REPORT

Complex Segmental Duplications Mediate a Recurrent dup(X)(p11.22-p11.23) Associated with Mental Retardation, Speech Delay, and EEG Anomalies in Males and Females

Roberto Giorda,[1,20,*] M. Clara Bonaglia,[2,20] Silvana Beri,[1] Marco Fichera,[5] Francesca Novara,[8] Pamela Magini,[9] Jill Urquhart,[10] Freddie H. Sharkey,[11] Claudio Zucca,[3] Rita Grasso,[4] Susan Marelli,[4] Lucia Castiglia,[5] Daniela Di Benedetto,[5] Sebastiano A. Musumeci,[6] Girolamo A. Vitello,[6] Pinella Failla,[7] Santina Reitano,[7] Emanuela Avola,[7] Francesca Bisulli,[12] Paolo Tinuper,[12] Massimo Mastrangelo,[13] Isabella Fiocchi,[13] Luigina Spaccini,[13] Claudia Torniero,[14] Elena Fontana,[14] Sally Ann Lynch,[15] Jill Clayton-Smith,[16] Graeme Black,[16] Philippe Jonveaux,[17] Bruno Leheup,[18] Marco Seri,[9] Corrado Romano,[6] Bernardo dalla Bernardina,[14] and Orsetta Zuffardi[8,19]

Submicroscopic copy-number variations make a considerable contribution to the genetic etiology of human disease. We have analyzed subjects with idiopathic mental retardation (MR) by using whole-genome oligonucleotide-based array *c*omparative *g*enomic *h*ybridization (aCGH) and identified familial and de novo recurrent Xp11.22-p11.23 duplications in males and females with MR, speech delay, and a peculiar electroencephalographic (EEG) pattern in childhood. The size of the duplications ranges from 0.8–9.2 Mb. Most affected females show preferential activation of the duplicated X chromosome. Carriers of the smallest duplication show X-linked recessive inheritance. All other affected individuals present dominant expression and comparable clinical phenotypes irrespective of sex, duplication size, and X-inactivation pattern. The majority of the rearrangements are mediated by recombination between flanking complex segmental duplications. The identification of common clinical features, including the typical EEG pattern, predisposing genomic structure, and peculiar X-inactivation pattern, suggests that duplication of Xp11.22-p11.23 constitutes a previously undescribed syndrome.

Mental retardation (MR), defined as cognitive impairment with onset before the age of 18, affects 2%–3% of the population in Western countries.[1] X-linked gene defects have long been considered important causes of MR on the basis of the observation that MR is significantly more common in males than in females.[2] Large cohorts of well-characterized families have been crucial to the identification of X-linked MR (MRX) genes, and MR-causing inactivating variants have now been described in about 10% of the genes on the X chromosome.[3] Autosomal and X-linked dominant gene mutations and subtle chromosomal rearrangements also have an essential role in the etiology of MR. Microarray-based comparative genomic hybridization (CGH) is becoming the method of choice for the identification of submicroscopic copy-number variations. In a recent study using high-resolution tiling X-aCGH, causal structural aberrations were detected in 4.6% of patients with idiopathic MR.[4] The Xp11 region carries almost 30% of all gene defects that underlie nonsyndromic MRX.[5]

As a result of a collaborative effort to share the results of individual diagnostic laboratories, we compared anonymous aCGH results of individuals with syndromic as well as nonsyndromic forms of MR. We screened 2400 subjects with isolated or syndromic MR for copy-number changes by whole-genome aCGH. Genomic DNA was extracted from probands' blood, EBV cell lines, and parents' blood with standard protocols. Array-based CGH was performed with the Agilent Human Genome CGH Microarray Kits 44K and 244B as previously described[6] or the Genechip Human Mapping 250K *Nsp* Array (Affymetrix). According to the manufacturer's protocols, the Affymetrix array was stained with the Fluidics Station 450 (Affymetrix) and scanned with a GeneChip scanner 3000 7G system (Affymetrix). Copy-number data were generated within the Affymetrix Genotyping Console via the CN4 algorithm with reference files from normal female samples previously run on the system. All possible copy-number alterations greater than 200 kb and not previously reported as CNVs were examined.

[1]Biologia Molecolare, [2]Citogenetica, [3]Neurofisiologia Clinica, [4]Neuroriabilitazione, Eugenio Medea Scientific Institute, Bosisio Parini, 23842 LC, Italy; [5]Laboratory of Genetic Diagnosis, [6]Unit of Neurology, [7]Unit of Pediatrics and Medical Genetics, Oasi Institute (Instituto di Ricovero e Cura a Caratterre Scientifico) for Research and Care in Mental Retardation and Brain Aging, Troina, 94018 EN, Italy; [8]Genetica Medica, Università di Pavia, Pavia, 27100 PV, Italy; [9]Unità Operativa Genetica Medica, Dipartimento di Scienze Ginecologiche, Ostetriche e Pediatriche, Policlinico Sant'Orsola Malpighi, Università di Bologna, Bologna, 40126 BO, Italy; [10]Biomedical Research Centre, Central Manchester Foundation Trust, Manchester, M13 9WL, UK; [11]Cytogenetics Department, Western General Hospital, Edinburgh, EH4 2XU, UK; [12]Dipartimento di Scienze Neurologiche, Università di Bologna, Bologna, 40126 BO, Italy; [13]Neurologia Pediatrica, Ospedale dei Bambini V. Buzzi, Milano, 20136 MI, Italy; [14]Neuropsichiatria Infantile, Policlinico G.B. Rossi, Università di Verona, Verona, 37129 VR, Italy; [15]National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, BT-29, Ireland; [16]Department of Clinical Genetics; Central Manchester Foundation Trust, Manchester, M13 9WL, UK; [17]Laboratoire de Génétique médicale, [18]Service de médecine infantile III et génétique clinique, Centre Hospitalier Universitaire Nancy, Nancy-Université, Nancy, 54500 Vandœuvre, France; [19]Instituto di Ricovero e Cura a Caratterre Scientifico C. Mondino, Pavia, 27100 PV, Italy
[20]These authors contributed equally to this work.
*Correspondence: roberto.giorda@bp.lnf.it
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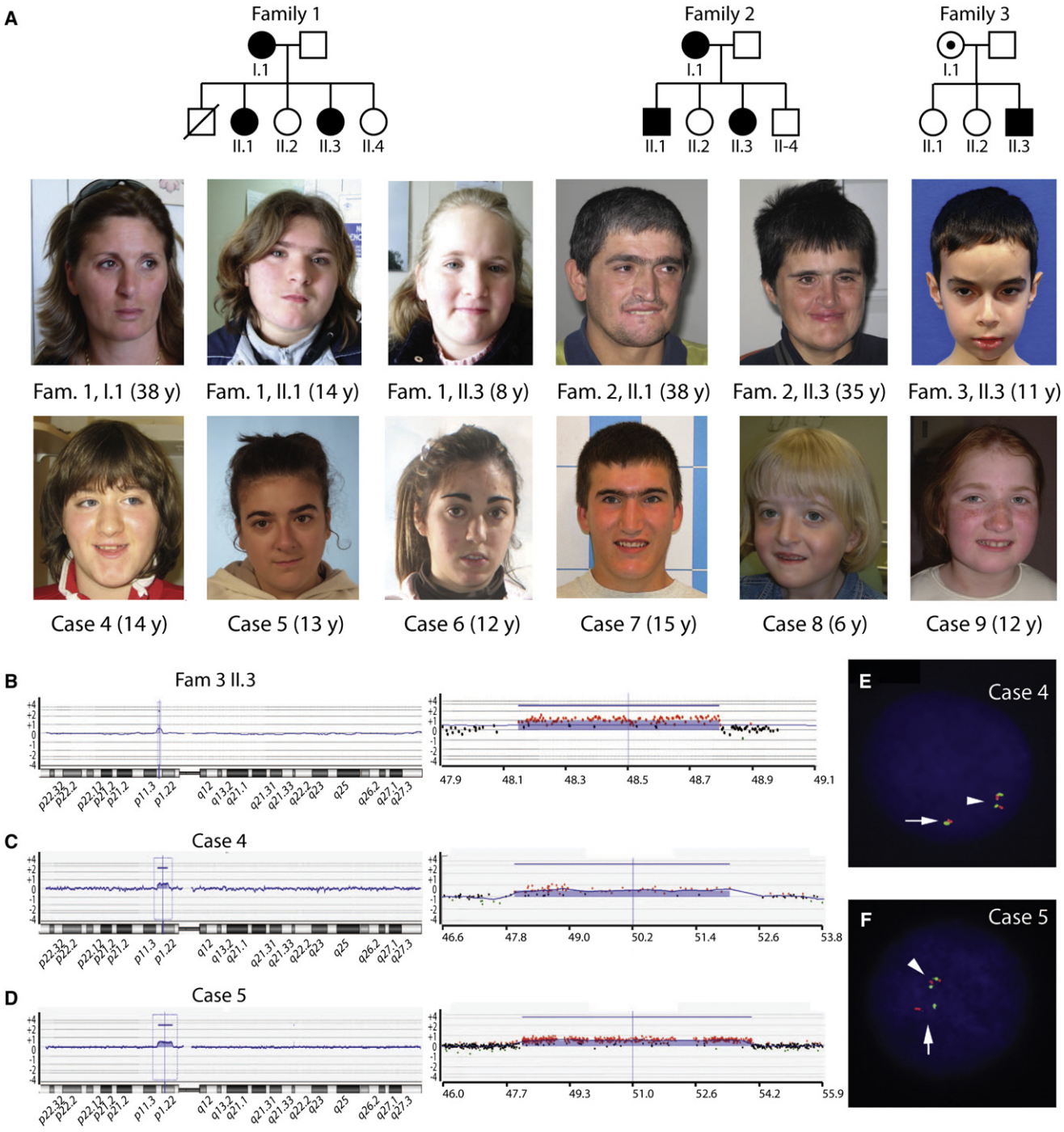

Figure 1. Analysis of Individuals with Duplications at Xp11.22-11.23
(A) Pedigree of the familial cases (subjects tested for the duplication are numbered) and clinical photographs of affected individuals. The age of each subject is indicated.
(B–D) Chromosome X aCGH profiles (left) and details (right, not enlarged to the same scale) of (B) family 3 II.3, 0.8 Mb duplication; (C) case 4, recurrent 4.5 Mb duplication; and (D) case 5, 6 Mb duplication. The March 2006 human reference sequence (NCBI Build 36.1) was used in all cases. Additional aCGH profiles are shown in Figure S1.
(E and F) Interphase FISH verification of the direct duplication in (E) case 4 and (F) case 5 (arrowheads). Normal chromosome X signals are indicated by arrows.

We identified two males and six females (0.33%) with a microduplication at Xp11.22-p11.23. The rearrangement was familial in three cases (Figure 1A): one female (family 1, II.1) and one male (family 2, II.1) shared a recurrent 4.5 Mb duplication with their affected mother and one affected sister (Figure 1A; Figure S1); the second male (family 3, II.3) inherited a smaller duplication (0.8 Mb) from his unaffected mother (Figures 1A and 1B); three

additional females carried 4.5 Mb de novo duplications (cases 4, 8, and 9) (Figure 1C), and two more had partially overlapping 6 and 9.2 Mb de novo duplications (cases 5 and 6) (Figure 1D).

Informed consent for further molecular and clinical characterization was obtained from subjects with Xp11.22-p11.23 duplication, their parents, or their legal tutors. The screening protocols were approved by all institutional review boards involved in the research. Genomic DNA from patients as well as from healthy controls was isolated from peripheral blood according to standard procedures. Specific target sequences were selected for quantitative PCR (qPCR) analysis with Primer Express software (Applied Biosystems, Foster City, CA). A control amplicon was selected with the same parameters in the mitogen-activated protein kinase 1 (*MAPK1* [MIM 176948]) gene on 22q11.2. The UCSC Genome Browser hg18 assembly (March 2006 human reference sequence, NCBI Build 36.1) maps and sequence were used as references. All primer sequences are available on request. Amplification and detection were performed as described[6] on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems) with SYBR Green PCR Master Mix (Applied Biosystems). No imbalances were detected by qPCR screening of 200 female controls via amplicons centered on the genes *PORCN* (MIM 300651), *GATA1* (MIM 305371), *SHROOM4* (MIM 300579), and *BMP15* (MIM 300247).

The presence of an Xp11.22-p11.23 duplication in all affected individuals and its absence in nonaffected family members (with the exception of the mother in family 3, who carries a much smaller duplication) and normal subjects demonstrate their causal relationship. All affected individuals (Table S1) show borderline to severe MR and speech delay. Poor speech articulation and a hoarse and/or nasal voice were often present. Early puberty was described in seven of the nine individuals examined, and six out of 12 were significantly overweight. Eight individuals had lower-extremity anomalies, including flat (*pes planus*) or arched (*pes cavus*) feet, fifth-toe hypoplasia, and syndactily. Case II.3 in family 3, carrying the smallest duplication, showed mild MR and language delay but no early puberty, excessive weight gain, or foot anomalies. In all other affected individuals, clinical features were apparently independent of sex and duplication size. Five subjects suffered from rare or isolated seizures. EEG recordings showed significant diffuse paroxysmal discharges in six individuals, all less than 15 years old; in five of them, discharges were associated with rolandic-like focal paroxysms. During wakefulness the discharges, probably related to secondary bilateral synchrony, were associated with subclinical absence seizures; during sleep, they became subcontinuous and produced a typical picture of *c*ontinuous *s*pike *w*ave during *s*low sleep (CSWS).[7] Subjects in family 2 presented EEG anomalies with diffuse sharp wave activities more evident during sleep or drowsiness. Once again, case II.3 in family 3 had a different EEG profile, characterized by rolandic-like paroxysms, and he showed rare seizures only in the first 6 months of his life.

Paternal origin of the duplication was demonstrated in all de novo female cases by polymorphic sequence-tagged site (STS) analysis (Table S2). Genotyping of polymorphic STSs was performed by amplification with primers labeled with fluorescent probes and subsequent analysis on a ABI 310 Genetic Analyzer (Applied Biosystems, Monza, Italy). Amplifications were performed with AmpliTaq Gold (Applied Biosystems) according to standard protocols. Chromosome X inactivation analysis of affected females, performed as described[8] on a polymorphic region of the androgen receptor (*AR* [MIM 313700]) gene, showed selective inactivation (defined as X-inactivation pattern $<$20:80 or $>$80:20) of the normal X chromosome in six subjects and random inactivation in three subjects (Table S3). The unaffected carrier mother in family 3 also had random inactivation. The X-inactivation pattern did not correlate with duplication size. Within families, in affected subjects the degree of X-inactivation did not seem to correlate with clinical phenotype severity.

We defined breakpoint junctions in eight individuals, including a previously described male with a de novo 4.5 Mb duplication[9] (case 7), by qPCR (Table S4) and long-range PCR amplification. For qPCR targets located in repeated portions of the chromosome, we used a BLAT search to select regions with significant homology to the target sequence and then performed multiple alignment with MAP; visual inspection of the aligned sequences allowed us to position the primers in target-specific regions. By q-PCR, we were able to narrow most of the breakpoint regions down to a size (5–10 Kb) amenable to long-range PCR amplification. We performed long-range PCRs with JumpStart Red ACCUTaq LA DNA polymerase (Sigma) by using target-specific primer pairs. Sequencing reactions were performed with a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and run on an ABI Prism 3130xl Genetic Analyzer.

Direct orientation was confirmed by interphase double-color Fluorescent in situ hybridization (FISH) (Figures 1E and 1F). FISH was performed on interphase cells according to standard procedures. Bacterial artificial chromosome (BAC) clones were selected from the human RPCI-11 library and provided by the BACPAC Resource Center (BPRC) at the Children's Hospital Oakland Research Institute in Oakland, CA. BAC DNAs were labeled either by biotin-16-dUTP or by digoxigenin with a nick translation kit (Roche). Double-color FISH was performed with RP11-8A2 (Xp11.23, AC027819.2 at 48,303,116–48,463,802 bp) and RP11-576P23 (Xp11.22, AL357894.6 at 50,147,678–50,302,524 bp) labeled with digoxigenin and biotin, respectively. The labeled probes were visualized with FITC-avidin (Vector, Burlingame, CA) or Rhodamin-conjugated anti-digoxigenin (Sigma). Hybridizations were analyzed with an Olympus BX71 epifluorescence microscope, and images were captured with the Power Gene FISH System (PSI, Newcastle upon Tyne, UK).

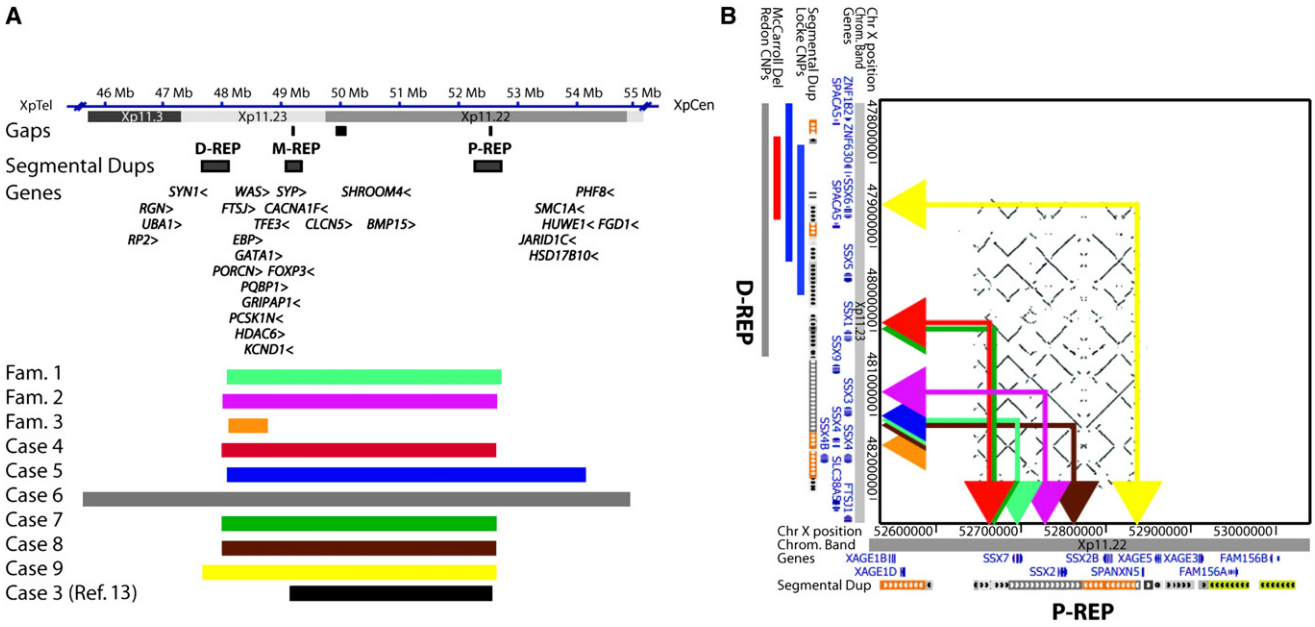

Figure 2. Schematic Structure of the Xp11.22-p11.23 Region
(A) Gaps in the genome assembly, segmental duplications, and Xp11.22-p11.23 duplications are shown. Disease-associated and copy-number-sensitive genes (not drawn to scale) are described in Table S6. Arrow colors correspond to line colors.
(B) Comparative analysis of the D-REP and P-REP regions performed with Pipmaker software.[38] Diagonal plot lines indicate stretches of sequence identity. The relative positions of chromosome bands, genes, segmental duplications, and copy-number variations are shown. Breakpoints are indicated by arrows when both are located within the SSX-REPs, and by arrowheads when one breakpoint is outside the regions shown. Detailed identity plots and junction sequences are shown in Figure S2 and Table S5.

The recurrent duplication is flanked distally by a segmental duplication (D-REP, 47.8–48.2 Mb) containing a cluster of genes and pseudogenes of the synovial sarcoma X breakpoint (*SSX*) and proximally by a complex repeat (P-REP, 52.1–53.1 Mb) rich in *SSX*, melanoma antigen (*MAGE*), and X antigen (*XAGE*) genes (Figure 2). Comparative analysis shows that their homology is limited to 300 Kb D-REP and 200 Kb P-REP regions containing *SSX* genes (Figure 2B). An additional segmental duplication rich in G-antigen (*GAGE*) genes (M-REP, 49.0–49.2 Mb) shows no homology with either D-REP or P-REP.

Sequence analysis of the junctions demonstrated that recurrent 4.5 Mb duplications are mediated by nonallelic homologous recombination (NAHR) or Alu-mediated recombination (Table S5 and Figure S2). Alu repeats also mediate the rearrangements in family 3 and case 5, for whom only one breakpoint maps to a complex repeat. Unlike other rearrangements caused by NAHR,[10] Xp11.22-11.23 duplication breakpoints do not cluster in defined hot spots.

Xp11.2 is a gene-rich, rearrangement-prone region within the critical linkage interval for several neurogenetic disorders harboring MRX genes.[11] All genes in the region, with the exception of a small cluster between 53.0 and 53.3 Mb, undergo X inactivation.[12]

Although X-inactivation pattern and clinical findings may be variable,[11] most females with chromosome X duplications are phenotypically normal because skewed X inactivation selectively inactivates the duplicated chromosome. On the contrary, in most females with Xp11.22-p11.23 duplication, the duplicated X chromosome is preferentially active, probably contributing to their clinical phenotype and suggesting that increased expression of a duplicated gene might act as a selective factor on dividing cells.

To our knowledge, only two cases with a recurrent 4.5 Mb duplication at Xp11.22-p11.23 have been reported: our case 7,[9] and one female with mild MR, language delay, repetitive behavior, and severe hypotonia[13] (case SK0306-004); the latter was detected in a group of families with simplex and multiplex autism spectrum disorders (ASD [MIM 209850]). To our knowledge, Xp11.22-p11.23 duplications have never been reported as copy-number polymorphisms in any published study. Additional polymorphic variations and pathogenic microdeletions and microduplications, probably facilitated by the complex pattern of direct and inverted repeats in the segmental duplications[14], have been described in regions adjacent to both D-REP and P-REP.[4,15,16]

Interestingly, a de novo 3.1 Mb duplication between M-REP and P-REP (49.0–52.1 Mb) was described in a girl (case 3 in Froyen et al.[4]) with moderate MR, speech delay, stereotypic movements, autistic features, no reported EEG abnormalities, and random X inactivation. Three of the eight genes (not counting *SSX*, *MAGE*, *GAGE*, and *XAGE*) contained in the duplicated region are involved in known inherited diseases (Figure 2A and Table S6); *SHROOM4* [MIM 300579] is an MRX gene.[17] In the affected male of

our family 3, a nonoverlapping 0.8 Mb duplication between 48.1 and 48.9 Mb, associated with X-linked recessive MR, speech delay, and EEG abnormalities, contains at least six genes involved in known diseases, including MRX genes *FTSJ1* [MIM 300499][18,19] and Polyglutamine-binding protein 1 (*PQBP1* [MIM 300463]).[20–23] The region between 48.1 and 52.6 Mb is duplicated in our subjects with recurrent Xp11.22-p11.23 duplication associated with dominant MR, speech delay, early puberty, foot anomalies, specific EEG pattern, seizures, and EEG paroxysms limited by pediatric age. A 0.1 Mb sequence between 48.9 and 49.0 Mb, excluded from both 0.8 Mb and 3.1 Mb duplications, contains the Synaptophysin gene (*SYP* [MIM 313475]), involved in MRX and epilepsy.[3] Overexpression of the bone morphogenetic protein 15 (*BMP15* [MIM 300247]) gene, selectively expressed in ovaries[24] and mutated in premature ovarian failure (POF4 [MIM 300510]),[25,26] could be responsible for early puberty in these patients. The duplications in cases 5 and 6 encompass a further region between 52.6 and 54.0 Mb containing eight genes; lysine-specific demethylase 5C (*KDM5C* [MIM 314690]),[27] *HSD17B10* (MIM 300256)[28,29], and *HUWE1* (MIM 300697)[30] are MRX genes. The duplication in case 6 spans the largest region and contains at least 30 additional genes. Among them, Synapsin 1 (*SYN1* [MIM 313440]),[31] *PHF8* (MIM 300560)[32,33], and *FGD1* (MIM 300546)[34,35] are associated with X-linked MR. The reciprocal Xp11.22-11.23 deletion has never been found and is probably not viable.

The phenotype of all subjects with recurrent 4.5 Mb and nonrecurrent larger duplications is remarkably similar despite the widely different number of genes, including MRX genes, involved, pointing to a key role of gene(s) in the common duplicated region in the defining clinical features of the syndrome. On the other hand, the level of MR can vary between borderline and severe, even in subjects with duplications of the same size and an identical X-inactivation pattern; behavioral signs can include shyness, stubbornness, autistic-like symptoms, or ASD as in the subject in Marshall et al.[13]. The EEG findings appear particularly suggestive for their pattern of secondary bisynchronism associated with atypical absences during wakefulness and continuous spike-wave pattern during sleep, persisting during adolescence even in the absence of clear epileptic seizures. Seizures and EEG paroxysms could be a prerogative of pediatric age; therefore, negative EEG results in adulthood cannot exclude the presence of EEG paroxysms in preadolescence. These findings appear to be so specific that it might be tempting to suggest a search for Xp11.22-p11.23 duplication in subjects with MR and speech delay associated with continuous spike waves during sleep, regardless of the presence of seizures.

The analysis of selected patients with borderline to severe MR, language delay, minor facial dysmorphic features, and a peculiar EEG pattern characterized by rolandic-like spikes and/or CSWS in the absence of obvious seizures will probably reveal additional cases of Xp11.22-p11.23 microduplication and demonstrate that the frequency of 0.33% we calculated was an underestimation. A similar experimental approach has recently yielded a prevalence of 0.64% for a recurrent 17q21.31 microdeletion in subjects with unexplained MR,[36] and 2% of male subjects with MR and progressive neurological symptoms[37] carry Xq28 duplications, including that of methyl-CpG-binding protein 2 (*MECP2* [MIM 300005]). It is also likely that genome-wide aCGH screening in patients without an obvious "chromosomal phenotype" will uncover new additional microdeletion and microduplication syndromes.

Supplemental Data

Supplemental Data include two figures and six tables and can be found with this article online at http://www.cell.com/ajhg/.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

BACPAC Resource Center (BPRC), Children's Hospital Oakland Research Institute, Oakland, CA, http://bacpac.chori.org/
BLAT search, http://genome.ucsc.edu/cgi-bin/hgBlat
MAP, Multiple Alignment Protocol, http://genome.cs.mtu.edu/map/map.html
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim
UCSC Genome Browser, http://genome.ucsc.edu/

References

1. Leonard, H., and Wen, X. (2002). The epidemiology of mental retardation: Challenges and opportunities in the new millennium. Ment. Retard. Dev. Disabil. Res. Rev. *8*, 117–134.
2. Lehrke, R. (1972). Theory of X-linkage of major intellectual traits. Am. J. Ment. Defic. *76*, 611–619.
3. Tarpey, P.S., Smith, R., Pleasance, E., Whibley, A., Edkins, S., Hardy, C., O'Meara, S., Latimer, C., Dicks, E., Menzies, A., et al. (2009). A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. Nat. Genet. *41*, 535–543.
4. Froyen, G., Van Esch, H., Bauters, M., Hollanders, K., Frints, S.G., Vermeesch, J.R., Devriendt, K., Fryns, J.P., and Marynen, P. (2007). Detection of genomic copy number changes in

patients with idiopathic mental retardation by high-resolution X-array-CGH: Important role for increased gene dosage of XLMR genes. Hum. Mutat. *28*, 1034–1042.
5. Ropers, H.H., Hoeltzenbein, M., Kalscheuer, V., Yntema, H., Hamel, B., Fryns, J.P., Chelly, J., Partington, M., Gecz, J., and Moraine, C. (2003). Nonsyndromic X-linked mental retardation: Where are the missing mutations? Trends Genet. *19*, 316–320.
6. Bonaglia, M.C., Giorda, R., Massagli, A., Galluzzi, R., Ciccone, R., and Zuffardi, O. (2008). A familial inverted duplication/deletion of 2p25.1–25.3 provides new clues on the genesis of inverted duplications. Eur. J. Hum. Genet. *17*, 179–186.
7. Tassinari, C.A., Bureau, M., Dravet, C., Dalla Bernardina, B., and Roger, J. (1992). Epilepsie avec pointesondes continues pedant le sommeil lent - Anterieurement decrite sous le nom d'ESES (epilepsie avec etat de mal electroencephographique pedant le sommeil lent). In Les Syndromes Epiteptiques de l'Enfant et le Adolescent, Second Edition, J. Roger, M. Bureau, C. Dravet, F.E. Dreifuss, A. Perret, and P. Wolf, eds. (Paris: John Libbey Eurotext), pp. 245–256.
8. Allen, R.C., Zoghbi, H.Y., Moseley, A.B., Rosenblatt, H.M., and Belmont, J.W. (1992). Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am. J. Hum. Genet. *51*, 1229–1239.
9. Bonnet, C., Grégoire, M.J., Brochet, K., Raffo, E., Leheup, B., and Jonveaux, P. (2006). Pure de-novo 5 Mb duplication at Xp11.22-p11.23 in a male: phenotypic and molecular characterization. J. Hum. Genet. *51*, 815–821.
10. Hurles, M.E., and Lupski, J.R. (2006). Recombination hotspots in non-allelic homologous recombination. In Genomic Disorders: the Genomic Basis of Disease, Chapter 24, J.R. Lupski and P. Stankiewicz, eds. (Totowa, New Jersey: Humana Press), pp. 341–355.
11. Ropers, H.H., and Hamel, B.C. (2005). X-linked mental retardation. Nat. Rev. Genet. *6*, 46–57.
12. Carrel, L., and Willard, H.F. (2005). X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature *434*, 400–404.
13. Marshall, C.R., Noor, A., Vincent, J.B., Lionel, A.C., Feuk, L., Skaug, J., Shago, M., Moessner, R., Pinto, D., Ren, Y., et al. (2008). Structural variation of chromosomes in autism spectrum disorder. Am. J. Hum. Genet. *82*, 477–488.
14. Stankiewicz, P., and Lupski, J.R. (2008). Genome architecture, rearrangements and genomic disorders. Trends Genet. *18*, 74–82.
15. Froyen, G., Corbett, M., Vandewalle, J., Jarvela, I., Lawrence, O., Meldrum, C., Bauters, M., Govaerts, K., Vandeleur, L., Van Esch, H., et al. (2008). Submicroscopic duplications of the hydroxysteroid dehydrogenase *HSD17B10* and the E3 ubiquitin ligase *HUWE1* are associated with mental retardation. Am. J. Hum. Genet. *82*, 432–443.
16. Qiao, Y., Liu, X., Harvard, C., Hildebrand, M.J., Rajcan-Separovic, E., Holden, J.J., and Lewis, M.E. (2008). Autism-associated familial microdeletion of Xp11.22. Clin. Genet. *74*, 134–144.
17. Hagens, O., Dubos, A., Abidi, F., Barbi, G., Van Zutven, L., Hoeltzenbein, M., Tommerup, N., Moraine, C., Fryns, J.P., Chelly, J., et al. (2006). Disruptions of the novel *KIAA1202* gene are associated with X-linked mental retardation. Hum. Genet. *118*, 578–590.
18. Freude, K., Hoffmann, K., Jensen, L.R., Delatycki, M.B., des Portes, V., Moser, B., Hamel, B., van Bokhoven, H., Moraine, C., Fryns, J.P., et al. (2004). Mutations in the *FTSJ1* gene coding for a novel S-adenosylmethionine-binding protein cause nonsyndromic X-linked mental retardation. Am. J. Hum. Genet. *75*, 305–309.
19. Hamel, B.C., Wesseling, P., Renier, W.O., van den Helm, B., Ropers, H.H., Kremer, H., and Mariman, E.C. (1999). A new X linked neurodegenerative syndrome with mental retardation, blindness, convulsions, spasticity, mild hypomyelination, and early death maps to the pericentromeric region. J. Med. Genet. *36*, 140–143.
20. Kalscheuer, V.M., Freude, K., Musante, L., Jensen, L.R., Yntema, H.G., Gécz, J., Sefiani, A., Hoffmann, K., Moser, B., Haas, S., et al. (2003). Mutations in the polyglutamine binding protein 1 gene cause X-linked mental retardation. Nat. Genet. *35*, 313–315.
21. Okuda, T., Hattori, H., Takeuchi, S., Shimizu, J., Ueda, H., Palvimo, J.J., Kanazawa, I., Kawano, H., Nakagawa, M., and Okazawa, H. (2003). PQBP-1 transgenic mice show a late-onset motor neuron disease-like phenotype. Hum. Mol. Genet. *12*, 711–725.
22. Lenski, C., Abidi, F., Meindl, A., Gibson, A., Platzer, M., Frank Kooy, R., Lubs, H.A., Stevenson, R.E., Ramser, J., and Schwartz, C.E. (2004). Novel truncating mutations in the polyglutamine tract binding protein 1 gene (*PQBP1*) cause Renpenning syndrome and X-linked mental retardation in another family with microcephaly. Am. J. Hum. Genet. *74*, 777–780.
23. Lubs, H., Abidi, F.E., Echeverri, R., Holloway, L., Meindl, A., Stevenson, R.E., and Schwartz, C.E. (2006). Golabi-Ito-Hall syndrome results from a missense mutation in the WW domain of the *PQBP1* gene. J. Med. Genet. *43*, e30.
24. Dube, J.L., Wang, P., Elvin, J., Lyons, K.M., Celeste, A.J., and Matzuk, M.M. (1998). The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. Mol. Endocrinol. *12*, 1809–1817.
25. Galloway, S.M., McNatty, K.P., Cambridge, L.M., Laitinen, M.P., Juengel, J.L., Jokiranta, T.S., McLaren, R.J., Luiro, K., Dodds, K.G., Montgomery, G.W., et al. (2000). Mutations in an oocyte-derived growth factor gene (*BMP15*) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat. Genet. *25*, 279–283.
26. Di Pasquale, E., Beck-Peccoz, P., and Persani, L. (2004). Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (*BMP15*) gene. Am. J. Hum. Genet. *75*, 106–111.
27. Jensen, L.R., Amende, M., Gurok, U., Moser, B., Gimmel, V., Tzschach, A., Janecke, A.R., Tariverdian, G., Chelly, J., Fryns, J.P., et al. (2005). Mutations in the *JARID1C* gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. Am. J. Hum. Genet. *76*, 227–236.
28. Ofman, R., Ruiter, J.P., Feenstra, M., Duran, M., Poll-The, B.T., Zschocke, J., Ensenauer, R., Lehnert, W., Sass, J.O., Sperl, W., et al. (2003). 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency is caused by mutations in the *HADH2* gene. Am. J. Hum. Genet. *72*, 1300–1307.
29. Lenski, C., Kooy, R.F., Reyniers, E., Loessner, D., Wanders, R.J., Winnepenninckx, B., Hellebrand, H., Engert, S., Schwartz, C.E., Meindl, A., et al. (2007). The reduced expression of the *HADH2* protein causes X-linked mental retardation, choreoathetosis, and abnormal behavior. Am. J. Hum. Genet. *80*, 372–377.

30. Froyen, G., Bauters, M., Boyle, J., Van Esch, H., Govaerts, K., van Bokhoven, H., Ropers, H.H., Moraine, C., Chelly, J., Fryns, J.P., et al. (2007). Loss of *SLC38A5* and *FTSJ1* at Xp11.23 in three brothers with non-syndromic mental retardation due to a microdeletion in an unstable genomic region. Hum. Genet. *121*, 539–547.
31. Garcia, C.C., Blair, H.J., Seager, M., Coulthard, A., Tennant, S., Buddles, M., Curtis, A., and Goodship, J.A. (2004). Identification of a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. J. Med. Genet. *41*, 183–186.
32. Laumonnier, F., Holbert, S., Ronce, N., Faravelli, F., Lenzner, S., Schwartz, C.E., Lespinasse, J., Van Esch, H., Lacombe, D., Goizet, C., et al. (2005). Mutations in *PHF8* are associated with X linked mental retardation and cleft lip/cleft palate. J. Med. Genet. *42*, 780–786.
33. Koivisto, A.M., Ala-Mello, S., Lemmelä, S., Komu, H.A., Rautio, J., and Järvelä, I. (2007). Screening of mutations in the *PHF8* gene and identification of a novel mutation in a Finnish family with XLMR and cleft lip/cleft palate. Clin. Genet. *72*, 145–149.
34. Pasteris, N.G., Cadle, A., Logie, L.J., Porteous, M.E., Schwartz, C.E., Stevenson, R.E., Glover, T.W., Wilroy, R.S., and Gorski, J.L. (1994). Isolation and characterization of the faciogenital dysplasia (Aarskog-Scott syndrome) gene: a putative Rho/Rac guanine nucleotide exchange factor. Cell *79*, 669–678.
35. Lebel, R.R., May, M., Pouls, S., Lubs, H.A., Stevenson, R.E., and Schwartz, C.E. (2002). Non-syndromic X-linked mental retardation associated with a missense mutation (P312L) in the *FGD1* gene. Clin. Genet. *61*, 139–145.
36. Koolen, D.A., Sharp, A.J., Hurst, J.A., Firth, H.V., Knight, S.J., Goldenberg, A., Saugier-Veber, P., Pfundt, R., Vissers, L.E., Destrée, A., et al. (2008). Clinical and molecular delineation of the 17q21.31 microdeletion syndrome. J. Med. Genet. *45*, 710–720.
37. Lugtenberg, D., Kleefstra, T., Oudakker, A.R., Nillesen, W.M., Yntema, H.G., Tzschach, A., Raynaud, M., Rating, D., Journel, H., Chelly, J., et al. (2009). Structural variation in Xq28: *MECP2* duplications in 1% of patients with unexplained XLMR and in 2% of male patients with severe encephalopathy. Eur. J. Hum. Genet. *17*, 444–453.
38. Schwartz, S., Zhang, Z., Frazer, K.A., Smit, A., Riemer, C., Bouck, J., Gibbs, R., Hardison, R., and Miller, W. (2000). PipMaker: A web server for aligning two genomic DNA sequences. Genome Res. *10*, 577–586.