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Mutations in Epilepsy and Intellectual Disability Genes in Patients with Features of Rett Syndrome

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Abstract

Rett syndrome and neurodevelopmental disorders with features overlapping this syndrome frequently remain unexplained in patients without clinically identified *MECP2* mutations. We recruited a cohort of 11 patients with features of Rett syndrome and negative initial clinical testing for mutations in *MECP2*. We analyzed their phenotypes to determine whether patients met formal criteria for Rett syndrome, reviewed repeat clinical genetic testing, and performed exome sequencing of the probands. Using 2010 diagnostic criteria, three patients had classical Rett syndrome, including two for whom repeat *MECP2* gene testing had identified mutations. In a patient with neonatal onset epilepsy with atypical Rett syndrome, we identified a frameshift deletion in *STXBPI*. Among seven patients with features of Rett syndrome not fulfilling formal diagnostic criteria, four had suspected pathogenic mutations, one each in *MECP2*, *FOXG1*, *SCN8A*, and *IQSEC2*. *MECP2* mutations are highly correlated with classical Rett syndrome. Genes associated with atypical Rett syndrome, epilepsy, or intellectual disability should be considered in patients with features overlapping with Rett syndrome and negative *MECP2* testing. While most of the identified mutations were apparently *de novo*, the *SCN8A* variant was inherited

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WEB RESOURCES

RettBase (URL: <http://mecp2.chw.edu.au>)

GeneMania (URL: <http://genemania.org/>)

from an unaffected parent mosaic for the mutation, which is important to note for counseling regarding recurrence risks.

Keywords

whole exome sequencing; Rett syndrome; *MECP2*; *CDKL5*; *FOXG1*; *STXBPI*; *SCN8A*; genetic; mutations; deletions

INTRODUCTION

Rett syndrome (RTT) is a clinically defined syndrome characterized by developmental regression followed by stabilization, partial or complete loss of purposeful hand skills and spoken language, gait abnormalities, and stereotypic hand movements [Neul et al., 2010]. Updated criteria for classical and atypical RTT were defined in 2010 [Neul et al., 2010]. Mutations in the gene methyl-CpG-binding protein (*MECP2*) have been reported in up to 95% of cases of classical RTT and in the majority of patients with milder forms of atypical RTT, such as the preserved speech variant [Neul et al., 2010; Neul et al., 2014; Renieri et al., 2009].

In patients with the early seizure (Hanefeld) variant and the congenital (Rolando) variant of RTT, mutations have been identified in cyclin-dependent kinase-like 5 (*CDKL5*) [Mari et al., 2005; Neul et al., 2010; Olson and Poduri, 2012] and in forkhead box G1 (*FOXG1*) [Florian et al., 2012; Kortum et al., 2011; Neul et al., 2010], respectively. Although patients with mutations in *CDKL5* or *FOXG1* have features that overlap with RTT and sometimes meet criteria for atypical RTT, they may have unique characteristics, including distinct epilepsy phenotypes [Fehr et al., 2013; Guerrini and Parrini, 2012; Seltzer et al., 2014].

We hypothesized that girls with features of RTT without *MECP2* mutations have mutations in other genes, including those associated with epileptic encephalopathies, intellectual disability (ID), or autism spectrum disorder. We also postulated that a greater number of RTT diagnostic or supportive features will increase the likelihood of a mutation in *MECP2* or related genes. We applied exome sequencing to a cohort referred to us as patients who were *MECP2*-negative with features of RTT to evaluate for mutations in known or new candidate genes, and to investigate potential underlying pathways that explain the phenotypic overlap.

METHODS

Cohort selection and phenotypic analysis

Eleven patients with features suggestive of RTT and negative initial clinical testing for mutations in *MECP2* were recruited by a RTT specialist (OK) to the Boston Children's Hospital Repository Core for Neurological Diseases. The protocol was approved by the Institutional Review Board at Boston Children's Hospital. We reviewed medical records and performed clinical phenotyping of features including epilepsy, EEG findings, MRI results, non-epileptic events, developmental history, physical examination, and genetic/metabolic evaluation. All available original EEG and MRI data were reviewed. We analyzed all 11

patients with respect to 2010 diagnostic criteria [Neul et al., 2010] and classified them as classical RTT, atypical RTT, or neither.

Review of clinical genetic testing

We reviewed all clinical genetic testing, including repeat *MECP2* gene testing that had not been available at the time of enrollment.

Exome sequencing

We performed exome sequencing for each proband using Illumina HiSeq with 100 bp paired-end reads (Agilent SureSelect XT Human All Exon v4). Coverage was >90% bases with minimum 20× read depth. (Details of the analysis pipeline are provided in the supplemental methods.) We screened the non-synonymous variants for known genes associated with RTT and variants (*MECP2*, *CDKL5*, *FOXP1*, *NTNG1*, *MEF2C*, *TOF4*) [Archer et al., 2006; Florian et al., 2012; Kobayashi et al., 2012; Kortum et al., 2011; Marangi et al., 2011; Nectoux et al., 2007; Neul et al., 2010; Olson and Poduri, 2012; Rajaei et al., 2011; Zweier et al., 2010], epilepsy, developmental delay (DD)/ID, and autism spectrum disorder [Carvill et al., 2013; Epi4K et al., 2013; Poduri et al., 2014; supplemental references]. If none were identified, we then screened for genes expressed in the brain and ideally involved in brain development or pathways overlapping with known genes as above. For candidate genes, we prioritized nonsense or frameshift mutations and single nucleotide changes predicted to be damaging (SIFT score < 0.05, PolyPhen2 score > 0.85).

After identifying potentially pathogenic variants, polymerase chain reaction (PCR) was performed in DNA from probands and their parents, when available, to confirm the variant and determine the inheritance pattern. Mutations were classified as presumed *de novo* if DNA from both parents was not available for sequencing, and apparently *de novo* if both parents were sequenced. Coordinates for exome data are listed in human genome build hg19. *De novo* changes and previously published disease-associated mutations were considered more likely to be disease-associated. We also considered *in silico* prediction tools and known phenotype-genotype correlations in determining whether variants were likely pathogenic, particularly in cases when full parental data or DNA were not available. For *MECP2* variants, we reviewed RettBase.

Mosaicism

TOPO TA cloning (Invitrogen, Carlsbad, CA) was used for one family with an identified variant suspected to be pathogenic but inherited to evaluate for mosaicism in a parent.

Pathway analysis

Pathway analysis was performed using GeneMania.

RESULTS

In a series of 11 patients with features suggestive of RTT, we identified a predicted pathogenic genetic mutation in each of six patients and a likely explanation in one other (Patient 7) (Table I). We describe the phenotypic features of the patients, including epilepsy,

non-epileptic events, developmental history, physical examination finding, and MRI findings. We classified patients as having classical RTT (three patients), atypical RTT (one patient), or features of RTT that do not fulfill formal diagnostic criteria (seven patients) (Table II and Supplemental Tables I-III) [Neul et al., 2010].

Here we present our genetic findings for each of these groups.

Patients with classical RTT

Of the three patients with classical RTT, two had mutations in *MECP2* (Table I). Patient 4 had had repeat *MECP2* sequencing in a research setting and later documented in the clinical record, not known to us at the time of enrollment; single-gene sequencing in the past and exome sequencing in this study identified the presumed *de novo* mutation *MECP2* c.316C>T (p.Arg106Trp). For Patient 6, we identified a previously undetected apparently *de novo* frameshift deletion, c.21delC (p.Ala8Argfs*36) in *MECP2*. No genetic etiology was identified in the third patient with classical RTT by clinical *MECP2* gene sequencing and deletion/duplication analysis in 2008 or research exome analysis (Patient 11).

Patient with atypical RTT

We identified a presumed *de novo* likely pathogenic mutation in the gene *STXBPI*, c.1658delC (p.Tyr554Thrfs*3), in Patient 10 with atypical RTT (Table I). This patient had apparently normal early development despite neonatal onset seizures then developmental regression at 6-7 months in the setting of infantile spasms with hypsarrhythmia. Early EEGs showed shifting asymmetry of epileptiform activity, and spasm-like movements occurred during focal seizures at 1 month of age. Her EEG at 3 years of age showed bifrontal epileptiform activity, intermittent bifrontal slowing, and poorly formed sleep features. Initially diffusely hypotonic, she later developed spasticity, acquired microcephaly (50th percentile at 2.5 months, -3.3 SD at 6 years), and small stature (height -3.5 SD at 6 years). At 8 years she cooed, said “hi”, and had two signs. She walked at 4 years with a wide-based, ataxic gait. She had purposeful hand use but not a pincer grasp. Hand automatisms included claspings and mouthing, not wringing. She had repetitive and self-injurious behaviors. Other features of RTT included impaired sleep patterns, peripheral vasomotor disturbances, and unprovoked laughing and screaming spells. MRI brain initially showed delayed myelination, but myelination normalized on follow-up imaging.

Patients with features of RTT that do not meet formal diagnostic criteria for RTT

Of seven patients with features suggestive of RTT that do not meet formal diagnostic criteria for RTT, suspected pathogenic mutations identified in four occurred in *MECP2*, *FOXG1*, *SCN8A*, and *IQSEC2*. Three patients were excluded from the RTT category due to grossly abnormal development in the first 6 months (Patients 3, 5, and 8). Three additional patients' features did not meet criteria due to lack of regression followed by recovery or stabilization (Patients 1, 2, and 9), and one patient did not have adequate supportive criteria (Patient 7).

Patient 2 had had a presumed *de novo* frameshift deletion, *MECP2* c.771_814del (p.Glu258Glyfs*58). Her deletion was identified by repeat *MECP2* sequencing and deletion/duplication analysis (performed in 2011), after redesigning primers for PCR due to a

polymorphism (IVS3-19delT) that was thought to mask the deletion on previous testing (performed in 2004). It was not detected by exome sequencing. She had developmental delay and plateau between 2-4 years, gait impairment, hand-wringing stereotypies, breathing disturbances including hyperventilation and air swallowing, epilepsy, long QT syndrome, scoliosis, bruxism, impaired sleep, acquired microcephaly, and low tone in infancy followed by development of increased tone in childhood. At 5 years 9 months, she had severe intellectual disability. Despite having many features associated with RTT and a diagnosis of RTT by her treating clinicians, she did not meet the formal diagnostic criteria due to developmental plateau but not regression.

Patient 9 had a pathogenic mutation in the gene *FOXG1*, c.565C>G (p.Leu189Val). She had severe global DD without regression, microcephaly, diffuse hypotonia, and hand dyskinesias. She had only a few words at 4.5 years of age. She had transient partial loss of purposeful hand skills related to dyskinesias and obsessive need to touch, an unsteady gait, sleep disturbance, and unprovoked shrieking spells. She did not have epilepsy; EEGs showed generalized slowing but no epileptiform activity. Cranial MRI showed mild hypomyelination and a small corpus callosum.

Patient 8 was found on exome sequencing to have a likely pathogenic heterozygous variant in *SCN8A*, c. 1588C>T (p.Arg530Trp). This variant was inherited from her father, who was mosaic for the mutation with the alternate (variant) allele. While in the patient's DNA, subcloning and resequencing was consistent with a heterozygous mutation (53/92 colonies with the alternate allele, 39/92 with the reference allele), in her father's DNA the alternate allele was present in only 17% (14/83 colonies) and the reference allele in 83% (69/83 colonies). These results are consistent with mosaicism in the father, with the alternate allele present in the heterozygous state in 34% of his cells. She had many of the typical features of RTT and ten of the supportive criteria (Table IIa and IIb) but abnormal development in the first 6 months. She briefly acquired a few words and motor skills before regressing between 2-4 years with a complete loss of language and ability to crawl or walk. She communicated through eye-pointing. She had periods of hyperventilation and breath holding, laughing spells, and bruxism when awake. Physical examination showed low muscle tone and poor head control in infancy, then later spasticity, delayed growth, and scoliosis. She had infantile spasms starting at 4 months and progressed into Lennox-Gastaut syndrome.

Patient 7 was found to have a predicted pathogenic *de novo* heterozygous variant in *IQSEC2*, a frameshift deletion, c.273_282del (p.Asn91Lysfs*112). This *de novo* *IQSEC2* mutation was identified both on research testing and independently on clinical gene panel testing. She had regression but did not lose purposeful hand skills, and she met all of the other major criteria for classical RTT as well as three supportive criteria (Table IIb) and hand stereotypies. She did not have epilepsy, microcephaly, or growth retardation. Cranial MRI showed delayed myelination.

DISCUSSION

Our data support the use of exome sequencing in patients with features of RTT negative for mutations in *MECP2*, though targeted testing using gene panels may be a reasonable first

step before exome sequencing. Genes associated with atypical RTT, epilepsy, ID, or autism spectrum disorder should be considered in patients not meeting criteria for RTT or when *MECP2* testing is negative, especially in patients with early onset epilepsy and/or autistic features. *FOXG1* is an example of a gene associated with atypical RTT or overlapping features as well as unique features [Florian et al., 2012; Kortum et al., 2011; Neul et al., 2010; Seltzer et al., 2014], confirmed by our study, but the list should be expanded to include *STXBPI*, *SCN8A*, and *IQSEC2*.

Clinical criteria supportive of classical RTT correlated with *MECP2* mutations in a relatively specific manner in our small series. Thus for patients with classical RTT, it is important to perform sequencing and deletion testing of *MECP2* thoroughly. As in our series, however, patients with *MECP2* mutations and associated neurodevelopmental disorders not meeting criteria for classical RTT are also described [Carney et al., 2003; Neul et al., 2010]. The only patient meeting criteria for atypical RTT had a mutation in *STXBPI*, emphasizing the genetic heterogeneity of RTT variants as recently reported for more severe clinical presentations [Neul et al., 2010; Neul et al., 2014]. The number of RTT criteria present, particularly of the supportive type, did not predict the identification of a likely pathogenic mutation.

We broaden the phenotypic spectra of *SCN8A* and *STXBPI*, established epilepsy genes [Deprez et al., 2008; Larsen et al., 2015; Ohba et al., 2014; Saitsu et al., 2008; Saitsu et al., 2010], to include features of RTT: hand stereotypies, breathing and muscle tone abnormalities, growth abnormalities, DD with or without regression, and non-specific features included in the supportive criteria for RTT. Regression in the setting of epileptic encephalopathy, however, differs from the pattern of regression in classical RTT in which regression precedes the onset of seizures (if they occur). Similar to patients with mutations in *CDKL5*, the gene most established in the early seizure variant of RTT, many patients with *STXBPI* or *SCN8A* mutations would not meet criteria for RTT due to abnormal development in the first 6 months or lack of developmental regression [Fehr et al., 2013; Larsen et al., 2015; Milh et al., 2011; Ohba et al., 2015]. Most mutations reported in *SCN8A* have been missense mutations, as in the patient reported here, with a proposed gain-of-function mechanism [Larsen et al., 2015; Ohba et al., 2014a]. As with other sodium channel mutations, functional testing would be required before concluding whether the mutation confers a gain or loss-of-function effect [Oliva et al., 2012].

For the patient with a mutation in *SCN8A*, we identified the mutation in mosaic form in the patient's unaffected father. Parental somatic mosaicism, as identified for Patient 8, has been previously reported, including in another patient with an *SCN8A* mutation as well as other epilepsy genes [Campbell et al., 2014; Carvill et al., 2013]. Counseling regarding recurrence risks should take into account this possible mechanism of inheritance from an apparently unaffected parent.

Based on this patient and prior literature, we suggest that mutations in the gene *IQSEC2*, known to cause X-linked ID with or without epilepsy in males, are associated with features of RTT in females, including developmental regression, hand stereotypies, and deceleration of head growth [Epi4K et al., 2013; Gandomi et al., 2014; Journal et al., 1990; Morleo et al.,

2008; Shoubbridge et al., 2010a; Shoubbridge et al., 2010b; Tran et al., 2014; Tzschach et al., 2015]. Previous data suggest that the *IQSEC2* expression pattern in the brain mirrors the expression profile of *CDKL5*, though a precise functional link is unknown [Morleo et al., 2007]. Since *IQSEC2* escapes X-inactivation [Tsuchiya et al., 2004], we hypothesize that either haploinsufficiency is sufficient to produce symptoms in females or alternately that the mutations may produce a dominant negative effect.

While all of the proteins encoded by genes identified in this study are not involved in a single pathway, there are some shared expression patterns (Supplemental Figure 1). Our study was too small to evaluate for evidence of novel genes for classical or atypical Rett syndrome.

Thus, we suggest that *MECP2* sequencing and deletion/duplication testing be performed for patients with classical RTT. For patients with features of RTT, including *MECP2*-negative classical RTT, clinical epilepsy panel testing or exome sequencing would be a logical next step to evaluate for mutations in related genes and potentially additional genes not yet associated with epilepsy, intellectual disability, and other features of RTT. In addition to the many reasons for which physicians and families benefit from a genetic diagnosis [Poduri et al., 2014; Sheidley and Poduri, 2012], in this era of evolving clinical trials that will require that patients are mutation-positive for enrollment, it is now more important than ever to identify these mutations as swiftly as possible.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Archer HL, Evans JC, Millar DS, Thompson PW, Kerr AM, Leonard H, Christodoulou J, Ravine D, Lazarou L, Grove L, Verity C, Whatley SD, Pilz DT, Sampson JR, Clarke AJ. NTNG1 mutations are a rare cause of Rett syndrome. *Am J Med Genet A*. 2006; 140:691–694. [PubMed: 16502428]
- Campbell IM, Yuan B, Robberecht C, Pfundt R, Szafranski P, McEntagart ME, Nagamani SC, Erez A, Bartnik M, Wisniewiecka-Kowalnik B, Plunkett KS, Pursley AN, Kang SH, Bi W, Lalani SR, Bacino CA, Vast M, Marks K, Patton M, Olofsson P, Patel A, Veltman JA, Cheung SW, Shaw CA, Vissers LE, Vermeesch JR, Lupski JR, Stankiewicz P. Parental somatic mosaicism is underrecognized and influences recurrence risk of genomic disorders. *Am J Hum Genet*. 2014; 95:173–182. [PubMed: 25087610]
- Carney RM, Wolpert CM, Ravan SA, Shahbazian M, Ashley-Koch A, Cuccaro ML, Vance JM, Pericak-Vance MA. Identification of MeCP2 mutations in a series of females with autistic disorder. *Pediatr Neurol*. 2003; 28:205–211. [PubMed: 12770674]

- Carvill GL, Heavin SB, Yendle SC, McMahon JM, O'Roak BJ, Cook J, Khan A, Dorschner MO, Weaver M, Calvert S, Malone S, Wallace G, Stanley T, Bye AM, Bleasel A, Howell KB, Kivity S, Mackay MT, Rodriguez-Casero V, Webster R, Korczyn A, Afawi Z, Zelnick N, Lerman-Sagie T, Lev D, Moller RS, Gill D, Andrade DM, Freeman JL, Sadleir LG, Shendure J, Berkovic SF, Scheffer IE, Mefford HC. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. *Nat Genet.* 2013; 45:825–830. [PubMed: 23708187]
- Deprez L, Weckhuysen S, Holmgren P, Suls A, Van Dyck T, Goossens D, Del-Favero J, Jansen A, Verhaert K, Lagae L, Jordanova A, Van Coster R, Yendle S, Berkovic SF, Scheffer I, Ceulemans B, De Jonghe P. Clinical spectrum of early-onset epileptic encephalopathies associated with STXBP1 mutations. *Neurology.* 2010; 75:1159–1165. [PubMed: 20876469]
- Epi4K Consortium, Epilepsy Phenome/Genome Project. Allen AS, Berkovic SF, Cossette P, Delanty N, Dlugos D, Eichler EE, Epstein MP, Glauser T, Goldstein DB, Han Y, Heinzen EL, Hitomi Y, Howell KB, Johnson MR, Kuzniecky R, Lowenstein DH, Lu YF, Madou MR, Marson AG, Mefford HC, Esmaeili Nieh S, O'Brien TJ, Ottman R, Petrovski S, Poduri A, Ruzzo EK, Scheffer IE, Sherr EH, Yuskaitis CJ, Abou-Khalil B, Alldredge BK, Bautista JF, Berkovic SF, Boro A, Cascino GD, Consalvo D, Crumrine P, Devinsky O, Dlugos D, Epstein MP, Fiol M, Fountain NB, French J, Friedman D, Geller EB, Glauser T, Glynn S, Haut SR, Hayward J, Helmers SL, Joshi S, Kanner A, Kirsch HE, Knowlton RC, Kossoff EH, Kuperman R, Kuzniecky R, Lowenstein DH, McGuire SM, Motika PV, Novotny EJ, Ottman R, Paolicchi JM, Parent JM, Park K, Poduri A, Scheffer IE, Shellhaas RA, Sherr EH, Shih JJ, Singh R, Sirven J, Smith MC, Sullivan J, Lin Thio L, Venkat A, Vining EP, Von Allmen GK, Weisenberg JL, Widdess-Walsh P, Winawer MR. De novo mutations in epileptic encephalopathies. *Nature.* 2013; 501:217–221. [PubMed: 23934111]
- Fehr S, Wilson M, Downs J, Williams S, Murgia A, Sartori S, Vecchi M, Ho G, Polli R, Psoni S, Bao X, de Klerk N, Leonard H, Christodoulou J. The CDKL5 disorder is an independent clinical entity associated with early-onset encephalopathy. *Eur J Hum Genet.* 2013; 21:266–273. [PubMed: 22872100]
- Florian C, Bahi-Buisson N, Bienvenu T. FOXG1-Related Disorders: From Clinical Description to Molecular Genetics. *Mol Syndromol.* 2012; 2:153–163. [PubMed: 22670136]
- Gandomi SK, Farwell Gonzalez KD, Parra M, Shahmirzadi L, Mancuso J, Pichurin P, Temme R, Dugan S, Zeng W, Tang S. Diagnostic exome sequencing identifies two novel IQSEC2 mutations associated with X-linked intellectual disability with seizures: implications for genetic counseling and clinical diagnosis. *J Genet Couns.* 2014; 23:289–298. [PubMed: 24306141]
- Guerrini R, Parrini E. Epilepsy in Rett syndrome, and CDKL5- and FOXG1-gene-related encephalopathies. *Epilepsia.* 2012; 53:2067–2078. [PubMed: 22998673]
- Journel H, Melki J, Turleau C, Munnich A, de Grouchy J. Rett phenotype with X/autosome translocation: possible mapping to the short arm of chromosome X. *Am J Med Genet.* 1990; 35:142–147. [PubMed: 2301467]
- Kobayashi Y, Ohashi T, Akasaka N, Tohyama J. Congenital variant of Rett syndrome due to an intragenic large deletion in MECP2. *Brain Dev.* 2012; 34:601–604. [PubMed: 22001500]
- Kortum F, Das S, Flindt M, Morris-Rosendahl DJ, Stefanova I, Goldstein A, Horn D, Klopocki E, Kluger G, Martin P, Rauch A, Roumer A, Saitta S, Walsh LE, Wiczorek D, Uyanik G, Kutsche K, Dobyns WB. The core FOXG1 syndrome phenotype consists of postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and corpus callosum hypogenesis. *J Med Genet.* 2011; 48:396–406. [PubMed: 21441262]
- Larsen J, Carvill GL, Gardella E, Kluger G, Schmiedel G, Barisic N, Depienne C, Brilstra E, Mang Y, Nielsen JE, Kirkpatrick M, Goudie D, Goldman R, Jahn JA, Jepsen B, Gill D, Docker M, Biskup S, McMahon JM, Koeleman B, Harris M, Braun K, de Kovel CG, Marini C, Specchio N, Djemie T, Weckhuysen S, Tommerup N, Troncoso M, Troncoso L, Bevtov A, Wolff M, Hjalgrim H, Guerrini R, Scheffer IE, Mefford HC, Moller RS, double dagger On behalf of the Euro ERESCCRP, Euro ERESCCRP. The phenotypic spectrum of SCN8A encephalopathy. *Neurology.* 2015; 84:480–489. [PubMed: 25568300]
- Marangi G, Ricciardi S, Orteschi D, Lattante S, Murdolo M, Dallapiccola B, Biscione C, Lecce R, Chiurazzi P, Romano C, Greco D, Pettinato R, Sorge G, Pantaleoni C, Alfei E, Toldo I, Magnani C, Bonanni P, Martinez F, Serra G, Battaglia D, Lettori D, Vasco G, Baroncini A, Daolio C,

- Zollino M. The Pitt-Hopkins syndrome: report of 16 new patients and clinical diagnostic criteria. *Am J Med Genet A*. 2011; 155A:1536–1545. [PubMed: 21671391]
- Mari F, Azimonti S, Bertani I, Bolognese F, Colombo E, Caselli R, Scala E, Longo I, Grosso S, Pescucci C, Ariani F, Hayek G, Balestri P, Bergo A, Badaracco G, Zappella M, Broccoli V, Renieri A, Kilstrup-Nielsen C, Landsberger N. CDKL5 belongs to the same molecular pathway of MeCP2 and it is responsible for the early-onset seizure variant of Rett syndrome. *Hum Mol Genet*. 2005; 14:1935–1946. [PubMed: 15917271]
- Morleo M, Iaconis D, Chitayat D, Peluso I, Marzella R, Renieri A, Mari F, Franco B. Disruption of the IQSEC2 transcript in a female with X;autosome translocation t(X;20)(p11.2;q11.2) and a phenotype resembling X-linked infantile spasms (ISSX) syndrome. *Mol Med Rep*. 2008; 1:33–39. [PubMed: 21479374]
- Nectoux J, Girard B, Bahi-Buisson N, Prieur F, Afenjar A, Rosas-Vargas H, Chelly J, Bienvenu T. Netrin G1 mutations are an uncommon cause of atypical Rett syndrome with or without epilepsy. *Pediatr Neurol*. 2007; 37:270–274. [PubMed: 17903671]
- Neul JL, Kaufmann WE, Glaze DG, Christodoulou J, Clarke AJ, Bahi-Buisson N, Leonard H, Bailey ME, Schanen NC, Zappella M, Renieri A, Huppke P, Percy AK, RettSearch C. Rett syndrome: revised diagnostic criteria and nomenclature. *Ann Neurol*. 2010; 68:944–950. [PubMed: 21154482]
- Neul JL, Lane JB, Lee HS, Geerts S, Barrish JO, Annese F, Baggett LM, Barnes K, Skinner SA, Motil KJ, Glaze DG, Kaufmann WE, Percy AK. Developmental delay in Rett syndrome: data from the natural history study. *J Neurodev Disord*. 2014; 6:20. [PubMed: 25071871]
- Ohba C, Kato M, Takahashi S, Lerman-Sagie T, Lev D, Terashima H, Kubota M, Kawawaki H, Matsufuji M, Kojima Y, Tateno A, Goldberg-Stern H, Straussberg R, Marom D, Leshinsky-Silver E, Nakashima M, Nishiyama K, Tsurusaki Y, Miyake N, Tanaka F, Matsumoto N, Saitu H. Early onset epileptic encephalopathy caused by de novo SCN8A mutations. *Epilepsia*. 2014; 55:994–1000. [PubMed: 24888894]
- Oliva M, Berkovic SF, Petrou S. Sodium channels and the neurobiology of epilepsy. *Epilepsia*. 2012; 53:1849–1859. [PubMed: 22905747]
- Olson H, Poduri A. CDKL5 mutations in early onset epilepsy: Case report and review of the literature. *J Pediatr Epilepsy*. 2012; 1:151–159.
- Poduri A, Sheidley BR, Shostak S, Ottman R. Genetic testing in the epilepsies-developments and dilemmas. *Nat Rev Neurol*. 2014; 10:293–299. [PubMed: 24733164]
- Rajaei S, Erlandson A, Kyllerman M, Albage M, Lundstrom I, Karrstedt EL, Hagberg B. Early infantile onset “congenital” Rett syndrome variants: Swedish experience through four decades and mutation analysis. *J Child Neurol*. 2011; 26:65–71. [PubMed: 21212452]
- Renieri A, Mari F, Mencarelli MA, Scala E, Ariani F, Longo I, Meloni I, Cevenini G, Pini G, Hayek G, Zappella M. Diagnostic criteria for the Zappella variant of Rett syndrome (the preserved speech variant). *Brain Dev*. 2009; 31:208–216. [PubMed: 18562141]
- Saitu H, Kato M, Mizuguchi T, Hamada K, Osaka H, Tohyama J, Urano K, Kumada S, Nishiyama K, Nishimura A, Okada I, Yoshimura Y, Hirai S, Kumada T, Hayasaka K, Fukuda A, Ogata K, Matsumoto N. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet*. 2008; 40:782–788. [PubMed: 18469812]
- Saitu H, Kato M, Okada I, Orii KE, Higuchi T, Hoshino H, Kubota M, Arai H, Tagawa T, Kimura S, Sudo A, Miyama S, Takami Y, Watanabe T, Nishimura A, Nishiyama K, Miyake N, Wada T, Osaka H, Kondo N, Hayasaka K, Matsumoto N. STXBP1 mutations in early infantile epileptic encephalopathy with suppression-burst pattern. *Epilepsia*. 2010; 51:2397–2405. [PubMed: 20887364]
- Seltzer LE, Ma M, Ahmed S, Bertrand M, Dobyns WB, Wheless J, Paciorkowski AR. Epilepsy and outcome in FOXG1-related disorders. *Epilepsia*. 2014; 55:1292–1300. [PubMed: 24836831]
- Sheidley BR, Poduri A. Genetics in clinical epilepsy: Issues in genetic testing and counseling. *J Pediatr Epilepsy*. 2012; 1:135–142.
- Shoubridge C, Tarpey PS, Abidi F, Ramsden SL, Rujirabanjerd S, Murphy JA, Boyle J, Shaw M, Gardner A, Proos A, Puusepp H, Raymond FL, Schwartz CE, Stevenson RE, Turner G, Field M, Walikonis RS, Harvey RJ, Hackett A, Futreal PA, Stratton MR, Gecz J. Mutations in the guanine

- nucleotide exchange factor gene IQSEC2 cause nonsyndromic intellectual disability. *Nat Genet.* 2010a; 42:486–488. [PubMed: 20473311]
- Shoubbridge C, Walikonis RS, Gecz J, Harvey RJ. Subtle functional defects in the Arf-specific guanine nucleotide exchange factor IQSEC2 cause non-syndromic X-linked intellectual disability. *Small GTPases.* 2010b; 1:98–103. [PubMed: 21686261]
- Tran Mau-Them F, Willems M, Albrecht B, Sanchez E, Puechberty J, Ende S, Schneider A, Ruiz Pallares N, Missirian C, Rivier F, Girard M, Holder M, Manouvrier S, Touitou I, Lefort G, Sarda P, Moncla A, Drunat S, Wieczorek D, Genevieve D. Expanding the phenotype of IQSEC2 mutations: truncating mutations in severe intellectual disability. *Eur J Hum Genet.* 2014; 22:289–292. [PubMed: 23674175]
- Tsuchiya KD, Grealis JM, Yi Y, Noel KP, Truong JP, Distech CM. Comparative sequence and x-inactivation analyses of a domain of escape in human Xp11.2 and the conserved segment in mouse. *Genome Res.* 2004; 14:1275–1284. [PubMed: 15197169]
- Tzschach A, Grasshoff U, Beck-Woedl S, Dufke C, Bauer C, Kehrer M, Evers C, Moog U, Oehl-Jaschkowitz B, Di Donato N, Maiwald R, Jung C, Kuechler A, Schulz S, Meinecke P, Spranger S, Kohlhasse J, Seidel J, Reif S, Rieger M, Riess A, Sturm M, Bickmann J, Schroeder C, Dufke A, Riess O, Bauer P. Next-generation sequencing in X-linked intellectual disability. *Eur J Hum Genet.* :2015. [Epub ahead of print].
- Vaher U, Nõukas M, Nikopensius T, Kals M, Annilo T, Nelis M, Ounap K, Reimand T, Talvik I, Ilves P, Piirsoo A, Seppet E, Metspalu A, Talvik T. De novo SCN8A mutation identified by whole-exome sequencing in a boy with neonatal epileptic encephalopathy, multiple congenital anomalies, and movement disorders. *J Child Neurol.* 2013; 29:NP202–206. [PubMed: 24352161]
- Veeramah KR, Johnstone L, Karafet TM, Wolf D, Sprissler R, Salogiannis J, Barth-Maron A, Greenberg ME, Stuhlmann T, Weinert S, Jentsch TJ, Pazzi M, Restifo LL, Talwar D, Erickson RP, Hammer MF. Exome sequencing reveals new causal mutations in children with epileptic encephalopathies. *Epilepsia.* 2013; 54:1270–1281. [PubMed: 23647072]
- Zweier M, Gregor A, Zweier C, Engels H, Sticht H, Wohlleber E, Bijlsma EK, Holder SE, Zenker M, Rossier E, Grasshoff U, Johnson DS, Robertson L, Firth HV, Cornelia K, Ekici AB, Reis A, Rauch A. Mutations in MEF2C from the 5q14.3q15 microdeletion syndrome region are a frequent cause of severe mental retardation and diminish MECP2 and CDKL5 expression. *Hum Mutat.* 2010; 31:722–733. [PubMed: 20513142]

Table 1a

Phenotype-genotype correlation of 11 patients with features of Rett syndrome and reported initial clinical testing negative for mutations in *MECP2*.

Patient	Meets 2010 criteria for classical RTT	Meets 2010 criteria for atypical RTT	Epilepsy and age of onset	Clinically consistent pathogenic mutation(s) based on clinical testing and exome sequencing
1	No	No	Yes, 4-5 weeks	None identified
2	No	No	Yes, 15 months	<i>MECP2</i> frameshift deletion
3	No	No	No	None identified
4	Yes	N/A	Yes, 4 years	<i>MECP2</i> missense mutation
5	No	No	No	None identified.
6	Yes	N/A	Yes, 12 years	<i>MECP2</i> frameshift deletion
7	No	No	No	<i>IQSEC2</i> frameshift deletion (likely pathogenic)
8	No	No	Yes, 4 months	<i>SCN8A</i> missense mutation
9	No	No	No	<i>FOXP1</i> missense mutation
10	No	Yes	Yes, 29 days	<i>STXBP1</i> frameshift deletion
11	Yes	N/A	Yes, 2 years	None identified

Table 1b

Identified known or suspected pathogenic mutations by genotypic subtype.

Genotypic sub-type	Gene	Patient #	Follow-up genetic testing	Whole exome sequencing (2013)	Inheritance	Evidence for pathogenicity
Classical Rett-syndrome gene	<i>MECP2</i>	2	c.771_814del (p.Glu258Glyfs*58) NM_004992.3 (2011)	Not seen	Presumed <i>de novo</i>	Frameshift
		4	c.316C>T (p.Arg106Trp)(2001, research report)	c.316C>T (p.Arg106Trp) NM_004992	Presumed <i>de novo</i>	Established pathogenic missense mutation [Neul et al., 2014; RettBase]
		6	Negative sequencing and deletion/duplication analysis (2008)	c.21delC (p.Ala8Argfs*36) frameshift deletion NM_001110792	Apparently <i>de novo</i>	Frameshift
Genes for variants of Rett syndrome	<i>FOXG1</i>	9	c.565C>G (p.Leu189Val) (2011)	c.565C>G (p.Leu189Val) NM_005249	Absent in mother, father not available	Missense SIFT 0.00 Polyphen2 0.84
Epileptic Encephalopathy genes	<i>STXBPI</i>	10	Not tested	c.1658delC (p.Tyr554Thrfs*3) NM_003165	Absent in mother, father not available	Frameshift
	<i>SCN8A</i>	8	Not tested	c.1588C>T (p.Arg530Trp) NM_014191	Mosaic in father (15–19% variant)	Missense SIFT 0.000 Polyphen2 0.999
X-linked Intellectual disability genes	<i>IQSEC2</i>	7	c.273_282del (p.Asn91Lysfs*112) NM_001111125.1 (2013)	c.273_282del (p.Asn91Lysfs*112) NM_001111125	Apparently <i>de novo</i>	Frameshift

All variants above were Sanger confirmed or confirmed by clinical testing. SIFT and Polyphen2 are *in silico* bioinformatics prediction tools for missense mutations. Year during which follow-up genetic testing was performed is indicated in parentheses.

Table IIa

Rett syndrome 2010 diagnostic criteria applied to the 11 patients in this series of patients with features of Rett syndrome.

Patient #	Age at last evaluation (years)	Required for all RTT	Main criteria					Exclusion criteria		Number of supportive criteria met (11 possible)	Meets 2010 criteria for classical RTT?	Meets 2010 criteria for atypical RTT?
			Regression followed by recovery or stabilization	Partial or complete loss of acquired purposeful hand skills	Partial or complete loss of acquired spoken language	Gait abnormalities: impaired or absence of ability	Stereotypic hand movements	Brain injury that causes neurological problems	Grossly abnormal psychomotor development in first 6 months of life			
1	9	No		Yes	No	Yes	Yes	Yes, suspected mitochondrial disorder	No	7	No	No
2	12	No		No	No	Yes	Yes	No	No	5	No	No
3	7	No		Yes	No	Yes	Yes	No	Yes	5	No	No
4	32	Yes		Yes	Yes	Yes	Yes	No	No	5	Yes	N/A
5	9.5	Yes		No	Yes	Yes	Yes	No	Yes	9	No	No
6	41	Yes		Yes	Yes	Yes	Yes	No	No	9	Yes	N/A
7	5.5	Yes		No	Yes	Yes	Yes	No	No	3	No	No
8	20	Yes		No	Yes	Yes	Yes	No	Yes	10	No	No
9	4.5	No		Yes	No	Yes	No	No	No	3	No	No
10	8	Yes		No	No	Yes	Yes	No	No	6	No	Yes
11	13	Yes		Yes	Yes	Yes	Yes	No	No	6	Yes	N/A

For classical Rett syndrome, required to have a period of regression followed by recovery or stabilization, all main criteria, and absence of exclusion criteria. For atypical Rett syndrome, required to have a period of regression followed by recovery or stabilization, at least 2 of 4 main criteria, and at least 5 of 11 supportive criteria. Patients 4, 6, and 8 were only evaluated at Boston Children's Hospital as adults (ages 20 - 40) so early information is limited. Patient 8 did not meet criteria due to grossly abnormal development in the first 6 months of life but otherwise would meet criteria for atypical RTT.

Table IIb

Summary of Rett syndrome supportive criteria in this series:

Patient #	Breathing disturbance when awake	Bruxism when awake	Impaired sleep pattern	Abnormal muscle tone	Peripheral vasomotor disturbance	Scoliosis/ kyphosis	Growth retardation	Small cold hands and feet	Inappropriate laughing/ screaming spells	Diminished response to pain	Intense eye communication – eye pointing,
1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No
2	Yes	Yes	Yes	Yes	No	Yes	No	No	No	No	No
3	No	Yes	Yes	Yes	No	No	Yes	No	Yes	No	No
4	No	No	Not at ages 27-32	Yes	Yes	Yes	Yes	Cold, not clear if small	No	Yes	No
5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No
6	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
7	Yes	No	No	Yes	No	No	No	No	Yes	No	No
8	Yes	Yes	Yes	Yes	No	Yes	Yes	Cold, not small	Yes	Yes	Yes
9	No	No	Yes	Yes	No	Yes	No	No	Yes	No	No
10	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No
11	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	No	No

* Sometimes the supportive criteria is “no,” because it is not well described in notes, but this does not exclude that some patients may have additional features.