Journal of Human Genetics (2015) 60, 313–317

www.nature.com/jhg

ORIGINAL ARTICLE

A novel *de novo* 20q13.32–q13.33 deletion in a 2-year-old child with poor growth, feeding difficulties and low bone mass

Meena Balasubramanian[1], Edward Atack[2], Kath Smith[2] and Michael James Parker[1]

Interstitial deletions of the long arm of chromosome 20 are rarely reported in the literature. We report a 2-year-old child with a 2.6 Mb deletion of 20q13.32–q13.33, detected by microarray-based comparative genomic hybridization, who presented with poor growth, feeding difficulties, abnormal subcutaneous fat distribution with the lack of adipose tissue on clinical examination, facial dysmorphism and low bone mass. This report adds to rare publications describing constitutional aberrations of chromosome 20q, and adds further evidence to the fact that deletion of the GNAS complex may not always be associated with an Albright's hereditary osteodystrophy phenotype as described previously.
Journal of Human Genetics (2015) **60,** 313–317; doi:10.1038/jhg.2015.22; published online 12 March 2015

INTRODUCTION

Reports of isolated subtelomeric deletions of the long arm of chromosome 20 are rare, but a few cases have been reported in the literature over the past 30 years.[1–13] Traylor *et al.*[12] provided an overview of these cases alongside six others with clinical similarities including skeletal and growth abnormalities, developmental delay and seizures.[12] Butler *et al.*[13] suggested that common abnormalities seen in 20q13 deletion patients were intellectual disability, speech problems, pre- and postnatal growth retardation, microcephaly, hypotonia, a high forehead and broad nasal bridge, a thin upper lip and a small chin, malformed ears, hypertelorism, a bulbous nasal tip and malformed hands and feet.[13] Solomon *et al.*[14] described a patient with a 0.7 Mb deletion at 20q13.33, and proposed that the deletion of *GTPB5* makes it a candidate gene for tracheoesophageal fistula/esophageal atresia.

We present a patient with a 2.6 Mb interstitial deletion of chromosome 20q, spanning a region more centromeric than the majority of cases previously published and within the region of deletion reported by Butler *et al.*,[13] and just proximal to the deletion reported by Solomon *et al.*[14] We describe the clinical phenotype of this child in the context of his microarray-based comparative genomic hybridization (arrayCGH) deletion at 20q13.32–q13.33, and define a possible minimal region of overlap for a common phenotype.

MATERIALS AND METHODS

This patient is a 2-year-old boy, first child of healthy, non-consanguineous, White North European parents. Pregnancy was complicated by concerns regarding oligohydramnios and poor growth. The 20-week anomaly scan showed a single umbilical artery and he was delivered by emergency cesarean section at 36+2 weeks gestation, owing to fetal distress. He did not require any resuscitation immediately after birth and Apgar scores were 9 and 9 at 1 and 10 min, respectively, of age. Birth parameters were: weight ~ 1.56 kg (0.4th–2nd centile), length ~ 40 cm (< 0.4th centile) and head circumference ~ 28.2 cm (< 0.4th centile). He spent the first 6 weeks of his life in the Special Care Baby Unit because of poor feeding and was diagnosed to have bilateral positional talipes, which resolved with physiotherapy. Cranial ultrasound at the time of birth showed a grade 1 subependymal hemorrhage. He was subsequently discharged home and continued to have significant problems with feeding and gastroesophageal reflux needing antireflux medications. His growth continued to remain well below the 0.4th centile, with some preservation of head circumference.

In terms of his development, he smiled at 2 months of age, sat at 7 months, walked at 13 months and there were no concerns with his vision, hearing and speech. On examination at 2 years of age, he was facially dysmorphic, with bilateral low-set ears, thin skin, reduced subcutaneous fat, grayish sclerae and a spooned-out appearance of his nails (Figures 1a and b). He had a mild degree of developmental delay, but there were no concerns with his vision, hearing or speech. Measurements included: weight ~ 6 kg (2 cm below 0.4th centile), height ~ 69.5 cm (1 cm below 0.4th centile) and head circumference ~ 42 cms (1.5 cm below 0.4th centile), with no evidence of asymmetry.

Investigations before arrayCGH included metabolic tests including urine organic and amino acids, plasma lactate, plasma acylcarnitine profile, serum amino acids, lipid profile, VLCFA (very long chain fatty acids), serum calcium and parathormone levels, which were all reported as normal. Echocardiogram and an abdominal ultrasound were also reported as normal. Cytogenetics showed a normal male karyotype (46,XY) and 11p15 methylation analysis and UPD7 (uniparental disomy on chromosome 7) to exclude Russell–Silver Syndrome (due to his small stature), methylation testing for Prader–Willi syndrome (in view of his feeding difficulties), all of which were negative. Skeletal survey demonstrated slender ribs and the long bones appeared osteopenic with normal bone age.

[1]Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, UK and [2]Sheffield Diagnostic Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, UK
Correspondence: Dr M Balasubramanian, Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, Western Bank, Sheffield S10 2TH, UK.
E-mail: meena.balasubramanian@nhs.net
Received 5 January 2015; revised 30 January 2015; accepted 4 February 2015; published online 12 March 2015

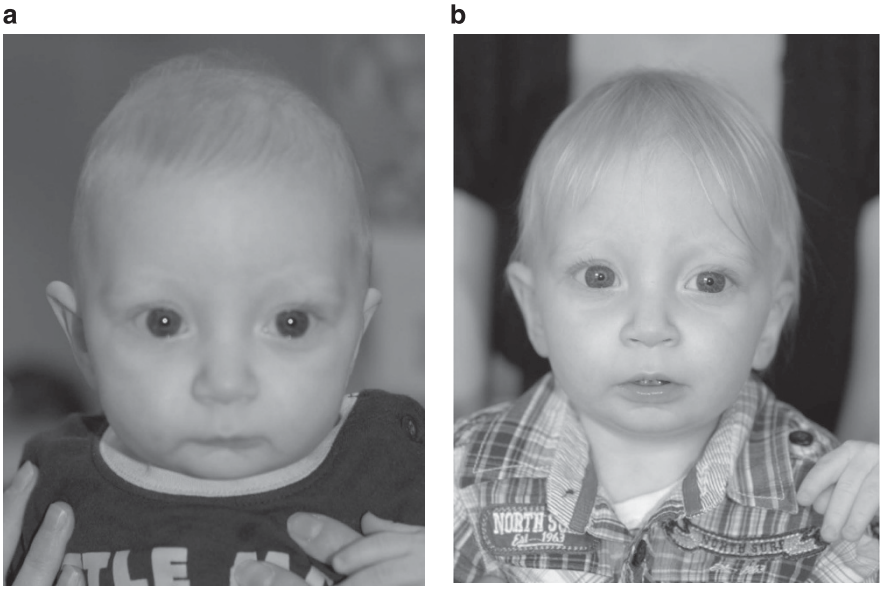

Figure 1 Patient as an infant (**a**) and at 18 months of age (**b**), demonstrating grayish sclerae, thin skin and facial dysmorphism. A full color version of this figure is available at *Journal of Human Genetics* online.

RESULTS

ArrayCGH and fluorescence *in situ* hybridization

ArrayCGH was performed on genomic DNA extracted from peripheral lymphocytes. This was applied to a BlueGnome oligonucleotide array, 8 × 60 K, International Standard Cytogenomic Array Consortium v2 configuration, according to the manufacturer's instructions, using Promega pooled control DNA as a reference. Slides were scanned using a Genepix Personal 4100A scanner (Axon Instruments) and analyzed using BlueGnome BlueFuse-Multi (version 3.2) analysis software. Confirmatory fluorescence *in situ* hybridization was performed using the BlueGnome RP3-492J12 BAC probe. The arrayCGH results showed a 2.6 Mb heterozygous interstitial deletion of chromosome 20q13.32–q13.33 (chr20: 56 571 799–59 230 205 Build GRCh37) (Figure 2a) including 21 HGNC genes (Table 1). Fluorescence *in situ* hybridization confirmed the deletion showing an absence of the RP3-492J12 probe on one chromosome 20 homolog (Figure 2b). Examination of the parents revealed that this was a *de novo* finding and the most likely cause of this patient's phenotype.

DISCUSSION

We report a 2-year-old boy with poor growth, feeding difficulties, lack of adipose tissue with poor weight gain, facial dysmorphism and radiological evidence of low bone mass without a history of fractures, with a 2.6 Mb interstitial deletion on chromosome 20q13.32–q13.33. This region contains 21 HGNC genes and 12 OMIM genes, one of which, *GNAS* is a DD2GP (associated with a developmental disorder) gene (https://decipher.sanger.ac.uk).[15] Interstitial deletions of the long arm of chromosome 20 are rare, but this has been reported with a proposed common phenotype comprising intellectual disability, speech problems, pre- and postnatal growth retardation, microcephaly, hypotonia, a high forehead and broad nasal bridge, a thin upper lip, small chin, malformed ears and hypertelorism.[5,13,15]

The patient reported here is perhaps more similar to the patients reported by Butler *et al.*,[13] and Geneviève *et al.*[5,13] and Butler *et al.*[13] described a 32-month-old girl of Peruvian origin with a 20q13.2–q13.33 deletion presenting with intellectual disability, low tone, absent speech, poor growth and a cleft lip.[13] Geneviève *et al.*[5] described two unrelated patients with a 6 Mb deletion of 20q13.2–q13.3 who presented with severe pre- and postnatal growth retardation, significant feeding difficulties, developmental delay, abnormal distribution of subcutaneous adipose tissue and facial dysmorphism with a non-Albright's hereditary osteodystrophy (non-AHO) phenotype. In fact, our patient described here has a very similar phenotype and looks facially very similar to patient 2.[5] Evidence from these manuscripts and our paper adds to the conclusion that interstitial deletion of 20q13.3 does not always result in an AHO phenotype, which is an important fact in the genetic counseling process, especially when the molecular karyotyping is carried out when young. Also, given the evidence that our patient did not have the AHO phenotype as with the patients described by Geneviève *et al.*,[5] we would hypothesize that the deletion was likely to be of paternal origin. Table 2 provides a comprehensive comparison of the patients reported with similar deletions in the literature with the patient described here and it is clear that interstitial deletions of 20q13.3 have a similar clinical presentation and a non-AHO phenotype. The deletion described by Solomon *et al.*[14] is a 0.7 Mb *de novo* deletion of 20q13.33, which is just distal to the patient reported here, and hence we would not expect a phenotypic overlap with this patient[14] (Figure 2c).

Table 1 provides a list of the HGNC genes involved with their corresponding OMIM number and gene function in 20q.[16,17] As is evident from the table apart from GNAS complex, the other genes are less commonly implicated in human disease and most of them are not associated with a phenotype in the heterozygous state. The genes with the lowest haploinsufficiency scores are: *STX16*, *VAPB*, *GNAS* and *SLMO2*, which have been discussed further. Of significance, the deleted region contains *GNAS* and *STX16*, part of the GNAS complex that is a complex imprinted locus that produces multiple transcripts through the use of alternative promoters and alternative splicing. We know that genomic imprinting, by which maternal and paternal alleles of some genes have differing levels of activity depending on parent of origin, have profound effects on growth and development in humans.

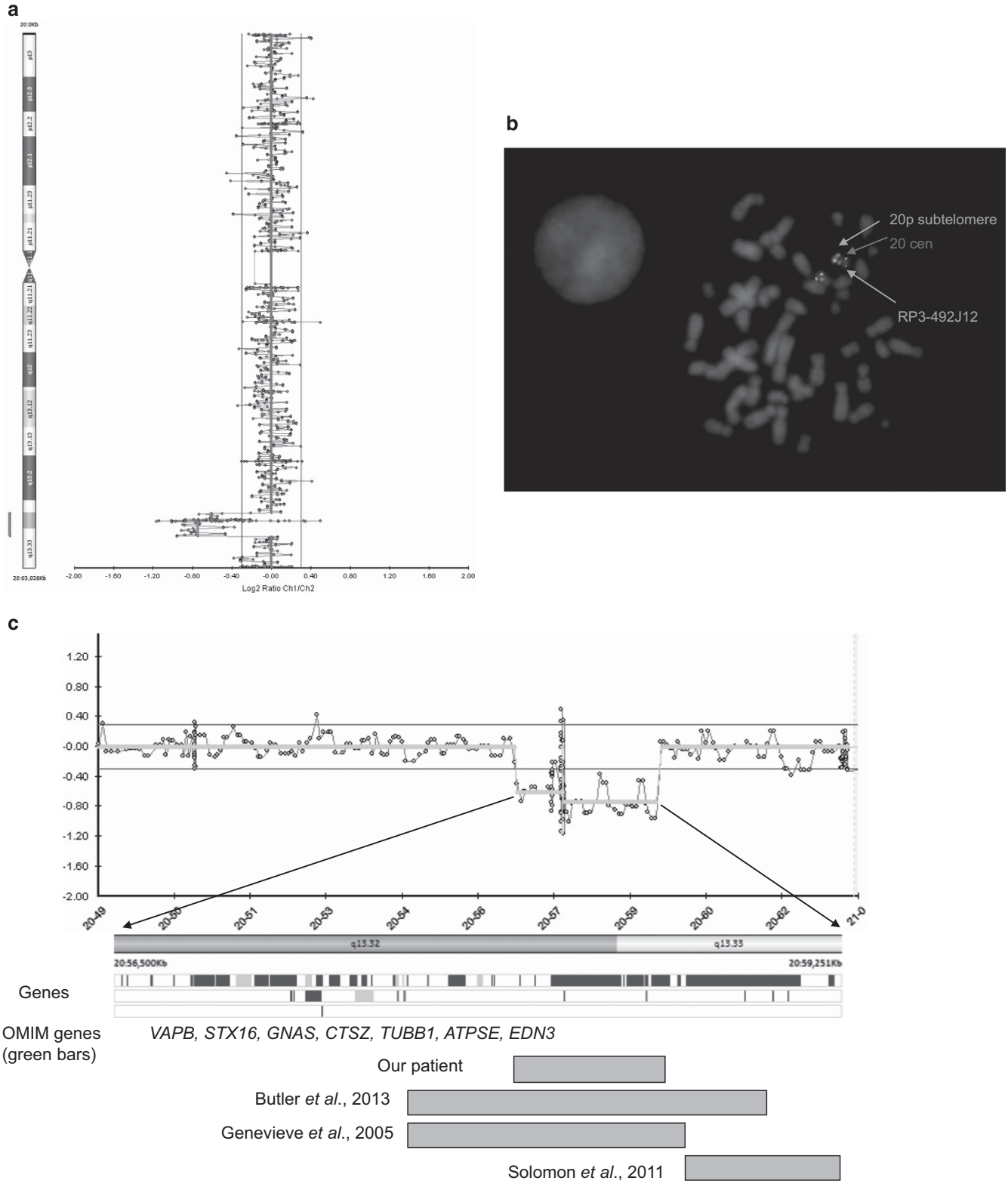

Figure 2 Array CGH profile showing deletion on chromosome 20q13.32–q13.33 (**a**), which was confirmed by metaphase fluorescence *in situ* hybridization (FISH) using BAC probe RP3-492J12 (**b**), and representation of the *de novo* 2.6 Mb deletion of 20q13.32–q13.33 (chr20: 56 571 799–59 230 205 Build hg19), showing the array profile of our patient. Shown here is a schematic of the genes in the region, blue bars represent HGNC genes, green bars represent OMIM genes. The OMIM gene names are given in order in the track below. Also shown, represented by the gray bars, are the deletions reported by Butler *et al.*[13] (chr20: 53 512 484–60 850 110 hg19), Genevieve *et al.*[5] (delineated by FISH) and Solomon *et al.*, 2011 (chr20: 59 671 821–60 329 092 hg18) (**c**). A full color version of this figure is available at *Journal of Human Genetics* online.

Table 1 Genes deleted from 20q13.32 to q13.33 Includes HGNC gene name, OMIM number, function according to uniprot and %HI.[16]

Gene name	*Description*	*OMIM*	*Function*	*%HI*
c20orf85				50.8
ANKRD60	Ankyrin repeat domain 60			93
RAB22A	Ras-related protein Rab-22A	612966	Has a role in endocytosis and intracellular protein transport	68.8
VAPB	Vesicle-associated membrane protein	605704	Microbiome	14
APCDD1L	Protein APCDD1-like			21
STX16	Syntaxin-16	603666	Involved in vesicular transport from the late endosomes to the *trans*-Golgi network	6.3
NPEPL1	Probable aminopeptidase		Probably catalyzes the removal of unsubstituted N-terminal amino acids from various peptides	67.8
GNAS	Neuroendocrine secretory protein 55	139320	Encodes for the G proteins that are a family of guanine-binding proteins that mediate signal transduction across cell membranes	20.5
NELFCD	Negative elongation factor C/D	605297	Essential component of the NELF complex, a complex that negatively regulates the elongation of transcription by RNA polymerase II	46.3
CTSZ	Cathepsin Z	603169	Exhibits carboxy-monopeptidase as well as carboxy-dipeptidase activity	81.7
TUBB1	Tubulin beta-1 chain	612901	Tubulin is the major constituent of microtubules	41
ATP5E	ATP synthase subunit epsilon, mitochondrial	606153	Mitochondrial membrane ATP synthase	37.7
SLMO2	Protein slowmo homolog 2		Platelet count	20.4
ZNF831	Zinc finger protein 831			88.4
EDN3	Endothelin-3	131242	Endothelins are endothelium-derived vasoconstrictor peptides	23.9
PHACTR3	Phosphatase and actin regulator 3	608725	Binds actin and PPP1CA; thus inhibiting the PP1 activity	21.2
SYCP2	Synaptonemal complex protein 2	604105	Major component of the axial/lateral elements of SCS during meiotic prophase	69.9
FAM217B	Protein FAM217B			87.3
PPP1R3D	Protein phosphatase 1 regulatory subunit 3D	603326	Acts as a glycogen-targeting subunit for PP1. PP1 is essential for cell division, and participates in the regulation of glycogen metabolism, muscle contractility and protein synthesis	71.3
CDH26	Cadherin-like protein 26		Cadherins are calcium-dependent cell adhesion proteins	98.2
c20orf197	Uncharacterized protein C20orf197			95

Abbreviations: %HI, % haploinsufficiency; PP1, protein phosphatase 1; SCS, synaptonemal complexes.

Table 2 Comparison of published cases with current patient

	Butler et al.[13]	*Geneviève et al.,*[5] *pt. 1*	*Geneviève et al.,* [5] *pt. 2*	*Solomon et al.* [14]	*Current patient*
Gender	F	F	F	M	M
Age	32 months	6 months	9 months	6 months	2 years
Height	<3rd centile	−6 s.d.	−5 s.d.	<3rd centile	<0.4th centile
Weight	<3rd centile	−4.5 s.d.	−4 s.d.	<3rd centile	<0.4th centile
OFC	<3rd centile	−4 s.d.	−2.5 s.d.	25th centile	<0.4th centile
Birth weight	1.47 kg (<3rd percentile)	1.32 kg (<0.4th centile)	1.57 kg (<0.4th centile)	NS	1.56 kg
Feeding difficulties	++	++	++	NS	++
Craniofacial features	Triangular face with open mouth	High forehead, small AF	High forehead, small AF	ND	Triangular face
Ears	Protruding, low-set	Simple, floppy, low-set	Simple, floppy, low-set	ND	Bilateral low-set
Eyes	Hypertelorism, ptosis, synophyrs	Enophthalmia, dysplastic iris	Enophthalmia, dysplastic iris	ND	Grayish sclerae
Nose	Flat, broad nasal bridge	Broad nasal bridge	Broad nasal bridge	ND	Normal
Upper lip/philtrum	Unilateral cleft lip	Short and prominent philtrum	Short and prominent philtrum	ND	Normal
Chin	Small, pointed	Small	Small	ND	Pointed
Bone age	Delayed	Delayed	Delayed	NS	Delayed
Subcutaneous fat distribution	NR	Abnormal	Abnormal	NR	Abnormal
AHO phenotype	No	No	No	No	No
ID	Moderate	Moderate-severe	Moderate	Speech delay	Mild
Hypotonia	+	+	+	−	+
Growth retardation	++	++	++	−	+
Deletion	7.3 Mb 20q13.2–q13.33	6 Mb 20q13.2–q13.3	6 Mb 20q13.2–q13.3	0.7 Mb 20q13.33	2.6 Mb 20q13.32–q13.33
Parental studies	Not done	Paternal origin	Paternal origin	*De novo*	*De novo*, likely paternal origin

Abbreviations: −, Absent; AF, anterior fontanelle; AHO, Albright's hereditary osteodystrophy; ID, intellectual disability; ND, not dysmorphic; NR, not reported; NS, not specified; OFC, occipital–frontal circumference; +, present; pt., patient.

Inactivating loss-of-function mutations in *GNAS* are known to cause pseudohypoparathyroidism (PHP type 1a), also known more commonly as AHO, whereas activating mutations in this gene are associated with the McCune–Albright syndrome and polyostotic fibrous dysplasia.[18] AHO is an autosomal-dominant condition characterized by obesity, short stature, round face, short bones in the fingers, especially the 4th and 5th metacarpals, and subcutaneous calcification. Intellectual disability is reported in about 70% of these patients.[19] In contrast, PHP type 1b is characterized by isolated renal parathyroid hormone resistance without any of the physical characteristics seen in AHO. PHP type 1b is caused because of methylation and imprinting defects of the GNAS complex from the maternal allele with subsequent loss of expression of Gs-α protein in proximal renal tubules (as only the maternal allele is expressed in the proximal renal tubules).[20] Another cause of PHP type 1b is because of microdeletions of *STX16*, a long-range control element of methylation at the GNAS locus.[21] Our patient did not have the AHO or PHP type 1b phenotype (in terms of the physical characteristics and the bone biochemistry) and hence is perhaps phenotypically more similar to the Peruvian patient reported Butler *et al.*,[13] with a non-AHO phenotype.

Of the other potential genes of interest, *VAPB* (vesicle-associated membrane protein-associated protein B) has a role in unfolded protein response, which is a process that suppresses the accumulation of unfolded proteins within the endoplasmic reticulum.[22] Heterozygous mutations in *VAPB* have been implicated in amyotrophic lateral sclerosis and late-onset, Finkel-type spinal muscular atrophy.[23] *SLMO2* is not a protein-coding gene and its function is not yet known. Of the other genes implicated in human disease within this deletion are, *EDN3* (Endothelin-3), mutations in which have been identified in Waardenburg type 4b (Shah-Waardenburg syndrome), congenital central hypoventilation syndrome (CCHS) and also, in some cases, of isolated Hirschsprung disease.[24,25] *TUBB1* codes for a major β-tubulin expressed in platelets and megakaryocytes and mutations in this gene are known to cause macrothrombocytopenia.[26] Therefore, although, the patient reported here does not have an AHO phenotype, and *GNAS* is the most likely candidate gene contributing to this phenotype in the deleted region.

In conclusion, this patient has clinical features that overlap those previously reported in association with 20q13 deletions. The 2.6 Mb interstitial deletion of chromosome 20q13.32–q13.33 is smaller than that previously reported by Butler *et al.*,[13] and therefore helps to narrow the minimum deleted region that may be responsible for the 'common' features of a 20q13.3 deletion syndrome. Further case reports of a similar nature are required to try and delineate the exact phenotypic variations in interstitial deletions within the long arm of chromosome 20.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGEMENTS

We thank the family for their participation in this report. This study was funded by the Sheffield Children's Hospital Charity.

1 Fraisse, J., Bertheas, M. F., Frère, F., Lauras, B., Rolland, M. O. & Brizard, C. P. Partial monosomy 20q: a new syndrome. Regional assignment of the ADA locus on 20q132 (author's transl)]. *La Sem. Hôpit.* **58**, 1366–1369 (1982).

2 Shabtai, F., Ben-Sasson, E., Arieli, S. & Grinblat, J. Chromosome 20 long arm deletion in an elderly malformed man. *J. Med. Genet.* **30**, 171–173 (1993).

3 Aldred, M. A., Aftimos, S., Hall, C., Waters, K. S., Thakker, R. V., Trembath, R. C. *et al.* Constitutional deletion of chromosome 20q in two patients affected with albright hereditary osteodystrophy. *Am. J. Med. Genet. A* **113**, 167–172 (2002).

4 Roberts, A. E., Cox, G. F., Kimonis, V., Lamb, A. & Irons, M. Clinical presentation of 13 patients with subtelomeric rearrangements and a review of the literature. *Am. J. Med. Genet. A* **128A**, 352–363 (2004).

5 Geneviève, D., Sanlaville, D., Faivre, L., Kottler, M. L., Jambou, M. & Gosset, P. Paternal deletion of the GNAS imprinted locus (including Gnasx1) in two girls presenting with severe pre- and post- natal growth retardation and intractable feeding difficulties. *Eur. J. Hum. Genet.* **13**, 1033–1039 (2005).

6 Ravnan, J. B., Tepperberg, J. H., Papenhausen, P., Lamb, A. N., Hedrick, J., Eash, D. *et al.* Subtelomere FISH analysis of 11 688 cases: an evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities. *J. Med. Genet.* **43**, 478–489 (2006).

7 Béri-Deixheimer, M., Gregoire, M. J., Toutain, A., Brochet, K., Briault, S., Schaff, J. L. *et al.* Genotype-phenotype correlations to aid in the prognosis of individuals with uncommon 20q13.33 subtelomere deletions: a collaborative study on behalf of the 'association des Cytogénéticiens de langue Française'. *Eur. J. Hum. Genet.* **15**, 446–452 (2007).

8 Kroepfl, T., Petek, E., Schwarzbraun, T., Kroisel, P. M. & Plecko, B. Mental retardation in a girl with a subtelomeric deletion on chromosome 20q and complete deletion of the myelin transcription factor 1 gene (MYT1). *Clin. Genet.* **73**, 492–495 (2008).

9 Bena, F., Bottani, A., Marcelli, F., Sizonenko, L. D., Conrad, B. & Dahoun, S. A *de novo* 1.1–1.6Mb subtelomeric deletion of chromosome 20q13.33 in a patient with learning difficulties but without obvious dysmorphic features. *Am. J. Med. Genet. A* **143A**, 1894–1899 (2007).

10 Petersen, M. B., Tranebjaerg, L., Tommerup, N., Nygaard, P. & Edwards, H. New assignment of the adenosine deaminase gene locus to chromosome 20q13 X 11 by study of a patient with interstitial deletion 20q. *J. Med. Genet.* **24**, 93–96 (1987).

11 Descipio, C., Morrissette, J. D., Conlin, L. K., Clark, D., Kaur, M. & Coplan, J. Two siblings with alternate unbalanced recombinants derived from a large cryptic maternal pericentric inversion of chromosome 20. *Am. J. Med. Genet. A* **152A**, 373–382 (2010).

12 Traylor, R. N., Bruno, D. L., Burgess, T., Wildin, R., Spencer, A., Ganesamoorthy, D. *et al.* A genotype-first approach for the molecular and clinical characterization of uncommon de novo microdeletion of 20q13.33. *PLoS One* **5**, e12462 (2010).

13 Butler, M. G., Usrey, K. M., Roberts, J. L., Manzardo, A. M. & Schroeder, S. R. 20q13.2–q13.33 Deletion syndrome: a case report. *J. Pediatr. Genet.* **2**, 157–161 (2013).

14 Solomon, B. D., Pineda-Alvarez, D. E., Hadley, D. W., Keaton, A. A., Agochukwu, N. B., Raam, M. S. *et al.* De novo deletion of chromosome 20q13.33 in a patient with tracheo-esophageal fistula, cardiac defects and genitourinary anomalies implicates GTPBP5 as a candidate gene. *Birth Defects Res. A* **91**, 862–865 (2011).

15 DECIPHER (DatabasE of Genomic variants and Phenotype in Humans Using Ensembl Resources) (2014). http://decipher.sanger.ac.uk. Accessed 30 Dec 2014.

16 Huang, N., Lee, I., Marcotte, E. M., Hurlesm, M. E. & Huang, F. Characterising and predicting haploinsufficiency in the human genome. *PLoS Genet.* **6**, e1001154 (2010).

17 Minocherhomji, S., Seemann, S., Mang, Y., El-Schich, Z., Bak, M., Hansen, C. *et al.* Sequence and expression analysis of gaps in human chromosome 20. *Nucleic Acids Res.* **40**, 6660–6672 (2012).

18 Aldred, M. A. & Trembath, R. C. Activating and inactivating mutations in the human GNAS1 gene. *Hum. Mutat.* **16**, 183–189 (2000).

19 Aldred, M. A., Bagshaw, R. J., MacDermot, K., Casson, D., Murch, S. H., Walker-Smith, J. A. *et al.* Germline mosaicism for a GNAS1 mutation and Albright hereditary osteodystrophy. *J. Med. Genet.* **37**, E35 (2000).

20 Bastepe, M., Frohlich, L. F., Hendy, G. N., Indridason, O. S., Josse, R. G., Koshiyama, H. *et al.* Autosomal dominant pseudohypoparathyroidism type Ib is associated with a heterozygous microdeletion that likely disrupts a putative imprinting control element of GNAS. *J. Clin. Invest.* **112**, 1255–1263 (2003).

21 Linglart, A., Gensure, R. C., Olney, R. C., Juppner, H. & Bastepe, M. A novel STX16 deletion in autosomal dominant pseudohypoparathyroidism type 1b redefines the boundaries of a *cis*-acting imprinting control element of GNAS. *Am. J. Hum. Genet.* **76**, 804–814 (2005).

22 Kanekura, K., Nishimoto, I., Aiso, S. & Matsuoka, M. Characterization of amyotrophic lateral sclerosis-linked P56S mutation of vesicle-associated membrane protein-associated protein B (VAPB/ALS8). *J. Biol. Chem.* **281**, 30223–30233 (2006).

23 Nishimura, A. L., Mitne-Neto, M., Silva, H. C., Richieri-Costa, A., Middleton, S., Cascio, D. *et al.* A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am. J. Hum.Genet.* **75**, 822–831 (2004).

24 Bidaud, C., Salomon, R., Van Camp, G., Pelet, A., Attié, T., Eng, C. *et al.* 1997. Endothelin-3 gene mutations in isolated and syndromic Hirschsprung disease. *Eur. J. Hum. Genet.* **5**, 247–251 (1997).

25 Bolk, S., Angrist, M., Xie, J., Yanagisawa, M., Silvestri, J. M., Weese-Mayer, D. E. *et al.* Endothelin-3 frameshift mutation in congenital central hypoventilation syndrome. *Nat. Genet.* **13**, 395–396 (1996).

26 Kunishima, S., Kobayashi, R., Itoh, T. J., Hamaguchi, M. & Saito, H. Mutation of the beta1-tubulin gene associated with congenital macrothrombocytopenia affecting microtubule assembly. *Blood* **113**, 458–461 (2009).