International Journal of Scientific Research and Engineering Development-– Volume 4 Issue 1, Jan-Feb 2021

Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

KCNJ11: Genetic Polymorphisms and Risk of Maturity Onset Diabetes of the Young (MODY) and early-onset type 2 diabetes

Said Trhanint[1*], Laila Bouguenouch[1, 2], Ihssane El Bouchikhi[1], Mohamed Ahakoud[1], Sana Abourazzak[2, 3], Hanan El ouahabi[2, 4], Hanane Latrech[5], Karim Ouldim[1, 2],

Lahsen El Ghadraoui[6], Nadia Maazouzi[6].

[1*]Laboratory of Medical Genetics and Oncogenetics, Hassan II University Hospital, Fez Morocco.

[2]Faculty of Medicine and Pharmacy of Fez, University of Sidi Mohammed Ben Abdellah, Fez, Morocco.

[3]Pediatric service of Hassan II University Hospital, Fez, Morocco.

[4]Endocrinology Service of Hassan II University Hospital, Fez, Morocco.

[5]Endocrinology Service of Mohammed VI University Hospital, Oujda, Morocco.

[6]Faculty of Sciences and Techniques, University of Sidi Mohammed Ben Abdellah, Fez, Morocco.

***Corresponding author:**

Dr. Said Trhanint
Laboratory of Medical Genetics and Oncogenetics,
Hassan II University Hospital, Fez, Morocco
Phone (or Mobile) No.: +212 675 055 110

----------**********----------

Abstract:

Background: KCNJ 11 gene encodes the Kir 6.2 subunit of ATP- sensitive potassium channel which is a critical regulator of pancreatic beta-cell insulin secretion. Genetic screening and analyses have helped several patient carrying mutations in the KCNJ11 and ABCC8 genes to shift from insulin treatment to oral sulphonylurea drugs.

The ATP-sensitive potassium channel plays a central role in stimulating the secretion of insulin in response to glucose by the beta-pancreatic cells. Heterozygous activating mutations in KCNJ11 have been reported as a cause of not only permanent neonatal diabetes, but also MODY and adult-onset diabetes in a number of studies.

Methods: We performed a Sanger sequencing investigation of KCNJ11 exon and promoter. We report a Moroccan family of three generations with several cases affected by this disease.

Results: The index case is a patient who presented hyperglycemia (12.94 mmol / L, without ketosis) requiring a continuous insulin infusion and with a normal body mass index (BMI). Genetic analysis revealed three variants V337I, E23K and S385C, previously reported in the literature. Two variants were also identified in the mother with type 2 diabetes, with a transmission model suggesting a dominant inheritance

Conclusions: Sequencing of the KCNJ11a gene leads to a correct molecular diagnosis of diabetes, while bioinformatics analysis has indicated the possible molecular causes of the dysfunction of KCNJ11. Knowledge of molecular diagnosis has made it possible to provide appropriate therapeutic care for this patient. Sanger sequencing is the most reliable method for MODY diagnosis. A correct molecular diagnosis helps in the optimal treatment of disease.

Keywords — MODY, KCNJ11, Polymorphisms, Early-onset type 2 diabetes.

----------**********----------

I. INTRODUCTION

Among the Diabetes Mellitus (DM) caused by genetic defects of the pancreatic beta cells, the most mentioned correspond to the MODY (Maturity-Onset Diabetes of the Young).[1] They are characterized by autosomal dominant transmission mutations that alter insulin secretion, with variable grade of hyperglycemia, generally of early appearance (before 25 years). This disorder does not present an association with Metabolic Syndrome or specific antibodies. It is estimated that this group could correspond to 5% of the total of diabetic patients.

At least 14 different genes have been described for the disease (Table 1). The most frequent are the heterozygous mutations of the Glucokinase gene (GCK) resulting in MODY type 2 and thenuclear hepatocyte factor 1-α gene (HNF-1 α) leading to MODY type 3.

The ABCC8 (ATP Binding Cassette Subfamily C Member 8) andKCNJ11 (Potassium Inwardly Rectifying Channel Subfamily J Member 11) genes encode the SUR1 and Kir6.2 subunits of the ATP-sensitive potassium channel (KATP) in the pancreatic cells, which has a direct role in the regulation of insulin release. Dominant and recessive activatingmutations in the KATP channel genes cause neonatal diabetes in the first six months of life, which can be permanent or transient.[2] Both forms respond to high-dose sulfonylurea therapy.[3] In contrast, recessive mutations inducing loss of function in ABCC8 and KCNJ11 are the most common cause of congenital hypoglycemic hyperinsulinism.[4] The ABCCC8 and KCNJ11 mutations are classified as a rare cause of MODY.

Many forms of diabetes have been reported in people who carry mutations in these genes. It may include type 2 diabetes,and also gestational diabetes.[5] This very large variability in the phenotype, particularly marked for the ABCC8 mutations, has not been convincingly explained to date. In addition, mutations in ABCC8 responsible for diabetes that may suggest MODY have been described in subjects with no known personal or family history of neonatal diabetes or neonatal hyperinsulinism.[6]

TABLE I: THE CAUSATIVE GENES FOR MATURITY-ONSET DIABETES OF THE YOUNG (MODY) AND PATHOPHYSIOLOGY ASSOCIATED WITH EACH MODY SUBTYPE

Subtype	Location	ID gene	Pathophysiology
MODY1	20q13.12	HNF4α	β-Cell dysfunction
MODY2	7p13	GCK	Glucose sensing defect
MODY3	12q24.31	HNF1α	β-Cell dysfunction
MODY4	13q12.2	PDX1	β-Cell dysfunction
MODY5	17q12	HNF1β	β-Cell dysfunction
MODY6	2q31.3	NEURO D1	β-Cell dysfunction
MODY7	2p25.1	KLF11	β-Cell dysfunction
MODY8	9q34.13	CEL	Pancreas endocrine and exocrine dysfunction
MODY9	7q32.1	PAX4	β-Cell dysfunction
MODY10	11p15.5	INS	Insulin gene mutation
MODY11	8p23.1	BLK	Insulin secretion defect
MODY12		ABCC8	ATP-sensitive potassium channel dysfunction
MODY13	11p15.1	KCNJ11	ATP-sensitive potassium channel dysfunction
MODY14	3p14.3	APPL1	Insulin secretion defect

(adapted from Online Mendelian Inheritance in Man-http: // www.ncbi.nlm.nih.gov/omim?term=MODY).

II. CASE REPORT

The patient is a 30-year-old, previously healthy, non-obese female with no past medical history. Her height and weight were 163 cm and 60 kg, respectively, and her body mass index was at 22.64.

Vital signs of the proband were within normal limits, her blood pressure was not elevated. Her screening lipid panel was within normal limits (cholesterol 3.55 mmol/L, LDL 1.91 mmol/L, HDL 1.4 mmol/L, and triglycerides 1.30mmol/L).And her glycated haemoglobin (HbA1c) ranged between 7% and 8%. Anti- GADA and Anti IA2 were absent (Tables 2).

TABLE 2: CLINICAL AND METABOLIC CHARACTERISTICS OF THE PATIENT.

Features	HNFA-MODY
Age at clinical diagnosis (years)	28
Age at molecular screening (years)	30
BMI (kg/m2)	22.64
HbA1c (%)	8%
FastingGlycaemiammol/L	12.94
Total Cholesterolmmol/L	3.55
HDLmmol/L	1.4
LDLmmol/L	1.91
Autoimmune markers (GAD/IA2)	Negative
Treatment	Insulin

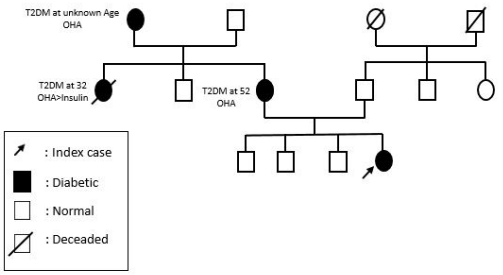

Fig. 1: Pedigree structure of the studied family.

III. METHODS

DNA extraction

Genomic DNA was extracted from blood samples using the Genomic DNA kit (Invitrogen).

DNA concentration and purity were determined using Nanovue spectrophotometer (Thermofisher Scientific).

Sanger sequencing

PCR amplification and Sanger sequencing standard protocols were carried out to detect the selected candidate variants of interest in the probandand their parents.In brief, the unique exon of KCNJ11and its promoter were amplified from DNA samples by polymerase chain reaction (PCR) using oligonucleotide primers designed by Primer3 software (available upon request). PCR products were first purified using the "exosap" kit.Then, theresulting amplicons were sequenced on automated ABI3500 dx (ABI Prism, Applied Biosystems, Massachusetts, MA, and USA) using the ABI prism Big Dye terminator v3.1 cycle sequencing kit. Sequence analysiswas performedusing SeqA5 software.

Bioinformatics analysis

The obtained sequences were compared to the KCNJ11 reference sequence (NM_000525.3) using "Nucleotide Blast alignment program" tool(http://blast.ncbi.nlm.nih.gov) (NCBI, Maryland, MD, and USA).The pathogenicity of the missense variants was assessedin silico by four prediction tools using a pathogenicityscore namely, Mutation taster, PolyPhen-2, Provean protein and SIFT.

IV. RESULTS

The clinical characteristics of patient are summarized in (Table 2).

We have sequenced the unique exon and promoter of the KCNJ11 region of this patient and their parents. Sequence analysis of KCNJ11 in the DNA of the proband did not reveal any mutation in the promoter, while

three variants were detected in the coding region (Table 3).Summarizes thefeatures of the commonKCNJ11 genetic variants foundin our patient, which arethe amino acid substitutions previously identified, E23K,S385C and V337I.

V. DISCUSSION

MODY13 is caused by a mutation in the KCNJ11 gene (OMIM: 616329), which was first reported by(Yorifuji et al. 2005),[7] and (Bonnefond et al. 2012)[8]who described a 4-generation French family with 12 members affected with MODY.

In our pedigree (Figure 1), all affected family members were overweight at diagnosis,suggesting a T2D.The three retained variants in our patient were located in the KCNJ11 gene which encodes the Kir6.2 subunitof the ATP-sensitive potassium channelprotein in the pancreatic beta cells. Insulin secretion is mediated through the ATP-sensitive potassium channel in pancreatic beta cells.The V337I and E23K variants of the *KCNJ11* gene, which have been identified in the index patient, are also found in her diabetic mother.

ConcerningKCNJ11 E23K. This variant is located in exon 1 of the*KCNJ11* gene. Substitution of A to G at nucleotide position 67 changesthe amino acid from lysine to glutamine (Lys23Gln) at theNH2-terminal tail of Kir6.2.

We verified the effect of this variant on the proteinfunction using bioinformatics prediction tools (PolyPhen-2, PROVEAN, sift and mutation taster). PROVEAN and SIFT programs show that this is a neutral variant. While the PolyPhen-2 program gives a score of 0, which means that this variant is benign. From these results, we could conclude that the E23K variant is a polymorphism with no pathogenic impact on carriers. Moreover, a previous study show this amino acid substitutiondoes not make any remarkable change in structureor function of the KCNJ11 protein.[9]However, the study of Haghvirdizadeh et al. has shown that the E23K variant can modify the charge at the ATP-binding region. At this level,twenty-four association studies and a recent meta-analysis showed a strong relationship between the E23K polymorphism and sensitivity to T2DM, while 21 studies did not confirm this conclusion.[10]

In the case of the variant c.1009G> A GTC → ATC, PROVEAN and SIFT gives a score of (-0.102) signifying that this variant is neutral. While Polyphen-2 gives a score of 0.004 showing a benign effect (Table 3). We could conclude, from these results, that the V337I variant is a polymorphism which has no pathogenic impact on subjects carrying KCNJ11-MODY.However, of thirteen studies on Diabetes Mellitus, three showed a strong association between this variant andType 2 diabetes.[11]Whereas the remaining studies showed noassociation with Type 2 diabetes,Type 1 diabetes, or Gestational diabetes mellitus.[10]In another study, the V337I polymorphism was associatedwith blood pressure among subjects with Type 2 diabetes.[12]

We also detected a KCNJ11 heterozygous variantc.1154C>G that results in S385C substitution.After bioinformatics analysis of the S385C variant, PROVEAN gives a score of -1.703, which means that the variant is neutral. However, since the score is close to the threshold score (-2.5), this suggests that this variant may have a pathogenic effect. While Polyphen-2 gives a score of 0.380 showing a benign effect, (Table 3) and MutationTaster gives "disease causing". These different findings lead us to suggest that the S385C variant would have a predisposing effect on monogenic diabetes.

KCNJ11 polymorphisms rs5219 (E23K), rs5215 (V337I) and rs41282930 (S385C) are three commonmissense polymorphisms that have been observed in *KCNJ11*geneof our index patient and has reported to increase the risk of T2D in multiplestudies including large scale genetic studies.[13, 14, 15]

TABLE 3: SUMMARY OF THE MUTATIONS REPORTED FOR THE KCNJ11 GENE

Number	**SNP**	**DNA level**	**Gene**	**Amino acid**	**Type de mutation**	**Family history**	**Sift**	**PolyPhen-2**	**PROVEAN**	**Mutation taster**
1	rs5215	c.1009G>A	Exon	V337I	missense	Yes mother	Tolerated	BENIGN with a score of 0.004	Neutral (-0.102)	polymorphism
2	rs5219	c.67A>G	Exon	E23K	missense	Yes mother	Tolerated	BENIGN with a score of 0.000	-	polymorphism
3	rs41282930	c.1154C>G	Exon	S385C	missense	No	deleterious	BENIGN with a score of 0.380	Neutral (-1.703)	Disease causing

VI. CONCLUSION

Our results suggest that genetic diagnosis of the KCNJ11 variants could help understand the molecular etiology, and more, provide personalized treatment for patients with this specific form of diabetes.

Finally, we recommend conducting a genetic study by exploring other MODY diabetes genes in patients with mild hyperglycemia or slightly elevated HbA1c, absence of specific complications, negative autoimmunity study and presence of DM over at least 2 generations.

ACKNOWLEDGEMENT

We thank all the patients and their families for their invaluable contribution to this study.

DECLARATIONS

Funding: This work was financially supported by Hassan II University Hospital and Faculty of Medicine and Pharmacy of Fez, University of Sidi Mohamed Ben Abdellah, Morocco.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: This study was approved by the Ethics Committee of Hassan II University Hospital of Fez (N°: 10/17) and conducted after obtaining written informed consent from patients or their parents (in case of children).

REFERENCES

[1] American Diabetes Association. 2014. « Diagnosis and Classification of Diabetes Mellitus ». *Diabetes Care* 37 Suppl 1 (janvier): S81-90. https://doi.org/10.2337/dc14-S081.

[2] Flanagan, S. E., E. L. Edghill, A. L. Gloyn, S. Ellard, et A. T. Hattersley. 2006. « Mutations in KCNJ11, Which Encodes Kir6.2, Are a Common Cause of Diabetes Diagnosed in the First 6 Months of Life, with the Phenotype Determined by Genotype ». *Diabetologia* 49 (6): 1190‑97. https://doi.org/10.1007/s00125-006-0246-z.

[3] Pearson, Ewan R., Isabelle Flechtner, Pål R. Njølstad, Maciej T. Malecki, Sarah E. Flanagan, Brian Larkin, Frances M. Ashcroft, et al. 2006. « Switching from Insulin to Oral Sulfonylureas in Patients with Diabetes Due to Kir6.2 Mutations ». *The New England Journal of Medicine* 355 (5): 467‑77. https://doi.org/10.1056/NEJMoa061759.

[4] Kapoor, R. R., S. E. Flanagan, C. James, J. Shield, S. Ellard, et K. Hussain. 2009. « HyperinsulinaemicHypoglycaemia ». *Archives of Disease in Childhood* 94 (6): 450 ‑ 57. https://doi.org/10.1136/adc.2008.148171.

[5] Babenko, Andrey P., Michel Polak, Hélène Cavé, KaneteeBusiah, Paul Czernichow, Raphael Scharfmann, Joseph Bryan, Lydia Aguilar-Bryan, Martine Vaxillaire, et Philippe Froguel. 2006. « Activating Mutations in the ABCC8 Gene in Neonatal Diabetes Mellitus ». *The New England Journal of Medicine* 355 (5): 456 ‑ 66. https://doi.org/10.1056/NEJMoa055068.

[6] Riveline, Jean-Pierre, Elise Rousseau, Yves Reznik, Sabrina Fetita, Julien Philippe, Aurélie Dechaume, Agnès Hartemann, et al. 2012. « Clinical and Metabolic Features of Adult-Onset Diabetes Caused by ABCC8 Mutations ». *Diabetes Care* 35 (2): 248 ‑ 51. https://doi.org/10.2337/dc11-1469.

[7] *Yorifuji, Tohru, KazuakiNagashima, KeijiKurokawa, Masahiko Kawai, Mariko Oishi, YoshiharuAkazawa, Masaya Hosokawa, YuichiroYamada, NobuyaInagaki, et TatsutoshiNakahata. 2005. « The C42R Mutation in the Kir6.2 (KCNJ11) Gene as a Cause of Transient Neonatal Diabetes, Childhood Diabetes, or Later-Onset, Apparently Type 2 Diabetes Mellitus ». The Journal of Clinical Endocrinology and Metabolism 90 (6): 3174 ‑78. https://doi.org/10.1210/jc.2005-0096.*

[8] Bonnefond, Amélie, Julien Philippe, Emmanuelle Durand, Aurélie Dechaume, Marlène Huyvaert, Louise Montagne, Michel Marre, et al. 2012. « Whole-Exome Sequencing and High Throughput Genotyping Identified KCNJ11 as the Thirteenth MODY Gene ». *PloS One* 7 (6): e37423. https://doi.org/10.1371/journal.pone.0037423.

[9] Harakalova, Magdalena, Jeske J. T. van Harssel, Paulien A. Terhal, Stef van Lieshout, Karen Duran, Ivo Renkens, David J. Amor, et al. 2012. « Dominant Missense Mutations in ABCC9 Cause Cantú Syndrome ». Nature Genetics 44 (7): 793 ‑ 96. https://doi.org/10.1038/ng.2324.

[10] Haghvirdizadeh, Polin, Zahurin Mohamed, Nor Azizan Abdullah, PanteaHaghvirdizadeh, Monir Sadat Haerian, etBatoul Sadat Haerian. 2015. « KCNJ11: Genetic Polymorphisms and Risk of Diabetes Mellitus ». *Journal of Diabetes Research* 2015: 908152. https://doi.org/10.1155/2015/908152.

[11] *Chavali, Sreenivas, Anubha Mahajan, RubinaTabassum, Om Prakash Dwivedi, Ganesh Chauhan, Saurabh Ghosh, Nikhil Tandon, et DwaipayanBharadwaj. 2011. « Association of Variants in Genes Involved in Pancreatic β-Cell Development and Function with Type 2 Diabetes in North Indians ». Journal of Human Genetics 56 (10): 695 ‑ 700. https://doi.org/10.1038/jhg.2011.83.*

[12] 12.Koo, B. K., Y. M. Cho, B. L. Park, H. S. Cheong, H. D. Shin, H. C. Jang, S. Y. Kim, H. K. Lee, et K. S. Park. 2007. « Polymorphisms of KCNJ11 (Kir6.2 Gene) Are Associated with Type 2 Diabetes and Hypertension in the Korean Population ». *Diabetic Medicine: A Journal of the British Diabetic Association* 24 (2): 178 ‑ 86. https://doi.org/10.1111/j.1464-5491.2006.02050.x.

[13] 13.Hansen, Sara K., Eva-Maria D. Nielsen, JakobEk, Gitte Andersen, Charlotte Glümer, Bendix Carstensen, Peter Mouritzen, et al. 2005. « Analysis of Separate and Combined Effects of Common Variation in KCNJ11 and PPARG on Risk of Type 2 Diabetes ». *The Journal of Clinical Endocrinology and Metabolism* 90 (6): 3629 ‑ 37. https://doi.org/10.1210/jc.2004-1942.

[14] 14.Hani, E. H., P. Boutin, E. Durand, H. Inoue, M. A. Permutt, G. Velho, et P. Froguel. 1998. « Missense Mutations in the Pancreatic Islet Beta Cell Inwardly Rectifying K+ Channel Gene (KIR6.2/BIR): A Meta-Analysis Suggests a Role in the Polygenic Basis of Type II Diabetes Mellitus in Caucasians ». *Diabetologia* 41 (12): 1511‑15. https://doi.org/10.1007/s001250051098.

Gloyn, Anna L., Michael N. Weedon, Katharine R. Owen, Martina J. Turner, Bridget A. Knight, Graham Hitman, Mark Walker, et al. 2003. « Large-Scale Association Studies of Variants in Genes Encoding the Pancreatic Beta-Cell KATP Channel Subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) Confirm That the KCNJ11 E23K Variant Is Associated with Type 2 Diabetes ». *Diabetes* 52 (2): 568 ‑ 72. https://doi.org/10.2337/diabetes.52.2.568.

