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Disruption of *MBD5* contributes to a spectrum of psychopathology and neurodevelopmental abnormalities

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Conflict of interest

The authors declare no conflict of interest.

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Abstract

Microdeletions of chromosomal region 2q23.1 that disrupt *MBD5* contribute to a spectrum of neurodevelopmental phenotypes, however the impact of this locus in human psychopathology has not been described. To characterize the structural variation landscape of *MBD5* disruptions and the associated psychopathology, 22 individuals with genomic disruption of *MBD5* (translocation, point mutation, and deletion) were identified through whole-genome sequencing or cytogenomic microarray at 11 molecular diagnostic centers. The genomic impact ranged from a single base pair to 5.4 Mb. Parents were available for 11 cases, all of which confirmed the rearrangement arose *de novo*. Phenotypes were largely indistinguishable between patients with full-segment 2q23.1 deletions and those with intragenic *MBD5* rearrangements, including alterations confined entirely to the 5′UTR, confirming the critical impact of non-coding sequence at this locus. We found heterogeneous, multi-system pathogenic effects of *MBD5* disruption and characterized the associated spectrum of psychopathology, which includes sensory integration disorder, anxiety, self-hugging, bipolar disorder and others. Importantly, unique features of the oldest assessed patient were early-onset dementia and behavioral regression. Analyses also revealed phenotypes that distinguish *MBD5* disruptions from seven well-established syndromes with significant diagnostic overlap. This study indicates that haploinsufficiency of *MBD5* causes diverse phenotypes, yields insight into the spectrum of resulting neurodevelopmental and behavioral psychopathology, and provides clinical context for interpretation of *MBD5* structural variations. Empirical evidence also suggests that disruption of non-coding *MBD5* regulatory regions is sufficient for clinical manifestation, highlighting the limitations of exon-focused assessments. These results suggest an ongoing perturbation of neurological function throughout the lifespan, including risks for neurobehavioral regression and early-onset dementia.

Keywords

MBD5; 2q23.1 microdeletion syndrome; chromosomal microarray; next generation sequencing; regression

Introduction

The increasing resolution of genomic technologies, including cytogenomic microarrays and next-generation sequencing, has led to remarkable advances in our understanding of the highly heterogeneous genetic etiology of neurodevelopmental conditions such as autism spectrum disorders (ASD). Among the largest known genetic risk factors contributing to ASD are recurrent structural variations such as chromosomal microdeletions and microduplications. However, interpretation of the associated clinical outcomes is complicated by the varying sizes of these dosage imbalances which typically disrupt many genes. For example, the 16p11.2 microdeletion and microduplication syndromes are associated with a spectrum of features including ASD, schizophrenia, obesity, dysmorphic

characteristics, and numerous other neuropsychiatric and behavioral disorders.[1, 2] Only a small number of recurrent chromosomal microdeletion syndromes have a single known contributing gene, such as *SATB2* in 2q33.1[3, 4] and *EHMT1* in 9q34.3[4, 5]. The localization of these contributing loci provides a route to understanding pathogenic mechanisms as well as deeply characterizing associated phenotypic features of these typically heterogeneous and complex syndromes. In the current study, we perform extensive phenotypic analyses of the diverse psychopathology associated with a single necessary and sufficient causal locus in the chromosome 2q23.1 microdeletion syndrome and describe the clinical features that overlap with multiple known syndromes.

The pathogenesis of 2q23.1 microdeletion syndrome was recently defined by Talkowski *et al*[6] and others[7–15] to be due to haploinsufficiency of a single gene, *MBD5* (methyl-CpG-binding domain protein 5), through characterization of the minimal region of overlap from non-recurrent genomic alterations in a cohort of 65 cases with chromosomal deletions or translocations. The mRNA isoform 1 of *MBD5* comprises 15 exons with exons 1–5 forming the non-translated 5′UTR. Monoallelic expression of this locus was found to be highly penetrant as both partial and complete deletions of *MBD5* involving coding and/or non-translated exons resulted in diverse neurodevelopmental phenotypes including ASD, intellectual disability, and seizures. In addition, no alterations of *MBD5* exons were observed in 7,878 controls or in the Database of Genomic Variants (DGV).[6]

The association of *MBD5* disruption with ASD and other neurobehavioral features is supported by its expression in the brain and the suggested function of its methyl-CpG-binding domain in heterochromatin and epigenetic regulation.[16, 17] In addition, sequencing of the *MBD5* coding region identified a missense mutation (p.79Gly>Glu) in this functionally critical domain that was significantly overrepresented in 747 ASD patients compared to 2,043 individuals from an exome sequencing study of patients with disorders of the heart, lungs and blood (NHLBI Exome Sequencing Project; initial $p = 0.012$, OR = 5.5).[6] Notably, the recent completion and revised variant calling in the final exome sequencing analyses of this mutation provides a more robust estimate of its association and effect size in ASD ($p = 0.0039$, OR = 5.2).[18] The methyl-CpG-binding domain is also shared with *MECP2*, a causal locus for Rett syndrome. Clinical features associated with *MBD5* deletions have considerable overlap with Rett syndrome and can also masquerade as Smith-Magenis (SMS), Cornelia de Lange (CdLS), Angelman (AS), Prader-Willi (PWS), Kleefstra, and Pitt-Hopkins (PTHS) syndromes.

We report here phenotypic characterization of 22 individuals with *MBD5* structural alterations collected through a multi-institution collaboration, revealing a complex clinical syndrome that involves a diverse range of neurodevelopmental features with variable severity. A significant inclusion in this cohort is the oldest assessed *MBD5* deletion patient who provides important confirmatory evidence that behavioral regression is a potential outcome. Interestingly, a subset of these patients with disruptions confined to the non-coding region of *MBD5* manifest a similar spectrum of clinical features as those with coding region rearrangements, confirming a pathogenic role for non-coding regulatory domains. We further identify considerable phenotypic overlap between *MBD5* disruptions and other

known diagnoses, and provide clinical context for syndrome differentiation with an emphasis on neurobehavioral considerations.

Methods

Subjects

Diagnostic screening of postnatal peripheral blood specimens from 17,477 consecutive patients tested in the Mayo Clinic Cytogenetics Laboratory from 2008–2012 using either the 4×44K or 4×180K oligonucleotide-based whole-genome cytogenomic microarray (Agilent Technologies, Santa Clara, CA) identified eight deletions spanning genomic segments including *MBD5* (Cases 1–8), six of which were of sufficient size for detection (>100 kb) and had cells available for confirmation by fluorescence *in situ* hybridization (FISH). Clinical phenotypic information was obtained from medical records following a Mayo Clinic IRB-approved protocol. A multi-institution collaboration of clinical diagnostic laboratories resulted in inclusion of additional patients not previously published from the Greenwood Genetic Center (Cases 9–12), Pathology Associates Medical Laboratories (Cases 13–14), Virginia Commonwealth University (Case 15), Fullerton Genetics Center (Case 16), and Boston Children's Hospital (Case 17) that were identified with different cytogenomic microarray platforms. Phenotypes were further considered in the analysis of two *MBD5* deletion cases reported by Motobayashi *et al*[19] (Motobayashi/Case 18) and Noh *et al*[20] (Noh/Case 19) as well an intragenic frameshift mutation c.150del or p.Thr52Hisfs*31 described by Kleefstra *et al*[5] (Kleefstra/Case 20) and an intragenic indel of -TC at position 148,942,435 in hg18 of exon 9 described by O'Roak *et al*[21] (O'Roak/Case 21), all of which were published after the Talkowski *et al* study[6]. The final patient, DGAP235 (Case 22), was enrolled in the Developmental Genome Anatomy Project (DGAP; www.dgap/harvard.edu) based on karyotypic detection of a *de novo*, balanced translocation 46,XY,t(2;5)(q22;q22), which was found to disrupt *MBD5* by massively parallel paired-end sequencing; this case was independent of two previously published translocations targeting *MBD5*.[6]

Genetic analysis of translocation case

Whole genome sequencing was performed using large-insert fragments in a mate-pair method that was previously optimized and applied to cases with abnormal karyotypes.[4, 22, 23] Sequencing paired-ends of fragments separated by 2,000 bases in genomic distance enabled high coverage of mapped inserts in a genome-wide manner with minimal individual nucleotide coverage required. In brief, genomic DNA was randomly sheared and size selected for 2 kb fragments. A cap adaptor containing an *EcoP*15I restriction site was ligated to the fragment ends and circularized with an internal adaptor containing a subject specific bar code and a single biotinylated thymine. The circularized fragments were restriction digested, junction fragments were isolated by binding the biotinylated adaptor to streptavidin beads, and an Illumina library was prepared directly on the beads. Multiplexed paired-end 25 cycle sequencing was performed on an Illumina HiSeq 2000 and 45.7% of all reads corresponded to DGAP235 (87,079,316 reads pairs). Quality control was assessed using FASTQC (Babraham Bioinformatics) followed by distributed parallel alignment of FASTQ read data to the human genome reference hg19 using BWA 0.5.9[24], merging of aligned BAM files with SAMtools 0.1.18[24], and coordinate and name-

sorting of aligned read-pairs using Picard Tools 1.5.8 (http://picard.sourceforge.net). A single-linkage clustering of anomalous read-pairs was competed with subsequent filtering of clusters based on size, mapping quality, uniqueness, and presence in a previous database of whole genome sequencing samples from a neurodevelopmental cohort[4] using a custom pipeline developed in C++ (BamStat) and MATLAB (Mathworks). The average mapped insert was 1,862 bp (standard deviation = 332 bp) and 94.1% of all reads aligned, yielding an average coverage of mappable inserts of approximately 39.2X (Supplementary Figures S1). See Supplementary Table S1 and Supplementary Results for library metrics, alignment information and bioinformatics analysis.

Phenotypic analysis

The developmental history and neurological, behavioral, and physical characteristics of the new patients were assembled. In addition, the original phenotypic data from the 65 cases reported by Talkowski *et al*[6] were examined. Age-dependent characteristics were considered relative to developmental stage. Individuals were considered to have "autistic-like symptoms" if autistic-like behaviors were specifically reported or a diagnosis of pervasive developmental disorder (PDD-NOS) was given. Phenotypic features were classified as present, absent, or not evaluated, and were reported in Table 3 only if present in more than one individual.

The clinical features of the new cohort (n = 22) were assessed for both overlap with and divergence from those cases in the published original cohort that had phenotypic data available (n = 48) to enable a more comprehensive phenotypic profile associated with *MBD5* disruption. A list of the 48 cases analyzed from the original cohort of Talkowski et al[6] is provided in the Supplementary Results. The phenotypic spectrum of the combined original and new *MBD5* disruptions (n = 70) was then compared and contrasted to well-established syndromes with diagnostic overlap (SMS, CdLS, AS, PWS, Rett, Kleefstra and PTHS). In each of these evaluations, cases were grouped by genotype, *i.e.* intragenic *MBD5* disruptions versus overlapping 2q23.1 deletions.

Results

Identification and molecular characterization of MBD5 disruption cases

The frequency of *MBD5* deletion was estimated to be 0.05% based on the identification of 8 cases out of 17 477 consecutive Mayo Clinic peripheral blood samples submitted for clinical testing by cytogenomic microarray. These 8 deletions, along with 10 cases from collaborators and 4 cases identified recently in the literature, define a new cohort that is independent of the subjects in Talkowski *et al*[6]. The new cohort (n = 22) includes one frameshift mutation, one indel mutation, one inverted translocation directly disrupting *MBD5*, three intragenic *MBD5* deletions, and 16 larger deletions. The rearrangements arose *de novo* in all cases for which parental testing was available (n = 11). However, two cases without parental genetic or phenotypic information involve brothers with similar deletions, suggesting likely parental inheritance or gonadal mosaicism. No bias in gender or age of diagnosis was observed in the 13 males and nine females ranging in age from two weeks to 69 years. The disrupted region varied from a single base pair point mutation to a 5.4 Mb

microdeletion, with 17 of the alterations being no more than 1.1 Mb (Figure 1a). Five of these alterations are within *MBD5*, all but one of which is confined entirely to the non-coding 5′UTR. In addition, Case 2 is a high percentage mosaic with the deletion present in 28/30 metaphases and 77.5% of interphase nuclei from a cultured specimen as well as 81.0% of interphase nuclei from a direct preparation.

Case 22 harbored a *de novo* translocation between chromosomes 2 and 5 that was balanced at karyotypic resolution. Whole-genome sequencing by large-insert jumping libraries revealed direct disruption of *MBD5* at the breakpoint on chromosome 2. The orientation of a small subset of reads relative to the reference sequence also supported the presence of a small local inversion at the chromosome 5 breakpoint on the der(2) chromosome, suggesting a translocation with an accompanying inversion (Figure 1b). We previously discovered that such microinversions are a pervasive feature of karyotypically balanced translocations.[22] Capillary sequencing confirmed the translocation breakpoints on both derivative chromosomes and the presence of a 169 bp microinversion of chromosome 5 on the der(2). The chromosome 2 breakpoints (der(2):148,732,432; der(5):148,733,228 in human genome build hg18) directly disrupt the 5′ non-coding region of *MBD5* that was previously shown to be interrupted in two independent translocations sequenced in Talkowski *et al*[6]. In sum, a total genomic imbalance of 795 bp was observed at the der(5) breakpoint (including both deleted sequence and duplication of microhomology), and the der(2) translocation and inversion events resulted in 41 bp of deleted sequence. The chromosome 5 breakpoint occurred in an intergenic region without annotated coding sequence within a 500 kb window of the breakpoint. Sequencing thus revised the interpretation of the karyotype from 46,XY,t(2;5)(q22;q22) to 46,XY,der(2)t(2;5)(q23.1;q23.1)inv(5)(q23.1q23.1),der(5)t(2;5)(q23.1;q23.1)dn. See Supplementary Table S2 for complete breakpoint information.

In the original cohort described by Talkowski *et al*[6] there were 12 intragenic *MBD5* deletions, two other translocations disrupting the same untranslated region of *MBD5* as the present case, and 41 larger 2q23.1 microdeletions encompassing *MBD5* and additional genes ranging in size from 38 kb to 19.3 Mb. After removal of the Talkowski *et al* cases without phenotypic information, the remaining 48 individuals were added to the 22 new cases for analysis. This combined cohort (n = 70) involved three translocations, one point mutation, one indel, and 65 deletions; 16 are intragenic *MBD5* alterations, all but one of which are confined to the 5′UTR. The following analyses are derived from the phenotypic features of these 70 individuals.

MBD5 disruption is associated with neurodevelopmental features and clinical variation including behavioral regression

Comparison of the original raw data from the *MBD5* disruption cases in Talkowski *et al*[6] to a new cohort of 22 cases demonstrated an independent confirmation of substantial genetic and phenotype overlap between the two groups. Combined assessment of these cohorts showed that *MBD5* disruption has a multi-system effect, leading to behavioral, growth/endocrine, craniofacial, skeletal, gastrointestinal, heart, urogenital and central nervous system anomalies.

The phenotypes are largely indistinguishable between intragenic *MBD5* and larger deletions, whether considering neurodevelopmental and behavioral abnormalities specifically or all other features (Table 1). However, a small subset of the characteristics may have resulted from loss of neighboring genes. This is suggested by features that were confined to larger deletion cases in both the new cohort and separately in the original cohort[6] that were assessed in at least 4 patients, specifically bruxism, hyperphagia, ataxia, obesity, brachycephaly, metopic ridging, optic nerve hypoplasia, strabismus, coarse facies, wide mouth, brachydactyly and hypoplastic genitalia. It is of note that only 2 of these twelve features are neurodevelopmental or psychiatric in origin, which further implicates *MBD5* as the causative gene for the majority of such phenotypes. Additional features potentially arising from genes near *MBD5* are demonstrated in the combined cohort analysis (Table 1). However, definitive genotype/phenotype correlations are challenging due to the relative rarity of intragenic *MBD5* disruptions (n = 16) compared to 2q23.1 microdeletions (n = 54), warranting further consideration in even larger cohorts.

Potential novel behavioral features linked with *MBD5* disruption are suggested in the new cohort including sensory integration disorder, anxiety, bipolar disorder and self-hugging, as well as a number of physical anomalies (Table 2). The associations of *MBD5* disruption with optic nerve hypoplasia and with social withdrawal were also potentially confirmed through observation of a second affected patient (Case 3 and Case 21, respectively). The new cohort further includes the oldest assessed *MBD5* disruption patient, a 44-year old man who had the novel features of early-onset dementia and cataracts (Case 3). Importantly, he demonstrated behavioral regression with worsening skin picking and obsessive-compulsive tendencies. Regression of motor and verbal skills was also noted in a second case in the new cohort (Case 19).[20]

Phenotypic presentation of MBD5 disruption overlaps with known syndromes

We analyzed extensive phenotypic data in a large cohort of subjects with *MBD5* disruptions to understand the consequences of multiple mutational mechanisms impacting transcription of this gene and to provide an assessment algorithm for clinicians to identify affected patients. However, devising an appropriate testing strategy presents a significant challenge due to substantial heterogeneity in both the type and severity of *MBD5*-related phenotypes which span cognitive, behavioral and physical domains. Moreover, *MBD5* disruption is a potent masquerader, resulting in a number of clinical features previously thought to be unique to other syndromes. Based on our analyses, it is clear that a first-tier diagnostic cytogenomic microarray test will aid in the partitioning of subjects into gene-specific disorders characterized by dosage imbalance. Such a recommendation would be consistent with recent guidelines.[25, 26] Absence of detectable dosage imbalance will then require clinicians to decide if karyotype or targeted gene-specific testing, such as sequencing for intragenic point mutations, small deletions or duplications, is appropriate based on the phenotypic gestalt. Of note, although whole exome sequencing is moving towards replacing cytogenomic arrays and gene or gene panel sequencing, targeted gene analysis could still have an important role in the future as UTR variants, including those in multiple cases in the current study, might not otherwise be detected.

To aid in determining the next diagnostic step in the absence of a detectable dosage imbalance, we present expansion of the clinical heterogeneity associated with *MBD5* disruption, as well as the distinguishing phenotypes or constellations of anomalies which would suggest additional diagnostic testing for *MBD5* versus targeting a classic syndrome with overlapping features including SMS[27–32], PWS[33–38], AS[39, 40], CdLS[41–44], Rett[45–47], Kleefstra[48–50], or PTHS[51–53]. Thus, the phenotypes in the combined cohort separated by intragenic *MBD5* versus larger 2q23.1 deletions that occurred in two or more cases were compared to those of other syndromes considered in the differential diagnosis (Table 3).

Neurodevelopmental and behavioral characteristics common to all of these syndromes include autistic-like actions, intellectual disability, developmental delay, speech delay, motor delay, hypotonia, sleep disturbances and seizures. There are also features confined solely to each of the syndromes in the differential diagnosis as detailed in the Supplementary Results. Although single characteristics can be helpful, the phenotypic diversity noted with *MBD5* disruptions remains a diagnostic challenge, as illustrated in the divergence of facial dysmorphism in *MBD5* deletion cases, ranging from isolated ear anomalies to multiple atypical characteristics (Figure 2). A clear understanding of how the breadth of phenotypes compares and contrasts for these multiple diagnoses is required. The differentiating and overlapping neurodevelopmental, behavioral, and other features presented in Table 3 are further described in detail for each syndrome in the Supplementary Results.

Discussion

Deletions encompassing *MBD5* are highly penetrant and result in a broad phenotypic spectrum that includes many neurodevelopmental and psychiatric traits. Our analyses of 22 cases with extensive clinical information confirm the phenotypes previously described in an independent cohort of 65 subjects described in Talkowski *et al*[6] and define the significant psychopathology associated with *MBD5* disruptions. When combined, these data demonstrate similar phenotypic traits between individuals with intragenic *MBD5* alterations and larger 2q23.1 microdeletions, consistent with *MBD5* being the necessary and sufficient pathogenic target in 2q21.3 microdeletion syndrome. The new cohort further expands the potential phenotypic spectrum to include the behavioral characteristics of sensory integration disorder, anxiety, bipolar disorder, and self-hugging, as well as the physical anomalies polydactyly, skin tags, diaphragmatic eventration, ventricular septal defect (VSD), spina bifida, cutis marmorata, flat occiput and short neck. The associations with optic nerve hypoplasia and with social withdrawal, each observed in a single patient in the original cohort[6], were also demonstrated by independent cases in this new cohort.

The potential for *MBD5* to perturb neurological function throughout life was demonstrated by the oldest assessed *MBD5* deletion patient at 44 years of age (Case 3). Such a delayed diagnosis is consistent with the difficulty in clinically identifying *MBD5* deletions and suggests potential longevity for affected individuals; a normal lifespan is further implied by a 69 year old male (O'Roak/Case 21), although his associated features are only known through 4 years of age. The 44 year old patient had early-onset dementia, cataracts and behavioral regression with a significant increase in skin picking and obsessive-compulsive activities. He thus represents the third case for which regression has been documented and

the first case with early-onset dementia. Regression also occurred in a second patient included within the new cohort (Noh/Case 19), a 2 year old girl with a 300 kb deletion overlapping two genes at 2q23.1. This child walked independently at 28 months of age but an examination at four years showed that she had lost a subset of her vocabulary and the ability to walk, standing only with support.[20] Another case of developmental regression was previously noted to occur suddenly at six years of age in a girl with a de novo approximately 4 MB deletion involving 15 genes at 2q23.1-q23.3. This child had progressive difficulties with fine motor skills and balance, worsening behavior, and loss of the ability to draw lines and circles.[9] It is of note that this child was originally tested for Rett and Angelman syndromes with normal results before the *MBD5* deletion was identified. These results merit careful evaluation of future cases for developmental and behavioral regression and emphasize the recommendation of cytogenomic microrarray as a first-tier test for all such cases.

Many features found in *MBD5* deletions such as intellectual disability, motor and speech delays, sleep disturbances, microcephaly, and seizures are non-specific and commonly found in multiple genetic syndromes. A more specific spectrum of behavioral and other phenotypes in individuals with *MBD5* disruptions may be diagnostically useful, although it remains challenging to differentiate from the profiles in other syndromes including SMS, CdLS, AS, PWS, Rett, Kleefstra, and PTHS. In fact, many patients with *MBD5* deletions previously reported in the literature were referred for genetic testing for one or more of these disorders, all of which were negative, prior to cytogenomic microarray providing an accurate diagnosis; this is illustrated well in a study of 2q23.1 microdeletion syndrome patients (n = 11) who had normal test results for AS (n = 8), SMS (n = 5) and Rett (n = 4) syndrome.[8] In the current cohort, the known additional testing included karyotype (n = 6), fragile X analysis (n = 6), *RAI1* sequencing for SMS (n = 2), *MECP2* and *CDKL5* sequencing for Rett syndrome (n = 1), methylation analysis and *UBE3A* sequencing for PWS/AS (n = 1), and *TCF4* sequencing for PTHS (n = 1). It is of note that guidelines indicate fragile X testing should be performed for any patient with intellectual disability[54], a feature also found in *MBD5* disruption, although there is no overlap of more specific phenotypes between these two syndromes which suggests that they should be clinically distinguishable.

The ability of *MBD5* deletions to manifest significant clinical heterogeneity that overlaps with previously defined syndromes in a highly penetrant manner confirms the advantage of using unbiased, high resolution genome-wide surveys such as cytogenomic microarrays when any of the above mentioned syndromes is suspected. However, cytogenomic microarrays do not detect balanced abnormalities or small pathogenic mutations, as occurred in cases 20 through 22, suggesting that implementation of whole-genome sequencing will also have a significant diagnostic impact and karyotyping may reveal additional balanced rearrangements. Our results further raise important issues about the current interpretation of variants of unknown significance and coding region-focused analyses such as exome sequencing as we found highly penetrant pathogenic rearrangements confined to the putative 5′UTR of *MBD5* (as in cases 5, 6, 16, 20 and 22) to be contributory towards diverse phenotypes. Therefore, a focused phenotypic annotation was undertaken in the current study

to complement the recent genetic characterization[6] of *MBD5* disruptions and to identify features that contrast with syndromes for which *MBD5* can be mistaken. This may help determine an appropriate testing strategy and avoid unnecessary medical expenses.

In summary, careful phenotyping of patients with *MBD5* disruptions confirms that abnormalities at this locus are highly penetrant risk factors for neurodevelopmental and other abnormalities. We also extend the associated clinical heterogeneity to a broad range of neuropsychiatric features including early-onset dementia, provide a differential diagnostic context, and suggest that this locus is potentially associated with risk for neurobehavioral regression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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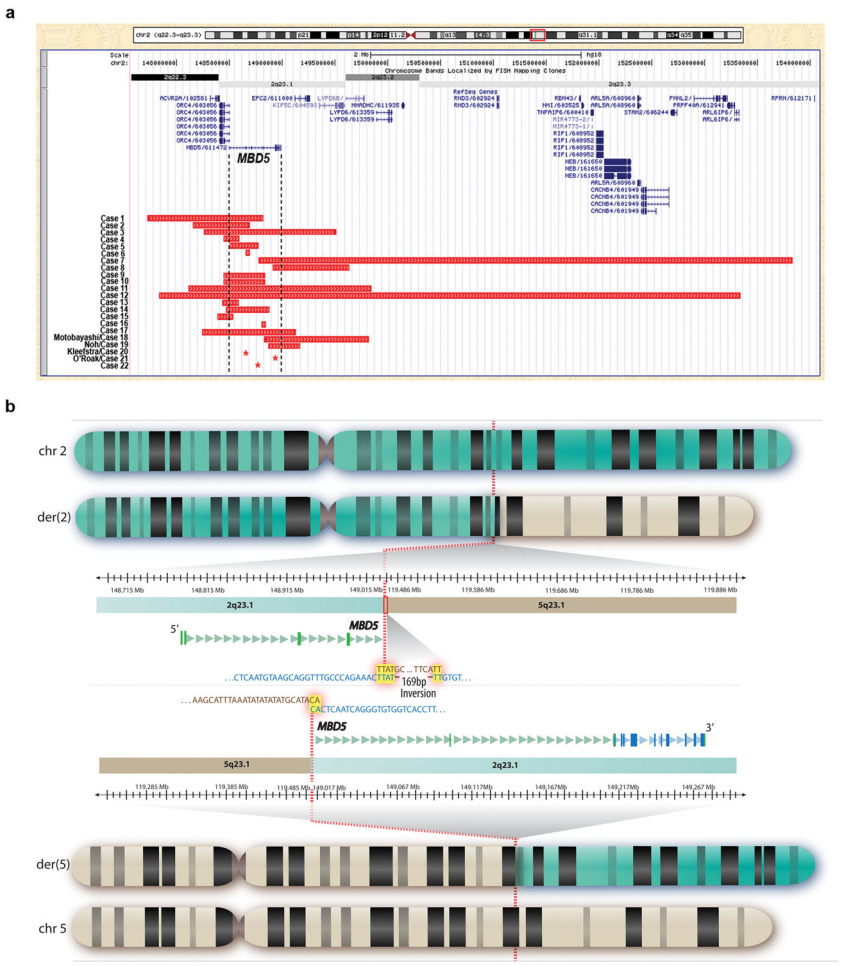

Figure 1.

Structural alterations disrupting *MBD5*. (**a**) UCSC genome browser (genome build hg18) demonstrating 22 cases of intragenic *MBD5* and larger 2q23.1 disruptions (red bars) identified by cytogenomic microarray. *MBD5* is at position 148,495,050–148,987,514 and contains 15 exons of which exons 1–5 are the non-coding 5′UTR and exons 6–15 are protein coding. Cases 5, 6, 16, 20 and 21 involve disruptions confined to the non-coding region of *MBD5* and Case 22 is confined to the coding region of *MBD5*, while all other cases overlap at least one other gene. Asterisks (*) denote a single base pair change resulting in a frameshift mutation in Case 20, a two base pair deletion in Case 21, and the translocation breakpoint in Case 22. (**b**) Whole genome sequencing of Case 22 delineated that an apparently balanced translocation between chromosomes 2 and 5 directly disrupted *MBD5* at 2q23.1 and did not affect any annotated functional sequence on chromosome 5. A local microinversion of 169 bases was also detected at the breakpoint of the chromosome 5 material on the der(2) chromosome. The GTG-banded idiograms depict the normal chromosomes 2 and 5 as well as the derivative chromosomes from the translocation. The breakpoint regions on the derivative chromosomes are expanded in the middle, providing genomic coordinates, cytogenetic bands, precise breakpoints (dotted red line), and surrounding nucleotide sequence of the junctions including microhomology (highlighted in

yellow) at the translocation and inversion breakpoints. The 5′ and 3′ non-coding UTR regions of the disrupted *MBD5* transcript are highlighted in green, the translated region is highlighted in blue, and exons are denoted by rectangles.

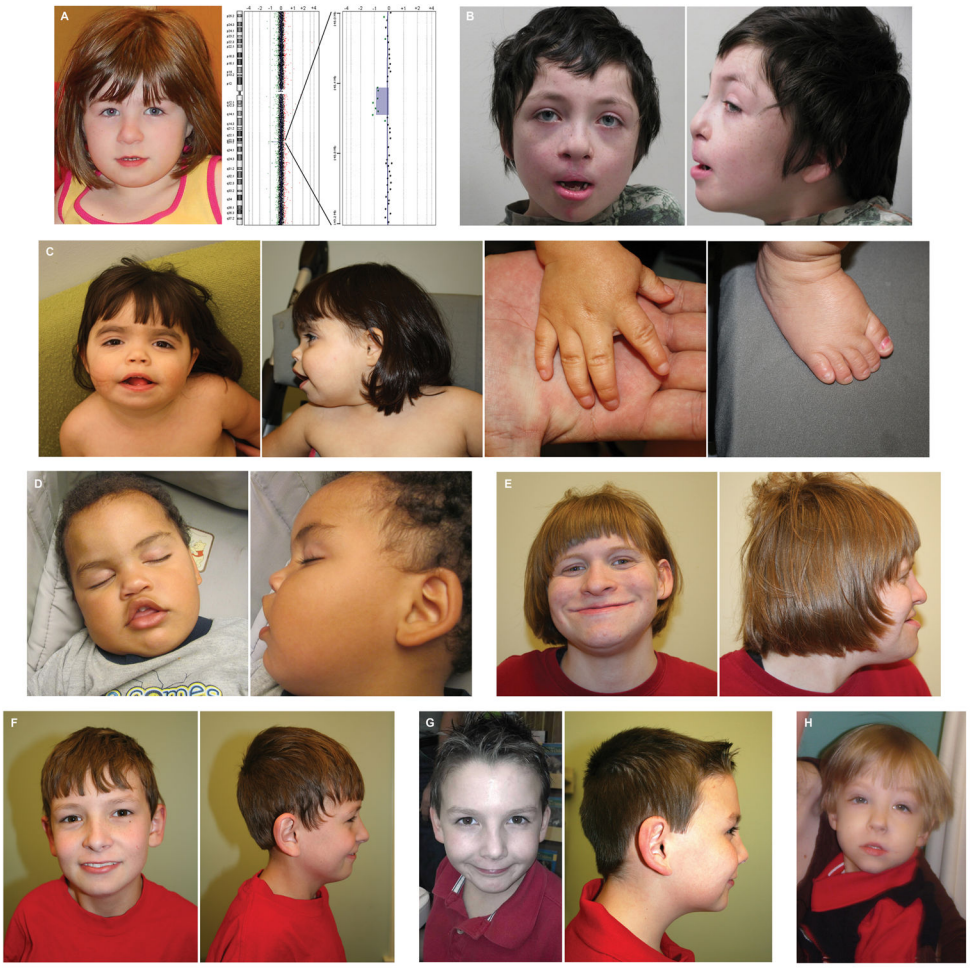

Figure 2.

Clinical features associated with *MBD5* disruption.

Patients with *MBD5* deletions have a broad range of physical features both in type and severity. A, Case 4 (6 year 9 month old female) has a round face, midface hypoplasia, flat nose and thin upper lip, which along with ID/DD, hearing loss, and unusual behavior of self-injury and altered sleep cycle, were highly suggestive of SMS. Cytogenomic microarray demonstrated a *de novo* deletion (153 kb) of the *MBD5* 5′ UTR while subsequent sequencing of the *RAI1* gene associated with SMS was negative. B, Case 15 (7 year 9 month old male) has brachycephaly, midface hypoplasia, depressed nasal bridge, a thin and tented upper lip, open mouth and dental crowding. C, Case 12 (3 year old female) has dysmorphic facies with synophrys, slightly downslanting palpebral fissures, tented upper lip, depressed nasal bridge with an upturned nose and somewhat prominent ears with attached lobes as well as a low posterior hairline, right supernumerary nipple, short neck, short thumbs and fifth fingers, and mildly dysplastic fifth toenails. D, Case 8 (2 year old male) has an open mouth with downturned corners, a depressed nasal bridge, and an overfolded helix. E, Case 11 (20 year old female) has a thin upper lip, a slightly smooth philtrum, mild epicanthal folds with slightly upslanting palpebral fissures and a prominent columella with a thickened nasal tip. F, Case 8 (14 year old male) has mildly prominent and overfolded ears with

thickened helices. G, Case 9 (12 year old male) is the brother of Case 8 and has similar atypical ears. H, Case 16 (7 year old male) has a thin upper lip, long philtrum, prominent nasal bridge, arched eyebrows, epicanthal folds and almond-shaped eyes.

Table 1

Phenotypes in Intragenic *MBD5* Disruptions and 2q23.1 Microdeletions in the Combined Cohort[1]

Features		*MBD5*		2q23.1	
		Number Reported[2]	Total Cohort[3]	Number Reported[2]	Total Cohort[3]
Behavioral					
	Aggression/Temper	3/3 (100%)	3/16 (19%)	10/11 (90%)	10/54 (19%)
	Anxiety	1/1 (100%)	1/16 (6%)	1/2 (50%)	1/54 (2%)
	Autistic-like behaviors	9/9 (100%)	9/16 (56%)	43/44 (97%)	43/54 (80%)
	Bruxism (teeth grinding)	--	0/16 (0%)	5/5 (100%)	5/54 (9%)
	Distractibility/Short attention span	5/5 (100%)	5/16 (31%)	15/15 (100%)	15/54 (29%)
	Hand flapping	2/2 (100%)	2/16 (13%)	2/3 (66%)	2/54 (4%)
	Hyperactivity	--	0/16 (0%)	2/4 (50%)	2/54 (4%)
	Hyperphagia	0/4 (0%)	0/16 (0%)	7/11 (64%)	7/54 (13%)
	Inappropriate happy demeanor	--	0/16 (0%)	5/5 (100%)	5/54 (9%)
	Insertion of hand in mouth or hand biting	1/1 (100%)	1/16 (6%)	6/6 (100%)	6/54 (11%)
	Obsessive-compulsive or routine-bound behaviors	--	0/16 (0%)	4/5 (80%)	4/54 (7%)
	Self-injurious behaviors including skin and eye picking	2/4 (50%)	2/16 (13%)	18/27 (66%)	17/54 (31%)
	Sleep disturbances	5/8 (63%)	5/16 (31%)	17/22 (77%)	17/54 (32%)
	Social withdrawl	1/1 (100%)	1/16 (6%)	1/2 (50%)	1/54 (2%)
	Sensory processing disorder	--	0/16 (0%)	2/3 (66%)	2/54 (4%)
Central Nervous System					
	Ataxia/Unusual gait	0/4 (0%)	0/16 (0%)	18/26 (72%)	18/54 (33%)
	Developmental delay/Intellectual disability	15/15 (100%)	15/16 (94%)	52/52 (100%)	52/54 (96%)
	Feeding difficulties	2/3 (67%)	2/16 (13%)	15/16 (94%)	15/54 (29%)
	Hypotonia/Abnormal muscle tone	3/4 (75%)	3/16 (19%)	22/23 (96%)	22/54 (41%)
	Motor delay	6/6 (100%)	6/16 (38%)	32/32 (100%)	32/54 (60%)
	Seizures	11/12 (92%)	10/16 (63%)	31/38 (82%)	31/54 (57%)
	Speech delay	7/10 (70%)	7/16 (44%)	37/37 (100%)	37/54 (69%)
Growth/Endocrine					
	Growth retardation	2/5 (40%)	2/16 (13%)	23/40 (56%)	23/54 (43%)
	Hirsutism	0/5 (0%)	0/16 (0%)	4/20 (20%)	4/54 (7%)

Features		*MBD5*		2q23.1	
		Number Reported[2]	**Total Cohort[3]**	**Number Reported[2]**	**Total Cohort[3]**
	Obesity	0/1 (0%)	0/16 (0%)	5/9 (56%)	5/54 (9%)
	Short stature	3/6 (50%)	3/16 (19%)	25/33 (70%)	25/54 (41%)
Craniofacial					
Cranium					
	Brachycephaly	0/6 (0%)	0/16 (0%)	12/28 (43%)	12/54 (22%)
	Metopic ridging	--	0/16 (0%)	2/4 (50%)	2/54 4%)
	Microcephaly	1/8 (13%)	1/16 (6%)	26/32 (81%)	26/54(48%)
	Plagiocephaly	--	0/16 (0%)	2/4 (50%)	2/54 (4%)
	Broad forehead	2/2 (100%)	2/16 (13%)	3/5 (60%)	3/54 (6%)
Ears/Nose					
	Nasal abnormalities	6/6 (100%)	6/16 (38%)	34/35 (97%)	34/54 (63%)
	Outer ear abnormalities	2/6 (33%)	2/16 (13%)	18/21 (86%)	18/54 (33%)
Eyes					
	Almond shaped eyes	1/1 (100%)	1/16 (6%)	2/2 (100%)	2/54 (4%)
	Astigmatism	1/1 (100%)	1/16 (6%)	2/4 (50%)	2/54 (4%)
	Hypotelorism	0/5 (0%)	0/16 (0%)	7/22 (36%)	7/54 (13%)
	Myopia/Hypermetropia/Corrective Lenses	2/2 (100%)	2/16 (13%)	6/9 (67%)	6/54 (11%)
	Optic nerve hypoplasia	--	0/16 (0%)	2/4(50%)	2/54 (4%)
	Palpebral fissures - downslanting	1/1 (100%)	1/16 (6%)	2/4 (50%)	2/54 (4%)
	Palpebral fissures - long	--	0/16 (0%)	3/4 (75%)	3/54 (6%)
	Palpebral fissures - upslanting	1/1 (100%)	1/16 (6%)	1/3 (33%)	1/54(2%)
	Strabismus	--	0/16 (0%)	2/5 (40%)	2/54 (4%)
	Synophrys	2/6 (33%)	2/16 (13%)	10/20 (50%)	10/54(19%)
	Thick/Arched eyebrows	1/1 (100%)	1/16 (6%)	17/22 (77%)	17/54 (31%)
Face					
	Coarse facies	--	0/16 (0%)	3/4 (75%)	3/54 (6%)
	Midfacial hypoplasia	1/6 (17%)	1/16 (6%)	14/26 (54%)	14/54 (27%)
Mouth/Chin					
	Dental abnormalities	4/7 (57%)	4/16 (25%)	13/26 (50%)	13/54 (25%)
	Downturned mouth corners or everted lower lip	2/4 (50%)	2/16 (13%)	16/22 (73%)	16/54 (29%)

Mol Psychiatry. Author manuscript; available in PMC 2016 February 17.

Features		*MBD5*		2q23.1	
		Number Reported[2]	**Total Cohort[3]**	**Number Reported[2]**	**Total Cohort[3]**
	Macroglossia or protruding tongue	2/7 (29%)	2/16 (13%)	6/24 (25%)	6/54 (11%)
	Micrognathia	3/5 (60%)	3/16 (19%)	13/24 (54%)	13/54 (25%)
	Open mouth	2/7 (29%)	2/16 (13%)	23/25 (92%)	23/54 (43%)
	Tented upper lip	1/5 (20%)	1/16 (6%)	17/24 (71%)	17/54 (31%)
	Thin upper lip	2/2 (100%)	2/16 (13%)	19/25 (76%)	19/54(36%)
	Wide mouth	0/6 (0%)	1/16 (6%)	14/18 (77%)	14/54 (27%)
Skeletal					
Extremities	Brachydactyly	0/4 (0%)	0/16 (0%)	12/27 (47%)	12/54(23%)
	Fifth finger clinodactyly	1/5 (20%)	1/16 (6%)	22/31 (72%)	22/54 (41%)
	Flat feet	3/3 (100%)	3/16 (19%)	3/4 (75%)	3/54 (6%)
	Sandal gap	3/6 (50%)	3/16 (19%)	8/24 (33%)	8/54 (16%)
	Short fifth digit	1/5 (20%)	1/16 (6%)	13/25 (52%)	13/54 (25%)
	Small hands or feet	1/5 (20%)	1/16 (6%)	22/30 (73%)	22/54 (41%)
Other	Hip dysplasia	--	0/16 (0%)	2/3 (67%)	2/54 (4%)
	Joint laxity	2/3 (67%)	2/16 (13%)	2/5 (40%)	2/54 (4%)
	Scoliosis	2/2 (100%)	2/16 (13%)	2/6 (33%)	2/54 (4%)
Gastrointestinal					
	Constipation	6/7 (86%)	6/16 (38%)	13/15 (87%)	13/54(25%)
	Gastroesophageal reflux	--	0/16 (0%)	3/3 (100%)	3/54 (6%)
Heart					
	Atrial septal defect	0/7 (0%)	0/16 (0%)	1/24 (4%)	1/54(2%)
	Ventricular septal defect	1/7 (14%)	1/16 (6%)	1/24 (4%)	1/54(2%)
	Pulmonary stenosis	0/7 (0%)	0/16 (0%)	1/24 (4%)	1/54(2%)
	Unspecified heart defect	0/7 (0%)	0/16 (0%)	1/24 (4%)	1/54(2%)
Urogenital					
	Hypoplastic genitalia	0/1 (0%)	0/16 (0%)	5/8 (63%)	5/54 (9%)

[1]The combined cohort is the new cases plus those cases from Talkowski et al with phenotype data (n = 70).

[2]Each denominator reflects the total number of cases with this feature specifically noted to be present or absent.

[3]Each denominator reflects the total number of cases in the cohort which may or may not represent assessment for the specific feature.

Mol Psychiatry. Author manuscript; available in PMC 2016 February 17.

Mol Psychiatry. Author manuscript; available in PMC 2016 February 17.

Table 2

New Features Potentially Associated with*MBD5* Region Disruptions

Case Number	Age (years)	Gender	Genes Affected[1]	Inheritance	New Features
1	19	F	3	*De novo*	--[2]
2	2	M	3	Unknown	--
3	44	M	5	*De novo*	Cataracts; Behavioral regression (worsening skin picking and obsessive-compulsive actions); Anxiety; Bipolar Disorder
4	5	F	2	*De novo*	Polydactyly; Skin tags; Ventricular septal defect
5	12	F	*MBD5* only	Unknown	--
6	<1	M	*MBD5* only	Unknown	Diaphragmatic eventration; Ventricular septal defect
7	25	F	20	Unknown	Spina bifida
8	2	M	4	*De novo*	--
9	14	M	2	Likely inherited[3]	Cutis mamorata; Sensory integration disorder; Anxiety
10	12	M	2	Likely inherited[3]	--
11	20	F	8	*De novo*	Flat occiput
12	3	F	22	*De novo*	--
13	5	F	2	Unknown	--
14	7	F	2	Unknown	--
15	7	M	3	Unknown	--
16	7	M	*MDB5* only	Unknown	Bipolar disorder
17	6	M	4	*De novo*	--
Motobayashi/18	5	M	4	*De novo*	--
Noh/19	2	F	2	*De novo*	Short neck
Kleefstra/20	>13	M	*MBD5* only	*De novo*	Self-hugging; Anxiety
O'Roak/21	13	M	*MBD5* only	*De novo*	--
22	2	M	*MBD5* only	Unknown	--

[1]Minimum number of affected RefSeq genes.

[2]Indicates no new phenotype observed in this case.

[3]Although no parental testing has been performed, Cases 9 and 10 are siblings indicating the deletion is likely inherited.

Table 3

Phenotypes in Intragenic *MBD5* Disruptions and 2q23.1 Microdeletions Versus Syndromes in the Differential Diagnosis

Features	SMS[1]	CdLS[1]	AS[1]	PWS[1]	Rett[1]	Kleefstra[1]	PTHS[1]	*MBD5*	2q23.1
Behavioral									
Aggression/Temper	+[2]	+	−	+	−	+	+	+	+
Anxiety	+	+	−	−	−	−	+	+/−[3]	+/−
Apathy/Catatonia after puberty	−	−	−	−	−	+	−	−	−
Autistic-like behaviors (stereotypic repetitive)	+	+	+	+	+	+	+	+	+
Bruxism (teeth grinding)	+	−	−	−	+	−	+	−	+
Distractibility/Short attention span	+	+	+	−	+	+	−	+	+
Eye contact (intense) and/or eye pointing	−	−	−	−	+	−	−	−	−
Hand-flapping	+	−	+	−	−	−	+	+	+
Hyperactivity	+	+	+	+	+	−	−	−	+
Hyperphagia	+	−	−	+	−	−	−	+	+
Inappropriate happy demeanor	−	−	+	−	+	−	+	−	+
Inconsolable crying	−	−	−	−	+	−	−	−	−
Insertion of hand in mouth or handbiting	+	−	−	−	−	−	−	+/−	+
Obsessive-compulsive or routine-bound behaviors	+	+	−	+	−	−	+	+/−	+
Panic-like attacks	−	−	−	−	+	−	−	−	−
Self-injurious behaviors including skin and eye picking	+	+	−	+	−	+	+	+	+
Sensory integration disorder	+	−	−	−	−	+	−	+/−	+
Sleep disturbances	+	+	+	+	+	+	+	+	+
Social withdrawal	−	+	−	−	−	−	−	+/−	+/−
Temperature intolerance	−	+	+	+	−	−	−	−	−
Central Nervous System									
Ataxia/Unusual gait	+	+	+	−	+	−	+	−	+
Breathing abnormalities	−	−	−	−	+	−	+	−	−
Developmental delay/Intellectual disability	+	+	+	+	+	+	+	+	+
Feeding difficulties	+	+	+	+	+	−	−	+	+
Generalized lethargy (infancy)	+	−	−	+	−	−	−	−	−
Hyporeflexia	+	−	−	+	−	−	−	−	−

Mol Psychiatry. Author manuscript; available in PMC 2016 February 17.

Features	SMS[1]	CdLS[1]	AS[1]	PWS[1]	Rett[1]	Kleefstra[1]	PTHS[1]	*MBD5*	2q23.1
Hypotonia/Abnormal muscle tone	+	+	+	+	+	+	+	+	+
Low-pitched cry in infancy	−	+	−	−	+	−	−	−	−
Motor delay	+	+	+	+	+	+	+	+	+
Oropharyngeal and gastroesophageal incoordination	−	−	−	−	+	−	−	−	−
Seizures	+	+	+	+	+	+	+	+	+
Speech delay	+	+	+	+	+	+	+	+	+
Tremulous movement of limbs	−	−	+	−	+	−	−	−	−
Velopharyngeal insufficiency/Vocal-fold abnormalities	+	−	−	−	−	−	−	−	−
Growth/Endocrine									
Delayed sexual maturation	+**	−	−	+	−	−	−	−	−
Failure to thrive	+	+	−	+	−	−	−	−	−
Growth retardation	+	+	−	−	+	−	+	+	+
Hirsutism	−	+	−	−	−	−	−	−	+
Hypercholesterolemia	+	−	−	−	−	−	−	−	−
Low thyroxine/Hypothyroidism	+	−	−	+	−	−	−	−	−
Obesity	+	+	+	+	−	+	−	−	+
Precocious puberty	+	−	−	−	−	−	−	−	−
Short stature	+	+	−	+	+	+	+	+	+
Craniofacial									
Cranium									
Brachycephaly	+	+	−	−	−	+	−	+	+
Flat occiput	−	−	+	−	−	−	−	−	−
Metopic ridging	−	−	−	−	−	−	−	−	+
Microcephaly	−	+	+	−	+	+	+	+/−***	+
Plagiocephaly	−	−	−	−	−	−	−	−	+
Broad forehead	+	−	−	−	−	−	−	+	+
Ears/Nose									
Nasal abnormalities	+	+	−	−	−	+	+	−	+
Outer ear abnormalities	+	+	−	−	−	+	+	+	+

Features	SMS[1]	CdLS[1]	AS[1]	PWS[1]	Rett[1]	Kleefstra[1]	PTHS[1]	*MBD5*	2q23.1
Eyes									
Almond-shaped eyes	–	–	–	+	–	–	–	+/–	+/–
Astigmatism	–	–	+	+	–	–	+	+/–	+
Coloboma of optic nerve	–	+	–	–	–	–	–	–	–
Deep-set eyes	+	–	–	–	–	–	+	–	–
Glaucoma	–	+	–	–	–	–	–	–	–
Hypotelorism	–	–	–	–	–	–	+	–	+
Iris anomalies	+	–	–	–	–	–	–	–	–
Long eyelashes	–	+	–	–	–	–	–	–	–
Microcornea	+	+	–	–	–	–	–	–	–
Myopia/Hypermetropia/Corrective Lenses	+	+	–	+	–	–	+	+	+
Nasolacrimal duct stenosis	–	+	–	–	–	–	–	–	–
Optic atrophy	–	+	–	–	–	–	–	–	–
Optic nerve hypoplasia	+	–	–	–	–	–	–	–	+
Palpebral fissures - downslanting	–	+	–	–	–	–	–	+/–	+
Palpebral fissures - long	–	–	–	–	–	–	–	–	+
Palpebral fissures - upslanting	+	+	–	–	–	+	+	+/–	+
Strabismus	+	+	+	+	+	–	+	–	+
Synophrys	+	+	–	–	–	+	–	+	+
Thick/Arched eyebrows	+	+	–	–	–	+	+	+/–	+
Face									
Coarse facies	+	–	–	–	–	+	+	–	+
Midfacial hypoplasia	+	–	–	–	–	+	–	+/–	+
Mouth/Chin									
Cleft palate	+	+	–	–	–	–	–	–	+
Dental abnormalities	+	+	–	–	–	+	+	+	+
Downturned mouth corners or everted lower lip	–	+	–	+	–	+	–	+	+
Long, smooth philtrum	–	+	–	–	–	–	–	–	–
Macroglossia or protruding tongue	–	–	+	–	–	+	–	–	+
Micrognathia	+	+	–	–	–	–	–	+	+

Features	SMS[1]	CdLS[1]	AS[1]	PWS[1]	Rett[1]	Kleefstra[1]	PTHS[1]	*MBD5*	2q23.1
Open mouth	+	−	−	−	−	−	−	+	+
Prognathia	+	−	+	−	−	+	+	−	−
Thin upper lip	−	+	−	+	−	−	−	+	+
Wide mouth	−	−	+	−	−	−	+	−	+
Neck/Thorax/Vertebrae									
Hypoplastic nipples	−	+	−	−	−	−	−	−	−
Short neck	−	+	−	−	−	−	−	−	−
Skeletal									
Extremities									
Brachydactyly	+	−	−	−	−	+	−	−	+
Clubbing of digits	−	−	−	−	−	−	+	−	−
Fifth finger clinodactyly	+	+	−	−	−	+	+	+/−	+
Flat feet	+	−	−	+	−	−	+	+	+
Lower limb amyotrophy	−	−	−	−	+	−	−	−	−
Sandal gap	−	−	−	−	−	−	−	+	+
Short fifth digit	−	−	−	−	−	−	−	+/-	+
Small hands or feet	+	+	−	+	+	−	+	+/−	+
Toe syndactyly	+	+	−	−	−	+	−	−	−
Upper limb reduction defects	−	+	−	−	−	−	−	−	−
Other						−	−		
Hip dysplasia	−	+	−	+	−	−	−	−	+
Joint laxity	−	−	−	−	−	+	−	+	−
Scoliosis	+	+	+	+	+	−	+	+	+
Gastrointestinal									
Abdominal bloating from excessive air swallowing	−	−	−	−	+	−	−	−	−
Bowel malrotation and obstruction	−	+	−	−	−	−	−	−	−
Constipation	+	−	+	+	+	−	+	+	+
Gastroesophageal reflux	−	+	+	−	+	+	+	−	+
Gallbladder dysfunction	−	−	−	−	+	−	−	−	−

Features	SMS[1]	CdLS[1]	AS[1]	PWS[1]	Rett[1]	Kleefstra[1]	PTHS[1]	*MBD5*	2q23.1
Heart									
Atrial septal defect	+	+	−	−	−	+	−	−	−
Ventricular septal defect	+	+	−	−	−	+	−	+/−	+/−
Pulmonary stenosis	+	+	−	−	−	+	−	−	−
Tetrology of Fallot	+	+	−	−	−	+	−	−	−
Unspecified congenital heart defect	+	+	−	−	−	+	−	−	−
Immune Function									
Immunoglobulin deficiency	+	−	−	−	−	−	−	−	−
Skin									
Cutis marmorata	−	+	−	−	−	−	−	−	+
Hypopigmentation	−	−	+	+	−	−	−	−	−
Urogenital									
Hypoplastic genitalia	−	+	−	+	−	+	+	−	+
Other urogenital anomalies	+	+	−	−	−	+	−	−	−

[1] Derived from references 27–53.

[2] Denoted as positive (+) when the feature was present in at least two cases.

[3] Denoted as potentially positive (+/−) when the feature was present in one case of *MBD5* plus one or more cases of 2q23.1 and vice versa.

Horizontal hash marks indicate features present in *MBD5* and/or 2q23.1 but not in the other syndromes.

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