An ABCC8 gene mutation and mosaic uniparental isodisomy resulting in atypical diffuse congenital hyperinsulinism

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ABSTRACT

Objective: Congenital hyperinsulinism may be due to diffuse or focal pancreatic disease. The diffuse form is associated with an increase in the size of β-cell nuclei throughout the whole of the pancreas and most commonly results from recessive $K_{\text{ATP}}$ channel mutations. Focal lesions are the consequence of somatic uniparental disomy for a paternally inherited $K_{\text{ATP}}$ channel mutation with enlargement of the β-cell nuclei confined to the focal lesion. Some “atypical” cases defy classification and show pancreatic β-cell nuclear enlargement confined to discrete regions of the pancreas. We investigated an atypical case with normal morphology within the tail of the pancreas but occasional enlarged endocrine nuclei in parts of the body and head.

Research Design and Methods: The $KCNJ11$ and $ABCC8$ genes encoding the $K_{\text{ATP}}$ channel subunits and microsatellite markers on chromosome 11 were analysed in DNA samples from the patient and her parents.

Results: A mosaic $ABCC8$ nonsense mutation (Q54X) was identified in the proband. The paternally inherited mutation was present at 90% in lymphocytes, 50% in normal pancreatic sections but between 64 and 74% in abnormal sections. Microsatellite analysis showed mosaic interstitial paternal uniparental isodisomy for chromosome 11p15.1.

Conclusion: We report a novel genetic mechanism to explain atypical histological diffuse forms of congenital hyperinsulinism due to mosaic uniparental isodisomy in patients with dominantly inherited $ABCC8$ (or $KCNJ11$) gene mutations.
Congenital hyperinsulinism (CHI) is characterised by the unregulated secretion of insulin from pancreatic β-cells. The inappropriate insulin secretion causes severe and persistent hypoglycaemia which is a potent cause of brain damage if inappropriately managed. The most common genetic causes of medically unresponsive CHI are recessive mutations in the genes ABCC8 and KCNJ11, encoding the two subunits (SUR1 and Kir6.2 respectively) of the pancreatic β-cell K\textsubscript{ATP} channel (1-3). About 50% of patients have germline mutations in either ABCC8 or KCNJ11 (4).

Histologically there are two main subtypes of CHI: diffuse and focal. These two discrete forms share a similar clinical presentation, but result from different pathophysiological and molecular mechanisms (5). Diffuse CHI is usually inherited as an autosomal recessive disorder, whereas focal CHI is sporadic and can potentially be cured by surgical removal of the focal lesion. The typical diffuse form affects all the β-cells and is most commonly due to recessive mutations in either of the genes encoding the two subunits of the K\textsubscript{ATP} channel. Typical diffuse disease is characterised by an increase in the size of the pancreatic β-cell nuclei throughout the entire pancreas. Focal hyperinsulinism results from a paternally inherited K\textsubscript{ATP} channel mutation together with somatic loss of the maternal chromosome 11p15 region (6-8). Outside the focal lesion there is no endocrine nuclear enlargement. The consequent imbalance in the expression of imprinted genes within the 11p15.5 region leads to focal islet cell adenomatous hyperplasia and the reduction to homozygosity of the K\textsubscript{ATP} channel mutation results in dysregulated insulin secretion within the focal lesion. Despite this classification into focal and diffuse disease there are still some cases which represent a diagnostic challenge as they cannot be easily classified into focal or diffuse disease (9; 10). Some of these “atypical” forms demonstrate pancreatic β-cell nuclear enlargement confined to discrete regions of the pancreas, which raises the possibility of mosaicism. The molecular pathophysiology of these “atypical” forms is unclear.

We report a novel case of CHI associated with a nonsense mutation in the ABCC8 gene that shows mosaic interstitial segmental paternal isodisomy. The histology of the resected pancreas showed pancreatic β-cell nuclear enlargement only in some sections of the pancreas. The Q54X mutation was heterozygous in unaffected sections of the pancreas, but present at between 64 and 74% in diseased tissue. This unique case suggests that some of the “atypical” histological diffuse forms of CHI may be due to somatic mosaicism.

**Clinical Case Presentation.** The patient was born to non-consanguineous parents at 36 weeks gestation with a birth weight of 3.21kg. She presented with symptomatic (fits) hypoglycaemia within three hours of birth and was diagnosed with hyperinsulinaemic hypoglycaemia (plasma glucose 1.2mmol/l, serum
insulin 22.4mU/l with undetectable fatty acids and ketone bodies, normal lactate and normal serum ammonia concentrations). The glucose infusion rate required to maintain a blood glucose concentration greater than 3.5mmol/L was 22mg/kg/min (N 4-6mg/kg/min). The patient underwent a trial of diazoxide (up to 20mg/kg/day) but showed no response. Subcutaneous infusion of octreotide and glucagon were used to stabilise her blood glucose concentrations.

**Histology.** A laparoscopic biopsy of the tail of her pancreas was performed at the age of 6.5 weeks. The biopsy measured 2.0x1.0x0.6cm. The frozen sections showed no focal lesion or evidence of diffuse disease (no enlarged endocrine nuclei). This was confirmed on serial paraffin sections (n=97). A biopsy from the region of the head of pancreas showed a lymphoid follicle and adjacent pancreas with very occasional enlarged endocrine nuclei in both frozen and serial paraffin sections (n=36). A ¹⁸ Fluoro-L-Dopa PET (Positron Emission Tomography) scan showed diffuse uptake of the tracer. One month later the patient underwent a near total pancreatectomy as she was unresponsive to medical therapy. The specimen measured 4.3x0.9x0.6cm and weighed 2.1g. Transverse blocks from the tail end to the head end (1-10) were taken and were processed into paraffin-wax. The 10 blocks were extensively sectioned and revealed no evidence of focal nodular endocrine hyperplasia. Blocks 1, 2 and 10 showed no histological abnormality. In particular no enlarged endocrine nuclei were detected. In contrast blocks 3-9 showed the presence of occasional enlarged endocrine nuclei (figure 1). Within four days post pancreatectomy she again developed hypoglycaemia. On this occasion hypoglycaemia was managed with subcutaneous octreotide (25-30mig/kg/day) injections in combination with 3 hourly day time feeds and continuous overnight feeds. On this regimen her blood glucose levels range between 4-7mmol/L.

Molecular genetic analysis
Sequencing analysis identified a novel nonsense mutation, Q54X (c.160C>T; p.Gly54X) in exon 2 of the *ABCC8* gene. This Q54X mutation results in a premature termination codon which is predicted to result in nonsense mediated decay of the mutant mRNA and/or production of a truncated protein lacking 1528 of the 1581 amino acid residues. The proband inherited the mutation from her clinically unaffected father who was heterozygous for the mutation (figure 2). Quantitative analysis of the mutation by real-time PCR showed the mutation was present at 91% (SD ± 3%) in the proband’s leukocyte DNA, 51% (SD ± 14%) in buccal cells and 53% in fibroblasts (SD ± 1%) (figure 3a). DNA samples extracted from the three unaffected sections of the pancreas (blocks 1, 2 and 10) were heterozygous for the mutation, whereas 7 samples resected from diseased pancreatic tissue (blocks 3-9) had on average a 68% mutation load (figure 3b).

Microsatellite markers on chromosome 11 were analysed in the proband and her parents. Markers spanning chromosome 11pter-11p15.2 and 11p14.2-11q25 were
heterozygous but D11S921, D11S902 and D11S1888 showed loss of heterozygosity within the chromosome 11p15.1 region (figure 4) in DNA extracted from blood and in pancreatic blocks 3-9. A multiplex ligation-dependent probe amplification (MLPA) assay was used to measure ABCC8 gene dosage relative to a series of control gene exons. The results showed normal diploid copy number. These data suggest that the loss of heterozygosity is likely to be the consequence of mosaic segmental paternal uniparental isodisomy. The interstitial region of UPD is between 493kb and 13.4Mb in size (figure 4) and encompasses the KATP channel genes which is consistent with the loss of heterozygosity observed in the patient’s leukocytes and within the regions of the pancreas that showed abnormal histology.

Mosaic paternal UPD of 11p15.5 has been identified in approximately 20% of patients with Beckwith-Wiedemann syndrome (BWS) (11). BWS is a congenital overgrowth syndrome which results from the dysregulation of imprinted genes at 11p15.5. The absence of syndromic features in our patient is consistent with the absence of UPD across the BWS locus. We recently reported a patient with telomeric mosaic segmental paternal UPD (11p14-11pter) which unmasked a recessive activating mutation in ABCC8 resulting in permanent neonatal diabetes in addition to syndromic features of BWS (12). To our knowledge this is the first reported case of interstitial UPD at 11p15.1. Although other genes are present in the region of UPD, no imprinted genes have been reported at 11p15.1 suggesting that this is not the disease mechanism. The patient’s phenotype is consistent with a recessively acting loss-of-function ABCC8 mutation, unmasked by paternal UPD within the affected areas of the pancreas. Whilst there are at least 40 reports of complete UPD unmasking a recessive mutation in various genes (13), to our knowledge a clinical phenotype has only been reported once before in association with mosaic UPD and a dominantly inherited mutation (14).

The mosaic interstitial paternal UPD is likely to be the consequence of somatic recombination between two non-sister chromatids during early embryogenesis. This post-zygotic interchromatid exchange would result in a cell line with paternal UPD, a reciprocal cell line with maternal UPD and a normal biparental cell line. Paternal UPD was present in lymphocytes and affected parts of the pancreas. We found no evidence for maternal UPD (Q54X mutation load <50%) in any tissues sampled. The paternal UPD cell line is unlikely to have a selective growth advantage since the chromosome 11p15.5 region, which contains genes involved in cell cycle regulation, is disomic. The maternal UPD cell line may therefore be present in other tissues which were not tested or it might have been present in the placenta.

Focal hyperinsulinism results from the inheritance of a paternal KATP channel mutation and somatic mosaicism within the lesion for paternal UPD of 11p15.1-15.5. The presence of paternal UPD in focal hyperinsulinism results in the inappropriate expression of imprinted genes at 11p15.5,
providing cells with a selective growth advantage. The UPD across 11p15.1 renders the paternally inherited $K_{ATP}$ channel mutation homozygous resulting in dysregulated insulin secretion within the focal lesion. In contrast, we describe a patient with atypical diffuse disease resulting from an earlier mitotic recombination leading to interstitial mosaic UPD in various tissues. The absence of focal disease in our patient can be explained by the lack of UPD at the imprinted region on 11p15.5. The presence of UPD at 11p15.1 results in a reduction to homozygosity of the $ABCC8$ mutation in some parts of the pancreas, similar to the situation associated with recessively inherited diffuse disease. This finding suggests that atypical forms of diffuse disease due to mosaic $K_{ATP}$ channel mutations could be associated with paternal or maternal inheritance.

In summary, we have identified a novel cause of congenital hyperinsulinism resulting from a nonsense $ABCC8$ gene mutation and mosaic paternal UPD of 11p15.1. Our findings suggest that “atypical” histological diffuse forms of CHI may be attributed to mosaic UPD in patients with inherited $ABCC8$ (or $KCNJ11$) gene mutations.

**RESEARCH DESIGN AND METHODS**

**KCNJ11 and ABCC8 sequencing.** Genomic DNA was extracted from peripheral leukocytes using standard procedures. The single exon of $KCNJ11$ was amplified by PCR as previously described (15). When no mutations were identified, the 39 exons of the $ABCC8$ gene encoding the SUR1 protein were amplified using previously described primers (16). Sequencing was performed in one direction using universal M13 primers and a Big Dye Terminator Cycler Sequencing Kit v3.1 (Applied Biosystems, Warrington, UK) and reactions analysed on an ABI 3730 Capillary sequencer (Applied Biosystems, Warrington, UK). Sequences were compared to the published sequence (($KCNJ11$ - NM_000525.3) ($ABCC8$ - NM_000352.2)) using Mutation Surveyor Software v2.61 (Biogene Ltd, Kimbolton, UK).

**Microsatellite analysis.** Mosaic paternal isodisomy was investigated with a panel of 20 microsatellite markers on chromosome 11, using DNA extracted from leukocytes from the proband and parents (12). Six of these markers were used to investigate UPD in the 10 sections of pancreatic tissue from the patient (15). Primer sequences are available on request.

**Multiplex Ligation-Dependent Probe Amplification (MLPA).** Gene dosage was analysed using MLPA. Exon-specific synthetic probes were designed for five exons (1, 8, 23, 31 and 39) of the $ABCC8$ gene (details available on request) which were used in conjunction with the MEN1 MLPA Kit (#P017) (MRC Holland, The Netherlands). MEN1 exon-specific and other control probes from the kit (n=24) were used as controls for the assay. The procedure was carried out according to manufacturer’s instructions. Briefly, 100-150ng of genomic DNA was used as a template. Following DNA
denaturation, probe hybridization, probe ligation and amplification, the products were separated according to size on an ABI 3130 (Applied Biosystems, Warrington, UK). The data were analysed using GeneMapper and GeneMarker analysis software v1.51 (Soft Genetics LLC, State College, PA, USA) to define the size and peak heights of the 29 probes. Two control samples and a positive control with a deletion of exons 1-8 (17) and a negative control were included in the assay. Dosage quotients were calculated using previously described methods (18).

**Real-time PCR quantification of the mutant allele.** Genomic DNA from leukocytes, fibroblasts, buccal cells and from 10 sections of pancreatic tissue was amplified using a mutation-specific TaqMan approach. Genomic DNA from the father who is heterozygous for the Q54X mutation was used in serial dilution to produce standard curves to determine linear range and accuracy of quantitation (primer and probe sequences are available on request). Reactions contained 5ul TaqMan Fast universal PCR mastermix no Amperase, 0.5ul Assays-by-Design (Applied Biosystems, Warrington, UK) probe and primer mix (corresponding to 36ul of each primer and 8ul of each probe), 2.5ul water and 2ul of DNA at a concentration of 100ng/ul. Amplification conditions were a single cycle of 95 C for 20s followed by 40 cycles of 95 C for 1s and 60 C for 20s. The relative level of mutant transcript relative to wild-type transcript was determined by the \( \Delta \Delta CT \) method (19). Each test was carried out in triplicate.

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Mosaic ABCC8 mutations and congenital hyperinsulinism

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FIGURE LEGENDS

Figure 1. H&E-stained sections of the proband’s pancreas showing uniform sized islet cell nuclei in an islet of Langerhans surrounded by exocrine acinar tissue (Normal) and an islet of Langerhans with enlarged endocrine nuclei identified by arrows (Abnormal)

Figure 2. A pedigree showing the inheritance of the novel Q54X ABCC8 mutation. The filled circle represents the proband who is affected with hyperinsulinism. The square with a dot represents the unaffected father who is a carrier for hyperinsulinism and the unfilled circle represents the unaffected mother. The genotype for each family member is provided, N/N denotes no mutation identified. Directly below each genotype is an electropherogram showing the sequencing results. A heterozygous c.160C>T mutation (arrow) resulting in the substitution of glycine (CAG) by a premature termination codon (TAG) at residue 54 (Q54X) of the ABCC8 gene is shown for the father. A mosaic mutation at the same position is present in the proband as shown by the increase in the mutant T allele relative to the wild type C allele. The mother is homozygous for the normal C allele at this position.

Figure 3. (a) Representation of the percentage mutant allele relative to wild type transcript present in DNA extracted from the proband’s buccal cells, leukocytes and fibroblasts. Error bars represent the standard deviation between runs. The percentage mutant allele is given above each bar (b) Representation of the percentage mutant allele relative to normal allele present in DNA extracted from 10 pancreatic samples from diseased and normal tissue. Testing was carried out in triplicate for each of the 10 samples and a mean average was plotted. Error bars represent the standard deviation between runs. The percentage mutant allele is given above each bar for the corresponding pancreatic section. Blue bars represent samples taken from areas of diseased pancreas, whereas tissue from normal parts of pancreas are represented by green bars.

Figure 4. (a) Results of microsatellite analysis on chromosome 11 for DNA extracted from peripheral lymphocytes of the proband and her parents. The distance between the marker and the telomere at 11p is given. Blue text represents markers which represent a region with no UPD, green text represents markers which are uninformative and red text illustrates markers which are informative for UPD. The alleles which are printed in bold and underlined are informative for UPD. (b) Representation of the position of the 20 markers analysed on chromosome 11. Blue sections represent regions with no UPD, the green section represents a region where microsatellites analysis provided uninformative results and the red section corresponds to the region with mosaic UPD. The distance in Mb is given for each of these regions. A region of chromosome 11 has been expanded to demonstrate the area of UPD in relation to the location of the K_ATP channel genes, KCNJ11 and ABCC8, and the imprinted locus at 11p15.5. Directly below the diagram are two
Mosaic ABCC8 mutations and congenital hyperinsulinism

electropherograms which demonstrate the results of microsatellite analysis for markers D11S921 and D11S935 for the proband and her parents. The X-axis indicates the product size (base pairs) and the Y axis the product quantity (arbitrary units). The results for marker D11S935 show normal bi-parental inheritance as demonstrated by the preferential amplification of the smaller product in the proband and parental DNA samples. In contrast the results for marker D11S921 illustrate mosaic UPD with a larger peak for the paternal allele compared to the maternal allele.
Figure 1
Figure 2

Mosaic ABCC8 mutations and congenital hyperinsulinism
Figure 3

a)

b)

Pancreatic Section
Mosaic ABCC8 mutations and congenital hyperinsulinism

Figure 4