Personalized and predictive medicine for pediatric diabetes through a genetic test using next generation sequencing

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Introduction

Monogenic diabetes (**MD**) accounts for at least 3% of all pediatric diabetes cases. **MD** is often misdiagnosed as type 1 or type 2 diabetes, because of its wide phenotypic spectrum. While clinical and biochemical parameters can suggest **MD**, a definitive diagnosis requires genetic analysis. We conducted a broad study to diagnose **MD** cases. Then, we designed a new diagnostic tool to obtain a comprehensive analytical instrument for the diagnosis of **MD**. A correct diagnosis of **MD** is crucial to optimize treatment and thereby improve metabolic control.

Objective and Methods

Diagnostic tool: This custom assay, designed based on liquid phase capture (Haloplex HS, Agilent, Santa Clara, CA, USA), allows for the trapping of all coding regions of the selected 42 genes and the respective splicing regions. Known enhancer regions and introns associated with diabetes were also included in the panel. All variants were confirmed by PCR and Sanger sequencing.

Results

Diagnostic panel

Here, we developed a new diagnostic panel of 42 genes. Mutations in several genes of this panel may lead to diabetes and/or congenital hyperinsulinism.

Table 1: Gene panel for the diagnosis of monogenic diabetes

Gene name	RefSeq	Chromosome	Theoretical coverage
HNF4A	NM_000457	Chr.20	100
GCK	NM_000162	Chr.7	100
HNF1A	NM_000545	Chr.12	100
PDX1	NM_000209	Chr.13	100
HNF1B	NM_000458	Chr.17	100
NEUROD1	NM_002500	Chr.2	100
KLF11	NM_003597	Chr.2	100
CEL	NM_001807	Chr.9	100
PAX4	NM_006193	Chr.7	100
INS	NM_000207	Chr.11	100
BLK	NM_001715	Chr.8	100
ABCC8	NM_000352	Chr.11	100
KCNJ11	NM_000525	Chr.11	100
SLC19A2	NM_006996	Chr.1	100
DNAJC3	NM_006260	Chr.13	100
PLAGL1	NM_001080954	Chr.6	100
GATA6	NM_005257	Chr.18	100
GATA4	NM_002052	Chr.8	100
SLC2A2	NM_000340	Chr.3	100
NKX2-2	NM_002509	Chr.20	100
NEUROG3	NM_020999	Chr.10	100
GLIS3	NM_152629	Chr.9	99.7
RFX6	NM_173560	Chr.6	100
MNX1	NM_005515	Chr.7	100
EIF2AK3	NM_004836	Chr.2	100
WFS1	NM_006005	Chr.4	100
IER3IP1	NM_016097	Chr.18	100
PAX6	NM_000280	Chr.11	100
FOXP3	NM_014009	Chr.X	100
STAT3	NM_139276	Chr.17	100
PCBD1	NM_000281	Chr.10	100
SIRT1	NM_012238	Chr.10	100
LRBA	NM_001199282	Chr.4	99.98
ZPF57	NM_001109809	Chr.6	100
PTF1A enhancer	hg19	Chr.10	96.6
INS intron	hg19	Chr.11	100
PPP1R15B	NM_032833	Chr.1	100
TRMT10A	NM_152292	Chr.4	100
KMT2D	NM_003482	Chr.12	98.87
KDM6A	NM_021140	Chr.X	100
RAP1A	NM_001010935	Chr.1	100
RAP1B	NM_015646	Chr.12	100
CISD2	NM_001008388	Chr.4	100
PTF1A	NM_178161	Chr.10	100

Custom-designed gene panel with 42 diabetes genes and known enhancer regions and introns with coverage of 99.89% of the targets. Chromosome: Chr.

The form to request insurance coverage for the analysis is available in "documents" on the webpage of the Swiss Society of Medical Genetics (SGMG), www.sgmg.ch). The request for the genetic analysis for monogenic diabetes is available at the following website http://www.hug-ge.ch/sites/interhug/files/structures/gr-demande__analyse/diagmol-std_e.pdf

Analysis by diagnostic panel

We have now analyzed the first 19 consecutive patients with the diagnostic tool and identified a monogenic disease in 53% of the subjects (Tbl 2).

Table 2: Genetic results obtained by the diagnostic panel

Patient ID	Gene	Gene defect	Protein effect	Pathogenicity*
1	*GCK*	c.1145G>A	p.Cys382Tyr	Likely pathogenic
2	*GCK*	c.43_46del	p.?	Pathogenic
3	*GCK*	c.1339dup	p.Arg447Profs*12	Pathogenic
4	*HNF1A*	c.526+2T>C	p.?	Likely pathogenic
5	*HNF1A*	c.608G>A	p.Arg203His	Likely pathogenic
6	*HNF1A*	c.809A>G	p.Asn270Ser	Pathogenic
7	*HNF1A*	c.1853_1854del	p.Pro618Argfs*30	Likely pathogenic
8	*ABCC8*	c.4516G>A	p.Glu1506Lys	Pathogenic
9	*PAX4*	c.652C>T	p.Arg218Cys	VUS
10	*EIF2AK3*	c.[2707C>T]; [2707C>T]	p. [Arg903*]; [Arg903*]	Pathogenic
11-19	*Neg*			

Deletions: del. Protein sequence: p. Coding DNA sequence: c.
We used the following analyses for the assessment of pathogenicity according to *Richards et al.

1. Exonic silent variants, if not located in the first or in the last codon of an exon, were discarded
2. All missense variants were evaluated according to their frequency in the general population (ExAC and gnomAD)
3. The pathogenic prediction was evaluated by different bioinformatics tools (SIFT, PolyPhen-2 and MutationTester)
4. The status regarding the pathogenicity according to ClinVar was searched
5. The conservation score by GERP was considered
6. The literature was checked whether the identified variants have been reported

Figure 1: Frequency of the genetic variants detected by the diagnostic panel

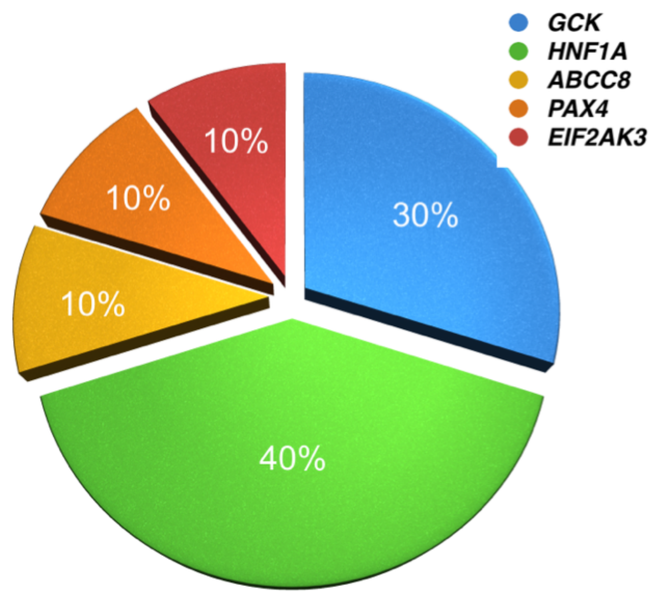
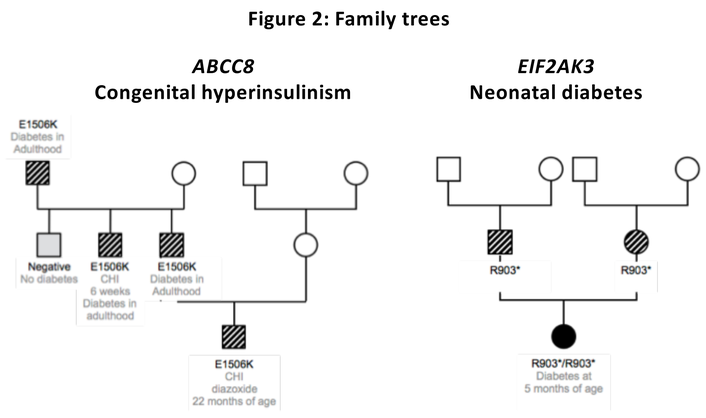


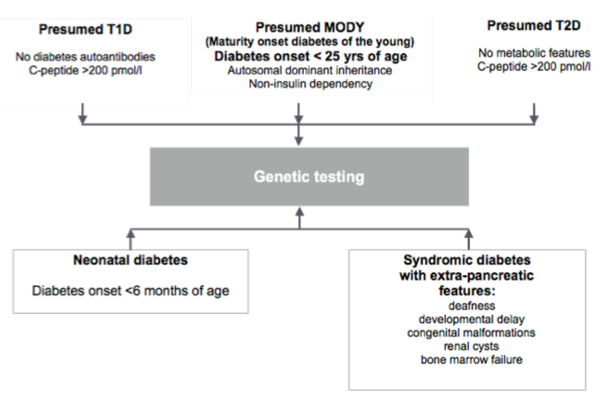
Figure 2: Family trees



Hatched symbols: subjects with identified *ABBC8* variant
Grey symbol: healthy subject negative for the *ABCC8* variant

Hatched symbols: heterozygous for *EIF2AK3* variant
Black symbol: homozygous for *EIF2AK3* variant
White symbols: healthy subjects without DNA analysis

Figure 3: An updated pathway for clinical decision-making for monogenic diabetes screening



For presumed autoantibody negative T1D cases, an additional indicator for genetic screening are persisting C-peptide levels after the honeymoon period of > 200 pmol/l with glucose > 8mmol/l, to avoid suppression of C-peptide levels by hypoglycemia. Type 1 diabetes: T1D; type 2 diabetes: T2D.

Conclusions

Our newly developed next generation diagnostic panel shows an actual pick-up rate of 53% in 19 consecutive patients, which is above the published rates of 21% to 37 % in the UK and 25% to 30% in France. The panel detects missense variants, insertions, and deletions, even large deletions extending from one exon to the entire gene. These results indicate that the three patients with *GCK* diabetes didn't need any treatment, the patients with *HNF1A* mutations could be switched to oral sulfonylurea or glinides. The child with congenital hyperinsulinism could successfully be treated by diazoxide. The child with neonatal diabetes due to the homozygous *EIF2AK3* mutation (Wolcott Rallison syndrome) needed insulin injections and multidisciplinary care to avoid fever, infections possibly preventing liver failure associated with the syndrome. These cases illustrate how applied precision medicine can tailor treatment to the needs of the individual patient with the aim to reduce short and long-term complications.

References

S. Kherra, J.L. Blouin, F. Santoni and V.M. Schwitzgebel. Precision medicine for monogenic diabetes: From a survey to the development of a next generation diagnostic panel, Swiss medical weekly (in press, 2017).
C.M. Stekelenburg, V.M. Schwitzgebel, Genetic Defects of the β-Cell That Cause Diabetes. Endocr Dev. 31, 179–202 (2016).
S. Richards *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*. **17**, 405–423 (2015).