Iranian J Publ Health, Vol. 43, No.7, Jul 2014, pp. 1011-1013

Letter to the Editor

Challenges for Molecular Diagnosis of Familial Early-Onset Diabetes in Unexplored Populations

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(Received 12 May 2014; accepted 26 May 2014)

Dear Editor in in Chief

Understanding the genetic causes of monogenic forms of diabetes is important for prognostic, follow up, and treatment of the patients (1). MODY (Maturity-onset diabetes of the young) is a form of monogenic diabetes clinically and genetically heterogeneous. Indeed, to-date there are at least 13 different forms of MODY, each one is due to mutations in a specific gene (MODY1-*HNF4A*, MODY2-*GCK*, MODY3-*HNF1A*, MODY4-*IPF1*, MODY5-*HNF1B*, MODY6-*NEUROD1*, MODY7-*KLF11*, MODY8-*CEL*, MODY9-*PAX4*, MODY10-*INS*, MODY11-*BLK*, MODY12-*ABCC8* and MODY13-*KCNJ11*) (2). Besides, MODY is not due to a recurrent mutation (1). Molecular diagnosis of MODY is often based on mutation screening using Sanger sequencing and MLPA (Multiplex ligation-dependent probe amplification) (3). In the Tunisian population and in other populations, the genetic causes of familial early-onset diabetes remain unknown (3).

In this report, we described the difficulties encountered in determining MODY genetic etiologies, after a mutation screening of *PAX4* (Paired box gene 4) gene in six MODY-X Tunisian families, using classical methods and suggesting some solutions.

The studied families had been previously found negative for mutations and deletions in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *INS*, *IPF1* and *NEUROD1* genes (3). The molecular analysis was carried out after taking informed written consent from all patients and after approval of the local ethics committee.

We chose to sequence *PAX4* gene because of the resemblance between the clinical characteristics of the six families' probands (Table 1) and the reported MODY9-*PAX4* patients (4, 5). Moreover, *PAX4* gene encodes a transcription factor which is part of the regulatory mechanism balancing the development of pancreatic beta cells (6).

The sequencing of all nine exons of *PAX4* showed four common polymorphisms and no causal mutations (Table 1). Thus, the six families were negative for an eighth gene out of thirteen (reported to be responsible for MODY forms). The familial early-onset diabetes observed in these patients could be explained by mutations in other reported genes (*ABCC8*, *KCNJ11*…) or in unknown genes. This clearly shows the difficulty and time consuming to determine the molecular causes behind MODY-X using Sanger sequencing based on clinical findings. Hence, the need for fast

and less expensive techniques that screen all 13 known MODY genes in one step. PCR enrichment in microdroplets combined with next generation sequencing, or targeted next generation sequencing assay could be used as a first step in the research of mutations in known MODY genes (7, 8). In case of mutation absence in these genes, whole exome and genome sequencing combined with high-throughput genotyping and linkage analysis could be useful to identify new genes responsible for monogenic diabetes (2). Otherwise, setting up a research strategy based on metabolomics and biomarkers might be of great interest for molecular diagnostic of MODY.

The genetic etiologies of familial early-onset diabetes are still unidentified in many populations. Undeniably, introducing a next generation sequencing-based strategy will reduce costs, increase the throughput, allow gaining precious time and elucidate new pathways underlying the pathophysiology of the disease.

Table 1: The clinical and molecular characteristics of Tunisian patients selected for *PAX4* mutation screening and MODY9 patients of other studies

Study	Patient	Age at diagnostic	Diabetic generations	BMI Kg/m²	Glycaemia mmol/l	HbA1c %	Traitement	*PAX4* SNPs	Consequence
Our study	F2	16	3	25,3	14,1	12.8	INS	rs327516 rs2233579 rs712701	c.-221G>C c.412+35G>A p.H321P
	F4	27	2	23,5	6,2	6	INS	rs327516	c.-221G>C
	F5	24	3	22,8	10,6	6.6	OHA-> INS	rs327516 rs2233579 rs712701	c.-221G>C c.412+35G>A p.H321P
	F6	29	3	18	12,2	14.9	Diet -> INS	rs327516 rs712701	c.-221G>C p.H321P
	F8	26	2	37,2	12,1	11.3	OHA-> INS+OHA	rs327516 rs2233578 rs2233579	c.-221G>C p.R133W c.412+35G>A
	F15	22	3	23,5	9	8.2	INS	rs327516 rs712701	c.-221G>C p.H321P
Thai study[4]	Proband	20	2	--	--	--	OHA	--	p.R164W
Japanese study[5]	Proband father	15 30	2 2	18 32	35 --	14.5 --	INS Diet	--	c.374-412del39

BMI = Body Mass Index, **HbA1c** = Glycosylated haemoglobin, **OHA** = Oral Hypoglycemic Agents, **INS** = Insulin

Acknowledgements

The authors declare that there is no conflict of interests.

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