Microdeletion of 9q22.3: A patient with minimal deletion size associated with a severe phenotype

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Abstract
Basal cell nevus syndrome (also known as Gorlin Syndrome; MIM109400) is an autosomal dominant disorder characterized by recurrent pathological features such as basal cell carcinomas and odontogenic keratocysts as well as skeletal abnormalities. Most affected individuals have point mutations or small insertions or deletions within the PTCH1 gene on human chromosome 9, but there are some cases with more extensive deletion of the region, usually including the neighboring FANCC and/or ERCC6L2 genes. We report a 16-year-old patient with a deletion of approximately 400,000 bases which removes only PTCH1 and some non-coding RNA genes but leaves FANCC and ERCC6L2 intact. In spite of the small amount of DNA for which he is haploid, his phenotype is more extreme than many individuals with longer deletions in the region. This includes early presentation with a large number of basal cell nevi and other skin lesions, multiple jaw keratocysts, and macrosomia. We found that the deletion was in the paternal chromosome, in common with other macrosomia cases. Using public databases, we have examined possible interactions between sequences within and outside the deletion and speculate that a regulatory relationship exists with flanking genes, which is unbalanced by the deletion, resulting in abnormal activation or repression of the target genes and hence the severity of the phenotype.

KEYWORDS
9q22.3 deletion, basal cell nevus syndrome, Gorlin syndrome, PTCH1

1 INTRODUCTION

Basal Cell Nevus Syndrome (BCNS, also known as Gorlin Syndrome; MIM109400) is an autosomal dominant disorder characterized by craniofacial and skeletal abnormalities and recurrent pathological features such as basal cell carcinomas (BCCs) and odontogenic keratocysts (Gorlin, 2004; Thalakoti & Geller, 2015). Ectopic calcification is also very prevalent. Affected individuals usually have pits on the palms and soles and have distinctive facial features including frontal bossing and macrocephaly. There is a wide variability in the manifestations of BCNS, and affected individuals can also develop other tumors and cancers including tumors of the central nervous system, particularly medulloblastoma (Evans & Farnon, 2002 [updated 2018]; Malbari & Lindsay, 2020). However, some affected individuals have minimal signs with very few BCCs or other lesions (Gorlin, 2004). BCNS is usually caused by a mutation in the PTCH1 gene (encoding patched 1; MIM601309) on the long arm of chromosome 9 (9q22.32), although similar phenotypes have been reported...
with mutations within SUFU (suppressor of fused homolog, located at 10q24.32; MIM607035) (Pastorino et al., 2009) and possibly PTCH2 (patched 2, located at 1p34.1; MIM603673) (Altaraihi et al., 2019; Fan et al., 2008; Fujii et al., 2013; Pastorino et al., 2009). These proteins regulate signaling through the hedgehog pathway, which mediates key developmental processes in the embryo and continues to be active in some cell types after birth.

The majority of cases of BCNS involve single base changes or small insertion/deletion (indel) variants of PTCH1 (Evans et al., 2017; Reinders et al., 2018; Soufrié et al., 2006; Wicking et al., 1997) (mutations listed at http://www.lovd.nl/ptch1). Up to 13% of BCNS cases have deletions of at least one exon (Evans et al., 2017) and a small proportion of these have larger deletions of chromosome 9 encompassing PTCH1 as well as additional material. The longest reported such deletion to date is 20.5 mb while the shortest is 0.35 mb (Boonen et al., 2005; Brickler et al., 2014; Cajalba et al., 2006; Cayrol et al., 2019; Gravelli et al., 2013; Isidor et al., 2013; Kieny et al., 2018; Muller et al., 2012; Redon et al., 2006; Reichert et al., 2015; Shimojima et al., 2009; Yamada et al., 2020). As with other microdeletion syndromes (Schmickel, 1986; Weise et al., 2012), most patients with 9q22.3 deletion have multiple signs and symptoms in addition to the manifestations of BCNS. These include hydrocephalus, premature fusion of the metopic suture or trigonocephaly, macrosomia, and global delay. These features may be attributed to the deletion of genes in addition to PTCH1. In particular in almost all reported cases the deletion encompasses all or part of the neighboring Fanconi anemia complementation group C (FANCC) gene (Gibson et al., 1994), potentially predisposing these individuals to the clinical manifestations of the recessive condition Fanconi anemia (MIM227645). Another gene that is frequently included in the deletion is TGFBR1 (9q22.33), associated with the dominant condition Loeys-Dietz syndrome type 1 (MIM609192) (Loeys et al., 2005) and a recessive condition involving susceptibility to multiple self-healing squamous epitheliomas (MIM132800) (Goudie et al., 2011). Most patients with 9q22.3 deletions have intellectual disabilities, in common with other microdeletions (Schmickel, 1986; Weise et al., 2012), although those with the smallest deletions may have normal intellectual function (for example, Case 10 from Muller and colleagues; Muller et al., 2012).

We report a patient with a short 9q22.3 deletion covering PTCH1 and several neighboring non-coding RNA genes, accompanied by severe BCNS and other manifestations of 9q22.3 microdeletion, but without intellectual disability.

2  |  CLINICAL REPORT

2.1  |  Clinical history

The patient is a 16-year-old boy, the second child of non-consanguineous parents with no relevant family history. The pregnancy was notable for a rapidly growing fetal head. He was delivered at 36 weeks by Caesarean section due to maternal hypertension. Birth weight was 3.76 kg (97th percentile for gestational age; Dobbins et al., 2012), length was 51.5 cm (75th percentile for full term; https://www.who.int/childgrowth/standards/en/), head circumference was 37.7 cm (99th percentile for full term; https://www.who.int/childgrowth/standards/en/). The baby was placed in a humidicrib and received oxygen for 3 days. At 6 weeks old, he was diagnosed with communicating hydrocephalus of unknown origin.

Surgery for hydrocephalus was undertaken at 7 months to insert a VP shunt. Figure 1(a) shows the patient at age 7 months immediately after surgery, where the large head is apparent, and Figure 1(b) shows the increasing head circumference over the first 2 years of life. Gross and fine motor developmental milestones were delayed along with speech. He crawled at 15 months of age and walked at approximately 18 months of age. At 2.5 years of age, he was not talking and was diagnosed with childhood apraxia of speech. He received physiotherapy, occupational therapy, and speech therapy and currently at age 16 years has no difficulty with speech. At 4 years of age, he was diagnosed with scoliosis and Sprengel deformity of the shoulders (https://rarediseases.info.nih.gov/diseases/7693/sprengel-deformity). X-ray examination revealed fused ribs and abnormalities in his spine. At 11 years 1 month MRI of the spine showed early disc degeneration in L3/L4 as well as upper thoracic asymmetry (convex left, centered at T3) and fused 4th and 5th ribs. Calcification of the falx was confirmed with MRI examination.

The presence of skin lesions was noticed by the parents from about 4 years of age. BCCs were first diagnosed clinically at age 10 years 3 months. Between then and age 16 years he had a total of 138 lesions removed, of which 58 were shown to be BCCs, 29 were trichoepitheliomas with the potential to progress to BCCs and the remainder were a range of other lesions, including skin tags and benign pigmented naevi. BCCs have been removed from arms, fingers, shoulders, back, neck, clavicle, scalp, popliteal fossa, groin, and abdomen. Keratocystic odontogenic tumors (KCOT) were first detected at 9 years 6 months. All permanent teeth were visible except for one wisdom tooth. Several teeth were displaced. A total of nine KCOT have been removed, along with eight teeth.

His height and weight tracked with the 97th centile for age from age 4 months (Figure 1(c),(d)). At age 16 years, his height is 184 cm (90th centile for age) and weight is 86 kg (97th centile for age). Puberty is currently well advanced. Head circumference at 16 years is 63.5 cm (>98th centile for adults; Figure 1(b)). He shows no sign of intellectual disability. He attends a local high school and has achieved his academic milestones with a grade point average above the average for his peers.

Father’s adult height is 176 cm and mother’s adult height is 168 cm. Predicted height for a male offspring is 178.5 cm (see Figure 1(c)). Father’s adult head circumference is 58 cm and mother’s adult head circumference is 55.5 cm. The parents and siblings show no signs of BCNS.
FIGURE 1  9q22.3 deletion patient. (a) Patient at 7 months of age, at the time of placement of the VP shunt. Note, dots on forehead are location marks from surgery. (b) Head circumference birth to 2 years. Circumference at 16 years is 63.5 cm. (c) Height charts; left birth to 2 years, right 2 to 16 years. Triangle—mother’s adult height; square—father’s adult height; open dot—predicted adult height. (d) Weight charts; left birth to 2 years, right 2 to 16 years. Data for growth charts were derived from CDC data from 2000 to 2007 (http://www.cdc.gov/growthcharts) as presented in spreadsheets available at https://www.seattlechildrens.org/about/community-benefit/obesity-program/education-for-healthcare-providers/excel-based-clinical-tools-assist-growth-charts/. Predicted adult height calculated from a formula provided in the same resource [Color figure can be viewed at wileyonlinelibrary.com]
2.2 | Treatment

To date, the treatment has consisted of regular skin surveillance, surgery to remove BCCs and KCOTs, together with avoidance of exposure to sunlight. The patient has been taking a daily supplement of nicotinamide (vitamin B3) for 2 years. Other clinical signs and symptoms are treated by conventional therapies. The possible use of hedgehog inhibitors as a way to manage the condition in the future has been reviewed, although there are undesirable side effects and hedgehog pathway inhibitors are contraindicated in children (Kieran et al., 2017; Robinson et al., 2017).

2.3 | Genetic analysis

Although the boy showed abnormalities in multiple systems, initial assessment at age 4 years suggested that he did not have any clinical syndrome warranting genetic testing. At 9 years 5 months, multiplex ligation-dependent probe amplification (MLPA) analysis of DNA was undertaken at West Midlands Regional Genetics Laboratory, UK, and it was determined that there was a heterozygous pathogenic deletion of exons 1 to 24 of the PTCH1 gene. He was given a diagnosis of BCNS. At age 10 years 10 months, further genetic testing was undertaken at Yale-New Haven Hospital, using chromosome microarray comparative genomic hybridization (CGH) (Agilent 180 K). The results revealed a 364Kb deletion (mapped to version GRCh37 of the human genome reference sequence) at 9q22.32, including the PTCH1 gene. The CGH microarray analysis of the parents was normal. Whole genome sequencing of the patient's DNA (performed by Genome One, Sydney, Australia) reported no other clinically significant variants relevant to the phenotype.

3 | METHODS

3.1 | Editorial policies and ethical considerations

The study was approved by Mater Misericordiae Ltd Human Research Ethics Committee (EC00332), approval number HREC/MML/45819 (V3), and conforms to the Australian Government National Statement on Ethical Conduct in Human Research (2007)—Updated 2018. All family members gave informed consent prior to participating in this research.

3.2 | Bioinformatic analysis

The patient's whole genome was initially sequenced by a commercial company (Genome One) from blood DNA and the FASTQ files were supplied. Reads were aligned to human genome reference sequence GRCh38 (GATK Resource Bundle) using bwa-mem version 0.7.12 (Li, 2013). Duplicate reads were marked using picard tools and indels were realigned using GATK 3.7 (McKenna et al., 2010). Single nucleotide variants (SNVs) and indels were detected with HaplotypeCaller from GATK 3.7. Structural variants including deletions, duplications, inversions, insertions, and translocations were detected with Delly version 0.7.7 (Rausch et al., 2012).

Genes within the deleted region were retrieved using Ensembl BioMart (http://www.ensembl.org/biomart/martview/). The significance of variants was determined using the variant effect predictor from Ensembl (http://www.ensembl.org/info/docs/tools/vep/index.html) which presents results of SIFT and PolyPhen analysis (McLaren et al., 2016), and using the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), an archive of reports of relationships among human variants and phenotypes (Landrum et al., 2018; Landrum & Kattman, 2018). Population allele frequencies and the common allele for variants were determined using the Genome Aggregation Database (gnomAD–Genomes) for Europeans, to most closely reflect the genetic backgrounds of the parents. The details were taken from dbSNP (https://www.ncbi.nlm.nih.gov/snp/). Bioinformatic analysis of regulatory relationships involving the deleted region used The GenoHancer Regulatory Elements section of GeneCards (https://www.genecards.org; accessed July 2020) which describes genomic regulatory elements related to the selected gene. GenoHancer is a database of genome-wide enhancer-to-gene and promoter-to-gene associations (Fishilevich et al., 2017). Regulatory elements are mined from The ENCODE Project (ENCODE Project Consortium, 2012), Ensembl regulatory build (Zerbino et al., 2015), FANTOM5 atlas of active enhancers (Andersson et al., 2014), VISTA enhancer browser (Visel et al., 2007), dbSUPER (Khan & Zhang, 2016), EPDnew (Dreos et al., 2013), UCNEbase (Dimitrieva & Bucher, 2013), and the craniofacial atlas (Wilderman et al., 2018). We also accessed the compendium of chromatin interactions provided by Jung and colleagues (Jung et al., 2019). The FANTOM5 dataset (Forrest et al., 2014), available at https://fantom.gsc.riken.jp/zenbu/ was used to determine promoter regions and gene expression levels. To examine the potential regulatory sequence FANCC-203, PacBio Iso-Seq cDNA-sequencing data from mixed cell lines were downloaded from https://downloads.pacbcloud.com/public/dataset/UHR_ISOSeq/. Full-length reads were aligned to the human genome (GRCh38) using Minimap2 (https://doi.org/10.1093/bioinformatics/bty191).

3.3 | Determination of parent of origin

Saliva samples were collected from the patient and his parents using OG-500 tubes (DNA Genotek, Ottawa, Canada) and DNA extracted according to manufacturer's instructions with the reagents supplied. Polymerase chain reaction (PCR) was used to amplify regions of the deletion containing potential segregating SNVs. The PCR contained 1 unit of MyTaq Taq polymerase (Meridian Bioscience, Sydney, Australia), 0.5 μM each primer, and 1 ng/μl template DNA in the manufacturer's MyTaq reaction buffer. PCR conditions were 1 min at 94°
followed by 30 cycles of 0.25 min at 94 C, 0.25 min at 60 C, and 0.5 min at 72 C. This was followed by 10 min at 72 C and the samples were then held at 10 C. PCR products were sent to the Australian Genome Research Facility, University of Queensland, Brisbane, Australia for cleanup and chain termination (Sanger) sequencing. Primers and variant alleles are listed in Table 1.

4 | RESULTS

4.1 | Genes within the deletion

To further understand the extent of the deletion, the whole genome sequence data were mapped to GRCh38 and the deletion was determined to extend from 9:95,403,395 to 9:95,804,680 (401,286 bases) (Figure 2(a), Supplementary Figure S1). Analysis of public databases showed that the deleted region contained the whole of the PTCH1 gene plus several long non-coding RNAs and two pseudogenes (Figure 2(b)). Unlike the majority of 9q22.3 micro-deletions, this deletion did not include the FANCC gene (encoding a protein of the Fanconi anemia DNA repair complex), although two non-coding transcripts attributed to FANCC were within the deleted region. FANCC-203 (ENST00000433644.2) is a short processed transcript wholly within the deletion; FANCC-212 (ENST00000636777.1) is a longer processed transcript where the promoter region and initial exon were within the deletion. In addition, the ERCc6L2 gene was beyond the distal breakpoint while a long non-coding RNA gene (LINC00476) was partially deleted. Analysis of three SNVs across the deleted region (indicated by arrows in Figure 2(a)) showed that the mother was heterozygous while the father was homozygous for the common allele. In each case, the patient had inherited the rare allele from the mother and no allele from the father, indicating that the deletion had occurred in the paternal chromosome (Figure 3).

One explanation for the variable phenotype is that the deletion exposed recessive variants in the haploid region. Given the extreme BCNS phenotype and since PTCH1, located at 9:95,442, 980–95,517,057, is the only known protein coding gene in the deletion (Figure 2(b)), we examined the variants in the remaining paternal PTCH1 allele. There were five variants that were different from the reference sequence, four being known SNVs where the proband carried an allele that is common in Europeans, and one was a novel variant. Four were intronic and one was in the 3’ UTR and all were considered benign or not reported as clinical variants. There were no protein coding variants in the maternal PTCH1 (Supplementary Table S1). Other genes within the deletion are transcribed into non-coding RNAs. These include FANCC-203 (ENST00000433644.2), which is highly expressed in cancer cell lines (FANTOM5 database; https://fantom.gsc.riken.jp/zenbu; see Supplementary Figure S2). FANCC-203 is transcribed into a variety of isoforms, not all annotated in ENSEMBL (Supplementary Figure S3). The FANCC-203 promoter is highly conserved and can act as a rare alternative promoter for the major FANCC gene, overlapping FANCC-212 (Figure 2, Supplementary Figure S3). The patient differed from the GRCh38 reference sequence for nine variants within FANCC-203, two in the promoter region (rs357521, T > A, frequency of A in Europeans = 0.38; rs357522, G > A, frequency of A in Europeans = 0.68) (Supplementary Table S2). The significance of these variants is unclear.

4.2 | Variants in members of the hedgehog signaling pathway

We then considered whether there might be detrimental variants in other members of the hedgehog signaling pathway by examining the sequence of hedgehog pathway genes listed previously (Onodera et al., 2017). Protein coding variants are shown in Supplementary Table S3. Although a number of coding sequence variants were present, the majority were known variants with appreciable frequencies in the general population, and in regions/codons that are not highly conserved. The patient was heterozygous for most of these. There were four deleterious/probably or possibly damaging variants. Three of these had conflicting predictions across SIFT, PolyPhen, and ClinVar. One was rs4667591 (in LRP2, MIM600073) where the patient was homozygous for the common allele and it seems unlikely that this would contribute to his phenotype. For rs1567047 (in FAT4, MIM612411), the patient was homozygous for the rarer allele (allele frequency 0.28). He was heterozygous for

| TABLE 1 | Variant genotyping |
|---------|-------------------|
| SNV ID  | Position         | Rare allele* | Frequency of rare allele* | Patient allele | Primers (5’–3’) |
|---------|-------------------|--------------|---------------------------|---------------|-----------------|
| rs45619434 | 9:95,480,278 | T            | 0.011                     | T             | GGGCTTGTGGGTGTTCCAGAGGCCCCACAAAAATTCACCA |
| rs184491760 | 9:95,626,888 | T            | 0.016                     | T             | CCCAGAAGACCTGGAACGGACGACGAGCCCTAAATGAA |
| rs182177904 | 9:95,800,040 | A            | 0.018                     | A             | AATTGGAAAAAGGCACAGGATCCCCACAGGAGGTCATGAGG |

Abbreviation: SNV, single nucleotide variant.

*The rare allele and its frequency were determined from the Genome Aggregation Database (gnomAD–Genomes) for Europeans, to most closely reflect the genetic backgrounds of the parents. The details are drawn from dbSNP (https://www.ncbi.nlm.nih.gov/snp/).
Of interest, he was heterozygous for rs121908120 (p.Phe228Ile), a predicted pathogenic variant in WNT10A (MIM606268) which has been associated with recessive syndromes affecting the teeth and skin (odontoonychodermal dysplasia MIM257980, tooth agenesis, selective, 4 MIM150400, Schopt-Schulz-Passarge syndrome MIM224750). Heterozygotes for Schopt-Schulz-Passarge syndrome have been reported to show dental anomalies (Bohring et al., 2009).

FIGURE 2  Chromosome 9 deletion region. (a) Ensembl genome browser image for Chr9:95,100,000-95,700,000. Deleted region is shown in red box. Arrows indicate single nucleotide variants used to identify parent of origin of the deletion. The gene model in Ensembl for FANCC extends across all transcripts showing links to the gene, but only two transcripts attributed to this gene, ENST00000433644.2 (FANCC-203) and ENST00000636777.1 (FANCC-212) lie within the deleted region; the main promoter for FANCC lies outside the deletion. See also Figure 4(a) and Supplementary Figure S3. (b) Genes within the deleted region, downloaded using Ensembl BioMart. PTCH1 is the only protein coding gene annotated in the region. IncRNA, long non-coding RNA [Color figure can be viewed at wileyonlinelibrary.com]

rs235768 (BMP2, MIM112261). Of interest, he was heterozygous for rs121908120 (p.Phe228Ile), a predicted pathogenic variant in WNT10A (MIM606268) which has been associated with recessive syndromes affecting the teeth and skin (odontoonychodermal dysplasia MIM257980, tooth agenesis, selective, 4 MIM150400, Schopt-Schulz-Passarge syndrome MIM224750). Heterozygotes for Schopt-Schulz-Passarge syndrome have been reported to show dental anomalies (Bohring et al., 2009).
4.3 | Regulatory sequences within the deletion

Another explanation for the severity of his condition would be disrupted interaction between regulatory sequences and their targets where one or other is within the deleted region. The compendium of long-range chromatin interactions published recently (Jung et al., 2019) indicated interactions of sequences within the deletion with ZNF169, FANCC, AL354861.3 (formerly known as DKFZP434H0512), ERCC6L2, LINC00476 (partly deleted), overlaps AL354861.3, and EIF4BP3 (Figure 4(b)). These results are summarized in Table 2 and presented in more detail in Supplementary Table S4, with ERCC6L2 and FANCC strongly supported as targets of enhancer activity within the deleted region.

5 | DISCUSSION

We have presented a patient with a microdeletion of chromosome 9 initially detected by MLPA as a deletion of PTCH1 and then confirmed by CGH and whole genome sequencing to cover approximately 400,000 bases. The deletion encompasses the PTCH1 gene and several long non-coding RNA genes and pseudogenes, but unlike most other cases of microdeletion 9q22.3 the deletion in our patient does not include the FANCC gene. The patient shows an extensive presentation of BCNS in addition to other features commonly seen in longer 9q22.3 deletions but rare in BCNS, such as hydrocephalus and macrosomia (Evans et al., 2017; Muller et al., 2012).

In general, microdeletions result in some form of intellectual disability. This is thought to relate to the high probability of deleting loci involved in neurological development (Schmickel, 1986; Weise et al., 2012). The deletion in our patient is among the shortest reported for this region and he has no intellectual impairment; his academic results are excellent. Although as a young child he had no speech, this was associated with his motor delay and was corrected with specialized speech therapy.

In spite of the short deletion and the presence of only one coding gene (PTCH1) within the deletion, our patient has a relatively severe presentation. For example, one 9q22.3 deletion patient (Boonen et al., 2005) had a deletion of 15 megabases (Mb) encompassing 87 genes, but had only 3 BCCs at the age of 21, while our patient with a deletion of less than 1 Mb containing only one coding gene had 58 BCCs and 29 trichoepitheliomas removed by age 16. There are several possible genetic explanations for phenotypic variability in microdeletion syndromes (Srour & Shevell, 2015) which can be applied here. First, the deletion may have unmasked a recessive mutation on the remaining (maternal) allele of PTCH1 or in the other non-coding transcribed sequences within the deletion. In our analysis, we did not find any potentially pathogenic variant in the remaining PTCH1 gene, so this explanation seems unlikely. It is more difficult to assess the likely consequences of variants in non-coding sequences, for example, the two variants in the promoter region of FANCC-203, but none were obviously pathogenic. Second, alleles at other genes that interact with PTCH1 may influence the phenotype. We looked at variants in the hedgehog pathway genes identified previously (Onodera et al., 2017) and found a number of heterozygous missense mutations that might contribute to the pathology in this patient. In particular, the variant in WNT10A has been associated with a tooth agenesis
syndrome, accompanied by skin abnormalities. Although generally recessive, this syndrome can manifest in heterozygous individuals (Bohring et al., 2009). It is possible that heterozygosity for the mutation in this gene could exacerbate the PTCH1 phenotype and this may explain some of our patient’s features.

Third, the phenotype may be influenced by epigenetic factors, which is more likely when a phenotype differs depending on the parent of origin as this suggests imprinting. We determined that the paternal chromosome was deleted in this patient. This is consistent with previous reports of 9q22.3 deletions that found all seven patients with overgrowth and macrocephaly had lost the paternal allele (Redon et al., 2006; Shimojima et al., 2009). The possibility of imprinting was suggested. The deletion presented here does not encompass any known imprinted regions but this explanation cannot

**FIGURE 4** Potential regulatory sequences affected by 9q22.3 deletion. (a) FANTOM5 gene expression data. Black box shows the deleted region. Red oval shows FANCC-203. Other ovals show promoter regions: black—PTCH1; blue—bidirectional promoter for ERCC6L2 and LINC00476 (see also Supplementary Figures S6 and S7); orange—FANCC. Potential enhancers are shown by the green symbols on the FANTOM5 enhancers track. (b) UCSC Genome Browser view. GeneHancer regulatory elements and histone modifications are shown. H3K4Me1 and H3K27Ac are often found near regulatory elements. H3K4Me3 is often found near promoters [Color figure can be viewed at wileyonlinelibrary.com]
be eliminated. However, in general, maternally expressed imprinted genes suppress growth while paternally expressed genes enhance growth (Ishida & Moore, 2013). Therefore, it would be expected that loss of the paternal allele of an imprinted gene would expose the maternal allele and decrease growth, which is in contrast to the reported cases. Yamada and colleagues report a mother and daughter who both have a 4 Mb deletion of 9q22.3 (Yamada et al., 2020). The daughter, who inherited the deletion from her mother and therefore carries the paternal sequence in the deleted region, showed postnatal overgrowth. The parental origin of the deletion in the mother is not revealed.

Finally, the deletion may cause imbalance between a regulator and its target. A recent article (Cappuccio et al., 2019) presented a patient with a microdeletion of chromosome 14 (14q21.1) encompassing a positive regulator of LRFN5, a gene outside the deletion that was reduced in expression in the proband. A similar relationship between a sequence within the deletion and its target in the diploid region may explain our patient’s presentation. Although there is only one coding sequence within the deletion, there are several other expressed sequences, described as long non-coding RNAs (lncRNAs) or pseudogenes (Figure 2(b)). LncRNAs have been shown to regulate expression (both by silencing and activation) of adjacent or overlapping genes (Gil & Ulitsky, 2020; Liu et al., 2018; Wang et al., 2011; S. Wang et al., 2016). Our analysis of enhancers and chromatin interactions indicated that the most likely targets of sequences within the deletion are FANCC and ERCC6L2 and haploinsufficiency for the deleted sequences may result in over- or underexpression of these and other potential target genes. PTCH1, ERCC6L2, LINCO0476, and FANCC are highly expressed in the osteosarcoma cell line SAOS-2 undergoing mineralization (Supplementary Figures S4–S7), and dysregulation of these genes could contribute to the skeletal phenotype in this patient. Loci containing let-7 family microRNAs are frequently within 9q22.3 deletions and have been implicated in aspects of the phenotype (Yamada et al., 2020) but these sequences, at around 9:94,220,000, are outside the deletion in the present patient. A recent article (Liu et al., 2020) showed that a sequence within the deletion (designated RP11-43505.2 in the article, which maps close to MT1P1 and likely overlaps with FANCC-203) is targeted by WNT signaling. Several WNT genes are targets of hedgehog signaling (Onodera et al., 2017), and their expression would likely be increased when one copy of PTCH1 is deleted. However, in this patient one copy of the target gene is also deleted, which may ameliorate or exacerbate the impact of the PTCH1 deletion.

Although microdeletion 9q22.3 cases usually have a deletion of the FANCC gene (Carajlo et al., 2019; Muller et al., 2012), the gene is intact in our patient and he has few manifestations of Fanconi anemia; in some characteristics his phenotype is opposite from those with biallelic FANCC mutations. For example, his height, weight, and head circumference have always been greater than the 90th centile for age (Figure 1), while Fanconi anemia patients are characterized by small stature, poor growth, and microcephaly (Schneider et al., 2015). In addition, bone marrow dysfunction means that they have low red blood cell counts, while our patient has recently been diagnosed with polycythaemia. One possibility is that a transcript from within the deletion region usually suppresses the activity of FANCC. His extreme phenotype may result in part from an imbalance between FANCC and

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**TABLE 2** Regulatory relationships between the deleted region and flanking sequences

| Target gene | Gene description | Target position | Found by
|-------------|------------------|----------------|-----------|
| **External targets of sequences within the deletion** | | |
| ZNF169 | Zinc finger protein 169 | 9:94,259,298–94,301,829 | Compendium of chromatin interactions |
| FANCC | Fanconi anemia complementation group C | 9:95,099,054–95,317,709 | EnhancerAtlas, Compendium of chromatin interactions |
| LINCO0476 | Long non-coding RNA 00476 | 9:95,759,231–95,876,049 | GeneHancer |
| ERCC6L2 | ERCC excision repair 6 like 2 | 9:95,871,264–96,121,154 | EnhancerAtlas, GeneHancer, Compendium of chromatin interactions |
| EIF4BP3 | Eukaryotic translation initiation factor 4B pseudogene 3 | 9:96,146,007–96,147,856 | GeneHancer |
| HSD17B3 | Hydroxysteroid 17-beta dehydrogenase 3 | 9:96,235,306–96,302,176 | Compendium of chromatin interactions |
| ZNF367 | Zinc finger protein 367 | 9:96,385,941–96,418,370 | Compendium of chromatin interactions |
| HABP4 | Hyaluronan binding protein 4 | 9:96,450,169–96,491,336 | Compendium of chromatin interactions |
| CDC14B | Cell division cycle 14B | 9:96,490,241–96,619,843 | Compendium of chromatin interactions |
| PRXL2C | Peroxiredoxin like 2C (AAED1) | 9:96,639,577–96,655,317 | Compendium of chromatin interactions |
| ZNF510 | Zinc finger protein 510 | 9:96,755,865–96,778,129 | Compendium of chromatin interactions |
| **Target within the deletion of flanking sequences** | | |
| FANCC-203 | Fanconi anemia complementation group C processed transcript (upstream) | 9:95,414,834–95,426,796 | GeneHancer |

Note: Some of the target genes are marked with a * indicating their potential role in the observed phenotype.
the putative regulatory transcript, leading to excess activity of FANCC, contrasting with Fanconi anemia C patients who have low levels of FANCC gene expression from both alleles (as it is a recessive condition). Since most 9q22.3 deletion patients are hemizygous for both loci, the balance between the two transcripts would be maintained with no manifestation of overexpression of FANCC. Notably, FANCC expression is associated with the FANCC-203 IncRNA promoter by a GeneHancer interaction (Figure 4, Supplementary Figure S3), and this relationship may be disrupted by the deletion of FANCC-203. The recent article concerning a microdeletion on chromosome 14 illustrated how deletion of a regulatory sequence can influence neighboring non-deleted gene (Capuccio et al., 2019). Another small 9q22.3 deletion was previously reported (Müller et al., 2012). This encompassed PTCH1 as well as the 5’ end of the FANCC gene including the promoter. The patient had similar phenotype but does not appear to be as severely affected as our patient, supporting the idea that deletion of FANCC-203 (ENST00000433644.2) or another regulatory sequence may cause increased expression of FANCC. This would not be found in the second patient who has the promoter region of FANCC deleted.

Enhancers for another flanking gene, ERCC6L2, were detected in the deleted region (Figure 4). This gene is expressed ubiquitously; FANTOM5 data show expression in a range of cell types (Supplementary Figure S6), including a number of immune cell lineages (granulocyte-macrophage precursors, plasmacytoid dendritic cells, adult and fetal thymus, common myeloid precursor) and some nervous system cells (cerebellum, pituitary). It is also highly expressed in a number of tumor cell types including the osteosarcoma line SAOS-2. The encoded protein, ERCC excision repair 6 like 2, is a helicase which may be involved in nuclear and mitochondrial nucleotide excision repair (Tummala et al., 2018). Patients with biallelic mutations of ERCC6L2 (MIM615715) have trilineage bone marrow failure, learning disabilities, and microcephaly (Tummala et al., 2014) as well as short stature and other features similar to Fanconi anemia (Jarviäho et al., 2018). Again, this phenotype contrasts with that of our patient, suggesting that negative regulators of this gene may be deleted and the resulting overexpression of ERCC6L2 may contribute to his condition. Both ERCC6L2 and FANCC are involved in DNA damage repair. Overactivation of one or both due to dysregulation may promote cell proliferation and could explain the macrosomia seen in this patient.

ERCC6L2 is encoded on the forward strand. It has a bidirectional promoter, with the reverse strand transcribed into a long non-coding RNA, LINC00476, that is expressed in CD4+ and CD8+ T lymphocyte lineages as well as SAOS-2 cells (Supplementary Figures S7). The deletion affects the 3’ end of one transcript attributed to this gene (LINC00476-205; ENST00000433656.2). A variant in this gene was associated with nicotine dependence by genome wide association study (Yin et al., 2017). The association of both ERCC6L2 and LINC00476 with neurological and behavioral deficits suggests that they are not important in this patient who has normal intellect and behavior. However, the 5’ end of LINC00476 would be brought closer to the main FANCC promoter (also on the reverse strand) on the deleted chromosome, which may contribute to illegitimate regulation of FANCC.

A recent case report presents a patient who was found to be heterozygous for a frame shift mutation (single base insertion) at the beginning of exon 11 of PTCH1 (p.Val502Gly fs'13) (Beltrami et al., 2020). Although the mutation affected only PTCH1, the patient had some manifestations of 9q22.3 deletion syndrome. The premature termination of translation would disrupt the sterol-sensing domain (amino acids 426–616) within the transmembrane sequence of patched 1. The authors showed that there was also an alteration in mRNA splicing. Thus either through premature termination, removal of the domain by alternative splicing or nonsense mediated decay, it is likely that this patient is haploinsufficient for the sterol-sensing domain as well as transmembrane domains 6–12, containing the large intracellular loop, the second large extracellular loop and the C-terminal cytoplasmic tail of the patched 1 protein. The sterol-sensing domain may be involved in interaction with the hedgehog ligand which is activated by addition of cholesterol and palmitoyl moieties, but has also been shown to bind sterols independently (Qi et al., 2019). This may indicate that patched 1 is involved in sterol translocation across the membrane. Reduced levels of PTCH1 as in our patient and the truncation of the protein before this point as in the patient discussed above (Beltrami et al., 2020) may explain the extensive phenotype, although other BCNS patients with truncating mutations which would remove this domain but with mild/late onset disease have also been reported (Reinders et al., 2018). Other authors (Wicking et al., 1997) have attributed all the congenital features of BCNS to haploinsufficiency, since the majority of mutations, including some that occur 5’ to the mutation in the patient of Beltrami and colleagues (Beltrami et al., 2020) would result in premature termination (see also (Reinders et al., 2018) and http://www.lovd.nl/). Therefore, it seems unlikely that the severity is due simply to the nature of the PTCH1 deletion.

An alternative explanation for the high number of skin and jaw lesions is that the patient received repeated doses of ionizing radiation prior to diagnosis of BCNS. This exposure, through X-ray and CT, was received when characterizing the skeletal and jaw phenotype and could have caused numerous additional lesions within the field of exposure and therefore contributed to the more severe lesion phenotype. Children with BCNS are at high risk of developing BCCs at the sites of radiation (reviewed by Kleinerman, 2009), but it is notable that our patient has had no BCCs in the jaw region. Since both flanking genes (FANCC and ERCC6L2) are involved in DNA repair, a dysregulation of these genes by mechanisms discussed above could interact with the radiation dose to exacerbate radiation sensitivity caused by the loss of PTCH1. Such exposure would not account for other features of his phenotype such as macrosomia, hydrocephalus, and skeletal anomalies.

We noted that there is an apparent duplication of sequences in the region with paralogues ZNF169 and ZNF510/782, NUTM2F and NUTM2G, YRDCP2 and YRDCP1, RNU6-79BP and RNU6-669P either side of the deletion. This may predispose the region to mispairing at meiosis leading to de novo deletion or duplication. The resulting deletions would be larger than that seen in our patient, although repair by gene conversion may have limited the extent.
Finally, in reviewing the literature it became clear that there is a long latency period between presentation of a 9q22.3 microdeletion patient for dysmorphic features or some other complication and detection of the deletion (Table 3). Our patient first presented at age 6 weeks but was not diagnosed with the microdeletion until after the age of 10 years. Others, with severe phenotypes, presented in childhood but the deletion was not detected for more than 30 years. In part, this is due to the development of technologies since the early descriptions of microdeletions in the 1980s (Schmickel, 1986) and it is to be hoped that increasing awareness of the complex phenotypes associated with microdeletions and the new whole genome sequencing approaches will ensure that diagnosis is now made quickly, reducing parental anxiety and potentially leading to more appropriate and more rapid treatment. In addition, early diagnosis of BCNS in these patients would ensure that doses of ionizing radiation were minimized, potentially preventing radiation-induced formation of skin and jaw lesions.

In summary, we present a patient with a short microdeletion within 9q22.3 who has a relatively severe presentation of BCNS (for example, early onset of multiple BCCs and jaw keratocysts) as well as manifestations usually associated with longer 9q22.3 microdeletions (macrosomia, obstructive hydrocephalus). We suggest that the extreme manifestations result from the imbalance of regulators within the deletion and their targets in the diploid region. Further examination of gene expression in this patient should clarify the relationship between the phenotype and the extent of the deletion.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Adam D Ewing performed the mapping and variant analysis. Seth W Cheetham analyzed the PacBio results for PTCH1 and FANCC-203. James J McGill, Michael Sharkey, and Rick Walker have been involved in diagnosis and clinical care of the patient, Jennifer A West and Malcolm J West were involved in review of the patient. Kim M Summers performed the laboratory and bioinformatic analysis and wrote the manuscript. All authors contributed to and approved the final version of the article.

DATA AVAILABILITY STATEMENT

Variant calls related to the deleted region and hedgehog pathway genes are provided in the Supporting Information. The raw sequence files that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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