

## LETTER TO THE EDITOR

# A missense *TCF1* mutation in a patient with mody-3 and liver adenomatosis

Antonio Marcondes Lerario,<sup>1</sup> Luciana Pinto Brito,<sup>1</sup> Beatriz Marinho Mariani,<sup>1</sup> Maria Candida B V Fragoso,<sup>1</sup> Marcel Autran Cesar Machado,<sup>2</sup> Roberto Teixeira<sup>2</sup>

<sup>1</sup>Unidade de Endocrinologia do Desenvolvimento, Laboratório de Hormônios e Genética Molecular (LIM-42), Disciplina de Endocrinologia, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil. <sup>2</sup>Laboratório de Investigação Médica de Transplante e Cirurgia Hepática (LIM-37), Cirurgia, Faculdade de Medicina, Universidade de São Paulo, SP, São Paulo, Brazil.

Email: amlerario@gmail.com

Tel.: 55 113069-7512

## INTRODUCTION

Maturity-onset diabetes of the young type 3 (MODY-3) is a non-ketotic form of diabetes mellitus with an autosomic dominant inheritance characterized by early onset (< 25 y) and a severe impairment in insulin secretion.<sup>1</sup> This disease is caused by heterozygous germline mutations of the *TCF1* gene. The product encoded by this gene is the hepatocyte nuclear factor 1 alpha (HNF-1 $\alpha$  – accession number NP\_000536) transcription factor, which has important physiologic roles in organs such as the pancreas, liver and kidneys.

Hepatocellular adenomas are benign neoplasms that usually develop as a solitary nodule and are associated with oral contraceptive use in 90% of cases. On the other hand, liver adenomatosis (LA) is characterized by the presence of multiple nodules (usually more than 5) and is considered to be a distinct disease, due to the higher prevalence in male sex and the unclear association with OCs.<sup>2</sup> LA has been reported in sporadic and familial forms. Although malignant transformation is rarely observed, spontaneous rupture and life-threatening bleeding may occur in large and subcapsular nodules.<sup>3</sup> For this reason, large nodules should be resected.

Since the 1970s, some reports observed the co-segregation of familial autosomic-dominant diabetes mellitus and LA, suggesting that a common genetic factor may be associated with both conditions.<sup>4,5</sup> Biallelic somatic *TCF1* inactivation has been observed in about 50% of hepatocellular adenomas, suggesting that this gene act as a tumor suppressor in liver.<sup>6</sup> Recently, germline *TCF1* mutations have been described in five familial cases of LA. In four of these families, a co-segregation of LA and diabetes could be clearly established. Interestingly, in all of these cases, the identified germline *TCF1* mutations were *nonsense* or *frameshift*.<sup>6-8</sup> In this report, we describe a MODY-3 patient with LA associated to a *TCF1* missense germline mutation.

## Case Report

A 27 year-old female who had a two-year history of non-insulin dependent diabetes mellitus (NIDDM) underwent

an abdominal ultrasonography for unrelated causes. Multiple hepatic nodules and liver enlargement were observed (Figure 1). The patient reported the use of oral contraceptives for the last 5 years and had a positive family history for diabetes mellitus. An abdominal CT-scan was performed and multiple hypervascularized hepatic nodules were observed, consistent with the diagnosis of LA. The largest nodule (18 cm) was resected and histological analysis revealed a liver adenoma.

## Genetic studies

An informed consent was obtained from the patient prior to DNA extraction. Genomic and tumoral DNAs were extracted from peripheral blood and paraffin-embedded tissues samples, respectively, according to standard protocols. The coding sequence of the entire *TCF1* gene was amplified by PCR. The amplified fragments were examined on 2% agarosis gel electrophoresis. The PCR products were directly sequenced using the BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA), in an ABI-PRISM 310 automatic sequencer (Perkin-Elmer corp), after a pretreatment with an enzymatic combination of exonuclease I and shrimp alkaline phosphatase (ExoSap-it – United States biochemical corp., Cleveland, OH).

## RESULTS

A single nucleotide substitution (CGT>CTT) was identified in heterozygous state in exon 4. This mutation encodes an arginine for leucine substitution in codon 263 (R263L - accession number NP\_000536). Sequence analysis of tumoral DNA identified a second (somatic) mutation of *TCF1* – the insertion of a cytosine residue in the polycytosine tract of exon 4, causing a frameshift (P291fsInC).

## DISCUSSION

In this paper, we report a rare association of MODY-3 and LA. Although co-segregation of LA and autosomic-dominant diabetes mellitus has been known since the 1970s,<sup>4</sup> only in recent years this phenotype was associated to *TCF1* germline mutations.<sup>6</sup> To date, only five unrelated families with LA and *TCF1* germline mutations have been reported so far,<sup>6-8</sup> in spite of MODY-3 being a relatively common genetic disease (~ 1% of NIDDM).<sup>9</sup> In most of familial cases, the diagnosis of LA was established on index cases after an

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**Figure 1** - Contrast-enhanced CT-scan showing multiple hypervascularized hepatic nodules, consistent with the diagnosis of LA.

acute hemorrhagic complication. In the majority of affected family members, the disease was mild and the diagnosis was established after ultrasound screening. Since large-scale imaging studies to evaluate the presence of LA in asymptomatic MODY-3 patients are not available, it is speculated that the prevalence of this condition may be underestimated.

In spite of the fact that the majority of MODY-3 patients are carriers of germline *missense* mutations,<sup>9</sup> only *nonsense* or *frameshift* mutations have been identified in the familial cases of LA.<sup>6-8</sup> Presumably, a severe impairment of the HNF-1 $\alpha$  function is required for the development of LA. The germline mutation identified in our case (R263L) has been recently described in a Korean MODY-3 family. Functional analysis demonstrated that this mutation causes a significant impairment of the HNF-1 $\alpha$  function.<sup>10</sup> We identified a second (somatic) *TCF1* mutation (P291fsInC) in the tumor tissue. Interestingly, P291fsInC was identified as the first-hit (germinative) mutation in two of the five described families. Our findings are in accordance with previous reports, in which the development of liver adenomas is associated to the inactivation of both *TCF1* alleles, either by allelic losses at chromosome 12q or by double mutational events.<sup>6,8</sup>

Although OCs are known to be a classical risk factor for the development of isolated liver adenomas (up to 90% of such tumor are associated to OCs use), it is not clear if it have a direct causal role in LA.<sup>2</sup> Recent data suggest that at least it may worsen the disease.<sup>5</sup> For this reason, we suggest that the use of OCs in MODY-3 patients should be monitored with care. In conclusion, this is the first report of LA associated to a *missense TCF1* mutation. The importance of molecular diagnosis of MODY patients is reinforced, and MODY-3 patients should be screened for LA. Also, the use of OCs in MODY-3 patients should be carefully monitored. Alternative forms of contraception should be considered.

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