Severe congenital encephalopathy caused by \textit{MECP2} null mutations in males: central hypoxia and reduced neuronal dendritic structure

Non-mosaic males with a 46,XY karyotype and a \textit{MECP2} null mutation display a phenotype of severe neonatal-onset encephalopathy that is distinctly different from Rett syndrome (RTT). To increase awareness of this rare disorder, we are reporting novel findings in a sporadic case, compare them to 16 previously reported cases and establish salient criteria for clinical diagnosis. The proband suffered from general hypotonia and hypoxia caused by hypoventilation and irregular breathing. He developed abnormal movements, seizures and electroencephalogram abnormalities. He failed to thrive and to reach any motor milestones and died at 15 months from central respiratory failure without a diagnosis. In a muscle biopsy, type II fibers were reduced in diameter, indicating central hypoxia. At autopsy, the brain was small with disproportionate reduction of the frontal and temporal lobes. Synaptophysin staining of synaptic vesicles was greatly reduced in cerebellar and spinal cord sections. Analysis of Golgi-stained pyramidal neurons from cortical layers III and V of the frontal and temporal lobes revealed drastically diminished dendritic trees. Post-mortem \textit{MECP2} mutation analysis on DNA and RNA from fibroblasts revealed a novel \textit{de novo} 9-nucleotide deletion including the intron 3/exon 4 splice junction. The two nucleotides flanking the deletion form a new splice site, and the aberrantly spliced transcript lacks seven nucleotides (r.378_384delTCCCCAG), causing a frameshift and premature termination codon (p.I126fsX11). Males with congenital encephalopathy, not females with RTT, represent the true human counterpart for the commonly studied \textit{MeCP2}-/-

Rett syndrome [RTT (MIM 312750)] is a mostly sporadic neurological disorder characterized by developmental delay and regression, loss of purposeful movements and development of stereotypic hand movements, deceleration of brain growth, autonomic dysfunction and seizures. RTT affects approximately one in 10,000 females in all populations. When hypotheses were debated to explain the sporadic occurrence limited to the female gender, the notion of X-linked dominant inheritance with male lethality was favored. In 1999, the cause of RTT was discovered (1). Affected females are heterozygous for inactivating mutations in the X-linked gene \textit{MECP2} (MIM 300005), encoding methyl-CpG-binding protein 2. Classic RTT is associated with random X-inactivation patterns, while favorably skewed X chromosome inactivation (XCI) generates an attenuated phenotype (2). An alternative hypothesis proposing exclusively male germ line-derived
MECP2 null mutations in males

Mutations (3) turned out to be partially true as the predominant type of MECP2 mutations, a C to T transition at CpG dinucleotides, originates in the male germ line (4, 5).

Affected boys, hemizygous for inactivating MECP2 mutations, are born alive, but they are underdiagnosed because they present with a completely different clinical picture. Historically, such males were discovered in families with inherited RTT in girls. Two families with more than one RTT female also included boys with severe congenital encephalopathy (CE) who died from respiratory insufficiency early in life. In the first family, one affected boy having a sister and maternal aunt affected with classic RTT was the first CE male in whom a MECP2 mutation [c.803delG] was demonstrated (2, 6, 7). In the second family, a Brazilian sibship with three girls [c.803delG] was demonstrated (2, 6, 7). In the second family, a Brazilian sibship with three girls affected with RTT, a male had died in the neonatal period. In this family, the most common truncating non-sense mutation (p.R168X), was transmitted to the RTT females from a carrier mother who was asymptomatic because of favorably skewed XCI, but the boy could not be studied for this mutation (2).

Subsequently, several female RTT cases were reported who had severely affected male siblings presenting with CE. MECP2 mutations included the most common missense mutation [p.T158M] (8), two single-nucleotide insertions (c.754_755insC) (9) and (c.754_755insG) (10) leading to a frameshift and premature stop codon at amino acid position p.258 (p.G252fsX6), a single-nucleotide deletion c.808delC (11), and a complex large genomic deletion removing the coding regions of exons 3 and 4 (12).

In all these families, the presence of one or more females affected with classic RTT called attention to the boys’ having inherited the same mutant MECP2 allele. Discovery of these familial male cases allowed the following conclusions: first, loss-of-function mutations in MECP2 that cause classic RTT in females are not prenatally lethal in boys. Second, in the absence of functional MeCP2 protein, intrauterine development proceeds normally and the brain structure appears grossly normal. Third, in most familial cases, the mothers were carriers of MECP2 mutations with favorably skewed XCI patterns, but the occurrence of germ line mosaicism is also well documented (Supplementary Table online).

Very recently, the CE phenotype has been recognized in sporadic and familial male cases in the absence of a female relative with RTT and the diagnosis was confirmed by the detection of MECP2 mutations. In Sweden, two brothers were identified with p.T158M whose mother was an asymptomatic heterozygote with skewed XCI (13). Sporadic severely affected males had de novo p.R294X (13) or c.803delG (14) mutations. Of four sporadic cases with progressive CE, reported from Australia and Alabama, three had a pathogenetic mutation: c.808delC, c.806delG and p.F157I (15). The fourth case had a MECP2 variant of unknown significance that was also present in his phenotypically normal mother who had a random XCI pattern.

The neuropathology of this severe progressive disorder has not been elucidated beyond the finding of greatly reduced brain weight. Individual cases, studied in more detail, had periventricular calcifications of the thalamus (8), thick and more complex perisylvian gyri with localized areas of unlayered polymicrogyria (16), or abnormal neuronal migration in the frontal lobe (15).

Here, we report, for the first time, dendritic structure abnormalities in cortical neurons in a sporadic male with CE and a novel de novo MECP2 mutation: a microdeletion removing a splice site led to an abnormally spliced mRNA with a frameshift and premature termination codon (p.I126fsX11). In the absence of a family history of RTT, he was not diagnosed during his lifetime. Comparison of his clinical and pathological features with those of 16 previously reported cases of CE and MECP2 null mutations allows a clinical delineation of this syndrome. This, in turn, should lead to increased awareness among clinicians and early mutation testing.

Materials and methods
Golgi staining and Sholl analysis
Cerebral tissue from the proband and an age- and sex-matched control was fixed in formalin and processed using the rapid Golgi technique (17). The control brain was obtained from archival material at Texas Children’s Hospital. There was no history of neurological disease in this infant, and the detailed neuropathological examination revealed a brain weight appropriate for a 15-month male with no pathological changes. Consent had been given by next of kin for use of the autopsy fixed brain for investigative purposes. Camera lucida drawings of apical and basal dendrites of neurons in two cortical layers from distinct brain regions were prepared and analyzed by using the Sholl method as described (17, 18).
Skin fibroblasts, genomic polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR Genomic DNA was purified by standard phenol–chloroform extraction from the proband’s skin fibroblasts and the mother’s blood, amplified and sequenced using standard techniques. To study the transcript, total RNA was extracted from the fibroblasts by using RNA Stat 60 (Teltest B, Friendswood, TX). Five micrograms of RNA were treated with DNaseI followed by reverse transcription using Superscript II (Invitrogen, Carlsbad, CA). PCR primers flanking intron 3 (F: 5′-AAGCAAAGGAAATCTGGCCG-3′ and R: 5′-TGTGTCGCTACCTTTTCGAA-3′) amplified a product of 116 bp. Studies were approved by the Stanford Institutional Review Board, Panel on Medical Human Subjects, and informed consent was provided by the proband’s parents. The fibroblasts are available (#GM21921) from the Human Genetic Mutant Cell Repository at the Coriell Institute, Camden, NJ.

Results
Medical history

The proband was born after an uneventful full-term pregnancy by normal vaginal cephalic delivery, aided by suction, to a 22-year-old G2P0A1 mother and 23-year-old father. The previous pregnancy was terminated because of a prenatal diagnosis of trisomy 21. The proband’s birth weight was 3075 g, and Apgar scores were eight at 1 and at 5 min. At 3 days of age, he had an episode of choking after feeding that led to cyanosis and apnea requiring chest compressions. He was admitted to hospital and treated with oxygen for 21 days and with ventilation for 6 days. He had consistent hypotonia and hypoventilation and developed seizures with an abnormal electroencephalogram (EEG). He was treated with caffeine for his apnea and phenobarbital for his seizures. He developed gastroesophageal reflux and failure to thrive, requiring nasal gastric tube placement, and later on, a gastrostomy tube for feeding.

At 5 months of age, he was readmitted for respiratory failure and was treated for bronchiolitis. His respiratory rate was highly variable, awake or asleep, occasionally dipping down to as low as 13–18 per min, with periodic breath-holding occurring about every 20 breaths. A sleep study revealed abnormal breathing patterns with 55% periodic breathing. A repeat sleep study showed periodic breathing 32% of the time. While his EEG was abnormal, no overt seizure activity was observed at that age. A medical genetics evaluation revealed a high narrow palate, myopathic hypotonic facies and general hypotonia (Fig. 1a,b). His genitalia were normal male, and both testes were descended. His final admission at 15.5 months of age was for bilateral pneumonia with fever and tonic seizures. He was treated with antibiotics for 9 days and died from central respiratory failure. A skin biopsy taken 2 days prior to his death yielded a fibroblast culture for further studies.

![Fig. 1. Clinical and muscle histology phenotypes. (a) Proband at 8 months of age and his parents. (b) Facial hypotonia and hand-to-mouth movement. (c) Brain magnetic resonance imaging showing mild frontal atrophy. (d) Quadriceps muscle biopsy at 5 months of age stained with hematoxylin/eosin showing irregular fiber size. (e) Muscle biopsy stained for myosin ATPase at pH 9.4 for determination of fiber types. The cross sections show marked variation in fiber size because of selective atrophy of type II fibers.](image-url)
Somatic and brain growth

The proband had severe postnatal growth failure. At 5 months of age, his weight was 4.88 kg (<third percentile) and his height was 63 cm (third percentile). Head circumference at 7 months of age was 39.5 cm (<second percentile). At 15 months of age, his height was 71.2 cm, which corresponds to the 50th percentile for a 7-month-old, with his head circumference at 42.1 cm (50th percentile for a 4-month-old). Thus, the deceleration of postnatal brain growth was more pronounced than that of body growth.

Neuromuscular and cognitive development

The presence of a high narrow palate suggested hypotonia in utero. During his lifetime, the proband did not reach any developmental milestones. He did not roll over or sit without support. He did not reach for objects. His vision and hearing were considered normal. At 5 months of age, he engaged in eye contact and focused on a target. He did laugh. He was able to put his hands in his mouth (Fig. 1b). No stereotypic movements were noted, but involuntary myoclonic movements of the extremities increased with age. At 15 months of age, he was able to raise his head and grab his legs. He could remain in a seated position for a few minutes if well supported. He did not track objects, but brought things to his mouth, although he would miss often. He was extremely hypotonic and deep tendon reflexes could not be elicited, although his extremities were hypertonic at times. Towards the end of his life, he had decreased motor activity, pinpoint pupils and marked bradycardia.

Laboratory and clinical investigations

Newborn screening tests, general chemistry panel, creatine phosphokinase and thyroid function studies were normal. Chromosome analysis revealed a normal male 46,XY karyotype. Molecular genetic tests excluded congenital myotonic dystrophy and Prader–Willi syndrome. Urine organic acid and plasma amino acids levels, acylcarnitine profile, and very long chain fatty acids were normal. Brain stem auditory response was normal. Initial EEG revealed sharp spikes over the central and temporal lobes, left greater than right. Follow-up EEGs were normal for age, and some were interpreted as showing immature activity. An EEG at 15 months of age revealed high voltage spiky complexes over the frontal/central areas, without evolution, and multifocal spike and slow complexes over the hemispheres bilaterally. Magnetic resonance imaging of the brain showed mild cerebral atrophy (Fig. 1c). A muscle biopsy from the quadriceps revealed type II fiber atrophy, most consistent with central hypotonia (Fig. 1d,e). Enzyme histochemical studies of muscle for succinic dehydrogenase and cytochrome oxidase activities were normal.

Autopsy and neuropathology

At autopsy, his body weighed 6670 g (<fifth percentile). There were no gross abnormalities in any organ system. Brain weight was 752 g (normal for age would be 1010 g). The frontal and temporal lobes appeared relatively small. The gyral pattern showed no abnormalities to suggest the presence of polymicrogyria (Fig. 2a). The cerebellum was grossly unremarkable. The brain stem appeared small but normally formed and histologically normal by routine hematoxylin and eosin staining. Coronal sections showed a cortex of normal thickness, and the architecture of subcortical gray matter structures appeared normal. White matter volume was decreased, and the corpus callosum appeared thin (Fig. 2b). Histologically, the cortex showed a normal laminar pattern. Synaptophysin staining, a measure for the number of synapses, was greatly reduced, compared with an age- and sex-matched control, in cerebellum (Fig. 2c,d) and spinal cord (Fig. 2e,f), as well as in cortex (not shown).

Analysis of dendritic processes by Sholl analysis of Golgi-stained neurons

Tissue from the formalin-fixed temporal and frontal lobes was sampled from this case and a control male brain and processed using the rapid Golgi technique (17). The apical and basilar dendrites of pyramidal neurons from layers III and V of the frontal and temporal cortex were subjected to Sholl analysis (17, 18). This quantitative method for morphometric neuronal studies uses concentric circles, 20 μ apart, to count the numbers of dendrites that extend for each distance from the soma, starting at the apical or basilar origin of the dendrites and extending to 320 μ from the cell soma. Camera lucida drawings of the neurons analyzed are shown in Fig. 3 and the results of the Sholl analyses in Fig. 4. In both brain areas, the dendritic arborization is significantly decreased in the proband compared with an age- and sex-matched control.
brain. Also, when compared with the previously published appearance of the pyramidal neurons in female RTT brains (17), the neurons analyzed from the male CE brain show a much more reduced dendritic branching pattern.

MECP2 mutation and abnormal transcript

Post-mortem MECP2 mutation analysis on DNA from skin fibroblasts revealed an 'IVS3-3-383del' mutation (Baylor DNA Diagnostic Laboratory). This novel mutation was not present in the mother’s blood when studied on two separate occasions (at Baylor and Stanford). As depicted in Fig. 5, the microdeletion removes three nucleotides of intron 3 and six of exon 4 and abolishes the splice site. The deletion probably occurred by slipped strand mispairing of a short tandem repeat (underlined) at the intron–exon boundary (indicated by ^) GTCCCCACAG^TCCCCAG

Fig. 2. Brain abnormalities at autopsy at 15 months of age. (a) Lateral view of the fixed brain showing a slightly foreshortened frontal lobe and a normal gyral pattern. (b) Coronal section at the level of the thalamus and lateral geniculate bodies, showing mild thinning of the corpus callosum and decreased white matter volume. (c–f) Immunohistochemical staining for synaptophysin showing a marked diminution of staining intensity in the proband (c and e) compared with an age- and sex-matched control (d and f) in samples of cerebellum (c and d) and spinal cord gray matter (e and f). The control brain was from a 12-month-old male with congenital heart disease and liver disease, who died of pulmonary hemorrhage while awaiting a liver transplant.
We hypothesized that the juxtaposition of the A at c.378-4 and the G at c.384 could create a new splice site TTGTCCCCAG^GGAAAAGCCT that has a score of 0.97 for the potential of a splice acceptor site (19). To test this hypothesis, we extracted RNA from the proband’s fibroblasts and sequenced reverse transcription polymerase chain reaction products. As predicted, the mutant transcript lacks the first seven nucleotides of exon 4 (r.378_384delTCCCCAG) and introduces a frameshift that leads to a premature stop codon (p.I126fsX11) (Fig. 5). This represents a novel mutation in MECP2.

Discussion  
CE caused by MeCP2 deficiency in males

The CE phenotype caused by loss-of-function MECP2 mutations in 46,XY males is distinctly different from RTT. Distinguishing neurological features of CE are (i) severe hypotonia, feeding problems and apneic episodes in the newborn period; (ii) respiratory insufficiency with irregular (Cheyne–Stokes) breathing; (iii) neonatal-onset, progressive deceleration of head growth which is disproportionately more severe than the significant retardation of somatic growth; (iv) axial hypotonia with limb rigidity; (v) movement disorders such as myoclonus, tremors, and dystonia; and (vi) lack of motor and cognitive development (Table 1, Supplementary Table online). Life span is drastically reduced with the majority of boys dying before 3 years of age from complications of respiratory arrest and chronic hypoxia. The oldest survivor, at 6 years, is ventilator dependent (10). Hypoventilation, with apneic episodes, occurs while awake or asleep in contrast to RTT females who appear to breathe normally during sleep, although a recent study documented breathing dysfunction during sleep in some RTT females as well (20). Stereotypic movements, the hallmark of RTT, are not prominent features of CE. Gastrointestinal reflux is common in both RTT and CE, and together with insufficient oral intake, often leads to placement of gastrostomy tubes.

In contrast to the strikingly abnormal clinical course of CE in postnatal life, the prenatal and birth histories of these boys are unremarkable, and the parameters of prenatal somatic development (height, weight and head circumference at birth) are normal for gestational age. Brain imaging studies were read as normal in most cases or as showing mild cerebral atrophy as in the case reported here. Few muscle biopsies were reported as abnormal, showing atrophy of type I and type II muscle fibers with mild complex I and II deficiency (16), or significant variability in fiber size, without evidence for grouping, and normal respiratory chain function (9). In our case, we found a reduction in fiber size affecting only type II fibers, consistent with central hypotonia.

The differential diagnosis of neonatal hypotonia and failure to thrive includes genetic disorders for which molecular tests are available such as Prader–Willi syndrome, spinal muscular atrophy,
congenital myotonic dystrophy, metabolic and mitochondrial disorders. Some types of CE caused by metabolic errors, such as urea cycle defects, are diagnosable by serum studies, e.g. ketoacidosis and hyperammonemia, and are treatable. The presence of breathing irregularity and hypoventilation, pointing to a brain stem abnormality, should raise the suspicion of CE and prompt MECP2 mutation analysis. The importance of diagnosing this disorder, despite a dismal prognosis and current lack of effective treatment, lies in excluding treatable disorders and providing recurrence risk estimates and prenatal monitoring of future pregnancies. Given the reported frequencies of maternal carrier status with skewed XCI (\(n = 6/14\) – Supplementary Table online), and of presumed maternal germ-line mosaicism (\(n = 4/14\) – Supplementary Table online), the recurrence risk may be significant.

Abnormal neuronal morphology and neurophysiology in CE and MeCP2-deficient male mice

In our proband, the first CE male for whom synaptophysin staining is reported, we found it to be greatly reduced in all gray matter areas studied. The monoclonal antibody against synaptophysin
binds to synaptic vesicles and provides an indication of the density of presynaptic terminals (21). In four female RTT brains, synaptophysin immunoreactivity was reported to be reduced by 20–40% in neocortical areas compared with controls (22).

Examination of neuronal structure with Golgi staining and Sholl analysis of RTT brains revealed reduced dendritic arborization in several brain regions (17, 23, 24). We are reporting the first studies of a male CE case in which the reduction of dendrites was strikingly more severe than in the female RTT cases.

The accurate mouse models for CE are male hemizygous MeCP2-/- knockout mutants. These mice have a delay in neuronal maturation, increased neuronal densities in cortical layers II/III and V, as well as thin dendrites with reduced numbers of dendritic spines (25). In contrast, cultured neurons that overexpress MeCP2 develop longer axonal and dendritic processes and an increased number of axonal and dendritic termini (26). The precise mechanism by which MeCP2 regulates dendritic arborization, spine development and/or turnover, as well as synaptic function, can best be studied in these available mouse models.

The breathing dysfunction seen in CE is replicated in male Mecp2-/- knockout mice that have increasingly frequent apneas until they die from respiratory arrest at 8–10 weeks of age (27). The number of tyrosine hydroxylase-producing neurons in the medulla is greatly reduced in these mutants, and the primary pathology appears to involve a defect of the noradrenergic and serotonergic modulation of the medullary respiratory network (27). Physiological studies in perfused working heart–brain stem preparations revealed a deranged vagal-pontine modulation of the respiratory rhythm. The results indicated impairment of sensory-mediated synaptic control of post-inspiration (28).

The mouse model provides opportunities for evaluating potential therapies. The number of apneic spells in Mecp2-/- mice was decreased by the administration of desipramine that increased the number of tyrosine hydroxylase-expressing cells in the mutant brain stem (29). The treated mice also had an extended life span. In addition, Mecp2-/- mice treated with an ampakine, a drug that facilitates activation of glutamatergic AMPA receptors, revealed restoration of normal breathing patterns (30).

Types and frequency of MECP2 mutations leading to CE in males

While paternally derived C>T transitions at one of eight CpG hotspots represent the majority of new MECP2 mutations in RTT females, these mutations are found in CE males only when inherited from a carrier mother with favorably skewed XCI. Therefore, the de novo mutations identified in CE males represent a different class. Whether sporadic or recurrent due to germ line mosaicism, they are predominantly frameshifts caused by single-nucleotide insertions or deletions that originate in the female germ line. The case presented here is unusual because he has a 9-nucleotide deletion, with consequences at the transcript level that strongly suggest a lack-of-function mutation. We predict that this mutation would cause classic RTT in a female, and reported evidence supports this interpretation. The point mutation c.378-2A>G, affecting the intron 3/exon 4 splice junction, was reported twice in early studies of RTT females (31, 32) and was interpreted to be disease causing, but its effect on the mRNA structure was not determined. When Bourdon et al. (33) studied the transcript generated...
by a similar c.378-2A>C mutation, they discovered activation of a cryptic splice site between c.384 and c.385. The resultant mutant transcript is lacking the exact same seven nucleotides as our case. Therefore, it is likely that the c.378-2A>G splice site mutation reported in the two RTT females (31, 32) leads to the same abnormal splice product. Although different at the genomic level, these two splice site mutations (c.378-2A>C and c.378-2A>G), which were identified in RTT females are identical at the mRNA and predicted protein levels to the deletion mutation in our CE proband, leading to premature truncation with loss of the transcriptional repression domain (TRD). Interestingly, all reported cases of de novo CE in males that have truncating mutations involve loss of the TRD.

The expected frequency of CE in males without a family history of RTT can be calculated as follows: de novo maternal origin is possible for 10–20% of female RTT cases that are due to mutations other than C>T transitions at CpG hotspots, including genomic deletions, and RTT occurs in approximately one in 10,000 female births. Therefore, maternal de novo mutations are expected at an incidence of between 1/50,000 and 1/100,000, and the same number should result in male CE cases. In 2003, Lynch et al. arrived at a similar estimate of 1/80,000 (34).

### Table 1. Salient clinical features in 17 males with CE and MECP2 null mutations

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<th>Clinical features</th>
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<td>Normal birth parameters (weight, length, and HC)</td>
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<td>Neonatal period</td>
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<td>Hypotonia and/or weak suck, feeding problems</td>
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<td>Apneic episodes</td>
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<td>Severe growth retardation</td>
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<td>Tremor, myoclonus, dystonia, and movement disorder</td>
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<td>EEG abnormalities</td>
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<td>Seizures</td>
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<td>Severe developmental delay</td>
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<td>Poor head control</td>
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<td>Normal</td>
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<td>Mild cerebral atrophy</td>
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<tr>
<td>Family member with mutation-positive RTT and/or CE</td>
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<sup>a</sup>Including two cases with information limited to uneventful pregnancy and term delivery.
<sup>b</sup>Reported as alive at 18 and 25 months.

MECP2 mutations in males causing phenotypes other than CE

Classic RTT occurs rarely in males and is usually associated with different types of mosaicism, such as coincidental occurrence of X chromosome aneuploidy with a MECP2 mutation and random XCI (35–37). Males with a RTT phenotype and a normal 46,XY karyotype may have somatic mosaicism for MECP2 mutations that arose post-zygotically (38–40). With no apparent selection against the MeCP2-deficient cells, the pattern of mosaicism in the affected boys resembles that of X-inactivation mosaicism in females with RTT, and in 47,XXY or 46,XX males who are heterozygous for a MECP2 mutation (reviewed in 41).

Non-mosaic male cases with a RTT phenotype are very unusual. Budden et al. (42) described a 46,XY boy with a p.S134C missense mutation, alive at 10 years, whose clinical course is typical for RTT. His sister is heterozygous for the same mutation and carries a diagnosis of autism, but does not meet diagnostic criteria for RTT, although her XCI pattern was random. Their mother is a phenotypically normal carrier with completely skewed XCI. Similarly, an 8-year-old boy with neonatal hypotonia and early-onset features of RTT, and his less severely affected
sister, inherited a c.397C>T (p.R133C) mutation from their mother. Most recently, a 4-year-old boy with a clinical course resembling RTT, and his asymptomatic mother with skewed XCI, were reported to have a distal truncating mutation: c.1158delG leading to p.388X (43). All three mutations, p.S134C, p.R133C and c.1158delG, have been identified in females with either classic or atypical (milder) RTT (http://mecp2.chw.edu.au). Undetected mosaicism can be excluded in the males with inherited mutations. Therefore, factors other than XCI may determine the variable severity of disease for these particular mutations in both hemizygous males and heterozygous females.

A Danish patient (44) has a mutation not previously reported in female RTT, a de novo c.816dup7, that causes a frameshift and, after a long stretch of missense amino acids, a premature termination codon at the end of the TRD. Clinically, he falls between the early-onset CE cases with TRD truncation and the less severe forms of mental retardation and spasticity whose truncating mutations are located more towards the C-terminus. He had failure to thrive with growth and developmental retardation from infancy and lost some skills at 1 year of age. He developed seizures with an abnormal EEG, stereotypic eye-rubbing movements, and severe constipation. It was his intense eye contact that raised suspicion of RTT and prompted MECP2 mutation screening. Studies of various tissues revealed no evidence for mosaicism. Mutant transcript levels were normal, but the transcript was not sequenced. While the boy is alive at the age of 14 years (J. B. Nielsen, personal communication), and now meets diagnostic criteria for RTT, some of his early symptoms are suggestive of CE, such as neonatal hypotonia and feeding difficulties leading to early-onset failure to thrive. It is possible that the predicted truncated protein has retained a nuclear localization signal (NLS) and may be partially functioning in contrast to the c.803delG mutation that leads to truncation within the NLS and caused typical MECP2-related CE in a male (2).

Other mutations in MECP2 that do not cause classic RTT in females have been reported in males from non-specific X-linked mental retardation families (45, 46). More recent studies of large series of sporadic or familial males with non-syndromic mental retardation, however, have not resulted in a high yield of MECP2 mutations (47). The frequency of causative mutations is estimated to be between one and four out of 1117 fragile X-negative-retarded males (48). While MECP2 mutation testing is indicated for males with the CE phenotype, i.e. neonatal apnea and lifelong respiratory insufficiency, progressive head growth deceleration, axial hypotonia combined with hypertonic extremities, movement disorders, seizures and lack of motor/cognitive development, it does not appear justified in cases having only non-syndromic developmental delay and cognitive impairment. While the heterogeneity of phenotypes associated with MECP2 mutations in males is quite remarkable (reviewed in 49), we wish to emphasize that MeCP2-deficient CE is a distinct and clinically recognizable disorder.

Supplementary material
Table S1. Comparison of clinical and molecular data on reported males with CE and MECP2 mutation.

Acknowledgements
We are grateful to the proband’s family for cooperating in our studies and to the physicians and other health care professionals involved in his care at Kaiser Permanente Oakland Medical Center and Oakland Children’s Hospital. Annie Chuang helped with the preparation of Fig. 4. The work was supported by the International Rett Syndrome Association (to UF).

References


