The application of Next Generation Sequencing MODY Gene Panel in Greek Patients

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Introduction: Maturity Onset Diabetes of the Young (MODY) constitutes a genetically and clinically heterogeneous type of Monogenic Diabetes (MD). It is characterized by autosomal dominant inheritance, early onset of diabetes (≤25 years), defect in the β-cell insulin secretion, positive family history of diabetes, absence of diabetic ketoacidosis, auto-antibodies (ICA, anti-GAD or IAA), insulin resistance. Patients usually have normal Body Mass Index [1]. To date, 14 different MODY subtypes have been reported each one with a distinct genetic etiology [2]. The most common MODY subtypes are MODY1-*HNF4A*, MODY2-*GCK*, MODY3-*HNF1A* and MODY5-*HNF1B*.

Objective: To identify the molecular defect of 49 MODY patients employing the methodology of Next Generation Sequencing (NGS) Targeted Gene Panel.

Patients and Methods: We studied 49 patients who met MODY criteria (Table 1). A panel of seven MODY genes (***GCK, HNF1A, HNF4A, HNF1B, INS, ABCC8*** and ***KCNJ11***) sized 29.45kb with 98.87% *in silico* coverage was designed by the Thermo Fisher Scientific Ion AmpliSeq Designer platform (version 5.6) according to hg19. NGS was performed on the Ion Torrent Personal Genome Machine (PGM) platform (Thermo Fisher Scientific, Waltham, MA, USA) using the Ion PGM™ Hi-Q™ View Sequencing Kit and ion 314™ chip v2. Bioinformatic tools were used to test the pathogenicity of the new variants detected. The pathogenic variants detected in the patients and the parent with the MODY phenotype when available, were also tested by Sanger sequencing.

Table 1. Means values of clinical characteristics of 49 MODY patients.

Gender	M:27/F:22
Age of diabetes (years)	15±9
Glucose (mg/dl)	200±116
HbA1c (%)	7.5±2.3
BMI (kg/m^2)	20.8±4.6
Birth Weight (gr)	3156±678

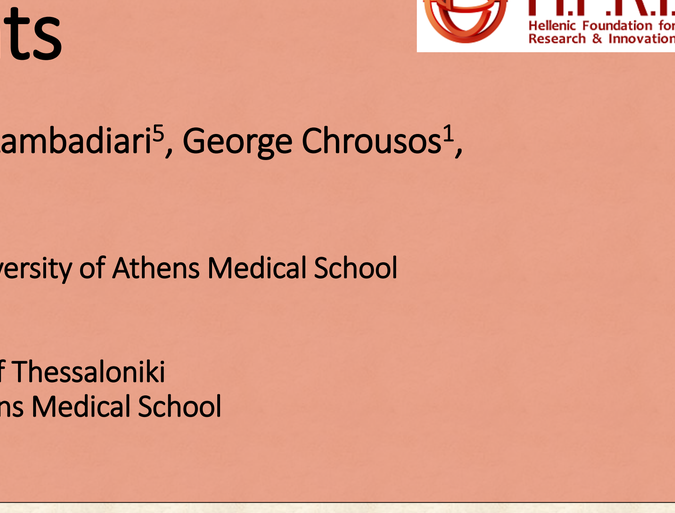

Figure 1. The minimum reads of depth of 49 MODY patients in the seven MODY genes of the panel.

Results: Thirteen pathogenic variants were identified in 12 of the 49 MODY patients tested (24%). The variants were: 2 nonsense, 10 missense and 1 splice site (Table 2). Four *novel* pathogenic variants were detected in the ***GCK* (p.Cys371X)**, ***HNF1A* (p.Asn402Tyr), *HNF4A* (p.Glu285Lys)** and ***ABCC8* (p.Met1513Thr)** genes. Four patients (33%) were found to be heterozygotes for *GCK* variants, two (16%) for *HNF1A* variants, one (8%) for *HNF4A* variant, one (8%) for *HNF1B* variant and five (42%) for *ABCC8* variants. **Interestingly, one patient was found to carry two different gene variants, one of the *GCK* gene (p.Tyr61X) and one of the A*BCC8* gene (p.Leu135Val)**. The combination of these two variants may lead to a reduced response of the β-cells at high glucose levels and a reduced insulin secretion. Two patients carried *de novo* pathogenic variants of the *GCK* gene (p.Ala259Thr) and *HNF4A* gene (p.Glu285Lys), respectively. No pathogenic variants were detected in the *KCNJ11* and *INS* genes.

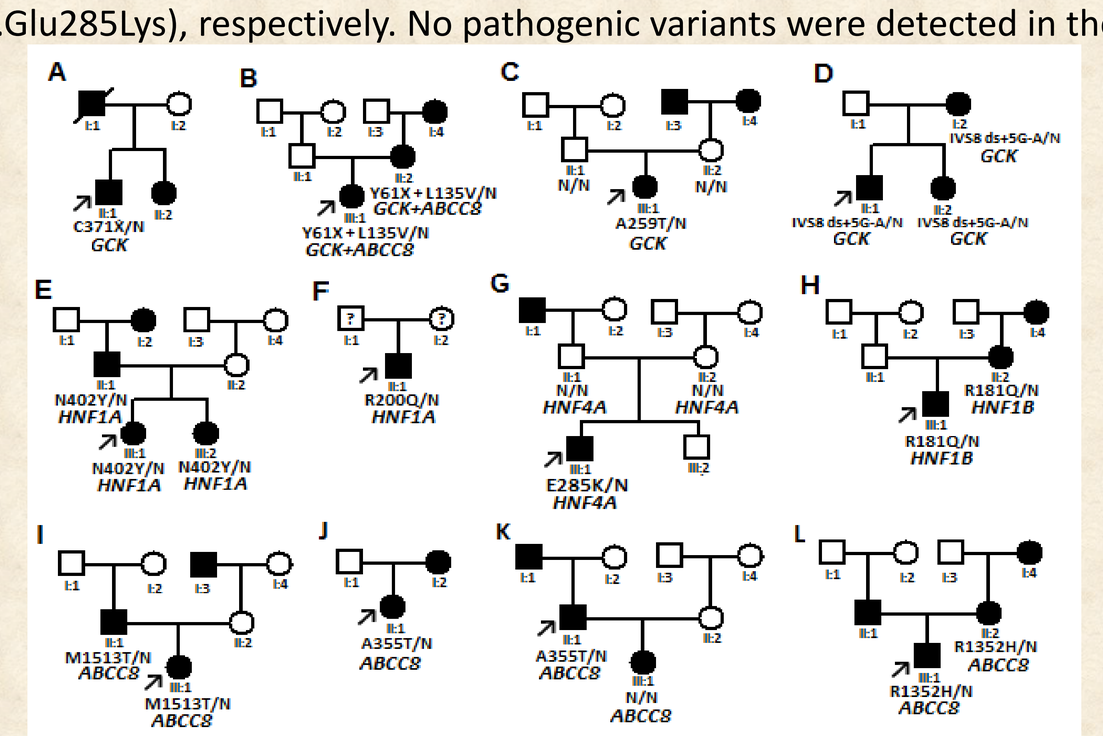

Figure 2. Pedigrees of the 12 patients were detected with a pathogenic variant.

Table 2. Heterozygous pathogenic variants detected by NGS in 12 MODY patients.

Number of patients	Gene	Reference Sequence	Variants		Reads of Depth		Novel
			in Protein	in cDNA	Normal Allele	Pathological Allele	
1	*GCK*	NM_000162	p.Cys371X	c.1113C>A	31	45	Yes
1*	*GCK*	NM_000162	p.Tyr61X	c.183C>A	159	172	No
1	*GCK*	NM_000162	p.Ala259Thr	c.775G>A	95	94	No
1	*GCK*	NM_000162	-	c.1019+5G>A	141	163	No
1	*HNF1A*	NM_000545	p.Asn402Tyr	c.1204A>T	194	209	Yes
1	*HNF1A*	NM_000545	p.Arg200Gln	c.599G>A	141	153	No
1	*HNF4A*	NM_000457	p.Glu285Lys	c.853G>A	19	15	Yes
1	*HNF1B*	NM_000458	p.Arg181Gln	c.542G>A	175	142	No
1	*ABCC8*	NM_000352	p.Met1513Th	c.4538T>C	50	63	Yes
2	*ABCC8*	NM_000352	p.Ala355Thr	c.1063G>A	150/227	139/215	No
1	*ABCC8*	NM_000352	p.Arg1352His	c.4055G>A	116	119	No
1*	*ABCC8*	NM_000352	p.Leu135Val	c.403C>G	185	197	No

*: one patient who carried two different pathogenic variants

Conclusions: The application of NGS targeted gene panel of 7 MODY genes offered genetic diagnosis in 24% of the patients tested and revealed four *novel* gene pathogenic variants and a digenic inheritance case. The majority (42%) of the detected pathogenic variants were in the *ABCC8* gene, indicating that MODY12 cases are probably more common than previously considered. Although a large number of MODY patients remain without the exact MODY type identification, the application of NGS methodology in diagnosis provides rapid results, is cost effective compared to Sanger sequencing and increases diagnostic accuracy. It is probable that the employment of a panel with more genes associated with monogenic diabetes will allow the molecular defect identification in more patients.

References:

[1] Brahm AJ, et al. Genetic Confirmation Rate in Clinically Suspected Maturity-Onset Diabetes of the Young. Can J Diabetes. 2016 ;40(6):555–60.

[2] Firdous P, et al. Genetic Testing of Maturity-Onset Diabetes of the Young Current Status and Future Perspectives. Front Endocrinol (Lausanne). 2018;9:253.

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