ARTICLE

Deletions flanked by breakpoints 3 and 4 on 15q13 may contribute to abnormal phenotypes

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Non-allelic homologous recombination (NAHR) between segmental duplications in proximal chromosome 15q breakpoint (BP) regions can lead to microdeletions and microduplications. Several individuals with deletions flanked by BP3 and BP4 on 15q13, immediately distal to, and not including the Prader–Willi/Angelman syndrome (PW/AS) critical region and proximal to the BP4–BP5 15q13.3 microdeletion syndrome region, have been reported; however, because the deletion has also been found in normal relatives, the significance of these alterations is unclear. We have identified six individuals with deletions limited to the BP3–BP4 interval and an additional four individuals with deletions of the BP3–BP5 interval from 34 046 samples submitted for clinical testing by microarray-based comparative genomic hybridization (aCGH). Of four individuals with BP3–BP4 deletions for whom parental testing was conducted, two were apparently *de novo* and two were maternally inherited. A comparison of clinical features, available for five individuals in our study (four with deletions within BP3–BP4 and one with a BP3–BP5 deletion), with those in the literature show common features of short stature and/or failure to thrive, microcephaly, hypotonia, and premature breast development in some individuals. Although the BP3–BP4 deletion does not yet demonstrate statistically significant enrichment in abnormal populations compared with control populations, the presence of common clinical features among probands and the presence of genes with roles in development and nervous system function in the deletion region suggest that this deletion may have a role in abnormal phenotypes in some individuals.

European Journal of Human Genetics (2011) 19, 547–554; doi:10.1038/ejhg.2010.237; published online 19 January 2011

Keywords: 15q13; segmental duplication; microdeletion; genotype–phenotype

INTRODUCTION

Segmental duplications (SDs) on proximal chromosome 15q mediate non-allelic homologous recombination (NAHR), resulting in deletion or duplication of the intervening material. Such recombination events result in Prader–Willi/Angelman syndrome (PW/AS), autism, and a recently described 15q13.3 microdeletion syndrome.^{1,2} The segmental duplications are grouped into breakpoints (BPs) on the basis of where breaks recur in these various genomic disorders. BP1 and BP2 are proximal to the PW/AS critical region and are the locations of the proximal BPs of class I and II deletions of the region, respectively. BP3 is the location of the distal BPs of the common PW/AS deletions.³ There are multiple additional clusters of SDs distal to BP3, two of which, BP4 and BP5, are commonly involved in the formation of supernumerary chromosomes from the region and are involved in the 15q13.3 microdeletion syndrome.^{2,4–6} Few deletions in 15q13 with the proximal break in BP3 have been reported. Deletions of the material between BP3 and BP4 have been thought to have minimal phenotypic effect based on inheritance patterns and phenotypes of similar severity among individuals with deletions spanning BP3–BP5 and those limited to the BP4–BP5 interval.^{2,7–9} In this study, we report clinical

details of five individuals with deletions that encompass the BP3–BP4 interval and compare them with cases in the literature to clarify the role this microdeletion may have in the development of an abnormal phenotype.

SUBJECTS AND METHODS

Subjects and controls

From May 2004 to August 2009, we tested 34 046 probands' samples submitted to Signature Genomic Laboratories. The most common clinical presentations were mental retardation, developmental delay, or multiple congenital anomalies. For individuals with 15q13 microdeletions described here, consent was obtained to publish photographs.

BAC-based microarray analysis

Microarray-based comparative genomic hybridization (aCGH) was performed on 24 380 of the probands' samples with a bacterial artificial chromosome (BAC) microarray (the SignatureChip; Signature Genomic Laboratories, Spokane, WA, USA).¹⁰ There have been multiple versions of the SignatureChip, with increasing genomic coverage in successive versions, and all versions have had coverage in the BP3–BP4 region on 15q13.1q13.2. Microarray analysis was performed as described.^{10,11}

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Received 16 July 2010; revised 26 October 2010; accepted 19 November 2010; published online 19 January 2011

Oligonucleotide-based aCGH

The remaining 9666 probands were tested by oligonucleotide-based aCGH with either a 105K-feature (SignatureChip Oligo Solution, custom designed by Signature Genomic Laboratories, made by Agilent Technologies, Santa Clara, CA, USA) or a 135-K feature (SignatureChip Oligo Solution version 2.0, custom designed by Signature Genomic Laboratories, made by Roche Nimble-Gen, Madison, WI, USA) whole-genome array using previously described methods.^{12,13} Samples from eight of the nine individuals with deletions encompassing the BP3–BP4 interval detected on BAC array were reanalyzed with the 105K-feature microarray. Samples from subject 8, whose deletion was initially detected by BAC array, and from subject 7, who had no previous BAC array analysis, were analyzed with the 135-K feature microarray. Results were displayed using custom aCGH analysis software (Genoglyphix; Signature Genomic Laboratories).

Fluorescence *in situ* hybridization

All deletions encompassing the BP3–BP4 interval reported here were visualized by metaphase fluorescence *in situ* hybridization (FISH) using BAC clone RP11-408F10, as previously described.¹⁴ Parental samples, when available, were also assayed with metaphase FISH.

RESULTS

Molecular analysis

During the study period, we identified 10 individuals with deletions encompassing the interval between BP3 and BP4 on 15q13 that did not extend proximally into the PW/AS commonly deleted region. Five of the subjects had deletions flanked by BP3 proximally and BP4 distally, four subjects had deletions flanked by BP3 proximally and BP5 distally, and subject 4 had an atypical deletion with the proximal BP in the interval between BP3 and BP4 and the distal BP within BP4 (chr15:27,359,655–28,801,860, UCSC March 2006 hg18 build) (Figure 1). Inheritance of the deletions was determined through parental FISH in seven cases: two BP3–BP4 deletions were apparently *de novo*, and two were maternally inherited; two BP3–BP5 deletions were apparently *de novo*, and one was maternally inherited; in the remaining three cases parents were unavailable for testing.

Clinical analysis

Clinical features of five of the subjects in our cohort and those in the literature are summarized in Table 1.

Subject 1. Subject 1 presented with dysmorphic features and borderline intellectual function and was found to have a maternally inherited BP3–BP4 deletion. Birth weight was 3.03 kg (10th percentile), length was 45.7 cm (<3rd percentile), and occipitofrontal circumference (OFC) was 34.5 cm (50th percentile). He had growth deficiency as an infant, with height and weight below the third percentile at 7.5 months (64.3 cm and 6.21 kg), whereas OFC was normal (45.5 cm, 75th percentile). Examination at 7.5 months showed a large anterior fontanel (4×8 cm), dysmorphic features, pectus excavatum, and hypotonia. He developed seizures at 18 months, which continued until the age of 4 years. A head CT scan at 18 months was normal. Testing at the age of 6 years using the Wechsler Intelligence Scale for Children showed an overall IQ of 71. At 13 years and 10 months of age, his height was 157 cm, weight was 46.6 kg, and OFC was 56.5 cm. He had speech apraxia and difficulty with pronunciation. He was dysmorphic (Figure 2, Table 1), hyperopic, and had amblyopia and accommodative esotropia. The subject's mother, who also has the deletion, is healthy, of normal height, and has normal developmental and cognitive abilities. The family history is significant for a maternal half brother and two maternal first cousins with learning disabilities, and a paternal uncle with porencephaly and mental retardation. Further maternal family members were not available for testing.

Subject 2. Subject 2 presented with developmental delay and hypotonia and was found to have an apparently *de novo* BP3–BP4 deletion. She had mild delays in acquiring skills, although physical therapy was discontinued at 3 years of age. Speech was also mildly delayed, but she had an extensive vocabulary at 4 years of age. She was not toilet trained and had occasional temper tantrums, but showed no significant behavioral problems. Hypotonia noted at 2 years of age was resolved by 4 years. Ophthalmological examination showed asymmetrical optic nerve cupping, which was considered to be a normal variant. At 4 years and 5 months of age, her height was 100 cm, weight was 14.7 kg, and OFC was 47 cm. She was non-dysmorphic.

Subject 3. Subject 3 presented with developmental delay, congenital heart defect, hypotonia, ataxic gait, and dysmorphic features and was found to have a maternally inherited BP3–BP4 deletion. Birth weight was 1.03 kg (75–90th percentile). He had a complex congenital heart defect, with obstruction of the left pulmonary veins, anomalous drainage of the right pulmonary veins into a scimitar vein leading to the inferior vena cava, and atrial and ventricular septal defects. He had bilateral cryptorchidia and inguinal hernias that required surgical correction. He had severe gastroesophageal reflux and required G-tube placement at 1 year of age, which he continued to use up to 6 years. He had hypogammaglobulinemia and frequent viral infections. Brain MRI at 21 months of age showed moderate dilatation of the lateral and third ventricles and prominent extra-axial CSF spaces. At 11.5 months, he had some head control, was able to roll over at 21 months, crawled by 3 years, and walked independently by 4 years. At 6 years of age, he had an ataxic gait, was not yet toilet trained, spoke simple sentences with a limited vocabulary, and had significant articulation problems. He had a disrupted sleep pattern, requiring melatonin for induction of sleep. He failed to thrive in early childhood, and at 2.5 years of age his weight was at the 3rd percentile and height at the 25th percentile. However, by 6 years of age his weight and height were at the 50th percentile. His physical examination showed a large head (OFC of 54 cm, 2 SD above the mean) and mild dysmorphic features (Figure 2, Table 1). He had significant hypotonia with hyperextensible joints. The subject's mother, who also has the deletion, is healthy and non-dysmorphic, and has normal developmental and cognitive abilities.

Subject 4. Subject 4 presented with developmental delays, hypotonia, seizures, and growth delay and was found to have an atypical 15q13.1q13.2 deletion that involves part of the BP3–BP4 region. Birth weight was 3.26 kg (25–50th percentile) and length was 50.8 cm (50–75th percentile). Neonatally, she had rotary nystagmus and severe hypotonia. Subsequently, she had slow growth and feeding difficulties. Seizures have been lifelong, starting within the first months of life. Head MRI and EEG were normal. Strabismus was treated with surgery and glasses. She held her head erect at 16 months, first walked with the assistance of a walker at 3.5 years, and only said 'mama' and 'dada' at 6 years and 9 months. She had food aversions and a history of constipation. At the age of 6 years and 9 months, her height was 96 cm, weight was 15.9 kg, and OFC was 48.5 cm. She had dysmorphic features (Figure 2, Table 1), severe hypotonia, and muscle weakness. She is adopted; the biological mother had vision problems and strabismus, and no information was available about the biological father.

Subject 5. Subject 5 presented with developmental delays and microcephaly and was found to have an apparently *de novo* BP3–BP5 deletion. Birth weight was 2.98 kg (10–25th percentile). Neonatal complications included meconium staining of the amniotic fluid,

Figure 1 Summary of microarray analysis of individuals with deletions of 15q13. At the top of the figure is a partial ideogram showing chromosome bands 15q13.1q14, with genomic coordinates corresponding to the hg18 build of the human genome. Plots show the deletions in 10 individuals with microdeletions at 15q13. Eight out of the 10 deletions were defined using a high-density 105-K oligonucleotide microarray (subjects 1–6, 9, 10), and two deletions were defined using a high-density 135-K oligonucleotide microarray (subjects 7 and 8). Vertical dashed lines indicate the approximate deletion sizes in the individuals. Red boxes represent segmental duplication blocks BP3, BP4, and BP5 that flank the BPs. Black, blue, and green arrows represent small- and medium-sized homologous duplicons contained within the segmental duplication blocks. The complex segmental duplication structure of this region has been simplified for illustrative purposes, and the orientations shown are according to the human reference genome. Blue boxes represent all known genes annotated in the Reference Sequence database in the deletion regions.

natal teeth, a heart murmur, and hypoglycemia. A newborn screen showed high serum immunoreactive trypsinogen (IRT) levels. The subject's maternal grandmother had cystic fibrosis (CF); preconceptional gene testing in the subject's mother revealed that she was a CF carrier ($\Delta F508$), whereas her father was negative for a panel of 70 mutations. Therefore, the subject had sweat testing, which was normal, and did not have *CFTR* mutational analysis. No CF symptoms were present, and she was considered to be a CF carrier. She rolled at 10 months, crawled at 14 months, cruised at 16 months, and spoke some sentences by 3 years. She showed a poor attention span, flapped her hands when excited, and had difficulty following direc-

tions; developmental preschool and therapies helped with these behaviors. She had poor growth initially and was at or below the third percentile from 6 to 26 months. Microcephaly has been persistent, and a head MRI at 2 years showed periventricular leukomalacia with volume loss within the parietooccipital regions, posterior thinning of the corpus callosum, and prominent extra-axial fluid in the posterior fossa, likely a mega cisterna magna. At the age of 4 years and 2 months, her height was 97 cm, weight was 15.5 kg, and OFC was 45.7 cm. She had dysmorphic features (Table 1) and developed bilateral breast buds by 16 months of age. Her parents have normal head sizes.

Table 1 Clinical features of individuals in the literature and this report with deletions involving BP3-BP4 at 15q13.1q13.2

Subject ID; age; gender	Deletion; inheri- tance	Growth percentiles	Development	Neurological	Eyes	Facial features	Other	Family history
van Bon, pt 19 (reference 7); 2 years 4 months; F	BP3-BP4; paternal	BW: <3rd; Wt: 9th; Ht: <3rd; OFC: <3rd	Delayed	Hypotonia; hearing impairment	NS	Hypertelorism; upslanting palpebral fissures; and prominent nasal root	Brachydactyly	Deletion in normal father, brother, and uncle; deletion not in brother with MR
Rosenberg, pt 11 (reference 8); F	BP3-BP4; maternal	Short stature; and microce- phaly	Mild MR	NS	NS	Minor dysmorphisms	Premature breast development	NS
Sharp 543/06 (refer- ence 2); 11 years 8 months; F	BP3-BP4; paternal	Wt: 97th; Ht: 25th; OFC: 50–75th	Delayed	NS	Coloboma	Flattened midface; anteverted nostrils; and prognathism; synophrys	Urteral ectasia	Father is normal
Subject 1; 13 years 10 months; M	BP3-BP4; maternal	FTT in early childhood; Wt: 25–50th; Ht: 25th; OFC: 90th	IQ 71, speech apraxia	Normal head CT scan; seizures; hypotonia; and mild-moderate conductive hearing loss	Hypertopia; amblyopia/esotropia	Frontal bossing; hypertelorism; broad nasal tip; upturned nares; flat philtrum; large ears; dolichocephaly; bifrontal nar- rowing; downslanting palpebral fissures; overcrowded teeth; high palate; and low ears	Large anterior fontanel, pectus excavatum; broad fingers and hands	Mother is normal; maternal half brother and 2 maternal first cousins (not tested) with LD; paternal uncle with MR
Subject 2; 4 years 5 months; F	BP3-BP4; <i>de novo</i>	Wt: 10–25th; Ht: 25th; OFC: 3rd	Mild delays	Hypotonia; occasional tantrums	Asymmetric optic nerve cupping	Nondysmorphic	NS	NS
Subject 3; 6 years 5 months; M	BP3-BP4; maternal	FTT in early childhood; Wt: 50th; Ht: 50th; OFC: 98th	Moderate delays, significant speech delays	Ventricle dilatation; prominent extra-axial CSF spaces; hypotonia; disrupted sleep; and ataxic gait	Nystagmus, resolved	Prominent forehead; bifrontal prominence; long face; and triangular chin	Complex CHD; cryptorchidism; inguinal hernias; finger camptodactyly, and clinodactyly; hyperextensible; severe GERD; frequent infections	Mother is normal
Subject 4; 8 years 9 months; F	Atypical, within BP3-BP4; unknown	Wt: 10–25th; Ht: <3rd; OFC: 2nd	Severely delayed	Normal MRI; seizures; severe hypotonia and weakness; and food aversion	Rotary nystagmus/stra- bismus	Short forehead; deep-set eyes; high palate; and crowded teeth	Fifth finger clinodactyly	Mother with visual problems and strabismus
Subject 5; 4 years 2 months; F	BP3-BP5; <i>de novo</i>	FTT in early childhood; Wt: 25–50th; Ht: 10th; OFC: <5th	Delayed	Hypotonia; periventricular leukomalacia; thinning of the corpus callosum; mega cisterna magna; and unusual hand movements	Normal	Natal teeth; small facial structure; prominent ears; and deep-set eyes	Premature breast development	Father with LD
van Bon, pt 17 (reference 7); 17 years; M	BP3-BP5; <i>de novo</i>	Wt: 90–95th; Ht: 3–10th; OFC: 3rd	Mild MR, dyspraxia	Hypotonia; profoundly hyperactive; and normal head imaging	NS	Upslanting palpebral fissures; synophrys; short nose with upturned nasal tip; smooth philtrum; and hypotelorism	Pubertas praecox; gynecomastia; shawl scrotum; scoliosis; fifth finger clinodactyly; brachydactyly	Half uncles with LD

Table 1 (Continued)

Subject ID; age; gender	Deletion; inheritance	Growth percentiles	Development	Neurological	Eyes	Facial features	Other	Family history
van Bon, pt 18 (reference 7); 8 years; M	BP3-BP5; unknown	Wt: 9th; Ht: 25th; OFC: 25th	Delayed	Hypotonia; friendly; and poor concentration	NS	Long, downslanting palpebral fissures; long eyelashes; arched eyebrows; large ears; and large mouth	Prominent finger pads and broad fingertips; short fifth fingers	Mother, brother, and half sibs with LD/DD
Sharp IMR338 (reference 2); F	BP3-BP5; maternal	Ht: 97th; OFC: > 97th	Delayed; speech difficulty	Seizures; white matter changes; and increased appetite	Squint; right optic pit – possible coloboma	Round flat face; upslanting palpebral fissures; epicanthal folds; and posteriorly rotated ears	Lax thumb joint; fifth finger clinodactyly; single-palmar crease; diabetes	Deletion present in mother and half sister, who are dysmorphic, have delays, and are epileptic
Helbig 40281601 (reference 20); F	BP3-BP5; unknown	NS	Normal cognition	Seizures	NS	NS	NS	NS
Ben-Shachar, pt F1-1 (reference 18)	BP3-BP5; unknown	Ht: 50th; Wt: 30th; OFC > 97th	DQ 58	Benign hydrocephalus; mild Chiari I; ASD; and stereotypic behaviors	NS	NS	NS	Adopted

Abbreviations: ASD, autism spectrum disorder; BW, birth weight; CHD, congenital heart defect; DD, developmental delay; DQ, developmental quotient; F, female; FTT, failure to thrive; GERD, gastroesophageal reflux disease; Ht, height; IQ, intelligence quotient; LD, learning disabilities; M, male; MR, mental retardation; NS, not specified; OFC, occipitofrontal circumference; pt, patient; Wt, weight.

DISCUSSION

We report clinical details of five individuals with deletions encompassing the BP3–BP4 interval on 15q13. These deletions can be *de novo* or inherited from clinically normal parents, and a comparison of these cases with each other and with those in the literature shows some phenotypic similarities. On the basis of these phenotypic similarities and gene content of the region, we propose that deletions of the BP3–BP4 interval can contribute to an abnormal phenotype. However, there is still phenotypic variability among subjects, and a consistent and recognizable phenotype is not present. This finding is similar to other recently described CNVs that have been associated with a wide spectrum of phenotypes ranging from severely affected to asymptomatic individuals, such as microdeletions of proximal 1q21.1 and TAR syndrome,¹⁵ microdeletions and microduplications of distal 1q21.1,^{16,17} microdeletions of 15q13.3,^{18–21} and microdeletions and microduplications of 16p11.2.^{22–24} One proposed explanation for the variable phenotypes is that the microdeletion or microduplication is primarily responsible for the disease, but other modifiers, genetic and environmental, will ultimately influence the phenotypic presentation.²⁵ An alternative hypothesis is that these loci are the modifiers for other yet-to-be-identified mutations in the genome. Variability in these other factors among individuals with the same microdeletion may explain their varying phenotypes. With this new understanding, closer scrutiny and broader interpretations are called for when examining novel genomic rearrangements that may be inherited from an apparently normal parent.

Previous reports have suggested that the deletions flanked by BP3 and BP4 are benign because they have been identified in healthy relatives of probands but not in all affected relatives (Table 1).^{2,7,9} Van Bon *et al*⁷ described individuals with deletions flanked by BP3 and BP5 to be similarly affected as those with deletions flanked by BP4 and BP5, and suggested that deletion of the BP3–BP4 interval did not contribute to the phenotype. However, a comparison of our subjects with those in the literature with deletions within the BP3–BP5 interval shows some common phenotypic features that may be attributable to the deletion of genes in the BP3–BP4 interval (Table 2). Some features are only present among individuals with deletions of the BP3–BP4 interval, such as hearing loss (2/7 among BP3–BP4 deletions *vs* 0/38 among BP4–BP5 deletions). Other features, although not exclusively found in individuals with deletions including the BP3–BP4 interval, are more prevalent among these individuals, including failure to thrive (4/6 *vs* 4/38), short stature (4/7 *vs* 4/42), microcephaly (4/7 *vs* 4/44), hypotonia (5/6 *vs* 13/27), renal abnormalities (1/7 *vs* 1/38), and premature puberty or abnormally early breast development (1/7 *vs* 1/38). Among individuals with BP3–BP4 or BP3–BP5 deletions, premature or abnormal breast development was seen in two females and in a male as precocious puberty and gynecomastia (Table 1), whereas a single individual with a deletion limited to BP4–BP5 has been reported with early pubarche.² Although a majority (5/6) of the individuals with deletions limited to BP3–BP4 have eye abnormalities, both structural and movement disorders, with five probands specifically reporting issues ranging from coloboma to nystagmus and strabismus, a similar variety of abnormalities has also been reported in individuals with deletions limited to BP4–BP5.^{2,7,18,21} Conversely, some features are more common among individuals with BP4–BP5 deletions, including behavioral concerns (36/50 among BP4–BP5 deletions *vs* 0/6 among BP3–BP4 deletions), macrocephaly (9/44 *vs* 1/7), and spinal abnormalities (1/38 *vs* 0/7), suggesting that genes in this region are contributing to the findings. Overall, the finding of phenotypic similarities among individuals with deletions incorporating the BP3–BP4 interval that are

Figure 2 (a) Subject 1 at 10 years. Note frontal bossing, bifrontal narrowing, hypertelorism, broad nasal tip with upturned nares, large and low-set ears, and downslanting palpebral fissures. (b and c) Subject 3 at 6 years. Note macrocephaly, prominent forehead, bifrontal prominence, long and oval face, and triangular chin. (d and e) Subject 4 at 8 years. Note microcephaly, short forehead, and deep-set eyes. (f) Subject 5 at 26 months. Note microcephaly, small facial structure, prominent ears, and deep-set eyes.

distinct from the BP4–BP5 deletion phenotype suggests that deletions of the BP3–BP4 interval may contribute to an abnormal phenotype in some individuals.

Haploinsufficiency of the four genes that lie in the ~1.1-Mb interval between BP3 and BP4 on 15q13.1 may explain the abnormal phenotypes seen in individuals with BP3–BP4 deletions. Although subject 4 is not haploinsufficient for all of these genes, other unidentified factors may be affecting her phenotype, especially given its relative severity in comparison with other subjects; therefore, all genes within the recurrently deleted interval should be critically assessed. Amyloid beta A4 precursor protein-binding family A, member 2 (APBA2, OMIM 602712; also known as *MINT2* or *X11L*) plays a role in nervous system development and is expressed only in neuronal tissue, encoding a neuronal adapter protein shown to interact with synaptic vesicle proteins and to regulate neurite outgrowth.^{26,27} Homozygous knockout mice have monoamine imbalances in the forebrain and impaired conflict resolution.²⁸ Apba2 also interacts with amyloid precursor protein and suppresses its amyloidogenic cleavage.²⁹ Tight junction protein 1 (*TJPI*, OMIM 601009) encodes zonula occludens-1, a scaffolding protein involved in regulation of cell growth in a cell density-dependent manner and in stabilizing tight junctions and connecting them to the cytoskeleton.³⁰ *Tjp1* has ubiquitous expression during development, with some later specialization in the CNS, eye, and heart.³¹ Knockout mice are embryonic lethal, although the heterozygous knockouts are reportedly normal.³² Deletion of *APBA2* or *TJPI* could affect development, particularly of the nervous system. Necdin-like gene 2 (*NDNL2*, OMIM 608243) is ubiquitously expressed in high levels in testes³³ and encodes part of the SMC5-6 protein complex that is responsible for resolution of DNA recombination.³⁴ Little is known about the fourth gene in the region, *FAM189A1*, although it likely encodes a transmembrane protein expressed in brain, eye, kidney, testes, and uterus (UniGene, <http://www.ncbi.nlm.nih.gov/uniGene>).

In addition to deletion of the genes between BP3 and BP4, genes within BPs may also be disrupted or deleted in these individuals.

Table 2 Summary of features in reported cases of deletions anywhere within BP3–BP5

	BP3–BP4 ^a	BP3–BP5 ^b	BP4–BP5 ^c
Low birth weight	1/5	0/3	3/16
Failure to thrive	4/6	1/5	4/38
Short stature	4/7	0/5	4/42
Microcephaly	4/7	2/5	4/44
Macrocephaly	1/7	2/5	9/44
Developmental delay and/or mental retardation	7/7	5/6	48/50
Hypotonia	5/6	3/4	13/27
Seizures or abnormal EEG	2/7	2/6	25/50 ^d
Behavioral concerns	0/6	5/5	36/50
Hearing loss	2/7	0/5	0/38
Abnormal brain imaging	1/3	3/4	8/28
Spinal abnormalities	0/7	1/5	1/38
Ocular features	5/6	1/5	11/37
Dysmorphic features	6/7	4/4	23/30
Heart defects	1/7	1/5	4/39
Renal abnormalities	1/7	0/5	1/38
Premature puberty or breast development	1/7	2/5	1/38

^aSubjects 1–4 in this report, as well as probands in the literature.^{2,7,8}

^bSubject 5 in this report, as well as probands in the literature.^{2,7,18,20}

^cPreviously reported probands.^{2,7,18–21,47,48}

^dIncludes 11 individuals from a cohort of individuals with seizures.²⁰

Within BP4 is *CHRFAM7A*, which is a fusion of exons E–A of *FAM7A* (several copies of which are also in BP4) to exons 5–10 and the 3' untranslated region of *CHRNA7*, which lies at the distal end of the BP4–BP5 region and extends into BP5.³⁵ Although the function of *FAM7A* is unknown, *CHRNA7* encodes the $\alpha 7$ subunit of the nicotinic cholinergic receptor that is hypothesized to contribute to the seizures and developmental delay seen in some individuals with 15q13.3 microdeletions extending to BP5.^{2,20,36} *CHRFAM7A* may be deleted in individuals with BP3–BP4 deletions, is likely to be deleted in subject

4, and is deleted in individuals with BP3–BP5 deletions. Studies have suggested an association between a 2-bp deletion polymorphism of *CHRFAM7A*, which disrupts the reading frame of the gene, and schizophrenia and dementia.^{37,38} However, it is unclear whether this gene's transcript is translated, and homozygous deletions of *CHRFAM7A* have been found in normal individuals.³⁹ Therefore, a deletion of this gene may not always cause an abnormal phenotype; however, as suggested by the association between psychosis and lower *CHRFAM7A* copy number, a deletion of the gene could predispose to neurocognitive disorders.³⁹

A comparison of the frequency of a deletion or duplication among cases with the frequency in controls is one method used to determine whether a genetic alteration predisposes individuals to an abnormal phenotype. No BP3–BP4 deletions were found in three control groups consisting of 2493 individuals,⁴⁰ 2026 individuals,⁴¹ and 3651 individuals (Evan Eichler, unpublished data), whereas another study with a control group of 2792 individuals did find one BP3–BP4 deletion.⁴² Combining these controls gives a similar frequency of deletions in cases referred for aCGH testing, and in controls (6/34046 cases that do not encompass the BP4–BP5 region vs 1/10962 controls, two-tailed $P=0.86$, χ^2 with Yates' correction). The Database of Genomic Variants has a single entry with a deletion of the region, found in a cohort of 506 healthy northern German individuals and 270 HapMap individuals.⁴³ Given that the frequency of these cases is rare ($\sim 1/5700$) in our population of individuals undergoing aCGH testing, a larger control set is needed for a more statistically powerful comparison.

Compared to other rearrangements of proximal 15q, BP3–BP4 deletions are relatively rare. In contrast to the 9/34046 (0.03%) individuals with deletions extending distally from BP3, our laboratory has detected 54 individuals with BP4–BP5 deletions out of 18517 individuals tested (0.29%), a 10-fold higher frequency. This difference is likely in part due to the genomic architecture of the region. Proximal 15q is polymorphic, with inversions and deletions found within the BPs, and inversions of segments between BPs,^{1,2,35,44,45} such as the inversion that is enriched in mothers of children with Angelman syndrome.⁴⁶ Some configurations of the region will be more likely to predispose to deletion following NAHR, when homologous SDs are in direct orientation with each other.⁴⁵ It has been estimated that out of $\sim 45\%$ of chromosomes 15 have within BP4 relative to the reference human genome that puts an ~ 300 kb SD in direct orientation with a homologous SD in BP5, NAHR between which would lead to a BP4–BP5 deletion.^{44,45} In contrast, BP3 has much smaller segments of high homology to BP4 and BP5 (around 30 kb), and many segments on BP3 have less homology to the corresponding segments in BP4 or BP5 than the homology between those segments in BP4 and BP5.^{1,2} There is, on average, $\sim 94\%$ sequence identity between BP3 and BP4/BP5, compared with $\sim 97\%$ sequence identity between BP4 and BP5.² BP3 has much larger segments of homology with BP1 and BP2, which explains why almost all chromosomal deletions causing PW/AS are flanked by BP3.¹ Larger control cohorts are necessary to determine whether the relative infrequency of BP3–BP4 deletions in our study population may be attributed to genomic architecture or to the absence of a clinically recognizable phenotype.

Individuals with deletions of the BP3–BP4 interval have common clinical features, including short stature or failure to thrive, microcephaly, hypotonia, and premature breast development in some individuals. BP3–BP4 deletions may be present in unaffected relatives, and two deletions have been reported in control cohorts, suggesting that additional factors have a role in the development of an abnormal

phenotype. Although current comparison with control populations does not demonstrate statistically significant enrichment of the BP3–BP4 deletion in abnormal individuals, the phenotypic similarities and gene content suggest that this deletion contributes to an abnormal phenotype in some individuals. Larger control cohorts and additional reports of individuals with BP3–BP4 deletions will further help to clarify the role that these deletions have in the development of abnormal phenotypes.

CONFLICT OF INTEREST

Jill A Rosenfeld, Justine Coppinger, Blake C. Ballif, Roger Schultz, Beth Torchia, Trilochan Sahoo, Bassem Bejjani, and Lisa G Shaffer are employees of Signature Genomic Laboratories, a subsidiary of Perkin-Elmer. The remaining authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Aaron Theisen (Signature Genomic Laboratories) for his critical reading of the manuscript.

- 1 Makoff AJ, Flomen RH: Detailed analysis of 15q11-q14 sequence corrects errors and gaps in the public access sequence to fully reveal large segmental duplications at breakpoints for Prader-Willi, Angelman, and inv dup(15) syndromes. *Genome Biol* 2007; **8**: R114.
- 2 Sharp AJ, Mefford HC, Li K *et al*: A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. *Nat Genet* 2008; **40**: 322–328.
- 3 Christian SL, Robinson WP, Huang B *et al*: Molecular characterization of two proximal deletion breakpoint regions in both Prader-Willi and Angelman syndrome patients. *Am J Hum Genet* 1995; **57**: 40–48.
- 4 Wandstrat AE, Leana-Cox J, Jenkins L, Schwartz S: Molecular cytogenetic evidence for a common breakpoint in the largest inverted duplications of chromosome 15. *Am J Hum Genet* 1998; **62**: 925–936.
- 5 Wang NJ, Liu D, Parokony AS, Schanen NC: High-resolution molecular characterization of 15q11-q13 rearrangements by array comparative genomic hybridization (array CGH) with detection of gene dosage. *Am J Hum Genet* 2004; **75**: 267–281.
- 6 Sahoo T, Shaw CA, Young AS *et al*: Array-based comparative genomic hybridization analysis of recurrent chromosome 15q rearrangements. *Am J Med Genet A* 2005; **139A**: 106–113.
- 7 van Bon BW, Mefford HC, Menten B *et al*: Further delineation of the 15q13 microdeletion and duplication syndromes: a clinical spectrum varying from non-pathogenic to a severe outcome. *J Med Genet* 2009; **46**: 511–523.
- 8 Rosenberg C, Knijnenburg J, Bakker E *et al*: Array-CGH detection of micro rearrangements in mentally retarded individuals: clinical significance of imbalances present both in affected children and normal parents. *J Med Genet* 2006; **43**: 180–186.
- 9 Menten B, Maas N, Thienpont B *et al*: Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports. *J Med Genet* 2006; **43**: 625–633.
- 10 Bejjani BA, Saleki R, Ballif BC *et al*: Use of targeted array-based CGH for the clinical diagnosis of chromosomal imbalance: is less more? *Am J Med Genet A* 2005; **134**: 259–267.
- 11 Ballif BC, Theisen A, Coppinger J *et al*: Expanding the clinical phenotype of the 3q29 microdeletion syndrome and characterization of the reciprocal microduplication. *Mol Cytogenet* 2008; **1**: 8.
- 12 Duker AL, Ballif BC, Bawle EV *et al*: Paternally inherited microdeletion at 15q11.2 confirms a significant role for the SNORD116 C/D box snoRNA cluster in Prader-Willi syndrome. *Eur J Hum Genet* 2010; **18**: 1196–1201.
- 13 Ballif BC, Theisen A, McDonald-McGinn DM *et al*: Identification of a previously unrecognized microdeletion syndrome of 16q11.2q12.2. *Clin Genet* 2008; **74**: 469–475.
- 14 Traylor RN, Fan Z, Hudson B *et al*: Microdeletion of 6q16.1 encompassing EPHA7 in a child with mild neurological abnormalities and dysmorphic features: case report. *Mol Cytogenet* 2009; **2**: 17.
- 15 Klopocki E, Schulze H, Strauss G *et al*: Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome. *Am J Hum Genet* 2007; **80**: 232–240.
- 16 Mefford HC, Sharp AJ, Baker C *et al*: Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. *N Engl J Med* 2008; **359**: 1685–1699.
- 17 Brunetti-Pierri N, Berg JS, Scaglia F *et al*: Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities. *Nat Genet* 2008; **40**: 1466–1471.
- 18 Ben-Shachar S, Lanpher B, German JR *et al*: Microdeletion 15q13.3: a locus with incomplete penetrance for autism, mental retardation, and psychiatric disorders. *J Med Genet* 2009; **46**: 382–388.

- 19 Pagnamenta AT, Wing K, Sadighi Akha E *et al*: A 15q13.3 microdeletion segregating with autism. *Eur J Hum Genet* 2009; **17**: 687–692.
- 20 Helbig I, Mefford HC, Sharp AJ *et al*: 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. *Nat Genet* 2009; **41**: 160–162.
- 21 Masurel-Paulet A, Andrieux J, Callier P *et al*: Delineation of 15q13.3 microdeletions. *Clin Genet* 2010; **78**: 149–161.
- 22 Bijlsma EK, Gijbbers AC, Schuurs-Hoeijmakers JH *et al*: Extending the phenotype of recurrent rearrangements of 16p11.2: deletions in mentally retarded patients without autism and in normal individuals. *Eur J Med Genet* 2009; **52**: 77–87.
- 23 McCarthy SE, Makarov V, Kirov G *et al*: Microduplications of 16p11.2 are associated with schizophrenia. *Nat Genet* 2009; **41**: 1223–1227.
- 24 Rosenfeld JA, Coppinger J, Bejjani BA *et al*: Speech delays and behavioral problems are the predominant features in individuals with developmental delays and 16p11.2 microdeletions and microduplications. *J Neurodevel Disord* 2010; **2**: 26–38.
- 25 Mefford HC, Eichler EE: Duplication hotspots, rare genomic disorders, and common disease. *Curr Opin Genet Dev* 2009; **19**: 196–204.
- 26 Okamoto M, Sudhof TC: Mints, Munc18-interacting proteins in synaptic vesicle exocytosis. *J Biol Chem* 1997; **272**: 31459–31464.
- 27 Zhang Y, Wang YG, Zhang Q *et al*: Interaction of Mint2 with TrkA is involved in regulation of nerve growth factor-induced neurite outgrowth. *J Biol Chem* 2009; **284**: 12469–12479.
- 28 Sano Y, Ornathanalai VG, Yamada K *et al*: X11-like protein deficiency is associated with impaired conflict resolution in mice. *J Neurosci* 2009; **29**: 5884–5896.
- 29 Saito Y, Sano Y, Vassar R *et al*: X11 proteins regulate the translocation of amyloid beta-protein precursor (APP) into detergent-resistant membrane and suppress the amyloidogenic cleavage of APP by beta-site-cleaving enzyme in brain. *J Biol Chem* 2008; **283**: 35763–35771.
- 30 Ebnet K: Organization of multiprotein complexes at cell-cell junctions. *Histochem Cell Biol* 2008; **130**: 1–20.
- 31 Kiener TK, Sleptsova-Friedrich I, Hunziker W: Identification, tissue distribution and developmental expression of tjp1/zo-1, tjp2/zo-2 and tjp3/zo-3 in the zebrafish, *Danio rerio*. *Gene Expr Patterns* 2007; **7**: 767–776.
- 32 Katsuno T, Umeda K, Matsui T *et al*: Deficiency of zonula occludens-1 causes embryonic lethal phenotype associated with defected yolk sac angiogenesis and apoptosis of embryonic cells. *Mol Biol Cell* 2008; **19**: 2465–2475.
- 33 Chibuk TK, Bischof JM, Wevrick R: A necdin/MAGE-like gene in the chromosome 15 autism susceptibility region: expression, imprinting, and mapping of the human and mouse orthologues. *BMC Genet* 2001; **2**: 22.
- 34 Taylor EM, Copsey AC, Hudson JJ, Vidot S, Lehmann AR: Identification of the proteins, including MAGEG1, that make up the human SMC5-6 protein complex. *Mol Cell Biol* 2008; **28**: 1197–1206.
- 35 Riley B, Williamson M, Collier D, Wilkie H, Makoff A: A 3-Mb map of a large Segmental duplication overlapping the alpha7-nicotinic acetylcholine receptor gene (CHRNA7) at human 15q13-q14. *Genomics* 2002; **79**: 197–209.
- 36 Shinawi M, Schaaf CP, Bhatt SS *et al*: A small recurrent deletion within 15q13.3 is associated with a range of neurodevelopmental phenotypes. *Nat Genet* 2009; **41**: 1269–1271.
- 37 Feher A, Juhasz A, Rimanoczy A, Csibri E, Kalman J, Janka Z: Association between a genetic variant of the alpha-7 nicotinic acetylcholine receptor subunit and four types of dementia. *Dement Geriatr Cogn Disord* 2009; **28**: 56–62.
- 38 Sinkus ML, Lee MJ, Gault J *et al*: A 2-base pair deletion polymorphism in the partial duplication of the alpha7 nicotinic acetylcholine gene (CHRFAM7A) on chromosome 15q14 is associated with schizophrenia. *Brain Res* 2009; **1291**: 1–11.
- 39 Flomen RH, Collier DA, Osborne S *et al*: Association study of CHRFAM7A copy number and 2bp deletion polymorphisms with schizophrenia and bipolar affective disorder. *Am J Med Genet B Neuropsychiatr Genet* 2006; **141B**: 571–575.
- 40 Itsara A, Cooper GM, Baker C *et al*: Population analysis of large copy number variants and hotspots of human genetic disease. *Am J Hum Genet* 2009; **84**: 148–161.
- 41 Shaikh TH, Gai X, Perin JC *et al*: High-resolution mapping and analysis of copy number variations in the human genome: a data resource for clinical and research applications. *Genome Res* 2009; **19**: 1682–1690.
- 42 Kirov G, Grozeva D, Norton N *et al*: Support for the involvement of large copy number variants in the pathogenesis of schizophrenia. *Hum Mol Genet* 2009; **18**: 1497–1503.
- 43 Pinto D, Marshall C, Feuk L, Scherer SW: Copy-number variation in control population cohorts. *Hum Mol Genet* 2007; **16**: Spec No. 2: R168–R173.
- 44 Flomen RH, Davies AF, Di Forti M *et al*: The copy number variant involving part of the alpha7 nicotinic receptor gene contains a polymorphic inversion. *Eur J Hum Genet* 2008; **16**: 1364–1371.
- 45 Makoff A, Flomen R: Common inversion polymorphisms and rare microdeletions at 15q13.3. *Eur J Hum Genet* 2009; **17**: 149–150.
- 46 Gimelli G, Pujana MA, Patricelli MG *et al*: Genomic inversions of human chromosome 15q11-q13 in mothers of Angelman syndrome patients with class II (BP2/3) deletions. *Hum Mol Genet* 2003; **12**: 849–858.
- 47 Miller DT, Shen Y, Weiss LA *et al*: Microdeletion/duplication at 15q13.2q13.3 among individuals with features of autism and other neuropsychiatric disorders. *J Med Genet* 2009; **46**: 242–248.
- 48 Baroy T, Misceo D, Braaten O *et al*: A *de novo* 15q13.2q13.3 deletion in a boy with an Angelman syndrome like phenotype. *Eur J Med Genet* 2010; **53**: 221–224.