Improved genetic testing for monogenic diabetes in the Swiss population by targeted next generation sequencing

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Introduction

Monogenic diabetes (MD) is a heterogeneous group of diabetes due to a single gene mutation and includes neonatal diabetes (NDM), Maturity Onset Diabetes of the Young (MODY) and rare forms of syndromic diabetes. Up to now, 13 genes have been identified related to MODY and 22 genes to NDM. MD remains undiagnosed in probably more than 90% of patients and traditional testing focuses usually only on one or a few genes.

The aim of the study was to identify mutations causing MD using a targeted next-generation sequencing (NGS) assay in the Swiss population with clinical suspicion of MD.

Method

Swiss endocrinologists were proposed to participate in the study and to send blood samples of patients with clinical suspicion of MD. Inclusion criteria were NDM, autoantibody negative type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) diagnosed before the age of forty-five without metabolic features and syndromic diabetes regardless of treatment. The analyses were performed by a targeted NGS assay sequencing coding and splicing regions of 323 potential diabetes genes using the Haloplex technology (Agilent technologies, Santa Clara, USA). All the identified variants were confirmed by Sanger sequencing.

Results

So far we have analyzed 141 diabetic probands by NGS. We identified 72 variants (72/141; **51%**) (Figure1) in 40 of the selected 323 potential diabetes genes. Thirty nine variants (39/72; **54%**) are found in one of the 13 putative MODY genes (*HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11*). The most frequent MODY variants are found in the *GCK* gene (30/72; **42%**) (Table1) and 33 variants (33/72; 46%) were found in miscellaneous genes known to be associated with T1DM, T2DM, NDM, glucose homeostasis or pancreas development (Figure2). 16 different mutations of *GCK* could be identified and 43.6% (7/16) were novel. 17 % (**12**/72) of the probands carried the p.Val203Ala missense mutation (Table2).

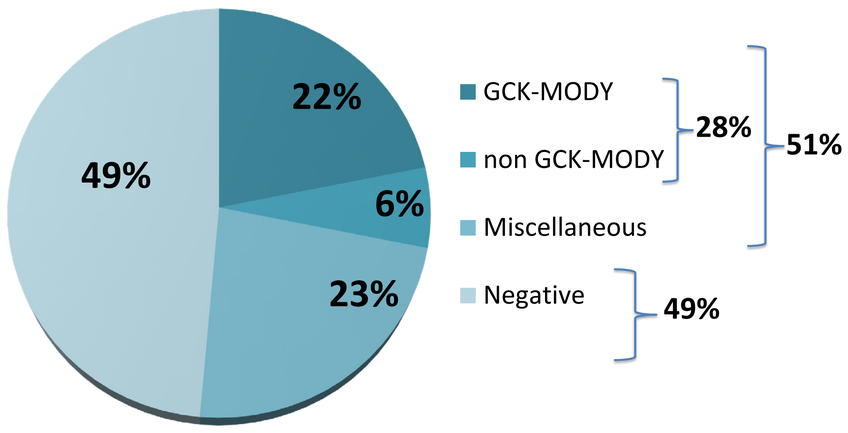

Figure 1:
22% of variants are *GCK*-MODY, 6% non *GCK*-MODY and 23% are variants in miscellaneous genes. Variants were identifed in 51% of the probands.

Table 1:
Number of variants found in MODY genes

MODY Subtypes	Number Probands	
GCK-MODY	**30**	**42%**
HNF4A-MODY	1	1.25%
HNF1A-MODY	1	1.25%
HNF1B-MODY	1	1.25%
KLF11-MODY	1	1.25%
BLK-MODY	3	4%
KCNJ11-MODY	2	3%
Miscellaneous	33	46%
TOTAL	72	100%

54%

Figure 2:
Distribution of variants found in potential diabetes genes other than MODY according to their molecular function (gene ontology categories analyzed with the Panther tool).

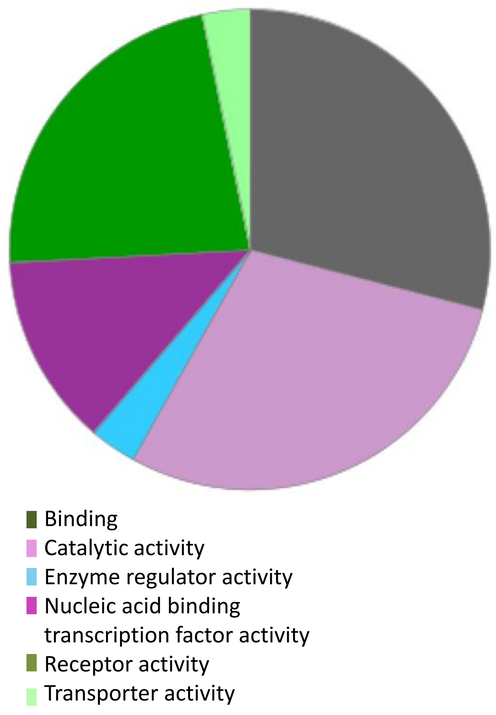

Table 2:
Identified *GCK* mutations
GDM: Gestationnal Diabetes Mellitus
(NCBI Reference Sequence: NM_000162)

Protein Position	Number Probands	Literature Clinical significance
p.Arg43His	1	Described/ MODY
p.Thr103Asn	1	Described/MODY
p.Ala119Thr	1	Novel/ Ala119Asp described
p.Ile163Asn	1	Novel
p.Ala188Thr	1	Described/ MODY
p.Arg191Trp	2	Described/ MODY, GDM
p.Val203Ala	12	Described/ MODY
p.Thr228Met	1	Described/ MODY, NDM
p.Cys230Phe	1	Novel
p.Ser263Pro	1	Described/ MODY
p.Gln287Leu	1	Described/ MODY
p.Arg303Gln	3	Novel/ Arg303Trp described
p.Gly448Ser	1	Novel
pLys15fs	1	Novel
IVS6+2T>A	1	Described/ MODY
p.M402fs	1	Novel
TOTAL	**30**	

Discussion and Conclusion

In this study MODY diabetes was diagnosed in 28% of the subjects in our cohort by NGS. We identified seven novel mutations in the *GCK* gene and one novel mutation in the *KLF11* gene (p.Asp370Gly).

In addition we identified 33 variants predicted to by pathogenic in 27 additional potential diabetes genes which will need further functional validation.

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M.G. Rees, JCI 2012. K.K. Osbak, Human Mutation 2009. C. Chambers, Pediar Diabetes 2015. S. Ellard, Diabetologia 2008. S.Ellard, Diabetologia 2013. E. De Franco, Lancet 2015.

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