ONLINE MUTATION REPORT

A novel mutation (R218Q) at the boundary between the N-terminal and the first transmembrane domain of the glycine receptor in a case of sporadic hyperekplexia

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Hyperekplexia or startle disease (STHE, OMIM 149400) is a rare neurological disorder characterised by neonatal generalised muscular hypertonia followed by a period of generalised stiffness and by an exaggerated startle response to sudden external stimuli that persists throughout life.¹ Hyperekplexia is usually inherited in an autosomal dominant manner although there is also evidence for a recessive form.¹⁻² Both forms are caused by mutations in the same gene, *GLRA1*, mapping on 5q31.2 and encoding for the $\alpha 1$ subunit of the inhibitory glycine receptor chloride channel.¹⁻¹⁰ Recently, it has been shown that glycine receptor beta subunit mutations can also lead to hyperekplexia.¹¹

Glycine receptor (GlyR) belongs to the ligand gated ion channel receptor superfamily (LGICs), which includes α -aminobutyric acid, acetylcholine, and 5-hydroxytryptamine receptors and are localised in the postsynaptic membrane. All share a common pentameric structure that spans the membrane, comprising homologous subunits arranged in a ring to form a central ion conducting pore. In the case of GlyR, the $3\alpha 1$ - 2β complex is selective for chloride ions.¹²

Each subunit is made up of a large glycosylated N-terminal extracellular domain, a short extracellular C-terminus, and four hydrophobic transmembrane spans (M1-M4), separated by two short hydrophilic loops M1-M2 (intracellular) and M2-M3 (extracellular) flanking the pore lining M2 domain, and by a long, extracellular, leucine rich M3-M4 loop.¹³⁻¹⁴

Considering both dominant and recessive hyperekplexia cases, 15, mostly missense, *GLRA1* mutations have been reported. Here we describe a novel *GLRA1* mutation that results in the substitution of the arginine at position 218 with a glutamine (R218Q). It lies at the boundary between the N-terminal and the first transmembrane domain and occurred de novo in a patient with hyperekplexia.

MATERIALS AND METHODS

Subjects

The proband is the third child of healthy, unrelated parents. Two older brothers are healthy. He was born at 40 weeks of gestation after an uneventful pregnancy; his birth weight was 4390 g and length 52 cm. Apgar scores were 7 and 10 at one and five minutes, respectively.

After two hours the infant developed cyanotic spells and spasms of the four extremities. Generalised twitching was elicited by touching and loud sounds. On examination the infant showed intermittent hypertonia with opisthotonus. Analysis of serum, urine, and cerebrospinal fluid as well as brain magnetic resonance imaging were normal. He continued having attacks of sudden, generalised jerks, mostly precipitated by sounds. In addition he had numerous episodes of sudden stiffness, resulting in falls without protective reflexes. During sleep, he often suddenly became stiff with respiratory arrest for 5 to 40 seconds. Tapping his nose resulted in a sudden jerk of his body.

Key points

- Hyperekplexia or startle disease (STHE, OMIM 149400) is a rare neurological disorder characterised by neonatal generalised muscular hypertonia followed by a period of generalised stiffness and by an exaggerated startle response to sudden external stimuli.
- It is caused by mutations in the *GLRA1* gene, which encodes the $\alpha 1$ subunit of the inhibitory glycine receptor chloride channel.
- Considering both dominant and recessive hyperekplexia, 15 mutations have been reported.
- Here we describe a novel *GLRA1* mutation, occurring de novo in a patient with hyperekplexia, which results in the substitution of the arginine at position 218 with a glutamine (R218Q). It lies at the boundary between the N-terminal and the first transmembrane domain and could inhibit the transduction of allosteric coupling from glycine binding to channel activation.

The clinical diagnosis of hyperekplexia was made. Treatment with clonazepam did not result in an effective improvement of symptoms. Psychomotor development appeared moderately delayed at 12 months of age and at 6 years of age the boy received special education in school.

The parents denied similar problems and their neurological examination was unremarkable.

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

Molecular studies

DNA was extracted from the proband and his parents by the phenol-chloroform technique. The whole coding region of *GLRA1* was amplified by nine sets of primers flanking all exons and PCR products were directly sequenced using an ABI PRISM 310 and the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA).⁴ According to published reports amino acid numbering starts from the alanine at position 29.

RESULTS AND DISCUSSION

The sequence analysis showed a heterozygous G→A base transition (nt 1030 GenBank XM_032738) in *GLRA1* exon 6 of the proband. This nucleotide change leads to an amino acid substitution at codon 218 (CGC→CAG) that replaces the highly conserved arginine at the boundary between the N-terminal and the M1 domain of the $\alpha 1$ subunit of the glycine receptor with a glutamine.³⁻¹⁵ This amino acid is invariant in glycine and GABA α receptor subunits from all species examined.¹⁵ The variation abolishes the restriction site *AccI*.

Figure 1 Schematic overview of the hyperkplexia producing mutations present in the $\alpha 1$ subunit of the human glycine receptor. The arginine (R) mutated at position 218 is shown in black.

Using *Acil*, the mutation was not found in the patient's parents or in 150 independent Italian controls. Paternity testing, performed in this patient according to Lynch,¹⁶ showed a paternity index of 100 that corresponds to a probability of paternity of 99.0%, suggesting the occurrence of a de novo mutational event.

Among patients with sporadic hyperkplexia, the present is the second case in which this phenotype has arisen from a de novo mutation rather than from the transmission of recessive alleles from unaffected heterozygous parents.^{2 4 6-8}

Seven missense mutations associated with dominant hyperkplexia have been described to date. They give rise to the substitution of amino acids located in the intracellular M1-M2 loop (one case), in the inner of the M2 domains (two cases), in the extracellular loop connecting segments M2-M3 (three cases), and within the large intracellular loop between M3 and M4 (one case) (fig 1). With the exception of the recurrent substitution of the arginine at position 271, which has been found in six out of 12 unrelated patients with dominant hyperkplexia investigated so far, the other mutations occurred no more than once.

The arginine 271 lies at the boundary between the M2-M3 extracellular loop and the M2 domain and plays a crucial role in the allosteric signal transduction pathway from the glycine binding process to the channel activation gate.^{13 14}

Among the *GLRA1* mutations associated with hyperkplexia in the heterozygous state, the R218Q substitution represents the first one which lies in the GlyR N-terminal region. Many studies have highlighted the role of this ectodomain for the ligand binding and the assembly properties of the glycine receptor. Disrupting a positive charge, R218Q might affect the correct protein folding and/or trafficking. More likely, considering the analogies of the arginine at position 218 with the arginine at position 271, the possibility exists that the R218Q substitution could inhibit the transduction of the allosteric coupling from glycine binding to channel activation, therefore suggesting that besides the M1-M2 and the M2-M3 loops the portion of the GlyR N-terminal region neighbouring to the M1 domain may also act as a structural component mediating this process.

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