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

Emma L Edghill1, Shihab Hameed2, Charles F Verge2, Oscar Rubio-Cabezas1,3, Jesús Argente3, Zdenek Sumnik4, Petra Dusatkova1, Simon T Cliffe5,6, Raoul CM Hennekam7, Michael F Buckley5,8, Khalid Hussain7, Sian Ellard1, Andrew T Hattersley1

1Institute of Biomedical and Clinical Science, Peninsula Medical School. Exeter, United Kingdom. 2Sydney Children’s Hospital and School of Women’s and Children’s Health, University of New South Wales. Randwick, NSW, Australia. 3Department of Endocrinology, University Pediatric Hospital “Niño Jesús”; Department of Pediatrics, Autonomous University of Madrid and CIBER Physiopathology of Obesity and Nutrition, Institute “Carlos III”. Madrid, Spain. 4Department of Pediatrics, Second Faculty of Medicine, University Hospital Motol. Prague, Czech Republic. 5Laboratory Services, Department of Haematology and Genetics, South Eastern Area. Sydney, NSW, Australia. 6Centre for Vascular Research, School of Medical Sciences, University of New South Wales. Sydney, NSW, Australia. 7Department of Endocrinology, Great Ormond Street Hospital for Children NHS Trust and the Institute of Child Health, University College. London, United Kingdom. 8Department of Human Genetics, Radboud University Nijmegen Medical Centre. GA Nijmegen, The Netherlands

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.

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 melanogaster 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 (r2 0.99). The expression levels of B2M, HNF4A and SLC29A3 transcripts were calculated

Received June 10th, 2009

Key words Diabetes Mellitus; Genes; Genetics; Infant, Newborn; SLC29A3 protein, human

Abbreviations PHID: pigmented hypertrichosis with insulin dependent diabetes

Correspondence Andrew T Hattersley
Institute of Biomedical and Clinical Science, Peninsula Medical School, Barrack Road, Exeter EX2 5DW, United Kingdom
Phone: +44-1392.6806; Fax: +44-1392.406.767
E-mail: andrew.hattersley@pms.ac.uk

Document URL http://www.joplink.net/prev/200907/30.html
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.

Acknowledgements The authors thank Annet Damhuis and Jonathan Locke for excellent technical support. This work was supported in Exeter by the European Union (Integrated Project EURODIASHM-CT-2006-518153 in the Framework Programme 6 (FP6) of the European-Community. Z.S. is supported by MHCRC grant 64203. O.R.C. is supported by an “Ayuda para Contratos post Formacion Sanitaria Especializada” from the “Instituto de Salud Carlos III” (FIS CM06/00013). S.E. is employed within the NIHR funded Peninsula Clinical Research Facility and A.T.H. is a Wellcome Trust Research Leave Fellow

Conflict of interest None to declare

References