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ARTICLE

Two novel members of the interleukin-1 receptor gene family, one deleted in Xp22.1–Xp21.3 mental retardation

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X-linked mental retardation is estimated to affect approximately 1 in 600 males. Although numerous genes responsible for syndromic mental retardation have been identified, the study of non-syndromic mental retardation suffers from intrinsic issues of genetic heterogeneity. During the investigation of three brothers with a contiguous gene deletion syndrome of Becker muscular dystrophy, glycerol kinase deficiency, congenital adrenal hypoplasia, and mental retardation, we found their dystrophin gene to be fused tail-to-tail with a gene encoding a novel member of the interleukin-1 receptor family, *IL1RAPL1*. This gene has a close relative in Xq22, which we call *IL1RAPL2*. Both *IL1RAPL1* and *IL1RAPL2* have novel C-terminal sequences not present in other related proteins, and are encoded by very large genes. The 1.8-megabase deletion in these patients removes not only the last exon of the dystrophin gene, the entire glycerol kinase and *DAX-1* genes, and the *MAGE-B* gene cluster, but also three exons encoding the intracellular signalling domain of *IL1RAPL1*. The literature contains multiple reports of patients with non-syndromic mental retardation in association with an Xp22.1–Xp21.3 microdeletion of a marker which lies within the *IL1RAPL1* gene. The gene is also wholly or partially deleted in patients with mental retardation as part of a contiguous deletion syndrome. We suggest that *IL1RAPL1*, and perhaps *IL1RAPL2*, are strong candidates for X-linked non-syndromic mental retardation loci, and that molecules resembling IL-1 and IL-18 play a role in the development or function of the central nervous system. *European Journal of Human Genetics* (2000) **8**, 87–94.

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Introduction

Contiguous gene deletion syndromes result from the removal of multiple genes by single continuous deletion events. They usually manifest as strictly overlapping combinations of symptoms which reflect the linear order of deleted genes. A well-characterised region where this happens is Xp22.1–Xp21.1. The disorder Duchenne muscular dystrophy (DMD) results from mutations in the 2.4-Mb dystrophin gene. Dystrophin gene deletions which extend telomerically (Xp22.1–p21.3, for example[1,2]) are associated with glycerol kinase deficiency (GKD), adrenal hypoplasia congenita (AHC), and non-specific mental retardation (MR). The genes responsible for GKD (encoding the enzyme glycerol kinase) and AHC (encoding DAX-1, an orphan nuclear receptor) have been identified,[3–6] largely as a result of mapping data derived from these contiguous gene deletion patients. The gene responsible for MR in these patients, however, has eluded identification.

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The issue of MR in Xp21 deletion syndrome is complicated by the fact that it can arise directly as a consequence of mutations in the dystrophin gene, being a recognised, if variable, feature of isolated DMD. However, the literature contains examples where MR due to Xp22.1–Xp21.3 deletion is present either without skeletal myopathy (with AHC,[7] in isolation[8–10]) or with the milder Becker muscular dystrophy (BMD), which is rarely associated with appreciable MR (patient 4482[11]). In addition, linkage studies[12–14] have localised a gene for non-specific X-linked MR to Xp22.1–Xp21.2. There is therefore clearly at least one gene in the Xp22.1–Xp21.3 region of which the disruption can cause (usually mild to moderate) non-syndromic MR.

The mammalian interleukin-1 receptor (IL1R) family currently comprises some six related cell-surface proteins. The function of four of these proteins is known; they represent pairs of receptors and receptor accessory proteins for the cytokines IL-1 and IL-18. IL-1 is an important pro-inflammatory cytokine which has wide-ranging effects in many cell types, and invokes both beneficial and harmful responses to immune challenge, while IL-18 collaborates with other cytokines (e.g. IL-12) to stimulate production of interferon-gamma.

We here describe two novel members of the IL1R family, both encoded by genes on the human X-chromosome. One of these is wholly or partially deleted in multiple cases of MR, whether in isolation or as part of a contiguous gene syndrome. We argue that loss of *IL1RAPL1* function is likely to contribute to the mental impairment in these cases.

Materials and methods

Patients

The three brothers in family 4482 are currently aged 12, 14, and 17 years. All three have a mild myopathy with a clear Becker-type course, glycerol kinase deficiency and adrenal hypoplasia. They all had bilateral undescended testes which have been surgically corrected. The eldest boy developed epilepsy at the age of 12; this was treated successfully for 2 years but seizures have recently recurred. The two younger boys have short stature, with heights below the third centile (the eldest is on the tenth centile). The boys have mild mental retardation – multiple IQ tests performed on the two younger boys yield quotients of 66–84 (mean 74) and 64–82 (mean 72), respectively; the eldest boy is expected to perform similarly.

Rapid amplification of cDNA ends (RACE) of dystrophin transcript

Muscle biopsy material from the youngest brother was made available by the Neuromuscular Unit at the Hammersmith Hospital. The tissue had been stored frozen in the mounting medium OCT. The tissue was separated from the medium by dissection and total RNA prepared by the method of Chomczynski and Sacchi.[15] This and a control muscle RNA sample were reverse transcribed using an oligo-d(T) adapter primer (5'-gactcgagtcgacatcgatttttttttttttttt-3') and amplified using a primer in exon 76 of the dystrophin gene (5'-ctctacagaggtccgacagca-3') together with the adapter primer (5'-gactcgagtcgacatcg-3') in a 3'-RACE reaction.[16] This primary RACE product was purified, digested with *Cla*I, which has a recognition site in the adapter sequence, and was ligated to a vectorette.[17] This vectorette library was then amplified using a nested dystrophin-specific primer (5'-gtcagcctatgctgctccgag-3') and the vectorette primer (5'-cgaatcgtaaccgttcgtacgagaatcgct-3'). The resulting products were purified and sequenced.

Bioinformatics

Segments of continuous sequence from clone 293I18 (accession number AC005748) were input into GRAIL; this returned three regions (exons 8, 9 and part of exon 10) as highly predicted exons with similarity to members of the IL1R family. Further genomic clones containing *IL1RAPL1* exons were identified using the complete cDNA sequence to perform a BLAST search[18] of high-throughput genome sequence (HTGS) and non-redundant (NR) databases at the National Center for Biotechnology Information (NCBI), using default parameters. Genomic clones containing *IL1RAPL2* exons were identified using the translation of the *IL1RAPL1* open reading frame to perform a TBLASTN search of the same databases; in some cases, sequence corresponding to a single exon was needed to maximise the significance of similarity. The incomplete contig sequence and mapping data available from groups at the Sanger Centre and the Baylor College of Medicine Human Genome Sequencing Center (HGSC) permitted a substantial amount of *in silico* mapping. Alignment and phylogenetic analysis was performed using CLUSTAL (neighbour-joining option).

5'- and 3'-RACE of IL1RAPL1 and IL1RAPL2

Nested amplifications were performed using Marathon human brain cDNA (Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. Primers used were 5'-caccaaagtggatcctgaccagtgg-3' (outer) and 5'-gaaatcctacctgatatgcttg-3' (inner) for 3'RACE of *IL1RAPL1*, 5'-ttccagctcaaagatgctccagccc-3' (outer) and 5'-ttctaactacgtaatttggggtc-3' (inner) for 5'RACE of *IL1RAPL1*, 5'-gaatatgatgcctatctctcttac-3' (outer) and 5'-ctttagactgtgacaatcctg-3' (inner) for 3'RACE of *IL1RAPL2*, 5'-cactgactagcatgttatggag-3' (outer) and 5'-gaatatagtctggagttagcacg-3' (inner) for 5'RACE of *IL1RAPL2*. Products were cloned into T-tailed pBluescript II SK- (Stratagene, La Jolla, CA, USA) or pCR2.1-TOPO (Invitrogen, Groningen, The Netherlands) and sequenced using dRhodamine dye-terminator methodology (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 377 automated sequencer.

Sequence tagged site (STS) content analysis

Lymphocyte genomic DNA from patient 4482 and two control samples was tested for the presence of various STSs by

amplification using the following pairs of primers. Dystrophin gene: exon 78 (5'-cctttctgatatctctgcc-3' and 5'-gagctgcaagtggagaggtgac-3', 232 bp), exon 79 (5'-gttgtcttcacccagaaatgg-3' and 5'-catgactgatactaaggac-3', 185 bp). *IL1RAPL1* gene: 4482UTR (intron 5, 3.2 kb 5' of exon 6; 5'-cactgtcttacccacagcaagcc-3' and ggctcctgcacccatgtaagaag-3'; 172 bp), intron 7 (2 kb 3' of exon 7; 5'-ggcacacagccaagaaggggtc-3' and 5'-gatgggtgggatagatcattcc-3', 432 bp), exon 8 (5'-caaatgcaagctaccaaccaag-3' and 5'-gaatatacgagctgctgccattg-3', 529 bp) exons 9 and 10 (across intron 9; 5'-caccaaagtggatcctgaccagtgg-3' and 5'-ttccagctcaaagatgctccagccc-3', 656 bp). In addition, primers corresponding to the previously described markers DXS1025, DXS1020, and DXS1098 (sequences obtained from Genome DataBase) were used.

Results

Characterisation of the patient's dystrophin transcript

Patient 4482 and his two brothers have been previously described as having a mild Becker-type muscular dystrophy (BMD), GKD and AHC. They have been shown to have an undefined distal deletion of the dystrophin gene which continues in a telomeric direction (Figure 1a), presumably removing their *GK* and *DAX-1* genes, but sparing the distal marker C7 (DXS28).[1,11] The boys also have mild but concordant MR (see Materials and methods for details).

We initially set out to define their deletion more precisely in order to assess its presumptive effect on their dystrophin protein. STSs designed to recognise 3' exons of the dystrophin gene showed that only the last exon (79) was missing. We then performed 3'RACE on muscle RNA from patient 4482 using nested primers in dystrophin exon 76. Whilst control muscle RNA gave products containing an intact dystrophin open reading frame and cognate 3'UTR sequence, duplicate 3'RACE reactions from the patient's muscle RNA gave a 1-kb band whose sequence diverged from the published dystrophin cDNA sequence immediately after exon 78 (Figure 1b). The novel sequence, termed 4482UTR (accession number AF181286), confers upon the patient's dystrophin protein six novel C-terminal amino acids (ALCCHT in single-letter code) instead of the normal three (DTM). The issue of the possible cause of myopathy in these boys is a complex one, as the subtlety of the qualitative effects on the protein raises the possibility that the loss of the highly conserved 3'UTR may be more critical; these matters will be the subject of a separate report.

4482UTR lies within a novel IL1R-like gene

We assumed that 4482UTR lay beyond the patient's deletion and was either the normal 3' exon for a novel gene, or was a usually functionless sequence behaving as a cryptic 3' exon. A BLAST search of the HTGS database revealed a region identical to 4482UTR in the fragmentary sequence of PAC

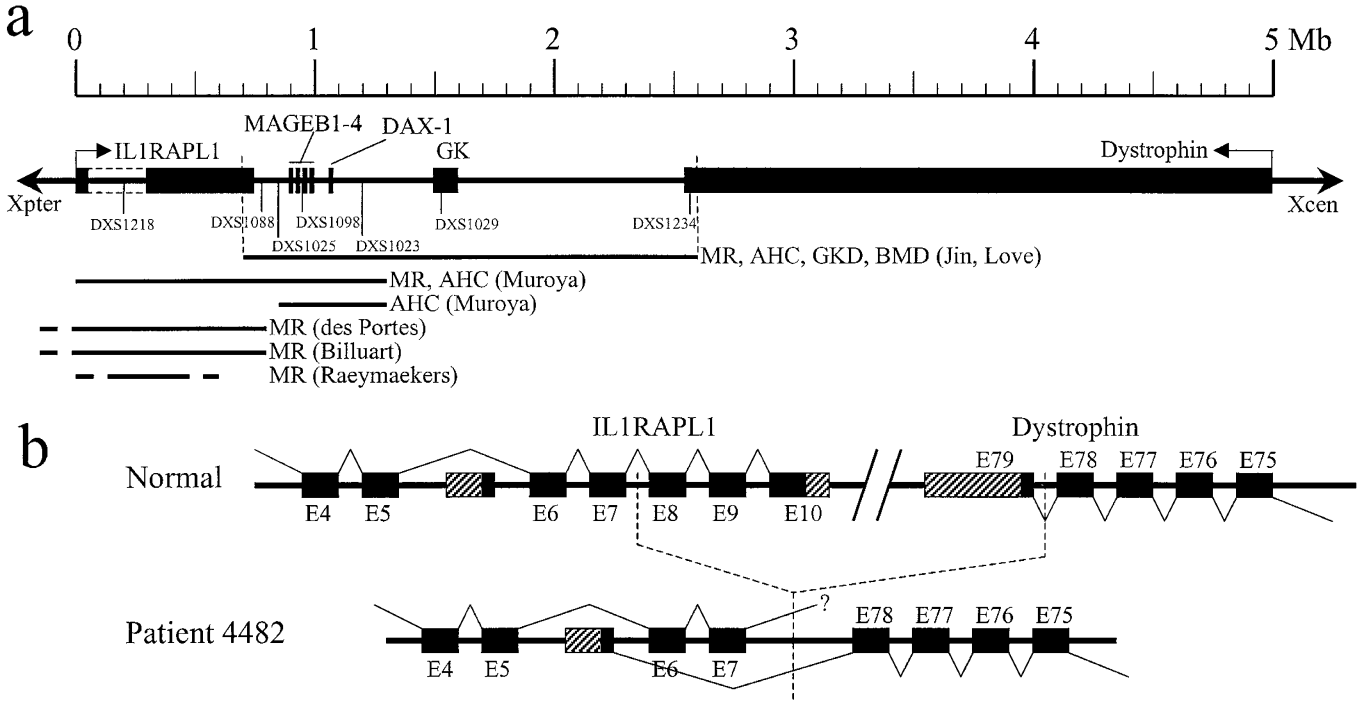

Figure 1 The nature and consequences of the deletion in patient 4482. (***a***) A map of about 5 Mb of Xp22.1–Xp21.1 region, showing the positions of known genes, some DXS markers, and the approximate extent of selected published deletions (see text). The map is based on data largely drawn from Ferrero et al[19] and the Baylor College HGSC website. (***b***) Diagram of the breakpoints in patient 4482, showing the transcriptional consequences of the deletion for the patient's dystrophin and IL1RAPL1 genes.

clone 293I18 (accession number AC005748; Muzny *et al*., unpublished direct submission to GenBank, 1998). This PAC has been mapped distal to the *DAX-1* gene but proximal to *DXS28*, placing it ideally with respect to the suspected extent of the deletion (in Xp22 bins 164–166).[19] The immediately adjacent genomic sequence contained a good consensus acceptor site that might mediate efficient splicing to exon 78 of the dystrophin gene in the patient.

As 5' and 3' RACE using normal human brain cDNA and primers in 4482UTR were unsuccessful, we tried a bioinformatic approach to assigning wild-type 4482UTR to a gene. The separate fragments of clone 293I18 sequence were analysed for potential exons using GRAIL. Amongst a number of weaker results, this yielded strong predictions for three sequences which were distantly related to members of the receptor family for cytokines interleukin-1 and interleukin-18. Indeed, when the three putative exons were arranged in order, they would together encode the entire transmembrane and intracellular domains of such a receptor, which we call *IL1RAPL1*.

Both 5' and 3' RACE from these exons yielded products from human brain cDNA, which were then cloned and sequenced (accession number AF181284). The products overlap and confirm that these exons are spliced into a single transcript in human brain. The transcript is not represented in expressed sequence tag (EST) databases, suggesting a low level or highly tissue-specific expression pattern. The 697-amino acid *IL1RAPL1* open reading frame shows modest but continuous similarity to members of the IL1R family (Figure 2).

BLAST searches of genomic databases using the complete *IL1RAPL1* cDNA sequence revealed that the open reading frame is probably encoded by 10 exons, eight of which lie in already sequenced genomic clones. Exons 1 and 5 were not recognised. Exons 6–10 all lie within clone 293I18, with 4482UTR mapping to the antisense strand of intron 5, 3 kb 5' of exon 6. The map positions of the genomic clones which contain exons 2–10 confirm that the *IL1RAPL1* gene is transcribed in a telomere-to-centromere direction and that these exons alone span a genomic region well in excess of 400 kb (Figure 3).

Refinement of the distal end of the deletion

We used STSs based on parts of the *IL1RAPL1* gene and three published proximal loci (see Materials and methods) to compare the STS content of the patient's genomic DNA with that of two control individuals. The results showed that the patient was deleted for *IL1RAPL1* exons 8, 9, and 10, and all three proximal markers. The distal junction of the deletion therefore lies in intron 7 of the *IL1RAPL1* gene, at some point at least 2 kb 3' of exon 7.

Thus in these brothers the *IL1RAPL1* and dystrophin genes are fused at introns 7 and 78 respectively, with the dystrophin transcript using an intronic sequence from the antisense strand of the newly apposed gene as its final exon (Figure 1b). Any residual transcript of their *IL1RAPL1* gene would encode only the extracellular domains of the receptor. We estimate the total size of the deletion to be about 1.8 Mb (Figure 1). Although we attempted 3'RACE of the *IL1RAPL1* transcript in order to establish the structure of any residual truncated transcript in the patients, this did not yield products from either patient or control lymphocyte RNA; normal brain tissue remains the only source from which 3'RACE of *IL1RAPL1* has been possible.

An IL1RAPL1-related gene on Xq

During BLAST searches of genomic databases using the *IL1RAPL1* cDNA sequence (see above) it became apparent that a close homologue of this gene lay on the long arm of the X-chromosome. We called this *IL1RAPL2*. In order to test whether this too was transcribed and correctly spliced, we performed 5' and 3'RACE from human brain cDNA using primers designed from putative *IL1RAPL2* exons. Sequence analysis of the overlapping products (accession number AF181285) confirmed that *IL1RAPL2* appears to represent a *bona fide* gene, encoding a protein with a continuous high homology to *IL1RAPL1* (Figure 2) and somewhat lower homology to other members of the IL1R family (see below). Strangely, however, 5'RACE never yielded a convincing first exon; sequence 5' to exon 2 varied between clones and often contained sequence of apparently mitochondrial origin. Bioinformatic methods also failed to identify nearby sequences with homology to the beginning of the *IL1RAPL1* coding sequence; as a result we do not know the N-terminal 30 amino acids of the predicted protein. All other exons are represented in finished genomic sequence; the gene has an identical exon/intron structure to *IL1RAPL1*, and similarly spans a region in excess of 400 kb (Figure 3). It lies in Xq22, between the genes for proteolipid protein (PLP) and thyroxine-binding globulin (TBG),[20] and is transcribed in a centromere-to-telomere direction.

Comparison of IL1RAPL1 and IL1RAPL2 with known IL1R family members

To our knowledge there are six previously characterized members of the IL1R family, plus an additional receptor which lacks a cytoplasmic domain. The family comprises the receptor for IL-1 (IL1R)[21] and its accessory protein (IL1RAP),[22] the receptor for IL-18 (IL18R, previously called IL1Rrp)[23] and its accessory protein (IL18RAP),[24] and two orphan receptors, T1/ST2[25] and IL1Rrp2.[26] These all share a common domain structure of tree N-terminal extracellular immunoglobulin-like domains, a transmembrane domain, and a C-terminal intracellular signalling domain (Toll/IL1R-related domain, TIR) which is related to the Toll family of receptors.[27] At least four of the genes map to a cluster in human 2q12,[28] although the *IL1RAP* gene maps to 3q28.

While *IL1RAPL1* and *IL1RAP2* share the overall domain structure, they have in addition a 120-amino acid C-terminal region which does not bear significant similarity to any other

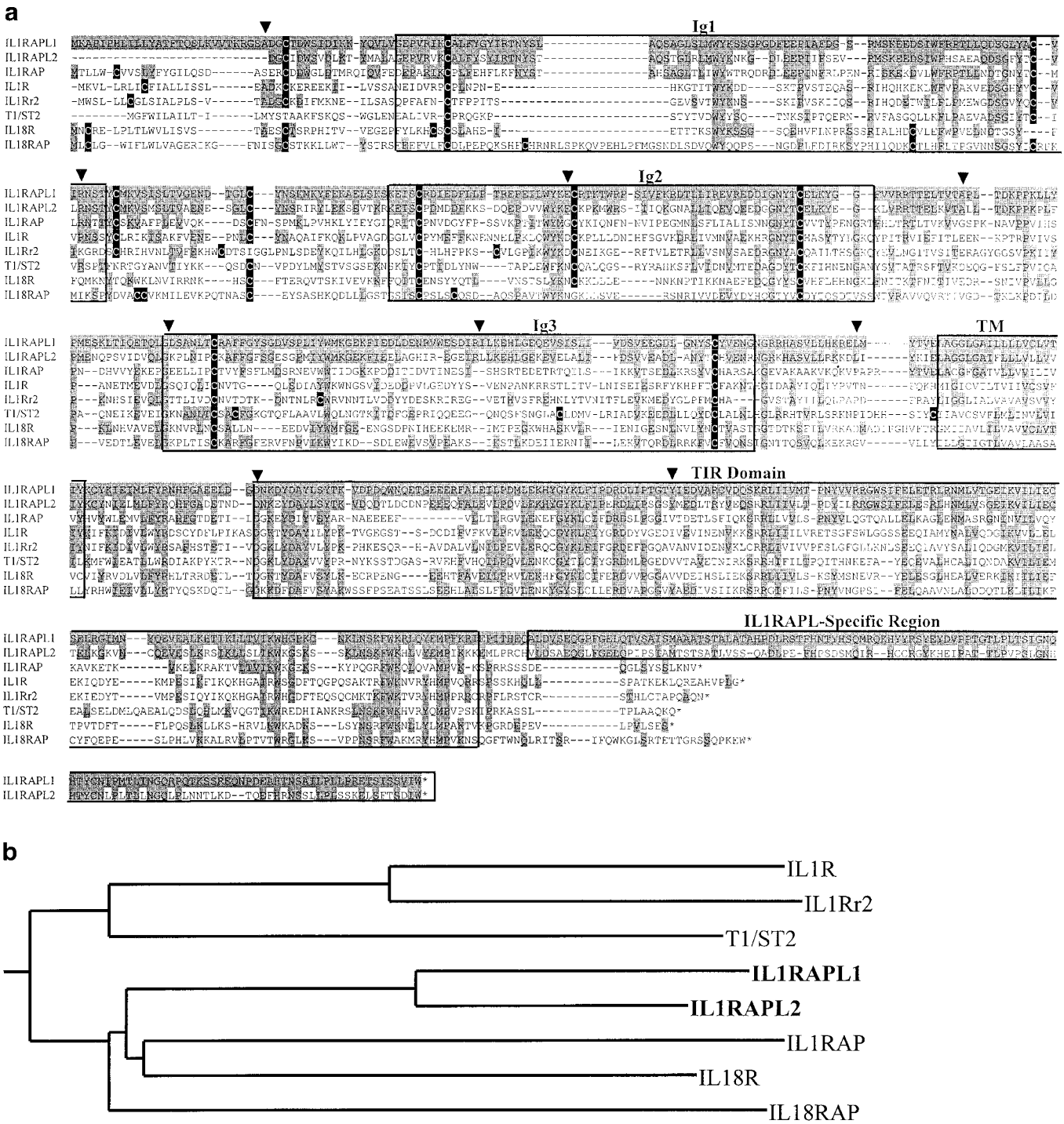

Figure 2 Relationship of IL1RAPL1 and IL1RAPL2 to known related proteins. (***a***) Alignment of the two proteins with other known members of the family. Residues identical to IL1RAPL1 are shaded. Extracellular cysteines are shown white on black. Domains are shown as boxes (Ig domains as defined by the IL1R crystal structure by Schreuder et al;[41] TIR domain as delineated by Rock et al.[27] Triangles represent known exon boundaries in both IL1RAPL1 and IL1RAPL2. (***b***) A phylogenetic tree of the receptors compared in (***a***). An alignment of the TIR domain was used to generate a neighbour-joining tree. Accession numbers: IL1RAPL1, AF181284; IL1RAPL2, AF181285; IL1RAP, AF029213; IL1R, X16896; IL1Rr2, U49065; T1/ST2, AB012701; IL18R, NM03855; IL18RAP, AF077346.

protein in the database. Phylogenetic analysis shows that the tree has extremely deep branches, with most family members being more-or-less equally related to each other (20–40% identity; Figure 2b). *IL1RAPL1* and *IL1RAPL2* are more similar to each other (65% identity) than to any of the other proteins. IL1RAP is marginally the next most closely related receptor (40% identity), with a particularly high degree of similarity (50% identity) in the otherwise poorly conserved Ig1 domain.

Discussion

IL1RAPL1 and mental retardation

We have described the disruption of *IL1RAPL1* in three related patients who have large deletions. These patients have a highly concordant form of mild MR, so the question arises as to whether the mental impairment is in any way attributable to the loss of *IL1RAPL1* function. Although MR is found in approximately one-third of cases of DMD, its occurrence in patients suffering from the milder BMD is not substantially higher than in the general population. It seems to us very unlikely that the mental impairment in these boys, however mild, is due to the subtle defect in their dystrophin protein. This said, their deletion removes the *GK* gene, the *DAX-1* gene, the entire MAGE-B cluster[29] (which maps between *DAX-1* and *IL1RAPL1*), and a large amount of additional DNA which may harbour as yet unrecognised genes. In order to explore the role of *IL1RAPL1*, we turned to the literature to examine its status in reported deletion cases.

The availability of the sequence data enabled us to place certain commonly used markers with respect to the two genes (Figure 3); this enabled us to position *IL1RAPL1* on previously described deletion maps and to judge its intactness in each case. Particularly useful was the finding that the popular polymorphic marker DXS1218 must lie within the *IL1RAPL1* gene.

Many patients with the classic Xp21 deletion syndrome (DMD, GKD, AHC, MR) have deletions whose distal ends extend as far as the marker C7 (DXS28), thereby probably removing the entire *IL1RAPL1* gene, but also have a severely disrupted dystrophin gene. The occurrence of MR in one-third of pure DMD patients means that we cannot consider these individuals. However, the literature contains a number of critically informative cases[7–10] without DMD, whose deletions are depicted in Figure 1a. In each case *IL1RAPL1* is the only known gene whose deletion status correlates with MR.

Although the number of apparent X-linked non-syndromic MR loci has now reached 59, it has been estimated from the residual overlaps that a mere 10 genes could account for all these loci.[30] Many of these loci (MRX2, 13, 21, 29, 32, 33, 36, 38, 43, 53, 54, 59) span the interval containing *IL1RAPL1*. Three studies[12–14] give particularly tight linkage of mild-to-moderate MR to the *IL1RAPL1* region in three families (maximum lod scores of 3.3 and 4.6 with the intragenic marker DXS1218 have been reported[12,14]).

We believe the cumulative case for the involvement of *IL1RAPL1* in deletion and non-deletion cases of MR to be strong. If one were to make the extreme (but not implausible) assumption that there is but one *MR* gene in the Xp22.1–Xp21.3 region, then the only gene which is known to lie within the minimum critical region (for both deletions and linked loci) is *IL1RAPL1* (see the summary in Figure 3 of the report by des Portes *et al*[10]). Furthermore, in Billuart *et al*[8] and des Portes *et al*[10] the deletions are estimated as spanning approximately 1 Mb. *IL1RAPL1* appears to be encoded by a very large gene; we have accounted for more than 400 kb, a distance which excludes not only two gaps of undefined size, but also the first intron, which is in many genes the largest.

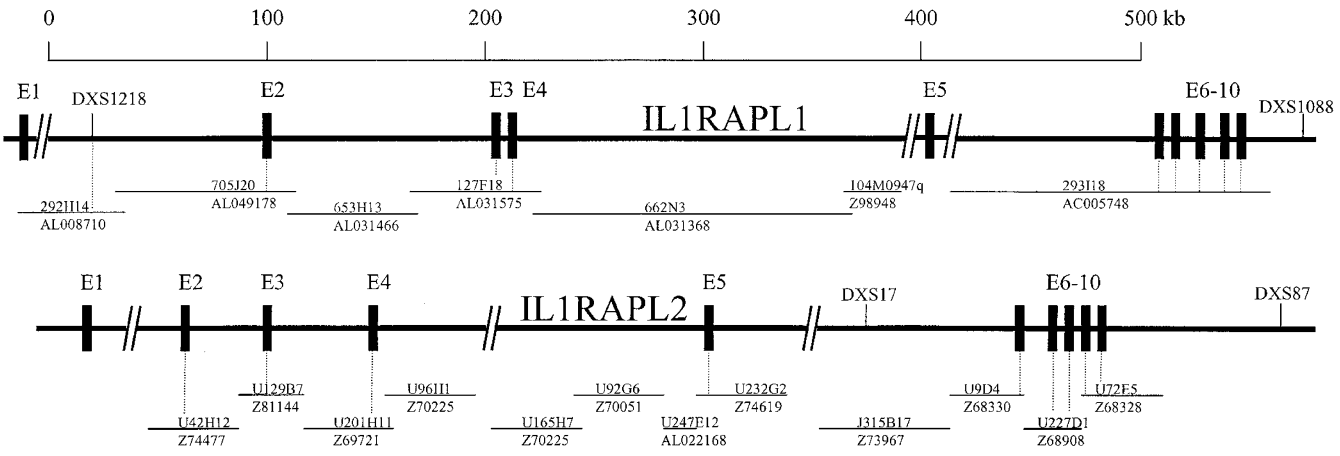

Figure 3 Gene structures of IL1RAPL1 and IL1RAPL2. Approximate maps of the two genes, generated from sequence and mapping data available from the Sanger Centre and Baylor College HGSC. IL1RAPL1 exons 1 and 5, and IL1RAPL2 exon 1 are not represented in the genome databases. Lines under maps represent the extent of sequenced clones, together with their name and accession number (correct and complete as of April 1999). The positions of four DXS markers are shown.

The *IL1RAPL1* gene therefore occupies at least half of this interval and may account for much of the remainder.

IL1RAPL2 and mental retardation

If *IL1RAPL1* is responsible for a subset of Xp-linked cases of MR, then suspicion must also fall on its closest relative, *IL1RAPL2*. We localise this to a region of Xp22 between the genes for PLP and TBG;[20] indeed, given the large size of the *IL1RAPL2* gene, it may occupy much of the approximately 1 Mb distance between these genes. Although no deletion syndromes are recognised in this area, a number of MR loci have been linked to Xq22 (although these often fail to exclude more distal regions). An interesting candidate disorder is the unusual Xq22-linked family with epilepsy and MR;[31] although this particular family shows X-linked dominant inheritance with male sparing, it is striking that one member of the family presented here and all six affected members of the MRX38 Xp21-linked family[13] also have a history of seizures.

Aetiology of mental retardation

MR affects about 1 in 30 of the population, with the excess of male cases reflecting a contribution by X-linked disorders of about 5% (1 in 600 of the population). To our knowledge, four genes have been previously implicated in non-syndromic X-linked MR. Three of these – *PAK3*,[32] *GDI1*[33] and *oligophrenin-1*[34] – are involved in the regulation of the low molecular weight GTPases rho and rab. The fourth, *FMR2*[35,36] is likely to be a transcriptional regulator.

Given that *IL1RAPL1* is clearly embedded in a family of proteins with well-established and closely related functions, certain predictions about its role can be made with confidence. None of these entails a clear functional connection between it and the four proteins mentioned above. *IL1RAPL1* is highly likely to be a cell-surface receptor or receptor accessory protein for a cytokine-like molecule. The ligand may be a known member of the IL-1 family (IL-1α, IL-1β, IL-1 receptor antagonist, IL-18) or may be a novel molecule, likely to resemble IL-1 and IL-18 as much as they resemble each other. The means of signalling is almost certain to be identical to that used by the IL1R family and the more distally related Toll protein (see, for example, Auron,[37] for an overview). Although most currently known downstream effects of IL1R family signalling are immunological in nature, recently generated knock-out mice for components of the IL-1 signalling pathway unexpectedly show severe developmental defects,[38,39] and the related Toll protein in *Drosophila* is involved in dorsoventral axis determination. A role for *IL1RAPL1* in the development and/or maintenance of cognitive function is therefore not implausible.

In conclusion, we describe two novel members of the IL1R family, both encoded by X-linked genes. The Xp22.1–21.3 gene is disrupted in several individuals with MR, including the ones described here. This suggests that signalling molecules resembling IL-1 and IL-18 are likely to play a key role in brain function. Future work is needed to assess the nature of this role, and to establish the extent of the contribution of defects in *IL1RAPL1* and its Xp22-linked relative *IL1RAPL2* to the aetiology of non-syndromic X-linked MR.

During the preparation of this manuscript, Carrié *et al* have published a report[40] describing the *IL1RAPL1* gene, together with a nonsense mutation in a patient with isolated MR. They also show that the microdeletion described by Raeymaekers *et al*[9] is intragenic. These results confirm our findings.

Acknowledgements

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