



ORIGINAL ARTICLE

Characterizing the phenotypic effect of Xq28 duplication size in *MECP2* duplication syndrome

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Individuals with methyl CpG binding protein 2 (*MECP2*) duplication syndrome (MDS) have varying degrees of severity in their mobility, hand use, developmental skills, and susceptibility to infections. In the present study, we examine the relationship between duplication size, gene content, and overall phenotype in MDS using a clinical severity scale. Other genes typically duplicated within Xq28 (eg, *GDI1*, *RAB39B*, *FLNA*) are associated with distinct clinical features independent of *MECP2*. We additionally compare the phenotype of this cohort ($n = 48$) to other reported cohorts with MDS. Utilizing existing indices of clinical severity in Rett syndrome, we found that larger duplication size correlates with higher severity in total clinical severity scores ($r = 0.36$; $P = 0.02$), and in total motor behavioral assessment inventory scores ($r = 0.31$; $P = 0.05$). Greater severity was associated with having the *RAB39B* gene duplicated, although most of these participants also had large duplications. Results suggest that developmental delays in the first 6 months of life, hypotonia, vasomotor disturbances, constipation, drooling, and bruxism are common in MDS. This is the first study to show that duplication size is related to clinical severity. Future studies should examine whether large duplications which do not encompass *RAB39B* also contribute to clinical severity. Results also suggest the need for creating an MDS specific severity scale.

KEYWORDS

clinical severity, genotype, *MECP2*, phenotype

1 | INTRODUCTION

Methyl CpG binding protein 2 (*MECP2*) duplication syndrome (MDS) is a rare X-linked genomic disorder primarily affecting males who are associated with interstitial chromosomal duplications at Xq28 encompassing the *MECP2* gene.¹ This gene encodes MeCP2, a critical regulator of neuronal gene transcription that is required for normal brain maturation.^{2,3} Loss of MeCP2 function is the primary cause of Rett syndrome (RTT), a distinct neurodevelopmental disorder affecting primarily females exhibiting some symptom overlap with MDS. Fewer than 200 cases of MDS have been described worldwide, however, it is probably underdiagnosed and estimated to account for at least 1% to 2% of all cases of X-linked intellectual disability (ID).¹ The core clinical phenotype of MDS involves infantile hypotonia, global developmental delays, choreiform movements, progressive spasticity, and recurrent respiratory infections.^{4–6} The shortest duplication sufficient for the core phenotype contains only *MECP2* and *IRAK1* genes^{7–9} and the pathogenic role of increased *MECP2* dosage is further supported by transgenic mouse models of MDS with overexpression of mouse or human *MECP2* that develop impaired coordination, seizures, and impairments in learning and memory.^{10–12} The spectrum of phenotypic features associated with MDS is gradually being defined, with the largest published cohort to date ($n = 59$) reporting high rates of constipation, stereotypic movements, epilepsy, decreased pain sensitivity, scoliosis, motor regression and specific facial dysmorphologies in addition to the aforementioned features.¹³

Our own prior studies show considerable inter-patient differences in severity of several symptom domains within MDS including ambulation, hand function, non-verbal communication, and susceptibility to infection¹⁴; furthermore, developmental regression occurs only in half of the population with highly variable age of onset.¹⁵ There is limited published information regarding factors that contribute to this observed variability in symptom severity. Although prior studies have suggested that duplication size is not related to the phenotype,^{4,13} this has not been examined within the context of a severity scale (as opposed to discrete symptoms). In addition, it is unknown whether specific phenotypic features contribute disproportionately to overall clinical severity and/or changes in severity within this population. This information would aid the development of a dynamic index of clinical severity with content validity for MDS as existing scales in use were created and validated for longitudinal studies in RTT.^{16,17} In addition, the degree to which presence/absence of additional salient genes (eg, *SRPK3*, *L1CAM*, *FLNA*, *GDI1*, *RAB39B*) within the duplicated interval contribute to severity has not yet been examined in detail. For example, other studies have noted that increased expression of *GDI1* (without duplication of *MECP2*) is associated with impaired cognition¹⁸ as well as autism.¹⁹ Increased dosage of *RAB39B* has also been associated with mild to moderate ID and behavioral problems.^{20,21} *SRPK3* is involved in muscle growth and homeostasis with overexpression causing muscle degeneration in mice.²² A neurological phenotype because of *L1CAM* overexpression has not been reported, but loss of function mutations are associated with corpus callosum abnormalities on neuroimaging²³ that are also seen in MDS.²⁴ Duplications in *FLNA* are

associated with bowel dysfunction that is also very common in MDS.^{25,26}

The recent report of phenotypic reversal after antisense oligonucleotide treatment in an animal model of MDS²⁷ suggests disease-modifying therapies may be possible. Clinical trials to test such future therapies will require standardized measures of clinical severity with content validity for MDS that can be applied by investigators across all study sites. Here, we present baseline data from our natural history study of MDS, including how these data compare to previous reports in the literature. We extend the prior studies by utilizing existing standardized indices of clinical severity for RTT while emphasizing relevant phenotypic features in MDS important for improving content validity of these scales. We additionally report whether duplication size or duplication of additional genes within the respective breakpoint intervals contribute to clinical severity. We hypothesize that differences in severity will be noted according to the presence/absence of additional genes within the duplicated interval.

2 | METHODS

2.1 | Participants

This cohort of patients is part of a broader longitudinal, natural history study of RTT, and Rett-related disorders including MDS. The RTT and related disorders natural history study consists of 14 sites around the United States. The sites are located in centers that have existing clinical and research specialists in these respective syndromes and are geographically spread around the country to facilitate enrollment. A total of 48 participants, including 5 females and 43 males, ranging between the ages of 1 to 28 years have enrolled in the study to date (Table 1). For each participant, all data is gathered in person through direct clinical exam (by a child neurologist or geneticist), and parent report. Participants are evaluated on a yearly basis. Given the smaller number of participants who have participated in longitudinal follow-up to date, only baseline data is being reported at this time. The families of all participants provided written informed consent and all procedures performed in the studies were carried out in accordance with the ethical standards of the respective institutional research committees. Data within the consortium is routinely checked for compliance. The natural history study is registered with Clinicaltrials.gov: NCT02738281.

2.2 | Scales

Clinical Severity Scale (CSS)—clinician rating: The CSS was developed for use in RTT and has been used to assess almost 2000 children, adolescents, and adults with RTT and related disorders who have been enrolled in the natural history study.³⁰ It is a composite score based on 13 individual ordinal categories measuring common clinical features during an in-person exam (eg, independent sitting, hand use, scoliosis, language, seizures, autonomic symptoms, onset of stereotypies, regression, head growth, etc.). Individual item scores range from 0 to 4 or 0 to 5 with 0 representing the least severe and 4 or

TABLE 1 Comparison of clinical features in the present study vs prior published studies in the literature

	Present study	Miguet et al ¹³	Lim et al ²⁸	Van Esch et al ²⁹
Number of patients	48	59	56	13
Gender	43 male/5 female	59 male	49 male/7 female	13 male
Age at diagnosis	4.16 years (50 months)	10 years (120 months)	36 months/male; 24 months/female	NR
Age at exam/self-report	9.01 years	11.7 years	7.9 years	
Abnormal development in first 6 months of life	31/48 (65%)	NR	NR	NR
Hypotonia	42/48 (88%)	57/58 (98.3%)	31/48 (64.6%) male; 4/7 (57.1%) female	12/12 (100%)
Recurrent Infections	27/48 (56%)	49/55 (89.1%)	38/49 (77.6%) males; 3/7 (42.9%) females	5/9 (55.6%)
Seizures	21/48 (43.8%)	35/59 (59.3%)	21/49 (42.9%) male; 3/6 (50.0%) female	4/9 (44.4%)
Constipation	39/48 (81%)	43/55 (78.2%)	39/49 (79.6%)	0/12 (0%)
Reflux	21/48 (44%)	34/51 (66.7%)	26/49 (53.1%) male; 3/7 (42.9%) female	NR
Drooling	37/48 (77%)	40/50 (80.0%)	NR	NR
Vasomotor disturbances	35/48 (73%)	15/31 (48.4%)	NR	NR
Breathing disturbances	12/48 (25%)	NR	NR	NR
Scoliosis and/or kyphosis	9/48 (19% with scoliosis as a diagnosis)	23/43 (53.5%); includes scoliosis and/or kyphosis	10/46 (21.7%) males; 0/5 (0%) female (scoliosis)	NR
Anxiety	14/48 (29%)	NR	NR	NR
Sleep problems	27/48 (43.8%)	NR	13/48 (27.1%) male; 1/7 (14.3%) female	NR
Bruxism	34/48 (71%)	33/46 (71.7%)	80%	NR
Regression	12/48 (25%)	12/31 (38.7%) (post seizure onset)	NR	NR
High pain tolerance	32/48 (67%)	29/37 (78.4%)	25/50 (50.0%)	NR
Low activity for age	29/48 (60%)	NR	NR	NR
Irritability	28/48 (58%)	NR	NR	NR
Early death (before age 25 years)	2/48 (4%)	9/59 (15.3%)	NR	6/11 (54.5%)

NR, Not Reported.

5 representing increasing severity.¹⁶ Lower total scores represent milder severity (see Appendices S1, S2, Supporting Information).

Motor Behavioral Assessment Scale (MBA)—clinician rating: The MBA has also been used to assess almost 2000 children, adolescents, and adults with RTT and related disorders who have been enrolled in the natural history study.³⁰ The current version of this scale consists of 34 items across three subscales: Behavior/social (irritability, aggression, poor eye gaze, sustained interest, etc.), Orofacial (bruxism, mouthing objects or hands, biting self, breath holding, hyperventilation, etc.), and motor (bradykinesia, dystonia, ataxia, chorea, etc.), the scores of which are based on current functioning. These are scored during a clinical interview and in-person exam by a specialist once per year. Items are captured on a 5-point Likert scale. Lower total scores indicate milder disease severity (see Appendices S1, S2).

Additional clinical features (constipation, reflux, drooling, bruxism, anxiety, sleep problems, irritability, etc.)

Salient clinical features were quantified through parent report and clinician observation according to a scale (none, occasional, frequent, very frequent, constant). For the purposes of this study, these were recoded to determine the presence or absence of these features. The number of infections was quantified over the preceding year at the in-person physician history. The presence of hypotonia, and developmental delays within the first 6 months of life was also quantified through physician exam.

2.3 | Cytogenetic and molecular analyses

Confirmation of duplication was required for entry into the study. Genetic analyses were performed by a variety of laboratories depending upon where the participant was originally diagnosed (eg, Baylor College of Medicine, University of Chicago, Signature Genomics, Greenwood Genetic Center, Athena, CHOP, Emory, Gene Dx, Duke, etc.). Most participants had their duplications detected through Array Comparative Genomic Hybridization (Array-CGH), while others (mostly older participants) had Multiplex-Ligation-dependent Probe Amplification (MLPA). Some had an earlier version of CMA (version 5), such that clearly delineated breakpoints could not be determined. Genomic positions are based on data from the human genome assembly GRCH37/hg19.³¹

3 | RESULTS

3.1 | Demographics

A total of 48 participants have enrolled in this study to date. The mean age of participants at evaluation is 9.01 years (SD = 6.79 years), and the mean age of diagnosis was 4.16 years (SD = 5.38 years). All participants in this study lived in the home; 58% of mothers and 52% of fathers had a bachelor's or advanced

degree and 58% of mothers and 75% of fathers were employed outside of the home.

3.2 | Clinical features

Table 1 lists the demographics of participants in this study, along with the frequency of salient clinical features as compared to prior published studies.^{5,13,28,29} When parents were asked to list their chief concern about their child, many reported that lack of effective communication (27%) was their most pressing concern, while 23% reported that seizures were their greatest concern. Of note is that 65% of parents reported that their children had significant delays within the first 6 months of life. In fact, 17 participants were diagnosed with MDS at age 12 months or younger, and 53% of the sample was diagnosed prior to age 2 years. Hypotonia, drooling, vasomotor disturbances (cold hands and/or feet, skin mottling), bruxism, and a high pain tolerance were commonly noted features in this cohort. A diagnosis of epilepsy was only noted in 21/48 participants (44%); and, 27/48 (56%) exhibited recurrent respiratory infections at the time of evaluation (27/48). In addition, only 12 participants had a documented regression at the time of their visit, with the age of those who had regressed being slightly older ($M = 11.87$ years, $SD = 6.08$) as compared to those who had not regressed ($M = 8.25$ years, $SD = 6.82$ years), although this is not statistically significant ($P = 0.11$).

3.3 | Clinical severity scale

First, descriptive statistics were conducted to examine CSS scores. Scores on the CSS ranged from 4 to 38 ($M = 16.57$, $SD = 7.34$). Next, because of the significant differences found between males vs females with MDS in prior studies,³² an ANOVA was used to test for any differences in CSS scores X gender. Significant differences were

noted in scores of males vs females, with males having significantly higher CSS scores ($M = 17.52$; $SD = 6.95$) than females ($M = 8.60$; $SD = 5.90$) ($F_{1, 47} = 7.55$; $P < 0.009$). Regardless of gender, the most commonly endorsed items were those that related to developmental functioning (eg, hand use, ambulation, non-verbal and verbal language, sitting) and stereotypies. Items that were least probably endorsed related to microcephaly and scoliosis.

3.4 | Motor behavioral assessment

A similar approach to analyses was followed as was described for the CSS above. Scores on the MBA ranged from 3 to 69 ($M = 33.09$; $SD = 14.03$). Significant differences were noted in the scores of males vs females, with males exhibiting higher (ie, more severe) scores ($M = 34.90$; $SD = 13.36$) than females ($M = 17.80$; $SD = 10.38$). Most commonly endorsed items related to developmental milestones (as noted with the CSS), bruxism, air/saliva expulsion, pain insensitivity, poor eye gaze, and apraxia/ataxia. Least endorsed items related to self-injury, aggression, breath holding, hyperventilation, myoclonus, and dystonia.

3.5 | Genetic breakpoints and duplication size

Information on specific breakpoints was available for 40 participants (see Figure 1). Duplication sizes ranged between 210 970 and 14 461 013 bp, with a mean size of 2 375 105 bp. Information regarding carrier status was available for 29 participants who chose to have additional genetic testing; $n = 24$ had inherited duplications (with a maternal carrier), while $n = 5$ had de novo duplications. Besides *MECP2* and *IRAK1*, the salient genes within duplication breakpoints with putative functions relevant to clinical aspects of MDS were *SRPK3*, *L1CAM*, *FLNA*, *GDI1*, and *RAB39B*.

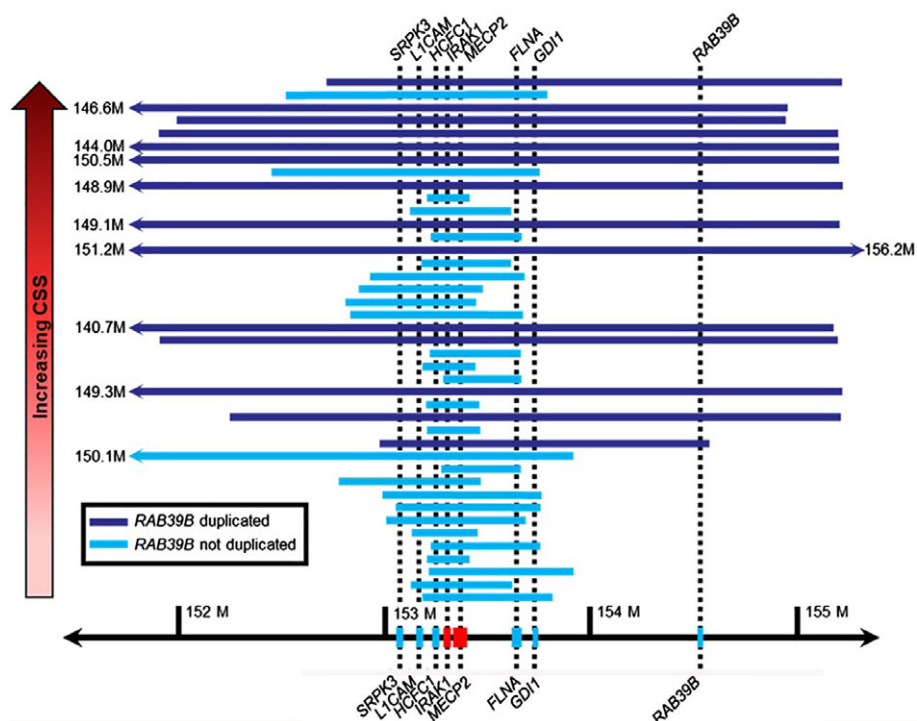


FIGURE 1 Xq28 duplication size X clinical severity score [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Mean total Clinical Severity Scale scores by the absence/presence of additional gene duplications within the BP interval controlling for duplication size

	SRPK3	L1CAM	FLNA	GDI1	RAB39B
Absence	12.33 (4.35) N = 15	11.75 (4.39) N = 12	13.64 (4.94) N = 11	12.72 (4.60) N = 18	13.08 (5.34) N = 26
Presence	17.88 (7.42) N = 25	17.54 (7.15) N = 28	16.62 (7.46) N = 29	18.32 (7.57) N = 22	20.86 (6.85) N = 14
F value	F = 2.78; P = 0.10	F = 3.13; P = 0.08	F = 0.148; P = 0.743	F = 2.81; P = 0.102	F = 8.88; P = 0.005

A larger duplication size significantly correlated with higher severity in total CSS score ($r = 0.36$; $P = 0.02$), and with greater severity in total MBA score ($r = 0.31$; $P = 0.05$). Because duplication size is significantly associated with higher CSS and MBA scores, ANCOVA's (controlling for duplication size) were performed to determine whether the presence/absence of individual genes within the breakpoint interval independently contributes to severity. Testing was performed for each of the five genes mentioned above using an ANCOVA test. For the CSS, the presence of the *RAB39B* gene in the duplication was associated with greater severity independent of duplication size. A trend toward greater severity was noted when the duplication involved *L1CAM*. These results (uncorrected values for $n = 5$ statistical ANCOVA tests) are summarized in Table 2. In addition, to address any concerns regarding multiple comparisons, the Benjamini-Hochberg procedure³³ was performed, and these results are detailed in Table S3. This test corrects for the false discovery rate. The results remain significant using this correction procedure that the presence of the *RAB39B* gene contributes to greater severity independently of duplication size. It is important to note that the false discovery rate was set at 0.10, based on the literature³³ and given the few studies that have been performed in this area. This is a conservative rate; however, even if the false discovery rate was set to 0.05, the results and interpretations of the findings would remain unchanged. The presence/absence of these genes did not have a significant effect on either the total MBA score or its domain-specific subscales. These results are summarized in Table 3, and corrected values are presented in Table S4 (results were unchanged with regard to significance or interpretation). Follow-up exploratory analyses were conducted to understand better the specific CSS items that seemed to differentiate the severity of participants with and without the *RAB39B* gene duplication. Follow-up ANOVA'S (including Bonferroni's correction for multiple comparisons) showed that microcephaly (partial $\eta^2 = 0.15$), and worse impairment in independent sitting (partial $\eta^2 = 0.19$), ambulation (partial $\eta^2 = 0.30$), and hand use (partial $\eta^2 = 0.12$) all accounted for the differences in severity.

4 | DISCUSSION

The results of this study extend the clinical characterization and genotype-phenotype correlations in MDS and establish the importance of duplication size and gene content as contributing to

phenotypic severity. Several large series of patients with MDS have now been described in the literature.^{5,13,28,29} This study examined some previously unreported features in MDS from those cohorts, including delays within the first 6 months of life (common in 65% of this cohort), breathing disturbances (25%), hypoactivity (also quite common at 60%), and behavioral features, such as anxiety (29%) and irritability (58%). Aggression and self-injury were not common amongst participants in this study. Consistent with previous cohorts, this study showed that hypotonia, constipation, bruxism, vasomotor disturbances (most often cold hands and/or feet, skin mottling), and high pain tolerance are common in MDS. As found in previous studies,³² clinical severity was milder in the females enrolled in this cohort as compared to the males. In contrast, while recurrent respiratory infections are reported to be quite common in MDS, they were seen less frequently in this cohort at the time of evaluation. This may, in part, reflect sampling bias perhaps because of lack of participation by patients perceived by parents to be too fragile to travel to a study site. Alternatively, it is possible that increased awareness of this aspect of the syndrome has resulted in more primary prevention practices including use of airway clearance devices, better hand hygiene, exposure avoidance, and more timely seeking of medical care such that respiratory infections are more probably to be milder or avoided altogether. The rate of epilepsy in this cohort was also lower compared to the recent French cohort,¹³ but was consistent with that of other cohorts.^{5,28} This could reflect the younger age of participants at the time of evaluation and will be important to track over time. In addition, it will be important to track any changes to regression status over time, as this feature was also less frequent in this cohort (also perhaps reflecting a younger age and/or potential sampling bias).

This study is the first to show that duplication size is related to phenotypic severity; the larger the duplication, the more severely affected the participant was on both indices of clinical severity. In further examining the results and the degree to which the presence/absence of specific genes impacts severity beyond duplication size, individuals with a duplication of *RAB39B* were more severely affected regarding having microcephaly, and worse motor impairments in terms of hand use, ambulation, and sitting independently. It is important to note that this was independent of duplication size alone, given that duplication size was statistically controlled for in the analyses. This is also the first study to show that duplications of both *MECP2* and *RAB39B* confer greater clinical severity.

TABLE 3 Mean grand total Motor Behavioral Assessment Scale scores by the presence/absence of additional gene duplications within the base pair interval controlling for duplication size

	SRPK3	L1CAM	FLNA	GDI1	RAB39B
Absence	26.53 (8.86) N = 15	25.08 (9.25) N = 12	27.36 (11.17) N = 11	27.11 (10.19) N = 18	27.92 (10.71) N = 26
Presence	33.52 (13.52) N = 25	33.39 (12.80) N = 28	32.24 (12.69) N = 29	34.00 (13.30) N = 22	36.43 (13.65) N = 14
F value	F = 0.885; P = 0.35	F = 1.77; P = 0.19	F = 0.172; P = 0.68	F = 0.742; P = 0.395	F = 1.06; P = 0.31

RAB39B is strongly expressed in the brain, encoding a small GTPase that regulates neuronal development.^{21,34} Similar to *MECP2*, *RAB39B* appears to be a dosage-sensitive gene and a prior study showed that duplications of *RAB39B* alone are associated with ID and behavioral problems,²¹ independently of duplication of the *MECP2* gene itself. It is not surprising then to see higher severity scores with duplication of *RAB39B* in our MDS patients. A caveat to these findings, however, is that in this study all but one of the cases with larger duplications and greater severity scores encompassed *RAB39B*. Only one participant with a large duplication that did not encompass *RAB39B* had a lower severity score. In future, larger scale studies, to continue to tease apart the degree to which an additional duplication of *RAB39B* contributes to clinical severity in MDS, it will be important to continue to examine the degree to which large duplications which do not encompass this gene contribute to clinical severity. In other words, if equally large duplications that do not encompass the *RAB39B* result in lower levels of clinical severity as was seen in one case in this study, then it can be stated with greater confidence that *RAB39B* independently confers greater severity in MDS. The specific associated features of motor impairments and microcephaly do not recapitulate findings in published reports of *RAB39B* duplication, however, the neurodevelopmental interactions are probably complex and make it challenging to predict phenotype when both genes are duplicated. Future studies should continue to examine these associations. Over time, it will be important to track whether those with MDS including duplication of *RAB39B* are more susceptible to developmental regression and whether the regression occurs at a younger age. Although the present study did not detect statistically significant differences in severity for the other genes in question, the relatively small sample size and lack of a disorder-specific assessment strategy are limiting factors in interpretation. Thus, future studies should continue to examine the degree to which the other genes contribute to clinical severity and specific clinical features. For example, duplications in *FLNA* have previously been associated with increased severity of gastrointestinal features²⁵ and while it was not clearly associated with increased overall severity in this study, the severity of gastrointestinal issues was not specifically measured (the presence/absence of constipation was noted, but not as part of the severity scale). In addition, mutations in *L1CAM* have been associated with cerebral ventricle dilation as well as hypoplasia of the corpus callosum³⁵ both relatively common findings on brain imaging in MDS²⁴ which was not systematically documented in this study. Finally, future studies should examine X-inactivation patterns in females with MDS (this could contribute to differing levels of severity).

It is important to note that the severity scales employed in this study were created for use in RTT, and although some construct validity is evident (eg, females had milder severity scores than males), future studies should focus on refinement of these scales to include additional items that are more specific to MDS. One of the severity scales employed, the Motor Behavioral Assessment, did not yield any significant results, perhaps because of the nature of and scaling of the items being less specific to MDS. Even within the CSS, some essential phenotypic characteristics specific to MDS (eg, respiratory infections and hospitalizations, later onset of regression, urinary retention, and bowel obstruction) are not captured, but would be essential for tracking developmental trajectories in MDS and should be part of the future

development of a more MDS-specific scale. For example, the age range of regression in RTT is more constricted and confined to younger ages as opposed to those with MDS who tend to regress at later ages.¹⁵

There are some important limitations to the present study that should be addressed in future work. Specifically, this cohort still represents a relatively smaller sample size (given the estimated prevalence of MDS) that is skewed toward a younger age range and because of the travel requirement is potentially biased toward individuals who are less clinically severe. In addition, in-person clinical assessments, especially when conducted on a yearly basis, can only provide a snapshot of a child's functioning. This becomes problematic when assessing medically complex neurodevelopmental disorders, such as MDS where patients may have day-to-day fluctuations in functional ability because of their medical comorbidities. For example, temporary loss of ambulation because of an acute seizure or decreased attentiveness and discomfort from severe constipation could easily bias clinical assessments for the day. Retrospective parental reporting of a patient's functioning is thus required to fill in the gaps; however, the long intervals of reporting common to the design of natural history studies to minimally inconvenience families creates significant potential for recall bias.³⁶ Future efforts employing modern technologies for remote observational data collection and parental reporting through ecological momentary assessment strategies could permit more frequent and timely assessments of patients in their home environment; thus, helping to minimize the burden of travel while more fully ascertaining the spectrum of severity in neurodevelopmental disorders such as MDS.

In summary, this is the first study of MDS to show that both duplication size and specific gene content play a role in clinical severity. These biomarkers in part explain the observed heterogeneity in clinical presentation. Future studies should refine and expand existing severity scales to include more MDS-specific items, especially given the impending human clinical trials that have been buoyed by the promising pre-clinical studies suggesting MDS could be a reversible disorder.²⁷ Such trials will greatly benefit from the biomarkers reported here for purposes of patient stratification; and will also require patient assessment tools that are standardized, dynamic, valid, and reliable to accurately measure outcomes. It is essential that these tools be refined with feedback from all salient stakeholders including clinicians, parents and patient advocacy groups as progress continues toward clinical trial readiness.

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CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

This paper adheres to the official RDCRN policy related to natural history studies. Available data will be released to the repository and will become available to the scientific community 1 year after publication of planned analyses, or after a period of 5 years from the date when the data were collected, whichever comes first.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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