REPORT

Mutations in the Gene Encoding the Sigma 2 Subunit of the Adaptor Protein 1 Complex, *AP1S2,* Cause X-Linked Mental Retardation

Patrick S. Tarpey, Claire Stevens, Jon Teague, Sarah Edkins, Sarah O'Meara, Tim Avis, Syd Barthorpe, Gemma Buck, Adam Butler, Jennifer Cole, Ed Dicks, Kristian Gray, Kelly Halliday, Rachel Harrison, Katy Hills, Jonathon Hinton, David Jones, Andrew Menzies, Tatiana Mironenko, Janet Perry, Keiran Raine, David Richardson, Rebecca Shepherd, Alexandra Small, Calli Tofts, Jennifer Varian, Sofie West, Sara Widaa, Andy Yates, Rachael Catford, Julia Butler, Uma Mallya, Jenny Moon, Ying Luo, Huw Dorkins, Deborah Thompson, Douglas F. Easton, Richard Wooster, Martin Bobrow, Nancy Carpenter, Richard J. Simensen, Charles E. Schwartz, Roger E. Stevenson, Gillian Turner, Michael Partington, Jozef Gecz, Michael R. Stratton, P. Andrew Futreal, and F. Lucy Raymond

In a systematic sequencing screen of the coding exons of the X chromosome in 250 families with X-linked mental retardation (XLMR), we identified two nonsense mutations and one consensus splice-site mutation in the *AP1S2* gene on Xp22 in three families. Affected individuals in these families showed mild-to-profound mental retardation. Other features included hypotonia early in life and delay in walking. *AP1S2* encodes an adaptin protein that constitutes part of the adaptor protein complex found at the cytoplasmic face of coated vesicles located at the Golgi complex. The complex mediates the recruitment of clathrin to the vesicle membrane. Aberrant endocytic processing through disruption of adaptor protein complexes is likely to result from the *AP1S2* mutations identified in the three XLMR-affected families, and such defects may plausibly cause abnormal synaptic development and function. *AP1S2* is the first reported XLMR gene that encodes a protein directly involved in the assembly of endocytic vesicles.

Mental retardation (MR) has a prevalence of ~2%–3% in developed populations and is more common in males than in females. Mutations in genes on the X chromosome are known to be a major cause of familial X-linked MR (XLMR), and the most common cause of XLMR is an expansion of an unstable trinucleotide repeat in the 5′ UTR of the *FMR1* gene in patients with fragile X syndrome.[1] Mutations in 60 other genes have also been implicated in XLMR.[2–4] However, there remains a substantial proportion of families with XLMR for which a clear causative mutation in a known gene has not yet been found. Each of the known XLMR genes accounts for a small proportion of families with nonsyndromic XLMR. The extent of the genetic heterogeneity associated with nonsyndromic XLMR compromises the search for additional underlying genes because linkage information from different families cannot be combined with confidence. As a consequence, many XLMR-affected families are mapped to large regions containing substantial numbers of potential disease genes.

As an alternative strategy to positional cloning and to obviate the requirement for small linkage intervals to which XLMR genes have been mapped, we have embarked on a systematic, high-throughput mutational screen of the X chromosome in 250 families with multiple cases of MR. Greater than 90% of these families could not be classified as having a known syndrome, although additional clinical abnormalities often accompanied the mental deficit. At least one individual from each of these 250 families was negative for an expansion of the trinucleotide repeat in the *FMR1* gene underlying fragile X syndrome. A screen of 60 other known syndromic and nonsyndromic XLMR genes was conducted by sequencing of the coding exons and splice junctions, and the 250 families are also negative for mutations in these. All families have been examined and are free of abnormalities revealed by conventional karyotype analysis at 500 G-banding resolution.
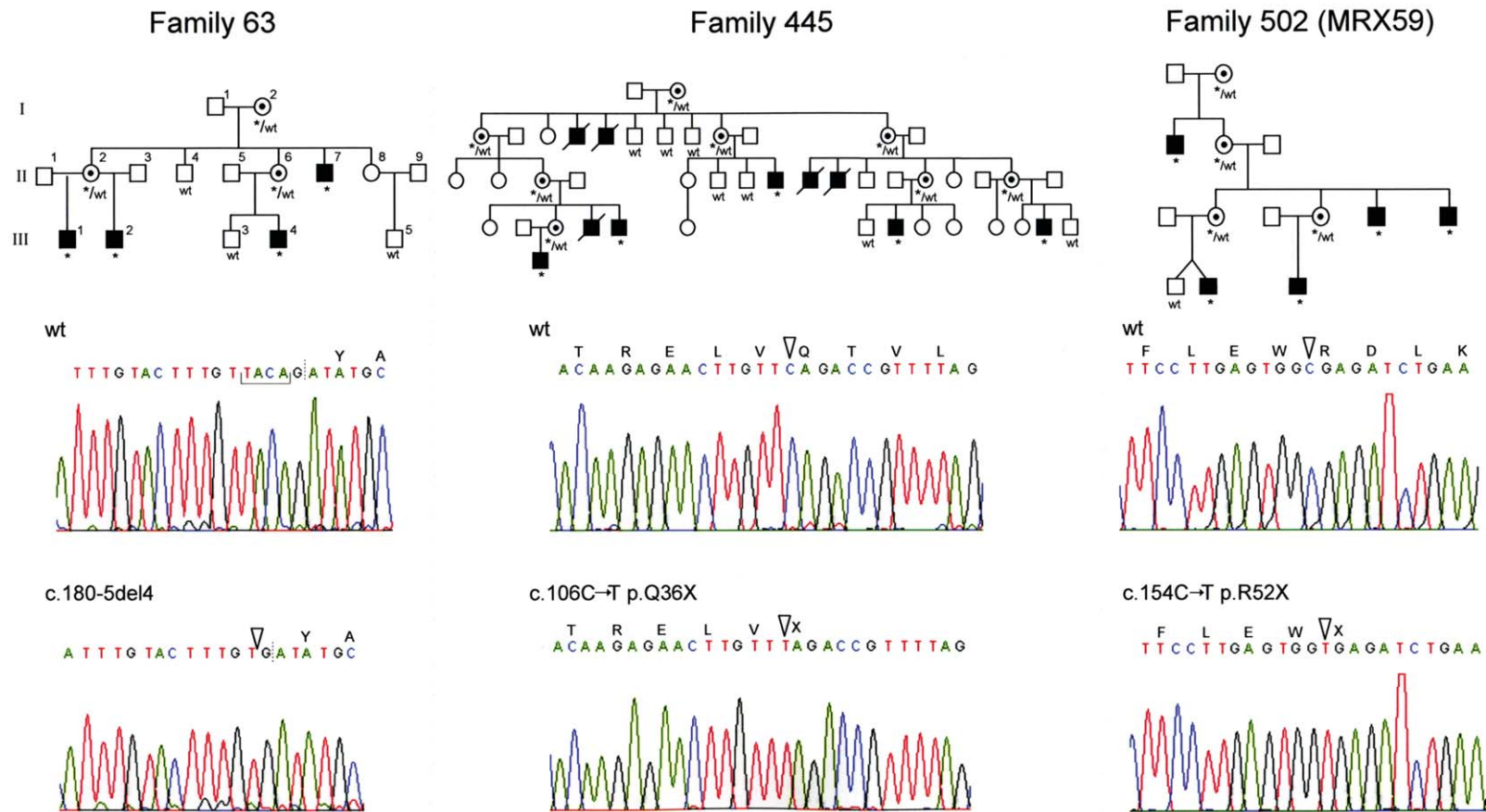
Our systematic mutational screen of the X chromosome is currently directed at the coding exons and splice junctions of 737 Vega annotated genes that are being examined for variants by bidirectional, PCR-based direct se-

Figure 1. Pedigrees of XLMR-affected families with mutations in *AP1S2*. Individuals with an asterisk carry the mutant allele in each family. Symbols with a dot represent obligate female carriers. Representative electropherograms of wild type (wt) and mutant sequences are shown below each pedigree.

quencing. Three deleterious mutations in *AP1S2* (MIM 603532; GenBank accession number NM_003916.3) on Xp22.2 were identified in the 250 families under investigation (figs. 1 and 2).

In family 445, previously described by Turner et al.,[5] there is a p.Q36X (c.106C→T) nonsense mutation. This family showed linkage to Xp22, with a LOD score of 4.8. In family 502 (MRX59), previously described by Carpenter et al.,[6] there is a p.R52X (c.154C→T) nonsense mutation. Family 502 (MRX59) showed linkage to Xp22, with a LOD score of 2.4. In family 63 (fig. 3), there is a 4-bp deletion preceding the splice-acceptor site of exon 3 that results in the replacement of the invariant A at position −2 with a T (c.180-5del4). *In silico* analysis with the use of the splice-site prediction software NNSPLICE (BDGP: Splice Site Prediction by Neural Network) indicated that this deletion reduced the strength of the consensus splice-acceptor site from 94% to 0%. This mutation is likely to cause skipping of exon 3, which would then introduce a translational frameshift, resulting in the inclusion of 3 novel aa, with termination at codon 64. RNA was not available for direct evaluation of this prediction. Family 63 showed linkage to Xp22, with a maximum LOD score of 1.9.

Available individuals from each family have been tested for the relevant mutation, and, in all three families, the mutation segregates completely with the disease (fig. 1). In family 63, the mutation was identified in all four affected males and three obligate females and was absent in two unaffected males. In family 445, the mutation was present in five affected males and eight obligate carriers and was absent in seven unaffected males. In family 502 (MRX59), all five affected males and four obligate carrier females carried the mutation, and the mutation was not found in the one unaffected male available for study. In family 445, expression of *AP1S2* was investigated in lymphocytes derived from an affected male (fig. 4). No obvious difference in expression was identified in an affected male harboring the R53X mutation, compared with a wild-type control, indicating that nonsense-mediated mRNA decay does not act on this premature stop codon containing mRNA. However, whether this and the other two *AP1S2* mutations represent partial or complete loss of function remains to be determined. No missense or synonymous (silent) variants were found in *AP1S2* among the full set of 250 families with XLMR. To evaluate further the significance of the truncating mutations, we sequenced *AP1S2* in 234 unaffected males and 246 unaffected females from the United Kingdom (in total, 726 alleles of *AP1S2*). No truncating variants were detected, and no missense or synonymous changes were observed, which demonstrated that the level of population variation in this gene is very low, further substantiating that the observed mutations in the three families with XLMR are disease causing. The difference between XLMR cases and controls for truncating mutations was significant ($P = .02$, by Fisher's exact test).

Clinical details of family 502 (MRX59) and family 445 have been published elsewhere and therefore will not be further reviewed here.[5,6] Family 63 includes four affected males (figs. 1 and 3). III-1 was delivered at term following an emergency cesarean section at 40 wk due to fetal distress. He was intubated at birth and was noted to have hypotonia as a neonate. He fed well, smiled appropriately, and was not thought to be dysmorphic. At age 6 mo, he
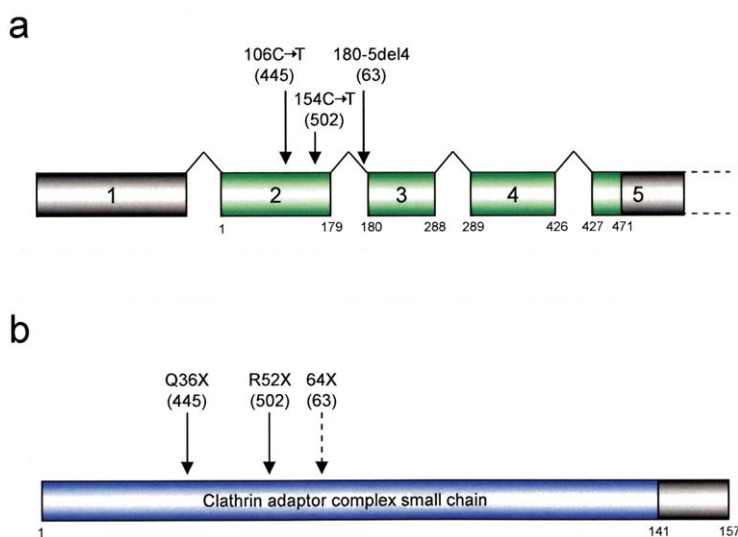
Figure 2. *a,* Schematic representation of the exon structure of *AP1S2,* with positions of mutations found in XLMR-affected families. *b,* Schematic representation of the coding sequence. The clathrin adaptor complex small-chain functional domain is marked, and positions of mutations are indicated. The dashed arrow indicates the predicted position of the translational stop codon, under the assumption of exon 3 skipping in family 63.

Figure 3. Photographs of the affected males in family 63. *a* and *b,* Individual II-7, aged 42 years. *c* and *d,* III-4, aged 24 years. *e* and *f,* III-1, aged 23 years. *g* and *h,* III-2, aged 11 years.

was noted to be hypotonic, with a bilateral convergent squint and marked motor delay. He started to walk at age 6 years. At age 7 years, he had his first seizure and has since been maintained on sodium valproate. As an adult, he lives in a residential home, requires full care, is obsessed with the washing machine, and spends much of the time watching the spin dryer. He has no intelligible speech. III-2 was born following elective cesarean section. His clinical features were similar to those of his older brother but were less severe. He started to walk at age 4 years and has developed simple language. He has attended a special school and continues to live at home. The family regards him as the least severely affected of the family members. III-4 was born at full term (42 wk). Neonatal hypotonia was noted. He sat at age 3 years and started to walk at age 6 years. He lives at home, his speech is limited, and he is capable of performing simple tasks. II-7 lived at home until age 30 years before moving to sheltered housing, where he is self-caring but needs supervision. He has limited language but is able to discuss simple concepts. He attends a day-care center 2–3 d per wk but does not go out alone. He has had aggressive outbursts.

The phenotypes of the three families with mutations in *AP1S2* are variable but exhibit many similarities, summarized in table 1. All affected males have MR. However, the degree of intellectual impairment ranges from mild to profound. Capacity for speech varies from none to simple but understandable language. None of the affected individuals have been capable of independent living, although several have managed in supportive-care homes. Many have required full-time care, including toileting and dressing. Obsessive behavioral routines have been reported for members of all three families, and aggressive outbursts have also been described. Many members of family 445 are microcephalic (head circumference <3rd percentile),

and two members of family 502 (MRX59) have also been reported to have microcephaly. Members of family 63 had head circumferences in the normal range. No significant dysmorphic features have been reported for all three families, although a long, thin face is a feature of both families 63 and 445. The obligate female carriers in all three families have normal intelligence.

Of particular note was marked hypotonia at age 6 mo in all affected members of the three families. All three families also report significant delay in walking but without other consistent features of neurological dysfunction. In family 63, walking was achieved between ages 4 and 6 years. In family 502 (MRX59), one affected male was reported to have started to walk at age 6 years. In family 445, one individual is reported to have started to walk at age 8 years. These features appear to be particularly char-
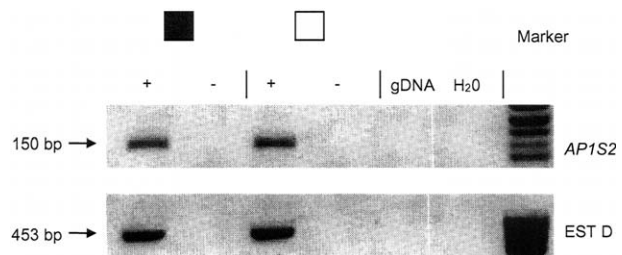
Figure 4. RT-PCR analysis of the *AP1S2* gene on lymphocyte-cell RNA in the proband from family 445 (*blackened square*) and a control individual (*unblackened square*). PCR amplification of *AP1S2* and the esterase D gene (EST D) shows no difference between affected and unaffected individuals. pUC19/*Hpa*II was used as a molecular weight marker. Experiments were performed in the presence (+) or absence (−) of reverse transcriptase. gDNA = genomic DNA.

Table 1. Selected Clinical Findings in Affected Males from Families with *AP1S2* Mutations

Characteristic	No. of Individuals with Characteristic		
	Family 63 (*n* = 4)	Family 445 (*n* = 7)	Family 502 (MRX59) (*n* = 5)
Degree of MR:			
Mild	1	2	2
Moderate	2	5	1
Profound	1	0	2
HC (percentile):			
<3	0	6	2
3–55	4	1	2
>55	0	0	1
Hydrocephalus	0	1	1
Childhood hypotonia:			
Absent	0	0	0
Present	4	7	5
Age at walking (years):			
1–2	0	1	0
3–4	1	1	4
>4	2	3	1
Not known	1	2	0
Speech:			
Absent	1	5	3
Minimal	1	2	0
Present	2	0	2
Aggressive outbursts:			
Absent	3	2	2
Present	1	5	3

NOTE.—HC = head circumference.

acteristic of the phenotype of *AP1S2* mutations. Delayed walking was reported in 47 (25%) of 190 evaluated families. Moreover, 3 (6%) of 47 families with delayed walking have mutations in *AP1S2*. Hypotonia was an infrequently reported clinical finding in the families with XLMR without *AP1S2* mutations.

The *AP1S2* gene is composed of five exons and encodes a small protein of 157 aa, 90% of which make up the clathrin adaptor complex small-chain domain. The *AP1S2* protein is involved in formation of clathrin-coated vesicles that form part of a well-characterized system of eukaryotic membrane protein trafficking (see Edeling et al.[7] for review). The system has two major components: clathrin, which provides structural integrity, and adaptor protein complexes, which determine selection of the vesicle cargo and promote clathrin-lattice formation onto the vesicle membrane.[8] There are five adaptor protein complexes associated with clathrin (AP1, AP2, AP3, AP4, and AP180), of which AP1 and AP2 are the best characterized. Both AP1 and AP2 adaptor complexes are composed of two large subunits (γ and $\beta1$ in the AP1 complex), one medium subunit ($\mu1$), and one small sigma subunit ($\sigma1$, or σ). *AP1S2* encodes the sigma 2 subunit of the heterotetrameric AP1 adaptor complex. The AP1 complex is preferentially associated with endosomes and the trans-Golgi network, whereas the AP2 complex is associated more with the cytoplasmic membrane. AP1 binds both YXXϕ (where X is any amino acid and ϕ is a large hydrophobic residue) and

[D/E]XXXL[L/I] acidic dileucine motifs found in vesicle cargo proteins, including transmembrane receptors.[7]

Aberrant endocytic processing through disruption of functional AP1 complexes is likely to result from the loss of function *AP1S2* mutations identified in the three families with XLMR. Such a deficit would plausibly have an impact on normal synaptic vesicle cycling and, thus, processing and trafficking of membrane proteins, including receptors and ion channels. AP1 has been shown to associate with synaptophysin, one of the most abundant synaptic vesicle proteins thought to play an important role in neurotransmitter release. The cytoplasmic tail region of synaptophysin interacts with γ-adaptin of AP1 in a yeast two-hybrid system, and these proteins have also been shown to colocalize in the perinuclear region of cultured hippocampal neurons.[9] Interactions between AP1 and the vesicular acetylcholine transporter, responsible for the transport of acetycholine from the cytosol into synaptic vesicles, have also been reported.[10] Disruption of the AP1 complex via mutations in *AP1S2* could feasibly impair these interactions and disrupt normal neurotransmitter processing within the synapse. This hypothesis is supported by *Drosophila* mutants with deficits in various endocytic proteins, including the clathrin assembly protein SNAP91 (AP180), which have altered synaptic development.[11] SNAP91, together with the AP1 and AP2 complexes, is critical for clathrin coat assembly under physiological conditions, and it has been suggested that SNAP91 interacts with adaptor protein complexes in regulating the uncoating of clathrin-coated vesicles.[7,12]

No previously identified XLMR gene has been centrally implicated in the general formation and processing of endocytotic vesicles. However, other proteins encoded by XLMR genes have been involved in the biology of synaptic vesicles. GDI1 is a Ras superfamily of small GTPases guanosine diphosphate (GDP)–dissociation inhibitor involved in vesicle trafficking via regulation of the GDP-GTP exchange reaction.[3] The protein DLG3/SAP102 has been reported to interact with the exocyst complex and may assist in the trafficking of N-methyl-D–aspartic acid receptors from the endoplasmic reticulum to the synaptic cell surface.[13] Moreover, *SYN1,* a known XLMR gene and a member of the synapsin family, encodes a neuronal phosphoprotein that associates with the cytoplasmic surface of synaptic vesicles.[14] Mutations in the clathrin/endocytic processing system have been reported in other human diseases. Autosomal recessive mutations in the adaptor protein ARH lead to defective endocytosis of low-density lipoprotein receptors in hypercholesterolemia (MIM 603813),[15,16] and autosomal recessive mutations in *AP3B1* result in Hermansky-Pudlak syndrome type 2 (MIM 203300).[17]

AP1S2 encodes a predicted protein of only 157 aa. The presence of three different truncating mutations in such a small gene, the complete segregation of mutations in affected individuals, the absence of the truncating variants in unaffected controls, and the previous evidence of link-

age to this region of the X chromosome strongly indicate that *AP1S2* is the gene responsible for MR in these three families. The small size of the gene may allow relatively straightforward introduction into diagnostic testing. To our knowledge, *AP1S2* is the first reported XLMR gene encoding a protein that is directly involved in vesicle assembly and processing.

Acknowledgments

Web Resources

The accession number and URLs for data presented herein are as follows:

BDGP: Splice Site Prediction by Neural Network, http://www.fruitfly.org/seq_tools/splice.html (for NNSPLICE)
GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *AP1S2* [accession number NM_003916.3])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *AP1S2,* hypercholesterolemia, and Hermansky-Pudlak syndrome type 2)

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