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SHORT COMMUNICATION

# Holoprosencephaly and preaxial polydactyly associated with a 1.24 Mb duplication encompassing *FBXW11* at 5q35.1

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**Abstract** Holoprosencephaly (HPE) is the most common developmental defect affecting the forebrain and midface in humans. The aetiology of HPE is highly heterogeneous and includes both environmental and genetic factors. Here we report on a boy with mild mental retardation, lobar HPE, epilepsy, mild pyramidal syndrome of the legs, ventricular septal defect, vesicoureteral reflux, preaxial polydactyly, and facial dysmorphisms. Genome-wide tiling path resolution array based comparative genomic hybridisation (array CGH) revealed a de novo copy-number gain at 5q35.1 of 1.24 Mb. Additional multiplex ligation-dependent probe amplification screening of a cohort of 31 patients with HPE for copy-number changes at the 5q35.1 locus did not reveal any additional genomic anomalies. This report defines a novel 1.24 Mb critical interval for HPE and preaxial polydactyly at 5q35.1. The duplicated region encompasses seven genes: *RANBP17, TLX3, NPM1, FGF18, FBXW11, STK10,* and *DC-UbP*. Since *FBXW11* is relatively highly expressed in fetal brain and is directly involved in proteolytic processing of GLI3, we propose *FBXW11* as the most likely candidate gene for the HPE and preaxial polydactyly phenotype. Additional research is needed to further establish the role of genes from the 5q35.1 region in brain and limb development and to determine the prevalence of copy number gain in the 5q35.1 region among HPE patients.

**Keywords** Array CGH · *BTRCP* · *FBXW11* · *FGF18* · Holoprosencephaly · Microduplication · 5q35.1

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## Introduction

Holoprosencephaly (HPE [MIM 236100]) is the most common developmental defect in humans affecting the forebrain and midface. The aetiology of HPE is highly heterogeneous and includes both environmental and genetic factors (Wallis and Muenke 2000). At least 12 different genetic loci have been associated with HPE (Dubourg et al. 2004). Causative mutations have mainly been identified in four genes: *Sonic Hedgehog* (*SHH* MIM:600725) at 7q36, *ZIC2* (MIM:603073) at 13q32, *SIX3* (MIM:603714) at 2p21, and transforming growth factor-beta-induced factor (*TGIF* MIM:602630) at 18p11.3 (Dubourg et al. 2004). Recently, Bendavid et al. (2006) found microdeletions encompassing these four genes in 16 out of 339 severe HPE cases (4.7%). Therefore, microdeletion of these genes may contribute to the aetiology of HPE. Here

we report on the identification of a de novo microduplication at 5q35.1 in a patient with lobar HPE and preaxial polydactyly. The microduplication defines a novel 1.24 Mb critical interval for HPE and preaxial polydactyly on human chromosome 5q35.1.

## Materials and methods

### Clinical report of the index patient

The 19-year-old patient was referred to our department in Nijmegen for genetic counselling. He presented with mild mental retardation, delayed motor development, and a mild pyramidal syndrome. He was born after an uncomplicated pregnancy and delivery. Right preaxial polydactyly (digitus minimus) was noted, as well as an asymmetric crying face due to aplasia of the right musculus anguli oris. The patient had a ventricular septal defect and at 5 years of age, he was operated on because of vesicoureteral reflux. He has had infrequent tonic–clonic seizures since the age of 6 years. Magnetic resonance imaging of the cerebrum revealed a lobar HPE (see Fig. 1). At 15 years of age, he was small (height 157 cm; < –2SD) with a relatively large occipitofrontal circumference (OFC) of 57 cm (+1SD). In addition, he exhibited an asymmetric face (left < right), synophrys, up-slanting palpebral fissures, finger-like thumbs, remnants of the polydactyly near the right thumb, and sandal gaps between the first and second toes (see Fig. 2). In addition, the tendon reflexes were brisk with a clonus of the ankles and two Babinski signs. Routine chromosome analysis was normal and subtelomeric multiplex ligation-dependent probe amplification (MLPA) failed to reveal any anomalies (SALSA MLPA kit P036, MRC Holland, Amsterdam, The Netherlands). Subsequently, the patient was included in a series of 100 mentally retarded patients tested by genome-wide tiling path resolution array-based comparative genomic hybridisation (array CGH) (De Vries et al. 2005).

### Array CGH and confirmation

The proband was tested for submicroscopic aberrations using array CGH. A tiling-resolution microarray consisting of 32.477 BAC clones, covering the entire human genome, was used. Microarray preparation, hybridisation, and data analysis, were described in detail previously (De Vries et al. 2005). Genomic DNA from the patient was hybridised in duplicate with dye-swap against a sex-mismatched reference pool. Parental samples were hybridised once against the same reference pool. Copy-number detection was performed automatically using a Hidden Markov model as described previously (De Vries et al. 2005). The array CGH results were confirmed using MLPA (Schouten et al. 2002). Fluorescence in situ hybridisation (FISH) using BAC clones also present on the array (RP11-768O14, CTD-2315O11, RP11-413I18, RP11-575K18) was used to determine the location of the aberration in the genome.

### Screening for copy-number changes at 5q35 among HPE patients using MLPA

In total, 31 DNA samples from patients with HPE were tested for copy-number changes in the 5q35.1 region using MLPA. Of these, 27 had HPE only and had previously been tested by sequencing analysis for mutations in *SHH, ZIC2, SIX3*, and *TGIF*. The remaining four patients both suffered from HPE and

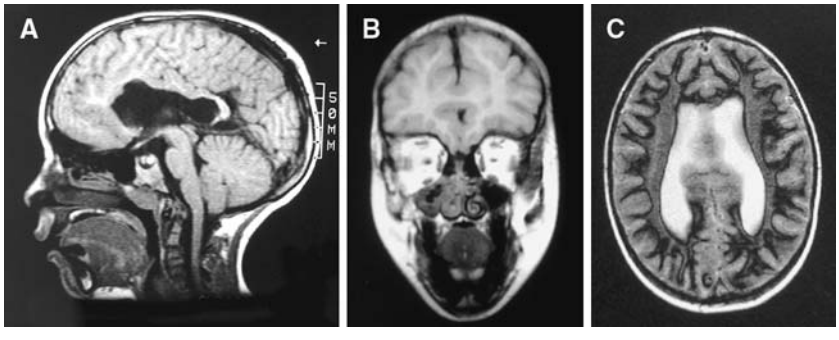


**Fig. 1a–c** T1-weighted magnetic resonance imaging (MRI) of the cerebrum at 6 years of age. The sagital T1-weighted image (**a**) shows the absence of the anterior part of the corpus callosum, characteristic of holoprosencephaly (HPE). Note that the frontal lobes are fused. The coronal T1-weighted image (**b**) and axial inversion recovery image (**c**) reveal that the interhemisperic fissure is absent in the frontal region, the frontal lobes are basely fused, and the grey matter is continuous between both the left and right hemispheres

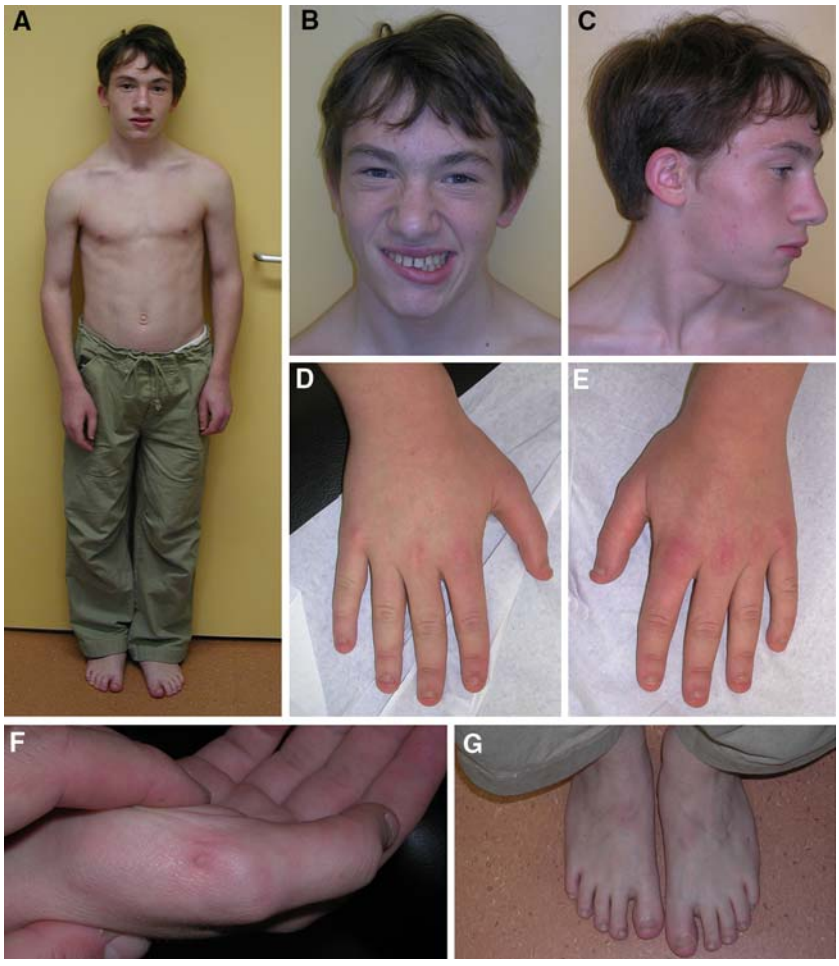


**Fig. 2a–g** Patient at 15 years of age. Note the small height: 1.57 m (–2 SD) with a relatively large skull (+1 SD), synophrys, up-slanting palpebral fissures, the asymmetric face (**a**–**c**), the finger-like thumbs (**d**, **e**), the remnants of the polydactyly near the right thumb (**f**), and the sandal gap between first and second toes (**g**)

ectrodactyly. A set of nine uniquely sized MLPA probes, hybridising to genes in the 5q35.1 region (*FGF18* and *FBXW11*), was created according to a protocol provided by MRC-Holland (http://www.mlpa.com/index.htm). Probe sequences are provided in Supplementary Table 1. Synthetic 5′ or 3′ half-probes were obtained from Biolegio (Malden, The Netherlands). Hybridisation, ligation and amplification of the MLPA probes were performed as described previously (Koolen et al. 2004; Schouten et al. 2002). Amplification products were identified and quantified by capillary electrophoresis on an ABI 3730 genetic analyzer, using GeneMapper software (Applied Biosystems, Foster City, CA). Data were normalised as described by Koolen et al. (2004).

## Results

A de novo copy-number gain at chromosome 5q35.1 was identified in the proband using a genome-wide 32K BAC microarray. Figure 3 represents the chromosome 5 array CGH plot showing the 1.24 Mb gain at 5q35.1 and the genes in the region. The centromeric breakpoint was within BAC clone CTD-2012P21 and RP11-768O14, and the telomeric breakpoint was within BAC clone CTD-2005E9 and CTD-2270L4. Based on the physical mapping positions as obtained from the May 2004 Freeze of the USCS Genome Browser, the size of the duplicated region was determined to be 1.24 Mb (170.5–171.8 Mb). In addition, the aberration was not reported as large copy-number variation in the database of genomic variants (http://www.projects.tcag.ca/variation/). Subsequent FISH analysis using intermediate BAC clones as probes yielded hybridisation signals on both chromosome 5 homologues. The signals on one of these homologs was subjectively larger, suggestive of a tandem microduplication of this region (data not shown). Array CGH and FISH experiments on parental samples did not reveal any aberration at 5q35.1, indicating a de novo anomaly in the patient. The duplication at 5q35.1 in the index patient was confirmed using specifically designed synthetic MLPA probes, hybridising to genes in the 5q35.1 locus (see

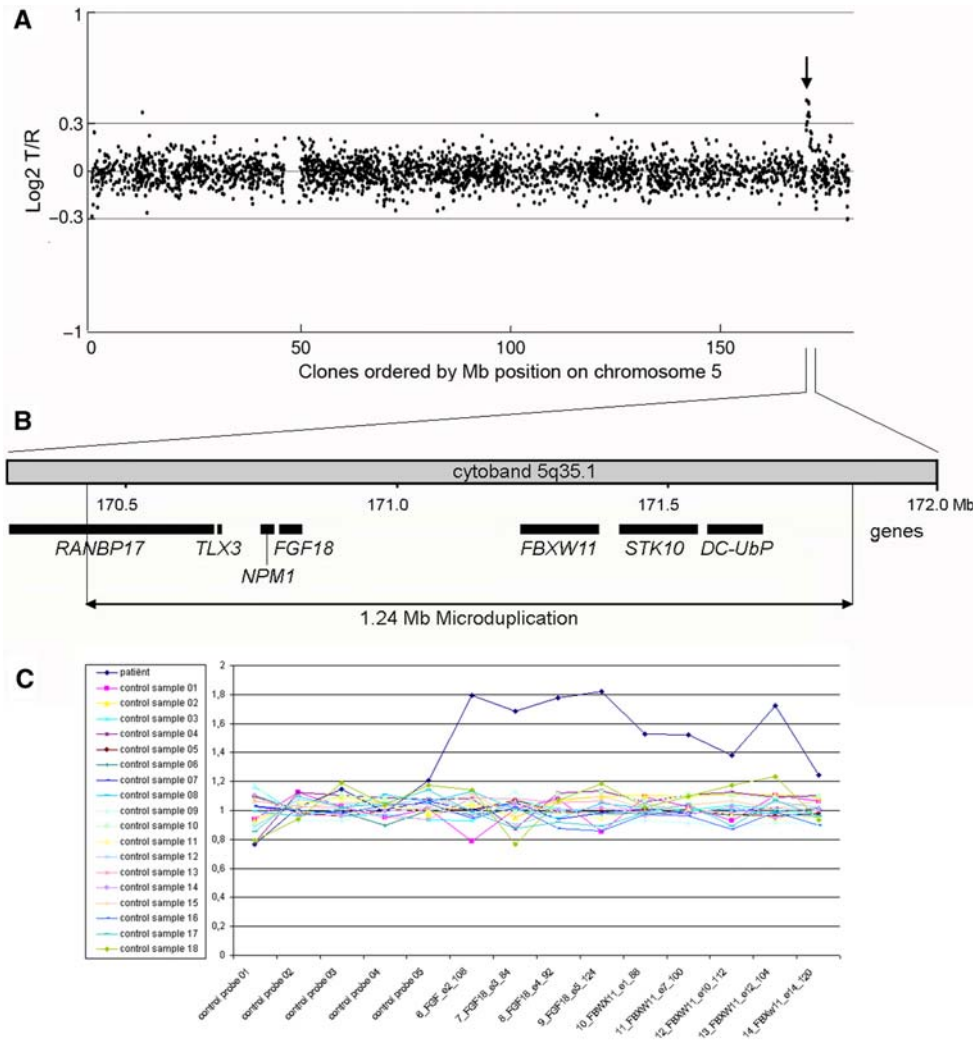


**Fig. 3** **a** Chromosome 5 array comparative genomic hybridisation (array CGH) plot showing the 1.24 Mb duplication at 5q35.1 (*arrow*). Individual BAC clones are shown as dots ordered by Mb position from pter to qter (custom track UCSC Genome Browser, May 2004 Freeze). Hybridisation intensities are represented as log2 transformed and normalised test over reference intensity ratios [$\log_2$(T/R)]. **b** Transcript map of the duplicated 5q35.1 genomic region. **c** Confirmation of array CGH data by multiplex ligation-dependent probe amplification (MLPA), using a specifically designed synthetic probe set containing nine probes corresponding to 5q35.1 (*FGF18, FBXW11*)

Fig. 3c). The same MLPA probe set was used to screen 31 additional patients diagnosed with HPE for copy number changes at 5q35.1; no additional anomalies were detected in these samples.

## Discussion

In this study we describe the detection of a de novo 1.24 Mb microduplication at 5q35.1 in a 19-year-old boy with HPE and preaxial polydactyly using a genome-wide tiling path resolution microarray. This finding suggests that one or more genes located within the duplicated region are sensitive to dosage alterations influencing brain development.

Clinical features commonly reported among 32 clinically well-described patients with microscopically visible duplications spanning cytoband 5q35.1 are low birth weight, developmental delay, mental retardation, microcephaly, down-turned palpebral fissures, hypertelorism, micrognathia, dysplastic ears and congenital heart defects (Groen et al. 1998; Lazjuk et al. 1985; Levy et al. 2002; Rodewald et al. 1980; Schinzel 2003; Schroeder et al. 1986). HPE was reported in two cases: a girl with a duplication of 5q32 → qter [46,XX,der(10)t(5;10)(q31.3;q26)] and a boy with a microscopically visible 5q32->qter duplication and a 5p15 → pter deletion, due to an inversion [46,XY, rec(5), dup q, inv(5)(p15q32)] (Lazjuk et al. 1985; Schroeder et al. 1986). Preaxial polydactyly has been described in terminal 5q duplications in two patients, whereas in one patient a duplication of the terminal thumb phalanges was noted. The phenotype of HPE is known to be variable, even among family members carrying the same mutation (Dubourg et al. 2004). The latter is in concordance with the absence of HPE and/or polydactyly in most patients with duplications encompassing the 5q35.1 region.

Based on our findings, we screened for copy-number changes of 5q35.1 in 31 patients with unexplained HPE using MLPA. However, MLPA analysis did not reveal additional aberrations in the 5q35.1 region.

Given that HPE and preaxial polydactyly are recurrent clinical findings in patients with terminal 5q duplication, the 1.24 Mb duplication in our patient defines a 1.24 Mb locus for HPE and preaxial polydactyly on human chromosome 5q35.1. The 1.24 Mb duplicated region in our index patient encompassed at least seven genes: *RANBP17, TLX3, NPM1, FGF18, FBXW11, STK10*, and *DC-UbP*. Several lines of evidence suggest that overexpression of the *FBXW11* gene is likely to be causative for HPE and limb malformation.

The *FBXW11* gene encodes the βTRCP2 protein, a F-box/WD40 repeat protein from the modular E3 ubiquitin protein ligase complex called SCFs (Skp1, Cdc53/Cull and F-box protein), which functions in phosphorylation-dependent ubiquitination (Kipreos and Pagano 2000). The *Drosophila* ortholog of βTRCP, Slimb, is required for the proteolysis of the transcription factor cubitus interruptus (ci), the key mediator of hedgehog signaling (Ming et al. 1998; Villavicencio et al. 2000). *Ci* is the ortholog of the GLI-Kruppel gene family in vertebrates. Interestingly, loss-of-function mutations in human *GLI2* are associated with pituitary anomalies and HPE-like features (Roessler et al. 2003), whereas mutations in *GLI3* can cause three distinct congenital syndromes: Greig cephalopolysyndactyly syndrome, Pallister–Hall syndrome, and postaxial polydactyly type A1 (Ming et al. 1998). Recently, it has been demonstrated that βTRCP is directly involved in proteolytic processing of GLI3 in vertebrates (Wang and Li 2006). Thus, duplication of the *FBXW11* gene is likely to result in increased βTRCP activity and consequently enhanced processing of GLI3 (Wang and Li 2006). Conversely, SHH is known to inhibit proteolytic processing of GLI transcription factors, and therefore acts to stimulate GLI-mediated transcription (te Welscher et al. 2002; Aza-Blanc et al. 1997). SHH is a crucial factor for the patterning of the ventral forebrain and is required for the separation of the primordial eye field and brain into two discrete hemispheres (Ming et al. 1998). Loss-of-function mutations in the *SHH* gene are associated with the development of HPE (Belloni et al. 1996; Roessler et al. 1996) and polydactyly (Lettice et al. 2002). It thus seems that reduced GLI activity, due either to inactivating mutations of *SHH* or *GLI*, or to overexpression of the *FBXW11* gene, contribute to the HPE and polydactyly phenotype. Additional evidence for the causative effect of duplication of the *FBXW11* gene is provided by the observation that duplications of the highly homologous *βTRCP2* gene at 10q24.32 are associated with split hand–split foot malformation (SHFM MIM 183600) (de Mollerat et al. 2003).

In summary, we define a novel 1.24 Mb critical interval at 5q35.1 for HPE and preaxial polydactyly. We propose *FBXW11* as the most likely candidate gene for HPE. Further research is warranted to unravel the role of this candidate gene in brain and limb development and to determine the prevalence of copy-number gain in the 5q35.1 region among HPE patients.

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