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RESEARCH ARTICLE

The array of clinical phenotypes of males with mutations in *Methyl-CpG binding protein 2*

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Abstract

Mutations in the X-linked gene *MECP2* are associated with a severe neurodevelopmental disorder, Rett syndrome (RTT), primarily in girls. It had been suspected that mutations in *Methyl-CpG-binding protein 2* (*MECP2*) led to embryonic lethality in males, however such males have been reported. To enhance understanding of the phenotypic spectrum present in these individuals, we identified 30 males with *MECP2* mutations in the RTT Natural History Study databases. A wide phenotypic spectrum was observed, ranging from severe neonatal encephalopathy to cognitive impairment. Two males with a somatic mutation in *MECP2* had classic RTT. Of the remaining 28 subjects, 16 had RTT-causing *MECP2* mutations, 9 with mutations that are not seen in females with RTT but are likely pathogenic, and 3 with uncertain variants. Two subjects with RTT-causing mutations were previously diagnosed as having atypical RTT; however, careful review of the clinical history determined that an additional 12/28 subjects met criteria for atypical RTT, but with more severe clinical presentation and course, and less distinctive RTT features, than females with RTT, leading to the designation of a new diagnostic entity, male RTT encephalopathy. Increased awareness of the clinical spectrum and widespread comprehensive genomic testing in boys with neurodevelopmental problems will lead to improved identification.

KEYWORDS

encephalopathy, genetics, male, *MECP2*, neurodevelopmental disorders, Rett syndrome

1 | INTRODUCTION

Rett syndrome (RTT) is a neurodevelopmental disorder occurring almost exclusively in females and results primarily from mutations in *Methyl-CpG-binding protein 2* (*MECP2*), the gene that encodes the transcriptional regulator MeCP2 (Amir et al., 1999; Hagberg, Aicardi, Dias, & Ramos, 1983; Rett, 1966). Two diagnostic entities have been identified based on the number of main and supportive diagnostic criteria (Neul et al., 2010): typical or classic RTT and atypical or variant RTT. RTT has been identified in boys who typically fall into two categories, those with 47XXY (Klinefelter syndrome; Schwartzman, Bernardino, Nishimura, Gomes, & Zatz, 2001; Vorsanova et al., 2001) or those with somatic mosaicism (Clayton-Smith, Watson, Ramsden, & Black, 2000). Rarely, atypical RTT has been noted in XY nonmosaic males with *MECP2* mutations associated with milder disease in females with RTT (Dayer et al., 2007; Neul et al., 2014). Despite the reports of males with *MECP2* mutations surviving, some recent publications continue to present a misconception that males with *MECP2* mutations did not survive pregnancy or had an early-onset severe encephalopathy that resulted in death at a very young age (Chen et al., 2017). Initial attempts to develop a mouse model lacking *Mecp2* failed due to intrauterine demise (Tate, Skarnes, & Bird, 1996). Subsequently, the same laboratory generated a mouse *Mecp2* null mouse using modified methodology, demonstrating that loss of MeCP2 function does not lead to embryonic lethality (Guy, Hendrich, Holmes, Martin, & Bird, 2001). Thereafter, several reports of affected human males emerged such that experienced clinicians recognize a spectrum of clinical involvement in males with *MECP2* mutations. These include mutations seen in females with RTT (Augenstein, Lane, Horton, Schanen, & Percy, 2009; Bianciardi et al., 2016; Kankirawatana et al., 2006; Schule, Armstrong, Vogel, Oviedo, & Francke, 2008; Villard, 2007), as well as mutations which appear to produce abnormal neurodevelopment only in males (Couvert et al., 2001; Dotti et al., 2002; Gomot et al., 2003; Klauck et al., 2002; Lambert et al., 2016; Moog et al., 2003; Orrico et al., 2000; Winnepenninckx, Errijgers, Hayez-Delatte, Reyniers, & Frank Kooy, 2002). Nonetheless, the misperception among those not familiar with RTT that males with *MECP2* mutations do not survive pregnancy or die within the first year of life unless afforded ventilatory support has been repeated in the published literature on animal models (Chen et al., 2017). Further, the suggestion that RTT occurs in males despite not meeting the established consensus criteria, or even having a duplication in *MECP2*, is still evident (Reichow, George-Puskar, Lutz, Smith, & Volkmar, 2015).

The occurrence of RTT predominantly in females is principally the result of de novo mutations, especially deamination of methylated cytosines, occurring in rapidly dividing germinal cells, namely sperm (Cuddapah et al., 2014; Girard et al., 2001; Thomas, 1996; Zhu et al., 2010). Such mutations cannot be transmitted to a male, explaining in part the lower frequency of affected males. Occasionally, RTT results from mutations in transmitting females who themselves do not fulfill the criteria for RTT, either having mild cognitive impairment or learning disability or being phenotypically normal, all related to unbalanced or skewed X chromosome inactivation (XCI; Augenstein et al., 2009; Schanen, 2001; Schanen & Francke, 1998). In other instances, the mutation may be a de novo event in the ovum.

Information gleaned from the RTT Natural History Study (RNHS; ClinicalTrials.gov: NCT00299312/NCT02738281) provides convincing evidence that the male phenotype for those with *MECP2* mutations, both with classic RTT or presenting with other clinical phenotypes is actually much broader than generally reported. From these two linked databases, we identified 30 males featuring widely varying phenotypes. In the future, the identification of additional males with similar clinical findings could result from more in-depth genetic assessment of individuals with neurodevelopmental abnormalities.

2 | METHODS

2.1 | Participants

The RNHS, RTT5201; CT.gov: NCT00299312, began enrolling participants in 2006. The RNHS is part of the Rare Diseases Clinical Research Network (RDCRN), established through the Office of Rare Diseases Research, National Center for Advancing Translational Sciences at the National Institutes of Health.

When it concluded in 2014, more than 1,200 individuals with RTT or with *MECP2* mutations or duplications had been identified. Participants recruited at four primary sites and four travel clinics across the United States provided a large cohort suitable for longitudinal information with the ultimate goal of conducting clinical trials. Of the total cohort in RTT5201, 22 males had *MECP2* mutations. In 2014, the continuation of the RTT NHS (RTT5211; CT.gov: NCT02738281) commenced. Nine males with a *MECP2* mutation have been enrolled in 5211 at the time of this analysis, one of these males having already been enrolled in RTT5201.

2.2 | Diagnosis

In RTT5201, a RNHS neurologist or geneticist (DGG, SAS, WEK, JLN, and AKP) with extensive clinical experience in RTT utilized the established criteria for diagnosis of RTT or other related phenotypes. In RTT5211, a neurologist or geneticist (TAB, JLN, SAS, EDM, or AKP) characterized nine males including the one male carried forward from RTT5201. All participants in the RNHS were required either to meet clinical criteria for RTT and/or to have a mutation in *MECP2*. The clinical diagnosis was determined from the information present within the RNHS database, and cases were classified as RTT (classic or atypical) if they met consensus criteria, neonatal encephalopathy if they showed impairment from birth, progressive encephalopathy if the presentation was delayed and worsening, or cognitive impairment if they did not show progressive worsening over the course of the study (Table 1).

2.3 | Genetic testing and mutation classification

Genetic testing consisted of Sanger sequencing of all four exons, and evaluation for large rearrangements if no sequence variants discovered. In 24 of the 30 cases, genetic information from the mother or sister of the affected individual was also available allowing for determination of inheritance pattern (Table 1).

TABLE 1 *MECP2* mutations and diagnostic categories of subjects

Case	Diagnosis age	MECP2 mutation	Mutation type	Inheritance pattern	Diagnostic category	Surviving	Age at death
1	5.8	c.397C>T, p.R133C	RTT-causing	De novo	Classic Rett – Mosaic	Yes	–
2	5	c.880C>T, p.R294X	RTT-causing	De novo	Classic Rett – Mosaic	Yes	–
3	11.9	c.exon 1 – 57-58ins	RTT-causing	Not tested	Cognitive impairment	Yes	–
4	0.8	c.378-3C>G, intronic	RTT-causing	Not tested	Progressive encephalopathy (male RTT encephalopathy)	No	4.2 years
5	2.2	c.397C>T, p.R133C	RTT-causing	Mother positive	Progressive encephalopathy	Yes	–
6	4.2	c.397C>T, p.R133C	RTT-causing	Mother positive	Progressive encephalopathy (male RTT encephalopathy)	Yes	–
7	0.9	c.507ins2bp, p.Q170fs	RTT-causing	De novo	Progressive encephalopathy (male RTT encephalopathy)	No	7.4 years
8	2.8	c.763C>T, p.R255X	RTT-causing	Not tested	Neonatal encephalopathy (male RTT encephalopathy) tracheostomy	No	9.7 years
9	0.6	c.806delG, p.G269 fs	RTT-causing	De novo	Neonatal encephalopathy (male RTT encephalopathy)	Yes	–
10	1.6	c.806delG, p.G269 fs	RTT-causing	De novo	Neonatal encephalopathy	No	3.3 years
11	0.8	c.808C>T, p.R270X	RTT-causing	De novo	Neonatal encephalopathy	Yes	–
12	3.3	c.1133-1134insT, p.A378fs	RTT-causing	De novo	Cognitive impairment	Yes	–
13	4.8	c.1145-*452del768p.L.382 fs	RTT-causing	Mother positive	Atypical Rett	No	5.3 years
14	3	c.1155-1200del46; p.L386 fs	RTT-causing	Mother positive	Atypical RTT	Yes	
15	4.7	c.1164-1207del44, p.P398fs	RTT-causing	Mother positive	Cognitive impairment	Yes	–
16	4.5	c.1164-1207del44, p.P398fs	RTT-causing	Mother positive	Cognitive impairment/progressive dystonia (male RTT encephalopathy)	No	13.1 years
17	18.1	c.1164-1207del44, p.P398fs	RTT-causing	Mother positive	Cognitive impairment/progressive dystonia (male RTT encephalopathy) Ventilatory dependent	No	29.8 years
18	2.9	c.1357C>T, p.P453X	RTT-causing	Mother positive	Progressive encephalopathy	No	4.8 years
19	2	c.353G>A, p.G118E	Likely pathogenic	De novo	Cognitive impairment	Yes	–
20	0.7	c.377A>T, p.N126I	Likely pathogenic	De novo	Neonatal encephalopathy	No	3.2 years
21	20.4	c.419C>T, p.A140V	Pathogenic	Not tested	Cognitive impairment	Yes	–
22	1.3	c.471C>G, p.F157L	Likely pathogenic	Not tested	Neonatal encephalopathy (male RTT encephalopathy) Ventilatory dependent	Yes	–
23	12.7	c.499C>T, p.R167W	Likely pathogenic	Mother positive	Cognitive impairment	Yes	–
24	2.2	c.527C>A, p.P176H	Likely pathogenic	Not tested	Progressive encephalopathy	Yes	–
25	2.3	c.917G>C, p.R306P	Likely pathogenic	De novo	Cognitive impairment/progressive dystonia (male RTT encephalopathy)	Yes	–
26	1.4	c.925C>T, p.R309W	Likely pathogenic	Presumptive (sister positive)	Cognitive impairment (male RTT encephalopathy)	Yes	–
27	3.5	c.964C>T, p.P322S	Likely pathogenic	Mother positive	Cognitive impairment	Yes	–
28	3.7	c.777C>T, p.A259A	Uncertain	Mother positive	Cognitive impairment/progressive dystonia	Yes	–
29	5.8	c.1100A>G, p.H367R	Uncertain	De novo	Progressive encephalopathy (male RTT encephalopathy)	Yes	–
30	4.3	c.1168-1173del6, p.390-391del	Uncertain	Mother positive	Cognitive impairment/progressive dystonia (male RTT encephalopathy)	Yes	–

Mutations were classified by evaluating the literature and RettBASE (http://mecp2.chw.edu.au) to determine if they had been previously identified in females with RTT. In those cases, they were classified as RTT-causing. Additionally, we classified those mutations that would result in a molecular change similar to that previously observed in a female with RTT as RTT-causing. The remaining *MECP2* sequence variants were evaluated in silico by determining the frequency of these variants in control populations from Exome Aggregation Consortium (ExAC, exac.broadinstitute.org) and 1000 Genomes (http://www.internationalgenome.org). The lack of variant detection or very rare detection rates support the possibility that these are deleterious variants. Further characterization was performed by analyzing the variants using a number of bioinformatic prediction tools (CLINSIG, SIFT, PolyPhen2_HDIV, PolyPhen2_HVAR, LRT, MutationTaster, MutationAssessor, FATHMM, PROVEAN, Meta-SVM, MetaLR, M-CAP, DANN) to assess the predicted deleterious effects on the protein. Variants were considered as Likely Pathogenic if a majority of the prediction tools indicted that the variants were "Damaging" or "Not-tolerated". We classified the remaining variants as "Uncertain".

Phenotypic evaluation: Each male was evaluated according to the following features: RTT main and supportive diagnostic criteria (Neul et al., 2010), clinical features including growth (Tarquinio et al., 2012), two global measures of RTT clinical involvement, the Clinical Severity Scale (CSS) and the Motor Behavioral Assessment (MBA; Cuddapah et al., 2014; Neul et al., 2008), and survival (Kirby et al., 2010; Tarquinio et al., 2015). Increasing overall scores on both the CSS and the MBA represent increasing severity.

3 | HUMAN STUDIES APPROVAL

Each site obtained and maintained Institutional Review Board (ethics) approval for the performance of these studies. The study clinicians verified all data at time of interview and examination. Parents or authorized caregivers provided approval for study conduct and publication of results prior to entry into the study. We registered these observational studies in ClinicalTrials.gov: NCT00299312 for RTT5201 and NCT02738281 for RTT5211.

4 | RESULTS

The 30 males with *MECP2* variants identified in the RNHS vary in age at diagnosis from 0.6 to 20.4 years (Table 1). The variants span the entire gene, with 18 RTT-causing mutations, nine pathogenic or likely pathogenic but not RTT-causing mutations, and three variants that upon further evaluation are likely benign (Table 1). Table 2 presents the bioinformatics evaluation of the likely pathogenic group.

4.1 | Classification of genetic variants in *MECP2*

The identified genetic changes were first classified as being clearly RTT-causing (n = 18) or not (n = 12; Table 1). Determination that a mutation was RTT-causing was based on whether the genetic change

TABLE 2 Results from predictive algorithm for likely pathogenic mutations in *MECP2*

Case	*MECP2* mutation	Ref SNP	ExAC	1000 g	Mutation type	CLINSIG	Prediction	SIFT	Polyphen2_HDIV	Polyphen2_HVAR	LRT	Mutation taster	Mutation assessor	FATHMM	PROVEAN	Meta SVM	Meta LR	M-CAP	DANN
19	c.353G>A, p.G118E	No record			Likely pathogenic	Not reported	12/12	D	D	D	D	D	M	D	D	D	D	D	0.998
20	c.377A>T, p.N126I	No record			Likely pathogenic	Not reported	2/2					D							0.994
21	c.419C>T, p.A140V	rs28934908	ND	2.28E-05	Pathogenic	Pathogenic	10/12	D	D	D	D	A	L	D	N	D	D	D	0.999
22	c.471C>G, p.F157 L	rs267608484	ND	ND	Likely pathogenic	Uncertain	12/12	D	D	D	D	D	M	D	D	D	D	D	0.999
23	c.499C>T, p.R167W	rs61748420	ND	ND	Likely pathogenic	Pathogenic	12/12	D	D	D	D	D	M	D	D	D	D	D	0.999
24	c.527C>A, p.P176H	rs61749701	7.99E-05	0.000265	Likely pathogenic	Uncertain	10/12	D	D	P	D	D	L	D	N	D	D	D	0.996
25	c.917G>C, p.R306P	rs61751443	ND	ND	Likely pathogenic	Not reported	10/12	D	P	B	D	D	M	D	N	D	D	D	0.996
26	c.925C>T, p.R309W	rs61751444	ND	ND	Likely pathogenic	Uncertain	12/12	D	D	D	D	D	M	D	D	D	D	D	0.999
27	c.964C>T, p.P322S	rs61751449	ND	ND	Likely pathogenic	Pathogenic	7/12	D	B	B	D	D	L	D	N	T	D	D	0.999

SIFT: D = damaging. PolyPhen2: D = probably damaging, P = possibly damaging, B = benign. LRT: D = Deleterious. MutationTaster: A = disease causing automatic, D = disease causing. MutationAssessor: H = high mutation prediction, M = medium mutation prediction. FATHMM: D = damaging. PROVEAN: D = damaging, N = neutral. MetaSVM: D = damaging, T = tolerated. M-CAP: D = damaging, T = tolerated. MetaLR: D = damaging. DANN: >0.996 is considered pathogenic.

had previously been identified in females with RTT (Cases 1, 2, 4, 5, 6, 8, 9, 10, 11, 14, 15, 16, 17, and 18) in the RettBASE (http://mecp2.chw.edu.au) genotype database, or were similar to previously reported RTT-causing mutations. Case 3 had a p.R20fs, identical to the p.R20fs seen in the reported c.59-60del (Chunshu, Endoh, Soutome, Kawamura, & Kubota, 2006). Case 5 had a p.Q170fs, similar to the reported p.Q170X mutation found in many individuals with RTT (RettBASE). Case 12 had a p.A378fs, which is the same frameshift reported in a different carboxy-terminal truncation, c. 1132del71, associated with RTT (PMID 11241840). Case 13 had a unique deletion starting at position c.1146; multiple reports of similar frame-shift deletions starting at position c.1145 have been identified in RTT (RettBASE), supporting the classification of this mutation as RTT-causing.

The remaining *MECP2* sequence variants were evaluated in silico (Table 2). Eight of the cases had *MECP2* mutations in which the majority of the prediction programs indicated deleterious effects of the variant and were considered "Likely Pathogenic" (Cases 19, 20, 22, 23, 24, 25, 26, and 27). Case 21 had a p.A140V mutation, which has been reported multiple times in boys with intellectual disability, neuropsychiatric, and movement abnormalities (Cohen et al., 2002) and is considered pathogenic although not associated with RTT.

Three subjects had sequence variation in *MECP2* that do not clearly disrupt function (Table 1). Two cases (Cases 28, 30) were identified as "Benign" by CLINSIG. One (case 28) involves a synonymous mutation (c.777C>T, p.A259A, refSNP rs1042870, ExAC 0.00039, 1000 Genomes 8×10^{-4}) inherited from his mother who appears normal. X-chromosome inactivation (XCI) analysis in her blood demonstrated a random (51:49) pattern. This mutation has been reported previously in both females and males and found not to be associated with RTT, and is listed as a silent polymorphism in RettBASE. Case 29 had a missense variant (p.H367R, not reported in refSNP, no record in ExAC or 1000 Genomes) that was predicted to be damaging in only a minority (3/12) of the prediction tools. The final subject (case 30) had a six base-pair, in-frame deletion (c.1168-1173del6, p.P390_391del, refSNP rs61753008, ExAC 8.5×10^{-5}, 1000 Genomes 5×10^{-4}). This variant was found in both the mother and grandmother and associated with a random (70:30) XCI pattern in the mother. The grandmother's XCI results were uninformative. The grandmother appears normal, but the mother has at least mild cognitive impairments. From this analysis, it is unclear whether any of these variants are causative, therefore we categorize them as "Uncertain".

4.2 | Individuals characterized as RTT

Two subjects were classified as classic RTT and were shown to have somatic mutations, leading to a cellular mosaic pattern of mutation. These individuals did not have the major exclusion criteria for classic RTT (poor early development, Table 3), and met all four main inclusion criteria (loss of hand skills, loss of spoken communication, hand stereotypies, and abnormal gait, Table 3). As classic RTT has been identified in males with somatic mutations in *MECP2* before (Clayton-Smith et al., 2000), this is not unexpected.

Two additional subjects (Cases 13, 14), both with RTT-causing *MECP2* mutations, were classified as having atypical RTT (Table 1). Both subjects had normal initial development followed by regression (Table 2), and clearly met three of the four main criteria (Table 3) and more than five of eleven supportive criteria (Table 4). Case 13 only showed transient hand stereotypies, and case 14 did not demonstrate any loss of hand skills.

4.2.1 | Subjects with RTT-causing mutations in *MECP2*

Sixteen subjects had germline (nonmosaic) *MECP2* mutations either found in or similar to those found in females with RTT, designated RTT-causing (Table 1). Inheritance from the mother occurred in eight, five were de novo, and in three cases parental mutational testing was not performed. Within this group, four were classified as neonatal encephalopathy, five with progressive encephalopathy, two atypical RTT, and five with cognitive impairment.

Eleven subjects in this group showed markedly abnormal early development and nine had a clear history of regression (Table 3). Seven had a clear history of lost hand skills, which ranged from the loss of pincer grasp to the loss of reaching for objects. Notably, regression of hand skills was observed very late in two subjects (cases 16 and 17), both of whom have the same carboxy-terminal truncating mutation inherited from their mothers. These subjects are related as uncle and nephew, and the younger individual has a sister with classic RTT, as previously reported (Augenstein et al., 2009). Of the nine who did not have any clear history of loss of hand skills, marked variation in the maximal hand skills gained was noted, ranging from no notable hand skills attained to pincer grasp. Only two of the sixteen gained and retained pincer grasp, and 5/16 either had no hand skills or lost all hand skills.

In general, acquisition of spoken language was markedly impaired in the 16 subjects with RTT-causing mutations (Table 3), with three attaining no vocalizations at all, eight gaining vocalizations, three gaining babbling, and one achieving words. Six showed loss of spoken language, with five ultimately having no utterances at all and only one regaining babbling. Eventually, all vocalization was completely absent in eight, seven with only vocalizations, and one able to babble.

Clear, persistent hand stereotypies were found in seven subjects and one had no hand stereotypies at all. Eight had transient stereotypies, with face/eye/nose rubbing in six. Only one of the 16 subjects attained and maintained independent normal gait (Case 3), whereas nine never gained any gait skills. Of the remaining six, four lost all acquired gait, and two retained independent dyspraxic gait. Thus, the majority of subjects in this group (13/16) eventually had no gait skills at all.

Many of the males with RTT-causing *MECP2* mutations had seizures, microcephaly, or supportive diagnostic features of RTT (Tables 4 and 5). The majority had periodic breathing, bruxism, sleep issues, abnormal muscle tone, growth failure, small hands or feet, abnormal pain response, seizures, or microcephaly. Interestingly, only one subject had eye pointing. Thirteen of the sixteen have at least five of the eleven supportive criteria required for the diagnosis of atypical RTT (Neul et al., 2010). The overall clinical severity as determined by the RTT Clinical Severity Scale (CSS) or the Motor Behavioral Assessment (MBA) was variable in this group, ranging from 12 to 42 (maximum score: 45) and 26 to 83 (maximum score: 136), respectively.

TABLE 3 RTT main criteria and clinical severity

Case	CSS	MBA	Poor early development	Regression	Loss of hand skills	Loss of communication	Hand stereotypies	Abnormal gait	Number major criteria
1	13	34	No	Yes	Yes (lost at age 2)	Yes	Yes (hand clapping, hand tapping)	Yes (dyspraxic but independent)	4/4
2	17	49	No	Yes	Yes (lost at 36 mo)	Yes	Yes (hand clapping)	Yes (dyspraxic but independent)	4/4
3	14	51	Yes	No	No (gained finger feeding, no pincer)	No (vocalizes)	Transient (rubbed nose; not persistent)	No (walks independently)	0/4
4	36	73	Yes	Yes	Yes (lost 12 months)	Yes (lost vocalizations 5 yrs, now no utterances)	Transient (rubbed face; not persistent)	Yes (not attained)	3/4
5	31	63	Yes	No	No (never attained)	No (only vocalizes)	Yes (hand flapping next to body)	Yes (not attained)	2/4
6	37	77	No	Yes	Yes (lost pincer 2.5 yrs)	No (never acquired)	Transient (rubbed eyes; not persistent)	Yes (lost supported gait 19 mo)	2/4
7	41	66	Yes	Yes	No (limited gain-reaching)	Yes (loss of babble; no utterances)	Transient (rubbed eye; not persistent)	Yes (not attained)	2/4
8	39	73	Yes	Yes	Yes (lost reaching for toy 8 mo)	Yes (babble lost 10 mo; no utterances)	Transient (hand mouthing 1.5 yrs; not persistent)	Yes (not attained)	3/4
9	34	57	Yes	No	No (never attained)	No (never acquired)	Yes (rare hand clapping)	Yes (not attained)	2/4
10	42	78	Yes	No	No (limited gain of reaching)	No (vocalizes)	Transient (rubbed nose; not persistent)	Yes (not attained)	1/4
11	38	71	Yes	No	No (never attained)	No (never acquired)	No	Yes (not attained)	1/4
12	12	36	No	No	No (gained pincer)	No (vocalizes)	Yes (hand wringing, washing)	Yes (independent dyspraxic gait)	2/4
13	42	83	No	Yes	Yes (lost finger feeding 18 mo)	Yes (lost words 30 months; now no utterances)	Transient (hand clapping 2.5 yrs; not persistent)	Yes (lost all at 48 mo)	3/4
14	17	26	No	Yes	No (gained cup hold, finger feed, pincer)	Yes (lost babble but regained)	Yes (hand clapping, tapping, finger flicking)	Yes (independent dyspraxic gait)	3/4
15	18	36	Yes	No	No (gained finger feed, no pincer)	No (only vocalizes)	Yes (squeezing, finger rubbing)	Yes (not attained)	2/4

(*Continues*)

TABLE 3 (Continued)

Case	CSS	MBA	Poor early development	Regression	Loss of hand skills	Loss of communication	Hand stereotypies	Abnormal gait	Number major criteria
16	23	45	No	Yes	Yes (lost pincer by 11 yo)	No (only vocalizes)	Transient (rubbed nose; not persistent)	Yes (lost supported gait)	2/4
17	33	78	Yes	Yes	Yes (lost all hand use 10 yo)	Yes (words lost; no utterances)	Yes (hand squeezing 10 yrs)	Yes (lost all gait)	4/4
18	27	61	Yes	Yes	Yes (lost finger feeding)	No (only vocalizes)	Yes (hand tapping, clapping)	Yes (not attained)	3/4
19	13	24	No	No	No (gained pincer grasp)	No (only vocalizes)	No	Yes (not attained)	1/4
20	36	61	Yes	No	No (never acquired)	No (never acquired; no utterances)	Transient (hand wringing 0.6 yr; not persistent)	Yes (not attained)	1/4
21	13	27	No	Yes	Yes (pincer 20 yrs)	No (multiple words)	Transient (hand wringing 5 yo; not persistent)	Yes (supported dyspraxic)	2/4
22	37	77	Yes	Yes	Yes (lost finger feeding 18 mo)	No (never acquired; no utterances)	No	Yes (not attained)	2/4
23	12	15	No	No	Yes (lost reaching 18 mo, regained, gained pincer grasp)	No (speaks in sentences)	Yes (hand clapping, finger rubbing)	No (walks/runs)	2/4
24	35	67	No	Yes	No (never acquired)	No (never acquired; no utterances)	Transient (hand mouthing 1.5 yrs; not persistent)	Yes (not attained)	1/4
25	25	57	Yes	Yes	No (gained finger feeding, no pincer)	Yes (lost vocalization at 8 mo)	Transient (hand clapping, tapping 1 yr; not persistent)	Yes (not attained)	2/4
26	9	21	No	Yes	Yes (lost pincer 36 mo)	No (only vocalizes)	Transient (hand flapping; not persistent)	Yes (dyspraxic but independent)	2/4
27	3	8	No	No	No (gained pincer)	No (speaks in sentences)	No	No (walks)	0/4
28	27	51	Yes	No	No (never attained)	Never acquired; no utterances	Transient (hand mouthing early; not persistent)	Yes (not attained)	1/4
29	19	34	Yes	Partial	No (gained pincer)	No (only vocalizes)	Yes (hand squeezing, clapping)	Yes (not attained)	2/4
30	11	44	Yes	Yes	No (never attained)	Yes (lost words 2.5 yrs; now vocalizes)	Transient (stared at hands at 3.5 yrs; not persistent)	Yes (not attained)	2/4

CSS = RTT clinical severity scale. MBA = motor behavioral assessment scale.

TABLE 4 RTT supportive criteria

Case	Periodic breathing	Bruxism	Sleep issues	Muscle tone	Peripheral vasomotor	Scoliosis	Growth failure	Small hands/ft	Screaming	Poor pain response	Eye pointing	Seizures	Microcephaly	No. supportive criteria
1	No	Yes	No	Yes	No	No	Yes	No	No	Yes	Yes	None	No	5/11
2	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes	No	Monthly	No	6/11
3	No	Yes	Yes	Yes	No	No	No	No	No	Yes	No	Daily	No	4/11
4	No	Yes	No	Yes	No	No	Yes	Yes	No	Yes	No	None	Yes	5/11
5	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	None	Yes	8/11
6	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes	No	Daily	Yes	7/11
7	Yes	No	Yes	Yes	No	<20°	Yes	Yes	No	Yes	No	Monthly	Yes	7/11
8	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes	No	Daily	Yes	7/11
9	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	No	None	Yes	6/11
10	Yes	No	Yes	Yes	No	<40°	Yes	Yes	No	Yes	No	Weekly	Yes	7/11
11	Yes	Yes	No	Yes	Yes	No	No	Yes	No	No	No	Daily	Yes	5/11
12	No	Yes	No	No	No	No	No	No	No	Yes	No	Monthly	No	2/11
13	Yes	Yes	Yes	Yes	No	<40°	Yes	Yes	No	Yes	No	Daily	Yes	8/11
14	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	Weekly	No	5/11
15	No	Yes	Yes	Yes	No	<20°	Yes	Yes	Yes	Yes	No	None*	Yes	8/11
16	No	No	Yes	Yes	Yes	Surq.	Yes	Yes	Yes	Yes	No	None	No	8/11
17	Yes	No	Yes	Yes	Yes	>60°	Yes	Yes	Yes	Yes	No	Daily	Yes	9/11
18	Yes	No	No	Yes	No	No	No	No	No	No	No	Daily	No	2/11
19	No	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	No	None	Yes	6/11
20	Yes	Yes	Yes	Yes	Yes	>60°	Yes	Yes	No	Yes	No	None	Yes	9/11
21	No	No	Yes	Yes	No	No	Yes	Yes	No	Yes	No	None	No	5/11
22	Yes	Yes	Yes	Yes	Yes	>60°	No	No	No	Yes	No	None	Yes	7/11
23	No	Yes	Yes	No	Yes	>40°	No	No	Yes	Yes	No	None	No	6/11
24	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	No	Daily	Yes	8/11
25	No	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes	No	None	Yes	6/11
26	No	Yes	No	No	No	No	No	No	No	Yes	No	None	No	2/11
27	No	Yes	Yes	No	Yes	No	No	No	No	No	No	None	No	3/11
28	No	Yes	Yes	Yes	Yes	<40°	Yes	Yes	No	Yes	No	Monthly	No	8/11
29	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	None	No	8/11
30	No	Yes	Yes	Yes	No	<20°	Yes	Yes	Yes	Yes	No	None	No	8/11

TABLE 5 Percentage of RTT supportive criteria by *MECP2* mutation type

Subject group	Periodic breathing % (*n*)	Bruxism % (*n*)	Sleep issues % (*n*)	Muscle tone % (*n*)	Peripheral vasomotor % (*n*)	Scoliosis % (*n*)	Growth failure % (*n*)	Small hands/ft % (*n*)	Screaming % (*n*)	Poor pain response % (*n*)	Eye pointing % (*n*)	Seizures % (*n*)	Microcephaly % (*n*)
Mosaic RTT (*n* = 2)	50 (1)	100 (2)	50 (1)	100 (2)	0 (0)	0 (0)	50 (1)	0 (0)	50 (1)	100 (2)	50 (1)	50 (1)	0 (0)
RTT causing (*n* = 15)	69 (11)	69 (11)	63 (10)	94 (15)	38 (6)	38 (6)	69 (11)	63 (10)	31 (5)	75 (12)	6 (1)	69 (11)	69 (11)
Early (*n* = 9)	78 (7)	78 (7)	56 (5)	100 (9)	33 (3)	22 (2)	78 (7)	67 (6)	22 (2)	78 (7)	11 (1)	67 (6)	89 (8)
Late (*n* = 6)	57 (4)	57 (4)	71 (5)	86 (6)	43 (3)	57 (4)	57 (4)	57 (4)	43 (3)	71 (5)	0 (0)	71 (5)	43 (3)
Likely pathogenic, non-RTT causing (*n* = 9)	33 (3)	89 (8)	89 (8)	67 (6)	78 (7)	33 (3)	44 (4)	33 (3)	22 (2)	89 (8)	0 (0)	11 (1)	56 (5)
Benign (*n* = 3)	0 (0)	100 (3)	100 (3)	100 (3)	67(2)	67 (2)	100 (3)	100 (3)	67 (2)	100 (3)	0 (0)	33 (1)	0 (0)

Previous work exploring the genotype–phenotype relationship in females with RTT revealed increased severity in early truncating mutations compared to late truncating mutations, and the presence of point mutations such as p.R133C conferring less severity (Cuddapah et al., 2014; Neul et al., 2008). Within this cohort of males with RTT-causing mutations, we did not observe decreased severity in boys with p.R133C mutations (Cases 5 and 6) compared to other mutations. However, observable differences are noted when comparing all early mutations (before codon 271) and late mutations (Figure 1). We chose codon 271 as the dividing point between early and late mutations because clear phenotypic differences exist in females with RTT (Cuddapah et al., 2014) and in mouse models (Baker et al., 2013) between those individuals with truncations before codon 270 and after codon 270. In general, males with early RTT mutations had a higher CSS score compared to those with late RTT mutations, with the exception of one outlier in each group (Case 3, an exon 1 mutation, and case 18, a very late truncating mutation). Furthermore, in the early mutation group, 8/9 were classified as either neonatal or progressive encephalopathy, whereas only 1/7 had progressive encephalopathy in the late mutation group. A greater percentage of subjects with early mutations had periodic breathing, bruxism, growth failure, and microcephaly, whereas a greater percentage of subjects with late mutations had sleep issues and screaming (Table 5).

4.2.2 | Subjects with non RTT-causing but likely pathogenic mutations in *MECP2*

Nine subject had mutations in *MECP2* that have not been seen in females with RTT but were identified as either pathogenic because identical mutations have been found in boys with intellectual disability and other clinical features (p.A140V, case 21; Venkateswaran, McMillan, Doja, & Humphreys, 2014), or because a majority of molecular prediction tools identify the mutations as being damaging or nontolerated (Table 2). Three individuals had identified inherited mutations, two clearly identified in mother and one presumably from mother because the same mutation was identified in a sister (Table 1). In this instance, testing the mother was not possible due to her inability to provide informed consent because of intellectual disability and neuropsychiatric features. Those mothers who tested positive were cognitively impaired according to previously obtained IQ testing. Three mutations were de novo and three parents were not tested.

Six of the nine in this group had normal initial development, and five had clear evidence of regression (Table 2). Two did not gain any hand skills, two achieved finger feeding, and five attained pincer grasp. Four had loss of hand skills, with one (Case 23) regaining the lost skill (reaching) and continuing to gain new skills (pincer). Three of the nine ultimately were able to maintain a pincer. Five subjects had 2/4 main criteria, but one did not have a history of regression and thus is excluded from the possibility of having a diagnosis of RTT.

Only one subject in this group lost vocalizations (Case 25). Of the eight with no history of language loss, three did not gain any spoken language at all and had no utterances, two only gained vocalizations, one had multiple words, and two could speak in sentences. Thus, the range of language abilities in this group is very wide. Gait was also highly variable in this group, with five not attaining any gait, one able

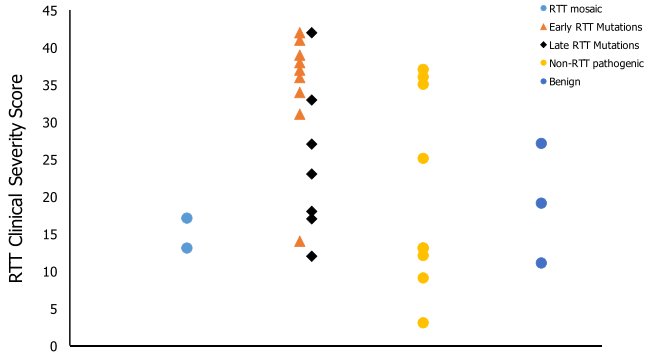

FIGURE 1 Distribution variation of CSS based on *MECP2* mutation type. Light blue circles = people with somatic mutations in *MECP2*. Orange triangles = people with early RTT-causing mutations in *MECP2*. Black diamonds = people with late RTT-causing mutations in *MECP2*. Yellow circles = people with pathogenic mutations in *MECP2* that do not cause RTT in females. Dark blue circles = people with benign variants in *MECP2* [Color figure can be viewed at wileyonlinelibrary.com]

to display dyspraxic supported gait, one with independent dyspraxic gait, and two with normal gait.

Three subjects had no evidence of hand stereotypies. One showed persistent hand clapping and finger rubbing, and five had transient stereotypies. In contrast to the face/eye/nose rubbing observed in subjects with RTT-causing mutations, most people with non-RTT causing pathogenic *MECP2* mutations had transient hand wringing/clapping/mouthing stereotypies.

Nearly all subjects in this group (8/9) had bruxism, sleep issues, and poor pain response (Tables 4 and 5). The majority also had abnormal muscle tone. Periodic breathing, scoliosis, and small hands or feet were only present in a third of the cases, and microcephaly in just over a half. Screaming was present in two subjects, and only one person had seizures. Seven of the nine had more than 5/11 supportive criteria.

4.2.3 | Subjects with uncertain variants in *MECP2*

The three individuals with uncertain genetic variants in *MECP2* had abnormal initial development, and only one had a clear history of regression. Case 29 had limited regression with transient loss of social smile between 18 and 36 months, but no clear loss of other developmental skills. Two of the three did not gain any hand skills, with the third acquiring pincer grasp. None had a history of loss of hand skills. One did not gain any spoken language, one only gained vocalization, and one lost words. Only one had persistent hand stereotypies, whereas the other two had nonpersistent transient stereotypies. Finally, none of these individuals gained any ability to walk.

All the subjects in this group had bruxism, small hands or feet, and poor pain response (Tables 4 and 5), but none had periodic breathing, eye pointing or microcephaly. Two had peripheral vasomotor abnormalities and screaming, and only one had seizures. All had 8/11 supportive criteria.

4.2.4 | Strict application of RTT diagnostic criteria leads to definition of new entity: male RTT encephalopathy

Aside from the two cases with somatic mosaic mutations in *MECP2* who clearly met all the criteria for classic RTT, only two of the remaining 28 were classified in the NHS database as atypical RTT, with the remainder classified as neonatal encephalopathy, progressive encephalopathy, or cognitive impairment. However, when the clinical criteria were strictly applied to these cases, we determined that 12 additional cases (RTT-causing mutations: Cases 4, 6, 8, 16, 17, 18; likely pathogenic mutations: Cases 7, 21, 22, 25, 26, 30) had a history of regression, at least two of the four major criteria (Table 3), and at least 5/11 supportive diagnostic criteria (Table 4).

The majority (8/12) of these individuals (classified as "male RTT encephalopathy) did not have normal initial development. Nine of the 12 had loss of hand skills and 5 had loss of spoken language. Only two had persistent hand stereotypies, whereas the remaining 10 had transient hand stereotypies. All 12 had abnormal gait, with 9 either not attaining gait or completely losing gait.

The most commonly present supportive criteria present were abnormal muscle tone and poor pain response in 11/12. Growth failure and small hands or feet were present in 8/12, and sleep issues in 7/12. Six individuals had period breathing, bruxism, or screaming, five had scoliosis, and four had peripheral vascular abnormalities. Interestingly, none had eye pointing, a very distinctive feature in typical RTT in females.

5 | DISCUSSION

Early publications had suggested that mutation in *MECP2* leads to embryonic male lethality or early postnatal demise; however, subsequent reports have provided a clearer profile of neurodevelopmental disorders in males including mutations not associated with RTT and mutations typically seen in females with RTT. Here, we present the clinical and genetic characterization of 30 boys and men with *MECP2* mutations from the RNHS. These results demonstrate a wide clinical variation in males with *MECP2* mutations; however, males with *MECP2* mutations display quite significant neurodevelopmental issues.

The previous failure to identify significant numbers of males with *MECP2* mutations that led to the assumption of embryonic lethality can be attributed to both an observational bias and the genetic mechanism of disease. First, the identification of males with *MECP2* mutations compared to the identification of females with RTT is relatively infrequent due in part to the lack of clear phenotypic features in the former when compared to the latter. In this work, we demonstrate that some consistent clinical features occur in males with *MECP2* mutations, including features similar to females with RTT. However, these features are variable and remain less distinctive than in females with RTT. Additionally, the mutagenic mechanism that results in common RTT-causing mutations is more likely to be present in females compared to males. The majority of RTT-causing *MECP2* mutations are typically de novo events arising in sperm, the rapidly dividing germinal cells, as a result of deamination of a methylated cytosine. Mutated sperm will only produce a female child. *MECP2* mutations in males occur from the much less common maternal inheritance or, perhaps, as a de novo event in the mother. Finding such mutations has been uncommon until recently. Given the higher rate of maternal inheritance observed in males with *MECP2* mutations compared to

females with RTT, genetic counseling regarding increased recurrence risk is critical.

The three cases presented here with *MECP2* variants classified as "Uncertain" present unique problems. In these cases it is not possible to assign causation definitively to the genetic changes observed in *MECP2*. A significant need is the ability to demonstrate clear loss of MeCP2 protein expression and/or function with variants in this group; however, clear molecular assays are lacking to characterize MeCP2 function. Additional genetic testing to evaluate other potential genetic causes through whole exome, whole genome, or targeted sequencing should be considered in these cases.

This study demonstrates that the severity of clinical involvement in males with *MECP2* mutations is remarkably broad, ranging from cognitive impairment to neonatal encephalopathy associated with early death. This divergence of the phenotype of males from females with RTT results in a decreased likelihood of detection on standard clinical investigations. Even when males with *MECP2* have some of the distinctive features of RTT, such as loss of spoken language, loss of hand skills, or hand stereotypies, these features may be significantly subtler than those seen in females with RTT, further limiting specific clinical identification. This notion is borne out by *MECP2* mutation studies of groups with neurodevelopmental delay that revealed mutations in 1.3–1.7% of males (Bourdon et al., 2003; Couvert et al., 2001; Donzel-Javouhey et al., 2006; dos Santos, Abdalla, Campos, Santos-Reboucas, & Pimentel, 2005; Kammoun et al., 2004; Moncla, Kpebe, Missirian, Mancini, & Villard, 2002; Moog et al., 2006; Orrico et al., 2000; Tejada et al., 2006; Ylisaukko-Oja et al., 2005; Yntema et al., 2002; Yntema et al., 2002), whereas few of these individuals were suspected as having *MECP2* mutations. We expect that as whole exome or genome sequencing becomes commonly used to characterize males with neurodevelopmental disorders, it is likely that the identification of *MECP2* mutations in these males will increase. A limitation of the present study is that the relatively small number of subjects identified at this time precludes comprehensive description of the entire range of clinical features, prognosis, and incidence. Increasing genetic identification of males with *MECP2* mutations will improve the delineation of this group. Another limitation is a more complete analysis of the behavioral features of these males, especially for males with less severe clinical presentations. Previous work has indicated that males with the p.A140V mutation show significant neuropsychiatric features such as mania and even psychosis. Unfortunately, it was beyond the scope and capabilities of this study to assess neuropsychiatric features in a systematic manner. Future work should evaluate these features in more depth.

A high proportion of males with *MECP2* mutations display greater clinical severity than females with RTT. Of the 28 subjects who did not have somatic mutations in *MECP2*, the majority (17/28) had abnormal initial development, a significant fraction (12/28) had neonatal or progressive encephalopathy, two were ventilator dependent, and nine died (mean age of death: 9.0 years). All of these features are uncommon and more severe than in females with RTT. The overall level of skills attained by these males was also much lower than those in females with RTT (Neul et al., 2014).

We are now able to begin to identify initial genotype–phenotype relationship in males with *MECP2* mutations. The presence of neonatal/progressive encephalopathy is much more common in males with RTT-causing *MECP2* mutations (56%), compared to either males with likely pathogenic non RTT-causing *MECP2* mutations (33%) or uncertain variants (33%). This relationship is clearer when we split the RTT-causing group into early (89%) and late (11%) *MECP2* mutations. The overall clinical severity, as measured by the CSS, also demonstrated that early *MECP2* mutations (both truncating as well as missense mutations) were associated with increased severity. Identification and characterization of additional subjects is needed to develop a deeper genotype–phenotype correlation.

Previous reports have indicated that very few males with *MECP2* mutations and unusual genetic features, such as somatic mutations or sex-chromosomal abnormalities such as Klinefelter syndrome (XXY), display the full clinical features required to make the diagnosis of RTT. Two of the boys in this report had somatic mutations in *MECP2*, met the diagnostic criteria for typical RTT, and displayed an overall phenotype identical to females with RTT. Additionally, we identified two XY boys with germline *MECP2* mutations who on initial evaluation met criteria for atypical RTT. However, when we carefully reviewed the historical and clinical information on all the cases we determined that an additional 12 subjects met the diagnostic criteria for atypical RTT. Critically, all had a history of regression. Although they met criteria for atypical RTT, they all had clinical features that are distinctive from girls and women with RTT. Notably, they displayed less frequent and more variable hand stereotypies, less frequent periodic breathing, and absence of characteristic eye pointing. As mentioned previously, the clinical course for these individuals appears more severe than observed in girls and women with RTT, with more impaired initial development, ventilatory requirement, and early death. We believe that these features make this clinical pattern distinctive enough to warrant assignment to a novel and distinctive diagnostic category, which we term "Male RTT encephalopathy". This diagnostic classification incorporates sufficient clinical features to assign a general diagnosis of RTT, but also acknowledges that the overall pattern and progression of disease is different from typical RTT seen in females with *MECP2* mutations. We propose that the criteria for the diagnosis of male RTT encephalopathy be (1) completely meeting criteria for RTT, meaning having clearly identified pattern of regression, displaying at least two of the four main criteria, and at least five of eleven supportive criteria, (2) mutation in *MECP2*, (3) male sex. It is important to note that the mere presence of a *MECP2* mutation in a male is not sufficient to make the diagnosis of male RTT encephalopathy, as 14/28 males in this study did not meet criteria for RTT and are not considered to have male RTT encephalopathy. In these cases, the diagnosis of neonatal encephalopathy/progressive encephalopathy/cognitive impairment with a *MECP2* mutation should continue to be applied.

6 | CONCLUSION

In males, mutations in *MECP2* are compatible with life and result in a spectrum of neurodevelopmental features. Detailed characterization of these males contributes to the delineation of a new diagnostic entity in a subset: male RTT encephalopathy. The latter includes the key RTT diagnostic feature, developmental regression, but also a more severe clinical course than typical RTT in females. Increased awareness of the clinical spectrum seen in males with *MECP2* mutations and

systematic application of whole exome or genome sequencing may aid in increased identification of other males and improve our understanding of their involvement in this condition.

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CONFLICT OF INTEREST

The authors have no conflicts to report.

ETHICS AND CONSENT TO PARTICIPATE

Each site (VUMC, UAB, BCM, Boston Children's Hospital, Children's Hospital of Philadelphia, University of Colorado School of Medicine, UCSD) obtained and maintained Institutional Review Board (ethics) approval for the performance of these studies. The study clinicians verified all data at time of interview and examination. Parents or authorized caregivers provided approval for study conduct and publication of results prior to entry into the study. We registered these observational studies in ClinicalTrials.gov: NCT00299312 for RTT5201 and NCT02738281 for RTT5211.

CONSENT FOR PUBLICATION

No personal identifying information, images, or videos are presented here. Subjects or their guardians consented to publication of results.

AVAILABILITY OF DATA AND MATERIALS

All data is stored at the Rare Diseases Clinical Research Network Data Management and Coordinating Center at the University of South Florida. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request, and are placed within dbGAP (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000574.v1.p1).

AUTHOR CONTRIBUTIONS

JLN and AKP conceived of study. JLN, AKP, TAB, EDM, WEK, DNL, SAS, DGG, BS, MK, AP, RR, SP, PH, TF, and SS enrolled and evaluated subjects and collected all data. JLN and AKP compiled and analyzed clinical data, JLN and JM analyzed genetic variant data. JLN and AKP wrote the manuscript, and all authors read, edited, and approved the final manuscript.

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