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ARTICLE

Structural variation in Xq28: *MECP2* duplications in 1% of patients with unexplained XLMR and in 2% of male patients with severe encephalopathy

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Duplications in Xq28 involving *MECP2* have been described in patients with severe mental retardation, infantile hypotonia, progressive spasticity, and recurrent infections. However, it is not yet clear to what extent these and accompanying symptoms may vary. In addition, the frequency of Xq28 duplications including *MECP2* has yet to be determined in patients with unexplained X-linked mental retardation and (fe)males with severe encephalopathy. In this study, we used multiplex ligation-dependent probe amplification to screen Xq28 including *MECP2* for deletions and duplications in these patient cohorts. In the group of 283 patients with X-linked mental retardation, we identified three Xq28 duplications including *MECP2*, which suggests that approximately 1% of unexplained X-linked mental retardation may be caused by *MECP2* duplications. In addition, we found three additional *MECP2* duplications in 134 male patients with mental retardation and severe, mostly progressive, neurological symptoms, indicating that the mutation frequency could be as high as 2% in this group of patients. In 329 female patients, no Xq28 duplications were detected. In total, we assessed 13 male patients with a *MECP2* duplication from six unrelated families. Moderate to severe mental retardation and childhood hypotonia was noted in all patients. The majority of the patients also presented with absent speech, seizures, and progressive spasticity as well as ataxia or an ataxic gait and cerebral atrophy, two previously unreported symptoms.

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We propose to implement DNA copy number testing for *MECP2* in the current diagnostic testing in all males with moderate to severe mental retardation accompanied by (progressive) neurological symptoms.

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Introduction

Genomic rearrangements including deletions and duplications are often related to disease. Cytogenetically visible deletions and duplications, are usually associated with a syndromic phenotype that comprises mental retardation (MR). Such complex phenotypes are brought about by the reduced or elevated expression of one or more dosage-sensitive genes from the respective deleted or duplicated regions. Often it is not clear which gene or genes are accountable for the mental disabilities observed in the patients with a large duplication or deletion. In few cases, one gene can be held responsible for the major phenotypic characteristics seen in these patients. For instance, the 9q-subtelomeric deletion syndrome can be attributed to haploinsufficiency of just one gene, *EHMT1*, that is located in the common deletion.[1] Severe mental retardation, hypotonia, seizures and recurrent infections in male patients can be the result of duplications in Xq28.[2] It is thought that elevated expression of specifically *MECP2* is responsible for this phenotype. Screening for copy number changes of *MECP2* in selected cohorts of patients has revealed *MECP2* duplications in 44 males with severe neurological problems from 21 families.[2–7] Besides apparently tandem duplications including Xq28, Sanlaville *et al*[8] reviewed 19 cases in which the Xq28-duplicated region is involved in a complex rearrangement. These included translocations to other chromosomes and Xp–Xq rearrangements. These patients and the patients with tandem duplications of *MECP2* share a similar phenotype including severe developmental delay, hypotonia, seizures and recurrent infections.

Although a specific phenotype has been described for patients with *MECP2* duplications, it is not yet clear to what extent the symptoms may vary. These could be related to other genes in the respective Xq28 duplications. In addition, the frequency of Xq28 duplications including *MECP2* has yet to be determined in male patients with idiopathic X-linked MR (XLMR) and (fe)male patients with severe encephalopathy. Therefore, we screened a 1.3 Mb region on Xq28 including the *MECP2* gene for copy number imbalances to determine the frequency and size of duplications in this region in different cohorts of MR patients. Two groups of patients were tested: (1) 283 male probands from families with an X-linked inheritance pattern but no significant linkage interval and initially diagnosed as idiopathic XLMR, and (2) 134 male and 329 female patients with mental retardation and severe, mostly progressive, neurological symptoms. In total, we identified six novel *MECP2* duplications in male patients from these two patient cohorts.

Materials and methods

Patients

The patient cohort of 283 retarded males was collected by the European XLMR consortium (www.euromrx.com). Karyotypes at a resolution of 550 bands were normal and expansions of the CGG repeat in the 5′-untranslated region of *FMR1* were excluded. The 463 patients with severe encephalopathy were ascertained by the DNA diagnostics division of the department of Human Genetics, Radboud University Nijmegen Medical Centre, the Netherlands. This group consisted of 134 male and 329 female patients, who had been referred for screening of intragenic mutations in the *MECP2* gene, because of features suggestive for Rett syndrome in women and severe encephalopathy in men. In none of the analyzed patients, point mutations in the *MECP2* gene have been found by direct DNA sequencing of the coding region. All DNA samples were isolated from whole blood by the salting out method as described by Miller *et al*.[9] Informed consent was obtained for all patients.

Multiplex ligation-dependent probe amplification

For testing of EuroMRX patients, 15 multiplex ligation-dependent probe amplification (MLPA) probes were used, which specifically match the coding sequence of 12 genes from the critical region in Xq28 (Table 1). MLPA probes were designed and MLPA reactions were performed as described earlier.[10] Additional probes elsewhere on the X chromosome and autosomes were used for quality control.[10] In addition, the commercial SALSA MLPA kit P015C for testing copy number variations of *MECP2* (MRC Holland, Amsterdam, The Netherlands) was used for validation and fine mapping. The patients with severe encephalopathy were tested in a DNA diagnostic setting using the commercial P015C MLPA kit.

Results

MLPA analysis

The index patient of each of the 283 EuroMRX families was tested for copy number variations in the Xq28 region with MLPA probes in the following genes: *TREX2*, *BGN*, *ATP2B3*,

Table 1 Probe sequences of MLPA probes

Gene	*Left probe (5′→3′)*	*Right probe (5′→3′)*
TREX2	cccgggccgagacctttgtctt	cctggacctggaagccactggg
BGN	ctgtcccatgggcttcggggtgaag	cgggcctactacaacggcatcag
ATP2B3	cacgcttagcagctttctcaccgccg	ccaaaccttgcctgggcactgggacc
DUSP9	cttcagcagattccaggccgagtgccct	cacctgtgtgagaccagccttgctggcc
PLXNB3	cacttggcactggcacctggccgaggcaca	ctctatgtcggcgcagtgaaccgcctcttc
ARHGAP4	ggaaagacctctgtccgccagggccttgggcc	agcatctaccacctctcccagtcctgggcccc
MECP2	gaagagaaagagggcaagcatgagcccgtgcagccatcagccca	ccactctgctgagcccgcagaggcaggcaaagcagagacatcag
MECP2	caccagttcctgctttgatgtgacatgtgactccccagaatacacc	ttgcttctgtagaccagctccaacaggattccatggtagctgggat
FLNA	tgcagtcagcctgaacggggcca	agggggcgatcgatgccaaggtg
FLNA	gcgcagctcgagtgcttggacaatggggatggc	acatgttccgtgtcctacgtgcccaccgagccc
FLNA	ccttcccctgccaggcatcgagcccacaggcaacatggtga	agaagcgggcagagttcactgtggagaccagaagtgctggc
GDI1	cctggctgcccgcccaggatacctgtctgactc	accctgggcgctgggctctggctgtttccaggtgttctg
PLXNA3	ctgggcaacaggccctccgtgccttcgtggtgacaga	caccacgcttacccacctggctgtgcaccgggtgactg
FAM3A	ccgaggcgctggagatggaaggctgtatcccgcggagaag	cacggccagctagcacggccagtgccaggaccgggccgag
GAB3	ggtccttagtgagcctggacagagcaaggagagggctcccca	ctccctaagccccacagccagctctgcatcaccacaccacgc

DUSP9, *PLXNB3*, *ARHGAP4*, *MECP2*, *FLNA*, *GDI1*, *PLXNA3*, *FAM3A* and *GAB3*. Three probands carried a duplication of at least the *MECP2* gene (Figure 1g; families A–C). The three duplications were confirmed by using the commercial MLPA probe kit from MRC Holland. In the 134 male patients with (progressive) neurological symptoms, three duplications of the *MECP2* gene were identified (Figure 1g; families D–F). All 329 female patients with a Rett syndrome-like phenotype were negative for duplications in the Xq28 region. Also 178 control male samples were negative for duplications in the Xq28 region.

Family A A duplication of about 400 kb was found in family A (D033 in the EUROMRX cohort; www.euromrx.com) spanning the genomic region starting proximal of the *ARHGAP4* gene to distal of the *FLNA* gene (Figure 1g). Family A consists of two brothers with severe mental retardation and one affected maternal uncle (Figure 1a). One other brother, III-8, was born prematurely at 25 weeks of gestation and died from a cytomegalovirus infection. All females were normal, except for the sister of the affected brothers who suffered from generalized epilepsy since the age of 12 years. Epilepsy was also present in another healthy maternal uncle and in a healthy brother of the maternal grandfather. The duplication was identified in both affected brothers and their mother, whereas the sister did not carry the duplication.

The index patient III-7 was born after a normal pregnancy and delivery with a birth weight of 3180 g. During the first six months he developed normally, after which his development stagnated. He could sit without support at the age of 18 months and walk without help at the age of 3 years and 3 months. At three and a half years, he spoke four to five words, after which his ability to speak got worse with the passing of time. Since the age of 5 years, he experienced seizures classified as multifocal discharge or Lennox–Gastaut syndrome, which were difficult to control with medication. Because of frequent aspiration pneumonia, he was fed by a percutaneous endoscopic gastrostomy tube since the age of 8 years. On examination at the age of 14 years, his height was 140 cm (−3.5 SD) and his head circumference 52.9 cm (−1.5 SD). He had a mildly flat midface, slightly enlarged ears (Figure 2d), mild clinodactyly and contractures of several joints, such as the knees, hips, elbows, shoulders and wrist joints. He did not speak and showed an apparent loss of passive understanding of language. After a period of suffering from an ataxic gait, he became wheelchair bound as of the age of 9 years. Neurological investigation showed pyramidal signs with exaggerated deep tendon reflexes, Babinski and Trömner signs, and jerky myoclonus. In addition, the patient presented with axial hypotonia and peripheral hypertonia. Laboratory investigation showed normal IgG and subclass IgG1 levels and a secondary hypothyroidism. Brain MRI showed a small periventricular defect and broadened Virchow–Robin spaces.

The affected brother, III-9, was born after normal pregnancy and delivery with a birth weight of 3250 g. His development was normal during the very first months, but thereafter his development stagnated as was seen in his brother. He was able to walk at the age of two and a half years, but after the age of 3 years his ability to walk regressed, because of slightly progressive truncal ataxia. At the age of 7 years only supported walking was possible. He never spoke a word. There were difficulties with eating and swallowing, but he had no aspiration pneumonia as his brother. At the age of 4 years, complex partial and grand mal types of seizures started. He showed a mildly flat midface and slightly enlarged ears (Figure 2e), mild contractures of knees with otherwise normal joints, hypotonia and mild jerky myoclonus.

Family B The Xq28 duplication in family B (P002 in the EUROMRX cohort; www.euromrx.com) had a minimum

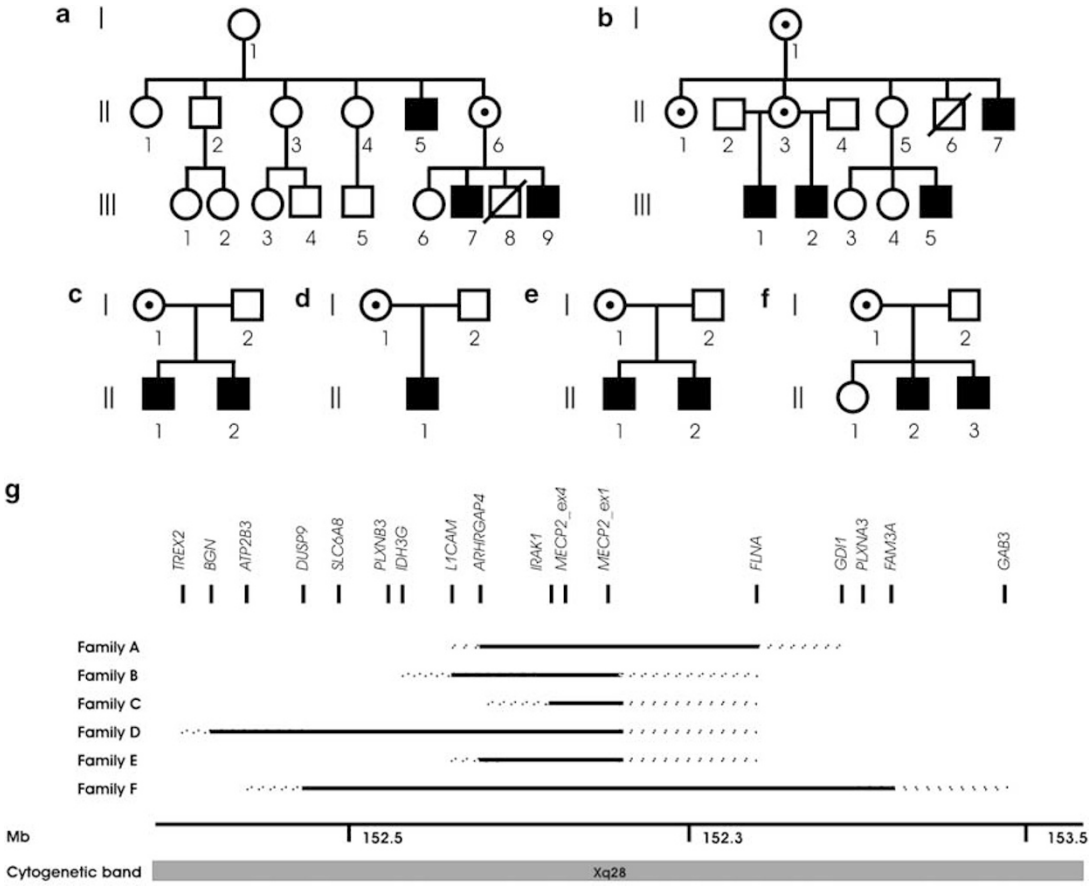

Figure 1 Schematic overview of the duplication mapping in the six affected families. (**a**–**f**) Pedigrees of the six affected families. (**g**) Detailed overview of the genomic region on the X chromosome surrounding *MECP2*. Genes tested by MLPA analysis are shown at the top. Black lines indicate the duplicated region and the dotted lines indicate the break point region between the last duplicated MLPA probe and the first probe with a normal copy number.

size of 260 kb and included *L1CAM*, *IRAK1* and *MECP2* (Figure 1g). Family B consists of four affected men in two generations and four carrier women (Figure 1b). Previous exclusion mapping excluded part of the X chromosome resulting in two possible disease-related regions: Xp11 and Xq28. All four affected boys were born after an uneventful pregnancy with normal birth parameters.

Patient II-7 showed delayed milestones with hypotonia. He could sit without support at the age of 12 months and walk alone at the age of 30 months. Initially, the only neurological abnormality was an ataxic gait, but in time progressive pyramidal signs with contractions of the hips and knees were reported. He had a moderate to severe mental deficiency with an estimated IQ below 50. He did not speak. At the age of three and a half years, he presented with motor stereotypies and gaze deficit. During the first years of life, no dysmorphic features were noted, but progressively, a more evident dysmorphic facial appearance with large ears, broad nose, coarse facial appearance, open mouth and drooling was noted. His fingers and toes were very slender. He had epileptic seizures since 8 years of age, which were of the tonic–clonic generalized type at the onset, but rapidly became tonic, a-tonic, or myoclonic. At the age of 12 years, he had a normal head circumference, but his cerebral MRI showed a moderate cerebral atrophy with a hypersignal of the white matter in the posterior parts of the cerebral hemispheres, associated with moderate superior vermis atrophy. After the age of 15 years, it was apparent that the neurological abnormalities got worse. The pyramidal signs progressed, the ataxia persisted and he developed mood disorders with aggressive behavior, very severe drug-resistant epilepsy with drop attacks, but no myoclonus, and frequent and daily falls needing the use of a helmet and a wheel chair. EEG recordings showed abnormal background activity, with slow waves and interictal generalized and/or focal discharges of the myoclonic type.

Patient III-1 seemed less severely affected. His development was delayed. He walked without support when he was 30 months old and did not speak. His total IQ was 50.

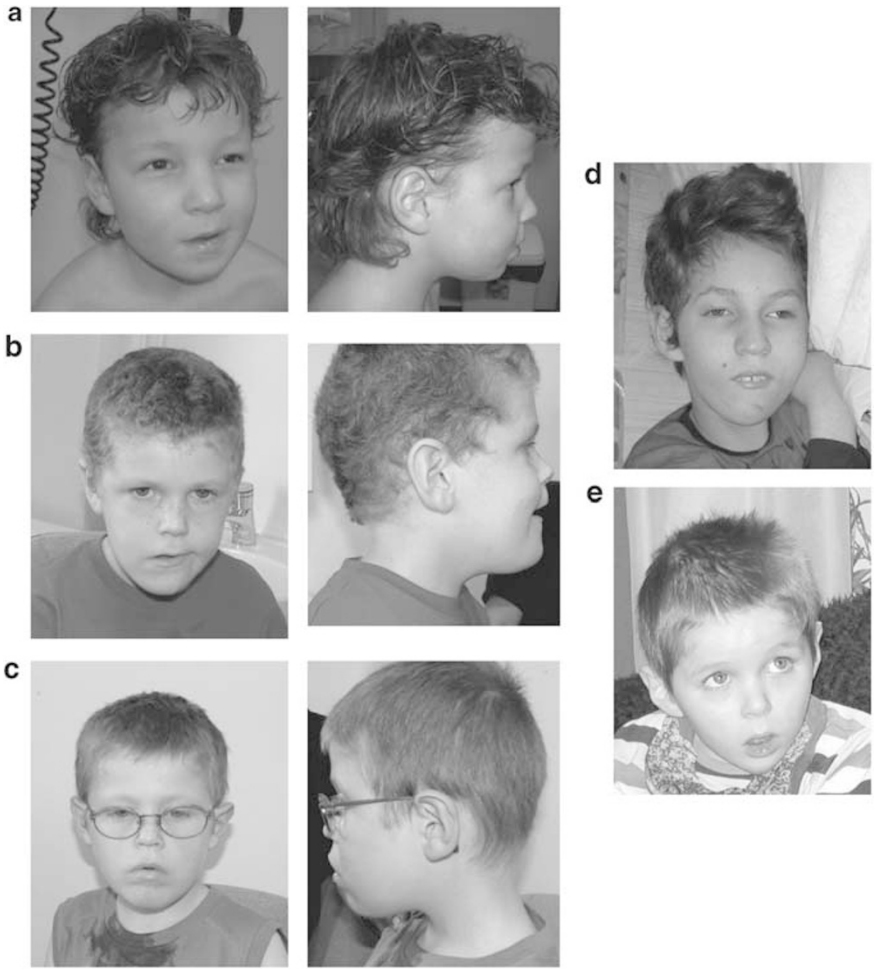

Figure 2 Photographs of five patients with a *MECP2* duplication. (**a**) Patient II-1 from family D at the age of 8 years. (**b**) Patient II-1 and (**c**) patient II-2 of family E at the age of 11 and 8 years, respectively. (**d**) Patient III-7 and (**e**) patient III-9 of family A at the age of 14 and 7 years, respectively. Note high forehead, small nose, deep-set eyes, large ears, eversion of lower lip and/or prominent chin in several patients.

He had a normal head circumference, slightly progressive dysmorphic features comprising large ears, broad nose, coarse facial appearance and slender fingers and toes, similar to those of patient II-7. He had mild ataxia, no pyramidal signs, and no mood disorders. Since the age of 18 years, he had developed generalized seizures. A cerebral MRI was performed at the age of 7 years and showed the same abnormalities as in his uncle, such as moderate cortical atrophy, hypersignal of the posterior part of the cerebral white matter but with a normal cerebellum.

Patient III-2 also had early-onset generalized hypotonia with delayed milestones. He could walk without support after 24 months, had moderate ataxia, could not speak, had dysmorphic facial signs similar to those of his uncle, and developed epilepsy, which started at the age of 10 years. The epilepsy was drug resistant with myoclonic seizures and drop attacks. EEG recordings and cerebral MRI showed abnormalities similar to those that were seen in patient II-7.

Patient III-5 had delayed milestones as well. At the age of 6 years he was mentally retarded, did not speak, had ataxia but had a normal facial appearance. No seizures have been observed yet.

Family C In family C (T065 in the EUROMRX cohort; www.euromrx.com), the duplication had a minimal size of 100 kb and included solely *MECP2* and *IRAK1* (Figure 1g). The two affected brothers were born after an uneventful pregnancy with normal birth parameters (Figure 1c). They both presented with moderate mental retardation, hypotonia, and macrocephaly. Metabolic studies and cerebral imaging were normal in the two boys.

The index patient II-1 was born at term with a weight of 3580 g, a height of 52 cm, and a head circumference of

34.5 cm. His development was delayed. He developed a social smile at the age of 2 months, was able to sit without support at the age of 17 months, started walking at the age of 2 years, and could only speak after the age of 5 years. At the age of 12 months, increased peripheral muscle tone and axial hypotonia was noted. Macrocephaly was obvious at two and a half years (head circumference of 52 cm, +2.4 SD). He developed ataxia at the age of 5 years. He experienced severe infections, such as pneumonia and meningitis. Since the age of 15 years, he suffered from absence type of seizures that did not respond to anti-epileptic treatment.

Patient II-2 was born at term with a weight of 3550 g, height of 49.5 cm and head circumference of 35.5 cm. His development was delayed as well. He could sit alone at the age of 18 months, started walking at the age of 2 years, and developed speech at the age of 6 years. Axial hypotonia was noted in infancy. His head circumference was 53.5 cm (+3.2 SD) at the age of 2 years and 10 months. He had ataxia and unsteady gait. Neither severe infections nor seizures were reported.

Family D In family D, a duplication of about 600 kb including the *BGN*, *ATP2B3*, *DUSP9*, *SLC6A8*, *PLXNB3*, *IDH3G*, *L1CAM*, *ARHGAP4*, *IRAK1* and *MECP2* genes was identified in an 8-year-old boy with a severe mental retardation (Figure 1g). He was born from healthy non-consanguineous parents and had a healthy older sister (Figure 1d). There were no other mentally retarded patients in the family. He was born after an uneventful pregnancy with a birth weight of 2500 g. His development was severely delayed. He started walking at the age of three and a half years. He developed no speech. As an infant, he had esophageal reflux and generalized hypotonia was noted. Up to the age of 5 years, he had several episodes of severe respiratory infections with, at one time, admission to the intensive care unit. He had no seizures. His height, weight and head circumference were normal. On physical examination at the age of 8 years, facial hypotonia with excessive drooling, lateral flaring of eyebrows (Figure 2a), clinodactyly, fetal pads and short and broad feet were noted. Tendon reflexes were close to normal. Metabolic screening including creatine measurements in urine and spinal fluid were normal. Cerebral MRI and CT-scan revealed cerebral atrophy.

Family E In family E, a duplication of about 200 kb including the *ARHGAP4*, *IRAK1* and *MECP2* genes was identified in a boy with severe neurological problems (Figure 1g). This boy had an affected brother as well (Figure 1e). Both patients were born after an uneventful pregnancy with normal birth parameters. They had severe psychomotor retardation. At the age of 8 years, patient II-1 had developed severe seizures and was also found to be hypothyroid for which he received L-thyroxine treatment. A brain MRI at the age of 10 years did not show abnormalities. At the age of 11 years (Figure 2b), he spoke some words, was hypotonic and could walk only a few steps, with an ataxic gait. He was incontinent. His height, weight and head circumference were all normal.

Patient II-2 had a weight of 12.5 kg (+1.5 SD), a height of 82 cm (+2 SD), and a head circumference of 46 cm (0 SD) at the age of 1 year. At the last clinical examination at the age of 8 years (Figure 2c), he was hypotonic, had developed no speech and was not toilet-trained. He could just walk unaided but with an ataxic gait. He had no seizures. His brain CT was normal. His EEG showed mild generalized dysrhythmia. Blood amino acids, lactate, pyruvate, and acid-base studies, were normal in both brothers.

Family F In family F, a duplication of about 900 kb including the *DUSP9*, *SLC6A8*, *PLXNB3*, *IDH3G*, *L1CAM*, *ARHGAP4*, *IRAK1*, *MECP2*, *FLNA*, *GDI1*, *PLXNA3* and *FAM3A* genes (Figure 1g) was identified in an eight-year-old boy with severe learning disability and epilepsy. This boy had an affected younger brother and a healthy older sister (Figure 1f). Both patient II-2 and patient II-3 were born to healthy unrelated parents after an uneventful pregnancy and did not have any perinatal problems. A maternal uncle died in infancy and a maternal granduncle was described as 'slow' but no further details are available.

Patient II-2 presented with severe learning disability, postnatal deceleration of head growth, bruxism, stereotypes, breath holding, recurrent severe respiratory infections, fluctuating dyskinesia, unsteady gait and loss of mobility at the age of 9 years. He was known with severe bowel constipation. At the age of 8 years, epilepsy started. EEG showed generalized spike waves and MRI showed ventricular dilatation and patchy signal abnormality in the deep white matter. Metabolic tests including creatine measurements in urine and cerebral spinal fluid were normal.

Patient II-3 had a milder phenotype. He started walking independently at the age of 3 years, developed minimal speech, and functioned in the moderate to severe learning disability range. Similar dysmorphisms as in his brother were noted. He had severe constipation problems as well. He had no postnatal fall off in head growth, severe infections, epilepsy, nor dyskinesia. MRI at 6 years showed general ventricular dilatation with signal change in the deep white matter, albeit less severe than in his brother.

Discussion

In a cohort of 283 mentally retarded patients with likely X-linked mode of inheritance, we identified three duplications in Xq28, which all included the *MECP2* gene. This indicates that the *MECP2* duplication incidence could be as high as 1% in this group of patients. Moreover, we

identified three duplications in 134 male patients with mental retardation and severe, mostly progressive, neurological symptoms, indicating that the mutation frequency could be as high as 2% in male patients with severe encephalopathy. In the cohort of 329 female patients with features suggestive of Rett syndrome, no *MECP2* duplications were identified. In addition, the female relatives from the affected male patients who carried a Xq28 duplication were all healthy. It thus appears that *MECP2* duplications do not lead to mental retardation in women. This is likely caused by skewing of X-inactivation, which has invariably been observed in women with a *MECP2* duplication until now.[2,4–6]

The duplications range from approximately 100–900 kb in size and all include at least the *MECP2* and the *IRAK1* genes. The overall severity of the phenotype does not seem to correlate with the size of the duplication (Table 2). The phenotype of patients from family C with the smallest duplication is as severe as that of the other patients. Yet, the phenotype of patients of family F, who had the largest duplication, was the most severe. One of the patients from the latter family died at the age of 10. Besides *MECP2*, there are four other genes duplicated in one or more families that are already involved in a specific phenotype. Loss-of function mutations in *SLC6A8* cause the creatine deficiency syndrome (OMIM 300352), which is characterized by MR, epilepsy, and expressive speech and language delay.[11] *SLC6A8* is duplicated in family D and F. In patients from these two families, creatine levels in urine and spinal fluid were normal. In addition, the phenotype does not seem to be different as compared with the other patients, although brachycephaly was only noted in patients from these two families. However, brachycephaly is a common consequence of hypotonia and is not always present in patients with a *SLC6A8* duplication.[4] Loss-of-function mutations in *L1CAM* result in hydrocephalus, MASA syndrome (mental retardation, aphasia, shuffling gait, and adducted thumbs), and spastic paraplegia.[12] Except for mental retardation, the characteristics of patients with *L1CAM* loss-of-function mutations were not present in any of the patients with a duplication of *L1CAM*. In addition, *L1CAM* is not X-inactivated,[13] which indicates that this gene is probably not dosage sensitive. *FLNA* gain-of-function mutations cause otopalatodigital syndrome I (OMIM 311300) or II (OMIM 304120), frontometaphyseal dysplasia (OMIM 305620), or Melnick–Needles syndrome (OMIM 309350).[14] The symptoms between patients with *MECP2* duplications and these four syndromes do not overlap, except for the presence of mental retardation. This shows that duplications of *FLNA* do not have the same effect as the gain-of-function mutations. Of note, *FLNA* is X-inactivated,[13] which indicates that the expression of this gene is dosage sensitive. *FLNA* loss-of-function mutations cause periventricular heterotopia (OMIM 300049),[15] a cerebral cortical neuron migration disorder that results primarily in seizures that do not respond to treatment.[16] However, drug-resistant epilepsy was not restricted to patients from family B and F, who have a *FLNA* duplication, which indicates that it is unlikely that duplication of solely *FLNA* is causing the epilepsy. *GDI1* loss-of-function mutations cause non-specific XLMR[17] and *GDI* is X-inactivated.[13] Moreover, duplications of *GDI1* alone result in moderate MR and microcephaly.[18] *GDI1* is duplicated only in family F. Interestingly, this is the family with the severest phenotype and the only one in which microcephaly is described in one of the two patients. Including the two patients from family F, five out of the eight patients with Xq28 duplication of whom the duplication is described in sufficient detail to be sure that *GDI1* is included, have microcephaly.[2,4,7,10] This supports a correlation between *GDI1* duplications and microcephaly. Moreover, the reversed correlation, that is, patients with microcephaly have a *GDI1* duplication, is true in five out of the six cases.

The main consistent clinical features in patients with a *MECP2* duplication are severe mental retardation, infantile hypotonia, progressive spasticity, seizures, absent speech and recurrent infections.[2] In the current study, all 13 affected males had infantile hypotonia and moderate to severe mental retardation. In addition, ataxia or an ataxic gait was described in almost all our patients, while it was not recognized as a symptom of the *MECP2* duplication phenotype before. In two patients pyramidal signs were present, but these developed rather late and were definitely not present at the onset of the disease. Seizures were observed in 54% of the patients. Remarkably, the epilepsy was drug resistant in families A, B and C, which was also noted in several previously reported male patients with a *MECP2* duplication.[5,6] In family A, epilepsy was also observed in unaffected family members, although the severity of the seizures was significantly milder in the unaffected individuals as compared with the male patients carrying *MECP2* duplications. The prevalence of infections in our patients is 23%, which is much lower in comparison to the 80% described in previously published work (Table 2). This indicates that patients with Xq28 duplications that include *MECP2* and *IRAK1* might be less prone to infections than thought earlier. On the other hand, it is also possible that infections during infancy are not always reported. Previously, microcephaly was reported in 36% of the patients with a *MECP2* duplication, whereas in our study only one patient had microcephaly. Moreover, two male patients from the same family presented with macrocephaly. Del Gaudio *et al*[4] described also one patient with macrocephaly, but his phenotype cannot be compared to that of our two patients, because he had three copies of *MECP2*. Our data suggests that microcephaly is not a consistent feature of patients with *MECP2* duplications. Cerebral atrophy is common in our patients and present in six out of the eight tested patients. This could be a result of epilepsy,[19] but only four of the examined

Table 2 Clinical description of the MR patients with a *MECP2* duplication

Family	*A*		*B*				*C*		*D*	*E*		*F*				
Patient	*III-7*	*III-9*	*II-7*	*III-1*	*III-2*	*III-5*	*II-1*	*II-2*	*II-1*	*II-1*	*II-2*	*II-2*	*II-3*	*This report* n/N	*Literature* n/N[a]	*Total* (%)
Main																
Age of last examination (years)	14	7	15	18	10	6	23	23	8	11	8	9	6			
Moderate to severe MR	+	+	+	+	+	+	+	+	+	+	+	+	+	13/13	63/63	100
Infantile hypotonia	+	+	+	+	+	+	+	+	+	+	+	+	+	13/13	57/57	100
Age of walking (years)	3.3	2.5	2.5	2.5	2	ND	2	2	4	3	3	6	3			
Absent speech	+	–	+	+	+	+	+	+	+	+	–	+	–	10/13	35/42	82
Progressive spasticity	+	+	+	–	–	–	+	–	–	–	–	+	+	6/13	19/22	71
Recurrent infections	–	–	–	–	–	–	+	–	+	–	–	+	–	3/13	47/58	70
Seizures	+	–	+	+	+	–	+	–	–	+	–	+	–	7/13	29/58	51
Ataxia/ataxic gait	+	+	+	+	+	+	+	+	ND	+	ND	ND	ND	10/10	–	–
MRI abnormalities	+	ND	+	+	+	ND	ND	ND	+	–	–	+	+	6/8	2/3	–
Death at <25 years of age	–	–	–	–	–	–	–	–	–	–	–	+	–	1/13	19/37	40
Facial abnormalities																
Microcephaly	–	–	–	–	–	–	–	–	–	–	–	+	–	1/13	22/58	32
Macrocephaly	–	–	–	–	–	–	+	+	–	–	–	–	–	2/13	0/58	3
Brachycephaly	–	–	–	–	–	–	–	–	+	–	–	+	+	3/13	13/38	31
Large ears	+	+	+	+	+	+	+	+	–	–	–	+	+	10/13	13/38	45
Flat midface	+	+	–	–	–	–	+	+	–	–	–	+	+	6/13	11/35	35
Broad nasal root	–	–	+	+	+	+	–	–	–	+	+	+	+	8/13	7/38	29
Hypertelorism	–	–	–	–	–	–	–	–	–	–	–	+	+	2/13	6/35	17
Other congenital abnormalities																
Swallowing problems	–	–	+	–	+	–	–	–	–	–	–	+	–	3/13	16/19	59
Genital abnormalities	+	–	–	–	–	–	–	–	–	–	–	–	–	1/13	24/38	49
Digital abnormalities	–	–	+	+	+	+	–	–	+	–	-	+	–	6/13	16/38	43

[a]2–8,10.
ND = not determined.

Table 3 Incidence of MECP2 duplications in males with MR selected by different criteria

Selection criteria	*Number of male patients*	*Number of MECP2 duplications*	*Frequency (%)*	*Reference*
Phenotype				
MR and spasticity	17	3	17.6	Van Esch *et al*[2]
Severe encephalopathy	134	3	2.2	This study
MECP2 testing[a]	122	2	1.6	Gaudio *et al*[4]
Chromosomal imbalances[b]	1380	5	0.4	Del Gaudio *et al*[4]
Genotype				
Linkage to Xq28	17	2	11	Friez *et al*[5]
X-linked inheritance	283	3	1.1	This study

[a]Patients sent to a diagnostic centre for *MECP2* mutation screening; no further information was disclosed by the authors.
[b]Males and females screened using a targeted microarray for disease relevant regions for deletions and duplications.

patients had both epilepsy and cerebral atrophy, which suggests that these symptoms are not related *a priori*. Based on the available MRI data, we could not determine a more specific cerebral phenotype, but it would be interesting to delineate such a correlation in an additional study. In our study, distinct facial features such as large ears and broad nasal root are frequently observed in patients, which suggests that there might also be a specific facial appearance associated with *MECP2* duplications. Combining all data, severe mental retardation and infantile hypotonia are

the two core features of patients with duplications of *MECP2*. Absence of speech, seizures, progressive spasticity, recurrent infections, and possibly ataxia present in various combinations, further define the clinical phenotype.

At present, 37 families have been reported with duplications in the Xq28 region including *MECP2*.[2–8,10] In addition, genomic segments proximal to the *MECP2* gene have been duplicated either to or from the autosomes 2, 10, 16 and 22.[20,21] Examination of the Xq28 duplication end points in 16 families did not provide any indication of a common mechanism for these genomic rearrangements.[22] Non-allelic homologues recombination was not involved in any of these duplications, although eight of the 32 break points coincided with low-copy repeats. Nevertheless, the presence of numerous repeats in the Xq28 region could induce genomic instability. In one patient, non-homologues end joining was demonstrated.[22] For two other patients, a two-step mechanism was suggested in which a part of Xq28 is inserted near *MECP2*, which is subsequently followed by breakage-induced replication with strand invasion of the normal sister chromatid resulting in a duplication of *MECP2* and a second telomeric duplication. Taken together, these data indicate that there are multiple mechanisms by which copy number changes can occur in Xq28, showing that this is a fragile genomic region in general.

Our results underline the importance of screening for *MECP2* gene duplications in male patients with moderate to severe mental retardation. In Table 3, the recently published data on the number of duplications found in different groups of patients are summarized. Patients selected for the specific *MECP2* duplication phenotype result in a mutation detection rate as high as 17%.[2] As our study shows, in male patients with severe encephalopathy, the mutation frequency is still more than 2%. In male patients referred for *MECP2* mutation analysis in regular DNA diagnostics, this is 1.6%.[4] This group of patients will overlap for a large part with our patients as male patients referred for *MECP2* mutation analysis usually have (progressive) neurological symptoms as well. Even in a group of male and female patients with a phenotype that is suspect for a chromosomal rearrangement, *MECP2* duplications were identified in 0.36% of the cases.[4] Analysis of MR patients with linkage to Xq28, regardless of the specific phenotype, showed that the incidence is 11%[5] and here we show that analysis of patients with XLMR without a specific linkage interval still results in a detection frequency of 1% for *MECP2* duplications. The mutation frequency of *MECP2* duplications is among the highest of the known X-linked mental retardation genes.[23] Therefore, we propose to implement DNA copy number testing for the *MECP2* gene in the current diagnostic testing in all male patients with moderate to severe mental retardation accompanied by (progressive) neurological symptoms.

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