

ARTICLE

Reciprocal deletion and duplication at 2q23.1 indicates a role for *MBD5* in autism spectrum disorder

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Copy number variations associated with abnormal gene dosage have an important role in the genetic etiology of many neurodevelopmental disorders, including intellectual disability (ID) and autism. We hypothesize that the chromosome 2q23.1 region encompassing *MBD5* is a dosage-dependent region, wherein deletion or duplication results in altered gene dosage. We previously established the 2q23.1 microdeletion syndrome and report herein 23 individuals with 2q23.1 duplications, thus establishing a complementary duplication syndrome. The observed phenotype includes ID, language impairments, infantile hypotonia and gross motor delay, behavioral problems, autistic features, dysmorphic facial features (pinnae anomalies, arched eyebrows, prominent nose, small chin, thin upper lip), and minor digital anomalies (fifth finger clinodactyly and large broad first toe). The microduplication size varies among all cases and ranges from 68 kb to 53.7 Mb, encompassing a region that includes *MBD5*, an important factor in methylation patterning and epigenetic regulation. We previously reported that haploinsufficiency of *MBD5* is the primary causal factor in 2q23.1 microdeletion syndrome and that mutations in *MBD5* are associated with autism. In this study, we demonstrate that *MBD5* is the only gene in common among all duplication cases and that overexpression of *MBD5* is likely responsible for the core clinical features present in 2q23.1 microduplication syndrome. Phenotypic analyses suggest that 2q23.1 duplication results in a slightly less severe phenotype than the reciprocal deletion. The features associated with a deletion, mutation or duplication of *MBD5* and the gene expression changes observed support *MBD5* as a dosage-sensitive gene critical for normal development.

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INTRODUCTION

Copy number variations (CNVs) have an important role in the genetic etiology of many neurodevelopmental disorders, including intellectual disability (ID) and autism. Studies show that neurodevelopmental syndromes associated with CNVs emerge from abnormal gene dosage, suggesting that strict regulation of gene dosage at some loci is required for proper neurodevelopment. These genes are labeled dosage-sensitive genes, and alteration in the expression of these genes can lead to considerable phenotypic effects. In this study,

we continue to delineate another dosage-sensitive gene associated with reciprocal deletion and duplication syndromes. We previously implicated the chromosome 2q23.1 region in genomic disease.¹ We reported that *methyl-CpG-binding domain 5 (MBD5)* is the major disease gene responsible for 2q23.1 deletion syndrome in 65 patients, with haploinsufficiency of *MBD5* resulting from a variety of 2q23.1 deletions.¹ Furthermore, we showed that the phenotypes associated with a deletion or mutation of *MBD5* were similar. Duplications of *MBD5* have been reported sporadically in the literature. Our group

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recently reported two patients with 2q23.1 duplications that included *MBD5*.² In a study, in which rare copy number variants in autism spectrum disorders were analyzed, one individual presented with a 2q23.1 duplication that included the *MBD5* gene.³ Before that report, three larger duplications in the 2q23 region were reported.^{4–6} Furthermore, the DECIPHER database has six duplications that involve *MBD5* (DECIPHER ID 251237, 251363, 248386, 251953, 254507 and 260771). Here, we report 23 new patients with microduplication of 2q23.1, for a total of 36 cases, establishing a duplication syndrome complementary to the microdeletion syndrome. We further demonstrate that *MBD5* is a dosage-sensitive gene and that improper *MBD5* dosage affects normal neurodevelopment and is likely a major player in the development of key clinical features present in both the 2q23.1 deletion syndrome and the 2q23.1 duplication syndrome.

MATERIALS AND METHODS

Subjects

We established an international collaboration to collect a cohort of individuals with duplications involving chromosome band 2q23.1 from microarray-based comparative genomic hybridization analyses. When available, peripheral blood was collected, and DNA, RNA, and lymphoblastoid cell lines (LCLs, human lymphocytes transformed with Epstein-Barr virus) were prepared according to standard methods. Phenotypic information was obtained from diagnostic referral data, medical records, clinical reports, genetics clinic evaluations, neurodevelopmental and/or psychological evaluations, parent surveys, photos, previously published articles and/or a clinical checklist sent to the referring physician or care provider. All samples and information were collected after informed consent was obtained and in accordance with local institutional review board approved protocols from Virginia Commonwealth University, The Hospital for Sick Children, The Mayo Clinic, Radboud University Nijmegen Medical Center and Nationwide Children's Hospital.

Phenotype analysis

Data were collected for characteristics related to subjects' medical and developmental history and neurological, behavioral and physical features (Table 1). Characteristics that were age dependent (behavior, sleep patterns and craniofacial, and skeletal abnormalities) were scored accordingly on the basis of development. Autistic-like symptoms were defined as having reported autistic-like behaviors, were specifically evaluated with the ADOS or ADI-R or were given a diagnosis of pervasive developmental disorder. Eye and pinnae abnormalities varied among subjects and were grouped in broad categories. A detailed phenotype analysis was performed on data collected from a subset of the 23 patients with 2q23.1 duplication syndrome and compared with the core characteristics associated with 2q23.1 deletion, partial or complete *MBD5*-specific deletions and *MBD5* mutations.¹ Ten subjects had in-depth clinical characterizations, and their features were compared in Table 1.

Expression analyses

LCLs from 2q23.1 duplication syndrome patients and controls were cultured according to standard methods. RNA was isolated from LCLs or lymphocytes collected from fresh blood, and cDNA was prepared according to published methods.⁷

Quantitative RT-PCR for assessment of *MBD5* mRNA expression was performed as previously described.¹ Briefly, predesigned Assays on Demand Gene Expression Products (Fermentus, Glen Burnie, MD, USA) Taqman minor groove binder (MGB) probes for *MBD5* (Hs02289233_m1) were used (Applied Biosystems, Inc.) with *GAPDH* (MIM 138400) (Hs99999905_m1) as the endogenous control. All cDNA samples were run in triplicate in 10 μ l reaction volumes. Taqman Universal PCR Master Mix (Applied Biosystems, Life Technologies, Inc., Grand Island, NY, USA), the probe, and deionized water were mixed together in a fixed ratio, and cDNA was then added to each well. PCR conditions were the Standard 7500 Run mode of the ABI Prism 7900 HT Sequence Detection System (Applied Biosystems). Cycle threshold (C_t) was

determined during the geometric phase of the PCR amplification plots, as recommended by the manufacturer. Relative differences in transcript levels were quantified according to the $\Delta\Delta C_t$ method and normalized to *GAPDH*. Data were analyzed with 7500 Fast System SDS Software (Applied Biosystems). Standard error was generated for each sample as previously described, and significance ($P < 0.05$) was determined by a one-sample *t*-test compared with theoretical mean of 1.0 ($P < 0.001$ for all deletion and duplication cases). Results are expressed as fold-change relative to 1.0 (normal control). We then compared mRNA expression of *MBD5* in 2q23.1 duplication subjects to that of our previously published *MBD5* expression studies in 2q23.1 deletion subjects.¹

Statistical analyses

Statistical analysis for gene expression data was performed with Prism 4 version 4.0b (GraphPad Software), as previously described.¹

RESULTS

The analyses presented here characterize the molecular genetic and phenotypic heterogeneity associated with the 2q23.1 microduplication syndrome. We provide data to support *MBD5* as the critical gene for both 2q23.1 deletion and duplication syndromes and demonstrate that appropriate dosage of *MBD5* is critical for normal development.

Refinement of the 2q23.1 duplication syndrome critical region

Given the establishment of *MBD5* haploinsufficiency and 2q23.1 microdeletion as a significant neurodevelopmental syndrome, we questioned whether duplication of this region also contributed to neurodevelopmental abnormalities. Thus, we searched for duplications of *MBD5* across academic and commercial testing databases and identified 36 cases with microduplications of 2q23.1, including 6 cases described in DECIPHER, 6 reported cases,^{2–6} and 23 new cases ranging from small duplications of 0.068 Mb to > 53 Mb (Figure 1) (Supplementary Table 1). Aligning the 2q23.1 duplications, the smallest region of overlap (SRO) was defined to one gene, *MBD5*, common to all duplications (Figure 1). No other genes were included in the SRO for all duplications or were present in duplications that did not also involve *MBD5*. Furthermore, the alignment of deletion and duplication regions revealed a common SRO that included only *MBD5* (Figure 1). Importantly, duplications disrupting any translated or untranslated exons of *MBD5* are not present in a control population of 13 991 individuals.⁸

All of the 2q23.1 duplications presented in this report are unrelated and are either *de novo* or of unknown inheritance with the exception of three cases that show transmission from mother to child (3/36 or 8.3% with maternal inheritance). GC19184a and GC19184b represent transmission from a mother to her son. The 8-year-old boy was referred for developmental delay, dysmorphic features and multiple joint contractures. GC3057a and GC3057b represent maternal inheritance from a mother to her 4-year-old daughter, referred for developmental delay and cerebellar ataxia. Cases 3424_3a and 3424_3b represent a reported case of a mother and her son, who presented with autism, mild ID and relative macrocephaly.³ Detailed clinical information on the mothers in these cases was not available; however, mothers were not considered to be affected by the evaluating clinicians.

Phenotype analyses show overlapping features between individuals with duplication or deletion of 2q23.1

In the literature, the phenotype associated with 2q23.1 duplications included developmental delay, seizures, hypotonia, mild dysmorphic features and behavioral problems with autistic features; however, a complete description of this disorder has not been elucidated.^{2–6}

Table 1 Clinical characteristics in individuals with 2q23.1 duplication

	SMS376	SMS381	SMS394	SMS397	SMS409	Chung 1 2012 ^a	Chung 2 2012 ^a	MGH2	Mayo1	Mayo2
Sex	F	M	F	M	M	M	F	F	F	M
Age of evaluation (years)	15	6	2, 5, 7, 9	7	7	8	5, 8, 17	2	12	1
<i>Neurological/behavioral</i>										
Developmental delay	+	+	+	+	+	+	+	+	+	+
Motor delay	+	+	+	+	+	+	+	+	+	+
Language impairment	+	+	+	+	+	+	+	+	+	+
Infantile hypotonia	+	+	+	+	+	+	+	+	+	+
Seizures	+	+	+	N	—	—	—	—	N	+
Behavioral problems	+	+	+	+	—	+	+	+	+	+
Sleep disturbances	N	N	+	+	+	N	—	—	N	N
Likeable/affable personality	+	+	+	+	+	+	+	+	N	N
Autistic-like features	+	+	+	+	+	+	+	+	+	+
<i>Craniofacial</i>										
Craniofacial anomalies	+	+	+	+	+	+	+	+	+	+
Midface hypoplasia	—	—	—	—	+	+	—	+	N	N
Arched eyebrows	+	—	N	+	+	+	+	—	N	N
Bilateral ptosis	+	—	+	+	+	+	+	—	N	N
Long eyelashes	+	—	N	+	+	+	+	—	N	N
Hypotelorism	+	—	—	+	+	+	+	+	N	N
Prominent nose	+	—	N	+	+	+	+	—	N	N
Wide mouth	+	—	N	+	+	+	+	+	N	N
Thin upper lip	+	—	N	+	+	—	—	+	N	N
Unspecified dental abnormalities	+	N	+	+	N	+	+	—	N	N
Small chin/micrognathia/retrognathia	—	—	+	+	+	+	—	+	N	N
Pinnae abnormalities	N	+	+	+	+	N	N	—	N	N
<i>Ocular</i>										
Strabismus	+	N	N	+	+	+	+	—	N	N
Visual impairment	—	N	N	+	—	—	+	—	N	N
<i>Extremities</i>										
Hand/foot abnormalities	+	+	+	+	+	+	+	+	N	N
Fifth finger clinodactyly	+	—	+	+	—	N	+	—	N	N
Large, broad first toe	N	N	N	+	+	N	+	—	N	N
Sandal gap, first-second toe	+	—	N	—	+	—	—	—	N	N

Abbreviation: N, not evaluated or not reported.

^aChung *et al*, 2012²

Given the large number of samples with more comprehensive phenotypic description and the common duplication of *MBD5* in all cases (Figure 1), we were able to provide a more detailed clinical characterization of 2q23.1 microduplication syndrome, comparing 34 features in 10 individuals for whom in-depth clinical information was available. Of the 28 most commonly reported features, all subjects had developmental delay, motor delay, language impairment and infantile hypotonia (Table 1). Behavioral problems and autistic-like symptoms were observed in all but one of the 23 new duplication cases, and all were noted to have affable personalities (Table 1). SMS397 was noted to be very affectionate and social and with an overdeveloped and musical ear, similar to that reported in Williams syndrome. SMS376 was described as a likeable teenager with high cognitive function despite her language impairment. While the subjects had craniofacial manifestations, these were generally mild anomalies and not consistent among all individuals (Figure 2). However, hypotelorism, downslanting palpebral fissures, arched eyebrows, bilateral ptosis,

long eyelashes, prominent noses, pinnae abnormalities and wide mouth were noted in most of the subjects evaluated (Table 1, Figure 2). Thin upper lip and small chin/micrognathia/retrognathia were also reported (Table 1). However, SMS381 was reported to have hypertelorism, telecanthus and full lips, indicating that considerable variability in the craniofacial features may be present. Subjects appear to have small noses with thick nasal tip when they are young (SMS397, SMS409), and when older, have large prominent noses (Chung 1 2012 and SMS376). Strabismus and periorbital fullness were frequently noted (SMS409, SMS376, Chung 2 2012 and SMS397) (Table 1, Figures 2 and 3). Unspecified dental abnormalities, such as widely spaced teeth, were also commonly noted (Table 1). Mild hand and foot abnormalities were seen in almost all subjects (Table 1, Figure 2). Notably, large, broad first toes were mentioned in all of the patients evaluated, and fifth finger clinodactyly was a common finding (Table 1, Figure 3). Two patients (3424_3b³ and MGH2) had relative macrocephaly (MGH2 with OFC 90th centile

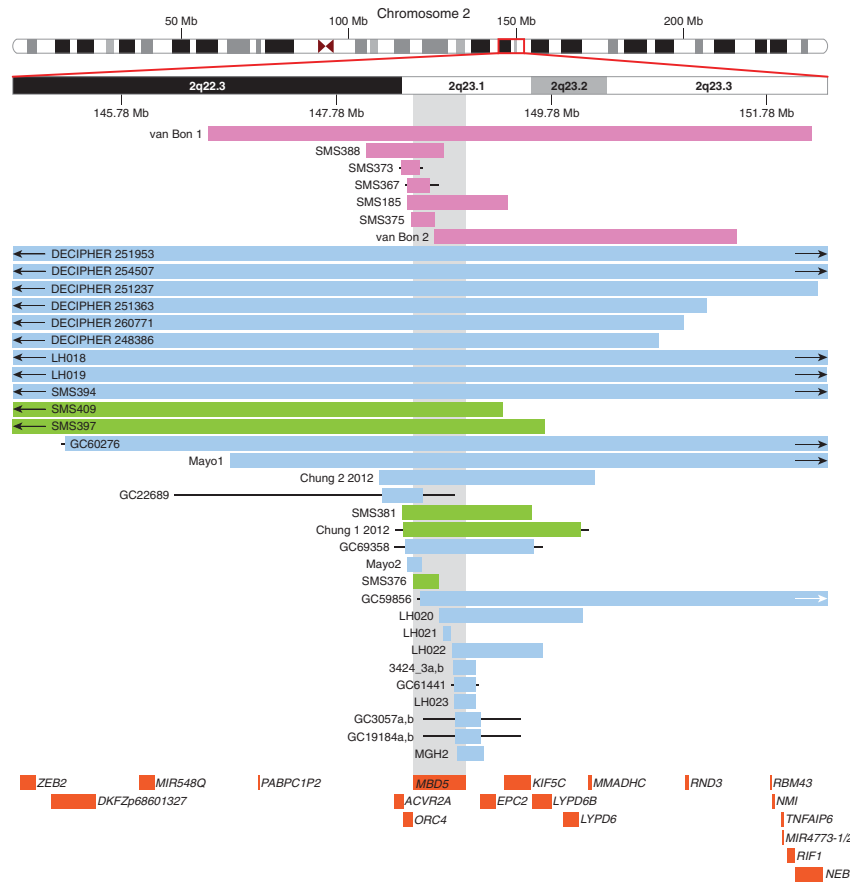


Figure 1 Delineation of the 2q23.1 critical region. Schematic representation of *MBD5*-containing duplications in this report and key variants previously reported in the literature. Duplications are in blue and green, while deletions are in pink. Three previously reported duplications that were cytogenetically defined; thus, no coordinates were given. Boxes represent the minimum size of the deletions/duplications and the horizontal lines extend through gaps in coverage to show the maximum deletion/duplication sizes. Green boxes represent individuals with *MBD5* expression studies reported here. Genes within the region are represented by orange boxes, and the shaded region shows the location of *MBD5*. All individuals are unrelated, and all cases are *de novo* with the exception of GC19184 (cases a and b) and GC3057 (cases a and b), 3424_3 (cases a and b) which represent parent/child pairs.³

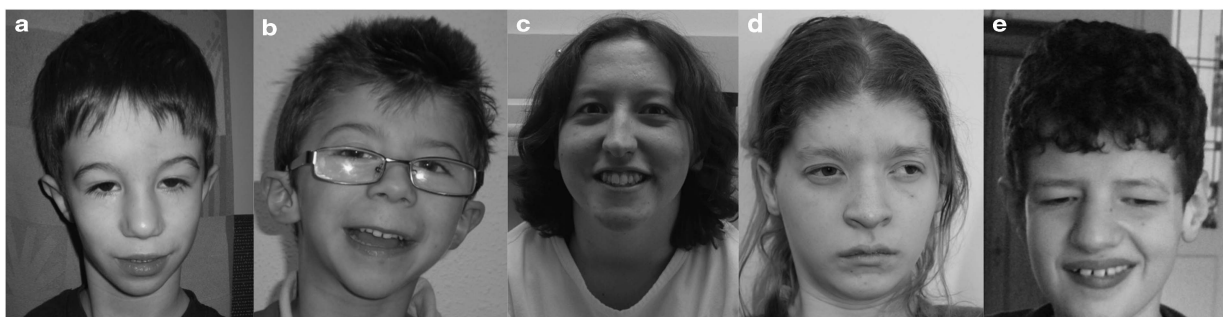


Figure 2 Features present in individuals with 2q23.1 duplication syndrome. Four individuals with 2q23.1 duplication are shown, (a) SMS409, 7 years; (b) SMS397, 7 years; (c) SMS376, 15 years; (d) Chung 2 2012, 17 years and (e) SMS381, 6 years. While some dysmorphic craniofacial features are present (high arched eyebrows, thin upper lip, periorbital fullness, downslanting palpebral fissures, triangular face), no significant craniofacial findings among individuals with 2q23.1 duplication are appreciated.

compared with height and weight of 30 and 40th centiles, respectively). No postnatal growth retardation, microcephaly, open mouth, small hands and feet, cardiovascular abnormalities or constipation were noted in the 2q23.1 duplication cases, although common features in the reciprocal deletion syndrome.

We compared the core phenotypes observed in three distinct categories of *MBD5* alteration, (1) *MBD5* point mutations, (2)

MBD5 deletions and (3) *MBD5* duplications (Table 2). All mutations, deletions and duplications share developmental delay, motor delay, language impairment, behavioral problems and autistic-like symptoms. Interestingly, seizures, which were seen in individuals with *MBD5* mutations, *MBD5*-specific deletions and 2q23.1 deletions, were rarely observed in *MBD5* duplications. In addition to the features noted above, the 2q23.1 deletion and duplication cases, some of

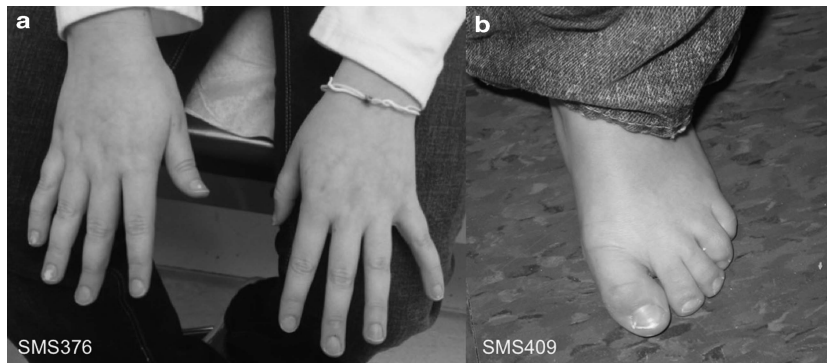


Figure 3 Hand and foot anomalies present in individuals with 2q23.1 microduplication syndrome. (a) Hands of SMS376. Note the fifth finger clinodactyly. (b) Foot of SMS409. Note the large, broad first toe.

Table 2 Prevalent phenotypic manifestations of *MBD5* alteration

Point mutations ^{1,11,24}	Deletions ¹	Duplications
Development delay ^a	Development delay ^a	Development delay ^a
Motor delay ^a	Motor delay ^a	Motor delay ^a
Language impairment ^a	Language impairment ^a	Language impairment ^a
Behavioral problems ^a	Behavioral problems ^a	Behavioral problems ^a
Autistic-like symptoms ^a	Autistic-like symptoms ^a	Autistic-like symptoms ^a
Withdrawal behaviors	Sleep disturbances ^b Infantile hypotonia ^b Heavy/arched eyebrows ^b Eye abnormalities ^b Prominent noses ^b Widely spaced teeth ^b	Sleep disturbances ^b Infantile hypotonia ^b Arched eyebrows ^b Eye abnormalities ^b Prominent noses ^b Widely spaced teeth ^b
Repetitive behaviors ^c	Repetitive behaviors (stereotypies) ^c	Likeable/affable personality
Seizures ^c	Seizures ^c Infantile feeding difficulties Thin upper lip Small chin/micrognathia/retrognathia Sandal gap Constipation	Bilateral ptosis Hypotelorism Strabismus Long eyelashes Wide mouth 5 th finger clinodactyly Large, broad first toe

^aPhenotype observed across *MBD5* mutation, deletion and duplication cases.

^bPhenotype observed across *MBD5* deletion and duplication cases.

^cPhenotype observed across *MBD5* mutation and deletion cases.

which are very large, spanning from 0.068 Mb to 53.7 Mb in size, have infantile hypotonia, arched eyebrows and nasal anomalies, with minimal clinical differences between the two groups. These data suggest that genes flanking *MBD5* may contribute to these additional features (Figure 1, Table 2). Overall, these findings further support an effect of *MBD5* gene dosage as the likely cause for the core phenotypes observed in 2q23.1 deletion and duplication syndromes.

Gene dosage of *MBD5*

We previously established haploinsufficiency of *MBD5* in promoter, 5'-noncoding exon, or coding exon deletions in 2q23.1 deletion subjects (Figure 4).¹ Here, we assessed the level of *MBD5* expression in cells from 2q23.1 duplication subjects and compared these levels to *MBD5* expression in cells from 2q23.1 deletion subjects (Figure 4). Data showed that *MBD5* mRNA expression levels were significantly

increased (1.52- to 1.83-fold) across all 2q23.1 duplications studied when compared with normal controls (Figure 4). The *MBD5* mRNA expression levels for the 2q23.1 deletions were 0.22- to 0.59-fold compared with normal controls (Figure 4).¹ The expression changes observed indicate that *MBD5* is a dosage-sensitive gene and that improper dosage of this gene could be the cause of the core phenotype that distinguishes 2q23.1 deletion and duplication subjects.

DISCUSSION

We report 23 new patients with a 2q23.1 duplication, which we believe represent a reciprocal duplication syndrome complementary to the 2q23.1 microdeletion syndrome. The microduplication sizes vary for all cases and range from 68 kb to > 53 Mb. Each duplication has distinct proximal and distal breakpoints, suggesting that the variants, similar to 2q23.1 microdeletions, are not mediated by low-copy repeats. As these deletions and duplications are likely not mediated by NAHR, they are likely caused by the genomic rearrangement mechanism known as fork-stalling and template-switching/microhomology-mediated break-induced replication.¹⁰ This mechanism also has been reported in microduplication syndromes, including Potocki-Lupski syndrome and Charcot-Marie-Tooth disease type 1A and hereditary neuropathy with liability to pressure palsies.¹⁰ Thus, it is possible that the duplications observed in the patients with this syndrome are mediated by this mechanism. Additional studies are in progress to understand the breakpoint sequences in several 2q23.1 deletion and duplication patients, which will help us understand the etiology of deletions and duplications in 2q23.1 syndromes.

All duplications encompass a region that includes either all or a portion of *MBD5*, an important protein in methylation patterning and epigenetic regulation.^{1,11,12} The resulting phenotype includes ID, language impairments, infantile hypotonia and gross motor delay, behavioral problems, autistic features, dysmorphic facial features (pinnae anomalies, arched eyebrows, prominent nose, small chin, thin upper lip) and minor digital anomalies (fifth finger clinodactyly and large broad first toe). In-depth clinical features for all 23 individuals were difficult to obtain; thus, having more individuals with ascertainment of in-depth clinical features of this disorder will contribute to our knowledge regarding the spectrum of phenotype present in individuals with *MBD5*-associated disorders. Both partial and whole-gene duplication of *MBD5* resulted in altered gene dosage, thus illustrating that *MBD5* is overexpressed in cells from 2q23.1 microduplication patients. We are aware that gene expression in LCLs in a neurodevelopmental disorder may have its limitations and may not be the best tissue for molecular and functional studies of a neurodevelopmental disorder. Analysis of *MBD5* in other model

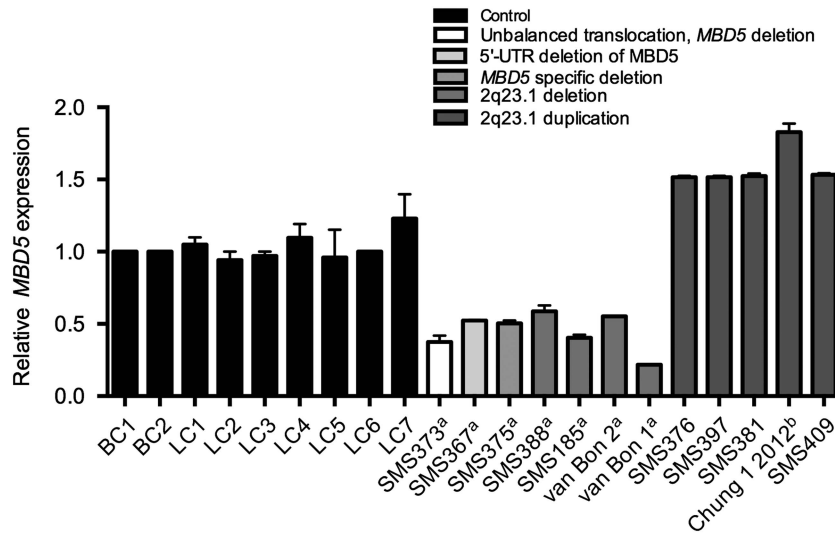


Figure 4 *MBD5* expression is altered by deletion, duplication or disruption of the *MBD5* gene. Quantitative real-time PCR expression analysis of *MBD5* is shown in LCLs or peripheral blood lymphocytes from individuals with 2q23.1 deletions, 2q23.1 duplications, and normal controls using predesigned assays from Taqman MGB probes for *MBD5* and *GAPDH*. *GAPDH* served as the endogenous control. Differences in transcript levels were quantified according to the $\Delta\Delta C_t$ method and normalized to *GAPDH*. Expression of all controls was normalized to 1.0. Acquired data were analyzed with 7500 Fast System SDS Software. Each bar represents mean (\pm SEM) of values from 3–10 independent experiments. Data show an expected range of expression for normal controls, 0.94- to 1.23-fold *MBD5* expression in lymphocytes (BC1-2) and LCLs (LC1-7). Samples from 2q23.1 deletion syndrome patients show 0.22- to 0.59-fold expression of *MBD5* ($P < 0.0001$ for all cases). Samples from individuals with 2q23.1 duplication show overexpression of *MBD5*, ranging from 1.52- to 1.83-fold compared with normal controls ($P < 0.0001$ for all cases). The expression changes observed indicate *MBD5* is a dosage-sensitive gene. ^aTalkowski *et al*, 2011; ^bChung *et al*, 2012.²

Table 3 Dosage-sensitive genes

Gene(s)	Deletion syndrome	Duplication syndrome
<i>MECP2</i>	Rett syndrome (OMIM # 312750)	Lubs X-linked mental retardation syndrome (OMIM # 300260)
<i>TBX1</i>	DiGeorge syndrome (OMIM # 188400)	Microduplication 22q11.2 syndrome (OMIM # 608363)
	Velocardiofacial syndrome (OMIM # 192430)	
<i>RAI1</i>	Smith-Magenis syndrome (OMIM # 182290)	Potocki-Lupski syndrome (OMIM # 610883)
<i>NSD1</i>	Sotos syndrome (OMIM # 117550)	Duplication <i>NSD1</i>
<i>ELN, GTF2, LIMK1, CLIP2</i>	Williams-Beuren syndrome (OMIM # 194050)	Williams-Beuren region duplication syndrome (OMIM # 609757)
<i>JAG1</i>	Alagille syndrome (OMIM # 118450)	Duplication 20p11
<i>UBE3A</i>	Angelman syndrome (OMIM # 105830)	Chromosome 15q11-q13 duplication syndrome (OMIM # 608636)
<i>SNRNP</i>	Prader-Willi syndrome (OMIM # 176270)	Chromosome 15q11-q13 duplication syndrome (OMIM # 608636)
<i>PLP1</i>	Pelizaeus-Merzbacher disease (OMIM # 312080)	Pelizaeus-Merzbacher disease (OMIM # 312080)
<i>PMP22</i>	Neuropathy, hereditary, with liability to pressure palsies (OMIM # 162500)	Charcot-Marie-Tooth disease Type 1A (OMIM # 118220)
<i>PAK2, DLG1</i>	3q29 deletion syndrome (OMIM # 609425)	3q29 duplication syndrome (OMIM # 611936)

systems, such as mouse and induced pluripotent stem cells, will provide insight into cellular requirement for normal *MBD5* dosage. Overexpression associated with a partial gene duplication is important to note because diagnostic laboratories might conclude that partial gene duplications are not pathogenic. However, the cases presented here illustrate that duplication of non-coding exons may be sufficient for overexpression and further illustrate the complexity of the effects of genomic alteration on transcription. Additional studies will be required to fully understand the transcripts that are produced in these variants.

The phenotype associated with a deletion, mutation or duplication of *MBD5* combined with the gene expression changes observed suggests that *MBD5* is a dosage-sensitive gene critical for normal development. Several genomic disorders are caused by CNVs involving one or more dosage-sensitive genes (Table 3). Thus, *MBD5* and chromosome 2q23.1

can be added to an expanding list of dosage-sensitive genomic regions that result in reciprocal deletion and duplication neurodevelopmental syndromes (Table 3).^{13,14} Some dosage-sensitive genes when deleted or duplicated result in similar phenotypes, such as *MECP2*.^{13,15} For other disorders, deletions and duplications involving the same dosage-sensitive genes result in significantly different clinical presentations, such as seen in Williams syndrome and chromosome 7q11.23 duplication syndrome.^{13,16} From the current study, it appears that *MBD5* falls into the former category of dosage-sensitive genes, but additional cases will need to be studied to determine if there are features which have not been identified or are underappreciated. The phenotypes observed in the microdeletion and microduplication syndrome are similar, with the 2q23.1 microduplication syndrome having a somewhat milder phenotype. The absence of seizures in the duplication cohort is a notable difference.

Previous studies have suggested that genomic duplications can present with a subtler phenotype. As we have shown in this study, there is significant potential for duplication carriers to go undetected.¹⁷ Furthermore, these carriers may transmit their duplication to their offspring, who may exhibit a more severe phenotype.¹⁸ We have three duplication cases in our cohort that exhibit maternal inheritance who all exhibit severe phenotypes in comparison to their mothers. Analysis of additional patient and parent samples will elucidate if there is a maternal inheritance bias or possible imprinting effect associated with this 2q23.1 region and the potential familial variable expressivity of the duplication syndrome. We previously implicated deletions of *MBD5* as clearly deleterious genomic alterations¹ and now provide data to support that duplications of *MBD5* also confer risk. Given the rare parental transmission of alterations of *MBD5* (whether deletion, mutation or duplication), these are not fully penetrant alterations. They were discovered by the assessment of non-recurrent CNVs and translocations across testing sites to identify the minimal region of overlap. By definition, these non-recurrent events are not polymorphisms. Moreover, the effect size reported previously¹ and in this study suggests disruption of the locus is rare in individuals without neurodevelopmental abnormalities. There is no evidence from any studies to date to suggest benign, common polymorphisms disrupt the exonic regions of *MBD5*; however, the data are insufficient to empirically determine the frequency of small recurrent events confined to intronic regions with current technical resolution; thus, the effect of intronic events within *MBD5* is not known at this time and requires additional study.

Further, we illustrate overlap between the features of 2q23.1 duplication and other syndromes such as Williams syndrome, fragile X syndrome, Rubinstein-Taybi syndrome, Smith-Magenis syndrome, Angelman syndrome and Prader-Willi syndrome. The cheerful demeanor and ease with strangers that is seen in Williams syndrome is also seen in some 2q23.1 duplication patients.¹⁹ Behavioral characteristics such as stereotypic movements and self-injurious behavior that are seen in 6/9 of the duplication subjects are also seen in fragile X syndrome and Smith-Magenis syndrome.^{20,21} The likeable and happy disposition of 2q23.1 duplication subjects is also present in the Angelman syndrome phenotype.²² The broad first toes, which are prevalent in the duplication subjects are also found in the Rubinstein-Taybi phenotype; however, broad thumbs were not observed.²³ These similarities between phenotypes suggest that the genes causative for these syndromes are possibly involved in overlapping molecular networks that, when altered, contribute to the observed phenotypes.

Overall, these findings suggest that precise gene dosage of *MBD5* is clearly required for normal development of speech/language and normal cognitive functions. Furthermore, these findings support a role for *MBD5* in neuronal development by mediating central nuclear functions important for regulation of gene dosage and expression. Finally, based on the similarity of *MBD5* disorders to other well-known syndromes, testing for *MBD5* dosage changes and/or mutation should be considered after molecular diagnosis for certain neurodevelopmental disorders proves futile.

CONFLICT OF INTEREST

Jill A Rosenfeld is an employee of Signature Genomic Laboratories, Perkin Elmer, Inc., Swaroop Aradhya is an employee of Gene Dx, Inc.,

and Stuart Schwartz is an employee of Laboratory Corporation of America.

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