Letters to the Editor

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A Novel Mutation (Gln266→His) in the Alpha1 Subunit of the Inhibitory Glycine-Receptor Gene (GLRA1) in Hereditary Hyperekplexia

To the Editor:

Hereditary hyperekplexia, or Startle disease (STHE; MIM 149400), is a rare dominant neurological disorder with high penetrance and variable expression, mainly characterized by infantile hypertonia and exaggerated startle response. Genetic and radiation hybrid mapping of the hyperekplexia region on distal 5q (Ryan et al. 1992) pointed to a candidate region that included the gene for glycine receptor (Warrington et al. 1992). Mutations in the α1 subunit of the inhibitory glycine-receptor gene (GLRA1) subsequently found both in familial and sporadic cases have been causally related to the disease (Shiang et al. 1993, 1994). The glycine receptor (GlyR) is a ligand-gated chloride-channel protein-mediating synaptic inhibition in the spinal cord and other brain regions. It is a pentameric complex comprising homologous α and β subunits, built from a large N-terminal region followed by four-membrane–spanning segments (M1–M4) (Grenningloh et al. 1990). The mutations, which were first identified in the GLRA1 gene, occur in the same base pair of exon 6 and result in the substitution of Arg271 of the mature polypeptide with an uncharged amino acid, either leucine, in one family, or glutamine, in three families (Shiang et al. 1993). The Arg271→Gln substitution is likely to be the most common, because it has subsequently been found in four other families (Schorderet et al. 1994; Shiang et al. 1994) and in a patient without a clear family history (Shiang et al. 1994). Two other mutations, Tyr279→Cys (Shiang et al. 1994) and Ile244→Asp (Rees et al. 1994) have been so far identified. It is interesting that homozygosity for the latter mutation was found in an apparently sporadic case, offspring of consanguineous heterozygous parents. Evidence for recessive forms of hyperekplexia in man is enhanced by recent findings in the recessive mouse mutants *spasmodic* (*spd*) and *oscillator* (spd^{ot}), resulting from an Ala52→Ser substitution and a microdeletion of 7 nt, respectively, in the homologous Glra1 gene (Buckwalter et al. 1994; Ryan et al. 1994). A very similar murine phenotype that exhibits autosomal recessive inheritance, *spastic* (*spa*), has been associated with an insertional mutation in the β subunit–encoding gene (Glyrb) of the glycine receptor (Kingsmore et al. 1994). The mutational repertoire underlying human hyperekplexia is thus likely to be incomplete because alterations causing either recessive or dominant forms not associated with the classical site mutation might remain undetected. Consistent with this hypothesis, several sporadic and familiar cases screened by either denaturing gradient-gel electrophoresis (Shiang et al. 1993, 1994) or SSCP in all exons all escaped detection of the mutation (Elmslie et al. 1994).

We studied an Italian family with individuals displaying a variable combination of clinical signs (fig. 1). The 1-year-old proband (IV-2) had excessive startle response, muscular hypertonia, and a continuing flexion state, whereas only startle response during early infancy was referred by her mother (III-3), aged 30 years. The proband's second cousin (IV-4) died when 45 d old from apnea following myoclonic fits, and her father (the proband's uncle, III-6) displays hypertonia and muscle stiffening. No history of infantile hypertonia was recorded in individuals II-2 or II-4.

GLRA1 exon 6 was amplified from genomic DNA from all the described family members (except IV-4) plus the proband's father (III-4) and asymptomatic maternal uncle (III-1). DNAs from 20 healthy individuals recruited outside the family were analyzed in parallel. Following direct sequencing of the amplified GLRA1 exon

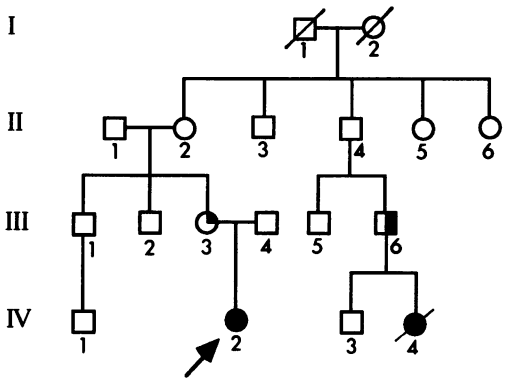

Figure 1 Pedigree of the family affected with hyperekplexia. Open symbols represent unaffected subjects; quarter-filled symbols represent subjects characterized by exaggerated startle response; half-filled symbols represent subjects characterized by exaggerated startle response and muscular hypertonia; and completely filled symbols represent subjects characterized by exaggerated startle response, muscular hypertonia, and myoclonic fits.

6, a G→C transversion was detected at nt 1178 of the published cDNA sequence in the proband and family members III-3, III-6, II-2, and II-4 (fig. 2). It is worth noting that no clinical signs were recorded anamnestically in II-2 or II-4. The severity of phenotypes in the pedigree seems to have become progressively worse over the three generations reported. This may be due to (*i*) considerably variable expressivity; (*ii*) predominant neonatal form with lessening of most clinical signs during the first year; or (*iii*) pure chance. The observed mutation changes the 266 CAG codon for glutamine to the CAC codon for histidine, leading to an uncharged polar amino acid being substituted by a charged basic residue. This sequence alteration was not found in either the proband's father (III-4) or her maternal uncle (III-1) or any of the external controls. The novel mutation described at codon 266 of the GLRA1 gene occurs in exon 6, five codons upstream of the codon where missense mutations have so far been identified in hyperekplexia patients. It is interesting that the affected amino acid belongs to the M2 membrane–spanning segment in the α1 subunit of the glycine receptor, which has been demonstrated to form the ion channel of GlyR (Bormann et al. 1993). The M2 segment of the α1 subunit is particularly rich in uncharged polar amino acids (Grenningloh et al. 1987) and is organized into an α-helix. The pivotal role of the entire M2 segment for channel function is suggested by its high conservation among human α1, α2, and α3 subunits and human and rat α1 subunits (Grenningloh et al. 1990; Bormann et al. 1993). It is likely that the substitution of the uncharged Gln266 with the charged His having a bulky basic R group may interfere electrostatically or physically with the α-helix formation. We speculate that disruption of the three-dimensional structure in the M2 segment of the α1 subunit would alter chloride permeability, leading to decreased receptor functionality in the heterozygote. However, only functional studies might elucidate the precise nature of the abnormality in the receptor displaying the observed Gln266→His substitution.

Structural modifications of the ion-channel region caused by the substitution of either a leucine or a glutamine for Arg271 in mature protein were shown to reduce binding and channel gating by agonists (Langosch et al. 1994; Rajendra et al. 1994). The murine *spd* mutation, which affects a residue located near the N terminus of the α1 subunit, was found to lower the agonist affinity of the receptors expressed in vitro (Ryan et al. 1994). The allelic murine spd^{ot} mutation, which causes a translational frameshift starting with residue 308 at the carboxy terminal of M3, was demonstrated to lead to a 90% reduction in strychnine binding (Buckwalter et al. 1994).

Although there is no easy interpretation for the widely variable expression of the Gln266→His mutation, which ranges from highly severe phenotype in two infants (IV-2 and IV-4) to major (III-6), minor (III-3) or no signs (II-2 and II-4) in adults of the family studied, it is compatible with typical dominant transmission. Also, the G→C transversion in codon 266 was not found in 20 unaffected individuals. This finding and the above arguments are consistent with a causal role for the mutation

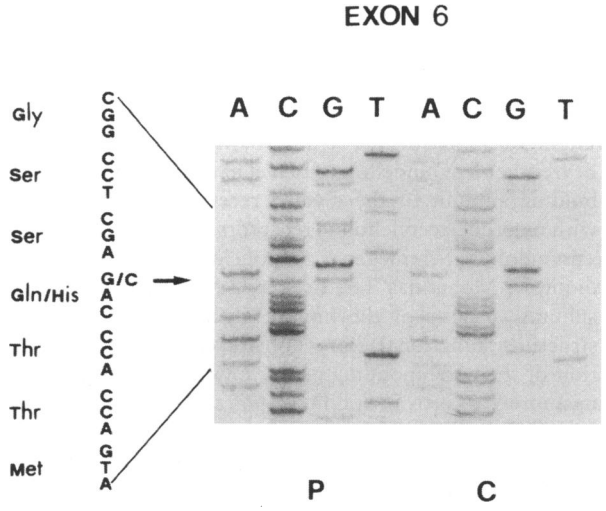

Figure 2 Direct dideoxynucleotide sequencing of exon 6 in the GLRA1 gene encoding the α1 subunit of GlyR. Genomic DNA from patients and controls was isolated from peripheral blood lymphocytes according to standard procedure, and exon 6 was amplified by PCR using the pair of primers described by Shiang et al. (1993). Direct sequencing of exon 6 visualizes both the normal and mutant alleles in the proband (P) but only the normal allele in an unrelated control (C). The affected region is depicted: an arrow marks the G→C transversion in codon 266 CAG in the heterozygous patient leading to substitution of Gln by His.

in codon 266 leading to substitution of Gln with His in the α1 subunit of GlyR in hyperekplexia in the family studied.

NICOLETTA MILANI,[1] LEDA DALPRÁ,[1] ALBERTO DEL PRETE,[2] ROBERTO ZANINI,[2] AND LIDIA LARIZZA[1]

[1]*Dipartimento di Biologia e Genetica per le Scienze Mediche, Facoltà di Medicina, Università di Milano, Milano; and* [2]*Dipartimento di Pediatria, Divisione di Patologia Neonatale, Ospedale di Circoli, Lecco, Italy*

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References

Bormann J, Rundstrom N, Betz H, Langosch D (1993) Residues within transmembrane segment M2 determine chloride conductance of glycine receptor homo- and hetero-oligomers. EMBO J 12:3729–3737

Buckwalter MS, Cook SA, Davisson MT, White WF, Camper SA (1994) A frameshift mutation in the mouse α1 glycine receptor gene (Glra1) results in progressive neurological symptoms and juvenile death. Hum Mol Genet 3:2025–2030

Elmslie FV, Rees M, Covanis A, Baxter P, Gardner-Medwin D, Curtis A, Burn J, et al (1994) Analysis of GLRA1 in familial and sporadic hyperekplexia. Am J Hum Genet Suppl 55:A358

Grenningloh G, Rienitz A, Schmitt B, Methfessel C, Zensen M, Beyreuther K, Gundelfinger ED, et al (1987) The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. Nature 328:215–220

Grenningloh G, Schmieden V, Schofield PR, Seeburg PH, Siddique T, Mohandas TK, Becker C-M, et al (1990) Alpha subunit variants of the human glycine receptor: primary structures, functional expression and chromosomal localization of the corresponding genes. EMBO J 9:771–776

Kingsmore SF, Giros B, Suh D, Bieniarz M, Caron MG, Seldin MF (1994) Glycine receptor β-subunit gene mutation in *spastic* mouse associated with LINE-1 element insertion. Nat Genet 7:136–141

Langosch D, Laube B, Rundström N, Schmieden V, Borman J, Betz H (1994) Decreased agonist affinity and chloride conductance of mutant glycine receptors associated with human hereditary hyperekplexia. EMBO J 13:4223–4228

Rajendra S, Lynch JW, Pierce KD, French CR, Barry PH, Schofield PR (1994) Startle disease mutations reduce the agonist sensitivity of the human inhibitory glycine receptor. J Biol Chem 269:18739–18742

Rees MI, Andrew M, Jawad S, Owen MJ (1994) Evidence for recessive as well as dominant forms of startle disease (hyperkplexia) caused by mutations in the α_1 subunit of the inhibitory glycine receptor. Hum Mol Genet 3:2175–2179

Ryan SG, Buckwalter MS, Lynch JW, Handford CA, Segura L, Shiang R, Wasmuth JJ, et al (1994) A missense mutation in the gene encoding the α_1 subunit of the inhibitory glycine receptor in the *spasmodic* mouse. Nat Genet 7:131–135

Ryan SG, Dixon MJ, Nigro MA, Kelts A, Markand ON, Terry JC, Shiang R, et al (1992) Genetic and radiation hybrid mapping of the hyperekplexia region on chromosome 5q. Am J Hum Genet 51:1334–1343

Schorderet DF, Pescia G, Bernasconi A, Regli F (1994) An additional family with Startle disease and a G1192A mutation at the α1 subunit of the inhibitory glycine receptor gene. Hum Mol Genet 3:1201

Shiang R, Ryan SG, Zhu Y-Z, Hahn AF, O'Connell P, Wasmuth JJ (1993) Mutations in the α_1 subunit of the inhibitory glycine receptor cause the dominant neurologic disorder hyperekplexia. Nat Genet 5:351–358

Shiang R, Ryan SG, Zhu Y-Z, O'Connell P, Wasmuth JJ (1994) Mutational and haplotype analysis of the a_1 subunit of the glycine receptor in hyperekplexia patients. Am J Hum Genet Suppl 55:A242

Warrington JA, Bengtsson U, Bailey SK, Lovett M, Wasmuth JJ (1992) A comparison of three methods to produce a high resolution physical map of 11 genes on the distal region of the long arm of human chromosome 5: radiation hybrid mapping, pulsed-field gel electrophoresis and fluorescent *in situ* hybridization. Am J Hum Genet Suppl 51:A248

Address for correspondence and reprints: Dr. Lidia Larizza, Dipartimento di Biologia e Genetica, Università di Milano, Via Viotti 5, 20133 Milano, Italy. E-mail: dibiogen@imiucca.csi.unimi.it

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Alterations of Chromosome 11q13 in Cervical Carcinoma Cell Lines

To the Editor:

In cervical cancer, evidence for the existence of a tumor-suppressor gene on chromosome 11 has been generated from studies with somatic cell hybrids (Stanbridge et al. 1981; Klinger and Kaelbling 1986), chromosome microcell transfer (Saxon et al. 1981; Koi et al. 1989), or deletion analysis of DNA markers (Srivatsan et al. 1991; Hampton et al. 1994; Mitra et al. 1994).

As suggested by somatic cell hybrids analysis, chromosome 11 harbors at least three distinctive tumor-suppressor genes (Weisman 1990), two on the short arm (Lichy et al. 1992) and one on the long arm (Misra and Srivatsan 1989). Loss of heterozygosity (LOH) analysis using 16 markers, 10 of which were microsatellite-based, placed the region of a putative tumor-suppressor