Refinement of the critical genomic region for hypoglycaemia in the Chromosome 9p deletion syndrome [version 1; peer review: awaiting peer review]

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Abstract

Background: Large contiguous gene deletions at the distal end of the short arm of chromosome 9 result in the complex multi-organ condition chromosome 9p deletion syndrome. A range of clinical features can result from these deletions with the most common being facial dysmorphisms and neurological impairment. Congenital hyperinsulinism is a rarely reported feature of the syndrome with the genetic mechanism for the dysregulated insulin secretion being unknown.

Methods: We studied the clinical and genetic characteristics of 12 individuals with chromosome 9p deletions who had a history of neonatal hypoglycaemia. Using off-target reads generated from targeted
next-generation sequencing of the genes known to cause hyperinsulinaemic hypoglycaemia (n=9), or microarray analysis (n=3), we mapped the minimal shared deleted region on chromosome 9 in this cohort. Targeted sequencing was performed in three patients to search for a recessive mutation unmasked by the deletion.

**Results:** In 10/12 patients with hypoglycaemia, hyperinsulinism was confirmed biochemically. A range of extra-pancreatic features were also reported in these patients consistent with the diagnosis of the Chromosome 9p deletion syndrome. The minimal deleted region was mapped to 7.2 Mb, encompassing 38 protein-coding genes. *In silico* analysis of these genes highlighted *SMARCA2* and *RFX3* as potential candidates for the hypoglycaemia. Targeted sequencing performed on three of the patients did not identify a second disease-causing variant within the minimal deleted region.

**Conclusions:** This study identifies 9p deletions as an important cause of hyperinsulinaemic hypoglycaemia and increases the number of cases reported with 9p deletions and hypoglycaemia to 15 making this a more common feature of the syndrome than previously appreciated. Whilst the precise genetic mechanism of the dysregulated insulin secretion could not be determined in these patients, mapping the deletion breakpoints highlighted potential candidate genes for hypoglycaemia within the deleted region.

**Keywords**
Chromosome 9p, Deletions, Hyperinsulinism, Hypoglycaemia,
Introduction
Monosomy of part of the short arm of chromosome 9 causes the complex congenital condition chromosome 9p deletion syndrome (MIM: 158170). These large contiguous gene deletions can occur in isolation or form part of an unbalanced translocation. The cardinal clinical features of the 9p deletion syndrome are craniofacial dysmorphisms, including trigonocephaly, midface hypoplasia, flat nasal ridge, long philtrum, short neck and developmental delay. Other common features include musculo-skeletal abnormalities, congenital heart defects, abdominal wall defects and disorders of sexual differentiation. A further rare feature is hypoglycaemia, which has been described in 3 of the >100 genetically confirmed cases.

The phenotypic heterogeneity observed between individuals with the chromosome 9p deletion syndrome is likely to reflect differences in the extent of the deletion, with individual features resulting from haploinsufficiency of a specific gene(s). An example is seen in males with 46,XY gonadal dysgenesis (MIM: 154230) which has been linked to disruption of the putative sex-determining genes DMRT1 and DMRT2 on 9p.

Recent efforts have focussed on defining the critical region for the 9p deletion syndrome but there have been some differences in results. Swinkels et al. refined the critical region to a 300 kb stretch of DNA on 9p22.3; however, this region did not overlap with the critical region mapped by Faas et al.1,4. Given the differences in the craniofacial features between the cohorts reported it seems likely that there is not a single ‘critical region’ for the 9p deletion syndrome but rather that the syndrome represents a phenotypically and genetically heterogeneous group of disorders with the extent of the deletion, and in some cases the reciprocal trisomy, determining the phenotype.

Congenital hyperinsulinism is a rare condition of hypoglycaemia due to dysregulated insulin production from pancreatic beta cells. Despite major advances in genetics the underlying cause of congenital hyperinsulinism is not identified in approximately 55% of patients6. Studying patients with congenital hyperinsulinism and the 9p deletion syndrome provides an opportunity to further unravel the genetic underpinnings of dysregulated insulin secretion in congenital hyperinsulinism.

In this study we investigated the clinical and genetic characteristics of 12 patients with congenital hypoglycaemia and a large deletion on chromosome 9p. We mapped the genomic breakpoints in all 12 patients which allowed for refinement of the critical region for hypoglycaemia to 7.2 Mb encompassing 38 genes. We sought to identify candidate genes for congenital hyperinsulinism in this region, an approach which has been successfully employed for gene discovery in other conditions.

Methods
Cohort
A total of 12 patients with large deletions of the short arm of chromosome 9 were identified (as described below) following referral for genetic testing for congenital hyperinsulinism or a history of neonatal hypoglycaemia. Informed consent for publication of the patients’ details was obtained. This study was approved by the North Wales Research Ethics Committee (517/WA/0327).

The DECIPHER database was searched for individuals who had hypoglycaemia and deletions of chromosome 9p which overlapped with the deletions identified in our cohort.

Calling deletions
In nine patients multiple syndromic features had prompted microarray analysis leading to the identification of a 9p deletion prior to referral for congenital hyperinsulinism genetic testing. No disease-causing variants were identified in the known genes. In the remaining three patients a deletion on 9p was detected using SavvyCNV (release 1) using off-target reads from the next-generation sequencing analysis of the known congenital hyperinsulinism genes. This technique calls 97.5% of true CNVs >1Mb.

Break points were mapped in patients 1-9 using off-target reads from the targeted next generation sequencing data. In patients 10-12 the breakpoints were mapped by microarray analysis. In 5/11 patients the 9p deletion formed part of an unbalanced translocation (Table 1).

Sequencing of the deleted region
To search for recessive mutations unmasked by the deletion, next generation sequencing was performed in three patients following targeted capture of the Chr9p24 region (patients 4, 5 and 6, Table 1). Illumina-compatible libraries were prepared after fragmentation of genomic DNA to ~200bp average size, then enriched for target regions using a custom RNA bait library designed against chr9:1-7,834,443 (GRCh37/hg19) with medium stringency against repetitive sequences (Prognosys Biosciences Inc., formerly of La Jolla, CA). Hybridization, capture, washing and amplification (15 cycles) were performed using a Rivia Targeted Enrichment Kit according to the manufacturer’s instructions (Rivia, formerly of La Jolla, CA). Libraries were sequenced on an Illumina HiSeq 2000 using 100 base paired-end reads.

Sequence data was analysed using an approach based on the GATK best practice guidelines. Reads were aligned to the GRCh37/hg19 human reference genome with BWA mem (version 0.7.15) followed by local re-alignment using GATK IndelRealigner (version 3.7.0). Large sections of the region are low complexity and while mean target coverage was 36X, 34X and 41X, only 56%, 56% and 58% of the minimal deleted region was covered at 10X or above in the three samples, respectively. Variants were called using GATK haplotype caller and annotated using Alamut Batch (Interactive Biosoftware version 1.11, Rouen, France) (an open-access equivalent is ANNOVAR). We excluded variants present in gnomAD at a frequency greater than 1 in 27,000 - the highest published prevalence of hyperinsulinism in an outbred population. Variants that were homozygous in internal controls
Table 1. Clinical and genetic characteristics of patients with Chromosome 9p deletion syndrome. PDA = Patent Ductus Arteriosus, VSD = Ventricular Septal Defect, ASD = Atrial Septal Defect, PFO = Patent Foramen Ovale. *Patient 10 and Patient 11 are siblings. **Genomic coordinates (GRCh37/hg19) of copy number variant detected by analysis of tNGS off-target reads (patients 1–9) or microarray analysis (patients 10–12).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Karyotype</th>
<th>Chromosome 9p deletion</th>
<th>Reciprocal Duplication</th>
<th>Gender</th>
<th>Birthweight (g)</th>
<th>Gestation (wks)</th>
<th>Current Age (yrs)</th>
<th>Hyperinsulinemic hypoglycaemia</th>
<th>Extra-pancreatic features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46,XY, del(9)(p22.1)</td>
<td>0-9,200,000</td>
<td>None detected</td>
<td>Male</td>
<td>4478</td>
<td>38</td>
<td>3</td>
<td>2 days</td>
<td>Metopic suture defect</td>
</tr>
<tr>
<td>2</td>
<td>46,XX,del(9)(p22.2)</td>
<td>0-14,000,000</td>
<td>None detected</td>
<td>Male</td>
<td>3440</td>
<td>38</td>
<td>2</td>
<td>20 weeks</td>
<td>Hypertelorism</td>
</tr>
<tr>
<td>3</td>
<td>46,XY,der(9)t(9;13)(p24.1;p22.3)</td>
<td>0-17,600,000</td>
<td>None detected</td>
<td>Female</td>
<td>3200</td>
<td>40</td>
<td>10</td>
<td>2 weeks</td>
<td>Small low set ears</td>
</tr>
<tr>
<td>4</td>
<td>46,XX,del(9)(p22.3)</td>
<td>0-9,320,017</td>
<td>Chr13:75000000-115200000</td>
<td>Female</td>
<td>3520</td>
<td>37</td>
<td>8</td>
<td>6 weeks</td>
<td>Macroglossia</td>
</tr>
<tr>
<td>5</td>
<td>46,XY,del(9)(p24.1)</td>
<td>0-7,300,000</td>
<td>None detected</td>
<td>Female</td>
<td>3670</td>
<td>38</td>
<td>9</td>
<td>Ongoing at 8 yrs</td>
<td>High arched palate</td>
</tr>
<tr>
<td>6</td>
<td>46,XX,del(9) (p24.1;p24)</td>
<td>0-7,200,000</td>
<td>Chr7:0-10,000,000</td>
<td>Female</td>
<td>3510</td>
<td>40</td>
<td>7</td>
<td>Ongoing</td>
<td>Long philtrum</td>
</tr>
<tr>
<td>7</td>
<td>46,XX,del(9)(p13)</td>
<td>0-7,600,000</td>
<td>None detected</td>
<td>Female</td>
<td>1700</td>
<td>39</td>
<td>2</td>
<td>Ongoing</td>
<td>Low set ears</td>
</tr>
<tr>
<td>8</td>
<td>46,XX,del(9)</td>
<td>0-12,400,000</td>
<td>None detected</td>
<td>Male</td>
<td>3670</td>
<td>34</td>
<td>2</td>
<td>Ongoing</td>
<td>Flat mid face</td>
</tr>
<tr>
<td>9</td>
<td>46,XX,del(9)(p13)</td>
<td>0-12,450,000</td>
<td>Chr13:107,452,410-110,155,270</td>
<td>Male</td>
<td>4160</td>
<td>38+1</td>
<td>11</td>
<td>Ongoing</td>
<td>High nasal bridge</td>
</tr>
<tr>
<td>10</td>
<td>46,XY,del(9)(p22.3)</td>
<td>0-17,600,000</td>
<td>None detected</td>
<td>Female</td>
<td>5050</td>
<td>41</td>
<td>20</td>
<td>Ongoing</td>
<td>Large mouth</td>
</tr>
<tr>
<td>11</td>
<td>46,XX,del(9)</td>
<td>0-12,450,000</td>
<td>None detected</td>
<td>Male</td>
<td>5050</td>
<td>42</td>
<td>8</td>
<td>Ongoing</td>
<td>Thin upper lip</td>
</tr>
<tr>
<td>12</td>
<td>46,XY,del(9)(p22.3)</td>
<td>0-10,955,813</td>
<td>None detected</td>
<td>Male</td>
<td>5050</td>
<td>42</td>
<td>8</td>
<td>Ongoing</td>
<td>None noted</td>
</tr>
</tbody>
</table>

**Blood glucose (mmol/L)**
- Patient 1: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 2: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 3: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 4: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 5: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 6: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 7: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 8: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 9: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 10: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 11: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9
- Patient 12: 2.9, 1.8, 2.5, 1.4, 1.8, 1.4, 1.8, 1.4, 2.7, 2.0, 0.7, 1.2, 1.9

**Insulin (pmol/L)**
- Patient 1: Not tested
- Patient 2: Not tested
- Patient 3: 97
- Patient 4: 28
- Patient 5: <6.0
- Patient 6: 111
- Patient 7: 96
- Patient 8: 42
- Patient 9: 51
- Patient 10: 47
- Patient 11: 53
- Patient 12: 100

**C-Peptide (pmol/L)**
- Patient 1: Not tested
- Patient 2: Not tested
- Patient 3: 228
- Patient 4: Not tested
- Patient 5: 980
- Patient 6: 540
- Patient 7: Not tested
- Patient 8: Not tested
- Patient 9: 364
- Patient 10: 629
- Patient 11: Not tested
- Patient 12: Not tested

**Treatment details**
- Patient 1: Diazoxide until remission (dose not available)
- Patient 2: Diazoxide 10.5mg/kg/day until remission
- Patient 3: Diazoxide 5mg/kg/day until remission
- Patient 4: Diazoxide 10mg/kg/day until remission (dose not available)
- Patient 5: Diazoxide 10mg/kg/day ongoing
- Patient 6: Diazoxide 10mg/kg/day ongoing
- Patient 7: Diazoxide 6mg/kg/day ongoing
- Patient 8: Diazoxide 10mg/kg/day ongoing
- Patient 9: Diazoxide 10mg/kg/day ongoing
- Patient 10: Diazoxide 10mg/kg/day ongoing
- Patient 11: Diazoxide 10mg/kg/day ongoing
- Patient 12: Diazoxide 10mg/kg/day ongoing

**Facial dysmorphism**
- Patient 1: Metopic suture defect, Hypertelorism
- Patient 2: Hypertelorism
- Patient 3: High arched palate, Small low set ears
- Patient 4: High arched palate, Small low set ears
- Patient 5: Metopic prominence, B-temporal narrowing, Prominent eyes, Low set ears, Long philtrum, Micrognathia
- Patient 6: Broad forehead, Brachycephaly, Low set ears
- Patient 7: Microcephaly, Up-slanting palpebral fissures, Prominent forehead, Lateral deviation, Thinner upper lip
- Patient 8: Sub mucosal cleft palate, Uvula bifida, Deep-seated ears, Hypertelorism, Small chin
- Patient 9: None noted
- Patient 10: Prominent forehead, Flat mid face, Wide base goitre, Low set ears
- Patient 11: Macroglossia, Small deep-seated ears
- Patient 12: Macroglossia, Small deep-seated ears
<table>
<thead>
<tr>
<th>Digits</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
<th>Patient 7</th>
<th>Patient 8</th>
<th>Patient 9</th>
<th>Patient 10</th>
<th>Patient 11*</th>
<th>Patient 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wide sandal gap</td>
<td>Normal</td>
<td>Long fingers and toes</td>
<td>4 limb postaxial polydactyly</td>
<td>Syndactyly 2&lt;sup&gt;nd&lt;/sup&gt; and 3&lt;sup&gt;rd&lt;/sup&gt; toes</td>
<td>Unilateral clinodactyly</td>
<td>Bilateral sandal gap</td>
<td>Broad and long fingers and toes</td>
<td>Normal</td>
<td>Normal</td>
<td>Broad fingers</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Cardiac</td>
<td>VSD</td>
<td>PDA</td>
<td>ASD</td>
<td>ASD, PDA</td>
<td>ASD, PDA</td>
<td>VSD</td>
<td>PDA, PFO/ ASD II</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other features</td>
<td>Abdominal wall hernia</td>
<td>Hypospadias, Micropenis</td>
<td>Large rocker-bottom feet</td>
<td>Telangiectasia</td>
<td>Delayed expressive speech</td>
<td>Mild gross motor delay (walked at 18 months)</td>
<td>Autosomal features</td>
<td>Moderate global developmental delay</td>
<td>Gastroesophageal reflux</td>
<td>Gastrostomy fed Obstructive sleep apnoea</td>
<td>Moderate bilateral conductive hearing loss</td>
<td>Nyctagmus with normal vision acuity</td>
</tr>
</tbody>
</table>
(n = 65) and intronic variants that were not predicted to affect splicing by the in silico tools MaxEntScan\textsuperscript{19}, SpliceSiteFinder-like\textsuperscript{20}, and NNSPLICE\textsuperscript{21} were excluded.

Evaluation of protein expression of candidate genes in deleted region

The expression of genes within the deleted region was assessed by the median transcripts per million value from the Genotype-Tissue Expression (GTEx) portal.

Results

Clinical characteristics

Clinical characteristics of the cohort are provided in Table 1. Hypoglycaemia (blood glucose <3.0 mmol/l) was diagnosed in 9/12 patients at birth and in three patients at the age of 3 days, 8 weeks and 20 weeks respectively. In 10/12 patient’s in our cohort and one patient reported in the literature\textsuperscript{4} detectable insulin at the time of hypoglycaemia confirmed a diagnosis of congenital hyperinsulinism which was treated with diazoxide. In the four remaining patients, two from our cohort (patients 3 and 12) and two from the literature, a diagnosis of congenital hyperinsulinism was not confirmed. In three of these patients insulin was either not measured or the results were not reported (patient 12, reference 5 and https://decipher.sanger.ac.uk/patient/249708). In the final patient (patient 3) insulin was measured at the time of hypoglycaemia but was suppressed (less than 6.0 pmol/L). The duration of hypoglycaemia varied considerably within our cohort with one child having transitory hypoglycaemia not requiring treatment yet another patient requiring ongoing diazoxide treatment at 8 years.

Two patients within the cohort (patients 10 and 11) were affected siblings; the remaining 10 patients were unrelated and had no family history of hypoglycaemia. Extra-pancreatic features previously reported in patients with Chromosome 9p deletions were observed in all individuals although there was no uniform phenotype. Common features reported in our cohort include cardiac anatomical defects in seven patients, facial dysmorphism in ten patients, digit/limb abnormalities in six patients and undervirilisation in four patients (Table 1).

The minimal deleted region for hypoglycaemia is 7.2 Mb

Analysis of sequence data confirmed deletions on chromosome 9p which ranged in size from 7.2 Mb to 19.2 Mb. These were aligned and compared to the deletions identified in the two patients reported in the literature and an individual listed on the DECIPHER database with a 9p deletion and hypoglycaemia\textsuperscript{4,5} (https://decipher.sanger.ac.uk/patient/249708). The minimal deleted region shared between the 15 patients spanned 7.2Mb (Chr9:0-7200000[hg19], 9p24.3-9p24.1) (Figure 1). In the patients in our cohort, sequence analysis of the known congenital hyperinsulinism genes did not identify a disease-causing variant.

The minimal deleted region for hypoglycaemia includes 38 genes

The 7.2Mb minimal deleted region on Chromosome 9 contains 38 protein-coding NCBI RefSeq genes (Table 2). Of these, SMARCA2, RFX3, CDC37L1 and UHRF2 have a gnomAD pLI score of >0.9 indicating that they are intolerant to loss-of-function variants\textsuperscript{17}. The three genes with the highest levels of expression in the pancreas are AK3, SMARCA2 and VLDLR all with a median transcripts per million value of >8 on the Genotype-Tissue Expression (GTEx) portal. Three further genes (KANK1, RFX3 and JAK2) are involved in pathways associated with insulin regulation according to the UniProt gene ontology database\textsuperscript{22}.

To test whether the deletion was unmasking a second recessively inherited mutation on the opposite allele we performed targeted capture followed by next generation sequencing of the minimal deleted region in three unrelated individuals. No rare
variants shared by all three samples were identified. We also searched for genes harbouring different rare variants in each of the three samples but did not identify any genes which met this criterion.

**Discussion**

Our cohort of 12 patients with 9p deletions and hypoglycaemia is the largest reported series and significantly widens the phenotypic spectrum over and above the three reported cases. In 10 of the 12 patients congenital hyperinsulinism was confirmed, whilst in two patients insulin was either not measured at the time of hypoglycaemia or was shown to be appropriately suppressed. Variability in extra-pancreatic phenotypes was observed in our cohort with specific features likely to be determined by the extent of the deletion in each patient and in five cases the reciprocal trisomy. This study allowed the refinement of the critical region for the Chromosome 9p deletion syndrome which features hypoglycaemia to 7.2 Mb. The critical gene(s)/regulatory region(s) within this locus are not known.

As insulin was appropriately suppressed in one patient in our cohort and was not measured in three further individuals we cannot be certain that hypoglycaemia results from dysregulated insulin secretion in all cases with a 9p deletion. If these four patients do have a different mechanism for hypoglycaemia compared to the congenital hyperinsulinism group, we would, however, not expect the size of the minimal deleted region for congenital hyperinsulinism calculated in this report to change given that the deletions in these patients were not critical for determining the boundaries on the 7.2 Mb region (Table 1 and Figure 1).

There are three possible mechanisms by which large deletions can cause disease: 1) disruption of a gene at the breakpoint 2) haploinsufficiency of a gene within the deletion and 3) unmasking a recessive mutation in a gene within the deleted region. The breakpoints for the deletions varied between patients in our cohort, making it unlikely that the disruption of a gene at a breakpoint is the cause of the hypoglycaemia in these patients. We performed targeted sequencing of the minimal deleted region to search for recessive mutations but did not identify any variants which could explain the phenotype. Although it is possible that our approach may have missed a mutation, given that only 56% of the minimal deleted region was captured at ≥10X coverage in three patients, from our data the most likely explanation is that haploinsufficiency of one or more genes within the minimal deleted region is responsible for the hypoglycaemia. If this is true we would expect this aetiology to be associated with variable penetrance given that patients without hypoglycaemia and deletions over this region have been reported. This variable penetrance would be similar to what is observed with the gonadal dysgenesis phenotype where 46,XY patients with 9p24 deletions and normal male external genitalia have been reported.

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**Table 2. Data on the genes within the minimal deleted region (Chr9:0-720000[hg19], 9p24.3-9p24.1).** pLI scores were obtained from gnomAD. Pancreatic expression was obtained from the Genotype-Tissue Expression (GTEx) portal (gtexportal.org). NA indicates the gene was not found in this database.

<table>
<thead>
<tr>
<th>NCBI RefSeq Gene</th>
<th>gnomAD pLI</th>
<th>Pancreatic expression GTEx (median transcripts per million)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WASHC1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>FOXD4</td>
<td>0</td>
<td>0.27</td>
</tr>
<tr>
<td>CBWD1</td>
<td>0</td>
<td>1.705</td>
</tr>
<tr>
<td>DOCK8</td>
<td>0</td>
<td>1.79</td>
</tr>
<tr>
<td>KANK1</td>
<td>0</td>
<td>8.72</td>
</tr>
<tr>
<td>DMRT1</td>
<td>0.74</td>
<td>NA</td>
</tr>
<tr>
<td>DMRT2</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>SMARCA2</td>
<td>1</td>
<td>13.4</td>
</tr>
<tr>
<td>VLDLR</td>
<td>0</td>
<td>8.84</td>
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<tr>
<td>KCNV2</td>
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<td>0.07</td>
</tr>
<tr>
<td>PUM3</td>
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<td>NA</td>
</tr>
<tr>
<td>RFX3</td>
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Interestingly, the deletions in four patients within our cohort do not overlap with the 3.5 Mb minimal deleted region defined by Faas et al. and only two of our patients had a deletion which overlapped with the 300 kb critical region identified by Swinkels et al. The majority of deletions in our patients were called from sequence data by savvyCNV which maps breakpoints with an estimated accuracy of ±200 kb. Even including this margin of error, not all of our patients have deletions which overlap with either of the previously identified critical regions. This is in keeping with the 9p deletion syndrome being a genetically and phenotypically heterogeneous collection of overlapping syndromes.

In conclusion, our study identifies 9p deletions as an important cause of hypoglycaemia and refines the critical region for this phenotype to 7.2 Mb. Whilst we highlight potential candidate genes the genetic mechanism for the hypoglycaemia in our patients remains unknown. Further studies are required to investigate the cause of hyperinsulinism in these patients and in those with other copy number variant (CNV) syndromes which feature congenital hyperinsulinism such as Turner’s syndrome where the causative gene(s) have also not been definitively identified. These large deletions can be screened for by targeted panels using an off-target CNV caller such as SavvyCNV.

Data availability
Underlying data
The genotype data could be used to identify individuals and so cannot be made openly available. Access to data is open only through collaboration. Requests for collaboration will be considered following an application to the Genetic Beta Cell Research Bank (https://www.diabetessgenes.org/current-research/genetic-beta-cell-research-bank/). Contact by email should be directed to the Lead Nurse, Dr Bridget Knight (b.a.knight@exeter.ac.uk).

Acknowledgments
This study makes use of data generated by the DECIPHER community. A full list of centres who contributed to the generation of the data is available from http://decipher.sanger.ac.uk and via email from decipher@sanger.ac.uk. Those individuals who carried out the original analysis and collection of the DECIPHER data bear no responsibility for the further analysis or interpretation of it in this study. This study makes use of data generated by the Genotype-Tissue Expression (GTEx) Project, which was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the analyses described in this manuscript were obtained from the GTEx Portal on 04/25/19.

References


