Short Report

**SLC29A3** mutation in a patient with syndromic diabetes with features of pigmented hypertrichotic dermatosis with insulin-dependent diabetes, H syndrome and Faisalabad histiocytosis

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**Abstract**

**Aims.** – Atypical forms of diabetes may be caused by monogenic mutations in key genes controlling beta-cell development, survival and function. This report describes an insulin-dependent diabetes patient with a syndromic presentation in whom a homozygous **SLC29A3** mutation was identified.  

**Methods.** – **SLC29A3** was selected as the candidate gene based on the patient’s clinical manifestations, and all exons and flanking regions in the patient’s genomic DNA were sequenced.  

**Results.** – A homozygous splice mutation (c.300+1G>C) resulting in a frameshift and truncated protein (p.N101LfsX34) was identified. The patient had insulin-dependent diabetes, congenital deafness, short stature, hyperpigmented patches on the skin, dysmorphic features, cardiomegaly, arthrogryposis, hepatosplenomegaly, anaemia with erythroblastopenia, and an inflammatory syndrome with fever and arthritis; she also presented with a fibrotic mediastinal mass. These clinical features overlapped with pigmented hypertrichosis with insulin-dependent diabetes (PHID), H syndrome, Faisalabad histiocytosis and sinus histiocytosis with massive lymphadenopathy (SHML), all of which are also caused by **SLC29A3** mutations.  

**Conclusion.** – This is the most severe case reported of **SLC29A3** mutations with cumulative features of all these syndromes. This extreme severity coincides with the most N-terminal location of the truncation mutation, thereby affecting all alternative transcripts of the gene. This case report extends the clinical variability of homozygous **SLC29A3** mutations that result in a spectrum of multisystemic manifestations.

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**Keywords:** Diabetes; Syndrome; Monogenic; Genetics; Diagnosis

**Résumé**

Une mutation dans le gène **SLC29A3** chez un patient diabétique présentant des caractéristiques de la dermatose hypertrichotique pigmentaire avec diabète insulinodépendant, du syndrome H et de l’histiocytose de Faisalabad.

**Objectifs.** – Les diabètes atypiques peuvent être causés par des mutations monogéniques dans des gènes importants dans le développement, la survie ou la fonction des cellules beta. Ce rapport décrit un patient atteint d’un diabète insulinodépendant syndromique, chez qui nous avons identifié une mutation homozygote du gène **SLC29A3**.

**Méthodes.** – Nous avons sélectionné le gène **SLC29A3** comme candidat en raison des manifestations cliniques du patient, et nous avons séquencé les exons et leurs régions flanquantes sur l’ADN génomique du patient.

**Résultats.** – Nous avons identifié une mutation homozygote affectant un site d’épissage (c.300+1G>C), qui entraîne un décalage du cadre de lecture et résulte en une protéine tronquée (p.N101LfsX34). Le patient était atteint de diabète insulinodépendant, surdité congénitale, petite taille,

Conclusion. – Il s’agit du cas le plus grave de patient porteur de mutation SLC29A3, qui cumule les caractéristiques de ces syndromes, ce qui coïncide avec la position N-terminale de la mutation de troncation, qui concerne tous les transcrits alternatifs du gène. Notre observation élargit la variabilité clinique des mutations homozygotes SLC29A3, qui résultent en un spectre étendu de manifestations multi-organes.

Mots clés : Diabète ; Syndrome ; Monogénique ; Génétique ; Diagnostic

1. Introduction

Insulin-dependent diabetes can occur within the context of various syndromic clinical presentations that may be caused by monogenic gene mutations. In recent years, several genes responsible for such monogenic syndromes have been identified, providing important information on the mechanisms involved in beta-cell function, growth, death and survival, as well as their alteration in diabetes. This knowledge has direct applications for optimizing diabetes treatment in patients whose diabetes is the result of such monogenic defects [1]. The present case report describes a patient who presented with a complex multisystemic syndrome with insulin-dependent diabetes in whom a mutation in the SLC29A3 gene encoding the equilibrative nucleoside transporter 3 (ENT3) was identified.

2. Case report

The patient, a Moroccan girl born to consanguineous parents, was referred to the paediatric clinic at Rabat at age 12 years for diabetes. The diagnosis had been made 12 months earlier based on the presence of polyuria–polydipsia and hyperglycaemia (blood glucose: 3.20 g/L) without ketonuria. The history revealed an uneventful pregnancy and a birth weight of 3800 g. Because of congenital deafness and dumbness, she was never sent to school. She had a history of ‘pericarditis’ at the age of 2 years and was treated for tuberculous pericarditis with no bacteriological or histological evidence. At the age of 11 years, 3 months after diabetes onset, she developed fever and changes in her general condition, which were reported as pulmonary tuberculosis and led to 8 months of antibacterial treatment once again.

On admission to hospital at age 12 years 8 months, she was still prepubertal, with a height of –3 SD and a weight of –2 SD. She also showed facial dysmorphism with hypertelorism, epicanthic folds, distal arthrogyrosis of the hands and distortion of the feet (Fig. 1A). Her skin was normal. Biological tests showed no systemic inflammation. HbA1c was elevated (8.3%), under insulin treatment (0.91U/kg/day). Her fasting C-peptide level was very low at 0.07 ng/mL (normal range: 0.78–4.10 ng/mL), indicating no residual beta-cell function. Islet autoantibody testing was not performed. After an initial and ongoing training of both the child and her mother, metabolic control improved and HbA1c remained stable near 6.5%. Screening for diabetes-related degenerative complications remained negative. There were no clinical signs of exocrine pancreas dysfunction. Growth continued along the –3 SD curve, reaching an adult height of 1.45 m. She achieved puberty at age 15 years.

At age 16 she was readmitted to the clinic for ankle arthritis and a fever that lasted 3 weeks. She was pale, and her liver and spleen were enlarged on examination. Inflammatory markers were raised, with an erythrocyte sedimentation rate (ESR) of 144 mm/h and C-reactive protein (CRP) of 138 mg/L. Blood count showed non-regenerative anaemia and thrombocytosis (haemoglobin: 6 g/dL; platelets: 713,000/mm³). Bacterial and viral serology, and tests for Koch’s Bacillus, rheumatoid factor, anti-DNA and antinuclear antibodies were all negative. Chest X-ray showed cardiomegaly and mediastinal widening (Fig. 1B) that was consistent with a cystic mass on thoracic computed tomography (CT). She was treated with steroids (2 mg/kg/day) and antibacillary drugs, both of which were stopped a month later because of a lack of biological improvement. The patient continued to take anti-inflammatory drugs and received blood transfusions on request. Bone marrow aspiration and biopsy showed erythroblastopenia. Thoracotomy was performed later for resection of the cystic mediastinal mass. Histological study confirmed a thin-walled cyst lined by fibrous unilaminated epithelium made up of elongated cells with eosinophilic cytoplasm and rounded nuclei with squamous metaplasia.

After the operation, the erythroblastopenia remained unchanged, thereafter requiring monthly transfusions of packed phenotype-matched red blood cells after immunization had been detected. Despite the use of chelators, ferritin remained very high (over 10 times greater than normal). Inflammatory markers persisted with an ESR fluctuating between 80 and 140 mm/h and a CRP between 60 and 130 mg/L. The mediastinal mass reappeared at age 18 due to incomplete surgical excision, albeit with no signs of compression. Simple monitoring was decided on as the follow-up.

The patient developed irregular hyperpigmented patches along the inner thighs and popliteal fossae and on her back and buttocks; these lasted a few weeks, evolving according to the variation in inflammatory markers (Fig. 1C). Histological examination of a skin biopsy from a hyperpigmented area showed hyperpigmentation of the basal cell layer and dermal elastosis (Fig. 1D). However, there was no evidence of inflammation (no inflammatory infiltrate and negative inflammatory markers on immunochemistry).

At age 18, the patient was treated with pulsed methylprednisolone (1.73 g/m²/day) for 3 days, followed by prednisone (2 mg/kg per os) for 3 months, but with no improvement. Oral cyclophosphamide (100 mg/m²/day) was then introduced for 2.5...
months, but again with no clinical or biological improvement. Treatment with cyclosporine was then given for 1 month and led to apparent improvement of the inflammatory syndrome, but was discontinued for cost reasons.

The clinical picture presented by the patient was strikingly homologous with several syndromes characterized by the variable occurrence of insulin-dependent diabetes, pigmented patches, growth retardation, deafness and inflammation: pigmented hypertrichotic dermatitis with insulin-dependent diabetes (PHID); H syndrome; Faisalabad histiocytosis; and sinus histiocytosis with massive lymphadenopathy (SHML). These are all caused by mutations in SLC29A3 (Table 1). For this reason, SLC29A3 was selected as the candidate gene in our case.

3. Methods

The patient’s genomic DNA was extracted from peripheral blood using the standard protocols. Sequencing of SLC29A3 coding regions and the corresponding exon-intron boundaries was performed on polymerase chain reaction (PCR)-amplified DNA using an Applied Biosystems 3730 DNA Sequencer (Foster City, CA, USA). HLA-DRB1*04 and *04 typing was performed as described elsewhere [2].

4. Results and discussion

A homozygous splice mutation (c.300+1G>C on NM_018344) resulting in a frameshift and truncated protein (N101LfsX34 on NP_060814) was identified; this had removed most of the transmembrane domains of protein (Fig. S1; see supplementary material associated with this article online). The same mutation was recently reported in two Egyptian siblings presenting with overlapping features of H syndrome, PHID and Faisalabad histiocytosis [3]. It is noteworthy that all three patients had the most extreme presentations compared with all other patients described with SLC29A3 mutations thus far, and these correlated with the extreme N-terminal position of the mutation. In particular, these three patients showed the combination of insulin-dependent diabetes, which is specific to PHID, hyperpigmentation that is commonly seen in PHID, and deafness, cardiac anomalies, arthrogryposis and anaemia, all of which are seen in H syndrome and Faisalabad histiocytosis. Our patient had severe anaemia with erythroblastopenia. Anaemia was also observed in one of the two Egyptian patients, and severe anaemia with erythroblastopenia has previously been reported in a patient with Faisalabad histiocytosis [4]. Our patient had no residual beta-cell function and had been treated by insulin from the time of diabetes onset. She was negative for HLA-DRB1*03 and *04. The absence of type 1 diabetes HLA risk alleles suggests a likely non-autoimmune process, as reported in most PHID patients [5,6]. In addition, our patient had a mediastinal mass, which has never been reported in any previous patients with SLC29A3 mutations. The mass appears to be similar to the orbital and scrotal masses that have been described in patients with Faisalabad histiocytosis/SHML, as well as the nasal infiltration described in Faisalabad histiocytosis [3,7,8]. A similar mutation (c.300+1G>A) resulting in a predicted similar
truncated protein (N101VfsX34) has also been identified in three patients with Faisalabad histiocytosis [8,9] with no evidence of the full-blown PHID and H syndrome seen in our patient and the Egyptian patients [3]. In particular, those three patients had neither diabetes nor pigmented patches. The predicted truncated proteins only differed in the first frameshifted amino acid at position N101 (L or V), which is unlikely to differentially alter protein function. Although both c.300+1G>C and c.300+1G>A were predicted to completely abolish the splice site, which was qualitatively confirmed by complementary DNA (cDNA) for the c.300+1G>A mutation [8], we hypothesize that very low normal residual splicing may occur for c.300+1G>A, but not – or at least much less so – for c.300+1G>C, which might then explain the different disease severity between the two mutations. Alternatively, the different clinical expressions related to these mutations may depend on additional genetic or environmental factors. The finding of a mediastinal mass with metaplastic cells in our patient was consistent with the observation of other cellular proliferations in Faisalabad histiocytosis, particularly in patients homozygous for the c.300+1G>A mutation; indeed, a report of early-onset cancer in a patient bearing this mutation supports the hypothesis that SLC29A3 deficiency may also predispose to neoplasia [8].

A more N-terminal mutation, c.243delA, was reported in a mild form of SLC29A3-associated disease [10]. In that case, the mild phenotype was interpreted to result from the restoration of a quasi-complete transcript as the result of frameshifts in alternative transcripts [10]. Most authors have rightly emphasized the variability of clinical presentations due to SLC29A3 mutations even in patients carrying the same mutations. For example, the homozygous G437R mutation has been associated with highly variable phenotypes, including within the same family [8]. Overall, our observation supports the idea that the extreme N-terminal location of truncation in c.300+1G>C is associated with the most extreme phenotype, with cumulative features of PHID, H syndrome and Faisalabad histiocytosis/SHML whereas, in contrast, other rare mutations, such as c.243delA and R363Q, result in remarkably mild clinical presentations [10,11]. All of these variant manifestations have now been grouped under ‘histiocytosis–lymphadenopathy plus syndrome’ (OMIM #602782). In addition, SLC29A3 mutations have recently been identified in dysostosesclerosis, a particular form of osteopetrosis that shares surprisingly few clinical features with the other syndromes caused by SLC29A3 mutations [12]. This points again to the wide variety of clinical

### Table 1

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>PHID</th>
<th>H syndrome</th>
<th>SHML/FH</th>
<th>Patients with c.300+1G&gt;C (N101LfsX34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>8 (6 families)</td>
<td>33 (23 families)</td>
<td>9 (3 families)</td>
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</tr>
<tr>
<td>Country of origin</td>
<td>India, Pakistan, Lebanese-Australian, North America</td>
<td>Bulgaria, Turkey, Israel, Japan, India, Algeria, Spain</td>
<td>Turkey, Pakistan, Palestine</td>
<td>Egypt</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
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<td>+++</td>
<td>+</td>
<td>+</td>
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<td>Hypertrichosis</td>
<td>+++</td>
<td>++</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Deafness/impaired hearing</td>
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<td>++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hepatosplenomegaly</td>
<td>+++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Short stature/growth</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Hepatitis</td>
<td>++</td>
<td>++</td>
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<td>Infiltrations/masses</td>
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<td>Nasal infiltration</td>
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<td>Orbital mass</td>
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<td>Scrotal mass</td>
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<td>Medial mass</td>
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<td>Mental retardation</td>
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</tbody>
</table>

**Main references**

[5,6] [14,15] [8] [3] [3] Present report

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Two patients had ‘mild forms’, one with R363Q mutation [11] and the other with c.243delA [10], and two patients with SLC29A3 mutations had clear clinical signs of dysostosesclerosis [12]. PHID: pigmented hypertrichosis with insulin-dependent diabetes; SHML: sinus histiocytosis with massive lymphadenopathy; +++: in >75% of cases; ++: in 25–75% of cases; +: in <25% of cases; –: absent; NR: not reported, no information or irrelevant.

* a Diabetes based on elevated HbA1c, but not insulin-treated at the time of study.

* b Erythroblastopenia reported only in our present patient and in one patient with FH.
manifestations caused by SLC29A3 mutations. ENT3 is a member of the SLC29 equilibrating nucleoside transporter family, which has a widespread tissue distribution and cellular localization mainly in mitochondria [13]. Its tissue distribution and role in mitochondrial metabolism is consistent with the variable phenotype observed in these patients in terms of both the severity and nature of the clinical manifestations. Our present observation further extends the clinical spectrum of SLC29A3 mutations and supports the possibility of some degree of phenotype–genotype correlation depending on the nature of the mutation.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A. Supplementary material

Supplementary material (Fig. S1) associated with this article can be found at http://dx.doi.org/10.1016/j.diabet.2013.03.007.

References