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ARTICLE

Recurrent Infections, Hypotonia, and Mental Retardation Caused by Duplication of *MECP2* and Adjacent Region in Xq28

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ABSTRACT

OBJECTIVE. Our goal was to describe the neurologic and clinical features of affected males from families with X-linked patterns of severe mental retardation, hypotonia, recurrent respiratory infection, and microduplication of Xq28 that consistently includes the *MECP2* (methyl-CpG binding protein 2) gene.

STUDY DESIGN. To identify duplications, multiplex ligation-dependent probe amplification of the *MECP2* gene was performed on male probands from families with X-linked mental retardation. The males either had linkage to Xq28 or had a phenotype consistent with previous reports involving Xq28 functional disomy. After detection of a duplication of *MECP2*, additional family members were tested to confirm the *MECP2* duplication segregated with the affected phenotype, and X-inactivation studies were performed on carrier females.

RESULTS. Six families with multiple affected males having *MECP2* duplications were identified by multiplex ligation-dependent probe amplification, and the carrier mothers were subsequently shown to have highly skewed X inactivation. In 5 of 6 families, the microduplication extended proximally to include the L1 cell adhesion molecule gene. The primary clinical features associated with this microduplication are infantile hypotonia, recurrent respiratory infection, severe mental retardation, absence of speech development, seizures, and spasticity.

CONCLUSIONS. Although many of the phenotypic features of our patients are rather nonspecific in cohorts of individuals with syndromic and nonsyndromic mental retardation, the proneness to infection is quite striking because the patients had normal growth and were not physically debilitated. Although the etiology of the infections is not understood, we recommend considering *MECP2* dosage studies and a genetics referral in individuals with severe developmental delay and neu-

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Key Words
mental retardation, hypotonia, recurrent infection, MECP2, duplication

Abbreviations
MECP2—methyl-CpG binding protein 2 gene
XLMR—X-linked mental retardation
L1CAM—L1 cell adhesion molecule gene
MLPA—multiplex ligation-dependent probe amplification
PCR—polymerase chain reaction
Ig—immunoglobulin
ANA—antinuclear antibody
CF—cystic fibrosis

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rologic findings, especially when a history of recurrent respiratory ailments has been documented.

MUTATIONS IN THE methyl-CpG binding protein 2 (*MECP2*) gene cause Rett syndrome and were implicated in the etiology of other related X-linked neurodevelopmental disorders.[1–4] Rett syndrome is typically characterized by normal postnatal development until 6 to 18 months of age, after which acquired speech and motor skills begin to regress. Purposeful hand movements are replaced by characteristic hand-wringing motions. Additional hallmark features include acquired microcephaly, profound mental retardation, seizures, and autistic behaviors. A wide array of *MECP2* single-base and small frameshift alterations were shown as the underlying molecular defect associated with most cases of typical Rett syndrome.[5,6] Individuals with Rett syndrome were also shown to have large deletions in *MECP2*, which is located in the gene-dense region of Xq28.[7,8]

In 2005, Sanlaville et al[9] reviewed the phenotypes associated with Xq28 disomy and included 2 of their own patients. In total, their report summarized 19 patients (16 males, 3 females) with functional disomy of Xq28 and a rather consistent clinical phenotype. These functional disomies resulted from Xq-Yq translocations, Xq-Xp rearrangements, or X-autosome translocations. Regardless of the location of the additional Xq28 material, virtually all affected individuals presented with severe developmental delay, microcephaly, growth retardation, hypotonia, hypoplastic genitalia, severe feeding problems, and recurrent respiratory infection.

More recently, Van Esch et al[10] showed small duplications at Xq28 in 3 families with X-linked mental retardation (XLMR) and in 1 additional family with a single affected male. The microduplications of Xq28 were variable in size but consistently included *MECP2* and L1 cell adhesion molecule (*L1CAM*), as well as 7 intervening genes. The clinical picture of the affected males consisted of severe mental retardation, absent speech, progressive neurologic problems (such as spasticity and seizures), mild facial dysmorphism, and severe recurrent respiratory infection and death before 25 years of age. An earlier case reported by Meins et al[11] was also compared, and additional expression studies in the Van Esch cohort indicated that overexpression of *MECP2* is most likely responsible for the observed neurologic phenotypes in these patients.[11] The overall clinical phenotype of these patients was comparable with that described for the 19 patients with Xq28 functional disomy.

Using multiplex ligation-dependent probe amplification (MLPA), we identified 6 X-linked families with microduplication of Xq28 including, at a minimum, *MECP2* to *L1CAM* in 5 families and *MECP2*, but not *L1CAM*, in the sixth family. Initially, probands from 17 X-linked families with linkage to Xq28 were tested, and from this cohort, 2 probands (K8210 and K8300) had results consistent with a duplication of *MECP2*. Concordancy studies indicated that the duplication segregated appropriately with the affected males and obligate carrier females. In rapid succession, the next 4 families were identified on the basis of the similarity of their clinical presentation to that of the first 2 families. Recurrent childhood infections, particularly pneumonia, served to distinguish the condition from other autosomal and X-linked hypotonia syndromes. Some affected boys showed facial hypotonia manifested by an open mouth with a tented upper lip. Hypotonia gave way to spasticity in childhood, and developmental milestones lagged from birth. Most affected males never acquired speech or unaided ambulation. Severe mental retardation was obvious in the early childhood years, and childhood or teenage death was typical.

METHODS

All molecular studies were performed on genomic DNA isolated from peripheral blood samples using standard isolation methodology. MLPA of the *MECP2* gene was performed as described by Schouten et al.[12] Test kits from MRC-Holland (Amsterdam, Netherlands) were used for all samples. The *MECP2* MLPA kit contained 20 probe pairs that targeted 4 *MECP2* exons, 6 X-linked control regions (including 1 L1CAM probe pair), and 10 autosomal control regions. Briefly, 100 to 200 ng of genomic DNA were denatured and hybridized with the probe mix overnight at 60°C. The following morning, the paired probes were ligated by using heat-stable Ligase-65 at 54°C for 15 minutes. The ligation was followed by a polymerase chain reaction (PCR) using a common M13 primer pair that hybridizes to the terminal end of each ligation product. The forward M13 primer was fluorescein phosphoramidites–labeled, and conditions for the PCR were as follows: 30 seconds at 95°C, 30 seconds at 60°C, and 1 minute at 72°C for 35 cycles. The resulting amplicons were separated by an Applied Biosystems 3100 capillary electrophoresis instrument and analyzed with Genescan software (Applied Biosystems, Foster City, CA).

Fragment analysis of the MLPA profiles was performed with peak heights and areas normalized to controls as previously described.[12] Data were analyzed by using a format based on Excel (Microsoft, Redmond, OR) with controls set to 1. Ratios of >1.5 were deemed indicative of duplication of the target sequence.

X-inactivation studies were performed by using the androgen receptor gene methylation assay described by Allen et al.[13] Genomic samples were digested with *Hpa*II restriction endonuclease, amplified by PCR, and compared against amplicons generated from an undigested genomic template. Fluorescently labeled fragments were separated on an Applied Biosystems 3100 instrument along with an internal size standard. Results were ana-

lyzed with GeneScan software, and X-inactivation ratios were calculated on the basis of peak area comparisons.

RESULTS

MLPA Findings

MLPA ratios after quantitation are displayed as profiles shown in Fig 1. Peaks 8, 10, 12, and 14 correspond with *MECP2* exons 4, 3, 2, and 1, respectively. Peak 6 corresponds with *L1CAM* and normally serves as an internal Xq28 control for *MECP2* deletions in female patients with Rett syndrome. For this study, *L1CAM* quantitation allowed partial determination of the size of the microduplication. Duplication of all 4 *MECP2* exons and the single *L1CAM* locus was apparent in 5 families (K8300, K8210, K8315, K9227, and K9228), indicating that the duplicated region is at least 200 kilobases (kb) in size. The sixth family (K9244) did not have an increased ratio at *L1CAM*, which indicates the microduplication has a proximal breakpoint in the intervening region between *L1CAM* and *MECP2*. Additional analysis of the 7 intervening genes (*LCA10*, *AVPR2*, *ARHGAP4*, *ARD1*, *RENBP*, *HCFC1*, and *IRAK1*) was not undertaken. For the 5 families with both *L1CAM* and *MECP2* (~200 kb apart), the involvement of the intervening genes was assumed. Preliminary data indicate that the size of these microduplications ranges from ~400 to 800 kb in length (data not shown).

CASE REPORTS

Partial pedigrees of the 6 affected families are given in Fig 2; clinical findings are summarized in Table 1. Clinical features and linkage studies on 2 of 6 families (K8300 and K8210) found to carry the microduplication were previously published.[14,15] These 2 families will be briefly summarized, and more detailed clinical descriptions of the 4 additional families will follow.

Pai et al[14] described a large, 4-generation family (K8300) with 5 affected males (only 1 surviving) and 10 carrier females with linkage to Xq28. The affected males all presented with severe mental retardation and a variety of other anomalies that were not consistently present in all 5 males. Seizure activity occurred in 4 of the affected boys, and no specific electroencephalogram patterns were noted. Four affected males had significant episodes of lower respiratory infection, and serum immunoglobulin (Ig)A and IgM levels were low in 1 of 2 males tested. This feature of their condition was considered remarkable given the fact these boys were not institutionalized and had normal growth parameters. The clinical picture in this family varied among the affected males and did not match any other known syndromic conditions.

Subsequently, a second family (K8210) with linkage to Xq28 was published by Lubs et al.[15] This 3-generation family had 5 affected males and 4 obligate carrier females. All affected males presented with severe mental retardation and progressive central nervous system deterioration. At the time of the report, 3 boys experienced swallowing dysfunction and gastroesophageal reflux and died before 10 years of age from secondary recurrent respiratory infections. Lubs et al[15] considered the clinical findings to represent a new XLMR syndrome, XLMR-hypotonia-recurrent infections.

K8315. Three brothers in K8315, 2 of whom were twins, were affected with recurrent infections, gastroesophageal reflux, severe mental retardation, spasticity, and seizures. The mother had 1 miscarriage of unknown gender, and there were no other males on the mother's side in the previous 2 generations.

II-2 was born at 38 weeks' gestation by cesarean delivery because of breech presentation. Intrauterine growth and measurements taken at birth were normal (birth weight: 3.3 kg [50th centile]; head circumference: 35 cm [50th centile]; length: 52.1 cm [50th centile]). Atonic seizures began at 7 years of age and were never

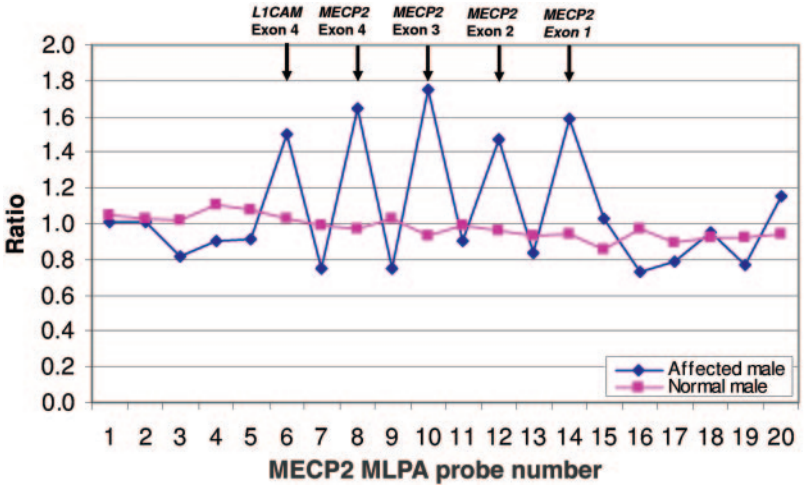

FIGURE 1
MLPA data profiles. A normal male is compared with a male with microduplication of Xq28 involving *MECP2* and *L1CAM*. Specific data points refer to 1 *L1CAM* and 4 *MECP2* targets of interest.

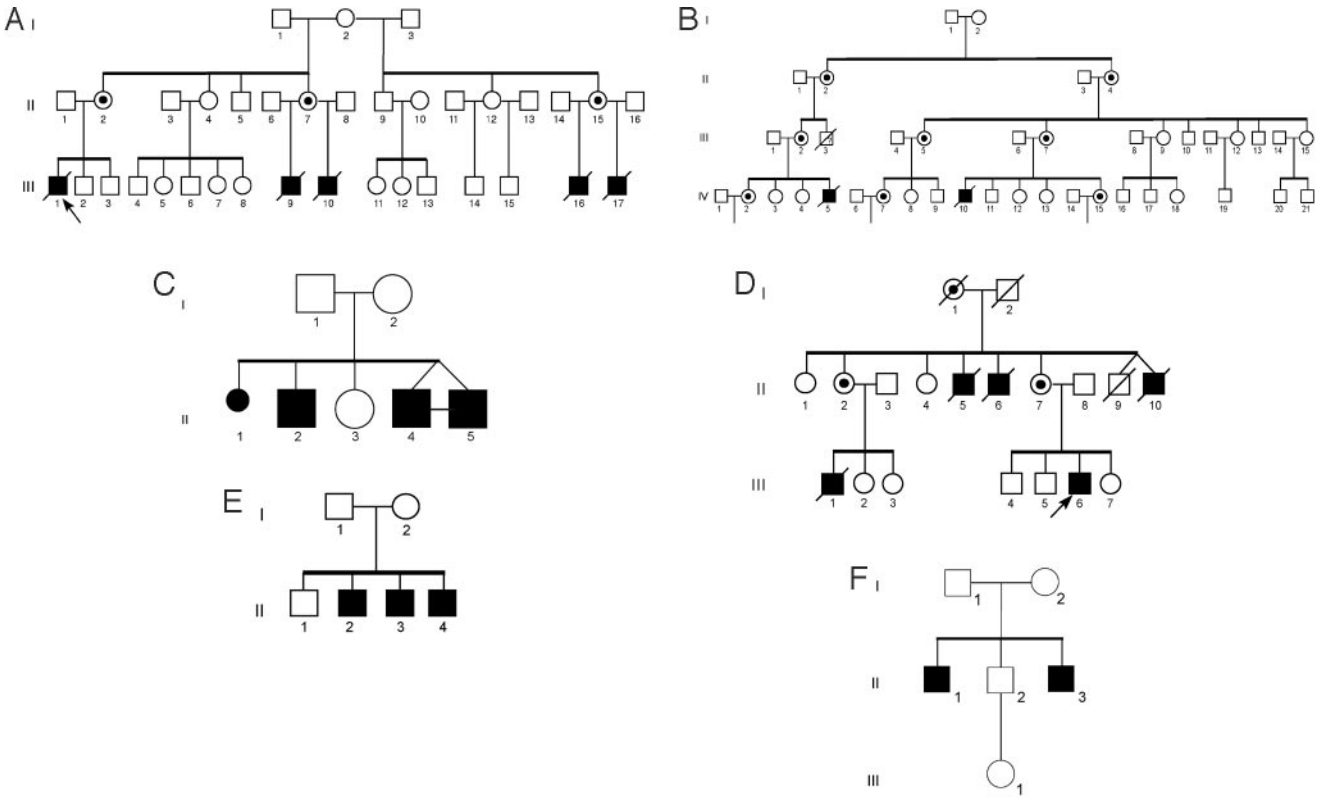

FIGURE 2
Partial pedigrees of 6 kindreds with Xq28 microduplication involving the *MECP2* gene. A, K8210; B, K8300; C, K8315; D, K9227; E, K9228; F, K9244.

TABLE 1 **Summary of Clinical Findings and Comparison With Patients Reported by Van Esch et al[10]**

	No. of Patients With Symptom/Total No. in Group						This Report, *n/N* (%)	Van Esch et al[10] Report, *n/N* (%)
	K8210	K8300	K8315	K9227	K9228	K9244		
Severe mental retardation	5/5	5/5	3/3	5/5	3/3	2/2	23/23 (100)	12/12 (100)
Microcephaly	0/5	1/3	0/3	1/5	0/3	0/2	2/21 (10)	2/10 (20)
Hypotonia	5/5	0/2	3/3	4/5	3/3	2/2	17/20 (85)	NR
Facial hypotonia	5/5	0/2	0/3	0/1	1/3	1/2	7/16 (44)	11/11 (100)
Small mouth	0/5	2/5	0/3	0/1	2/3	0/2	4/18 (21)	NR
Excessive drooling	3/5	0/2	3/3	1/1	2/3	2/2	10/15 (69)	NR
Gastrointestinal reflux	5/5	0/2	3/3	1/1	2/3	2/2	13/16 (81)	NR
Swallowing dysfunction	5/5	0/2	3/3	1/1	3/3	2/2	14/16 (88)	NR
Absent speech	4/4	1/2	3/3	5/5	3/3	2/2	18/19 (95)	10/12 (83)
Limited speech	0/4	1/2	0/3	0/5	0/3	0/2	1/19 (5)	2/12 (17)
Never walked	2/3	1/3	0/3	4/5	0/3	0/2	7/19 (37)	7/12 (58)
Limited ambulation	1/3	2/3	3/3	1/5	3/3	2/2	12/19 (63)	5/12 (42)
Spasticity[a]	1/1	1/1	3/3	—	0/3	2/2	7/10 (70)	9/9 (100)
Seizures	2/5	4/5	3/3	3/5	1/3	2/2	15/23 (65)	4/9 (44)
Severe and recurrent infections	5/5	4/5	3/3	5/5	3/3	2/2	22/23 (96)	5/9 (56)
IgA deficiency	0/2	1/2	1/1[b]	0/1	2/2	0/2[b]	4/10 (40)	NR
Death at <25 y of age	3/5[c]	4/5[d]	1/3[e]	4/5[f]	0/3[g]	0/2	12/23 (52)	6/11 (55)

NR indicates not reported.
[a] Appears in later childhood or teen years.
[b] One with decreased IgM.
[c] Two alive at ages 14 and 17 years.
[d] One alive at age 15 years.
[e] Two alive at age 17 years.
[f] One alive at age 14 years.
[g] Three alive at age 3, 5, and 13 years.

completely controlled with anticonvulsants. He experienced recurrent respiratory infections: Over a 7-month period in his eighth year, 8 episodes of pneumonia were documented. Examination at 16 years old showed a head circumference of 57.3 cm (80th centile), prominent eyes, simple helices, wide alveolar ridges, prominent

fingertip pads, and generalized spasticity with contractures. Serum IgA and IgM levels were low. Results of other extensive laboratory studies (fluorescence in situ hybridization 15, chromosomes, electromyogram, nerve conduction, and urine organic acids) were normal. An electroencephalogram showed bifrontal spikes and generalized spike and wave activity. An MRI scan showed a single area of gray matter heterotopia and mild Dandy-Walker variant.

II-4, the first born of monozygous twins, weighed 1610 g (50th centile) at 32 weeks' cesarean delivery. He experienced gastroesophageal reflux, chronic otitis media, and repeated respiratory infections during childhood. At 15 years old, he had a head circumference of 56 cm (60th centile), prominent eyes, eversion of the lower eyelids, flat midface, prominent nasal bridge, spasticity, and decreased musculature of the legs. He used a wheelchair for mobility.

II-5, the second born of twins, had a birth weight of 2190 g (95th centile) at 32 weeks' gestation. He required a ventilator for respiratory distress and had 6 episodes of pneumothorax in the newborn period. Cranial ultrasound showed agenesis of the corpus callosum. Recurrent respiratory infections were common during his childhood. At 15 years old, he had a head size of 56 cm (60th centile); long, thin face; eversion of the lower lids; prominent eyes; flat midface; prominent nasal bridge; and narrow palate. He also had spasticity and drooled excessively.

K9227. This kindred includes 5 affected males in 2 generations. By history, they had severe developmental failure, infantile hypotonia, seizures, recurrent bronchitis and pneumonia, and episodes of cyanosis and abdominal distension during infancy. In all except II-6, growth during the first few years seemed deficient but improved beginning at ~4 years of age. Only 1 affected male (II-5) learned to walk, and none developed significant speech. Death from pneumonia occurred at ages 11/2 years (III-1 and II-10), 16 years (II-5), and 29 years (II-6).

III-6, the only surviving affected male, is now 14 years old. Developmental milestones were severely delayed; he is nonverbal and can take a few steps using ankle-foot orthoses and a forward-facing walker. He has experienced recurrent episodes of pneumonia since infancy. Myoclonic seizures began at 9 years old. He now has a head circumference at the 50th centile, facial asymmetry, and hypotonia. He has normal reflexes but has had surgery for tight heel cords. Testing of Ig levels indicated normal levels.

K9228. K9228 has 3 affected brothers (Figs 2 and 3). II-2 was born at term after breech presentation. His weight was 3390 g (50th centile), length was 49.5 cm (25th–50th centile), and head circumference was 35 cm (50th centile). He sat at 7 months of age, walked at 2 years,

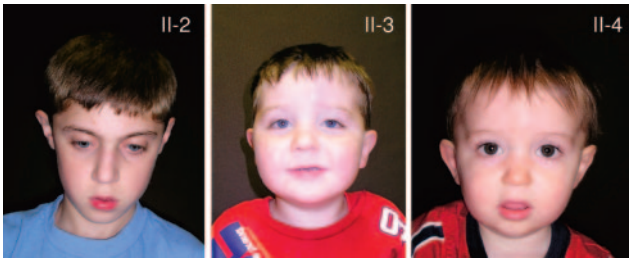

FIGURE 3
II-2, II-3, and II-4 from kindred 9228 at ages 11, 4, and 2 years. They are normocephalic; II-2 has a hypotonic face and small mouth; II-3 has small mouth.

and never spoke words. He had hypotonia, seizures, gastroesophageal reflux requiring gastrostomy tube placement, recurrent otitis media, recurrent pneumonia, and bronchospasm. He developed complex partial seizures at 5 years of age. Examination at age 11[7/12] years showed a head circumference of 54 cm (50th centile), weight of 40 kg (60th centile), and height of 147 cm (50th centile). The ears had upturned lobes, and the mouth appeared small. Hands, feet, and digits appeared long (hand and middle finger measurements were 17.2 cm [75th centile]). The feet were flat and everted. Genitalia showed Tanner stage 2 development. There was gait ataxia, head shaking, and some involuntary movements of the limbs. He had severe mental retardation and scored <20 on the Vineland Adaptive Behavior Scale.

II-2 has undergone extensive immunologic investigations because of the selective IgA deficiency with mildly decreased IgM and mild elevation of total IgG levels. He also has had problems responding to polysaccharide antigens. He required 2 Prevnar boosters followed by Pneumovax to achieve significant protective titers to *Streptococcus pneumoniae*. In addition, he required reboosting to *Haemophilus influenzae*, type b. His tetanus titers were protective at >7. He is varicella immune. He has normal numbers and percentages of T cells but has persistent decrease in the T-cell response to *Candida* (<20% of normal). T-cell functional studies demonstrated good proliferative capacity to mitogens. He has no evidence of complement deficiency and has a normal nitroblue tetrazolium. His most recent test results in 2005 showed an IgG level of 1809 mg/dL, IgA level <10 mg/dL, and an IgM level of 33 mg/dL. The IgE level was normal at 1.3 mg/dL. The IgG subclasses were normal when tested at 8 years old. His complete blood cell count showed no evidence of anemia, thrombocytopenia, or leucopenia. The antinuclear antibody (ANA) was negative.

II-3 weighed 3.9 kg (90th centile) at term birth. He crawled at 15 months, began walking at 16 months, and never developed speech. Hypotonia was noted by 6 months, and he experienced recurrent otitis media, sinusitis, pneumonia, and bronchospasm. At age 4[2/12] years, he had a head circumference of 51.5 cm (50th

centile), weight of 19.5 kg (75th centile), and height of 101 cm (25th centile). There was mild facial asymmetry; upturned earlobes; small nose with narrow alae nasi; thickened, flat, and everted feet; unsteady gait; involuntary hand movements; and normal prepubertal genitalia. He has not had seizures and scored 43 on the Vineland Adaptive Behavior Scale.

Immunologic studies have shown a poor response to polysaccharide antigens such as pneumococcal polysaccharide and *Haemophilus*. After 2 attempts with pneumococcal vaccination with Prevnar, interleukin-3 showed measurable titers in only 2 of 12 serotypes tested. After repeats of Pneumovax and Prevnar vaccines at 4 years old, he developed protective titers in 8 of 12 serotypes. He has a nonspecific ANA (1:80 titer) with negative Smith antibodies and negative double-stranded DNA. In infancy he had decreased T-cell proliferation to *Candida*, but that normalized by 4 years old. Serum Igs were normal (IgM: normal; IgG: normal; and IgA: low normal [52 mg/dL]).

II-4 weighed 3.7 kg (75th centile) at term birth. At 20 months he had no speech and could crawl and pull to a stand. He has not had seizures; he drooled and had recurrent bronchospasm. Head circumference was 47.5 cm (20th centile), weight was 11.1 kg (25th centile), and height was 81 cm (15th centile). The face had a triangular shape, and the nose and mouth were small. The superior helices were unfolded. Two fingers had periungual fungal infection. He scored 62 on the Vineland Adaptive Behavior Scale.

II-4 required 4 Prevnar vaccinations, as well as a Pneumovax, but eventually showed an excellent response to 8 of 12 serotypes assessed. At 3 years old he had a negative ANA. His complete blood cell count showed no evidence of anemia, thrombocytopenia, or leukopenia. Ig levels were normal (IgG: 1202; IgA: 109; and IgM: 86 mg/dL). The *H influenzae* total IgG was low at 0.43, with >1 considered to be indicative of protection. The T-cell functional studies showed normal lymphocyte proliferation to tetanus, phytohemagglutin, concanavalin A, and pokeweed mitogen.

K9244. Two brothers in K9244 had childhood hypotonia, recurrent infections, severe mental retardation, gastroesophageal reflux, seizures, and spasticity (Figs 2 and 4). Development was globally delayed; neither developed speech, and both had an unsteady gait and osteoporosis.

II-1 was born at term gestation by cesarean delivery, weighing 3 kg (25th centile). He had coarctation of the aorta, which was repaired at 1 month of age. All developmental milestones were delayed. He began walking at 3 years of age and never developed speech. He experienced recurrent pneumonia and had a tracheostomy placed at 30 years of age. He had gastroesophageal reflux, excessive drooling, and seizures since infancy. He has undergone surgery for peripheral vascular occlusive disease of the legs. At 33 years of age, measurement showed a head circumference of 57.7 cm (75th centile), weight of 59 kg (3rd centile), and height of 160 cm (3rd centile). He was nonverbal and nonambulatory. Strabismus, spasticity, and normal genital development were noted.

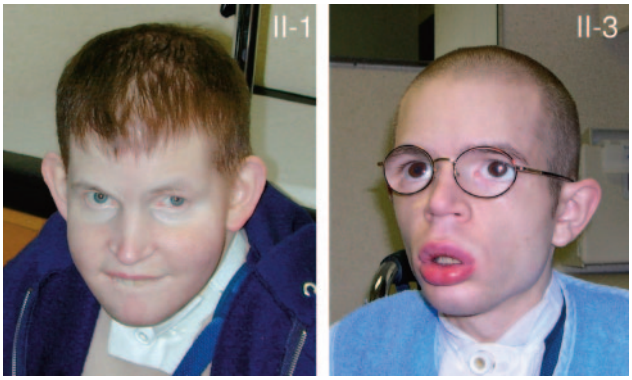

FIGURE 4
II-1 and II-3 from kindred 9244 at ages 33 and 25 years. Both are normocephalic; II-1 has strabismus; II-3 has prominent lips; both have tracheostomies.

II-3 was delivered by cesarean section at term gestation, weighing 3.3 kg (50th centile). He developed seizures at 6 months of age and had delay of all developmental milestones. He has experienced gastroesophageal reflux and recurrent respiratory infections, and has a feeding tube and tracheostomy. At 25 years of age, examination showed he was severely mentally retarded, drooled excessively, and was nonverbal and nonambulatory. His lips were prominent and head circumference was 55.2 cm (15th centile).

DISCUSSION

Recurrent respiratory infections, especially recurrent pneumonia, serve to distinguish this syndrome clinically from other XLMR-hypotonia syndromes. Most of the 18 patients described have required mechanical ventilation on ≥1 occasions. Four of 5 survivors >15 years old have tracheostomies. Meningitis occurred in 1 patient, pyelonephritis occurred in 1, and upper respiratory infections including otitis media were common. Contributing factors include gastrointestinal reflux and swallowing dysfunction. Comprehensive immunologic evaluations were performed only on the males from K9228. Peripheral leukocyte counts and differentials were normal. IgA levels were decreased or undetectable in 4 of 10 individuals tested. IgM levels were decreased in 2 individuals.

Hypotonia is a common clinical presentation in infants and young children who have developmental delay. Common genetic conditions associated with infantile hypotonia may have autosomal (lissencephaly, Prader-Willi syndrome, myopathies) or X-linked (Pelizaeus-Merzbacher, Coffin-Lowry, Lowe, Allan-Herndon-Dudley, α-thalassemia mental retardation) genetic etiol-

ogies. Microduplications in Xq28 that include *MECP2* must now be included among the X-linked etiologies. Facial hypotonia manifested by tented upper lip, open mouth, and drooling occurred in about half of the patients. Van Esch et al[10] also noted in their study that all 11 patients examined had facial hypotonia. The infantile hypotonia associated with Xq28 microduplication progresses to spasticity in childhood. This progression is seen in other X-linked hypotonia syndromes, especially Pelizaeus-Merzbacher and Allan-Herndon-Dudley syndromes.[16]

Malformations do not contribute significantly to the morbidity associated with this syndrome. One patient had coarctation of the aorta, 2 had inguinal hernias, 1 had agenesis of the corpus callosum, and 1 had a gray matter heterotopia and Dandy-Walker variant on MRI scan. Two affected males had microcephaly, and the lower face, including the mouth, appeared small in 4 patients. Only 3 males (2 in K9244 and 1 in K9227) reached 25 years of age. The 3 affected males in K9228 are alive at ages 3, 5, and 13, and 2 males in K8210 are alive at ages 14 and 17 years. One male in K8300 is alive at 15 years, and the 2 surviving males in K8315 are on ventilators at 17 years old.

Female carriers of the duplication show marked skewing (>90:10) of X inactivation. None have shown evidence of cognitive impairment, hypotonia, or recurrent infections. This finding is the same as in another XLMR-hypotonia syndrome, the α-thalassemia mental retardation syndrome, caused by mutations in the *XNP* gene located in Xq13. Van Esch et al[10] pointed out that although several genes are duplicated in their Xq28 patients, *MECP2* duplication is likely the genomic change responsible for the clinical findings in their patients. This is based on their patients having increased levels of *MECP2* expression and the same overexpression in another patient with an Xq28 microduplication involving *MECP2* and the same clinical features shown by Meins et al.[11] Although the extent of the Xq28 duplications are not known in our subjects, all included the *MECP2* gene and, therefore, would be compatible with Van Esch et al's argument. It should be noted that a nonpathogenic duplication of Xq28 sequence that does not include *MECP2* was recently shown.[17] This particular duplication was considered polymorphic because it was subsequently detected in 2 of 30 control individuals.

Most of the attention directed toward *MECP2* has been in regard to the diagnosis of Rett syndrome in females.[18] Separate reports have identified pathogenic *MECP2* alterations in individuals with PPM-X syndrome (X-linked psychosis, pyramidal signs, and macroorchidism) and in patients with X-linked mental retardation and progressive spasticity.[19,20] Other studies have suggested that *MECP2* mutations that do not lead to typical Rett syndrome in females may be a common cause of nonsyndromal mental retardation in males.[21–25] These mutations do not seem to be as common as originally proposed, and the current findings suggest that duplication of *MECP2* may potentially affect more males with developmental delay. Genetic testing for *MECP2* abnormalities is readily available in a number of laboratories at the present time, but it should be noted that not all diagnostic laboratories that offer *MECP2* sequencing analysis offer deletion and duplication studies. This may be an important factor to consider when deciding where to submit samples for the appropriate testing. *MECP2* dosage studies are typically less expensive than sequencing-based testing and should be pursued directly for individuals with clinical presentations similar to those shown in this study rather than starting with sequencing analysis, which is the standard protocol for Rett syndrome testing. Furthermore, individuals with such duplications may have a more predictable phenotype that could potentially lead to earlier diagnosis. Duplications such as those present in these patients are not visible on high-resolution chromosome analysis. Detection is best accomplished by using specific intragenic probes such as those included in the *MECP2* MLPA kit or array–comparative genomic hybridization that has probes for this region of Xq28.

Larger-scale studies in cohorts, with significant phenotypic similarities to the patients with *MECP2* duplication presented to date, are needed to determine the frequency of Xq28 microduplications in individuals with severe mental retardation, as well as the range of clinical variability. Additional work is also necessary to determine the significance of the other genes that are duplicated, and the role their expression may play in the clinical presentation of those affected individuals. Of particular interest is a better understanding of the immune dysfunction that coincides with Xq28 microduplication. Identification of other microduplications that encompass *L1CAM* and other adjacent genes, but do not include *MECP2*, may be especially helpful in delineating the clinical impact of *MECP2* duplication. Historically, novel submicroscopic duplications were difficult to detect and are typically overlooked by standard cytogenetic and molecular studies. The use of newer molecular techniques such as array-CGH and MLPA, which are designed to detect these types of abnormalities, will certainly have an impact on better diagnosis for moderately sized genomic dosage alterations.

Two studies in mice have associated *MECP2* overexpression with a progressive neurologic phenotype. The first study used transgenic techniques to rescue an MeCP2- deficient strain, which ultimately led to the discovery that neuronal MeCP2 overexpression results in severe motor dysfunction.[26] Second, Collins et al[27] developed a transgenic line designed to address in more depth the significance of *MECP2* overexpression. These mice were considered to be a better model for studying *MECP2* overexpression, because they had fewer con-

founding genetic effects when compared with the mice generated by Luikenhuis et al[26]. These mice have a double dose of *MECP2* and a progressive neurologic decline similar to our patients with *MECP2* duplication.[27] To date, these mice are the best supporting evidence that increased levels of *MECP2* are associated with the features shown in our patients. Interestingly, mice with only subtle *MECP2* increases in expression demonstrated better motor coordination and cerebellar learning when compared with wild-type littermates, although they did all eventually demonstrate signs of neurologic deterioration. These findings are bolstered by the report of increased levels of *MECP2* expression in one individual with autism and another with pervasive developmental disorder.[28] It is tempting to speculate that, in addition to genomic duplications, other epigenetically influenced mechanisms could lead to greater *MECP2* expression and a subsequent abnormal neurologic presentation.

The susceptibility of affected males with Xq28 duplication to respiratory infection raises the question regarding the use of prophylactic antibiotics. Short-term and long-term antibiotic prophylaxis is recommended in certain circumstances in which an individual has a generalized vulnerability to serious infections (eg, immunodeficiency syndromes), compromised organ function (eg, asplenia), or exposure to specific pathogens (eg, *Neisseria meningitidis* exposure).[29–31] Prophylactic antibiotics were shown to reduce respiratory tract infections and mortality in adults receiving intensive care.[32] In the United Kingdom, it is recommended that all patients with cystic fibrosis (CF) <2 years of age receive long-term flucloxacillin from the time of diagnosis to prevent infection with *Staphylococcus aureus*.[33] A large study in the United States, however, concluded that routine prophylaxis should not be administered to healthy young children with CF and found that antistaphylococcal prophylaxis leads to more frequent infection. Recently, it was suggested that prophylaxis be used in CF from diagnosis up to 3 years of age, a period when prophylactic antibiotics reduce infection without increasing risk of resistance.[33] The use of prophylaxis may also be beneficial in patients with Xq28 microduplication; however, before such therapy can be recommended, more information needs to be discerned regarding the types of infection in these patients, especially the colonizing organisms involved. Gastroesophageal reflux and immune dysfunction may be contributing factors. Currently, early detection of infection and aggressive therapy with pathogen-specific antibiotics seems prudent.

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Recurrent Infections, Hypotonia, and Mental Retardation Caused by Duplication of MECP2 and Adjacent Region in Xq28

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