2 rs560887** **IVS4-9G>A WFS1 rs10010131** **c.3475-7C>T ADAMTS9 rs199940595**
15_0010	*PPARA* *AMT*	c.56T>C c.631G>A	p.Leu19Pro p.Glu211Lys	c.986C>T(p.Thr329Ile) SDC3 rs2282440 c.668A>G (p.Gln223Arg) LEPR rs1137101 c.2836G>A (p.Ala946Thr) IFH1 rs1990760 c.441-26T>C G6PC2 rs560887 c.2911G>A(p.Gly971Arg) IRS1 rs1801278 IVS4-9G>A WFS1 rs10010131 c.973C>T (p.Arg325Trp) SLC30A8 rs13266634 c.67G>A (p.Glu23Lys) KCNJ11 rs5219 c.1052G>A (p.Cys351Tyr) ANKRD55 rs76363118 c.10046G>A (p.Cys3349Tyr) HECTD4 rs753421097 c.1052C>T (p.Ser351Leu) TBC1D4 rs780021277
E/1853	*FFAR4* *MOB2* *CILP2* *ABCC8*	c809G>AT c.758G>A c.23055C>A c.3329+8T>C	p.Arg270His p.Gly253Ala p.Arg1019Trp	c.668A>G (p.Gln223Arg) LEPR rs1137101 c.1337T>C (p.Leu446Pro) GCKR rs1260326 c.2836G>A (p.Ala946Thr) IFH1 rs1990760 c.441-26T>C G6PC2 rs560887 IVS4-9G>A WFS1 rs10010131 c.973C>T (p.Arg325Trp) SLC30A8 rs13266634 c.11G>A (p.Ser4Asn) RREB1 rs116821447 c.385+7G>T BCL11A rs775429960 c.397-3C>T PPARG rs370830238
15/1356	*GCK* *KCNK16*	c.898G>T c.253_255delGGCinsA	p.Glu300Lys p.Gly85Lysfs*51	c.668A>G (p.Gln223Arg) LEPR rs1137101 c.1337T>C (p.Leu446Pro) GCKR rs1260326 c.441-26T>C G6PC2 rs560887 c.973C>T (p.Arg325Trp) SLC30A8 rs13266634 c.67G>A (p.Glu23Lys) KCNJ11 rs5219 c.2069G>C(p.Ser690Thr) PCSK1 rs6235 c.499G>T (p.Ala167Ser) HADH rs370306695 c.1113+9T>C WARS rs149903755
E/2179	*THADA* *FTO* *SLC5A2*	c.1214A>G c.191A>G c.1035_1062del28	p.Asp405Gly p.Glu64Gly p.Val346Alafs*17	c.668A>G (p.Gln223Arg) LEPR rs1137101 c.1337T>C (p.Leu446Pro) GCKR rs1260326 c.2836G>A (p.Ala946Thr) IFH1 rs1990760 c.441-26T>C G6PC2 rs560887 IVS4-9G>A WFS1 rs10010131 c.973C>T (p.Arg325Trp) SLC30A8 rs13266634 c.67G>A (p.Glu23Lys) KCNJ11 rs5219 c.272G>T (p.Arg91Leu) NOTCH2 rs143195893 c.1126T>C (p.Ser376Pro) ANKRD55 rs77017041

Patients with two or more mutations in different genes

CONCLUSIONS

In this study we found, in association with known heterozygous/homozygous SNPs associated with diabetes, rare and pathogenetic variants in the 66% of cases. Interestingly, in 40% of positive cases, we identified, in addition to MODY genes, two or more mutations in other different genes. These results suggest a complex aetiology of MODY, in contrast with reports that consider it caused by mutations in single genes. The advent of high-throughput sequencing (HTS) has made simpler to identify that monogenic disease could present digenic (DI) or more complex inheritance. The complexity of DI transcends the genetics. To construct a compelling proof that the inheritance is digenic rather than monogenic may require a multidisciplinary team that can apply techniques to understand the two/more genes and proteins specifically and their interaction. This approach, in formidable way, can contribute not only to a correct genetic counseling, but especially for the choice of the personalized treatment. In fact, patients with diabetes often are treated similarly, with little consideration of individual characteristics that might affect clinical outcome and therapeutic response. Our study provides a highly sensitive method for identification of variants in new causative genes associated with diabetes and draws the best way for a tailored medicine.

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