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Recurrent 200-kb deletions of 16p11.2 that include the *SH2B1* gene are associated with developmental delay and obesity

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Purpose: The short arm of chromosome 16 is rich in segmental duplications, predisposing this region of the genome to a number of recurrent rearrangements. Genomic imbalances of an approximately 600-kb region in 16p11.2 (29.5–30.1 Mb) have been associated with autism, intellectual disability, congenital anomalies, and schizophrenia. However, a separate, distal 200-kb region in 16p11.2 (28.7–28.9 Mb) that includes the *SH2B1* gene has been recently associated with isolated obesity. The purpose of this study was to better define the phenotype of this recurrent *SH2B1*-containing microdeletion in a cohort of phenotypically abnormal patients not selected for obesity. **Methods:** Array comparative hybridization was performed on a total of 23,084 patients in a clinical setting for a variety of indications, most commonly developmental delay. **Results:** Deletions of the *SH2B1*-containing region were identified in 31 patients. The deletion is enriched in the patient population when compared with controls ($P = 0.003$), with both inherited and de novo events. Detailed clinical information was available for six patients, who all had developmental delays of varying severity. Body mass index was ≥95th percentile in four of six patients, supporting the previously described association with obesity. The reciprocal duplication, found in 17 patients, does not seem to be significantly enriched in our patient population compared with controls. **Conclusions:** Deletions of the 16p11.2 *SH2B1*-containing region are pathogenic and are associated with developmental delay in addition to obesity. ***Genet Med*** **2010:12(10):641–647.**

Key Words: *16p11.2, microdeletion, SH2B1, developmental delay, obesity*

The ability to identify copy number changes using array comparative genomic hybridization (CGH) or single nucleotide polymorphism (SNP) genotyping arrays has facilitated the identification of several new microdeletion or microduplication syndromes in recent years, and these techniques are now the first-line tests for the detection of genomic imbalances. Examples of recently identified syndromes include 17q21.31, 15q24, and 17q23.1q23.2 microdeletion syndromes, in which patients present with recognizable patterns of shared clinical features including dysmorphic facies, developmental delays, and other features.[1–5] In addition, several recurrent genomic imbalances that are associated with incomplete penetrance and highly variable expressivity have been identified.[6] The 1q21.1 microdeletion, for instance, is enriched in patients with developmental delay or intellectual disability compared with phenotypically normal controls, but dysmorphic features and congenital anomalies are so variable that the deletion cannot be recognized based on clinical presentation.[7,8] Similarly, the 15q13.3 microdeletion can be associated with intellectual disability, epilepsy, or schizophrenia,[9–12] and this deletion is often present in unaffected parents.

Certain genomic regions are particularly prone to rearrangements leading to copy number changes due to the presence of flanking segmental duplications. The proximal short arm of chromosome 16 is one such region, and several distinct, recurring imbalances of 16p have been associated with abnormal phenotypes (Fig. 1). The largest of these is deletion of 16p11.2–p12.2, which is associated with common clinical features despite variability in the size of the deletion.[13–15] This deletion ranges from 7 to 9 Mb in length and has a common distal breakpoint (located ~21.4 Mb from the telomere), but inconsistent proximal breakpoints. Developmental delays (particularly speech delay), feeding difficulties, recurrent ear infections, and similar dysmorphic facial features are frequent findings in patients with this deletion. Duplications and deletions of an approximately 600-kb region in 16p11.2 (~29.5–30.1 Mb from the telomere), which partially overlaps the centromeric end of the 16p11.2p12.2 deletion, have been associated with variable

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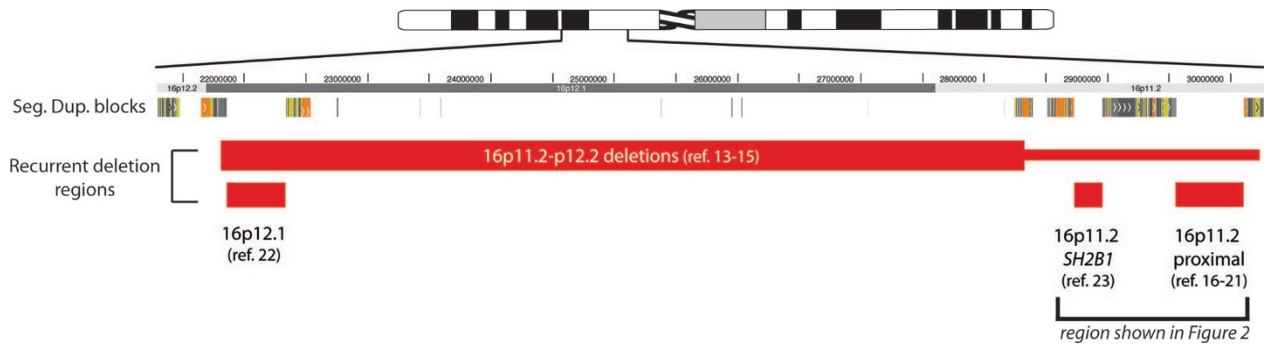

Fig. 1. Genomic context for the 16p11.2 region. An ideogram of chromosome 16 is shown at the top with the 16p11.2–p12.2 region (chr16:21,800,000–30,200,000) shown below in greater detail. Segmental duplications are represented by orange (>99% sequence similarity), yellow (98–99% similarity), and gray (90–98% similarity) boxes. Red boxes represent the recurrent deletions that are discussed in the text. Large 16p11.2–p12.2 deletions have the same distal breakpoint but variable proximal breakpoints, represented by the thinner red bar. Note that each of the recurrent deletion regions is flanked by segmental duplication blocks, which are thought to facilitate nonallelic homologous recombination.

phenotypes including autism, intellectual disabilities, behavioral disorders, congenital anomalies, and schizophrenia.[16–21] A 520-kb deletion in 16p12.1 (~21.9–22.4 Mb from the telomere) has been found to be a risk factor for neurodevelopmental disease, with more severe clinical features in individuals who carry an additional large copy number change.[22] Finally, a novel, ~200-kb region of deletion in 16p11.2 (~28.7–28.5 Mb from the telomere) has been recently reported in association with obesity.[23] The *SH2B1* gene (OMIM #608937), which plays a role in leptin and insulin signaling, is within the common region of deletion in these individuals.

We report a larger series of patients with imbalances that include the *SH2B1* gene region in 16p11.2. We found that deletions in this region were significantly enriched in patients with abnormal phenotypes referred for array CGH testing, compared with phenotypically normal controls. We observed not only a high frequency of obesity in individuals with this deletion but also developmental delay or intellectual disability and other variable phenotypic features.

MATERIALS AND METHODS

Subjects and controls

Subjects included in this study were identified by array CGH that was performed in a clinical setting at Signature Genomic Laboratories, Spokane, WA, between November 2007 and December 2009 or at Seattle Children's Hospital, Seattle, WA, between November 2007 and May 2009. Patients with imbalances that include the 200-kb *SH2B1* critical region (28.7–28.9 Mb) were identified from all patients tested at the two sites during these time intervals, regardless of the indication for testing. Patients with imbalances extending distal to the minimal 200-kb critical region were included; however, patients with deletions that also extended through the proximal 600-kb critical region (29.5–30.1 Mb) were not included in our analysis. Referring physicians of patients with imbalances identified from Signature Genomic Laboratories were contacted to help coordinate release of additional clinical information for publication. Clinical information for patients with imbalances identified at Seattle Children's Hospital was collected from the hospital's electronic medical record. The approval for these projects was obtained from the Institutional Review Board at Seattle Children's Hospital and from the Institutional Review Board-Spokane. One additional patient was identified in a study of patients with epilepsy.[24] Body mass index (BMI) for age and percentile was calculated using The Centers for Disease Control and Prevention BMI calculator (http://apps.nccd.cdc.gov/dnpabmi/) for child and teen.

For control comparisons, we combined data from three large published datasets: 2493 controls evaluated using Illumina HumanHap300 (14 probes within the critical region) or HumanHap 550 (18 probes) SNP genotyping arrays[25]; 3181 controls evaluated using Affymetrix 6.0 SNP genotyping arrays (72 probes)[12]; and 2026 controls evaluated using Illumina HumanHap 550 genotyping arrays (18 probes).[26]

Methods

For the patients evaluated at Signature Genomic Laboratories and Seattle Children's Hospital, multiple different array platforms were used. Earlier testing was performed using a bacterial artificial chromosome microarray (SignatureChipWG™ version 1.0.1 and version 2.0 or SignatureSelect® version 2.0; Signature Genomic Laboratories, Spokane, WA) as previously described.[27] More recent testing was performed using oligonucleotide-based microarrays containing either 105K- or 135K-features (SignatureChipOS™ version 1.1 manufactured by Agilent Technologies, Santa Clara, CA; SignatureChipOS™ version 2.0 manufactured by Roche NimbleGen, Inc., Madison, WI) as previously described.[28] All array designs and genomic coordinates are based on NCBI build 36.1. All deletions were visualized by fluorescence in situ hybridization using a probe made from bacterial artificial chromosome RP11–22P6. Visualization of the duplications with this probe, even by interphase analysis, was not possible because of their size. Analysis of metaphase cells showed hybridization of the probe to both homologues of 16p only, consistent with tandem duplication in all patients, and excluding insertion of the duplicated region into another region of the genome. For a subset of deletion cases for which DNA was available, we performed high-density oligonucleotide array CGH using a custom array (Agilent Technologies, Santa Clara, CA) with 1980 probes in the 16p11.2 region (chr16: 28.0–30.2 Mb; average probe spacing 1100 bp).

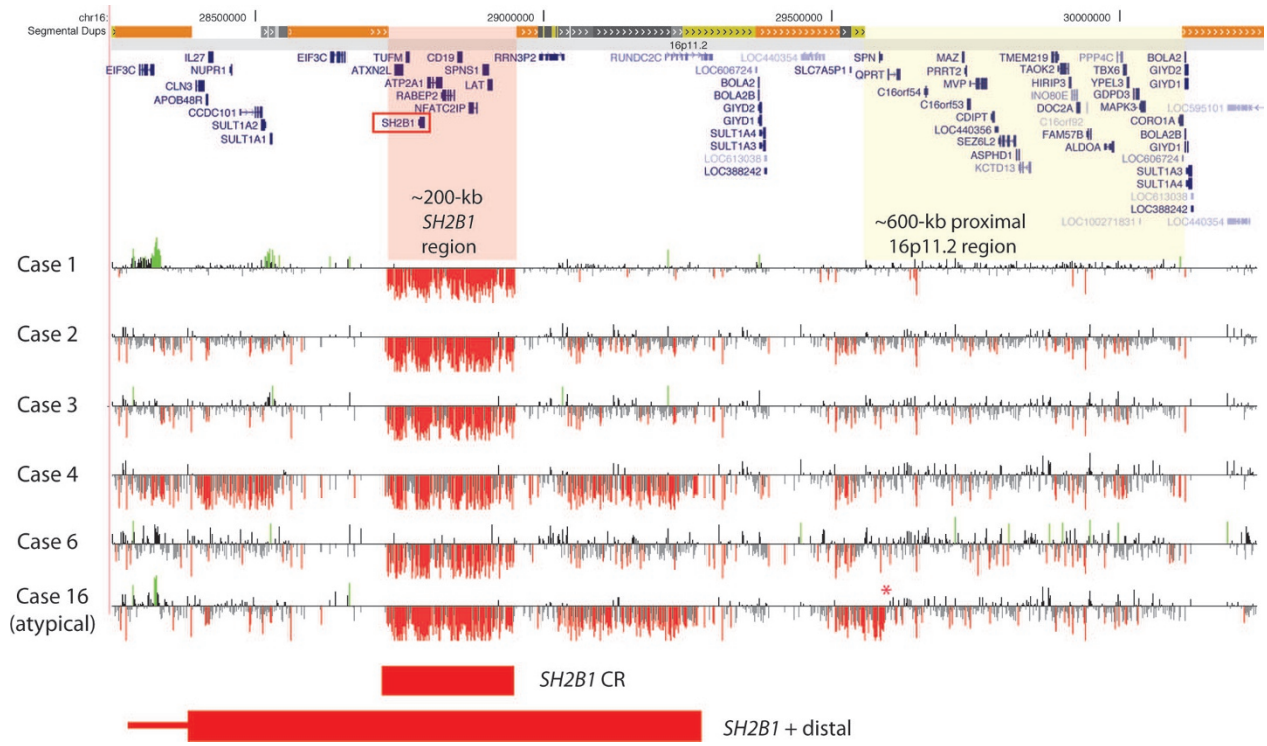

Fig. 2. A UCSC genome browser representation of the 16p11.2 region (chr16:28,000,000–30,500,000) and array CGH data for a subset of patients. Red shading represents the ~200-kb *SH2B1* critical region, and yellow shading represents the more proximal 16p11.2 region that has been associated with multiple developmental phenotypes. Segmental duplications are represented as described earlier. Gene names are in blue. High-density array CGH data are shown for individuals 1–4, 6, and one patient with an atypical deletion. For each individual, deviations of probe $\log_2$ ratios from 0 are depicted by gray and black lines. Those exceeding a threshold of 1.5 SD from the mean probe ratio are colored green and red to represent relative gains and losses, respectively. Red rectangles represent the minimally deleted regions of the two sizes of deletions detected in this study; the thin red bar represents variable breakpoint of the larger deletion. The asterisk (*) represents the atypical proximal breakpoint seen in a single case. Frequency of the ~200-kb (*SH2B1* critical region; includes the one atypical deletion) deletions and larger (*SH2B1* + distal) deletions in cases and controls (ctrls) are noted in the Table 1 along with *P* values.

Table 1 Number of cases and controls with SH2B1 deletions or duplications

	Deletions			Duplications		
	Cases	Controls	*P*	Cases	Controls	*P*
SH2B1 CR	22	1		14	3	
SH2B1 + distal	9	0		3	0	
Total	31	1	0.003	17	3	0.4

RESULTS

Among a total of 23,084 patients analyzed by Signature Genomic Laboratories or Seattle Children's Hospital, 48 genomic imbalances in 16p11.2 that include the *SH2B1* gene were identified. We will refer to this region as the 200-kb *SH2B1* region, which is distinct from another previously described centromeric 16p11.2 region of imbalance (29.5–30.1 Mb from the telomere) that has been associated with a variety of phenotypes (Figs. 1 and 2). Imbalances of two different sizes were included in our study (Fig. 2 and Table 1). The 200-kb region that encompasses *SH2B1*, located 28.7–28.9 Mb from the telomere, was common to all 48 patients (31 with deletions, 29 of which are unrelated, and 17 with duplications). Nine of the patients (seven unrelated) with a deletion and three with a duplication had a larger imbalance of ~500–600 kb that extended in the telomeric direction (28.4–28.9 Mb). Moreover, patients with imbalances that extended through the entire proximal 600-kb 16p11.2 (29.5–30.1 Mb) critical region were not included.

The overall frequency of deletions in our series of patients with abnormal phenotypes in these two cohorts is 0.13% (0.13% [29/21,820] at Signature Genomic Laboratories and 0.16% [2/1,264] at Seattle Children's Hospital). In contrast, evaluation of control studies revealed a single 200-kb deletion in 7,700 subjects (0.013%).[12,25,26] Therefore, deletions in this region are significantly enriched in patient populations when compared with control populations ($P = 0.003$, Fisher's exact test). The calculated frequency for duplications is 0.07% in cases compared with a 0.04% frequency in controls (3/7,700). Therefore, duplications are not significantly enriched in cases when compared with controls ($P = 0.4$). Inheritance was determined in 13 patients with deletions and in 5 patients with duplications, with both inherited and de novo cases observed. Deletions were

paternally inherited in three cases, maternally inherited in five cases, and de novo in five cases (38%). Duplications were maternally inherited in four cases and de novo in one case. Clinical information on parents was not available.

Additional genomic imbalances were present in six patients with 16p deletions and in six patients with duplications. In two cases with a 16p11.2 deletion and one additional imbalance, the second imbalance was known to cause disease: deletion of 15q11.2q13.1 (20,366,669–26,193,909)[29] and maternally inherited duplication of 15q11.2q13.1 (22,577,151–26,079,398).[30] In a third case, the second imbalance, duplication of 1q21.1 (genomic coordinates 145,031,795–146,193,043), is sometimes associated with an abnormal phenotype.[7,8] In the remaining three cases, the second hits were each of uncertain clinical significance and included deletion of 13q31.3 (92,880,558–93,044,066); duplication of 1q42 (234,314,080–235,417,931); and duplication of 9p24.3 (204,166–779,985). Indications for array CGH testing were variable and did not differ significantly between deletion cases and duplication cases. The most common indication given on the test requisition was developmental delay or mental retardation, followed by congenital anomalies and dysmorphic features.

Deletions

Additional clinical information was obtained for six patients with deletions (Table 2): five from Signature Genomic Laboratories and Seattle Children's Hospital and one patient identified in an epilepsy cohort (Patient 6).[24] All patients had developmental delays or intellectual disabilities, regardless of the size of their deletion. Four of the six patients had a BMI at or above the 95th percentile. All growth parameters including height, weight, and BMI were at or above the 95th percentile in three of these four patients, whereas weight and BMI were above the 95th percentile and height was above the 90th percentile in the fourth. Besides the common features of developmental delays and overgrowth or obesity, no other consistent phenotypic findings were observed, although many patients displayed a variety of congenital anomalies or dysmorphic features (Table 2). Age at diagnosis of the deletion ranged from 3 to 15 years. None of these six patients had a second genomic imbalance.

Case reports

Patient 1

According to medical records, this patient carried diagnoses of autism and schizophrenia. This individual was living in a foster home due to a difficult social situation and was held back in school in the third grade. Mild dysmorphic features were noted, including small palpebral fissures bilaterally (−4 SD). Bilateral fifth finger clinodactyly was also noted. No congenital defects were documented. Growth parameters were within normal limits.

Patient 2

This patient was born at 37 ½ weeks gestation. Parents first became concerned about language development at the age of 2 years. Significant speech and motor delays were noted. Independent walking was achieved at the age of 17 months. Additional workup included normal fragile X testing and normal brain magnetic resonance imaging (MRI). There also was a history of lack of body hair in infancy and sparse coarse hair in early childhood without abnormality of the nails. Height was between the 90th and 95th percentile, and weight was above the 95th percentile. BMI for age was above the 99th percentile. The deletion was inherited from the father, who completed high

Table 2 Clinical phenotype of patients with isolated 16p11.2 deletions

Patient no.	Weight [kg] (%)	Height [cm] (%)	BMI [kg/m^2] (%)	Developmental delay	Behavioral problems/ADHD	Seizures	Autism	Speech delay	Congenital anomalies or dysmorphism	Other medical problems	Inheritance
1	30.3 (25–50)	139.7 (50–75)	15.6 (25–50)	+	+	–	+	–	Small palpebral fissures	Schizophrenia	Unknown
2	20.9 (>95)	105 (90–95)	19.5 (>99)	+	–	–	–	+	Not noted	Abnormal hair	Paternal
3	117.8 (>>99)	160 (>95)	46 (>>99)	+	+	–	–	–	Low anterior hairline, deep set eyes	Bipolar disorder, precocious puberty, hypothyroidism	Unknown
4	13.3 (25–50)	93.2 (25–50)	15.9 (25–50)	+	–	–	–	–	Not noted	Not noted	De novo
5	29.5 (>99)	114.9 (>99)	22.3 (>99)	+	–	–	+	+	Not noted	Macrocephaly	De novo
6	90.5 (>99)	183.5 (>95)	27 (95)	+	–	+	–	+	Iris coloboma	Not noted	Unknown

All patients have the same 200-kb deletion with the exception of patient 4 whose deletion extends toward the telomere and is ~570 kb.
ADHD, attention deficit hyperactivity disorder; BMI, body mass index.

school and some college. No other information is available on the father.

Patient 3

This patient was born at 36 weeks gestation. Apgar scores were 4 and 9 at 1 and 5 minutes. A developmental evaluation at the age of 5 years showed global developmental delays. Gross motor skills were assessed at the 2nd percentile at that time. Intellectual disability and mild dysmorphic features including deep-set eyes and a low anterior hairline were noted. Behavioral issues including food hoarding and stealing were also noted. Additional medical problems included precocious puberty, asthma, Hashimoto thyroiditis and hypothyroidism, and a history of multiple episodes of otitis media. According to medical records, there was a diagnosis of bipolar disorder, and the patient was living in a foster home. All growth parameters were above the 95th percentile including height, weight, and BMI (46 kg/m^2, >99th percentile). Additional genetic testing had included methylation study for Prader-Willi that was negative.

Patient 4

This patient was the product of a full-term pregnancy. This patient initially presented with global developmental delays, hypotonia, and abnormal body odor. Mild left plagiocephaly and one anteriorly displaced ear, but no other dysmorphic features were noted. There was a negative metabolic evaluation and a normal brain MRI. An echocardiogram did not reveal any abnormalities. Height, weight, and BMI were in the 25th–50th percentile.

Patient 5

This patient was born full-term. Speech and social delays were initially noted at the age of 2 years. By the age of 3½ years, both motor and speech delays and features of autism were noted. There was a history of recurrent otitis media, but hearing examination was normal. Growth parameters were always at or greater than the 95th percentile, and BMI was above the 99th percentile. There was macrocephaly, with a head circumference at the 98th percentile. Except for posterior ear pits, no other dysmorphic features were present. A brain MRI was normal and *PTEN* mutation testing was normal.

Patient 6

This patient first experienced afebrile seizures at the age of 1½ years. Febrile and afebrile seizures persisted for many years, despite treatment with valproic acid. The patient had developmental and speech delay and iris coloboma. Brain MRI, echocardiogram, and renal ultrasound were all normal. Family history is positive for intellectual disability in the mother and maternal half brothers, and epilepsy in a paternal half brother. Array CGH has not been performed on these individuals. Birth weight and length were at the 50th percentile. BMI was always at or above the 95th percentile. At the time of the last evaluation, height, weight, and BMI were all at or above the 95th percentile.

Breakpoint analysis

To determine whether there were significant differences in breakpoints among cases, we performed high-density oligonucleotide array CGH using a custom array targeted to the 16p11.2 region to refine breakpoints in 20 cases for which there was sufficient DNA. DNA was available for 16 cases with the smaller deletion and 4 cases with the larger deletion. In 15 of 16 cases with the ~200-kb deletion, both breakpoints lie within the flanking segment duplications and seem to be identical within the resolution of the array (Supplemental Digital Content 1, Figure, http://links.lww.com/GIM/A119). The most likely substrates for nonallelic homologous recombination in these cases are directly oriented 36-kb duplicated blocks of DNA with >99% sequence identity. One case seems to have an atypical proximal breakpoint within unique sequence in the proximal 16p11.2 critical region (Fig. 2, atypical case and Supplemental Digital Content 1, Figure, http://links.lww.com/GIM/A119). Of the four cases with larger deletions, all seem to have the same proximal breakpoint, and there seem to be two similar but variable breakpoints within the distal segmental duplication (Supplemental Digital Content 1, Figure, http://links.lww.com/GIM/A119). None of the differences in breakpoints correlated with any specific phenotypic feature.

Duplications

Additional clinical information was obtained for five patients with duplications that all involved the smaller 200-kb *SH2B1* critical region. Two of these patients carried a diagnosis of autism spectrum disorder. In one of these patients with a maternally inherited duplication, height was near the 50th percentile and weight was at the 5th percentile. In the second patient with autism spectrum disorder, there were additional findings of seizures and aggressive behavior. Height was near the 75th percentile, weight was between the 75th and 90th percentile, and BMI was in the 93rd percentile. Two other patients with duplications shared similar congenital defects, including cleft palate and cardiovascular findings (atrial and ventricular septal defects in one and patent ductus arteriosis, left ventricular dilation, and patent foramen ovale in the other). One of these patients also had mild microcephaly, growth delay (weight <5th percentile and height between the 10th and 15th percentile), and possible expressive language delay, whereas the other was a newborn with microphthalmia, bilateral optic nerve and chorioretinal colobomas, iris colobomas, and long fingers and toes. The duplication was de novo in the latter patient. The fifth patient with a duplication had attention deficit hyperactivity disorder, conduct disorder, ataxia, abnormal speech, flat philtrum, malar hypoplasia, fifth finger clinodactyly, and mild 2–3 toe syndactyly. Height and weight for this patient were above the 95th percentile, whereas BMI was at the 92nd percentile. Thus, although two of these patients were overweight, none of them were obese, and two had weight at or below the 5th percentile.

DISCUSSION

Chromosome 16p11.2 is rich in segmental duplications, accounting for the high frequency of recurrent chromosomal imbalances in this region of the genome. Here, we report recurrent deletion of a 200-kb region in 16p11.2 that is significantly enriched in a patient population with a variety of different clinical findings compared with phenotypically normal controls, supporting a role for this deletion in the pathogenesis of abnormal phenotypes. In addition, the de novo occurrence of deletions in 40% of patients also suggests pathogenicity. The deletion has a minimal region of overlap of 200 kb at coordinates 28.7–28.9 Mb in these patients and includes the *SH2B1* gene among others. It is distinct from a previously described and more proximal 600-kb 16p11.2 deletion, located at coordinates 29.5–30.1 Mb, that has been associated with a variety of abnormal phenotypic findings, including autism and intellectual disabilities. We provide detailed clinical information on six patients with the 200-kb *SH2B1*-containing deletion.

There are a few previous reports of patients with 200-kb *SH2B1* deletions. The first reported case, included in a study of imbalances involving the more proximal 600-kb 16p11.2 region, was considered an "atypical 16p11.2 deletion."[19] The patient and his father who also had the deletion had developmental problems and minor dysmorphisms. No growth parameters were reported. Two additional patients with 200-kb *SH2B1* deletions are described in the Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER; https://decipher.sanger.ac.uk/application/; DECIPHER cases 249980 and 251199).[31] Both have developmental delay and one is obese, whereas the other has dysmorphic features, hyperactivity, and seizures.

A recent study identified five patients with *SH2B1*-containing deletions among a cohort of 300 Caucasians with severe early-onset obesity.[23] Three of these patients had the 200-kb *SH2B1* deletion, whereas two of them had a larger deletion that also included the more proximal 600-kb 16p11.2 region. The latter two patients had developmental delay in addition to obesity. Two more cases with the 200-kb *SH2B1* deletions were identified in a replication cohort of 1062 patients with isolated obesity, and the deletion cosegregated with obesity in one of these families with available samples. In addition, the frequency of the deletion in patients with severe obesity was significantly greater than in controls. On the basis of these findings, the authors concluded that a significant association exists between deletions that include *SH2B1* and obesity. Supporting this assertion is the fact that disruption of *Sh2b1* in mice, which encodes an adaptor protein involved in leptin and insulin signaling, results in obesity and severe insulin resistance.[32] Bochukova et al. also found striking similarities between the human leptin-receptor-deficient phenotype and the phenotype in their patients with *SH2B1* deletions. Of note, deletions of the more proximal 600-kb region of 16p11.2 alone have also been associated with obesity.[33]

In contrast to the study by Bochukova et al. in which only patients with severe obesity were studied, the patients presented here were ascertained for a variety of different abnormal phenotypes. We cannot specifically address the frequency of the deletion in obese individuals because our patient population was not restricted to this phenotype; however, four of our six patients with detailed clinical information that allowed us to calculate BMI were obese. Given that the prevalence of obesity is 16.4% in the general pediatric population[34] and 19.3% in children with learning disabilities,[35] our results support the previous finding that this deletion plays a role in obesity. Although Bochukova et al. observed severe obesity with excess weight predominantly due to fat mass, we observed more generalized overgrowth, with height, weight, and BMI at or above the 95th percentile in three of six patients. In addition, developmental delay or intellectual disability was the most common indication for testing in our patient population. In the obese patient population, developmental delay was attributed to the deletions that also included the more proximal 600-kb 16p11.2 region. Most of the patients in our study had only the minimal 200-kb *SH2B1*-containing deletion, and patients with deletions that extended through the entire more proximal 16p11.2 deleted region were specifically excluded here. Therefore, we conclude that in addition to obesity, deletion of the minimal 200-kb *SH2B1*-containing region also predisposes to developmental delay or intellectual disability. These findings may be due to the difference in patient populations between the two studies.

In addition to developmental delay and obesity, a variety of different clinical indications and phenotypic findings were present less frequently in our patient population. Congenital anomalies, hypotonia, dysmorphic features, encephalopathy, and seizures were included in the indication for testing in only one or two patients each. Without detailed clinical information on more patients with *SH2B1*-containing deletions, the true frequency of these findings is uncertain; however, a number of other imbalances have been found to be associated with diverse phenotypes (reviewed in Ref. 6).

Additional genes contained in this 200-kb region include *ATXN2L*, *TUFM*, *ATP2A1*, *RABEP2*, *CD19*, *SPNS1*, *NFATC2IP*, and *LAT*. There are few published reports that implicate some of these genes in the etiology of human disease. *TUFM* (OMIM #602389) is a mitochondrial elongation factor and one patient with a homozygous missense mutation leading to infantile encephalopathy has been reported.[36] The indication for array CGH testing in two patients in this study included "encephalopathy." One patient was found to have a 16p11.2 deletion and the other a duplication. Mutations in *ATP2A1* (OMIM #108730) have been identified in two families with autosomal recessive inheritance of Brody myopathy (OMIM #601003), an impairment of muscle relaxation in the setting of exercise. *LAT* (OMIM #602354) seems to play a role in T-cell development. *CD19* (OMIM #107265) is involved in B-cell signaling, and homozygous mutations have been identified in a few families with antibody deficiencies. Therefore, based on currently known functions of these other genes in the region of deletion, it is unclear how they might contribute to the phenotypes in these patients.

We note that six of the 31 patients (19.4%) with the 200-kb *SH2B1* 16p11.2 deletion also had a second genomic imbalance that was either clinically significant or of uncertain clinical significance. The frequency of patients in our series with two "hits" is therefore similar to that recently reported by Girirajan et al.[22] in a series of patients with deletions of 16p12.1. It is possible that the combined effect of two genomic imbalances modifies the phenotype, but we do not currently have enough information to address this hypothesis. We also identified a number of patients with a reciprocal *SH2B1*-containing duplication; however, the clinical significance of this reciprocal duplication is harder to assess. The duplication does not seem to be enriched in patients compared with controls, which makes it more difficult to assign pathogenicity to this copy number change. Developmental delay or intellectual disability was not as frequent when compared with patients with deletions. Congenital malformations were present in two of five patients for whom we had additional clinical information and included a cleft palate and cardiac defects. Two of the patients also had a diagnosis of autism spectrum disorder. Speech delays were present in three of five patients with the duplication. The DECIPHER database contains two additional patients (cases 2014 and 249152) with this duplication and both had speech and developmental delays and one had seizures. Given the lack of enrichment of the duplication in patients compared with normal controls, and the inconsistent phenotypic presentations, the clinical significance of the duplication remains uncertain.

In conclusion, we have further characterized the phenotypic features associated with a recurrent 200-kb deletion encompassing the *SH2B1* gene. Significant enrichment in a patient population when compared with controls supports its pathogenicity in causing a variety of phenotypes. Our findings also support the association between the recurrent 200-kb 16p11.2 deletion and obesity. In addition, we observe developmental delay and various other neurodevelopmental features in all patients for which phenotypic information was available.

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