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LETTER

Mutations in the *SLC29A3* Gene Are not a Common Cause of Isolated Autoantibody Negative Type 1 Diabetes

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Dear Sir:

Recessive mutations in the *SLC29A3* gene have recently been shown to result in diabetes [1, 2]. In the last year eleven families with the H syndrome (OMIM#612391) and five families with pigmented hypertrichosis with insulin dependent diabetes (PHID) have been described, resulting from seven different recessive *SLC29A3* mutations. The most common feature in all but two cases is pigmented hypertrichosis. Hyperglycaemia is an overlapping feature of the two syndromes although it is much rarer in the H syndrome where it is present in 1/15 subjects [1, 3] compared to 5/6 subjects with PHID [2, 4, 5]. The median age of diagnosis for diabetes was 12 years (range: 4-15 years), all patients were insulin treated with only 1/5 testing positive for GAD autoantibodies. It is not known if milder mutations in the *SLC29A3* gene can cause autoantibody negative type 1 diabetes without associated syndromic features.

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Abbreviations PHID: pigmented hypertrichosis with insulin dependent diabetes

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The *SLC29A3* gene encodes ENT3, a member of the equilibrative nucleoside transporter family (SLC29), which mediates intracellular trafficking of nucleosides [6]. In man the *SLC29A3* gene is most highly expressed in uterus [7]. *In vivo* studies of the *Drosophila melanogatser* ortholog of *SLC29A3* (*ENT1*) have shown it interacts the insulin signalling pathway, although the molecular basis of the interaction has yet to be characterised [2]. In addition, it has been detected in total human pancreas but it is not known if it is expressed in the exocrine component or in the islets [7].

In order to determine whether the *SLC29A3* gene is expressed in endocrine pancreas, we quantified *SLC29A3* transcripts by real-time PCR in human islet, pancreas and uterine RNA, relative to that of the *HNF4A* gene, which has documented expression in the beta cell [8]. *B2M* was used as an endogenous control. Probes to *SLC29A3* mRNA were targeted to the exon 5-6 junction of the *SLC29A3* gene (NM_018344.4) and were validated by standard curve analysis over eight 1.10 serial dilutions (r^2 0.99). The expression levels of *B2M*, *HNF4A* and *SLC29A3* transcripts were calculated

from average crossing points of triplicate samples, using the comparative Ct (ΔΔCt) method [9]. Compared to uterine *SLC29A3* mRNA levels there were 17% and 2% expression in the pancreas and islets respectively (relative to *B2M*). Moreover *SLC29A3* mRNA makes up only a small proportion of the beta cell transcriptome representing 0.4% of transcripts detected for *HNF4A*. Therefore *SLC29A3* is detectable in both exocrine and endocrine pancreas, although the expression is lower in the latter.

Early-onset diabetes is a feature of both the H syndrome and PHID. We hypothesised that mutations in the *SLC29A3* gene could cause isolated diabetes in children and screened 47 cases diagnosed at a median of 5 years (range: 1-16 years) with autoantibody negative type 1 diabetes (antibodies tested at or soon after diagnosis; glutamic acid decarboxylase and/or islet antigen 2), and without pigmented hypertrichosis. Mutations in the *HNF1A, HNF4A, KCNJ11* and *INS* genes were excluded by sequence analysis in all subjects.

We amplified the 6 exons of *SLC29A3* (primer sequences available on request), including the exon/intron boundaries and non-coding exon 1. We did not identify any pathogenic *SLC29A3* mutations, but the common non-synonymous polymorphisms rs2277257, rs780668, rs2252996, and rs2487068 were present at a minor allele frequency of 71%, 7%, 9% and 6%, respectively.

We have shown that *SLC29A3* is expressed in the human islet and recessive mutations are likely to result in beta cell failure, however mutations in this gene are not a common cause of isolated autoantibody negative diabetes diagnosed in children under 17 years.

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Conflict of interest None to declare

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