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Brief Research Communication

Analysis of the Genomic Structure of the Human Glycine Receptor α2 Subunit Gene and Exclusion of This Gene as a Candidate for Rett Syndrome

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The gene that encodes the human α2 subunit of the inhibitory glycine receptor (GLRA2) is located on the X chromosome (Xp22.2) in a candidate region for a number of neurological disorders. Recently, an exclusion mapping strategy identified this region to be concordant in familial Rett syndrome (RTT) patients. Based on its established expression pattern and known function, GLRA2 was selected as a candidate gene for Rett syndrome. Major gene rearrangements were excluded based on Southern analysis using the GLRA2 cDNA as probe. To identify more subtle mutations, we determined the genomic structure for GLRA2, which consists of nine exons and a putative alternatively spliced exon 3. The exon-intron boundaries were sequenced in order to design primer sets for polymerase chain reaction (PCR) amplification of all exons and their immediately flanking intronic regions. PCR products amplified from genomic DNA isolated from 40 RTT patients were subsequently characterized by heteroduplex analysis, and no mutations were detected. Characterization of the intron-exon structure of GLRA2 will facilitate future mutational analysis of this gene for other neurological disorders mapping to human Xp22.2. Am. J. Med. Genet. 78:176–178, 1998.

KEY WORDS: Rett syndrome; glycine receptor α2 subunit; Xp22.2

INTRODUCTION

Exclusion mapping studies using families where maternal inheritance of the Rett syndrome (RTT) mutation is clearly demonstrated showed that two pairs of affected half-sisters and an affected aunt-niece pair are discordant for the region between DXS1053 in Xp22.2 and DXS1222 in Xq22.3 [Ellison et al., 1992; Schanen et al., 1997]. Hence, the candidate regions for the RTT gene include Xp22.2-pter and Xq22.3-qter, with the exception of the pseudoautosomal regions. Human Xp22.2 is also a candidate region for a number of neurological disorders including Aicardi syndrome (MIM 304050), Partington syndrome (MIM 309510), X-linked Charcot-Marie-Tooth neuropathy (MIM 302801), and X-linked mental retardation syndromes (MRX1, MIM 309530).

The glycine receptor α2 subunit gene (GLRA2) maps to human Xp22.2 and therefore is a good candidate for any one of the neurological disorders described above [Siddique et al., 1989; Econs et al., 1990; Grenningloh et al., 1990]. The glycine receptor is a multisubunit, ligand-gated ion channel that mediates inhibitory neurotransmission [reviewed in Betz, 1992]. The four developmentally regulated ligand-binding α subunits assemble with various structural β subunits to form chloride channels that display functional and pharmacological differences [reviewed in Matzenbach et al., 1994]. Because the α2 subunit gene is abundantly expressed in embryonic and neonatal spinal cord and brain, mutations in GLRA2 might interfere with normal neuronal differentiation and lead to severe mental retardation [Malosio, 1991]. Given the expression pattern of GLRA2 and the functional importance of inhibitory neurotransmission, we hypothesized that the α2 subunit is a reasonable candidate for RTT. In search of rearrangements and/or mutations in GLRA2 in RTT, we screened genomic DNA from RTT patients by Southern analysis and heteroduplex analysis (HA).

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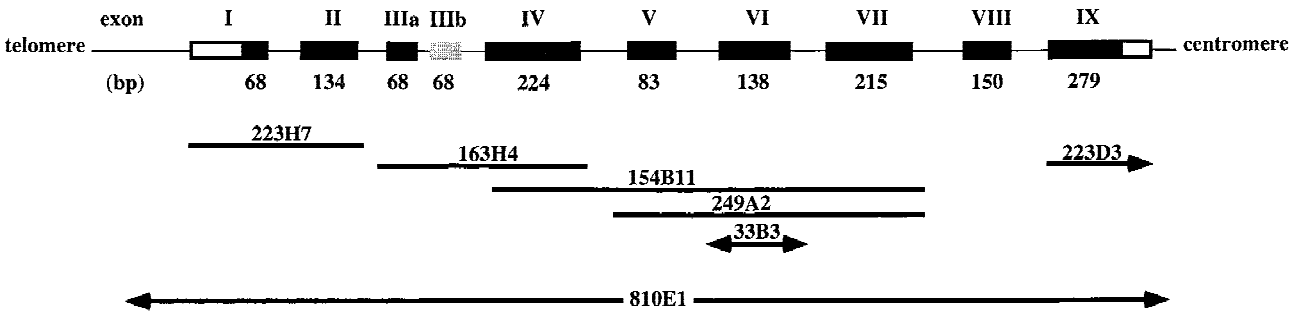

Fig. 1. Exon-intron organization of the human glycine receptor α2 subunit gene. Top: The nine exons coding for the GLRA2 are depicted by solid boxes, and the untranslated exon sequences which are known are shown by open boxes. The size in base pairs (bp) for the coding regions in each exon is indicated. Exon 3b, 68 bp, is shown by a shaded box. Exon and intron sizes are not drawn to scale. Exon I is telomeric to exon IX. Bottom: The position of six cosmids and one YAC (810E1) are shown. The exact ends of cosmids 33B3 and 223D3 are unknown. The coding sequence for GLRA2 maps between DXS7168 and PIGA.

The genomic organization of the GLRA2 gene was analyzed to identify exon-intron boundaries for proper amplification of exons in HA.

METHODS, RESULTS, AND DISCUSSION

Southern analysis was performed using the GLRA2 cDNA as a probe and RTT DNA from six unrelated patients. After restriction analysis with 22 different enzymes, no detectable abnormalities were identified.

Using the cDNA encoding the GLRA2 gene as a probe, the Lawrence Livermore X-chromosome cosmid library (LLOXNC01) was screened to isolate clones that would together correspond to the entire genomic locus. This screen resulted in 11 cosmid clones with partial overlap and redundancy. Based on the deduced primary sequence and exon-intron architecture of the mouse glycine receptor α2 subunit, which shares 99% amino acid identity with the human counterpart, primers were designed to yield 10 sequential products using the polymerase chain reaction (PCR) from human GLRA2 cDNA template. The PCR products were subsequently used as probes to order six nonredundant cosmid clones and to obtain nearly complete coverage of the genomic locus (Fig. 1). None of the cosmids contained exon 8. The yeast artificial chromosome (YAC) clone, 810E1, was shown to contain the entire genomic locus by PCR amplification of exon 1 and 9. Analysis of sequence tagged sites (STSs) derived from GLRA2 exons and other Xp22.2 markers that have been ordered established that the direction of the GLRA2 transcript is telomere to centromere.

Exon-intron boundaries were deduced for the entire coding region, except for exon 8, by double-stranded sequencing using the ordered cosmids as template. The boundaries for exon 8 were identified by inverse PCR using Hae III digested YAC 810E1 fragments as template. The inverse PCR products were cloned and sequenced. The human GLRA2 is encoded by 9 exons. A putative exon 3b, separated by less than 100 base pairs downstream from exon 3a, may generate alternative splice isoforms of the human α2 subunit as previously described for the rat and murine α2 subunits [Kuhse et al., 1991; Matzenbach et al., 1994]. These results reveal a high degree of structural conservation of the α2 subunit between rodents and human. The consensus dinucleotide GT and AG were identified at all the donor and acceptor splice sites (Table I).

Primer pairs were designed in intronic sequences for PCR amplification of each exon (Table II). PCR products amplified from genomic DNAs of 40 RTT patients were subjected to hetenduplex analysis (HA) on nondenaturing mutation detection enhancement (MDE) polyacrylamide gels (AT Biochem Malvern, PA). No band shifts, indicative of polymorphisms nor mutated nucleotides, were observed for any of the amplified fragments. Unfortunately, the HA was not possible

TABLE I. Exon-Intron Junctions of the Human GLRA2 Subunit Gene

Exon no	Exon length (bp)	5′ Splice donor		3′ Splice acceptor	
I	68[a]		ATGAAC	CTTCAG	gtaggtgaaacgactttgcatgttg
II	134	gctaaaagattaatgatgtgtttag	GACGGC	TTAAAG	gtaggttccacttaaacttacgtta
IIIa[b]	68	gcaatattctcattgcattctgcag	GTCCTC	ACCATG	gtaagtgctgcaatgccactggcaa
IIIb[b]	68	aaattttctattgttcaacttgcag	GGCCTC	ACAATG	gtgagtgggactgagcattgaagcc
IV	224	acacttgtccacggcatttctgtag	GACTAC	TATCAG	gtaagcctccattggctgcacatgt
V	83	tttaatttttttttttgtttgctaag	ACTCAC	AGAGTT	gtaagtcaccactgttgaaatgact
VI	138	tttcactatgatttctttacctcag	TTGGGT	ACACTG	gtaagtttctttttttttttttttttc
VII	215	ttaaatgatcatttcctccttctag	GAAAGT	CCAAAG	gtaagaaatcttgcttgataacaga
VIII	150	gtgtgtgtctctctctctctctcag	GTCTCC	AATAAG	gtatgattgcccctcagttcagaca
IX	279[c]	gcttcctctgtctttattccgtcag	GAAGAA	AAATAG	

[a]Length from the first nucleotide of the open reading frame.
[b]IIIa and IIIb are putative alternatively spliced exons.
[c]Length to the stop codon of the open reading frame.

TABLE II. PCR Primer Pairs for Amplification and HA of the Human GLRA2 Subunit Gene

Exon no.	PCR product length (bp)	Forward primer (5′–3′)	Reverse primer (5′–3′)	Annealing temperature (°C)
I	298	CACTTTGTCCTAGCATCTTTCTGG	TACCACCCTCCCACATTATTAGG	58
II	273	GTTAGCTCTCAAGGGATGCAAATG	CCCTGAAAATGTTTTTCAGTTAAC	58
IIIa	203	ATTGCACAGATGTTAATGGAGCTG	CAGTTTGTGTTCACAGCATAATTG	55
IIIb	202	CAATTATGCTGTGAACACAAACTG	ATGTCTCACAAATATGCCACAGTG	55
IV	345	GAAATGCAAATAGAACTCCTGTGC	CAGCTTTACATTAGCGGAGAAATG	58
V	280	TGTCACTTTGCTTAATGCCAAGTG	GGAATGAGTTGTAAGGGTCCTTTC	55
VI	357	TGACTGAGCGTATGTCTGCTTTAG	GCCTGGTAAATATGCAGCAATTAG	58
VII	373	AAACAACGTGGGATAATGGAATTG	CCAACATTTCTGTCCTCACAGAAC	58
VIII	320	TCCTGGCAGGCTTTCATAGTC	TCTTGCTTATTGTGTGCTCTTCTG	58
IX	444	TCTAAAGAATTTTAAGCATCTTCC	CCTCTGTCAAAGTGATATAAAGAC	55

with exons 5 and 6, probably due to the high T/A content immediately preceding exon 5 and following exon 6. Hence these exons were screened for mutations by sequencing. DNA from somatic cell hybrids retaining either the maternal or paternal X chromosome from two RTT patients (one familial and one sporadic) was used for PCR amplification and sequencing of all the exons of the GLRA2 gene [Ellison et al., 1992]. These PCR products were sequenced employing the same primer set that was used for amplification. A single-base substitution, in exon 7, was detected by sequence analysis in three of the four alleles sequenced. Compared to the α2 subunit cDNA and cosmid clone sequences, the base change is a C-to-T transition at nucleotide 747 in the coding region, which does not lead to an alteration of the amino acid (His). Analysis of this sequence in 15 independent chromosomes demonstrated a C in six alleles and a T in nine alleles, confirming that the variation is a polymorphism. No other variants were noted.

Based on the data presented in this study, the GLRA2 gene is very unlikely to be mutated in RTT given the absence of major gene rearrangements and mutations that lead to alterations in the coding region. These data do not exclude a promoter mutation or loss of transcription of one allele in RTT patients. However, given the large number of RTT patients evaluated in the HA study (40) this possibility is less likely. The characterization of the exon-intron structure of the GLRA2 gene will facilitate future mutation analysis of this gene as a candidate for the neurological diseases that map to Xp22.2.

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