Supplementary Information

Highly penetrant spectrum of psychopathology and diagnostic overlap with multiple syndromes resulting from disruption of *MBD5*

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SUPPLEMENTARY RESULTS

BIOINFORMATIC ANALYSIS OF WHOLE-GENOME SEQUENCING

Fastq files were aligned, merged, and sorted, followed by single linkage clustering of anomalous read-pairs which identified 13,407 candidate clusters. Subsequent filtering narrowed the clusters of interest to 368, with an average mapping quality of both reads in a pair of ≥20. This set was further gated on a minimum cluster size of >20% of expected diploid jumping coverage (at least 4 read-pairs, n=193); a maximum cluster size metric of ≤2x haploid coverage (≤78 read-pairs, n=191) and a minimum cluster uniqueness of ≥90% (n=95). Finally, each cluster was further screened for potential mapping artifacts or polymorphisms by searching for other observations in a set of k=16 whole-genome sequencing samples of a neurodevelopmental cohort from previous experiments. Genome-wide, 15 putative translocation clusters remained with less than four observations in other samples (under 25% heterozygosity). Furthermore, only one filtered cluster supported a chimeric event between chromosomes 2 and 5, the karyotypically detected abnormality. The single remaining cluster of interest comprised 27 read-pairs in such orientation relative to the reference genome that they supported a balanced reciprocal translocation event

with a local inversion at the chimeric junction disrupting *MBD5* on 2q23.1. Analyses revealed 4 bp of microhomology at the der(2) translocation breakpoint, 2 bp of microhomology at the der(2) inversion breakpoint, and 2 bp of microhomology at the der(5) translocation breakpoint. The translocation and inversion events were confirmed by capillary sequencing. These results led to the revision of the clinical karyotype interpretation from 46,XY,t(2;5)(q22;q22)dn 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.

CO-OCCURING CNVs

A small deletion of unknown significance at chromosome 22q11.21 was detected in Case 6 (genomic coordinates 19,062,437-19,835,417 in hg18) while a *de novo* deletion from chromosome 5q11.2 to 5q12.2 was observed in Case 11 (genomic coordinates 58,401,783-63,433,812 in hg18).

LIST OF CASES FROM TALKOWSKI ET AL INCLUDED IN OR EXCLUDED FROM THE CURRENT PHENOTYPE ANALYSIS

Cases with phenotypic data in the original cohort of Talkowski et al were included in the current phenotype analysis (n = 48): CHB70, CHB71, CHB74, CHB90, Chung 1, De Vries 2, DG142, GC13268, GC13619, GC16547, GC16886, GC22710, GC23032, GC24009, GC25123, GC31738, GC31835, GC35827, GC45915, GC47335, GC47437, GC59850, GC60793, GC62805, Jaillard 1, Jaillard 2, Koolen 1, LH008, NCH 1, SMS185, SMS361, SMS368, SMS367, SMS373, SMS375, SMS388, van Bon 1, van Bon 2, van Bon 3, van Bon 4, van Bon 5, van Bon 6, van Bon 7, van Bon 8a, van Bon 8b, van Bon 9, van Bon 10, and Wagenstaller 1 (Patient 29195).

Cases without phenotypic data in the original cohort of Talkowski et al were excluded from the current phenotype analysis (n = 17): LH001, LH002, LH003, LH004, LH005, LH006, LH007, LH009, LH010, LH011, LH012, LH013, LH014, LH015, LH016, and LH017.

DIFFERENTIAL DIAGNOSIS OF *MBD5* DISRUPTIONS AND SMITH-MAGENIS SYNDROME (SMS)

SMS results from deletion or mutation of *RAI1* at chromosome 17p11.2. The behavioral hallmarks of SMS including sleep disturbances and self-injurious manifestations such as nail, skin, and eye picking are also seen in intragenic or larger *MBD5* deletions. The more specific behavioral characteristics of sensory integration issues, as well as a tendency for insertion of hands into the mouth, aggression, and short attention span, are also shared in common. Individuals in both syndromes can further manifest autistic-like behaviors, intellectual disability, developmental delay, seizures, feeding difficulties, speech delay, hypotonia, small hands and feet, short stature, scoliosis, constipation and congenital heart defects. An unusual self-hugging behavior common in SMS was noted in the *MBD5* deletion-containing Case 20. Overlapping craniofacial characteristics include midfacial hypoplasia, coarse facies with age, optic nerve hypoplasia, strabismus, broad forehead, synophrys, an open-mouth posture, dental anomalies, brachycephaly, micrognathia and cleft palate.

A case that highlights the strong similarity in features between *MBD5* disruption and SMS is an almost 7 year old girl (Case 4) with a 153 kb *de novo* deletion overlapping a portion of *ORC4* and the 5'UTR of *MBD5*. This child's facial features (**Figure 2**), behavior and neurodevelopmental phenotype significantly raised a clinical suspicion for SMS, prompting

RAI1 gene sequencing despite the already known abnormal cytogenomic microarray result. No *RAI1* sequence variants were identified.

Although there are many traits in common, certain features may distinguish the two syndromes. Lethargy, hyporeflexia, velopharyngeal insufficiency/vocal card abnormalities, hypercholesterolemia, low thyroxine/hypothyroidism, immunoglobulin deficiency and precocious puberty noted in SMS have not been reported in *MBD5* disruptions. Plagiocephaly and joint laxity are present in *MBD5* disruptions but are not associated with SMS. Eye phenotypes also diverge; astigmatism and hypotelorism are common only in *MBD5* disruptions, while microcornea and anomalies of the iris appear to be characteristic only of SMS. In addition, eyes in individuals with SMS are deep-set with mildly upslanting palpebral fissures while those in *MBD5* disruptions have been described as upslanted, downslanted or long, when reported.

DIFFERENTIAL DIAGNOSIS OF *MBD5* DISRUPTIONS AND PRADER-WILLI (PWS) OR ANGELMAN (AS) SYNDROMES

While PWS and AS involve alteration of the same genetic region at chromosome 15q11.2-q13 but with markedly different phenotypic effects, both syndromes share many features with *MBD5* disruptions. All three syndromes are associated with autistic-like behaviors, intellectual disability, developmental delay, seizures, sleep disturbances, hyperactivity, speech delays, hypotonia, strabismus, feeding difficulties and scoliosis. Additional hallmark characteristics of AS but not PWS include an inappropriately happy demeanor, hand-flapping, a protruding tongue, microcephaly and ataxia as well as a flat occiput, the latter of which was also noted in the *MBD5* deletion-containing Case 11. PWS is further defined by later onset hyperphagia resulting in obesity, obsessive-compulsive disorder and self-injurious behaviors as well as short

stature, hip dysplasia, myopia, cryptorchidism and small hands and feet. All of these hallmarks for both AS and PWS have been reported in *MBD5* disruptions, further illustrating the significant ability of *MBD5*-associated phenotypes to masquerade as other disorders.

Distinguishing *MBD5* disruption phenotypes not generally present in either PWS or AS include insertion of hands into the mouth and eye anomalies, in particular hypotelorism and synophrys, as well as skeletal limb abnormalities including fifth finger brachydactyly or clinodactyly and a sandal gap. Features not present in *MBD5* disruptions that occur in AS are hypopigmentation and tongue thrusting, while PWS can include failure to thrive, generalized lethargy in infancy and hyporeflexia.

DIFFERENTIAL DIAGNOSIS OF *MBD5* DISRUPTIONS AND CORNELIA DE LANGE SYNDROME (CdLS)

CdLS results from deletion or mutation of either *NIPBL* at chromosome 5p13.2, *SMC1A* at chromosome Xp11.22, or *SMC3* at chromosome 10q25.2. The self-injurious behaviors and aggression that *MBD5* shares in common with PWS and SMS also occur in CdLS. The downturned corners of the mouth, thin upper lip and micrognathia observed in individuals with *MBD5* deletions are considered classic for CdLS. Thick, arched eyebrows with synophrys and hirsutism are additional overlapping features, especially in individuals with larger 2q23.1 deletions, as are autistic-like behaviors, intellectual disability, developmental delay, speech delay, sleep disorders, seizures, hypotonia, feeding difficulties, strabismus, dental anomalies, short stature, brachycephaly, microcephaly, cardiac defects, cryptoorchidism and gastroesophageal reflux. Cutis marmorata which is often observed in CdLS was also reported in

the *MBD5* deletion-containing Case 8. Another *MBD5* deletion patient (Case 7) was originally diagnosed with CdLS due to having many of the above listed features.

While small hands and feet manifest in both *MBD5* disruptions and CdLS, one of the most divergent features is the severe upper extremity reduction defects common in CdLS but not reported in *MBD5* disruptions. Other distinguishing features of CdLS include long eyelashes, toe syndactyly, low-pitched cry in infancy, optic nerve colobomas, glaucoma, hypoplastic nipples, optic atrophy and nasolacrimal duct stenosis. Conversely, the behavioral features of hand-flapping, hyperphagia, hand insertion into the mouth and open-mouth as well as other phenotypes such as midface hypoplasia, macroglossia, wide mouth, and joint laxity are only observed with *MBD5* disruptions and not in CdLS.

DIFFERENTIAL DIAGNOSIS OF *MBD5* DISRUPTIONS AND RETT SYNDROME

Rett syndrome results from deletion or mutation of *MECP2* at chromosome Xq28 and is classically associated with developmental regression, particularly loss of speech and acquired microcephaly. *MBD5* deletion patients can have speech delay that is typically non-regressive as well as microcephaly which is usually congenital. It is of note that regression of motor skills, speech and/or behavior has only been identified in three *MBD5* deletion cases to date, two of which are in the new cohort in the present study (Cases 3 and 19). Autistic-like behaviors, intellectual disability, developmental delay, seizures, sleep disturbances, ataxia, speech delays, hypotonia, bruxism, strabismus, scoliosis, feeding difficulties and constipation are also present in both conditions.

Despite common behavioral problems in *MBD5* disruptions, the inconsolable crying, intense eye contact, panic-like attacks, lower limb amyotrophy, abdominal bloating from

excessive air swallowing, gallbladder dysfunction and oropharyngeal and gastroesophageal incoordination known to occur in Rett syndrome have not been described with *MBD5* disruption. Of greatest use in distinguishing the two syndromes is the wide range of dysmorphic facial features as well as skeletal anomalies and ophthalmic conditions that define *MBD5* disruptions while normal facial features without skeletal or eye anomalies other than strabismus are characteristic of Rett syndrome.

DIFFERENTIAL DIAGNOSIS OF *MBD5* DISRUPTIONS AND KLEEFSTRA SYNDROME

Kleefstra syndrome results from deletion or mutation of *EMHT1* at chromosome 9q34.3. The many neurological and behavioral features that Kleefstra syndrome has in common with SMS also occur in *MBD5* disruptions. These include the general neurological characteristics of autistic-like behaviors, developmental delay, intellectual disability, speech delay, motor delay, hypotonia and seizures as well as the more specific traits of self-injurious behavior, aggression, sensory integration disorders and sleep disturbances. In addition, there are many physical features shared by patients with Kleefstra syndrome, SMS, and *MBD5* disruptions including microbrachycephaly, synophrys, midfacial hypoplasia, coarse facies, dental anomalies, congenital heart defects, and gastroesophageal reflux. The obesity and hypogenitalia found in Kleefstra syndrome and *MBD5* disruptions are also reminiscent of PWS, while the protruding tongue is more suggestive of AS.

Distinguishing physical features include the many ophthalmic and skeletal abnormalities found in *MBD5* disruptions as well as wide mouth, open mouth and constipation which are not present in Kleefstra syndrome. Ataxia, obsessive-compulsive behaviors and hand-flapping are common in *MBD5* disruptions but are not generally features of Kleefstra syndrome. An

important differentiating behavioral factor is that of social withdrawal which has only been described in two cases of *MBD5* disruption and is significantly less severe than the apathy and catatonia occurring after puberty that are hallmarks of Kleefstra syndrome.

DIFFERENTIAL DIAGNOSIS OF *MBD5* DISRUPTIONS AND PITT-HOPKINS SYNDROME (PTHS)

Pitt-Hopkins syndrome results from deletion or mutation of *TCF4* at 18q21.2. Like many of the other syndromes mentioned above, PTHS and *MBD5* share in common autistic-like behaviors, developmental delay, intellectual disability, motor delay, seizures, hypotonia, sleep disturbances, microcephaly and speech delay. More specific mutual behavioral and neurological features are self-injury, aggression, hand-flapping, and obsessive-compulsive behaviors.

The intermittent hyperventilation that is found in PTHS but not in *MBD5* disruption is one of the most distinguishing features. Also divergent between the two syndromes is clubbing of the fingers and toes in PTHS but not *MBD5*, although *MBD5* disruption does have abnormalities of the limbs such as brachydactyly or clinodactyly of the fifth digits and small hands and feet. Features found with *MBD5* disruptions but not PTHS include brachycephaly, plagiocephaly, prominent forehead, synophrys, midfacial hypoplasia, open mouth and congenital heart defects.

FEATURES CONFINED SOLELY TO EACH OF THE SYNDROMES IN THE DIFFERENTIAL DIAGNOSIS OF *MBD5* DISRUPTIONS

Only patients with SMS have velopharyngeal insufficiency/vocal cord abnormalities, hypercholesterolemia, precocious puberty, iris anomalies and immunoglobulin deficiency, while

only those with AS have tongue thrusting. Similarly, only patients with CdLS have long eyelashes, nasolacrimal duct stenosis, optic atrophy, hypoplastic nipples, upper limb reduction defects, bowel malrotation and obstruction, low-pitched cry in infancy, colobomas of the optic nerve and glaucoma, while only patients with Rett have lower limb amyotrophy, abdominal bloating from excessive air swallowing, gallbladder dysfunction, intense eye contact and/or eye pointing, inconsolable crying, panic-like attacks, and oropharyngeal and gastroesophageal incoordination. Finally, only patients with Kleefstra have apathy/catatonia while only those with PTHS have clubbing of the digits.

Table S1. Sequencing metrics

Metric	**Result**
Total Reads	174,158,632
Total Reads Forward Strand	87,535,837 (50.3%)
Total Reads Reverse Strand	86,622,795 (49.7%)
Mapped Reads	163,934,000 (94.1%)
Both reads mapped	154,640,599 (88.8%)
Singletons	9,293,401 (5.3%)
Proper-Pairs	65,298,138 (79.7% of Mapped)
Proper-Pairs Mean Insert Size	1862.47 bp
Proper-Pairs Insert Size SD	332.019 bp
Average Jumping Coverage	39.23x
Average Physical Coverage	1.32x

Table S2. Translocation breakpoint sequences

Karyotype **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**

Expanded Karyotype **46,XY,t(2;5)(2pter-148,732,432:: chr5:119,513,903-119,514,070::chr5 119,514,075-qter; 5pter-119,513,863::chr2 148,733,228-qter)dn [hg18]**

der(2)

GGGGCATAAATGCCCACCAATATCCCAGGAGGCCCACAAAAATTTGCAGCTGCTTCGTTGTGGTGGGCTGGAGAT
ATGCCTGAATCTCATCAACGATAGCCTCTGGGATGGCCTTTGTCTTACCCGACCAGATAACTCCCAAGAATTTGACA
AATGAAGCAGGCCCTTGGACCTTAGATTTGTTGACGGCCCAACTGCATGCTGCCAAATGTCGCCACAAGAGGGATG
CCACCACTTCTAAATCTGCAAGAGAATCAGAGGTTACCATATCATCAATATAATGGAATAGGTGGACCCATTCTGGC
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CTGTAAAAGTTCATTGTTGCCTGTCCCATGTGAAGGCGAACTGTTCCTGGCTCTCTGGAGTGATATCGATGGAGAA
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TGATACAGCTTCATGCAAAGAGGGTGTTACTTTTTTCAGTTCCCAATAGTCCACCATCATCCACCAGTTTCTATCAGG
CTTTCTAACTGGCCACACTGGAGAACTGTAGGGGCTATGGGTGCCACGCACTATCTGCACCTCTTCCAGCTTTTTGT
CTCAGTTATCTCTGTATGTCCATCCGGCAAGCAGTATTGATGGGTGGAAGTAACCAGTCAGGGTTGTGGCAGAACC
TGAGGCTGGTGATGTGTATGTCCGTGCAGCACCAGCTACACCACATGCACTCGGAGTCTGAATTCCCTGGCTGTAG
TTTGTAATGCCAAGCCATGTAAAATGTCCACCCCCAGAATGTATTCAGGTATGGGAGAAACACACACAGTATATAA
ACGGGGAGTCAAACAACGGATGCCAAGGTACAGAGATACAGGTTTCACTTTCACTGACTCGCTTTCATAACCCTCA
ATGTAAGCAGGTTTGCCCAGAAAC::**TTAT**::GCAATAATTTTACTTTCTGTTATCAATTATTTGGCCAATATTTTATTTA
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AGCACCATTTGAAAATACTCTGGTTCTATATTTTTCATTGTGTACGAAATCCTGACAATAATTTTTATATTTTAAAGCA
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TTAACATACAAAAGAGCCATGAAGTAGACAAAAGGCTAGGTTTCTTACAAGAAAAGAAATAGAATAGAATAAGCT
TAAAATATATAGTTCAGTAAAAAAATAAGCACACAGCACTAATTGTGCTACTCTATTGCACTTTTATTAGTTAGGATT
CACTTGACTATAAATTATAGAAATCTAACTCAAA

der(5)

AAAGAAAATAGTACAAATGGATAACCAAGACTAAGAATGTGTATAAAAATGCTTTAATTATTTCTTTAATAAAAATA
GGAAAAAAATTATAATTTCCCCTCTGTGGTCTTAGTATTGGTGAGAGACTAAACATGGAATTAAATAAAATATTTAA
AAAATTTCTGATACTGCATATTCTCACTTATACATGGGAGTTAAATGATGAGAACACACGGACACATAGAGGGGAA
CAACAGATCCTGGGGCCTACCTGAGGGTGAAGGGTGAGAGGAGAGAAAGGATCAGGGAAAATAACTAATGGGT
GCCAGGCTTAATACCTGGGTGACAAAATAATCTGTACAACAAACCCCATGACACAAGTTTATCTGTATAACAGACCT
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CAGGCAATTATGCATGTATTATATATGTGGCATTCTCAAGTAAGGTTTTGAGTAAAATTTGTATGATATTAATTACCC
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GAAGAATGAAAAAAATTAAATTTGAATGGGCAACTTAGAAGCATTTAAATATATATATGCATA::**CA**::CTCAATCAG
GGTGTGGTCACCTTGCCCTTGTGCCAACTGCCCGCTCACCTGCAACCAACGTGCTGCATCCCTCAGAGACTGGAAGT
GTAGTTCTTCTAGTGCAGTCAAAAATGCCCATCCAACTCTGCAAGCAAAGACTCGTTCATTCTCAGTGCTCTGTGCTT
CCAGCTGCTTCAGCATATTCTCCATGCTTGCTGGGGACCCATCTACCTCTGCCCATGTTTCCCTCAGAGCCCATCCAA
GCAGCACAGCTGCCACCGTGTATCACAACCCATGTTGTGGATACATGGCCGACCCAGAATCAGTGGGGACCAAAG
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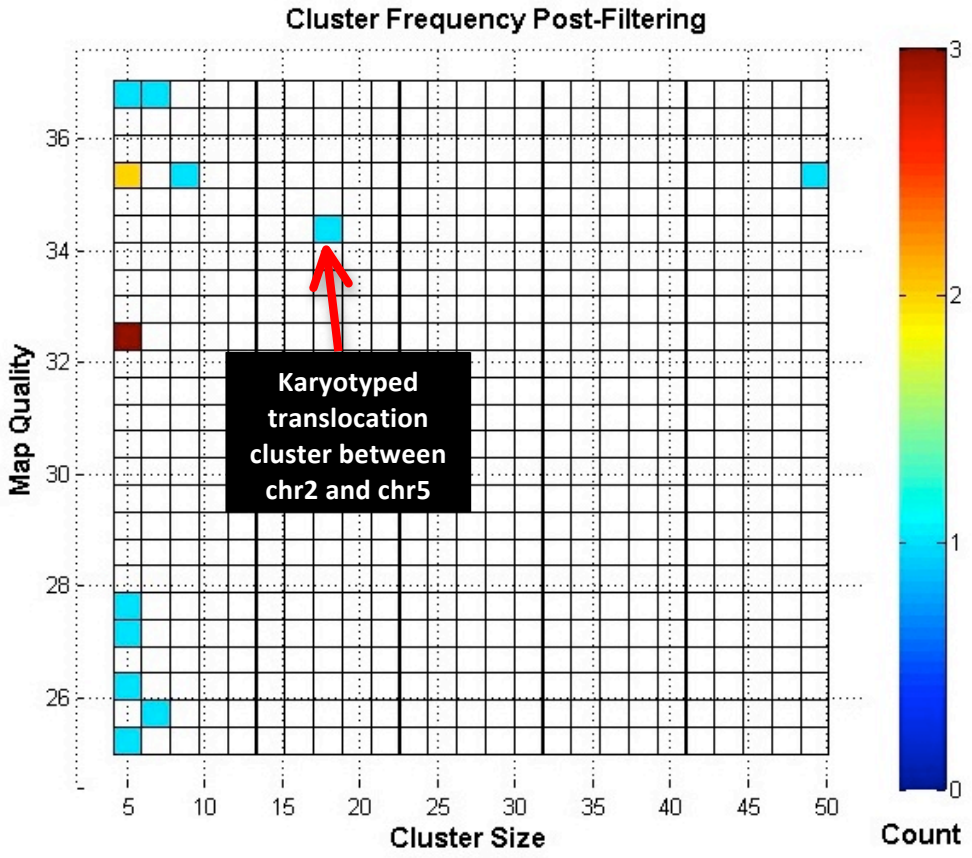
TGAGCCATATGTGCAGCATTACCGGGGTAATTATACCTTTTACAGACAATAGTGGCTTTGAGCCAAGCATGAGCTC
ACATAGGTCATCATCTAATGCTCCTCACGTGGTGTGGTTGCATAATGTGCAGAGCTGTGCACCTGCACCACAAACCC
GCTGAGTCATGCTGC

Note: " :: " indicates breakpoint including microhomology (bolded and underlined)

Figure S1.


Cluster Frequency Post-Filtering
Map Quality
36
34
32
30
28
26
Karyotyped translocation cluster between chr2 and chr5
5
10
15
20
25
30
35
40
45
50
Cluster Size
3
2
1
0
Count