Unstable expansion of the CAG trinucleotide repeat in MAB21L1: report of a second pedigree and effect on protein expression

Russell L Margolis, O Colin Stine, Christopher M Ward, Mary L Franz, Adam Rosenblatt, Colleen Callahan, Meeia Sherr, Christopher A Ross, Nicholas T Potter

Abstract

MAB21L1, originally termed CAGR1, is the human homologue of the *C elegans* cell fate determining gene *mab21*. MAB21L1, mapped to 13q13, contains a highly polymorphic 5' untranslated CAG repeat that normally ranges from six to 31 triplets in length. A pedigree has been previously reported in which the repeat length is expanded to 45-50 triplets and is transmitted unstably between generations; the expansion did not correlate to a clinical phenotype but did exhibit somatic mosaicism. We now report a second pedigree with an expanded and unstably transmitted MAB21L1 CAG repeat of similar length. The expansion is not clearly associated with a clinical phenotype, though the complexity of the pedigree renders any conclusion concerning phenotype-genotype relationships speculative. The expansion did not result in decreased expression of MAB21L1 protein. The length, C-G rich composition, somatic mosaicism, and unstable transmission of the expanded CAG repeat in MAB21L1 resemble the premutations observed in other genes, such as FMR1 and MDPK, in which longer expanded repeats are associated with a clinical phenotype. This raises the possibility that longer expansions in the MAB21L1 repeat may also be associated with a clinical phenotype.

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Division of Neurobiology, Department of Psychiatry and Behavioral Science, Johns Hopkins University School of Medicine, Baltimore, MD, USA
R L Margolis
C M Ward
M L Franz
A Rosenblatt
C Callahan
M Sherr
C A Ross

Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD, USA
C Stine

Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA
C A Ross

Developmental and Genetic Center, The University of Tennessee Medical Center, Knoxville, TN, USA
N T Potter

Correspondence to:
Dr Margolis, Department of Psychiatry, Meyer 2-181, 600 N Wolfe Street, Baltimore, MD 21287, USA.

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Trinucleotide repeat expansions are now known to result in at least 13 diseases. Of these, eight are CAG repeats that encode glutamine and lead to neurodegeneration.[1–3] The other expansions, of various types, induce disease by several different mechanisms. For instance, a CGG expansion in the 5' untranslated region of FMR1 causes fragile X syndrome type A through a decrease in gene transcription.[4] Expansion of the GAA repeat in an intron of frataxin causes Friedreich's ataxia,[5] perhaps through anomalies of RNA splicing, loss of protein expression, and subsequent mitochondrial dysfunction.[6] CTG expansion in the 3' untranslated region of MDPK leads to myotonic dystrophy,[7–9] presumably through poorly understood effects on the expression of MDPK and surrounding genes.[10]

We have described a human gene, originally termed CAGR1 and now known as MAB21L1 (GenBank U38810), mapping to 13q13 and containing a highly polymorphic 5' untranslated CAG repeat.[11] Of particular interest, MAB21L1 is homologous to the *mab21* gene found in the nematode *C elegans*. Mutations in *mab21* result in mutant nematodes with abnormal differentiation of neuronal and epithelial cells, and consequent dysmorphic development and abnormal behaviour.[12] The gene therefore appears essential for determining cell fate in part of the *C elegans* sensory apparatus, and perhaps in other regions.

The CAG repeat in MAB21L1 normally ranges from six to 31 triplets in length as ascertained in 1610 chromosomes,[11 13] with a heterozygosity of 88% and no evidence of meiotic or somatic instability. In addition, Potter[13] detected an allele with 50 triplets in a proband with idiopathic mental retardation and early signs of attention deficit/hyperactivity disorder. Alleles in three other subjects from two previous generations of this family were also expanded, with repeat length ranging from 45 to 51 repeats. Repeat length tended to increase in subsequent generations, but the long allele was not associated with a phenotypic abnormality. The original description of MAB21L1[11] also included a single subject from an independent pedigree (No 508) with a repeat of 46 triplets. Analysis of genomic DNA derived from peripheral leucocytes showed somatic mosaicism of the expanded repeats in both pedigrees.

We have subsequently obtained additional information about pedigree 508 (fig 1). The proband, who has bipolar affective disorder type II (DSM IV criteria), ataxia, dystonia, and abnormal eye movements, has been examined on multiple occasions and is described in detail elsewhere.[14] She was originally ascertained through the Baltimore Huntington's Disease Program (BHDP), where she was evaluated for possible Huntington's disease (HD). The proband's father died from what was said to have been Huntington's disease (he was not examined in the BHDP and records are limited), and his brother and several cousins had genetically confirmed HD. A second cousin once removed had, by report, clinical HD, an expansion in the HD gene, and two normal MAB21L1 alleles. However, the proband has tested negative for HD (multiple tests on multiple blood samples), dentatorubral pallidoluysian atrophy, and spinocerebellar

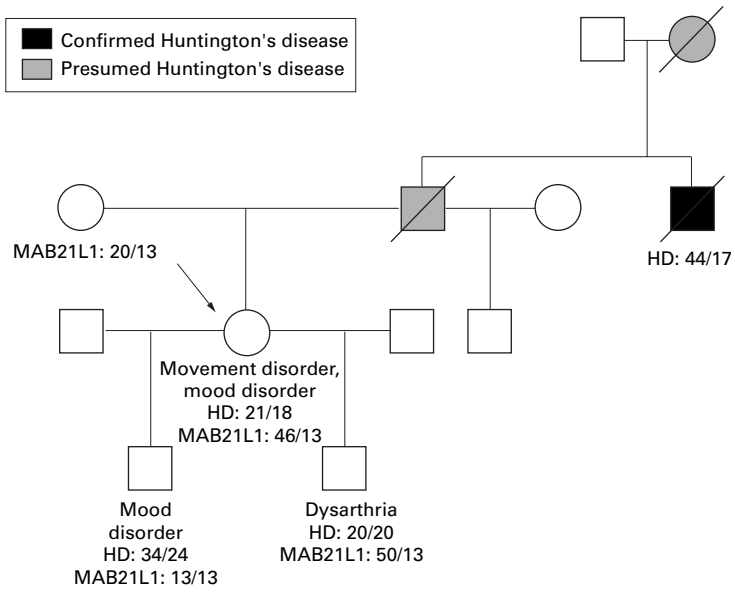

Figure 1 A portion of pedigree 508.

ataxia types 1, 2, 3, and 6. Her mother, who does not suffer from a movement disorder, affective disorder, or dementia (by her report on interview), has two MAB21L1 alleles of typical length, one of which appears to have been transmitted to the proband. The proband's sons were both examined in detail, using a structured neuropsychiatric interview and examination. One son has bipolar affective disorder type I (DSM IV criteria), no movement abnormality, and normal MAB21L1 alleles. The other son has mild dysarthria but no other neurological or psychiatric signs or symptoms. He apparently received the expanded MAB21L1 allele from his mother; the allele expanded on transmission to 50 triplets.

To examine the influence of the expanded MAB21L1 allele on protein expression, we generated a polyclonal antiserum in rabbits against the carboxyl terminal 14 amino acids of the protein (AREILTNPKSLEKL, residues 346-359 of human MAB21L1) coupled to a KLH carrier. Sera were screened for cross reactivity with the original peptide ligand by direct ELISA. Reactive sera were further purified by peptide affinity chromatography on cyanogen bromide activated Sepharose 4B columns (Sigma). On immunoblot analysis, the purified antisera detected a protein of approximately 38 kDa protein in extracts from each of 16 different regions of the human CNS. This reactivity was completely abolished by preincubation of the antiserum with homologous peptide before immunoblotting.[15] Western blots of protein extracts from lymphoblastoid cell lines derived from pedigree 508 were probed with this affinity purified antiserum. The results (fig 2) indicate that the level of MAB21L1 protein in lymphoblasts from the two family members with an expanded MAB21L1 repeat is not decreased.

Data from the pedigree reported here confirm the previous finding that abnormally long alleles of MAB21L1 are unstably transmitted. There is no clear correlation of the expanded allele to a

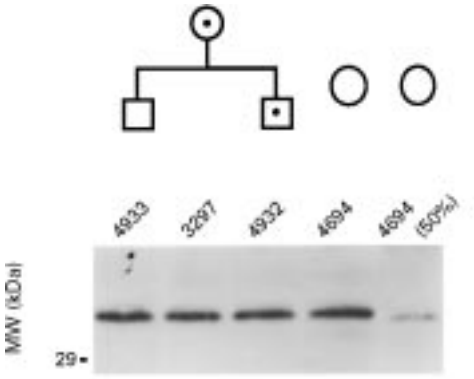

Figure 2 MAB21L1 protein expression in members of pedigree 508. Subject identification numbers are listed above each lane; 150 µg of protein extracted from lymphoblastoid cell lines was subjected to polyacrylamide gel electrophoresis and transferred to nitrocellulose as previously described.[18] Blots were probed with a 1:100 dilution of the affinity purified MAB21L1 C-terminal antiserum and visualised by chemiluminesence using the Phototope-HRP Western Blot Detection Kit (New England Biolabs, Beverly, MA) following the manufacturer's instructions. There were no consistent differences in protein levels between the two family members with expanded MAB21L1 alleles (the proband, 3297, and her son, 4932) and two other family members (the other son of the proband, 4933, and a first cousin twice removed, 4694). A deliberate 50% underloading of protein from family member 4694 results in an obviously reduced signal. The experiment was performed three times with nearly identical results.

clinical phenotype, and the expanded allele does not appear to lead to a major loss of protein expression. While the expanded alleles may represent a genotypic variant with no functional consequence, the length of the expansion, the nature of the repeat (C-G rich), its unstable transmission, and the presence of somatic mosaicism are similar to premutations observed in the FMR-1 and MDPK genes.[7 16 17] That longer expansions have not been detected may reflect the relative rarity of the moderate expansions that we have observed or, possibly, the embryonic lethality of a long expansion. Detection of additional pedigrees with MAB21L1 expansions may help distinguish among these possibilities.

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