REFERENCE LABORATORY GENETICS

ENAC ISO15189 Nº 1065/LE2112

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GENETIC STUDY OF MATURITY ONSET DIABETES OF THE YOUNG (MODY) BY MASSIVE SEQUENCING (NGS)

Request No.:	000		
Client:	-		
Analysis code:	20124		
Patient Name:	xxx		
Date of Birth:	N/A	**Patient Ref.:**	xxx
Gender:	N/A	**Sample Type:**	Whole blood
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: N/A.

RESULT AND INTERPRETATION

The presence of a heterozygous likely pathogenic variant in the *GCK* gene that could explain the maturity onset diabetes of the young (MODY) hypothesis, has been identified. (See Recommendations).

The complete list of studied genes and coverage details is available in Table 1. (Methodology)

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
GCK	NM_000162.5:**c.661G>A p.(Glu221Lys)**	Heterozygosis	Autosomal Dominant	**Likely Pathogenic**

* Nomenclature according to HGVS v15.11
^ Based on the recommendations of the *American College of Medical Genetics and Genomics (ACMG)*

The ***GCK*** variant **c.661G>A p.(Glu221Lys)** is a *missense* that predicts an amino acid change from Glutamic acid to Lysine at position 221 of the protein. It is described in the HGMD database (CM970635) as a pathogenic variant and in ClinVar database (ID: 36241) as a pathogenic variant/Likely Pathogenic associated with maturity onset diabetes of the young (MODY). The variant is described in the dbSNP population database (rs193922317). The bioinformatic predictors (SIFT, Mutation Taster and Polyphen-2) estimate that the change would have a pathogenic effect. In the scientific literature consulted, the variant is reported in several articles

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in patients with maturity onset diabetes of the young (MODY) (PMID: 10694920, 30592380, 23295292, 28170077).

Based on these data, the variant is classified as a **Likely Pathogenic Variant.**

The GCK gene (OMIM: 138079) encodes the glucokinase protein. Pathogenic variants in the *GCK* gene are associated with late-onset non-insulin-dependent diabetes mellitus (OMIM: 125853), entity with an autosomal dominant inheritance pattern and with MODY type 2 (OMIM: 125851) entity with an autosomal dominant inheritance pattern.

RECOMMENDATIONS

In order to establish co-segregation with the disease and thus confirm the pathogenicity of a likely pathogenic variant, it must be studied in affected and unaffected relatives.

Genetic counselling should be offered to the patient by the prescriber physician. If additional information regarding the results or genetic counselling is required, the physician can contact our team at genetics@referencelaboratory.es.

CLINICAL NOTES AND OBSERVATIONS

Maturity-onset Diabetes of the Young (MODY)

Group of monogenic disorders due to primary defects in insulin secretion, with A: D inheritance pattern corresponding to non-autoimmune forms of diabetes. 40 genes related to the etiopathogenesis of diabetes have been described and 13 of which are causes of MODY. 20% of patients with MODY condition do not have pathogenic variants in the six most frequently implicated genes: *GCK* (Glucokinase gene, with expression in pancreatic b cells, hepatocytes and central nervous system), *HNF* (hepatocyte nuclear transcription factor and its three subunits *HNF1A*, *HNF4A* and *HNF1B*), Activating mutations in genes KCNJ11 and ABCC8, which form the ATP-sensitive K+channel (KATP channel), have been shown to cause transient or permanent neonatal diabetes and a neonatal hypoglycemia due to hyperinsulinism. Other genes than those mentioned may have overlapping clinical phenotypes. The exact incidence in the general population is unknown. It is estimated 1%. In cases where the family variant is known, prenatal diagnosis is possible, as well as the use of assisted reproduction techniques. El diagnóstico prenatal en sangre materna (NIPT) no es posible en la actualidad en ninguna de estas entidades. Currently prenatal diagnosis in maternal blood (NIPT) is not possible in any of these entities.

GCK Gene MODY 2

Due to GCK gene variants, located at 7p13. More than 600 variants have been described in this gene; some in a few patients and even in single families. These are variants in heterozygosis with loss of gene function. Heterogeneous phenotype with complete penetrance. It is a frequent entity, with a prevalence in the general population estimated at 1.1 /1000 live births.

Clinical condition: Moderate fasting hyperglycemia with 6.5% glycosylated hemoglobin (present at birth, which increases little with age). Hyperglycemia is usually stabilized, so clinical complications are rare. Low risk of vascular complications. Retinopathy may exist, although it is not frequent. Hyperglycemia may have a similar degree in other affected family members, so they may not be diagnosed. They are usually asymptomatic people with normal weight and height. Sometimes the diagnosis appears by chance or on

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pregnancy controls. The presence or absence of b cell antibodies makes it possible to distinguish the Diabetes type 1 from a GCK MODY.

Prenatal diagnostic aspects

If the fetus inherits a maternal variant in the *GCK* gene, maternal treatment is not necessary since endogenous insulin secretion will be established according to glucose level, as in the mother. En este caso el crecimiento del feto será normal. Si, por el contrario, el feto no presenta la variante materna, se necesita tratar a la madre, ya que el riesgo de macrosomía fetal es del 50%. In this case, fetal growth will be normal. If, on the contrary, the fetus does not have the maternal variant, the mother should be treated since the risk of fetal macrosomia is 50%.

The risk of recurrence for the patient is 50% in each pregnancy without being able to predict the clinical severity.

METHODOLOGY

DNA extraction and quantitative and qualitative evaluation of the DNA obtained.

Capture and enrichment of exonic regions and flanking intronic areas of genes contained in the REFLAB MedExome (Roche) sequencing panel with the Roche NimbleGen SeqCap EZ HyperCap Library™ technology.

Massive sequencing with the NextSeq™(Illumina) sequencer.

Identification of the variants of interest in regard to the reference genome (hg19) after filtering, according to specific quality criteria. Annotation of the obtained variants with a specific bioinformatic software: Alamut Visual™ (Interactive Biosoftware), Ingenuity Variant Analysis™ (QIAGEN), Variant interpreter™ (Illumina) and VarAFT™. The used reference databases have been the population databases dbSNP, 1000genomes, EXAC and gnomAD; the clinical databases Human Gene Mutation Database (HGMD version 2019.3), ClinVar and LOVD; the disease specific databases, if applicable, and Reference Laboratory Genetics´own databases. The bioinformatic analysis to evaluate the possible impact of the variants of interest on the structure and functionality of the protein has been carried out with the bioinformatic programs Mutation Taster, SIFT and PolyPhen-2. These analyses are only a predictive tool; they were not experimentally proven.

The nomenclature used to define the variants follows the criteria of the *Human Genome Variation Society (HGVS)* (http://www.HGVS.org/varnomen).

Classification of variants based on the recommendations of the *American College of Medical Genetics and Genomics (ACMG)* (Richards S. *et al*., 2015). Only those variants that, based on current information, are considered pathogenic, likely pathogenic or of uncertain clinical significance, are reported. (The complete list of identified variants is available upon request).

The obtained average reading depth was 173,90x being > 20x in 99,30% of the regions analysed.

The reported INDEL variants are confirmed by Sanger sequencing.

LIMITATIONS: The results obtained do not exclude variants outside the analysed regions of the genome or genetic anomalies not detectable by massive sequencing such as large rearrangements, large deletions/duplications (Copy Number Variant; CNV), insertions / deletions of> = 10 nucleotides, variants in repetitive regions or with a high percentage of GC, and variants in genes with pseudogenes with highly homologous sequences.

It is not possible to rule out the presence of variants in other unanalysed genes.

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Table 1. List of reported genes and coverage details

Gene	NM	10x %	Exons with coverage < 100%*
BLK	NM_001715	100,00	-
GCK	NM_000162	100,00	-
HNF1A	NM_000545	100,00	-
HNF1B	NM_000458	100,00	-
HNF4A	NM_175914	100,00	-
KCNJ11	NM_000525	100,00	-
KLF11	NM_003597	100,00	-
NEUROD1	NM_002500	100,00	-
PAX4	NM_006193	100,00	-
PDX1	NM_000209	100,00	-
SLC2A2	NM_000340	100,00	-

*Due to the current intrinsic limitations associated with massive sequencing technology, some gene exons analysed may be insufficiently covered. If it is considered appropriated by a medical specialist, it would be possible to sequence exons with coverage below 100% using the Sanger method or other alternative molecular technique.

IMPORTANT NOTE

The information contained in this report is based on current scientific knowledge and the results obtained from the application of the technology in this report, are detailed. Due to continuous advances, the documented information may be modified in the future as a result of the emergence of new scientific evidence.

The genetic/genomic studies carried out by Reference Laboratory S.A. are exclusively intended for qualified health professionals for their interpretation. The results obtained are not, per se, a medical consultation, diagnosis or treatment, nor should they be interpreted as such. Only a specialized professional can correctly interpret the results and offer a diagnosis or prescribe a treatment to a patient based on these. Consequently, no information obtained from our studies can be used to replace the advice and diagnosis of a specialized professional.

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