

# A Novel (Paternally Inherited) Duplication 13q31.3q32.3 in a 12-Year-Old Patient with Facial Dysmorphism and Developmental Delay

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## Key Words

Duplication 13q · Facial dysmorphism · *GPC5* · *GPC6* · Learning difficulties · 13q31.3q32.3

## Abstract

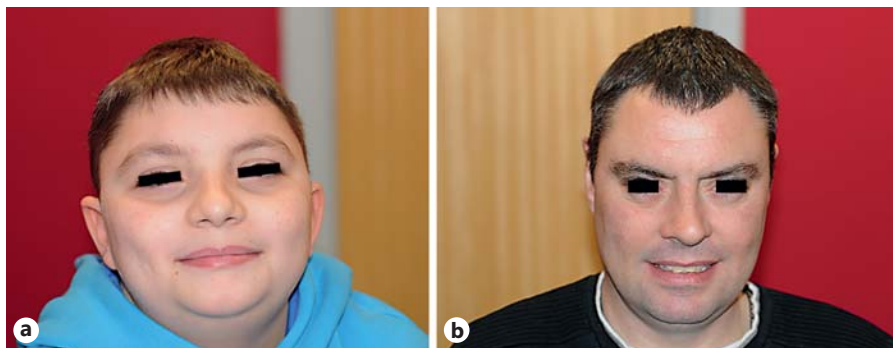
We report a 12-year-old boy referred to the Clinical Genetics service in view of facial dysmorphism, learning difficulties and autistic spectrum disorder. 60K arrayCGH revealed an 8.2-Mb duplication on chromosome 13q31.3q32.3, which was paternally inherited. This specific duplication on chromosome 13 has not been previously reported in the medical literature, and there are no familial or de novo patients with the same duplication breakpoints. This region contains 24 OMIM genes, including the glypicans *GPC5* and *GPC6*, and the *ZIC2* gene. We discuss the relevance of this chromosome imbalance and discuss the impact of this duplication on our patient's phenotype. Given that the duplication on 13q was paternally inherited, and although initially thought to be of uncertain significance, on exploring the family history further, it became apparent that the father had learning difficulties as a child and previous surgery for congenital diaphragmatic hernia. Here we explore the phenotype in association with this novel duplication on chromosome 13q and add to the existing literature on array findings within this region.

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Chromosome 13 is known for its paucity of genes and has long been studied for its variable phenotypic penetrance in cases of full trisomy 13 (Patau syndrome, lethal in neonates). Phenotype-genotype correlation studies involving cases of partial trisomy for segments of chromosome 13 suggest that certain trisomic regions give rise to certain phenotypes. Early reviews of partial 13q duplication cases grouped duplications as either proximal (pter-q22.1) or distal (qter-22.2), assigning certain phenotypes to only one group [Rivas et al., 1984; Tharapel et al., 1986]. Duplication of the proximal part of chromosome 13 is more phenotypically variable and less studied, with possible association with severe developmental retardation and minor dysmorphic traits [Schinzel, 2001]. Clinical interpretation can be challenging, especially when reciprocal regions of monosomy are present elsewhere in the genome due to aberrations arising from parental balanced translocations [Tharapel et al., 1986; Wei et al., 2012].

High-resolution arrayCGH analyses of partial 13q duplication cases have allowed potential clinically pathogenic candidate genes to be identified. Interrogation of these candidate genes attempts to link genotype with phenotype. The clinical variability of the more established del(13)(q32) syndrome supports the possibility of further contiguous gene syndromes which encompass numerous dosage variable genes.

**Fig. 1.** Facial appearance of the proband (**a**) and father (**b**), demonstrating similar facial dysmorphism including wide, elongated palpebral fissures, a prominent nose and elongated columella of the nose.



Here we present a father and son, both with a dup(13)(q31.3q32.3) identified by 60K arrayCGH. This region contains 24 duplicated OMIM genes; 2 of them, *GPC5* and *GPC6*, belong to the glypican family of genes. We explore the family history and discuss the relevance of this duplication and how this might contribute to the phenotype. We further discuss the differences in the clinical phenotype between the proband and his father, and their duplication in relation to others in the published literature.

## Materials and Methods

ArrayCGH was performed on genomic DNA extracted from peripheral lymphocytes from our proband and his father. This was applied to Oxford Gene Technologies 60 mer oligo-array, printed in  $8 \times 60K$  International Standard Cytogenomic Array (ISCA) Consortium configuration, according to manufacturer's instructions, using Promega pooled control DNA as a reference (Promega Corporation, Madison, Wis., USA). Slides were scanned using a Genepix Personal 4100A scanner (Axon Instruments) and analysed using BlueGnome BlueFuse-Multi (Version 3.0) analysis software (BlueGnome, an Illumina company, Cambridge, England, UK).

## Clinical Phenotype

The proband is a 12-year-old boy referred to the Clinical Genetics service with learning difficulties, autistic spectrum disorder and unexplained muscle cramps with no evidence of a myopathy. He is the second child of healthy, nonconsanguineous White European parents. His mother had a previous miscarriage at 10-weeks gestation and a still birth of unknown cause at 7-months gestation. He has an older sibling who is fit and well.

Antenatal scans of the proband were reported as normal, and there were no concerns during the pregnancy. He was born at term with a birth weight of 3.28 kg (9–25th centile) and was well immediately after birth. He showed developmental delay; he sat up at 18 months of age, walked at 2 years of age and was delayed with his speech. He currently attends a mainstream school but is scheduled

to attend a secondary school for children with learning disabilities. He had previously been examined by the Paediatric Neurology department in view of muscle cramps, but all results of metabolic studies were reported as normal.

On examination, he showed a dysmorphic appearance, including elongated, wide palpebral fissures, elongated columella, bilateral low-set ears, short-webbed neck, and a box-shaped body habitus. His growth parameters at 12 years of age included height of 144.6 cm (25th centile), weight of 48.7 kg (91st–98th centile) and head circumference of 51.5 cm (0.4th–2nd centile).

On further enquiry, following the knowledge that this duplication was paternally inherited, his father was also noted to have similar dysmorphism (fig. 1a, b). His growth parameters were height of 164 cm (2nd–9th centile), weight of 66.6 kg (25–50th centile) and head circumference of 55 cm (9th centile). His father had mild-moderate learning difficulties as a child but was now living independently with his family. Interestingly, he was diagnosed with a congenital diaphragmatic hernia, which needed surgery 17 days after birth.

## Results

60K arrayCGH was performed as part of ongoing investigations to identify a cause for the proband's learning difficulties. This showed an 8.2-Mb duplication on chromosome 13q31.3q32.3 between basepairs 92,776,868 and 101,047,505 (genome build GRCh37) confirmed as being paternally inherited (fig. 2).

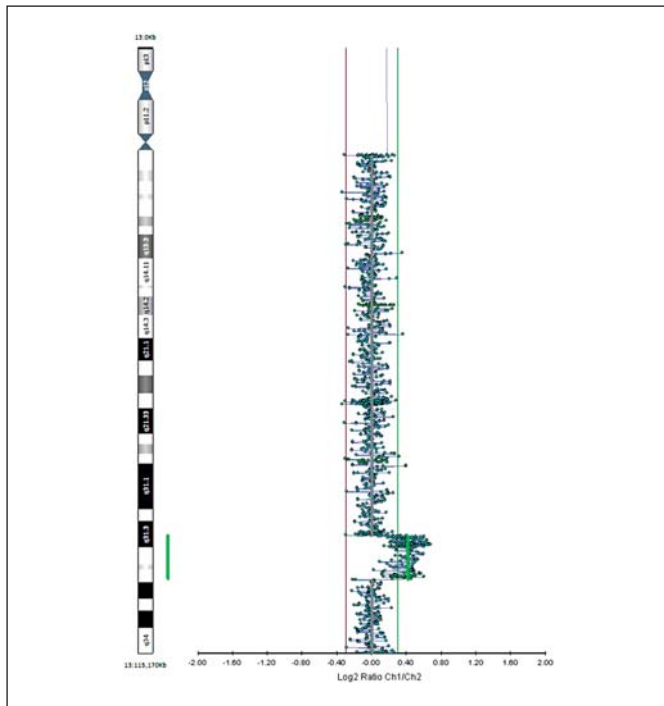
The duplication contains 29 genes (listed here from q31.1 to q32.3); *GPC5*, *GPC6*, *DCT*, *TGDS*, *GPR180*, *SOX21*, *ABCC4*, *CLDN10*, *DZIP1*, *DNAJC3*, *UGGT2*, *HS6ST3*, *OXGR1*, *MBNL2*, *RAP2A*, *IPO5*, *FARP1*, *RNF113B*, *STK24*, *SLC15A1*, *DOCK9*, *UBAC2*, *GPR18*, *GPR183*, *TM9SF2*, *CLYBL*, *ZIC5*, *ZIC2*, and *PCCA*. Of these, 24 are OMIM genes (all of those listed above excluding *TGTS*, *CLDN10*, *RNF113B*, *UBAC2*, and *ZIC5*).

The 24 OMIM genes were ranked according to their haploinsufficiency score (HI score). Since the probability of haploinsufficiency considers the dosage sensitivity of a

**Table 1.** Duplicated OMIM genes (HI scores of <15) in the region of duplication [genome build Hg19/GRCh37]

OMIM gene, No.	HI score*	Description
<i>ZIC2</i> #603073	4.9	cerebellum protein
<i>SOX21</i> #604974	10	expressed in mammalian brain
<i>PCCA</i> #232000	11.1	mutations cause propionic acidemia
<i>GPC5</i> #602446	11.5	modulate morphogens such as sonic hedgehogs (SHHs) and <i>ZIC2</i>
<i>GPC6</i> #604404	11.5	
<i>STK24</i> #604984	12.2	MAP kinase pathway
<i>HS6ST3</i> #609401	14.7	morphogen (mouse models only)

\* 1 = high, 99 = low.



**Fig. 2.** Log<sub>2</sub> ratio plot from 60K oligonucleotide arrayCGH for chromosome 13, highlighting an 8.4-Mb duplication (green bar) between basepairs 92,776,868 and 101,047,505 – with breakpoints 13q31.3 and 13q32.3, seen in the proband and his father.

gene [Huang et al., 2010], we applied the same rationale to duplication. Albeit nonempirical, we assume it only reasonable to expect that HI scores are more successful predictors of pathogenicity (for duplications) compared to only the size of the interval or the number of genes within the interval. Genes with a relative high HI score (<15) were scrutinised for previous reference in the literature associated with cytogenetic or arrayCGH dupli-

cation (the score of <15 was selected due to the spread of HI scores exhibited across all of the duplicated genes) and 7 genes were chosen to be scrutinised for further discussion (table 1). Of these 7 genes, *PCCA*, *STK24*, *SOX21*, and *HS6ST3* were not deemed important for further discussion (either due to the known function of the gene, or its function only being described in lesser mammals).

Although this rationale feels commonsensical, it does have limitations. HI scores have only been validated in relation to allele loss and empirically should only be used for such. However, triplosensitive phenotypes in association with copy number gain can be clinically harder to interpret compared to copy number loss, and this method allows the laboratory scientist the opportunity to perform gene interrogation based on an established dataset (HI scores). For very large duplications containing large numbers of genes, this can be very useful.

That said, the 29 genes were also investigated for their known protein function, particularly expression in brain or neuronal tissues as well as citations in the medical literature associated with developmental delay, intellectual disability and/or dysmorphism. Apart from *GPC5*, *GPC6* and *ZIC2*, no association was identified. *IPO5* and *DOK9* have been described as pathogenic candidate genes in the noninflammatory corneal disorder keratoconus; however, as our proband and his father did not present with any clinical eye problems, these genes were not investigated further. Our discussion, therefore, focuses predominantly on *GPC5*, *GPC6* and *ZIC2*.

## Discussion

ArrayCGH analysis has revolutionised the diagnostic pathway for children with developmental delay/learning disability and has led to an increase in diagnoses made in

**Table 2.** Cases of del(13q) reported in the literature similar in size and location to the proband's duplication (includes pure and impure duplications, detected by arrayCGH or conventional karyotyping)

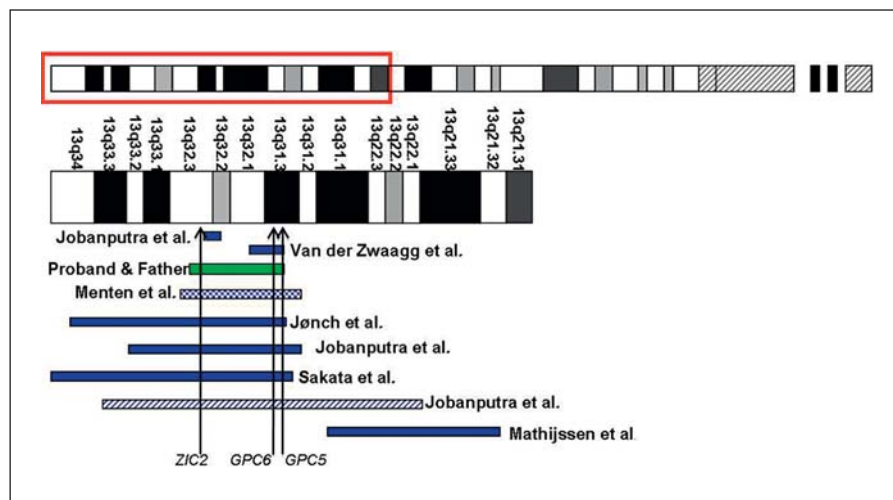
Reference	Duplication	Size (Mb)	Basepairs or loci	Other aberration or rearrangement	Clinical features
Jobanputra et al., 2012 Patient 1	13q32.2q32.2	0.564	99,309,736–99,873,865	0.384-Mb dup at 2q37.3	2-vessel cord on sonogram, normal at birth, prenatal diagnosis performed due to advanced maternal age
Jobanputra et al., 2012 Patient 2	13q31.2q32	18.7	not given	none	unilateral postaxial polydactyly, ASD, AVD, macrocephaly, dilation of renal pelvis, poor developed white matter, temporal lobe hypoplasia
Jobanputra et al., 2012 Patient 3	13q22.1q33.1 (noncontinuous/ segmental)	27.09	not given	none	developmental delay, anterior temporal arachnoid cyst
Jønch et al., 2012	13q31.3q34	22.5	91,675,986–114,211,064	del(13)(q34)	termination of pregnancy at 16 + 6 for diaphragmatic hernia
Mathijssen et al., 2005	13q21.31q31.1	21	RP11-234023–RP11-564H19	none	
Menten et al., 2006	13q31q32.3 60% mosaic (+ 30% normal cell line)	13.1	RP11-388D4–RP11-564N10	none	autistic behaviour, developmental delay, broad thumbs, nasal speech, strabismus, trigonocephaly
Sakata et al., 2008	13q31-qter	Cytogenetic G-band and FISH spectral karyotyping only		der(9)t(9;13)(p23;q31)	glaucoma, bilateral polydactyly, eye anomalies, Kawasaki disease, hydronephrosis, bilateral retained testis
van der Zwaag et al., 2009	13q31.3q32.1	5.58	89,665,466–95,252,224	invdup(13)	macrocephaly, broad nasal bridge, frontal bossing, postaxial bilateral polydactyly, rotated ears

children referred to the Paediatric/Clinical Genetics services. An ongoing challenge is the interpretation of novel chromosomal imbalances where deletions and duplications can be a diagnostic conundrum. Here we report a family with paternally inherited trisomy for the region of chromosome 13 between breakpoints 13q31.3 and q32.3 in a 12-year-old male, referred for clinical dysmorphism and developmental delay. We review previously published duplicated 13q cases in relation to this novel finding, which includes the genes *GPC5*, *GPC6* and *ZIC2*.

Trisomy 13 has a severe clinical presentation but can present with varying phenotypic penetrance for certain abnormalities, for example, polydactyly is not always present. Trisomy for smaller regions of chromosome 13 allows for genotype-phenotype studies which can elucidate candidate genes responsible for certain phenotypes.

Two of the genes duplicated in our proband and his father are *GPC5* and *GPC6*, 2 of the known 6 glypican family members (*GPC1–GPC6*). Gene structure is highly conserved across this family, with 14 cysteine residues preserved in all of the 6 family members [Veugelers et al., 1999]. Glypicans are understood to modulate activity of heparan sulphate-binding growth factors [Campos-Xavier et al., 2009; Filmus et al., 2008]. Mutations in *GPC3*, *GPC5* and *GPC6* have been linked with Simpson-Golabi-Behmel syndrome, omodysplasia and spina bifida, respectively, all of which encompass defects associated with growth and/or neural regulation [Bassuk et al., 2013]. In vivo studies show glypicans to regulate signalling of Wnts, Hedgehogs (Hhs), fibroblast growth factor receptors (FGF's) and bone morphogenic proteins (BMPs) [Topczewski et al., 2001; Yan and Lin, 2007] and suggest that

**Fig. 3.** The duplicated region of chromosome 13q in the proband and his father (green bar) compared to patients described in the published literature (blue bars). The patient described by Menten et al. [2006] (blue-checked bar) had a 60% mosaic duplication. The patient described by Jobanputra et al. [2012] (blue diagonally lined bar) had a segmental region of duplication. The approximate positions of the genes *ZIC2*, *GPC5* and *GPC6* are indicated with vertical arrows.



these genes may play an important role in developmental pathways.

Patient 1, described by Jobanputra et al. [2012] (table 2), had a duplication of approximately 0.564 Mb discovered via prenatal invasive testing, performed due to advanced maternal age and a 2-vessel cord identified on sonogram. The child was healthy at birth and was seen to develop normally, although formal developmental testing was not performed. The child's duplication does not cover the *GPC5* or *GPC6* genes, or the *SOX21* gene (located distal to *GPC6*) which is expressed in mammalian brain, suggesting that duplication of *GPC5* and/or *GPC6* may be important in developmental pathways.

van der Zwaag et al. [2009] described a 5.58-Mb duplication containing both *GPC5* and *GPC6* in a child with macrocephaly, broad nasal bridge, frontal bossing, and postaxial bilateral polydactyly. The group suggests *GPC5* and *GPC6* are candidate genes for polydactyly; however, neither our proband nor his father present with polydactyly. In comparison, our proband carries a larger duplication and presents with dysmorphic features: elongated, wide palpebral fissures, elongated columella, bilateral low-set ears, short-webbed neck, and a box-shaped body habitus.

The duplicated region we report here also includes the *ZIC2* gene (fig. 3). The classical feature of holoprosencephaly seen in del(13q) is attributed to haploinsufficiency for *ZIC2*, the likely cause of brain malformation [Brown et al., 1998]. Holoprosencephaly due to *ZIC2* mutations has a distinct face and forebrain phenotype [Solomon et al., 2010]. *ZIC2* encodes a transcription factor involved in both axial and midline establishment, and in the develop-

ment of the dorsal telencephalon [Cheng et al., 2006; Warr et al., 2008]. Duplications of *ZIC2*, however, are not reported to be associated with holoprosencephaly, and it is suggested that *ZIC2* duplications alone are not responsible for developmental delay [Jobanputra et al., 2012]. This highlights that the interpretation of gene duplication compared to gene deletion can be more challenging. Indeed the *ZIC2* gene has the lowest HI score of the 29 genes in our proband's duplication. This emphasises that the reasonable expectation of HI scores to be more successful predictors of duplication pathogenicity [compared to the size of the interval or the number of genes within the interval (see results section for further description)] should be approached with caution.

The most similar duplication described in the literature to that seen in our proband (in size and location) is described by Menten et al. [2006]. The study describes a 13.1-Mb mosaic duplication in a child with autistic behaviour, developmental delay and broad thumbs. Two other CNVs, similar in size and location to the duplication in the proband and his father in this case report are described on the DECIPHER (<http://decipher.sanger.ac.uk>) and via email from decipher@sanger.ac.uk and ISCA (dbVar, <http://www.ncbi.nlm.nih.gov/dbvar/>) databases. DECIPHER entry CHG000221 is a 10.52-Mb duplication between basepairs 89,678,237 and 100,197,340 (genome build GRCh37) containing, amongst others, the *GPC5* and *GPC6* genes. ISCA entry nssv578657\_unk is an 8.32-Mb duplication between basepairs 93,865,876 and 102,189,455 (genome build GRCh37) and contains the *GPC6* and *ZIC2* genes. Developmental delay and intellectual disability are described in both cases, with further

phenotypic information provided for DECIPHER patient CHG000221, which include broad thumbs, wide nasal bridge, anteverted nares, and long philtrum. The inheritance information has not been provided for either CNV.

Diaphragmatic hernia was reported in the father of the proband in this case report and noted in a 13q31.3q34 duplication described by Jønch et al. [2012]. Therefore, given the overlapping duplications described above and the phenotypic manifestations in our proband and his father, it appears that this duplication on 13q is pathogenic and mainly described in association with developmental delay, intellectual disability, facial dysmorphism, and possible presentation at birth with diaphragmatic hernia. Further case reports are required to expand on this phenotype and describe these overlapping duplications as a separate entity in the medical literature.

However, the varying clinical features between our proband and his father, in relation to previously reported cases of partial dup 13q (bearing in mind, some of these

may have been ascertained by G-banding analysis), does add weight to the uncertainty surrounding novel array findings in patient cohorts with learning disability and/or developmental delay. ArrayCGH findings of unknown clinical significance can be difficult to interpret without further published material regarding the region of the genome in question. That said, variable phenotypic penetrance and clinical presentation will continue to be seen in cases of partial and full duplication and deletion of chromosome 13. Our findings should be helpful when counselling families with similar duplications or in trying to interpret prenatal array findings associated with this region of chromosome 13.

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