Non-specific X-linked semidominant mental retardation by mutations in a Rab GDP-dissociation inhibitor

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Non-specific X-linked mental retardation (MRX) is a very common disorder which affects ∼1 in 600 males. Despite this high frequency, little is known about the molecular defects underlying this disorder, mainly because of the clinical and genetic heterogeneity which is evident from linkage studies. Recently, a collaborative study using the candidate gene approach demonstrated the presence of mutations in GDIα, a Rab GDP-dissociation inhibitor encoded by a gene localized in Xq28, associated with non-specific mental retardation. GDIα is mainly a brain-specific protein that plays a critical role in the recycling of Rab GTPases involved in membrane vesicular transport. The study presented here was designed to assess the prevalence of mutations in the GDIα in mentally retarded patients and to discuss the clinical phenotypes observed in affected individuals. Mutation screening of the whole coding region of the GDIα gene, using a combination of denaturing gradient gel electrophoresis and direct sequencing, was carried out in 164 patients found negative for expansions across the FRAXA GCC repeat. In addition to the nonsense mutation recently reported in MRX48, we have identified a novel missense mutation in exon 11 of the GDIα gene in one familial form of non-specific mental retardation. In this family (family R), all affected males show moderate to severe mental retardation, and the X-linked semidominant inheritance is strongly suggested by the severe phenotypes in males with respect to mildly affected females or unaffected obligatory carriers. This study showed that the prevalence of GDIα mutations in non-specific mental retardation could be estimated to be 0.5–1%, and molecular diagnosis and genetic counselling in some cases of non-specific mental handicap can now be provided.

INTRODUCTION

Non-syndromic X-linked mental retardation (MRX) is a common and vastly heterogeneous group of disorders which affect ∼1 in 600 males (1). Despite this high frequency, little is known about the molecular defects underlying these disorders, mainly because of the clinical and genetic heterogeneity which is evident from linkage studies. Genetic mapping data on individual families identified >65 MRX loci with several overlaps between intervals of assignment. However, recent reviews reported by Gedeon et al. (2) and the European XLMR consortium (3) showed that these loci are clustered in 8–10 non-overlapping regions, suggesting the involvement of 8–10 X-linked genes in non-specific mental retardation. Recently, we have reported a large French family (MRX48) affected with a non-specific MRX which is linked to Xq28 (4). Five other unrelated families with MRX have been linked to this region (reviewed in ref. 4). Among the candidate genes for MRX located in the Xq28 region and highly expressed in brain was that encoding GDP-dissociation inhibitor α (GDIα), a member of the accessory proteins family involved in the regulation of the activity of Rab GTPase proteins implicated in vesicle targeting and fusion events (5). Recently, a collaborative investigation of this gene (6,7), set up by D.T.’s laboratory and involving our group, showed its involvement in non-specific mental retardation in two families affected with MRX mapped to Xq28 (MRX48 and 41). In order to evaluate the prevalence of non-specific mental retardation related to GDIα, we screened 164 non-fragile X mentally retarded individuals for point mutations.

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in the whole coding region of the GDIα gene by using a combination of denaturing gradient gel electrophoresis (DGGE) and direct sequencing analyses. In our cohort of patients with mental retardation, we included patients from MRX16 and MRX48 families, known to be located in Xq28. In addition to the nonsense mutation reported in MRX48, we identified a novel missense mutation in an additional family (family R). Additional evidence that the novel mutation of the GDIα gene is significant was provided by the co-segregation of this base substitution with the disease in the large French family. These data showed that investigation of GDIα could now be proposed as an additional tool to establish an accurate and reliable molecular diagnosis in non-specific mental retardation.

RESULTS

Mutation analysis

Coding exons of the GDIα gene from 164 patients with non-specific mental retardation were screened for mutations by DGGE. No abnormalities were detected in exons 1–2 and 4–10. Analysis of exon 3 revealed a polymorphism at codon 73 in 24 out of 164 (14%); DNA sequencing revealed a change from AA T to AAC at position 377 (position +1 of the cDNA corresponds to 158 nucleotides upstream of the ATG), which did not alter the amino acid (asparagine) residue (data not shown). The frequency of this polymorphic variant among the normal subjects studied is estimated to be 12% (12/100 normal chromosomes).

In addition to the nonsense C→T mutation at position 366 of the cDNA reported in MRX48 (6,7), the screening revealed the presence in one mentally retarded patient of an abnormal migration pattern of exon 11. Further inquiry revealed that this patient is one of the members of a large French family (family R) affected with MRX. The sequence of the region revealed a G→C substitution at cDNA position 1426 (Fig. 1). This change causes an arginine to proline substitution at amino acid 423 of GDIα. The missense mutation creates a unique StyI restriction site. This event was used to study the segregation of this base substitution in the large family of this patient. Figure 2 shows that the mutation is in perfect co-segregation with the mental retardation phenotype. This base substitution was absent in >100 normal controls.

It is interesting to point out that our investigation did not reveal any mutation in the MRX16 family (8), known to be located in Xq28, in a genetic interval encompassing GDIα.

Figure 1. Fluorescence sequence analysis of exon 11 of the GDIα gene. The underlined nucleotide indicates the position of the mutation R423P (G→C at position 1426).

Figure 2. Pedigree of the family R and results of StyI restriction enzyme analysis of exon 11 amplified samples. Digestion by StyI yields two fragments corresponding to the co-migrating 126 and 127 bp bands when the G→C substitution (R423P) is present. III-3, III-7 and IV-7, affected males; II-2, III-2, III-13, IV-1 and IV-8, heterozygous females; III-4, IV-2 and IV-9, normal males. Restriction enzyme analyses confirm the sequence interpretation and indicate co-segregation of the mutation with the phenotype in family R.
Clinical features
In the two MRX families (MRX48 and R), all affected males show moderate to severe mental retardation. No statural growth deficiency, or minor facial or other abnormal physical features were observed. Seizures were observed only in one affected patient of family R. The only common feature of these two families is the X-linked semidominant inheritance which is strongly suggested by the severe phenotype in males with respect to mildly affected female or unaffected obligatory carriers, and no male-to-male transmission. Clinical data of the MRX48 family were reported previously (4). One of the affected individuals of the family R herein reported, IV-7, underwent clinical examination and psychometric work-up in a department of pediatric neurology; for the other affected males, thorough anamnestic data were collected from physicians, social workers and relatives. The proband, individual IV-7, was born at term after a normal pregnancy with a good neonatal adaptation. Birth weight was 3120 g, height 48.5 cm, OFC 33 cm. Developmental skills were delayed: he sat at 12 months, walked at 24 months and he had no language at 30 months. At 3 years old, psychometric evaluation showed a severe mental retardation with a developmental quotient (DQ) of 25 and some autistic features. Clinical assessment, including growth chart, hearing, vision and skin examination was normal. Neurologic examination showed a global hypotonia, with normal muscular strength and osteotendinous reflexes. In individual IV-7, brain magnetic resonance imaging (MRI), electroencephalogram (EEG), biochemical blood and urine screening (thryoid-stimulating hormone, free thyroxine, amino acid chromatography) were normal. At 5 years, he had poor language with only few words, and many stereotypes such as rocking and handtapping. He was referred for special education in an institution. Individuals III-3, III-5 and III-7 have severe mental retardation with poor language, they are not able to read and write; III-3 and III-5 have been withdrawn from their family after judicial decisions and they attend sheltered workshops. III-7 is under guardianship and lives at home; he was treated for seizures a few years ago. Individual III-4 was also withdrawn from his family and attended special schools. However, unlike his brother, he was considered as unaffected because he can read and write; he works efficiently and is very well integrated in society. Consistent with these data is the normal IQ recently evaluated in this individual. Other males of this family (III-6, IV-2 and IV-9) attended normal school and have a good social behaviour. Consistent with these data is the normal IQ recently evaluated in this individual. Other males of this family (III-6, IV-2 and IV-9) attended normal school and have a good social behaviour. 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In addition to R70X (C→T at position 366) shown to segregate with the disease in the MRX48 family, the novel missense mutation predicts a change of an arginine to proline (R423P) and would lead to a drastic amino acid substitution (change of type). Initially, one would speculate that an arginine to proline mutation affects the protein conformation and possibly the binding to Rab GTPase. Moreover, we believe that this novel missense mutation is pathogenic because (i) it co-segregates with the disease trait in the large family R; (ii) it was localized in a conserved residue participating in one of the α-helices surrounding the Rab-interacting site in Caenorhabditis elegans, Drosophila melanogaster and Saccharomyces cerevisiae (5, 9) and (iii) it was not found in >100 control chromosomes. In addition, pairwise linkage analysis carried out with informative markers in Xq28 (DXS8011, DXS8061, F8c and DXS1073) and with the mutation in GDIα (used as a biallelic marker with allele frequencies of 0.01 and 0.99) indicated a significant linkage between the disease and the mutation, with a two-point maximum LOD score of 3.01 at θ = 0. The multipoint linkage analyses with six point runs confirm the significant linkage with Xq28 markers.

To date, in addition to GDIα, the only genes known to be involved in non-specific mental retardation are FMR2 and the gene coding for oligophrenin-1. The FMR2 gene, involved in the very rare cases (0.11%) of FRAXE syndrome (10, 11), encodes a protein of unknown function, but with significant similarity to the proto-oncogene product AF-4, also known as a serine/proline-rich protein (12–15). The oligophrenin 1 gene, recently identified by Billuart et al. (16), was found mutated in unrelated individuals affected with non-specific mental retardation. This gene is highly expressed in fetal brain and encodes a protein which contains a domain typical of a rho GTPase-activating protein (rhOGAP) (17, 18). In view of the genetic heterogeneity of non-specific mental retardation, one should expect an increasing number of genes to be involved, and oligophrenin followed by a number could be used as a nomenclature system to facilitate designation and classification of these genes. Therefore, we propose the name oligophrenin 2 for GDIα.

The heterogeneity of clinical expression in obligate carrier females could be explained by a skewed inactivation of the X chromosome. This hypothesis was tested in MRX48 and no correlation was observed between the clinical severity and a skewed inactivation pattern in lymphocytes (4). However, a different pattern of inactivation in lymphocytes and in brain remains possible, and the lack of correlation in lymphocytes cannot rule out the influence of X inactivation in the clinical phenotype of heterozygous females. The semidominant inheritance of the mental retardation condition associated with the nonsense and missense mutations in MRX48 and the family described here, respectively, and the recessive inheritance of the phenotype in MRX41 could be related to differences in X
inactivation patterns of neuronal precursor cells, although this cannot yet be tested experimentally.

In the proband from MRX16 family mapped to Xq28, no relevant mutation was detected in GDIα either by DGGE analysis or by direct sequencing of PCR products corresponding to the 11 coding exons. Although these investigations cannot exclude the presence of a mutation that might lie in the promoter or intronic sequences, an alternative hypothesis could be the involvement in mental retardation of a second locus localized in Xq28.

In summary, in our cohort of 164 male samples referred for fragile X syndrome testing and found to be negative for expansions across the FRAXA GCC repeat, the frequency of mutations in the GDIα gene associated with mental retardation could be estimated as ∼0.5–1% of the tested sample set (5–10 times less than the frequency of fragile X syndrome in the population referred to our diagnosis laboratory for fragile X testing). It appears that mutations in the GDIα gene are associated with non-specific X-linked semidominant mental retardation. Absence or alteration of GDIα could modify interaction with the Rab3 proteins, especially Rab3 and 3C, and change the amount of Rab3 available for cycling between the active and the inactive state. Lack of GDIα may not only be responsible for a general change in synapse functionality but also of more subtle and specific alterations in more limited regions of the brain. Other proteins involved in this pathway are probably potential candidate genes for mental retardation. For the population studied, we conclude that the GDIα-associated mental retardation is a significant form of mental retardation for which genetic diagnosis could be now undertaken.

### MATERIALS AND METHODS

#### Patients

One hundred and sixty four patients with idiopathic mental retardation were identified by examining the fragile X case record of J.C. Kaplan’s molecular diagnosis unit at the Cochin Institute. Male patients were selected on the basis of: (i) a low IQ or, if too young for psychological assessment, the presence of significant developmental delay and (ii) no recognizable syndrome whether chromosomal, Mendelian, acquired or environmental. Fragile X syndrome was excluded by Southern blot analysis using DNA digested with EcoRI–EagI endonucleases and the StB12-3 probe corresponding to the FRAXA locus (19). Control samples were from normal male individuals from Duchenne muscular dystrophy families. Ethics committee approval and patient or family consent were obtained.

#### Mutation analysis

**Denaturing gradient gel electrophoresis (DGGE).** DNA was extracted from peripheral blood leukocytes, and the 11 exons and the flanking intronic sequences of the GDIα gene were PCR amplified separately from genomic DNA using the primers listed in Table 1, with psoralen clamps (20). DGGE conditions were chosen according to the Meltmap program, kindly provided by L. Lerman et al. (21). The denaturants were 7 M and 40% formamide, and gels were run at 60°C. PCR products were subjected to electrophoresis as described in Table 2.

<table>
<thead>
<tr>
<th>Table 1. Parameters for amplification of the GDIα gene fragments</th>
</tr>
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<tbody>
<tr>
<td>Fragment</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Exon 1</td>
</tr>
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**Table 2. Parameters for DGGE conditions**

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<tr>
<th>Fragment</th>
<th>Gradient (%)</th>
<th>Running time (h) at 160 V</th>
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<tr>
<td>Exon 1</td>
<td>40–90%</td>
<td>9.5</td>
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<tr>
<td>Exon 2</td>
<td>40–90%</td>
<td>8.3</td>
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<tr>
<td>Exon 3</td>
<td>30–80%</td>
<td>6</td>
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<tr>
<td>Exon 4</td>
<td>30–75%</td>
<td>6.75</td>
</tr>
<tr>
<td>Exon 5</td>
<td>40–90%</td>
<td>6.7</td>
</tr>
<tr>
<td>Exon 6</td>
<td>35–70%</td>
<td>6.5</td>
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<tr>
<td>Exon 7</td>
<td>20–70%</td>
<td>6.6</td>
</tr>
<tr>
<td>Exon 8</td>
<td>40–90%</td>
<td>8.9</td>
</tr>
<tr>
<td>Exon 9</td>
<td>40–90%</td>
<td>8.3</td>
</tr>
<tr>
<td>Exon 10</td>
<td>40–90%</td>
<td>8.3</td>
</tr>
<tr>
<td>Exon 11</td>
<td>30–75%</td>
<td>7.1</td>
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**Mutation identification.** PCR products showing an abnormal migration pattern on DGGE analysis were sequenced directly on an automated sequencer (ABI 377; Perkin-Elmer) using the Dye Terminator method. Every sequence variation was checked by restriction analysis of genomic DNA. Familial segregation was also checked.

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