Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

Unexpected finding of a whole *HNF1B* gene deletion during the screening of rare MODY types in a series of Brazilian patients negative for *GCK* and *HNF1A* mutations

Renata P. Dotto [a,1], *Fernando M.A. Giuffrida* [b,1], *Luciana Franco* [a], *Andreia L.G. Mathez* [a], *Leticia S. Weinert* [c], *Sandra P. Silveiro* [c], *Joao R. Sa* [d], *Andre F. Reis* [a,*], *Magnus R. Dias-da-Silva* [a]

[a] *Laboratory of Molecular and Translational Endocrinology, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil*
[b] *Universidade do Estado da Bahia (UNEB), Salvador, Brazil*
[c] *Endocrinology Unit – Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil*
[d] *Diabetes Center, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil*

ARTICLE INFO

Article history:
Received 7 April 2016
Accepted 17 April 2016
Available online 26 April 2016

Keywords:
MODY
Diabetes mellitus
MLPA
HNF1B
Mutation

ABSTRACT

Thirty-two patients with diabetes negative for point mutations in *GCK* and *HNF1A* underwent further molecular screening of *GCK*, *HNF1A*, *HNF4A*, and *HNF1B* by MLPA analysis. We described the first Brazilian case of MODY5 due to a heterozygous whole-gene deletion in *HNF1B*, who developed rapidly progressive renal failure and death.

1. Introduction

An ever growing number of Maturity-Onset Diabetes of the Young (MODY) subtypes have emerged, there being at least 14 described to date. More common forms such as *GCK*, *HNF1A*, and *HNF4A* comprise the majority of MODY cases [1–9].

Genetic testing for MODY has become a challenge, especially for rarer subtypes and for the completeness of mutation screening using traditional methods, either Sanger sequencing or multiplex ligation-dependent probe amplification (MLPA) [10]. Among them, MODY caused by hepatocyte nuclear factor-1 homeobox B (*HNF1B*) mutations has been described more often lately, due to the larger number of tested individuals. Besides, HNF1B-MODY has a markedly heterogeneous phenotype, making clinical selection of individuals for genetic testing more difficult [11]. Routine genetic

* *Corresponding author at:* Universidade Federal de São Paulo (UNIFESP), Diabetes Center, R. Estado de Israel 639-Vila Mariana, São Paulo, SP 04022-001, Brazil. Tel.: +55 11 55764856.
E-mail address: afreis2005@gmail.com (A.F. Reis).
[1] These authors contributed equally to this paper.
http://dx.doi.org/10.1016/j.diabres.2016.04.035
0168-8227/

testing for MODY is run in many labs in such a way that when mutations are not identifiable by Sanger, testing is completed by adding MLPA screening, aimed at detecting gross gene insertions and deletions.

In this article, we aim to investigate large mutations in MODY genes by applying MLPA technique in a cohort of cases negative for point mutations in *GCK* and *HNF1A* genes. We were able to diagnose the first Brazilian patient with an *HNF1B* whole-gene deletion, whose familial phenotype was not typically associated with this molecular diagnosis.

2. Methods

2.1. Subjects

We recruited 32 clinically defined MODY patients [1] and 20 healthy non-diabetic control subjects. MODY patients have been followed at the Diabetes Centre of UNIFESP and the Endocrinology Unit of UFRGS. Peripheral blood samples were collected after signed written informed consent was obtained (local Ethics Committee number – 853607).

2.2. Molecular analysis

Genomic DNA was extracted from blood leucocytes using in-house methodology as previously described [12]. Genetic analysis of *GCK* and *HNF1A* by Sanger Sequencing was performed as previously described [10]. Thirty-two suspected MODY cases negative for point mutation were further tested using MLPA (P241-D2 MODY®) probemix containing probes for *GCK*, *HNF1A*, *HNF1B*, and *HNF4A* genes, then analyzed using Coffalyser ® software.

3. Results

One out of 32 MODY suspected subjects showed a genetic defect detected by MLPA. The observed genetic disruption comprised the deletion of all exons 1 to 9 of *HNF1B*, yielding the mutation p.Met1_Trp557del, as demonstrated in Fig. 1. No mutation was found in his mother and sister.

The patient was a 39-year-old male, body mass index (BMI) 18.1 kg/m^2, who had been admitted to the emergency room of Hospital São Paulo at UNIFESP. He reported a diabetes diagnosis during a routine examination 15 years before, when he was instructed to use insulin. He also reported diabetes in his mother and maternal grandfather, but he was unaware of age at diagnosis in both. His mother, with BMI 28.8 kg/m^2, has been irregularly treated with metformin and insulin. The patient himself had used insulin irregularly during the past 14 years, but never showed weight loss or signs/symptoms of ketoacidosis, even when missing insulin injections for several days. In the years preceding hospital admission, he made more regular use of insulin (approximately 30 units of NPH and 10–14 units of regular per day), with capillary glucose values between 80 and 150 mg/dL (both pre and post-prandial). His irregular medical follow-up and insulin-treatment compliance in the months prior to hospital admission evolved to progressive asthenia, which aggravated in the last few days, and ended up with his hospitalization. He reported sudden worsening of vision, watery diarrhoea, vomiting, and nausea. Upon admission, the patient was dehydrated and showed poor general appearance. Retinal examination showed bilateral vitreous haemorrhage, already in reabsorption. His laboratory tests are summarized in Table 1. After 5 days of intensive medical care, the patient showed rapid clinical deterioration with sepsis and passed away.

4. Discussion

In this paper, we describe the analysis of a series of individuals negative for common types of MODY gene mutations, and the successful molecular strategy of adding the MLPA technique. Interestingly, this is the first Brazilian patient with HNF1B-MODY to be diagnosed, who did not show typical clinical pattern of this MODY subtype, in regard to renal cysts and hypoplasia, or urogenital morphological alterations. Other extra-renal phenotypes such as pancreatic hypoplasia, exocrine dysfunction, and gout, although not systematically investigated, were not reported in the patient either. Besides, he showed a slightly low magnesium level, a finding that can bear a relationship to HNF1B-caused renal disease. Hypomagnesaemia is actually a common finding, being frequently overlooked [13]. His molecular diagnosis has been made post mortem by adding MLPA analysis to the routine genetic test of MODY.

HNF1B-MODY has been described as having a very heterogeneous phenotype, despite morphologic kidney abnormalities being the main finding. This fact has led some authors to devise scores employing clinical and laboratory data. Those scores have been proposed as tools to clinical screening of candidates to molecular diagnosis [14,15]. By applying this score to the present patient, yet limited to using only available data, we identified a score 8, which is still lower than the proposed threshold. Another interesting finding was the familial history of diabetes in his mother, which initially contributed to the clinical suspicion of MODY. She came out negative for mutations both by Sanger and MLPA analyses. These findings altogether suggest the patient to carry a *de novo* mutation, found in ~50% of HNF1B-MODY patients [15], and his mother to have type 2 diabetes, being thus a phenocopy. DNA samples from the father were unavailable, but there was no paternal history of diabetes or renal disease.

Our HNF1B-MODY patient showed marked renal function deterioration with probable onset in adulthood, even though this complication could also be related to diabetes itself, since he showed advanced diabetic retinopathy. Nevertheless, progressive loss of renal function has been described in *HNF1B* patients [16]. Musetti et al. studied 67 adults with unknown aetiology renal failure and kidney morphological abnormalities, mainly renal cysts and dysplasia, or familial history of nephropathy, and found *HNF1B* mutations in 9% [17]. In fact, *HNF1B* is a member of the superfamily of transcription factors, and plays a role in pancreas, liver, bile ducts, kidneys, lungs, and genital tract [16,18,19].

From a genetic standpoint, our study demonstrates that combining genetic testing tools may be useful in the diagnosis of suspected MODY cases. Besides, large deletions and