Analysis of GLRA1 in hereditary and sporadic hyperekplexia: a novel mutation in a family cosegregating for hyperekplexia and spastic paraparesis

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Abstract
Hyperekplexia is a rare condition characterised by the presence of neonatal hypertonia and an exaggerated startle response. Mutations have been described in GLRA1, the gene encoding the $\alpha 1$ subunit of the glycine receptor, in dominant families with hyperekplexia and in a single sporadic case, thought to represent an autosomal recessive form of the disease. In this study the coding region of the GLRA1 was analysed in eight probands with hyperekplexia by restriction digest and sequencing. Two familial cases were found to possess the previously described G1192A (R271Q) mutation in exon 6. In an additional family in which hyperekplexia cosegregates with spastic paraparesis, a novel A to G transversion at nucleotide 1206 in exon 6 was detected that changes a lysine at amino acid 276 to a glutamate (K276E). In four sporadic cases no mutations were found. In addition, one familial case did not have a mutation in the coding region of the gene.

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Key words: hyperekplexia; glycine receptor; GLRA1.

Hyperekplexia or startle disease is characterised by the presence of an exaggerated startle response to unexpected auditory, visual, or sensory stimuli. Affected subjects frequently present at birth with hypertonia. Hyperekplexia is usually inherited in an autosomal dominant manner. Linkage studies localised the gene to distal chromosome 5q.¹ Subsequently, mutations were detected in exon 6 of the gene encoding the α_1 subunit of the glycine receptor, GLRA1.² The previously described mutations are listed in table 1. All occur in large dominant pedigrees²⁻⁵ except for one which may represent a recessive form of the disease.⁵ In this study, GLRA1 was analysed in eight probands with hyperekplexia. In three cases the families clearly display dominant inheritance; four cases are sporadic. In one family (family R) the family history is uncertain and it is possible that they may represent an autosomal recessive form of the disease. The pedigrees of the patients under investigation are shown in the figure. In the case of family N, lower limb spasticity was found to cosegregate with the hyperekplexia trait⁷ and is linked to markers in the GLRA1 region on chromosome 5.

DNA was extracted from each proband and their relatives and intronic oligonucleotide primers flanking all exons of the GLRA1 gene^{2,3} were used to amplify individual exons from each proband. Exon 6 PCR products from all probands were investigated for the presence of the most common mutations at nucleotide position 1192 by restriction digestion with *Xho*I. G to A and G to T mutations at this position abolish an *Xho*I restriction site. Probands G and M were heterozygous for this site. Sequence analysis of exon 6 in both cases showed them to be heterozygous for a G to A base transition resulting in an arginine to glutamate amino acid change. Restriction fragment analysis of exon 6 PCR products from all available family members showed that these mutations segregated completely with the disease trait (data not shown).

Exon 6 PCR products from probands not possessing the 1192 mutation were subjected to sequence analysis (sequencing primers shown in table 2) using paramagnetic bead technology and the Applied Biosystems Prism single stranded sequencing kit. A heterozygous A to G base transition was detected at nucleotide 1206 in family N predicted to cause a Lys276Glu amino acid change in the mature

Table 1 Previously reported GLRA1 mutations in hyperekplexia

Exon	Base change	Amino acid substitution	Mode of inheritance	Reference
6	G1192A	Arg271Glu	Dominant	2,3,4
6	G1192T	Arg271Leu	Dominant	2
7	A1216G	Tyr279Cys	Dominant	3
6	T1112A	Ile244Asn	Recessive	4

Table 2 Sequencing primers used in the analysis of GLRA1

Primer	cDNA Nucleotide No (22)	Sequence (5'-3')
Exon 1 - F	62-78	GCAGGTCTGGACGCTCTC
Exon 1 - R	281-264	TGCTTTGTAGTCCACGAG
Exon 2 - F	382-400	GCTCGCTCCGCAACCAAGC
Exon 2 - R	470-453	GGGCCTGATCCTGGCATC
Exon 3 - F	486-503	CCAGTGAACGTGAGCTGC
Exon 3 - R	537-520	CAGCAATGGAACCAAGC
Exon 4 - F	559-576	TCAACATCTTCCTGCGGC
Exon 4 - R	762-744	AGAGGACATTCCCATTCC
Exon 5A - F	781-800	TGACACTGGCCTGCCCATG
Exon 5A - R	Intronic	GATTTCTGCCTATCCCATTG
Exon 5B - F	Intronic	TGCTTCTGAGTGGTGACTG
Exon 5B - R	983-965	GTGCTTGGTGCAGTATCTC
Exon 6 - F	1131-1150	GCGGCAGATGGGTTACTACC
Exon 6 - R	1203-1187	GCAGAGATGGCTCGAGAG
Exon 7 - F	1209-1222	GTGTCCTATGTAAAGCCATTG
Exon 7 - R	1341-1323	GCTTCCTCCTGAATCGGAG
Exon 8 - F	1424-1443	CAAGGATGGCATCTCAGTCA
Exon 8 - R	1495-1477	GACTTAAGATGGTGACAGGAG

F = forward, R = reverse.

Hyperkplexia pedigrees. Closed symbols indicate affected members and open symbols unaffected subjects. In family N the hyperkplexia trait cosegregates with spastic paraparesis.

protein. This change results in the loss of a *StyI* restriction site and was shown to segregate consistently with the hyperkplexia/spastic paraparesis trait in this family (data not shown). This mutation was not observed in any of the other families investigated and in addition was not observed in 100 normal chromosomes. PCR products from all other exons were sequenced in all affected subjects; no further mutations were detected.

We have therefore detected mutations in *GLRA1* in three out of eight probands with hyperkplexia, all of whom originate from families in which there is a clear autosomal dominant pattern of inheritance. It is possible that the remaining five cases could represent new dominant mutations, or an alternative, autosomal recessive form of the disease as has been suggested previously.⁵

The finding of a mutation in *GLRA1* in a family in which hyperkplexia cosegregates with lower limb spasticity raises the possibility that other related phenotypes may be attributable to mutations in *GLRA1*. Autosomal dominant pure hereditary spastic paraparesis is known to display genetic heterogeneity and there are families in which the trait appears unlinked to any of the three mapped loci on chromosomes 14, 2, or 15.⁸⁻¹¹ *GLRA1* may therefore represent a candidate gene for these unlinked families.

Note added in proof

A further mutation in exon 6 of *GLRA1* which changes a glutamine to a histidine at position 266 has recently been described in a dominant Italian family.¹²

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