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SHORT REPORT

A mutation (V260M) in the middle of the M2 pore-lining domain of the glycine receptor causes hereditary hyperekplexia

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We investigated the molecular basis of hyperekplexia (STHE), an inherited neurological disorder characterised by neonatal hypertonia and an exaggerated startle response, in a kindred and identified a novel missense mutation in the pore-lining M2 domain of the α_1 subunit of the glycine receptor (*GLRA1*). Sequencing analysis of all exons of the *GLRA1* gene revealed a G1158A base transition in affected, heterozygous patients. The base transition results in a valine to methionine substitution at codon 260 in the middle of the M2 transmembrane domain. The location within the M2 domain suggests for this substitution a likely role in altering ion channel properties. *European Journal of Human Genetics* (2001) **9,** 873–876.

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Introduction

Hyperekplexia or startle disease (STHE; OMIM 149400) is a rare autosomal dominant or recessive neurological disorder, with high penetrance and variable expression. It is characterised by neonatal hypertonia and an exaggerated startle response to unexpected stimuli, particularly auditory, followed by a period of generalised stiffness.[1] Different mutations in the coding region of the α_1 subunit of the inhibitory glycine receptor chloride channel gene (*GLRA1*) have been shown to cause STHE.[1,2]

The glycine receptor (GlyR) is a member of the ligand-gated ion channel receptor superfamily ($LGIC_S$), which also includes γ-aminobutyric acid (GABA), acetylcholine (nACh) and 5-hydroxytryptamine ($5\text{-}TH_3$) receptors and are localised in the postsynaptic membrane.[3] All share considerable sequence and structural homology and consist of five subunits arranged to form an ion channel pore, which in the case of GlyR, is selective to Cl^-. Each α_1 subunit is made up of a long NH_2-terminal extracellular domain, a short C terminus and four hydrophobic transmembrane spans (M1–M4), including the pore-lining M2 domain.[4] Many of the STHE mutations studied to date cause single-point substitution of residues in either the short intracellular (M1–M2) or extracellular (M2–M3) loops flanking the luminal M2 domain and are crucial in inhibiting the transduction of the allosteric coupling from ligand binding to channel activation.[5] Here we report, in a kindred with hereditary hyperekplexia, a novel amino acid substitution (V260M) occurring in the middle of the GlyR M2 domain.

Materials and methods

Subjects

The pedigree of the family is shown in Figure 1. The index patient (II, 1) at the age of 1 month was affected by stiffness of the four limbs, misdiagnosed as spasticity, and by sustained startle responses to sudden, unexpected tactile or sound stimulations, such as nose tapping, abrupt displacement and loud noise. The number of the attacks varied from day to day, depending on the environmental stimulations. They never occurred while the child slept. During attacks, consciousness remained

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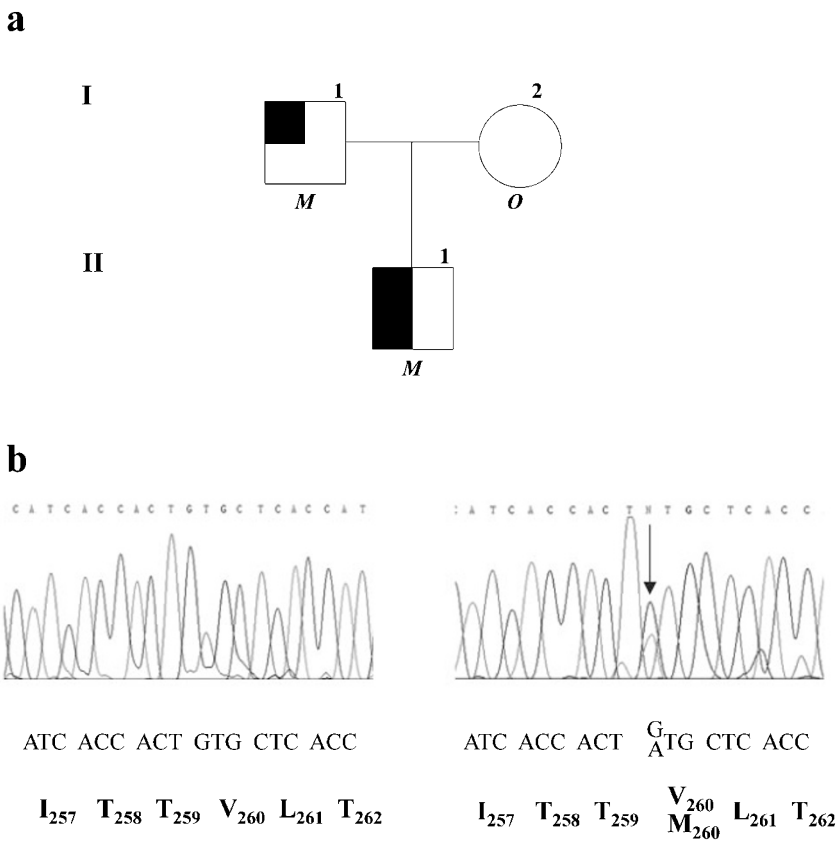


Figure 1 (**a**) Pedigree of the examined Italian family with STHE. Half-filled symbol represents subject characterised by exaggerated startle response and muscular hypertonia; quarter-filled symbol represents subject characterised by exaggerated startle response. M=heterozygous for the G/A mutation in *GLRA1* exon 6; 0=homozygous for the wild type. (**b**) GLRA1 exon 6 partial double-stranded sequencing and deduced amino acid sequences of Individuals I-2 (left) and II-1 (right). The arrow indicates the position of the G/A mutation leading to the valine (V)/methionine (M) substitution at codon 260.

clear. By the age of 12 months, the stiffness abated significantly, but startle responses persisted. Psychomotor development was normal. On examination, the child was alert, with weight/length ratio at the 50° percentile and head circumference at 50° percentile. Muscle tone increased during manipulation, becoming again normal during sleep. Magnetic resonance imaging of the head was normal. No treatment was started. His parents were instructed to stop the stronger startle responses by flexing the baby's head and trunk over his legs.[6] His father at first denied similar problems, but, interestingly, once the genetic study had been performed, he admitted to having suffered from abnormal startle responses and a sort of rigidity during early infancy. His neurologic examination was unremarkable.

Collection and analysis of blood samples was performed after appropriate informed consent.

Molecular studies

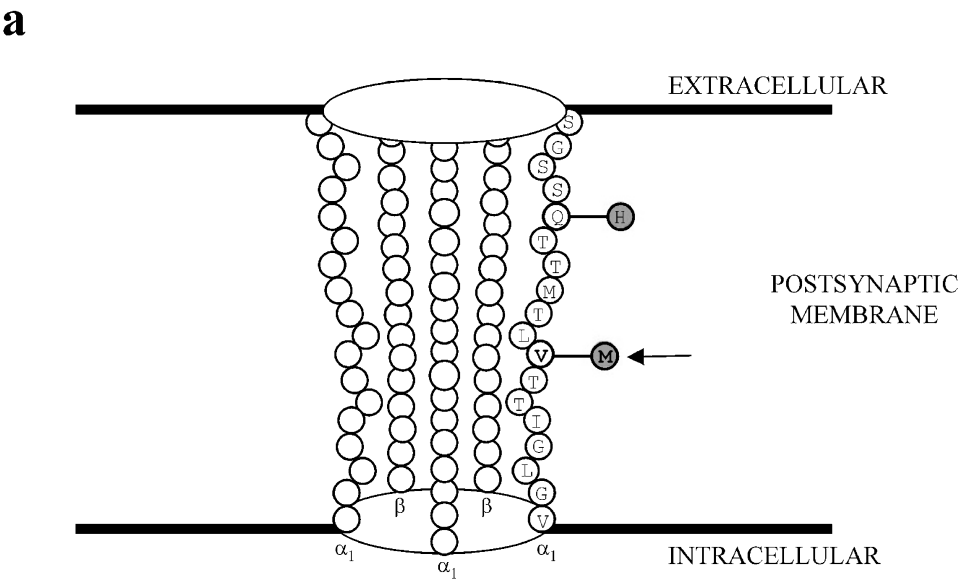
DNA was extracted from the members of the kindred by phenol-chloroform technique and nine sets of primers flanking all exons were used to amplify the entire GLRA1 coding sequence.[7] PCR products were directly sequenced using an ABI PRISM 310 sequencer and the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA, USA).

Results

Sequence analysis of exon 6 in the propositus and his father showed them to be heterozygous for a G to A base transition at nucleotide position 1158 of the *GLRA1* gene (Figure 1a). This mutation results in a valine to methionine substitution at codon 260 (Figure 1b). It lies near the centre of the highly conserved, key functional M2 domain (Figure 2a). None of the 150 Italian independent controls showed this mutation.

Discussion

Using genetic approaches, several mutations have been described that cause hyperekplexia; gene structure-func-



b

	M2 (1' … 18')
GlyR α_1	APARVGLGITT**V**LTMTTQSSGSRA
GABA$_A$R α_1	VPARTVFGVTTVLTMTTLSISARN
nAChR α_7	G-EKISLGITVLLSLTVFMLLVAE
5-HT3R α_1	G-ERVSFKITLLLGYSVFLIIVSD

Figure 2 (**a**) Schematic representation of the pentameric structure containing three α_1 and two β subunits, which makes up the pore-lining M2 domain of the glycine receptor channel. Amino acids of the α_1 subunit are shown as circles with their single-letter code. Shaded residues attached by stems represent amino acid substitutions producing STHE. The arrow marks the substitution presented in this work. (**b**) Alignment of residues in the second membrane-spanning (M2) region of the α_1 subunits of the glycine, GABA and 5-hydroxy-tryptamine receptors and of the α_7 subunit of the acetylcholine receptor. To facilitate comparison among the M2 domains of different LGIC members, a universal numbering system (see ref [10]) has been used. The residues mutated in STHE are shaded. The valine (V) mutated at position 8 is shown in bold and underlined.

tion analyses of some of these mutant alleles have provided further insight into the biology of ion channels although many questions concerning gating and permeation remain unresolved.[8]

The only dominant STHE mutation which predicts a missense mutation to a residue that is not located in either the M1 to M2 or the M2 to M3 loop is the substitution of a glutamine by a histidine at residue 266,[9] at position 14 of the M2 domain using the terminology of Miller which assigns position 1 to the amino terminus of the M2 domain (the intracellular end) (Figure 2b).[10] This mutation primarily manifests itself as a large reduction in single-channel open time without associated major changes in ligand-binding parameters, or permeation properties, such as conductance and anion-to-cation selectivity.[11]

In the present study we found in a kindred with STHE, a valine to methionine substitution at position 8. In the other ligand-gated ion channels, this position is occupied by the same amino acid, or by another similar non polar residue (Figure 2b). It immediately precedes a leucine highly conserved in all the members of the LGIC family. The hyperekplexia mutation we found, like to the other STHE mutations investigated to date, should produce a 'decrease of function' phenotype and, although only functional studies might elucidate the precise nature, the location of this substitution in the middle of the M2 domain suggests a role in altering ion channel properties. Its identification could help shed further light on the physio-pathology of the fast neurotransmission mediating $LGIC_S$.

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