



RESEARCH ARTICLE

REVISED Refinement of the critical genomic region for congenital hyperinsulinism in the Chromosome 9p deletion syndrome [version 2; peer review: 3 approved]

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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

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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,

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REVISED Amendments from Version 1

Following the requests of reviewers, the title of the manuscript has been changed to highlight that 9p deletions cause Congenital Hyperinsulinism. The description of the clinical characteristics of the cohort have been updated to confirm that hypopituitarism was excluded as a cause of hypoglycemia in these patients. The results section has been updated to clarify which patients had been screened for mutations in the known hyperinsulinism genes. The manuscript now includes discussion on the disruption of imprinted genes by large deletions. [Figure 1](#) has been updated to include patient 3, the ID for the patient listed on Decipher and the size of the minimal deleted region. The SDS birthweights for the cohort have been added to [Table 1](#) and the age at remission for patient 3 has been included. [Table 2](#) has been expanded to show whether disruption of genes residing within the minimal deleted region are known to cause human disease.

Any further responses from the reviewers can be found at the end of the article

Introduction

Monosomy of part of the short arm of chromosome 9 causes the complex congenital condition chromosome 9p deletion syndrome (MIM: 158170)¹. These large contiguous gene deletions can occur in isolation or form part of an unbalanced translocation². 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 differentiation³. A further rare feature is hypoglycaemia, which has been described in 3 of the >100 genetically confirmed cases⁴⁻⁶.

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 9p⁷.

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.*^{3,8}. 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 cells⁹. Despite major advances in genetics the underlying cause of congenital hyperinsulinism is not identified

in approximately 55% of patients¹⁰. 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 conditions¹¹.

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 cohort⁶.

Calling deletions

In nine patients multiple syndromic features had prompted microarray analysis leading to the identification of a 9p deletion. 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 >1Mb¹².

Break points were mapped in patients 1-9 using off-target reads from the targeted next generation sequencing data. In these patients analysis of the known hyperinsulinism genes did not identify a mutation. In patients 10-12 the breakpoints were mapped by microarray analysis, DNA was not available for targeted sequencing in these individuals. 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).

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 INGS off-target reads (patients 1–9) or microarray analysis (patients 10–12).

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11*	Patient 12
Karyotype	46,XY,del(9)(p22.1)	Not performed	46,XX,del(9)(p22.2)	46,XY,der(9)t(9;13)(p24;q22.3)	46,XX,del(9)(p23)ish del(9)(pter-)	46,XX,der(9)t(8;9)(q24.1;p24)	Not performed	Not performed	46,XX,del(9)(p24.1)	46,XY,der(9)t(9;13)(p23;q33.3)	46,XY,der(9)t(9;13)(p23;q33.3)	46,XY,del(9)(p23)
Chromosome 9p deletion*	0-19,200,000	0-14,000,000	0-17,600,000	0-9,806,011	0-9,330,617	0-7,800,000	0-7,200,000	0-7,600,000	0-7,600,000	0-12,450,000	0-12,450,000	0-10,955,813
Reciprocal Duplication*	None detected	None detected	None detected	Chr13:75000000-115200000	None detected	Chr8:117800000-146400000	Chr7:0-10,000,000	None detected	None detected	Chr13:107,452,410-115,105,270	Chr13:107,452,410-115,105,270	None detected
Gender	Male	Male	Female	Assigned Female	Female	Female	Female	Male	Female	Male	Male	Male
Birthweight (g)	4478	3440	3200	2960	3520	3670	3510	3000	1700	3650	4160	5050
Gestation (wks)	38	38	40	37	38	40	39	37	34	38+1	41	42
Birth weight SDS	2.87	0.86	-0.10	0.16	1.32	0.99	0.93	0.26	-1.22	1.27	1.49	2.93
Current Age (yrs)	3	2	2	10	8	9	7	8	2	11	20	8
Hyperinsulinaemic hypoglycaemia												
Age at onset	3 days	2 days	20 weeks	Birth	Birth	Birth	Birth	8 weeks	Birth	Birth	Birth	Birth
Age at remission	26 days	43 weeks	7 months	4 years	1.4 years	1.3 yrs	6 weeks	Ongoing at 8 yrs	Ongoing at 2.8yrs	Ongoing	Ongoing	1 week (no follow-up)
Blood glucose at onset of hypoglycaemia (mmol/L)	2.9	1.8	2.5	1.4	1.8	1.8	1.4	2.7	2.0	0.7	1.2	1.9
Insulin (pmol/L)	97	28	<6.0	111	96	42	51	47	53	100	190	Not tested
C-Peptide (pmol/L)	228	Not tested	Not tested	980	540	Not tested	Not tested	Not tested	364	629	Not tested	Not tested
Treatment details	Diazoxide until remission (dose not available)	Diazoxide 10.5 mg/kg/day	No treatment (dose not available)	Diazoxide 5 mg/kg/day until remission	Diazoxide 7mg/kg/day until remission	Diazoxide until remission (dose not available)	Diazoxide 10mg/kg/day until remission	Diazoxide 2mg/kg/day ongoing	Diazoxide 6mg/kg/day	Diazoxide 10mg/kg/day ongoing	Diazoxide 10mg/kg/day ongoing	No treatment

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11*	Patient 12
Extra-pancreatic features												
Facial dysmorphism	Meiotic suture defect Hypotelorism High arched palate Small low set ears	Not noted in infancy	Meiotic suture synostosis Low set ears High narrow palate Hypotelorism	Meiotic prominence Bi-temporal narrowing Prominent eyes Low set ears Long philtrum Micrognathia	Broad forehead Brachycephaly Low set ears	Microcephaly Up-slanting palpebral fissures High nasal bridge Large mouth Thin upper lip	Sub mucosal cleft palate Uvula bifida Deep-seated ears Hypertelorism Small chin	None noted	Prominent forehead Flat mid face Wide base gait Low set ears	MacroGLOSSIA Small deep-seated ears	MacroGLOSSIA Small deep-seated ears	MacroGLOSSIA
Digits	Wide sandal gap	Normal	Long fingers and toes	4 limb postaxial polydactyly Syndactyly 2 nd and 3 rd toes	Unilateral clinodactyly Bilateral sandal gap	Broad and long fingers and toes	Normal	Normal	Broad fingers	Normal	Normal	Normal
Cardiac	VSD	PDA	ASD	ASD, PDA	ASD, PDA	VSD	PDA, PFO/ASD II	-	-	-	-	-
Other features	Abdominal wall hernia Hypospadias Micropenis Large rockerbottom feet Telangiectasia	Delayed expressive speech Mild gross motor delay (walked at 18 months) Autistic features	Moderate global developmental delay Gastrooesophageal reflux Gastrostomy fed Obstructive sleep apnoea Moderate bilateral conductive hearing loss Nystagmus with normal vision acuity	Severe under virilisation with dysgenetic testes Perineal hypospadias Widely spaced nipples Congenital bilateral glaucoma Abnormal corpus callosum Small cerebellum Severe developmental delay Large CSF spaces Slow weight gain	Tracheo-oesophageal fistula	Widely spaced nipples Skeletal dysplasia Seizures	Haemorrhages: cortical, subcortical cerebellar and brain marrow Significant developmental delay	Glandular hypospadias Developmental delay Cranial MRI: Occipital encephalomalasia and perifocal gliosis	Developmental delay (motor and speech) Lymphedema in both legs Hepatomegaly Small umbilical hernia	Developmental delay (motor and speech) Muscular hypotension Hepatomegaly Umbilical hernia	Developmental delay (motor and speech) MRI showed delayed myelination Cryptorchidism	Global developmental delay (motor and speech) MRI showed delayed myelination Cryptorchidism

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 guidelines¹³. Reads were aligned to the GRCh37/hg19 human reference genome with *BWA mem* (version 0.7.15)¹⁴ followed by local re-alignment using *GATK IndelRealigner* (version 3.7.0)¹⁵. 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*).¹⁶ We excluded variants present in *gnomAD*¹⁷ at a frequency greater than 1 in 27,000 - the highest published prevalence of hyperinsulinism in an outbred population¹⁸. Variants that were homozygous in internal controls (n = 65) and intronic variants that were not predicted to affect splicing by the *in silico* tools *MaxEntScan*¹⁹, *SpliceSiteFinder-like*²⁰, and *NNSPLICE*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 all patients cortisol deficiency was excluded clinically and biochemically at diagnosis and no patients had evidence of growth failure. In

10/12 patient's in our cohort and one patient reported in the literature⁴ 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^{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](#)).

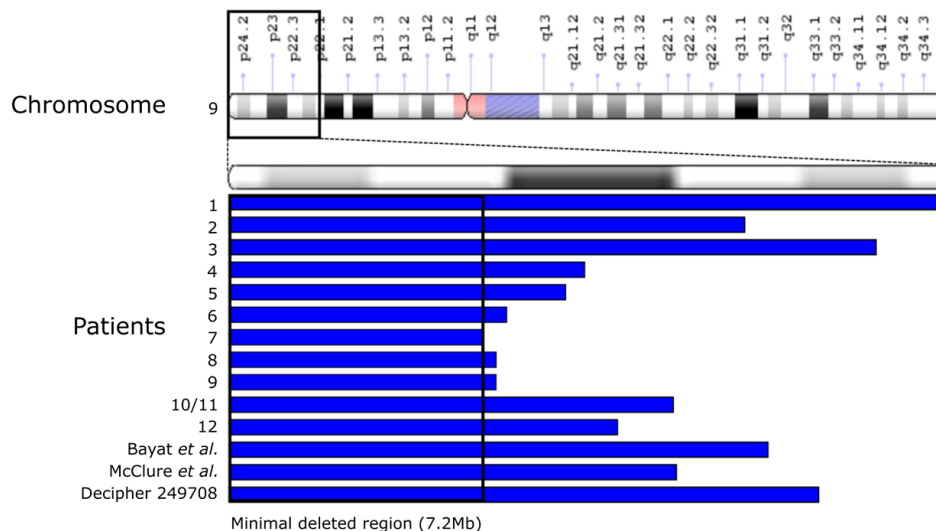


Figure 1. Diagram showing the deletions in our patients (patients 10 and 11 are siblings) and three other reported patients with 9p deletions and hypoglycaemia. Details of the size of the individual deletions are provided in [Table 1](#). A list of the NCBI RefSeq genes within the 7.2Mb minimal deleted region is provided in [Table 2](#).

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 variants¹⁷. The three genes with the highest levels of

Table 2. Data on the genes within the minimal deleted region (Chr9:0-7200000[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. When disease-causing mutations have been reported details of the associated syndrome and the inheritance of mutations are provided.

NCBI RefSeq Gene	gnomAD pLI	Pancreatic expression GTEx (median transcripts per million)	Disease-causing gene OMIM ID (Phenotype, Inheritance)
<i>WASHC1</i>	NA	NA	-
<i>FOXD4</i>	0	0.27	-
<i>CBWD1</i>	0	1.705	-
<i>DOCK8</i>	0	1.79	# 243700 (Hyper-IgE recurrent infection syndrome. Recessive)
<i>KANK1</i>	0	8.72	# 612900 (Cerebral palsy, spastic quadriplegic, 2)
<i>DMRT1</i>	0.74	NA	-
<i>DMRT3</i>	0	0	-
<i>DMRT2</i>	0.01	0.02	-
<i>SMARCA2</i>	1	13.4	# 601358 (Nicolaidis-Baraitser syndrome. Dominant)
<i>VLDLR</i>	0	8.84	* 192977 (Cerebellar hypoplasia and mental retardation with or without quadrupedal locomotion 1. Dominant)
<i>KCNV2</i>	0	0.07	* 607604 (Retinal cone dystrophy 3B. Dominant)
<i>PUM3</i>	NA	NA	-
<i>RFX3</i>	1	1.18	-
<i>GLIS3</i>	0	3.52	# 610199 (Diabetes mellitus, neonatal, with congenital hypothyroidism, Recessive)
<i>SLC1A1</i>	0	1.47	# 222730 (Dicarboxylic aminoaciduria, Recessive)
<i>SPATA6L</i>	0	2.04	-
<i>CDC37L1</i>	0.97	6.83	-
<i>PLPP6</i>	NA	NA	-
<i>AK3</i>	0	24.6	-
<i>RCL1</i>	0.06	6.52	-

NCBI RefSeq Gene	gnomAD pLI	Pancreatic expression GTEx (median transcripts per million)	Disease-causing gene OMIM ID (Phenotype, Inheritance)
<i>JAK2</i>	0.65	2.43	# 600880 (Budd-Chiari syndrome, Somatic)
<i>INSL6</i>	0	0	-
<i>INSL4</i>	0	NA	-
<i>RLN2</i>	0	0.151	-
<i>RLN1</i>	0.01	0.0404	-
<i>PLGRKT</i>	0	5.03	-
<i>CD274</i>	0.2	1.2	-
<i>PDCD1LG2</i>	0	0.167	-
<i>RIC1</i>	NA	NA	# 618761 (Catifa Syndrome, Recessive)
<i>ERMP1</i>	0	4.7	-
<i>MLANA</i>	0	0.0957	-
<i>KIAA2026</i>	0.66	4.04	-
<i>RANBP6</i>	0	5.44	-
<i>IL33</i>	0	1.57	-
<i>TPD52L3</i>	0.2	0	-
<i>UHRF2</i>	1	6.91	-
<i>GLDC</i>	0	0.0397	# 605899 (Glycine encephalopathy, Recessive)
<i>KDM4C</i>	0	5.15	-

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 database²².

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 four 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 3) unmasking a recessive mutation in a gene within the deleted region and 4) disruption of an imprinted gene. None of the genes within the deleted region are known to be imprinted and 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 $\geq 10X$ 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 reported³. 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^{3,7}.

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.*⁸ and only two of our patients had a deletion which overlapped with the 300 kb critical region identified by Swinkels *et al.*³. The majority of deletions in our patients

were called from sequence data by savvyCNV⁸ 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^{23,24}. These large deletions can be screened for by targeted panels using an off-target CNV caller such as SavvyCNV¹².

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.diabetesgenes.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.

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Version 2

Reviewer Report 18 August 2020

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Nicola Brunetti-Pierri 

Telethon Institute of Genetics and Medicine, Naples, Italy

Banjerjee and colleagues have adequately addressed all the previously raised issues.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical Genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 11 August 2020

<https://doi.org/10.21956/wellcomeopenres.17783.r39816>

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Yao Yang 

Department of Genetics and Genomic Sciences, Icahn Institute for Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

I have reviewed the revised manuscript and response. Authors have addressed my comments. I don't have any further comments, and would suggest to accept this manuscript for indexing.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 11 August 2020

<https://doi.org/10.21956/wellcomeopenres.17783.r39815>

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Raphael Del Roio Liberatore Junior 

Pediatric Endocrinology-Pediatric Diabetology and Metabology Section, Department of Pediatrics, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirao Preto, Brazil

It is ready to be indexed.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pediatric Endocrinology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 24 June 2020

<https://doi.org/10.21956/wellcomeopenres.16912.r38978>

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Yao Yang 

Department of Genetics and Genomic Sciences, Icahn Institute for Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

In the manuscript "Refinement of the critical genomic region for hypoglycemia in the Chromosome 9p deletion syndrome", authors studied 12 patients with chromosome 9p deletion syndrome who had a history of neonatal hypoglycemia. Study of such cases might be helpful to understand the disease mechanism.

The study was well designed and the manuscript was well structured and written. I will recommend to index the MS after comments below are addressed.

1. Figure 1 illustration of deletion seems not consistent with the deletion data shown in table 1. Either one should have error.
2. Did author test those genes associated with hypoglycemia to rule out pathogenic variants on those genes for these cases?
3. The NGS data for the sequencing didn't achieve enough depth and coverage to rule out the third possible mechanism mentioned by author: unmasking a recessive mutation. I understand authors already discussed a little bit about this, but a more comprehensive analysis of NGS data with enough depth and coverage will strength the conclusion of this paper. However, I wouldn't say this is necessary to index.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clinical Molecular Genetics, Bioinformatics, NGS

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 24 Jul 2020

Sarah Flanagan, University of Exeter Medical School, Exeter, UK

In the manuscript "Refinement of the critical genomic region for hypoglycemia in the Chromosome 9p deletion syndrome", authors studied 12 patients with chromosome 9p deletion syndrome who had a history of neonatal hypoglycemia. Study of such cases might be helpful to understand the disease mechanism.

The study was well designed and the manuscript was well structured and written. I will recommend to index the MS after comments below are addressed.

1. Figure 1 illustration of deletion seems not consistent with the deletion data shown in table 1. Either one should have error.

We thank the reviewer for their appraisal of our manuscript. We are grateful for highlighting the discrepancies between table 1 and figure 1. A new version of figure 1 has now been uploaded which includes patient 3 (omitted by mistake in the previous version). The tables and figure have now been double checked.

2. Did author test those genes associated with hypoglycemia to rule out pathogenic variants on those genes for these cases?

We apologise that this was unclear. We had excluded all known causes of congenital hyperinsulinism in 9 of the 12 patients. In the three remaining patients samples were unavailable to targeted next-generation sequencing. We have updated the methods to reflect this point.

3. The NGS data for the sequencing didn't achieve enough depth and coverage to rule out the third possible mechanism mentioned by author: unmasking a recessive mutation. I understand authors already discussed a little bit about this, but a more comprehensive analysis of NGS data with enough depth and coverage will strengthen the conclusion of this paper. However, I wouldn't say this is necessary to index.

We agree with the reviewer that it has not been possible to rule out a missed recessive mutation given the depth/coverage of the sequencing, which we hope is reflected in the text of our discussion.

Competing Interests: None

Reviewer Report 18 June 2020

<https://doi.org/10.21956/wellcomeopenres.16912.r38980>

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Nicola Brunetti-Pierri 

Telethon Institute of Genetics and Medicine, Naples, Italy

Banjerjee and colleagues report 12 patients with 9p deletions and hypoglycemia. They also defined the minimally deleted interval trying to pinpoint the gene responsible for the metabolic defect.

The paper is well written and provides novel information. However, there are several issues that require attention:

1. There are discrepancies between Fig. 1 and Table 1:a) Patient 3 is reported to have a 17.6 Mb deletion on table 1 (larger than the deletion of patient 2) but according to figure 1, the deletion is smaller than patient 2.b) Deletion size is similar between patients 6-7-8-9 and yet patient 9 is shown in fig-1 to have a larger deletion. Patient 4 and 5 deletions should be similar in size according to the table.
2. In Fig. 1: patient 12 is not shown. Why there is a single base for 9/10?
3. Table 1: why there is a 'not applicable' for the age of hypoglycemia remission of patient 3? Is it not available?
4. Indicate on the table that the glucose levels were at the time of onset of the hypoglycemia.
5. Please add whether each patient was AGA, SGA or LGA according to their birth weight and gestational age.
6. Dose of diazoxide is not included in all cases.
7. Please change age at diagnosis to 'onset' to make clear it refers to the hypoglycemia.
8. Fig. 1 should include the size of the deletions and an Ensembl/USC snapshot of the genes included in the minimally deleted interval.
9. Is any of the genes in the interval imprinted? Imprinting would be a fourth mechanism that the authors can include in the discussion. Moreover, there are defects of imprinted genes known to cause hyperinsulinism (e.g., 11p15 region) and neonatal diabetes.
10. Discuss whether haploinsufficiency for the genes in the interval are responsible for any known syndromes and whether these syndromes have been associated to hypoglycemia.
11. Can the authors estimate the frequency of the hypoglycemia among all reported cases of 9p deletions? This estimate would help understanding whether mutations on the undeleted allele might be implicated. Given the apparently higher frequency of hypoglycemia in 9p deletion cases, one would predict that the underlying mechanism for hypoglycemia it cannot be due to unmasking of a recessive allele.
12. Add DECIPHER ID for the case included on fig. 1.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical Genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 24 Jul 2020

Sarah Flanagan, University of Exeter Medical School, Exeter, UK

Banerjee and colleagues report 12 patients with 9p deletions and hypoglycemia. They also defined the minimally deleted interval trying to pinpoint the gene responsible for the metabolic defect. The paper is well written and provides novel information. However, there are several issues that require attention

1. There are discrepancies between Fig. 1 and Table 1:a) Patient 3 is reported to have a 17.6 Mb deletion on table 1 (larger than the deletion of patient 2) but according to figure 1, the deletion is smaller than patient 2.b) Deletion size is similar between patients 6-7-8-9 and yet patient 9 is shown in fig-1 to have a larger deletion. Patient 4 and 5 deletions should be similar in size according to the table. Fig. 1: patient 12 is not shown. Why there is a single base for 9/10?

We thank the reviewer for their thorough review of our manuscript and for highlighting the discrepancies between the table and the figure. We had mistakenly uploaded a previous version of figure 1 for peer review which had not included patient 3, this patient had been included in the table. A new figure has been uploaded and the data double checked.

2. Table 1: why there is a 'not applicable' for the age of hypoglycemia remission of patient 3? Is it not available?

The table has been updated to show that the child had normal glycaemia at the age of 7 months.

3. Indicate on the table that the glucose levels were at the time of onset of the hypoglycemia.

The table has been updated as requested.

5. Please add whether each patient was AGA, SGA or LGA according to their birth weight and gestational age.

The birth weight standard deviation score for each patient has been added to table 1. We would prefer to add SDS scores than AGA/SGA/LDA.

6. Dose of diazoxide is not included in all cases.

We had included all of the information that we have available to us regarding diazoxide dose. When the dose is unavailable we had noted this in the manuscript.

7. Please change age at diagnosis to 'onset' to make clear it refers to the hypoglycemia.

The table has been updated as requested.

8. Fig. 1 should include the size of the deletions and an Ensembl/USC snapshot of the genes included in the minimally deleted interval.

We have added the size of the minimal deleted region to the figure as requested. The size of each of the individual deletions is recorded in table 1. We felt that annotating them to the figure reduced clarity. Due to the minimal deleted region containing 38 protein coding genes (listed in table 2) we also felt that these would be difficult to include in figure 1 in a way that could be clearly read. We have not updated the legend to help highlight that this information is available within the tables.

9. Is any of the genes in the interval imprinted? Imprinting would be a fourth mechanism that the authors can include in the discussion. Moreover, there are defects of imprinted genes known to cause hyperinsulinism (e.g., 11p15 region) and neonatal diabetes.

We agree that disruption of imprinted genes is a possible mechanism of disease in individuals with large CNVs. We had checked the genes in the minimal deleted region but none are known to be imprinted (Baran et al The landscape of genomic imprinting across diverse adult human tissues. *Genome Res.* 2015 Jul;25(7):927-36). We have now added a sentence to the discussion to reflect this point.

10. Discuss whether haploinsufficiency for the genes in the interval are responsible for any known syndromes and whether these syndromes have been associated to hypoglycemia.

Further information to highlight which of the genes within the deleted region are reported to cause disease when disrupted have now been included in table 2. With the exception of *SLC1A1* none of these genes have been associated with hypoglycaemia. The OMIM record

for *SLC1A1* list fasting hypoglycaemia as a feature however this information is taken from a case report published in 1974 (Teijema et al: Dicarboxylic aminoaciduria: An inborn error of glutamate and aspartate transport with metabolic implications, in combination with a hyperprolinemia, *Metabolism* 23:115, 1974) prior to discovery of the *SLC1A1* gene (Bailey et al Loss-of-function mutations in the glutamate transporter *SLC1A1* cause human dicarboxylic aminoaciduria. *J. Clin. Invest.* 121: 446-453, 2011).

11. Can the authors estimate the frequency of the hypoglycemia among all reported cases of 9p deletions? This estimate would help understanding whether mutations on the undeleted allele might be implicated. Given the apparently higher frequency of hypoglycemia in 9p deletion cases, one would predict that the underlying mechanism for hypoglycemia it cannot be due to unmasking of a recessive allele.

We do not believe that it would be accurate to offer a prediction on the frequency of hypoglycaemia in the 9p deletion syndrome based on published cases as this does not provide an unbiased cross-sectional cohort. We agree with the reviewer that unmasking of a recessive allele is unlikely, we hope we have addressed this point adequately in the discussion.

12. Add DECIPHER ID for the case included on fig. 1.

The ID for the Decipher case has now been included on figure 1.

Competing Interests: None

Reviewer Report 19 December 2019

<https://doi.org/10.21956/wellcomeopenres.16912.r37375>

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Raphael Del Roio Liberatore Junior

Pediatric Endocrinology-Pediatric Diabetology and Metabology Section, Department of Pediatrics, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirao Preto, Brazil

The authors studied 12 patients with 9p deletion syndrome who had hypoglycaemia in the neonatal period in an attempt to find a genetic cause to explain hypoglycaemia.

Hypoglycaemia is a very uncommon symptom of 9p deletion syndrome and hyperinsulinism has recently been described in patients with 9p deletion syndrome as a cause for hypoglycaemia in these patients. Thus, the study proposal is appropriated and justified.

Regarding clinical aspects, the authors report that patients 03 and 12, although presenting hypoglycaemia, did not have confirmation of hyperinsulinism. In addition, these 2 patients did not

require treatment.

The experimental approach is quite elegant and complete, with the aim of finding a genetic cause for hypoglycaemia in 9p deletion syndrome patients. Unfortunately, this cause was not found.

However, here are some considerations:

1. In the fourth paragraph of the introduction, the authors report that in 55% of cases of congenital hyperinsulinism, no genetic causes are found. Thus, I believe that major contribution of this research could be that candidate genes present in the lost region of chromosome 9 do not explain the occurrence of congenital hyperinsulinism. I believe that patients 03 and 12 may be excluded from the sample and the title could may be changed to: Refinement of the critical genomic region for congenital hyperinsulinism in chromosome 9p deletion syndrome.
2. Still regarding patients 03 and 12, it is not clear what was the cause of the episode(s) of hypoglycaemia. These patients may have hypopituitarism, especially patient 03, who may have changes in the medial line.
3. It was not clear in the manuscript if the genes known to be related to congenital hyperinsulinism have being studied.
4. Interestingly, although 9p deletion syndrome is described as gonadal dysgenesis and sex reverse, only patients 1, 4, and 8 had genital alterations. I don't know if the authors paid attention to this fact.

I would be absolutely satisfied with the answer to these considerations that even reduce the importance and elegance of the present study.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pediatric Endocrinology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 24 Jul 2020

Sarah Flanagan, University of Exeter Medical School, Exeter, UK

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We thank the reviewer for their constructive appraisal of our manuscript. We would like to keep patients 03 and 12 within the cohort as we believe that it is important to report that there may be variability in the biochemical hypoglycaemic screens in these patients. That said, we agree that there is overwhelming evidence that these deletions result in hyperinsulinemic hypoglycaemia in the majority of patients and as such we have now altered the title as suggested to reflect this point.

2. Still regarding patients 03 and 12, it is not clear what was the cause of the episode(s) of hypoglycaemia. These patients may have hypopituitarism, especially patient 03, who may have changes in the medial line.

Thank you for highlighting this point; cortisol deficiency was excluded in all patients clinically and biochemically at diagnosis and no patients had evidence of growth failure. The paper has now been updated to reflect this.

3. It was not clear in the manuscript if the genes known to be related to congenital hyperinsulinism have being studied.

We apologise that this was unclear. We have now updated the methodology section to clarify which patients within our cohort underwent targeted next-generation sequencing.

4. Interestingly, although 9p deletion syndrome is described as gonadal dysgenesis and sex reverse, only patients 1, 4, and 8 had genital alterations. I don't know if the authors paid attention to this fact.

We agree it is interesting that of the 7 individuals in our cohort with a 46XY karyotype only 3 had gonadal dysgenesis. The variable penetrance with this phenotype is included within the discussion.

Competing Interests: None
