Difficulties in the diagnosis aetiology of A- β- ketosis-prone diabetes in a North-African adult

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Abstract: Ketosis-prone diabetes (KPD) is a heterogeneous syndrome characterized by patients who present with diabetic ketoacidosis or unprovoked ketoacidosis but do not necessarily have the typical phenotype of autoimmune type 1 diabetes. In this case report, we expose the difficulties in the diagnosis aetiology of A- β- ketosis-prone diabetes (KPD) in a 22-year-old North-African woman presenting with diabetic ketoacidosis. Her initial glycaemia was at 17.2 mmol/L and glycated haemoglobin (HbA1c) was moderately increased at 7.6% with no clinical evidence of other precipitating illnesses or stressful events. Her baseline and glucagon-stimulated serum C-peptide levels were below the detection limit at the time of admission. All anti-pancreatic antibodies were negative and pancreatic imaging was normal. Direct sequencing of HNF1A, HNF4A, INS, IPF1, NEUROD1 and PAX4 genes were performed and showed three mutations in HNF1A (I27L), NEUROD1 (A45T) and PAX4 (H321P). HLA typing showed the genotype DQB1*03-DRB1*11/DQB1*03-DRB1*13. Based on that data, type 1A diabetes mellitus (DM) could be excluded. However, criteria of fulminant type 1 DM are mostly filled but difficult to be confirmed and the MODY (Maturity Onset diabetes of the young) hypothesis could not be ruled out as other genes may be involved. Our observation highlights the difficulties of understanding the aetiology of A- β- KPD with sudden-onset although clinical, imaging, immunologic and genetic data are available.

Keywords: Ketosis-prone diabetes, Diabetic Ketoacidosis, Type 1 diabetes mellitus, MODY, Etiology, Genetics.

INTRODUCTION
Clinical forms that do not fit the traditional categories defined by the American Diabetes Association (ADA) have been emerging in recent decades [1]. Until recently, clinical cases with unprovoked diabetes ketosis in young individuals are often due to type 1 diabetes mellitus (T1DM) [2]. However, the absence of autoimmunity and/or the presence of a significant beta cell function in some of these cases observed in recent data from longitudinally followed cohorts have raised the question of possible atypical cases of diabetes mellitus with ketosis-prone onset [3]. These subtypes were gathered under a novel heterogeneous syndrome called “Ketosis-Prone Diabetes” (KPD). This syndrome includes 4 clinically and pathophysiologically distinct subtypes based on the presence or absence of β-cell auto-immunity (A) and the presence or absence of β-cell functional reserve (β) [4]. Although, the resulting “Aβ” classification system of KPD has proven to be highly accurate and predictive of clinical outcomes such as glycaemic control and insulin dependence, the underlying pathophysiology and genetic abnormalities of some subtypes remain unclear especially in cases of sudden-onset DM and total beta-cell destruction without autoimmunity stigma (A-β-) [5].
We discuss here the clinical and molecular findings in a North-African young woman from Tunisia who presented with a sudden-onset A- β- subtype of KPD.

CASE PRESENTATION
A healthy 22-year-old Tunisian woman complained of abdominal pain, nausea and polyuria, which had begun three days before coming to hospital. She had a strong family history of diabetes mellitus in maternal grandmother, mother, aunt and uncle. She had no past medical or surgical history. She was not on any medications prior to admission and did not smoke, or drink alcohol. She had not lost any significant weight and denied any recent infections. Evaluation in the emergency center revealed Diabetic Ketaoacidosis (DKA) with no clinical evidence of other precipitating illnesses or stressful events.

On physical examination, her BMI (Body mass index) was at 23 kg/m². She had a diffuse abdominal swelling without hemodynamic instability. Laboratory tests revealed no evidence of acute infection, cardiac ischemia, or cerebrovascular disease, renal pancreatic or liver dysfunction. The arterial pH was 7.29, anion gap 29, bicarbonate 17 mmol/L, and glucose 17.2 mmol/L with positive urine ketones and glucose. Her TSH level was at 1.88 mIU/L (0.35–5.5), HbA1c was moderately increased at 7.6%, without haemoglobinopathy on haemoglobin (Hb) electrophoresis. The patient was admitted to the hospital and received standard treatment for DKA (iv fluids and insulin). She recovered uneventfully and was discharged on the third hospital day. She had a diffuse abdominal pain, nausea and polyuria, which had begun three days before coming to hospital. She had no past medical or surgical history. She was not on any medications prior to admission and did not smoke, or drink alcohol. She had not lost any significant weight and denied any recent infections. Evaluation in the emergency center revealed Diabetic Ketaoacidosis (DKA) with no clinical evidence of other precipitating illnesses or stressful events.

After obtaining local ethics committee approval and written informed consent from our patient, genomic DNA was extracted from peripheral blood using Flexigene DNA kit (QiagenInc). HLA typing for DQB1 and DRB1 loci was carried out by INNO-LiPA HLA-DQB1 Update and INNO-LiPA HLA-DRB1 Plus kits respectively (INNOGENETICS® Gent, Belgium) according to the manufacturer’s recommendations. The results were interpreted using LiRAS™ software for INNO-LiPA HLA (INNOGENETICS®) and revealed the genotype DQB1*03-DRB1*11/DQB1*03-DRB1*13.

We also screened HNF1A, HNF4A, INS, IPF1, NEUROD1 and PAX4 genes by direct sequencing of all exons and exon-intron boundaries. Polymerase chain reaction (PCR) was performed in a 50 µl volume containing 50 ng of genomic DNA, 20 pmol of each primer and 1 U of Recombinant DNA polymerase (Invitrogen, Carlsbad, CA, USA) in ABI 9700 thermocycler (Applied Biosystems, USA). PCR conditions, primer sequence and bloc cycler program are available on request. PCR cycling conditions were denaturation at 94°C for 10 min followed by 40 cycles of denaturation at 94°C for 30 s, annealing temperature for 30 s and extension at 72°C for 45 s, with a final extension at 72°C for 7 min. Purified PCR products were sequenced on both strands, by BigDye Ver. 1.1 (Applied Biosystems) in ABI 9700 thermocycler (Applied Biosystems, USA). Sequencing products were purified by Wizard®MagneSil™ Sequencing Reaction Clean-Up System kit (Promega) before capillary electrophoresis on an ABI 310 DNA Analyser (Applied Biosystems). Results were analyzed using Seqscape v2.0 software.

Moreover, to search for any deletion or duplication in GCK, HNF1B, HNF1A, HNF4A genes, MLPA technique was performed according to the manufacturer’s recommendations in a thermocycler ABI 9700 using the “SALSA MLPA KIT P241-B1 MODY” (from MRC-Holland, Netherland). No novel or rare mutation was found in HNF1A, HNF4A, INS, IPF1, NEUROD1 and PAX4 genes. However, three mutations rs1169288-HNF1A (I27L), rs1801262-NEUROD1 (A45T) and rs712701-PAX4 (H321P) have been identified in the patient (Table 1). Neither deletions nor duplications of the studied genes were detected.

DISCUSSION
Our patient presented with negative auto-antibodies which argues against the hypothesis of classical T1DM. Otherwise, we remarked that her clinical and biological features could meet the diagnostic criteria required for the diagnosis of fulminant T1DM which are:

- Evidence of ketosis or ketoacidosis within about 7 days of hyperglycaemia onset,
- plasma glucose level greater or equal to 16.0 mmol/L (≥ 288 mg/dL) and glycohaemoglobin less than 8.5% at initial visit [6],
- and urinary C-peptide excretion less than 10 µg/d or fasting serum C-peptide level less than 0.3 ng/mL (< 0.10 nmol/L) [6], or less than 0.5 ng/mL (< 0.17 nmol/L) after intravenous glucagon or after meal at onset [7].

However, the flu-like symptoms preceding the disease onset in the Japanese survey were not detected in our patient [8].

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The common aetiology of fulminant T1DM has not yet been identified, although, hypothesis of viral infection was suggested because of the abrupt onset [9]. Researchers suggest that beta cells undergo specific damage in autoimmune T1DM, which may be promoted by the selective recognition of beta cell antigens by T cells. In contrast, both beta and alpha cells are damaged in fulminant T1DM, indicating that a cytotoxic mechanism that operates against both beta and alpha cells is involved in the pathogenesis of fulminant T1DM [10]. The role of class II HLA molecules in presenting antigenic peptides to T cells has been extensively studied but their role in fulminant T1DM is unclear. As suggested in type 1A DM, they could be involved in activating anti-islet T-cell reactivity because lymphocytic infiltration has been observed in the exocrine pancreas of patients with fulminant T1DM [22], which may explain why serum amylase is invariably elevated at disease onset[8].

Despite the lack of evidence for an immune pathogenesis, fulminant T1DM has been reported to be associated with specific HLA genes, as is classical T1ADM. Japanese patients with fulminant T1DM had more commonly the class II HLA DR4-DQ4 haplotype (encoded by DRB1*0405-DQB1*0401) [11]. Our patient had the genotype DQB1*03-DRB1*11/DQB1*03-DRB1*13 which was not described in previous fulminant T1DM cases. Some studies in Tunisian patients reported that DRB1*11-DQB1*0301 haplotype was lower in T1ADM patients than in control subjects and even protective from T1ADM [12,13]. Taking into account all these clinical and genetic data, we are not able to exclude definitely the diagnosis of fulminant T1DM.

Otherwise, the studied genes were chosen as part of a suspected MODY diabetes diagnosis (OMIM#606391). The hypothesis of MODY was based on her early age (22 year-old), familial history of diabetes (on 3 generations), absence of auto-antibodies for type 1 diabetes, low levels of C-peptide (indicating a probable default in insulin secretion) and absence of obesity. Actually, these are points of resemblance between MODY diabetes and fulminant T1DM making a diagnosis difficult especially when we know that 80% of MODY cases are misdiagnosed as type 1 or type 2 DM [14]. Absence of rare or novel mutations in the studied genes does not definitely argue against a MODY type in the patient since mutations in other genes could exist. That’s why; there is a need for a suitable strategy of genetic exploration in the case of MODY patients [15].

Furthermore, the three mutations detected in our patient were previously reported to be associated to

Table-1: Polymorphisms found in our patient after mutation screening of HNF1A, HNF4A, INS, NeuroD1, IPF1 and PAX4 genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>ID</th>
<th>Nucleotide change</th>
<th>Amino-acid change</th>
<th>Status</th>
<th>Reported to be associated to diabetes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNF1A</td>
<td>rs1169289</td>
<td>c.51 C&gt;G</td>
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<td></td>
<td>rs1169288</td>
<td>c.79 A&gt;C</td>
<td>I27L</td>
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<tr>
<td></td>
<td>rs56348580</td>
<td>c.864&gt;G&gt;C</td>
<td>G288G</td>
<td>Heterozygote</td>
<td>No</td>
<td></td>
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<tr>
<td></td>
<td>rs55834942</td>
<td>c.1545&gt;G&gt;A</td>
<td>T515T</td>
<td>Heterozygote</td>
<td>No</td>
<td></td>
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<tr>
<td>HNF4A</td>
<td>rs736824</td>
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<tr>
<td></td>
<td>rs745975</td>
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<td></td>
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<td></td>
<td>rs3746574</td>
<td>c.1217-145T&gt;C</td>
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<td>No</td>
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<td></td>
<td>rs3746575</td>
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<td></td>
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<td></td>
<td>rs2273618</td>
<td>c.827-88T&gt;C</td>
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<td>No</td>
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<td></td>
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<tr>
<td>INS</td>
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<td>c.-115 G&gt;C</td>
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<td>No</td>
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<tr>
<td></td>
<td>rs3842741</td>
<td>c.-159 A&gt;G</td>
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<tr>
<td></td>
<td>rs689</td>
<td>c.-23T&gt;A</td>
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<td></td>
<td>rs5506</td>
<td>c.187+11T&gt;C</td>
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<tr>
<td></td>
<td>rs3842753</td>
<td>c.*22 A&gt;C</td>
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<td>NeuroD1</td>
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<td>c.133A&gt;G</td>
<td>A45T</td>
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<td>No</td>
<td>Kavoura et al. [18]</td>
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<tr>
<td>PAX4</td>
<td>rs327516</td>
<td>c.13+175G&gt;C</td>
<td>H321P</td>
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<td>No</td>
<td>Biason-Lauber et al. [21]</td>
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<td></td>
<td>rs712701</td>
<td>c.962A&gt;C</td>
<td></td>
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<td>Yes (DT1)</td>
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<tr>
<td></td>
<td>CM051949</td>
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</table>


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diabetes. HNF1A-rs 1169288 (I27L) was found with a higher frequency in women with gestational diabetes compared to a control group [16] and in another study it was associated with insulin resistance, but not beta cell function [17]. Kavvoura et al., in a meta-analysis, mentioned that NEUROD1-rs1801262 (A45T) was likely a susceptibility factor to T1DM particularly in subjects of Asian descent [18] and as a susceptibility factor to T2DM in a Chinese population [19]. Although, this mutation does not affect the regulation of the human insulin gene eprromoter and does not seem to have a direct role in the pathogenesis of either type 1 or type 2 diabetes [20].

In addition, the C/C genotype of PAX4-rs712701 (H321P) was found to be associated to T1DM and to limit the proliferation of beta cells under glucose stimulation [21].

In conclusion, this case highlighted the difficulties in the diagnosis aetiology of A- β- ketosis-prone diabetes. A worldwide survey should be carried out in the future in order to improve the understanding of this form of diabetes.

Abbreviations:
GCK : Glucokinase
HNF1A : Hepatocyte Nuclear factor 1 homeobox A
HNF4A : Hepatocyte nuclear factor 4-alpha
IA2 : Islet Antigen 2
INS : insulin gene
IFP1 : insulin promoter factor 1
NEUROD1 : Neurogenic differentiation 1
PAX4 : Paired box gene 4
MLPA: Multiplex Ligation-dependent Probe Amplification
TSH: Thyroid-stimulating Hormone

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