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Mutations in the polyglutamine binding protein 1 gene cause X-linked mental retardation

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We found mutations in the gene *PQBP1* in 5 of 29 families with nonsyndromic (MRX) and syndromic (MRXS) forms of X-linked mental retardation (XLMR). Clinical features in affected males include mental retardation, microcephaly, short stature, spastic paraplegia and midline defects. PQBP1 has previously been implicated in the pathogenesis of polyglutamine expansion diseases. Our findings link this gene to XLMR and shed more light on the pathogenesis of this common disorder.

XLMR is a prominent unsolved problem in clinical genetics. Based on the distribution of linkage intervals in 125 unrelated families, we recently showed that roughly one-third of all mutations underlying MRX are clustered on proximal Xp[1]. This observation prompted us to search for mutations in families with linkage intervals overlapping this region.

In 5 of 29 families studied, we detected mutations in *PQBP1* that cause frameshifts in the fourth coding exon (**Supplementary Methods** online), which contains a stretch of six AG dinucleotides in the DR/ER repeat (**Fig. 1** and **Supplementary Fig. 1** online). In two families (family N9 (not previously reported) and family SHS with Sutherland-Haan syndrome (MRXS3; ref. 2)), all affected males carry an extra AG dinucleotide (3898_3899dupAG), whereas in two others (family N45 (not previously reported) and family MRX55; ref. 3), two AG dinucleotides are deleted (3896_3899delAGAG). A single AG unit (3898_3899delAG) is deleted in affected males of family N40 (ref. 4). In all families, these mutations segregated with the disease and were present in all obligate heterozygotes that we tested. Except for one, all obligate heterozygotes that we examined have random X-chromosome inactivation (data not shown) and have IQs in the normal range. Apart from a single-nucleotide polymorphism (IVS2–3C→T), we found no sequence variation in control X chromosomes.

The duplication observed in families N9 and SHS and the deletion found in families N45 and MRX55 give rise to almost the same frameshift (**Fig. 1b**). Still, there is considerable inter- and intrafamilial phenotypic variation (**Supplementary Table 1** online). For example, males with SHS show mental retardation, short stature, microcephaly, brachycephaly, spastic diplegia, small testes and anal stenosis or atresia, whereas there is no spastic diplegia or small testes in family N9, with an identical mutation. In both families the disease is not progressive. In family MRX55, in whom the predicted mutant protein differs by only two amino acids, affected individuals are moderately retarded but have no other clinical signs, except for a somewhat smaller body size in one individual (height was 159 cm, ≥2 s.d. below normal at the age of 20 years). In contrast, in addition to mental retardation, all affected individuals in family N45 have microcephaly, one has anal atresia and another has complete situs inversus. Some of this clinical variability may be due to differences in the genetic background. Family MRX55 is from Morocco, families N9 and N45 are from the Netherlands and family SHS has English ancestry.

In family N40, all affected males have congenital heart defects in addition to severe mental retardation, microcephaly, spasticity, short stature, cleft or highly arched palate and other craniofacial abnormalities[4]. The mother of two of the affected individuals has a corrected atrial septal defect. Facial features coarsened with age.

Several *PQBP1* splice variants have been described[5]. All but one very rare variant contain exon 4, which is mutated in the five families. The three different types of mutations cause frameshifts that lead to premature stop codons, resulting in truncated PQBP1 proteins that lack several important domains.

The particularly severe clinical phenotype seen in family N40 may be due to the fact that the C-terminal end of the predicted truncated protein is entirely different from that of the mutant proteins in the other families (**Fig. 1b**) and may give rise to aberrant protein-protein interactions.

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Published online 23 November 2003; doi:10.1038/ng1264

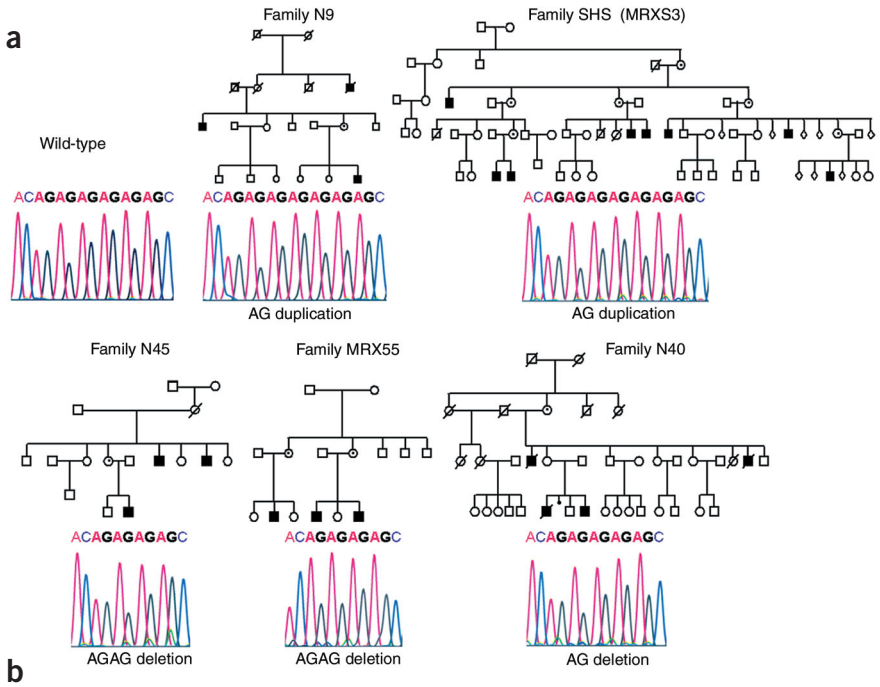

Figure 1 Mutations in *PQBP1* affecting the AG stretch of the DR/ER repeat. (**a**) Sequence chromatogram from one affected individual is shown together with the pedigree of the respective family. (**b**) Partial nucleotide and amino acid sequences of wild-type and truncated mutant PQBP1 proteins. The wild-type and mutant AG stretches are shown in red letters. Mutant amino acids are in italics and underlined in family N40. Lengths of wild-type (WT) and truncated mutant proteins are indicated.

In lymphoblastoid cell lines of all four affected individuals tested, the concentration of *PQBP1* transcripts was markedly reduced, with almost undetectable expression in family N40 (**Supplementary Fig. 2** online). This can be explained by nonsense-mediated mRNA decay. Given the location of the mutations relative to the terminal exon 6, all *PQBP1* splice variants except *PQBP1* isoform b, which contains intron 4 and 5, probably undergo degradation mediated by nonsense-mediated mRNA decay.

To study the effects of these mutations at the protein level, we carried out cell-transfection experiments (**Supplementary Methods** online). Full-length PQBP1 protein localized predominantly to the nucleus, consistent with reports from the literature[6–8], but the mutant proteins were dispersed throughout the cell (**Fig. 2**). Taken together, the observations of lowered *PQBP1* mRNA levels and dispersion of the truncated PQBP1 proteins throughout the cell suggest that interaction of PQBP1 with nuclear target proteins is compromised.

Premature stop codons in the DR/ER repeat of PQBP1 lead to deletion of the C2 domain. This domain has been shown to bind to U5-15kDa[9], a component of the nuclear pre-mRNA splicing machinery[10]. Loss of the C2 domain and its interactions may contribute to both the cellular displacement of the mutant proteins and the pathogenesis of MRXS and MRX.

PQBP1 has a WW domain, which interacts with the large subunit of RNA polymerase II (Pol II) in a phosphorylation-dependent manner[11] and with NpwBP (also called WBP11 and SNP70; refs. 12,13). NpwBP colocalizes with mRNA splicing factors and may thus have a role in the regulation of pre-mRNA processing[12,13]. But repeated experiments did not find a strong impact of mutant forms of PQBP1 on Pol II phosphorylation (data not shown). This may be explained by tight regulation of Pol II in cellular growth and differentiation. Still, it is conceivable that even subtle changes in Pol II phosphorylation may have crucial effects on the development and function of the brain. The PRD (DR/ER repeat) stretch is also responsible for binding to Brain-2

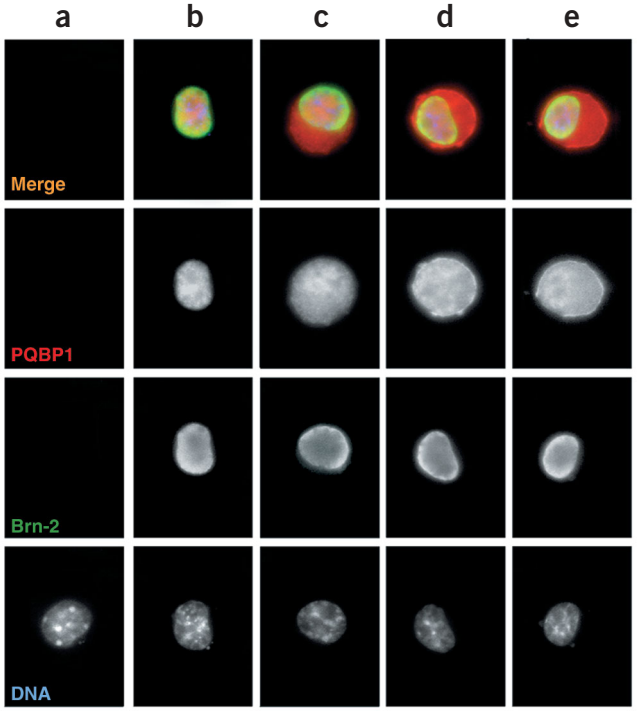

Figure 2 Subcellular localization of recombinant PQBP1 (red), Brn-2 (green) and of truncated mutant PQBP1 (red) proteins in Neuro2A cells. Top row, Merged images of DNA (blue) and protein channels; second row, Myc-tagged PQBP-1; third row, Brn-2; bottom row, DNA staining. (**a**) Cell transfected with empty vector. (**b**) Cell transfected with full-length PQBP1 and Brn-2. Both proteins localized to the nucleus. (**c**) Cell expressing Brn-2 and mutant PQBP1 protein with an AG duplication. (**d**) Cell expressing Brn-2 and truncated mutant PQBP1 with an AGAG deletion. (**e**) Cell expressing Brn-2 and truncated mutant PQBP1 with an AG deletion. All PQBP1 mutant proteins have lost their predominant nuclear localization, whereas Brn-2 is still restricted to the nucleus.

(Brn-2; ref. 7). Coexpression of mutant PQBP1 and Brn-2 proteins showed the expected dispersed signal for truncated PQBP1 (**Fig. 2c–e**), whereas Brn-2 still localized to the nucleus (**Fig. 2b–e**), suggesting that cytoplasmic mutant PQBP1 may no longer interact with this transcription factor.

In all but one of the genes known to be associated with MRX, mutations are rare[14,15]. We found mutations in *PQBP1* in 5 of 29 families, including one family with MRX[3] and two families with different forms of MRXS[2,4]. PQBP1 has been implicated previously in a variety of neurodegenerative disorders due to its direct interaction with huntingtin, ataxin and other relevant polyglutamine-containing proteins. Our observations indicate that PQBP1 is also a key player in the etiology of XLMR. Ongoing studies aim to investigate the causes of the notable inter- and intrafamilial variability and to elucidate the functional role of PQBP1 in XLMR and other neurological disorders.

GenBank accession numbers. *PQBP1* mRNA including untranslated exon 1b, NM_005710; *PQBP1* mRNA including untranslated exon 1a, AB016533; *PQBP1* complete coding region, AJ242829; PQBP1 protein, CAB44309; genomic clone including *PQBP1* untranslated exon 1, AF207550.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

We thank I. van der Burgt, C. de Die-Smulders, R. Robledo and M. Siniscalco for providing blood from affected individuals; M. Vingron for discussion; R. Reinhardt for support with mutation search; R. McEvilly for the rat Brn-2 cDNA clone; H. Madle and S. Freier for establishing lymphoblastoid cell lines; P. Nierle for help in the initial stage of the mutation screening; and M. Klein, V. Suckow, Z. Kijas, B. Lipkowitz, K. Lower, O. Mckenzie, S. Kübart and M. Mangelsdorf for technical assistance. This work was supported by a grant from the Australian National Health and Medical Research Council to J.G., by the Deutsches Humangenom-Programm, by the Nationales Genomforschungsnetzwerk and by the 5th EU Framework.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 26 September; accepted 31 October 2003
Published online at http://www.nature.com/naturegenetics/

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Hypomethylation of D4Z4 in 4q-linked and non-4q-linked facioscapulohumeral muscular dystrophy

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The autosomal dominant myopathy facioscapulohumeral muscular dystrophy (FSHD1, OMIM 158900) is caused by contraction of the D4Z4 repeat array on 4qter. We show that this contraction causes marked hypomethylation of the contracted D4Z4 allele in individuals with FSHD1. Individuals with phenotypic FSHD1, who are clinically identical to FSHD1 but have an unaltered D4Z4, also have hypomethylation of D4Z4. These results strongly suggest that hypomethylation of D4Z4 is a key event in the cascade of epigenetic events causing FSHD1.

Epigenetic changes of DNA have been investigated as causes of monogenic disorders, tumorigenesis and aging and are suspected to be important for common multifactorial diseases also. Monogenic diseases associated with epigenetic phenomena are caused either by mutations in chromatin remodeling factors[1] or by position effect variegation mechanisms mostly involving regulatory elements[2]. An epigenetic role is also prominent in imprinting disorders[3].

FSHD1, which progressively and variably affects muscles of the face, shoulder and upper arm[4], has such a suspected epigenetic etiology. We previously mapped FSHD1 to 4qter and showed that it is caused by contraction of the polymorphic D4Z4 repeat array[5,6] (**Supplementary Fig. 1** online). In healthy individuals, D4Z4 consists of 11–150 units on both chromosomes, whereas individuals with FSHD1 carry one 4q array of 1–10 units. About 5% of individuals with FSHD1 do not have a contraction of D4Z4 and are considered to have phenotypic FSHD1 (ref. 7).

Several observations suggest an epigenetic etiology in FSHD1. First, the subtelomere of chromosome 10q contains a nearly identical polymorphic D4Z4 repeat, but size reductions of this repeat are not pathogenic. Second, exchanges between the homologous repeat arrays on 4q and 10q are frequently observed, but these exchanged repeats are only pathogenic when the contracted form resides on chromosome four[8]. Finally, D4Z4 contractions on 4qter are necessary but not sufficient to cause FSHD1, because the actual pathogenicity is only associated with one of two alleles (4qA) of the 15–20-kb 4qter moiety located immediately distal to D4Z4 (ref. 9).

The presentation of FSHD1 requires that there is a contracted D4Z4 repeat located on chromosome 4q and in *cis* with only one of

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Published online 23 November 2003; doi:10.1038/ng1262