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Screen for MAOA Mutations in Target Human Groups

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Abstract

Brunner et al. [1993: Am J Hum Genet 52: 1032–1039–1993: Science 262:578–580] described males with an MAO-A deficiency state resulting from a premature stop codon in the coding region of the *MAOA* gene. This deficiency state was associated with abnormal levels of amines and amine metabolites in urine and plasma of affected males, as well as low normal intelligence and apparent difficulty in impulse control, including inappropriate sexual behavior. In the present study, disruption of the MAOA gene was evaluated in males with mental retardation with and without a history of sexually deviant behavior, as well as normal controls, healthy males, and patients with other diseases (Parkinson disease, Lesch-Nyhan syndrome). When available, plasma samples were evaluated first for levels of 3-methoxy, 4-hydroxyphenolglycol (MHPG), a metabolite of norepinephrine which serves as the most sensitive index of MAO-A activity in humans. Blood DNA from individuals with abnormally low MHPG, and from other individuals for whom metabolite levels were not available, were screened for nucleotide variations in the coding region of the MAOA gene by single-strand conformational polymorphism (SSCP) analysis across all 15 exons and splice junctions, and by sequencing, when indicated by either altered metabolites or SSCP shifts. No evidence for mutations disrupting the MAOA gene was found in 398 samples from the target populations, including institutionalized mentally retarded males (N = 352) and males participating in a sexual disorders clinic (N = 46), as well as control groups (N = 75). These studies indicate that MAOA deficiency states are not common in humans.

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Keywords

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INTRODUCTION

The monoamine oxidases, MAO-A and MAO-B, are isozymes encoded in two distinct genes, MAOA and MAOB, on human chromosome Xp11.4–11.3 [reviewed in Chen et al., 1995]. These enzymes are critical in the degradative deamination of a number of biogenic amines which serve as neurotransmitters, e.g., norepinephrine, serotonin, dopamine, and histamine, or which can act as "false" transmitters that disrupt neurotransmission [reviewed in Murphy and Kalin, 1980; Weyler et al., 1990]. Both isozymes have a wide range of activity levels in human tissues, with MAO-B activity being measured in platelets and lymphocytes [Bond and Cundall, 1977] and MAO-A activity in cultured skin fibroblasts [Hotamisligil and Breakefield, 1991]. No variations in the amino acid sequence of either isozyme have been associated with different levels or kinetics of enzyme activity [reviewed in Chen et al., 1995]. Thus, the coding sequence of these genes appears to be remarkably conserved, which is surprising, since the MAOs have overlapping substrate specificity and tissue distribution and can thus compensate for each other. Brunner et al. [1993a,1993b] described a family from the Netherlands manifesting an X-linked, recessive syndrome characterized by altered amine metabolites, low normal intelligence, and loss of impulse control associated with deviant behavior. This condition mapped to the chromosomal region bearing the MAO genes by linkage analysis. MAO-B activity in platelets from affected males was normal, but MAO-A activity in fibroblasts was below the level of detection. This MAO-A deficiency was caused by a single base-pair substitution (C to T) in exon 8 of the MAOA gene, converting the codon for glutamine to a stop codon. The resulting truncated protein lacked the cofactor binding site and hence had no activity.

Given that loss of MAO-A activity is compatible with human life and not associated with a disease state, a search was undertaken to determine how frequent this deficiency state might be in males, including both healthy controls and other patient populations (Parkinson disease, Lesch-Nyhan syndrome), and in populations manifesting mental retardation (MR) and/or deviant behavior. Blood samples were screened by high pressure liquid chromatography (HPLC) for plasma levels of 3-methoxy,4-hydroxyphenolglycol (MHPG), a catecholamine metabolite, previously reported to correlate highly with the magnitude of MAO-A, but not MAO-B inhibition [Pickar et al., 1981; Sunderland et al., 1985]. Those with very reduced plasma MHPG levels were then further screened by DNA analysis for nucleotide variations in the coding region of the MAOA gene.

MATERIALS AND METHODS

This sample set included four subgroups: 1) Forty-six male subjects (age range 21–56 years) with paraphilic disorders, including pedophilia, who were attending a sexual disorder clinic (SDC) in Baltimore, Maryland [Berlin et al., 1991], were screened for plasma MHPG levels. Except for two individuals with no detectable levels, the others fell within normal range. The

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11 individuals with the lowest levels were screened by single-strand conformational polymorphism (SSCP) analysis and sequencing. 2) Of 30 males with MR from the Greenwood Genetics Center (GGC), Greenwood, South Carolina, five were classified as having severe MR, 14 had mild-moderate MR, and 11 were not evaluated for severity of MR. All had some family history of MR ranging from evidence of X-linked MR to one first-degree relative with MR. Two of the patients were also noted to have behavioral problems. All of the males had been tested for a mutation at the FMR1 locus and were found to be negative by molecular analysis. 3) Two hundred and seventy-one male subjects from the Tainan Educational Nursing Institute (TE) were evaluated. Plasma MHPG levels ranged from 7.05–49.02 pmoles/ml. The IQ of 90 subjects ranged from below 10–70, with one subject at 93. The degree of mental retardation for those whose IQ values were not available ranged from "medium" to "very severe." Subject TE175, who showed the lowest level of MHPG (7.05 pmol/ml) among subjects from TE, was diagnosed as having "very severe" mental retardation, cerebral palsy, and partially disabled legs. Subject TE170, who showed the second lowest level of MHPG (7.72 pmol/ml), had an IQ of 42, with retained testicle and epilepsy. 4) Fifty-one institutionalized male subjects from the New England region (MB/KS) with mental retardation of unknown etiology were studied. Ages ranged from 3–70 years (7 males were less than age 16 years). All were clinically judged to have moderate to severe mental retardation, often associated with significant behavioral problems [Butler et al., 1993, 1995; Butler and Singh, 1993]. In all these subjects there was a family history of mental retardation and, although pedigree data were often imprecise, in many there was evidence supporting classic X-linked transmission. Many of these males had cytogenetic testing excluding fragile X syndrome, and none had the phenotypic features typical of this condition.

Single-Strand Conformation Polymorphisms

The polymerase chain reaction (PCR) was used to amplify each exon from approximately 100 ng of genomic DNA extracted from blood samples using a Nucleon Extraction kit (Scotlab, Woburn, MA). PCR reactions contained 1 x reaction buffer (Boehringer-Mannheim, Indianapolis, IN), 200 μM dATP, dCTP, dGTP, and dTTP, 100 ng of the appropriate primer, and 1.25 units of Taq polymerase, as modified from Orita et al. [1989]; these were then layered with 200 μl of mineral oil. After a preliminary denaturation at 94°C for 2 min, the DNA then underwent 30 cycles of amplification consisting of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, followed by a final extension step at 72°C for 10 min. Ten microliters of this reaction were resolved on a 1.5% agarose gel to check for DNA purity. One microliter of the reaction product was used as template in a second PCR reaction containing α-^{32}P-dGTP (3,000 Ci/mmol; Amersham/Searle, Arlington Heights), 50 μM dGTP, and the same primers as in the previous reaction. An aliquot of the "hot" PCR reaction was diluted 1:50 in 10 mM EDTA/0.1% SDS. Three microliters of the diluted sample were then mixed with three microliters of a solution containing xylene cyanol, bromophenol blue, and formamide (US Biochemicals, Cleveland, OH), and boiled for 5 min. The samples were then cooled on ice, and loaded immediately onto a nondenaturing 6% (or 8% for exons 14 and 15) polyacrylamide/5% glycerol gel. SSCP gels were run at room temperature overnight at 5–10 W. Gels were then dried under vacuum and placed on Kodak X-AR film with an intensifying screen overnight at −80°C.

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PCR

Double-stranded PCR reactions contained: 100 ng genomic DNA, 100 ng of the appropriate primers [see Tivol et al., 1996], 1 x PCR buffer (Boehringer-Mannheim), 200 μM dGTP, dATP, dTTP, and dCTP, and 1.25 units of Taq polymerase overlaid with mineral oil. The samples underwent an initial denaturation at 94°C for 2 min. This was followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min. This was finally followed by 72°C for 10 min.

Sequencing

Double-stranded PCR products were used as template for double-stranded PCR sequencing. Four microliters of the PCR product were added to 2 μl of shrimp alkaline phosphatase (Gibco, Gaithersburg, MD), 1 μl of a 1:10 dilution of exonuclease 1 (Gibco), and 3 μl of H2O. This was incubated at 37°C for 15 min, and then at 85°C for 15 min. One microliter of the appropriate primer was then added and the mix was heated to 99°C for 5 min, placed in an ice-water bath for 5 min, and then quick-spun. Dideoxy sequencing was carried out per the manufacturer's instructions (USB Sequenase Kit, Cleveland, OH).

Metabolites

Plasma and platelet-rich plasma samples were collected using acid-citrate-dextrose solution as the anticoagulant [Murphy et al., 1976]. Plasma MHPG was analyzed by HPLC, using electrochemical detection [Scheinin et al., 1983].

RESULTS

In previous studies, levels of MHPG in plasma have proven to be the most sensitive indicator of levels of MAO-A activity in humans (Table I). Although the range of MHPG levels in the normal population is wide (4–38 pmol/ml in 142 individuals, Murphy personal communication), most have a narrow range (14.8 ± 0.8 pmol/ml, mean ± SD). Individuals with loss of MAO-A activity caused by inhibition with clorgyline [Pickar et al., 1981], or a known genetic deficiency of MAO-A and not MAO-B [Brunner et al., 1993a,b], or of both MAO-A and MAO-B [Sims et al., 1989; Lenders et al., 1996], have MHPG levels well below normal range. In 322 samples from males institutionalized with mental retardation, none showed MHPG levels below normal range (Table I). Interestingly, two severely mentally retarded males from different families in this group showed elevated levels of the trace amines, m-tyramine and phenylethylamine, the latter of which was within the range for documented MAO-B-deficient subjects [Murphy and Sims, unpublished observations; Lenders et al., 1996]. In contrast, no plasma MHPG was measurable (<0.1 pmol/ml) in two males out of 46 from a sexual disorders clinic (Table I). However, these latter samples were collected and analyzed only once, and it was not possible to obtain repeat samplings from these two individuals to confirm this finding.

As another mode of screening, blood DNA was extracted and analyzed by SSCP analysis or sequencing of the coding region of all 15 exons and splice junctions of the MAOA gene [Chen et al., 1991; Grimsby et al., 1991]. SSCP analysis was carried out on 32 control males and 11 Lesch-Nyhan males [Tivol et al., 1996; Costa et al., 1980]; on 32 males with

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Parkinson disease [Hotamisligil et al., 1994]; on 30 mentally retarded males with abnormal behavior (from the Greenwood Genetics Center; no plasma samples available for MHPG analysis); and on nine males from the sexual disorders clinic (Table II). SSCP analysis revealed only previously described normal polymorphisms [Tivol et al., 1996]. Double-stranded sequencing was then carried out on PCR fragments generated across the entire coding sequence and splice junctions of MAOA for eight controls [as reported in Tivol et al., 1996], the two mentally retarded males with undetectable levels of MHPG from the SDC, and two MR individuals (TE) with MHPG levels at the low end of the normal range (7–8 pmol/ml). Again, no novel nucleotide changes were observed beyond those polymorphisms noted in Tivol et al. [1996].

DISCUSSION

The monoamine oxidases, MAO-A and MAO-B, have a critical role in neurotransmitter metabolism and, thereby, neuronal communication. Recent studies in humans and mice have shown that complete loss of either MAO-A or MAO-B activity is compatible with life and reproductive fitness [Brunner et al., 1993b; Lenders et al., 1996; Cases et al., 1995]. However, in humans, even partial inhibition of MAO can lead to sensitivity to dietary biogenic amines. In particular, inhibition of MAO-A activity and intake of certain food substances have been associated with hypertensive crises which can be life-threatening [reviewed in Murphy et al., 1983; Berry et al., 1994]. The present study was undertaken to determine the frequency of disrupting mutations in the MAOA gene in human males. In addition to three control groups, neurologically normal, individuals diagnosed with Lesch-Nyhan syndrome, and individuals diagnosed with Parkinson disease, the study focused on individuals with mental retardation and sexual dysfunction, with or without deviant behavior, based on the reported phenotype of documented MAOA-deficient males in a family in the Netherlands [Brunner et al., 1993a,b]. No evidence of MAO-A deficiency as determined by plasma MHPG levels or disrupting mutations in the coding region of the MAOA gene (SSCP and sequencing of genomic DNA) was found in any of these 441 males with MR and/or deviant behavior, or in an additional 174 controls. Plasma MHPG levels are a very sensitive indicator of MAO-A activity, and based on drug studies in humans, would fall below the normal range at levels 10% normal activity. Only two of the targeted individuals screened for MHPG had abnormally low levels. Also, the *MAO-A* gene was screened only for mutations in the coding region and splice junctions. SSCP analysis alone should detect >80% of single base-pair changes and a greater percentage of more substantive changes. The two individuals with nondetectable MHPG could have mutations in noncoding regions of the gene that disrupt functional expression, as could other individuals analyzed for *MAO-A* mutations (but not screened for metabolites). This study supports a relatively low incidence of the MAO-A deficiency state in the human population.

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TABLE I

Plasma MHPG Levels

Subjects	Range of values (pmol/ml)
Groups	
General population (N = 142)	4.2–38.1[a]
X-linked mental retardation (MB/KS; N = 51)	8.1–36.0
Sexual disorder clinic (N = 46)	0.0[b]–21.7
Mental retardation (TE; N = 271)	7.0–49.0
Gene-related or drug-induced reductions in MAO activity	
Clorgyline-treated depressed patients (N = 10)[c]	1.8–4.3
MAO-A/B deficiency (N = 5)	0.1–0.4
MAO-A deficiency (N = 2)	1.1–2.1
Selegiline-treated patients (N = 18)[c]	2.6–8.7

[a]Mean ± SD = 14.8 ± 0.8.

[b]Two individuals with no MHPG peak on HPLC-EC.

[c]Clorgyline used at doses which selectively inhibit MAO-A; selegiline at concentrations which inhibit both MAO-B (completely) and MAO-A (partially).

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TABLE II

DNA Analysis of MAOA Exons[*]

Groups	Number analyzed	SSCP	Full sequence
Controls[a]	32	32	8
Lesch-Nyhan	11	11	3
Parkinson disease	32	32	ND
X-linked MR			
GGC	30	30	ND
TE	2[b]	ND	2
SDC (BG)	11	9	2[c]

[*] ND, not done; MR, mental retardation; GGC, Greenwood Genetics Center; TE, Tainen Educational Nursing Institute; SDC, sexual disorder clinic; BG, collected by B.D.G.

[a] From Tivol et al. (1996]. For 80–100% of the coding region.

[b] Individuals TE175 and TE170 with levels of plasma MHPG <8 pmol/ml.

[c] Two individuals with no detectable plasma MHPG.