

Case Report

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

Ines Slim^{1,2,3*}, Abdelbasset Amara^{2,4,5*}, Asma Baba¹, Molka Chadli-Chaieb^{1,2}, Koussay Ach^{1,2}, Saloua Jemni-Yaacoub⁶, Ali Saad⁴, Philippe Froguel^{7,8,9}, Moez Gribaa⁴, Martine Vaxillaire^{7,8,9}, Larbi Chaieb^{1,2}

¹Department of Endocrinology and Diabetes, Farhat Hached University Hospital, Sousse, Tunisia

²Unit of Molecular Endocrinology, Faculty of medicine, University of Sousse, Tunisia

³Department of Health Sciences, University of Leicester, 22-28 Princess Road West, Leicester, LE1 6TP, United Kingdom

⁴Laboratory of Human Cytogenetics, Molecular Genetics and Reproductive Biology, Farhat Hached University Hospital, Sousse, Tunisia

⁵Higher Institute of Biotechnology of Monastir, University of Monastir, Tunisia

⁶Blood Transfusion Centre, Sousse, Tunisia

⁷European Genomic Institute for Diabetes (EGID) FR 3508, F-59000 Lille, France

⁸CNRS UMR8199, Pasteur Institute of Lille, Lille, France

⁹Lille 2 University, Lille, France

*Both authors contributed equally to this work

*Corresponding Author:

Dr. Abdelbasset Amara

Email: abdelbasset.amara@mailpost.tn

Abstract: Ketosis-prone diabetes (KPD) is a heterogeneous syndrome characterized by patients who present with diabetic ketoacidosis or unprovoked ketosis 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 “A β ” classification system of KPD has proven to be highly accurate and predictive of clinical outcomes such as glycaemic control and insulin dependence, the underlying physiopathology and genetic abnormalities of some subtypes remain unclear especially in cases of sudden-onset DM and total beta-cell destruction without autoimmunity stigmata (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 Ketoacidosis (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). HbA_{1c} 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 on a regimen of NPH insulin twice daily with rapid human insulin before meals. All anti-GAD (Glutamic Acid Decarboxylase), anti-IA2 (Islet antigen 2), and anti-islet cell antibodies were negative. Serum pancreatic lipase and hepatic enzymes levels were normal. Pancreatic tomodensitometry was normal. There was no clinical evidence of diabetic retinopathy, neuropathy, or nephropathy. Her baseline and glucagon-stimulated serum C-peptide levels, performed one week after DKA, were below the detection limit (<0.1 ng/mL) at the time of admission and remain undetectable one year later. Her mean dose of insulin was titrated from 28 IU/day, at the admission, up to 48 IU/day (0.8 IU/kg/day) one year later. No recurrence of ketosis has been occurred.

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 *HNFI1A*, *HNFI4A*, *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 μ l 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 cyler 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*, *HNFI1B*, *HNFI1A*, *HNFI4A* 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 *HNFI1A*, *HNFI4A*, *INS*, *IPF1*, *NEUROD1* and *PAX4* genes. However, three mutations rs1169288-*HNFI1A* (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 μ g/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].

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

Gene	ID	Nucleotide change	Amino-acid change	Status	Reported to be associated to diabetes	Reference
<i>HNF1A</i>	rs1169289	c.51 C>G	L17L	Heterozygote	No	Chiu <i>et al.</i> [17]
	rs1169288	c.79 A>C	I27L	Heterozygote	Yes (DT2)	
	rs56348580	c.864:G>C	G288G	Heterozygote	No	
	rs55834942	c.1545 G>A	T515T	Heterozygote	No	
<i>HNF4A</i>	rs736824	c.50-30T>C		Homozygote	No	
	rs745975	c.50-5C>T		Homozygote	No	
	rs3746574	c.1217-145T>C		Homozygote	No	
	rs3746575	c.1217-67C>G		Heterozygote	No	
	rs2273618	c.827-88T>C		Homozygote	No	
<i>INS</i>	rs3842738	c.-115 G>C		Heterozygote	No	
	rs3842741	c.-159 A>G		Heterozygote	No	
	rs689	c.-23T>A		Homozygote	No	
	rs5506	c.187+11 T>C		Heterozygote	No	
	rs3842753	c.*22 A>C		Homozygote	No	
<i>NeuroD1</i>	rs1801262	c.133A>G	A45T	Homozygote	Yes (DT1)	Kavvoura <i>et al.</i> [18]
<i>PAX4</i>	rs327516	c.13+175G>C	----	Homozygote	No	Biaison-Lauber <i>et al.</i> [21]
	rs712701 or CM051949	c.962A>C	H321P	Homozygote	Yes (DT1)	
<i>IPF1</i>	No mutation					

*MAF source: 1000 genomes (dbSNP database) <http://www.ncbi.nlm.nih.gov/projects/SNP/>

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 defect 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

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 promoter 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

IPF1 : insulin promoter factor 1

NEUROD1 :Neurogenic differentiation 1

PAX4 :Paired box gene 4

MLPA: Multiplex Ligation-dependent Probe Amplification

TSH: Thyroid-stimulating Hormone

Acknowledgements

We appreciate the helpful technical guidance of Mrs. Faouzia Galai, Mrs. Sihem Sassi, Mrs. Ahlem Msakni, Mrs. Safa Bouker, Mr. Julien Philippe and Mrs. Aurélie Dechaume.

Duality of interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. American Diabetes, A. (2013) Diagnosis and classification of diabetes mellitus. *Diabetes care*, 36 Suppl 1, S67-74.
2. Kitabchi, A.E., Umpierrez, G.E., Miles, J.M. & Fisher, J.N. (2009) Hyperglycemic crises in adult patients with diabetes. *Diabetes care*, 32, 1335-1343.
3. Maldonado, M., Hampe, C.S., Gaur, L.K., D'Amico, S., Iyer, D., Hammerle, L.P., Bolgiano, D., Rodriguez, L., Rajan, A., Lernmark, A. & Balasubramanyam, A. (2003) Ketosis-prone diabetes: dissection of a heterogeneous syndrome using an immunogenetic and beta-cell functional classification, prospective analysis, and clinical outcomes. *The Journal of clinical endocrinology and metabolism*, 88, 5090-5098.
4. Brooks-Worrell, B.M., Iyer, D., Coraza, I., Hampe, C.S., Nalini, R., Ozer, K., Narla, R., Palmer, J.P. & Balasubramanyam, A. (2013) Islet-specific T-cell responses and proinflammatory monocytes define subtypes of autoantibody-negative ketosis-prone diabetes. *Diabetes care*, 36, 4098-4103.
5. Balasubramanyam, A., Nalini, R., Hampe, C.S. & Maldonado, M. (2008) Syndromes of ketosis-prone diabetes mellitus. *Endocrine reviews*, 29, 292-302.
6. Hanafusa, T. & Imagawa, A. (2007) Fulminant type 1 diabetes: a novel clinical entity requiring special attention by all medical practitioners. *Nature clinical practice. Endocrinology & metabolism*, 3, 36-45; quiz 32p following 69.
7. Umpierrez, G.E., Smiley, D. & Kitabchi, A.E. (2006) Narrative review: ketosis-prone type 2 diabetes mellitus. *Annals of internal medicine*, 144, 350-357.
8. Imagawa, A., Hanafusa, T., Uchigata, Y., Kanatsuka, A., Kawasaki, E., Kobayashi, T., Shimada, A., Shimizu, I., Toyoda, T., Maruyama, T. & Makino, H. (2003) Fulminant type 1 diabetes: a nationwide survey in Japan. *Diabetes care*, 26, 2345-2352.
9. Vreugdenhil, G.R., Schloot, N.C., Hoorens, A., Rongen, C., Pipeleers, D.G., Melchers, W.J., Roep, B.O. & Galama, J.M. (2000) Acute onset of type I diabetes mellitus after severe echovirus 9 infection: putative pathogenic pathways. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 31, 1025-1031.
10. Sayama, K., Imagawa, A., Okita, K., Uno, S., Moriwaki, M., Kozawa, J., Iwahashi, H., Yamagata, K., Tamura, S., Matsuzawa, Y., Hanafusa, T., Miyagawa, J. & Shimomura, I. (2005) Pancreatic beta and alpha cells are both decreased in patients with fulminant type 1 diabetes: a morphometrical assessment. *Diabetologia*, 48, 1560-1564.
11. Imagawa, A., Hanafusa, T., Uchigata, Y., Kanatsuka, A., Kawasaki, E., Kobayashi, T., Shimada, A., Shimizu, I., Maruyama, T. & Makino, H. (2005) Different contribution of class II HLA in fulminant and typical autoimmune type 1 diabetes mellitus. *Diabetologia*, 48, 294-300.
12. Abid Kamoun, H., Hmida, S., Kaabi, H., Abid, A., Slimane Houissa, H., Maamar, M., Mojaat, N., Ben Hamed, L., Dridi, A., Kamoun Zribi, M., Nagati, K., Haddad, A. & Boukef, K. (2002) HLA polymorphism in type 1 diabetes Tunisians. *Annales de genétique*, 45, 45-50.
13. Stayoussef, M., Benmansour, J., Al-Irhayim, A.Q., Said, H.B., Rayana, C.B., Mahjoub, T. & Almawi, W.Y. (2009) Autoimmune type 1 diabetes genetic susceptibility encoded by human leukocyte antigen

- DRB1 and DQB1 genes in Tunisia. *Clinical and vaccine immunology* : CVI, 16, 1146-1150.
14. Malecki, M.T. (2010) The search for undiagnosed MODY patients: what is the next step? *Diabetologia*, 53, 2465-2467.
 15. Amara, A., Chadli-Chaieb, M., Chaieb, L., Saad, A. & Gribaa, M. (2014) Challenges for molecular diagnosis of familial early-onset diabetes in unexplored populations. *Iranian journal of public health*, 43, 1011-1013.
 16. Shaat, N., Karlsson, E., Lernmark, A., Ivarsson, S., Lynch, K., Parikh, H., Almgren, P., Berntorp, K. & Groop, L. (2006) Common variants in MODY genes increase the risk of gestational diabetes mellitus. *Diabetologia*, 49, 1545-1551.
 17. Chiu, K.C., Chuang, L.M., Ryu, J.M., Tsai, G.P. & Saad, M.F. (2000) The I27L amino acid polymorphism of hepatic nuclear factor-1alpha is associated with insulin resistance. *The Journal of clinical endocrinology and metabolism*, 85, 2178-2183.
 18. Kavvoura, F.K. & Ioannidis, J.P. (2005) Ala45Thr polymorphism of the NEUROD1 gene and diabetes susceptibility: a meta-analysis. *Human genetics*, 116, 192-199.
 19. Liu, L., Jia, W., Zheng, T., Li, M., Lu, H. & Xiang, K. (2006) Ala45Thr variation in neuroD1 gene is associated with early-onset type 2 diabetes with or without diabetic pedigree in Chinese. *Molecular and cellular biochemistry*, 290, 199-204.
 20. Hansen, L., Jensen, J.N., Urioste, S., Petersen, H.V., Pociot, F., Eiberg, H., Kristiansen, O.P., Hansen, T., Serup, P., Nerup, J. & Pedersen, O. (2000) NeuroD/BETA2 gene variability and diabetes: no associations to late-onset type 2 diabetes but an A45 allele may represent a susceptibility marker for type 1 diabetes among Danes. *Danish Study Group of Diabetes in Childhood, and the Danish IDDM Epidemiology and Genetics Group. Diabetes*, 49, 876-878.
 21. Biason-Lauber, A., Boehm, B., Lang-Muritano, M., Gauthier, B.R., Brun, T., Wollheim, C.B. & Schoenle, E.J. (2005) Association of childhood type 1 diabetes mellitus with a variant of PAX4: possible link to beta cell regenerative capacity. *Diabetologia*, 48, 900-905.
 22. Sekine, N., Motokura, T., Oki, T., Umeda, Y., Sasaki, N., Hayashi, M., Sato, H., Fujita, T., Kaneko, T., Asano, Y. & Kikuchi, K. (2001) Rapid loss of insulin secretion in a patient with fulminant type 1 diabetes mellitus and carbamazepine hypersensitivity syndrome. *Jama*, 285, 1153-1154.