Genotype-phenotype correlations in hyperekplexia: apnoeas, learning difficulties and speech delay

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Congenital hyperekplexia is a rare, potentially treatable neuromotor disorder. Three major genes of effect are known, and all three affect glycinergic neurotransmission. Two genes encode for subunits of the postsynaptic inhibitory glycine receptor, GLRA1 encoding the α1 subunit and GLRB encoding the β subunit. The third, SLC6A5, encodes the cognate presynaptic glycine transporter 2. Ninety-seven individuals had a clinical diagnosis of hyperekplexia confirmed by genetic testing: 61 cases had mutations in GLRA1, 24 cases in SLC6A5 and 12 in GLRB. Detailed retrospective clinical analysis ascertained that all gene-positive cases present in the neonatal period (occasionally prenatally) and that clonazepam is the treatment of choice (95% found it to be efficacious). We confirm that hyperekplexia is predominantly a recessive condition but dominant cases are seen (16%). We found no genetic evidence for ‘major’ or ‘minor’ forms of hyperekplexia on a population basis. Thirty-five gene-negative cases were studied for comparison, their cardinal feature was presentation after the first month of life (P < 0.001). In addition to the characteristic ‘stiffness, startles and stumbles’ of hyperekplexia, apnoea attacks (50 of 89) and delayed development (47 of 92) were frequently reported. Patients with SLC6A5 mutations were significantly more likely to have had recurrent infantile apnoeas (RR1.9; P < 0.005) than those with GLRA1 mutations. Patients with GLRB and SLC6A5 mutations were more likely to have developmental delay (RR1.5 P < 0.01; RR1.9 P < 0.03) than those with GLRA1 mutations; 92% of GLRB cases reported a mild to severe delay in speech acquisition. Molecular modelling of pathogenic mutations demonstrates specific patterns of protein disruption that can be used to predict phenotype severity. The developmental delay in hyperekplexia, and speech acquisition in particular, may represent failure of developmental neural networks or subtle neurogenic migration defects in the absence of presynaptic glycine release. We recommend early genetic testing for symptomatic neonates and possibly preconception counselling for those at risk for GLRB and SLC6A5 mutations, because of the more challenging phenotype.

Keywords: hyperekplexia; genetics; phenotype; correlation; treatment options

Abbreviation: GlyR = glycine receptor; GlyT = glycine transport

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Introduction

Hyperekplexia is a neurogenetic condition characterized by hypertonia that is predominantly seen in the trunk or lower limbs and easily-provoked startle responses to tactile or acoustic stimuli (Harvey et al., 2008; de Koning-Tijssen and Rees, 2009; Thomas et al., 2010). The generalized stiffness can be prominent enough to permit infants to be rotated between the vertical and horizontal plane with barely a change in posture (Suhren et al., 1966). These startle attacks can be prolonged, with many minutes of axial rigidity. It is a rare condition with < 100 published cases confirmed by genetic testing since the first gene of effect (GLRA1) was described (Shiang et al., 1993). Surprisingly, for such a clinically recognizable condition, classical (congenital) hyperekplexia was first described as late as 1958 by Kirstein and Silfverskiold, with exaggerated hypnagogic jerks in addition to sudden falls. Additional features were linked to this unidentifed hereditary condition, including epilepsy in some affected family members and sensitivity to alcohol and phenobarbitone, which attenuated the startle response (Kok and Bruyn, 1962; Suhren et al., 1966; Gastaut and Villeneuve, 1967). Periodic limb movements in sleep and a characteristic head-retraction response complete the phenotype (Bakker et al., 2009). Onset of symptoms after infancy should prompt investigations for an acquired cause—pontine injury (Bakker et al., 2006; Davies et al., 2010) or rarely, anti-GlyR antibodies (Hutchinson et al., 2008; Mas et al., 2011). This is an important distinction, as treatment for classic hyperekplexia is based on medication to combat hypertonia and startle rather than true disease modification.

Hyperekplexia is predominantly caused by mutations in the genes encoding the postsynaptic inhibitory glycine receptor (GlyR; GLRA1 and GLRB) and presynaptic glycine transport (GlyT2; SLC6A5) (Shiang et al., 1993; Rees et al., 1994, 2006; Chung et al., 2010, 2013; Carta et al., 2012; James et al., 2013). There is rare (<1% of cases), but recognized genetic heterogeneity through association with mutations in gephyrin (GPHN; Rees et al., 2003), and collybistin (ARRHGEF9; Harvey et al., 2004). The phenotypes associated with these mutations are more devastating: homozygous deletions or missense mutations in GPHN (also associated with molybdenum co-factor deficiency) are characterized by untreatable seizures and neonatal death. GlyRs are heteropentameric (2α1:3β) inhibitory ligand-gated ion channels that facilitate fast chloride-mediated responses predominantly in the brainstem and spinal cord (Grudzinska et al., 2005; Yang et al., 2012). By contrast, GlyT2 is a sodium and chloride dependent transporter, which is involved in the reuptake of glycine from the synaptic cleft into glycineric neurons, thereby maintaining the pool of glycine for presynaptic vesicular replenishment. Hyperekplexia has some notable landmarks: (i) the description of mutations in GLRA1 by Shiang et al. (1993) was the first chanelopathy associated with ligand-gated ion channels; (ii) mutations in SLC6A5 (Rees et al., 2002) defined the first neurological disorder conclusively linked to a defect in presynaptic transporter for a classical fast-acting neurotransmitter; and (iii) the first demonstration of gain-of-function, tonic activation of the GlyR channel as a novel disease mechanism in ligand-gated ion channels (Chung et al., 2010). Similar phenotypes in mice, cattle and dogs have been described with mutations in GlyR α1 subunit, β subunit and GlyT2 genes (Gomeza et al., 2003; Harvey et al., 2008). These have helped to guide the choice of candidates for human gene screening.

The mainstay of treatment in hyperekplexia has been clonazepam, an allostERIC potentiator of GABA_A receptors, which is efficacious for patients with GLRA1, GLRB and SLC6A5 mutations (Andermann et al., 1980; Ryan et al., 1992; Bakker et al., 2009; Chung et al., 2010, 2013; Carta et al., 2012; James et al., 2013). Although potentially treatable, hyperekplexia is not necessarily a benign condition; there have been reports of an increased incidence of sudden infant deaths in hyperekplexia families and startle attacks when ambulant (combined with a variable hypertonic spastic gait) can lead to sudden falls and subsequent head injuries and fractures (Giaccoa et al., 1994). It remains unclear whether the sudden deaths are all attributable to hypertonic/apnoeic attacks, or whether the children who died prematurely had even inherited the familial mutation(s). As one of the global reference centres for hyperekplexia diagnosis and genetics, we wanted to ascertain whether there may be genotype–pheno-type correlations in hyperekplexia.

Materials and methods

Since 1994, we have received referrals for 230 families where a clinical diagnosis of hyperekplexia has been suspected. Our screening process, referral criteria and review procedure for gene-negative patients are described elsewhere (Harvey et al., 2008; Davies et al., 2010). A strict narrow definition of hyperekplexia was used when identifying the gene-negative cases and cases were not included if they had anything more than non-specific abnormalities at MRI. The mutations were identified through Sanger sequencing of the exons, splice sites and flanking DNA sequence up to 40 bp after the splice site. All samples were screened for large insertions and deletions through MLPA (multiplex ligation-dependent probe amplification) analysis in all three genes. Clinical ascertainment was reviewed and approved by the Local Research Ethics Committee, the South West Wales REC. We have used a range of functional analyses for estimating pathogenicity as previously published (Rees et al., 2002, 2003, 2006; Chung et al., 2010, 2013; Carta et al., 2012). Structural modelling of GlyR α1 and GlyT2 wild-type and variant proteins was performed using a homology modelling pipeline (http://membraneproteins.swan.ac.uk/modelling/), assembled with the Biskit structural bioinformatics platform (Grünberg et al., 2007), which scans the entire Protein Data Bank for candidate homologues, as described in Chung et al. (2010). The receptor homology modelling has recently improved with publication of the GluCl homologue for GlyR α1 providing 50% homology (69% sequence coverage), which permits us to describe novel associations in this study (Hibbs and Gouaux, 2011).

We contacted all referring clinicians of patients with a positive genetic diagnosis using a standard pro forma to establish both a standardized quality data set and to acquire a clinical update on the individual cases (Supplementary material). In addition 35 unresolved cases were included for comparison. The pro formas included details of reported co-morbidities (such as neonatal apnoeas and seizures) and theoretically possible complications such as anxiety or deafness. Data were enhanced with information from clinic letters and in some cases the authors interviewed and examined the patients. We analysed the data by comparing GLRA1, GLRB and SLC6A5 mutations, dominant versus recessive or compound heterozygous inheritance and sporadic versus...
familial cases. When comparing characteristics we used a single typical index case from each family. The chi-squared test was used using Yates’ correction when needed.

Results

One hundred and ten cases positive for GLRA1, GLRB or SLC6A5 mutations were identified from our database and we received pro forma replies for 94 cases (86% return). The sex mix was equal and the cases ranged from 2 years 5 months to 47 years 3 months at time of last contact (mean age 14 years and 4 months). A further 35 gene-negative cases were included for comparison. The cases with no pro forma return were often referred >10 years ago and the custodian clinicians (and in some cases, the institutions) were no longer clinically active. The non-pliers were proportionate. Three additional cases were obtained from colleagues in Liege, Belgium. There were 61 cases with GLRA1 mutations (14 dominant missense (23%), 24 recessive missense (39%), 23 recessive nonsense (38%)); 24 with SLC6A5 mutations (eight recessive missense (33%), 14 recessive nonsense (58%) and two not confirmed (8%)); and 12 GLRB mutations [one dominant missense (8%), two recessive missense (17%), nine recessive nonsense (75%)] (Table 1).

Phenotypic characteristics

Every patient exhibited an exaggerated startle in response to tactile or auditory stimuli; whereas the pattern of hypertonia was surprisingly varied. All GLRB cases demonstrated clear hypertonia—compared with GLRA1 (74%, \( P < 0.05 \)) and SLC6A5 cases (84%, not significant) with many cases explicitly having normal or reduced tone (Table 1). Every gene-positive case had symptoms from birth, indeed some exhibited startle in utero during the last trimester—in contrast, 54% of the gene-negative cases had onset after the first month of life, often into childhood (\( P < 0.001 \)). Recurrent injurious falls were common (>50% of the gene-positive cases) in contrast with the gene-negative group where it was reported in less than a quarter (\( P < 0.001 \)). Seizures were a definite feature in seven cases and probable in another five: all 12 cases were due to recessive mutations. This produces an uncorrected prevalence estimate of epilepsy in hyperekplexia of 7–12%. No children were reported as having cardiac arrhythmias, autonomic abnormalities, metabolic deficiencies or hearing difficulties. There were sporadic reports of ocular apraxia and congenital extraocular eye movement disorders (all GLRB cases). We received many reports of hypersalivation and relative failure to thrive. Oro-facial tactile stimulation of feeding appears to be a potent trigger for hypertonic attacks, which can result in failure to thrive.

Apnoeas and learning difficulties

Recurrent apnoea attacks are commonly seen in hyperekplexia ranging from a feature of a third of dominant GLRA1 cases, to 80% of those with SLC6A5 and 89% GLRB mutations (GLRA1 versus SLC6A5 \( P < 0.004 \), GLRA1 versus GLRB \( P < 0.03 \)). There is a striking difference between the prevalence of apnoea attacks in recessive GLRA1 hyperekplexia; missense mutations are associated with 71% of cases and nonsense mutations with 22% (\( P < 0.0002 \)). Delays in gross motor and speech acquisition were commonly reported—with a striking gene of effect pattern seen. Eight of 12 children with GLRB mutations reported motor delay and 11 of 12 had delays in speech acquisition (GLRA1 versus SLC6A5 RR1.5, \( P < 0.01 \), versus GLRB RR1.9, \( P < 0.02 \)). Global developmental delays were predominantly a feature of the recessive hyperekplexias—but observed twice as frequently in GLRB and SLC6A5 (where they were equivalent at >80%) than in recessive GLRA1. There is a stark difference between this finding and the reported rate of learning difficulties at 8% in dominant GLRA1 (the first reported cases).

Twenty-one kindreds had learning difficulties and recurrent apnoea attacks, nine of which had SLC6A5 mutations and seven with GLRB mutations. Ten kindreds had learning difficulty without a previous history of apnoea; all had GLRA1 mutations, \( P < 0.001 \); and all, with one exception, were caused by recessive or compound heterozygous mutations. Patients with recessive or compound heterozygote mutations (independent of the gene involved) were also more likely to exhibit developmental delay or learning difficulties (\( P < 0.02 \), 51% with recessive inheritance compared with 10% dominant.

Clonazepam and referral pattern

Sixty-five cases had been treated with the GABAAR potentiator clonazepam (67%), 96% of these had a satisfactory subjective improvement in either hypertonia, startle or both. As expected, dose-related sedating side-effects limited its utility in some cases. There was no pattern formed by looking at those who had a dramatic or equivocal response to clonazepam. Of the 17 individuals with SLC6A5 mutations, 14 had been tried on clonazepam and 13 found a sufficient and sustainable benefit from this therapy; similarly all GLRB cases responded well to clonazepam (one to nitrazepam).

Gene-negative cases

The 35 cases without mutations in the three genes of effect had a number of similarities with the genetically proven cases: the majority had exaggerated startle (86%), were hypertonic (69%), and apnoeas and falls were seen albeit at a lower frequency than other cases (23% RR2.4 \( P < 0.001 \), 23% RR2.7 \( P < 0.001 \)). Importantly, just under half of cases reported that startle symptoms began after the first month of life and not soon after birth; which we suspect may represent a novel sub-phenotype. However, we have not identified a genetic explanation for later-onset cases (\( P < 0.001 \)).

Structural modelling

Modelling of GLRA1 mutations in glycine receptor homologues

The wealth of GLRA1 mutations permitted sub-categorization into pathogenic classes. This brought stronger correlations than primarily analysing by phenotypic criteria (Fig. 1). Hypertonia, although not a universal feature of GLRA1 mutations, can be associated with almost any structural change. Developmental delay is
invariably associated with a conformational change at the cytoplasmic end of M4 with mutations affecting the extracellular domain residues in addition to M2 changes (such as E103K and R65W). Similarly those with delays in speech acquisition not only have this conformational change at M4, but a secondary impact on M2. Of note there is a phenotypic cluster of cases of classic hyperekplexia with hypertonia and no learning difficulties and an excellent response to clonazepam, which have a similar structural profile (M147V, I244N, P250T and R271Q).

Reduced current

The most frequently occurring mutation (dominant R271Q) is adequately expressed at a cell surface level, but produces a reduced current and cases invariably escape without learning difficulties and without prominent apnoea attacks. Within this class homozygous E103K, compound heterozygous E103K and del634-635CT produce the most severe phenotypes. The narrowing of the channel at M2 is most marked in these cases with a major conformation change at the end of M4 (Fig. 2).

The R65L/del ex 4–7 compound heterozygote, which causes a reduced current, has a particularly benign phenotype with an excellent response to clonazepam. Although it has the predicted change in orientation of the extracellular end of M1, with a narrowing at the extracellular end of M2 (in keeping with other mutations in the class) these changes are much less dramatic. These minor changes explain why it is trafficked so efficiently (identical to wild-type) but as the R65 residue is an important glycine binding determinant the current generated is reduced. R65W, however, (below) is not expressed adequately at a cell surface level.

Reduced cell-surface expression

The R65W mutation results in drastically reduced number of functional channels and cell-surface receptors (Chung et al., 2010). Mutations that produce channels that are only poorly expressed at the cellular membrane demonstrate more substantial conformational changes with effects on M3 as well as M2 and M4. Examples include the compound heterozygous R65W and homozygous D165G mutations: D165G shows an additional loss of helical structure at the cytoplasmic end of M2. In R65W, the cytoplasmic end of M3 projects towards M2, while in D165G, the helix is extended at the extracellular end of M3 and shortened.

### Table 1 Distribution of clinical traits dependent on gene mutation and method of inheritance

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<th>Inheritance / mutation</th>
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<th>Hypertonia</th>
<th>Apnoeas</th>
<th>Falls</th>
<th>Delayed development</th>
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at the cytoplasmic end. Both R65W and D165G lead to a conformational change at the cytoplasmic end of M4 (Fig. 2).

Receptor trafficking deficits
A failure to traffic the receptor to the membrane produces a moderate to severe phenotype. The cytoplasmic ends of M3 and M4 appear to be critical in trafficking defects; such as in the homozygous R252C mutation. Along with a narrower channel at the extracellular end of M2, this also produces a significant conformational change at the cytoplasmic end of M4. A more dramatic example of the misalignment of M2 and the significant extension and reorientation of the cytoplasmic ends of M3 and M4 is the homozygous G254D (Fig. 2A). These trafficking mutants all have recurrent neonatal apnoeas.

Modelling of SLC6A5 mutations in glycine transporter 2 homologues

A common effect at T578
There are fewer SLC6A5 variants and yet a clear pattern emerges between changes in molecular structure and phenotype. In the pathogenic hyperekplexia variants, the position and orientation of the T578 side chain is notably different to that of wild-type GlyT2, which markedly changes the predicted interactions with one of the two Na⁺ ions and the glycine ligand. This change occurs with all the variants (except the truncated variants where the residue is not encoded). In all but the S256R variant, T578 is predicted to form an alternative threonine side chain oxygen interaction with the glycine ligand H2 atom (such as in P243T, Y705C and E248K, Fig. 3). The side chain oxygen of T578 of the P243T and Y705C variants is predicted to be within the interaction range of Na⁺ 1 in addition to the glycine ligand. In contrast to the normal protein, other parts of the T578 side chain are within 3 Å of Na⁺ 1 in all the variants and so may impede the passage of this Na⁺ ion. L574 is introduced into the Na⁺ 1 binding site in all variants, with predicted spatial overlap. More notably D577 is also introduced into the Na⁺ 1 binding site of all variants where it is coded in the protein, except for E248K (Fig. 3), which interestingly, is associated with a milder phenotype with no learning difficulties.

SLC6A5 compound heterozygosity
The analysis of compound heterozygote variants reveals a similar pattern with regard to the changes in position of key residues...
being consistent with more severe phenotypes. The S256R/Y656H combination displays introduction of T578 to interaction range of Na⁺1.

These changes are consistent with those of the single mutations associated with neonatal apnoeas. D577 is not introduced to the binding site of Na⁺1 of S256R/Y656H, similar to the E248K variant; neither case is associated with learning difficulties. The F547S/Y656H combination shows all of the above changes, as well as the introduction of D577 to the binding site of Na⁺1 and is likewise associated with a panoply of startle, falls, learning difficulty, neonatal apnoeas and impaired motor development.

No molecular modelling is presented for GLRB as the vast majority of mutations are deletions, nonsense or splice site changes. Molecular modelling of missense mutations including P169L and Y470C has been recently described (Chung et al., 2013).

Discussion

This is the first description of how the hyperekplexia genotypes affect the associated clinical phenotypes. We report definitive indications for gene-specific phenotypic differences and an association of recessive inheritance with increased risk of learning difficulties and developmental delay, particularly speech acquisition. We also highlight the risk of severe recurrent neonatal apnoeas with hyperekplexia (particularly associated with mutations in GLRB and SLC6A5), which has hitherto not been recognized. The inclusion of gene negative cases identified startle at birth and falls as the cardinal features of classic hyperekplexia. Furthermore the scale of this cohort permits molecular modelling to explain phenotypic variation between pathogenic mechanisms and partially within each class. We also acknowledge some limitations to our study: it has proved impossible for us to examine each patient individually and so we are dependent on the information provided by the referring clinicians. Patients of differing ages are being compared: some are too young for certain features (learning difficulties or falls for example) to exhibit themselves. Similarly, there is a recall bias from older patients as their neonatal apnoeas may be forgotten, under-reported or ignored. It is very probable that a lack of familiarity with this condition leads to under-reporting and misdiagnosis (or an over-diagnosis of seizures). Furthermore we are more likely to be referred cases when there is greater diagnostic doubt and a more complicated phenotype. Despite this being the largest description of hyperekplexia families and cases by a significant margin, it is quite possible that we are unable to recognize or prove the existence of rarer but relevant co-morbidities and characteristics.

Recessive versus dominant inheritance

Hyperekplexia was initially misrepresented as demonstrating solely autosomal dominant inheritance, because these families were easier to identify and study, which exacerbated an ascertainment bias. Autosomal dominant inheritance accounts for 16% of the cases in this study. This may be an underestimate; we are more likely to receive requests for a genetic diagnosis when the clinical pattern is ambiguous, such as a lack of family history. However, it...
is important to recognize the importance of recessive inheritance in the context of genetic counselling. In addition consanguineous families may also harbour other undetected recessive mutations that may explain the extended phenotypes.

Parental sampling has identified hyperekplexia as a highly penetrant condition, although occasionally incomplete penetrance has been reported (Zoons et al., 2012). There is a small degree of variability within families, for example, a family from the UK with autosomal dominant inheritance due to the common GLRA1 R271Q mutation exhibited mild learning difficulties, until the present generation, where an affected individual completed mainstream schooling. However, this may represent the success of early diagnosis and prompt treatment with clonazepam (rather than chlordiazepoxide which her ancestors used;
Lindahl, 2005). Of the 12 pairs of siblings who share the same genotype, there appears to be an extensive degree of homology. When the core features of hypertonia, startle, falls, learning difficulty, response to drugs and apnoea are compared there is 4% discordance, driven mainly by the ambiguity due to variable questionnaire entries. There is heterogeneity, however, derived from the variation in the physical characteristics during development (worst in the first year) and over the course of a day (improved in sleep, with alcohol and benzodiazepines—provoked by external stimuli). However, we cannot exclude other considerations such as in vivo pathophysiological mechanisms, incomplete genetic penetrance or the occurrence of digenic or multigenic cases.

**Hyperekplexia and apnoeas**

Neonatal apnoeas have been recognized in hyperekplexia following a case report (Kurczynski et al., 1983) and were estimated to occur in 10% of patients (Giacola et al., 1994). Apnoeas occurred in 56% of all patients in our study, but in >80% of cases with GLRB/SLC6A5 mutations. These events are often associated with the triggered tonic, abdominal splinting attacks and can be life-threatening. A patient who was compound heterozygous for SLC6A5 mutations (Y491C and Q630X) suffered 47 respiratory arrests in an 8-week period (Rees et al., 2006). The explanation as to why apnoeas were regarded as a feature of ‘sporadic’ hyperekplexia (Bakker et al., 2006, 2009) lies within the inheritance patterns; patients with GLRB/SLC6A5 mutations are significantly more likely to describe serious neonatal cyanotic attacks and the majority of GLRB/SLC6A5 mutations are recessive—presenting sporadically. The same trend applies for GLRA1 mutations where sporadic index cases have been linked to recessive mutations (Rees et al., 2002; Chung et al., 2010).

**Learning difficulties and developmental delay**

Forty per cent of people with GLRA1 mutations had neither neonatal apnoeas nor delayed development, in contrast to cases with SLC6A5/GLRB mutations where just one person had neither co-morbidity. It has been incorrectly stated that the cognitive profile of people with hereditary hyperekplexia is unaffected or mildly impaired—although recognizing that ‘sporadic’ cases were more complex (Zhou et al., 2002); and late motor milestones have been previously described (Tsai et al., 2004) in a family with recessive GLRA1 hyperekplexia. MRI is ubiquitously normal in classical hyperekplexia. It is likely that the apnoea attacks do not directly cause cognitive impairment: 40% of the cases with learning difficulty or developmental delay had no previous apnoea attacks (all had GLRA1 mutations). The developmental delay demonstrated in some patients may represent subtle alterations in neuronal migration and targeting within the context of an environment where glycine is depleted from presynaptic terminals, or incomplete neural networks due to the lack of GlyR activation during brain development. The lack of GlyR activation during embryonic and infantile brain development will produce direct widespread glycnergic consequences and indirect effects on other neural networks such as gamma-aminobutyric acid (GABA) and N-methyl-D-aspartic acid (NMDA).

The phenomenon of last trimester intrauterine startle first described by Leventer et al. (1995) is confirmed in this study. It suggests that the glycnergic synaptopathy is apparent months before birth—during the time of greatest neurocortical development and the developmental switch from GlyR alpha-2 homopentamer receptors to GlyR alpha/beta heteropentameric receptors. In the neonatal brain many inhibitory synapses initially are mixed GABAergic and glycnergic, which changes during CNS maturation. Castaldo et al. (2004) suggested that the glycnergic synapse and GlyRs may well participate in cognitive development. GlyRs have been described in septal cholinergic neurons, which play a major role in learning and memory by means of their connections with the hippocampus and cerebral cortex, and their function can be modulated by psychotropic medication (Laube et al., 2002). Our finding that speech acquisition is preferentially affected implicates glycnergic pathways in expressive language. However, many patients reported later developmental ‘catch-up’, suggesting a sophisticated compensatory mechanism that plausibly may involve upregulation of inhibitory GABA, or downregulation of antagonistic glutamate systems.

**Gene-negative cases**

Hindsight allows us to reappraise previously published gene-negative patients. Shiang et al. (1995) described 17 patients: 11 had definite autosomal dominant inheritance and uncomplicated hyperekplexia without learning difficulty, whereas five of the six gene-negative patients had developmental delay. We now know that one had compound SLC6A5 mutations (Carta et al., 2012) and another has a recessive mutation in GLRB (Chung et al., 2013). The 35 gene-negative cases form a looser grouping with exaggerated startle co-presenting with occasional hypertonia, falls and apnoea. It leads us to speculate that the gene-negatives fall into the following categories: (i) genuine hyperekplexia where intragenic DNA variations in GLRA1, GLRB and SLC6A5 remain undetected (ENCODE consortium et al., 2012); (ii) classical hyperekplexia with genes yet undiscovered; (iii) neonatal/early-infancy acquired hyperekplexia that is a phenocopy of genetic hyperekplexia e.g. autoantibody driven hypertonia and startle such as described by Hutchinson et al. (2008) with autoantibodies against GlyR or GlyTs; and (iv) other undiagnosed or unrecognized neuromotor disorders presenting as mimics, sharing common features such as excessive startle or hypertonia.

**Modelling explains pathogenic class differences**

The large number of GLRA1 cases identified and the delineation of their functional consequences permits a degree of classification by pathogenic mechanism. Broadly there are four groups of equal size, with mutations causing: (i) adequate cell-surface expression but reduced current; (ii) limited cell-surface expression of poorly-functioning channels; (iii) trafficking defects; and (iv) prematurely truncated GlyR subunits (Table 2). The first class has the least complicated hyperekplexia (learning difficulties and apnoeas are...
more scarce) and the other three are similar in severity. The fifth class is rarer, producing a tonic-opening leaky channel (Chung et al., 2010).

It has been suggested that mutations that lead to a complete lack of function may be more likely to exhibit a severe phenotype with learning difficulties, such as R218Q, or the nonsense mutation Y202X in GLRA1 (Table 2; Brune et al., 1996; Castaldo et al., 2004). Y202X does have an extended phenotype in keeping with mutations in the truncated class, but surprisingly R218Q has a much attenuated phenotype. Modelling of R218Q predicts a narrowing at extracellular end of M2, with an extended end to cytoplasmic M3 and a major conformational change to the cytoplasmic end of M4: dramatic changes but not as severe as others within its class.

### Hyperekplexia treatments

A prompt diagnosis is of importance, as the vast majority of cases respond well to clonazepam, which may offer more relief from startling than hypertonia (Tijssen et al., 1997). Physical activity (for hypertonia) and patient education should form part of a treatment regime for anyone with hyperekplexia (such as the ‘Hyperekplexia Society’ on Facebook). The major non-pharmacological treatment for infants with hyperekplexia is the Vigevano manoeuvre (Vigevano et al., 1989). Understanding that there appears to be a difference in clinical presentation (between GLRB/SLC6A5 and GLRA1 hyperekplexia) means that we must recommend both early genetic testing for suspected neonates and preconception counselling for couples who have a family history of hyperekplexia.

### Conclusions

This study represents, by far, the largest cohort of hyperekplexia cases reported, supported by 20 years of genetic hierarchical analysis and functional characterization. We firmly establish that the constellation of symptoms caused by failure of glycinergic synaptic transmission can be generated by mutations affecting presynaptic and postsynaptic proteins. However, mutations in GLRB and SLC6A5 cause repeated apnoea attacks in the majority of cases and most either report developmental delay (with speech delay prominent) or learning difficulties. Sporadic cases of this type are explained by recessive mutations, with consanguinity a risk factor and early treatment may help alleviate the morbidity due to recurrent apnoeas. Hyperekplexia remains a clinical diagnosis, but we argue that prompt genetic analysis will help estimate prognosis, assist in preconception counselling and aid the planning of safer neonatal care. For those patients that remain gene-negative we can now begin a process of phenotypic reassessment and further stratification of hyperekplexia in preparation for second generation genetic analysis.

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### Supplementary material

Supplementary material is available at Brain online.
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