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ments and from studies of a gphp knock-out mouse model (12, 19, 20). Heterogeneous roles for gephyrin have been implicated by protein-protein interactions with a range of determinants such as RAFT1, tubulin, profilin, collybiuin, and the dynein light chains 1 and 2 (17, 21–24).

The preliminary structure of the gephyrin gene (GPHN) has been described in mice and humans (25–27), although there is a degree of ambiguity in the number of exons due to the presence of multiple transcript isoforms, and it is uncertain that all exons have been located and identified. Indeed, GPHN expression is not restricted to rat brain and spinal cord but is also found in liver, kidney, lung, and retina (28–30). Gephyrin knock-out mice and reconstitutive cell culture assays have demonstrated that gephyrin expression in nonneuronal tissue is a requirement for the biosynthesis of molybdenum cofactor (30, 31). Striking homologies are observed between the primary structure of GPHN and proteins involved in bacterial, plant, and invertebrate molybdenum cofactor biosynthesis. Gephyrin, therefore, has a synaptic function as a postsynaptic anchoring and clustering protein in neurons while facilitating a highly conserved metabolic purpose in nonneuronal tissues (32).

The genetic and structural basis of the functional dichotomy is not established; however, in rats, a putative explanation may lie in the generation of distinct transcript isoforms of gephyrin constructed from alternative splicing of eight exonic “cassettes” within four regions of GPHN (25, 28, 33). Apart from conferring neuronal and nonneuronal characteristics to the mature polypeptide products, the purpose of the transcript heterogeneity pattern is unclear, and the number of isoform combinations in human tissue remains unestablished.

In addition to major disruption of molybdenum cofactor biosynthesis, gephyrin-deficient mice display a neuromotor phenotype, which resembles human hereditary hyperekplexia. This neurological condition (Mendelian Inheritance of Man: 149400 and 138491) can be caused by dominant and recessive mutations in the GLRA1 and GLRB receptor genes and is characterized by an abnormal, persistent startle response to unexpected stimuli, neonatal hypertonia, and a chronic accumulation of injuries caused by unprotected, startle-induced falls (34–40). Furthermore, autoimmunity to GPHN was detected in a patient with Stiff-Man syndrome, a disorder that has a degree of phenotypic overlap with hyperekplexia (41). Collectively, this indicates that GPHN is a candidate gene for neurological and metabolic disorders, and support for the latter has recently emerged with a description of multiple transcript isoforms, and it is uncertain that all exons have been located and identified.

In response to the candidacy of gephyrin for hyperekplexia, we pursued the definitive genomic structure of gephyrin by a combination of in silico BAC contig construction and multisite RT-PCR methods. Using a yeast two-hybrid (YTH) screen, with the GlyR β subunit intracellular loop as bait, we attempted to identify further GlyR-interacting proteins that might be implicated in hyperekplexia. The results of this screen, together with the extensive search for all human GPHN transcripts in both neuronal and nonneuronal tissues, suggest the presence of one new GPHN exon and at least 11 neurological transcript isoform combinations that redefine the genomic organization of GPHN. Having established the number of exons in the GPHN gene, we completed a systematic exon by exon mutation analysis of GPHN in a cohort of hyperekplexia patients devoid of GLRA1 and GLRB mutations. One novel mutation (N10Y) and several SNPs are reported; however, the functional effect of the mutation remains elusive despite assays for receptor targeting and clustering. We also present evidence that GlyR-gephyrin binding is dependent on the presence of an intact C-terminal Moεa homology domain and that the heterogenous GPHN linker region is not the physical determinant for gephyrin-GlyR binding.

MATERIALS AND METHODS

RT-PCR Analysis of GPHN Alternative Splicing—A GPHN gene-specific primer (Table I) was used in a first-strand cDNA synthesis reaction (SuperScript II; Invitrogen) from total RNA derived from adult brain, fetal brain, cerebellum, and human spinal cord (Clontech). In addition to neurological tissue, cDNA was synthesized from a selection of nonneurological tissues including heart, liver, lung, kidney, trachea, fetal liver, pancreas, and placenta (Clontech). In the first instance, conserved primers were designed in constitutional regions surrounding the candidate regions of alternative splicing, namely regions C12, C3, C4/5, and C6/7 (Table I). To validate the initial RT-PCR methodology, downstream nested primers within the GPHN spliced exons were used in conjunction with primers from upstream invariant portions of the GPHN message (Table I). In the presence of multiple PCR products, DNA fragments were cloned into pGEM-Easy vectors (Stratagene), and the transformants were PCR-screened and assessed for size differences by molecular screening agarose (Roche Applied Sciences) and 10% non-denaturing polyacrylamide gels (Sigma). Minipreparations of clones were digested with EcoRI, and inserts were sequenced using ABI 3100 technology.

YTH Screening—To identify GlyR β subunit interactors in human brain, we cloned the large intracellular loop of the human GlyR β subunit into the plasmid vector pYTH9 (43, 44). In this manner, the large TM3-TM4 intracellular loop (known to harbor a gephyrin binding motif) (8) was fused to the GAL4 DNA binding domain. pYTH9 is advantageous for YTH screening, since the bait plasmid can be stably integrated into the yeast genome at the trp1 locus. An adult human brain cDNA library in pACT2 (HL4004AH; Clontech) was be screened by transformation of library DNA into the pYTH9-GlyR β yeast strain. Transformed yeast were plated on selective dropout media lacking leucine, tryptophan, and histidine, supplemented with 10 μM 3-aminobenzyladenine (Sigma), miniprepped, and sequenced. True interactions were checked by retransformation of library plasmids into yeast containing pYTH16 (an episomal version of pYTH9) and pYTH16-GlyR β. Library plasmids that were capable of autoactivation (i.e. activated lacZ with pYTH16 alone) were excluded from further analysis.

Constructs, Human Embryonic Kidney (HEK) Cell Transfection, and Confocal Microscopy—For the pDSRed-GlyR β construct, the large intracellular loop of the human GlyR β subunit was amplified using the primers BRDSRed1 (5′-GCTGACTACACAGGCTGCCAGGACGGGT-3′) and BRDSRed2 (5′-AACGGATCCTTGCATAAAGATCAGAGTTCC-3′) and cloned into the EcoRI and BamHI sites of pDSRed-N1 (Clontech). For the pEGFP-gephyrin construct, the entire coding region of the rat gephyrin P1 isoform (28) was amplified using the primers G1 (5′-CGCTGATCACTACATGGCGACCGAGGGA-3′) and G14 (5′-TGGCTCGAGTCATAGCCGTCCGATGA-3′), cut with EcoRI and BamHI, and cloned into the BigIII and SalI sites of pEGFP-C2 (Clontech). The N10Y mutation was introduced into pEGFP-gephyrin by site-directed mutagenesis, using 27-mer oligonucleotides and the QuickChange kit (Stratagene). Collybiuin CDNAs were amplified from postnatal (P0) rat brain first-strand cDNA using the primers CB1 (5′-GTTGAGATCCGGCTTCAACCAGCGCGG-3′) and CB3 (5′-TGAAGATTCGTTCCTCTTCTGTGATC-3′) and cloned into the BamHI and EcoRI sites of the vector pBK5 (Stratagene). The pBK5mycCB2SH3 construct (pBK5mycCB2SH3-S) encodes the collybiuin II isoform (23). All amplifications were performed using Pfu Turbo proofreading DNA polymerase (Stratagene), and DNAs for transfection were made using the Plasmid Maxi Kit (Qiagen). All constructs were sequenced using the BigDye ready reaction mix (PerkinElmer Life Sciences) and an ABI 310 automated DNA sequencer (Applied Biosystems).

HEK cells (ATCC CRL11268) were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units ml−1 penicillin G, and 100 μg ml−1 streptomycin at 37 °C in 5% air-5% CO2 (45). Exponentially growing cells were electroporated (400 V, infinite resistance, 125 microfarads; Bio-Rad Gene Electro-
SSCP gels were silver-stained as previously described (47).

Samples were cooled and applied to 10% nondenaturing gels (49:1; Sigma) and run at 75 V for 12 h. Analysis was carried out using the Transgenomics dHPLC. Variant dHPLC profiles suggestive of sequence heterogeneity were recommended by predicted helicity profiles across the DNA fragment.

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**RESULTS**

**GlyR β Subunit YTH Screen**—In an attempt to identify further GlyR-interacting proteins that may be implicated in hyperekplexia, we carried out a YTH screen of an adult human brain cDNA library, using the GlyR β subunit TM3-TM4 intracellular loop as bait. The cDNA library had an original complexity of 3.5 × 10^6 independent cDNA clones, and 8 × 10^4 cDNAs were screened. Twelve positive clones were recovered, which were specifically interacted with the GlyR β subunit bait. These represented eight independent cDNAs encoding variants of the human homologue of GPHN (Fig. 1). No other interacting proteins were found. Some of our gephyrin cDNAs (e.g. Geph1) were full-length, which allowed us to predict the entire coding sequence of the human gephyrin polypeptide. Structurally, the N- and C-terminal domains of gephyrin show high similarity to E. coli proteins (MogA and MoeA), a Drosophila protein (CINAMON), and an Arabidopsis thaliana protein (CNX1), all of which are involved in the synthesis of a molybdoenum-containing co-factor essential for molybdoenzyme activity. Analysis of the phylogenetic conservation within the human GPHN open reading frame reveals a 94% homology to rodent gphn sequence and a 91% homology to chicken sequence, assuming the exclusion of all alternatively transcribed exons. In comparison with rodents, this is translated into a 99.7% amino acid conservation (Fig. 2) within the constitutional 736-amino acid reading frame. Degeneracy is observed at two sites, I240L and A242R, amino acid changes that lie immediately 5' adjacent to the C3 linker insertion site (30). The amino acid conservation between rodents and a human gephyrin sequence is reduced to 98.7%, with all but one of the nine degenerate changes occurring at the MoeA domain, and a reversal of the rodent I240L and A242R changes is observed (Fig. 2).

Interestingly, several of the GlyR β-interacting GPHN clones (Geph3–8) were missing all of the N-terminal MogA homology domain (amino acids 16–169) as well as most of the “linker” segment of gephyrin (amino acids 170–322; Fig. 1). This suggests that an intact C-terminal MoeA domain is required for...
strong interactions with the GlyR subunit and that the binding site on gephyrin for GlyRs does not reside in the MogA homology domain or the linker region.

**Transcript Heterogeneity of Human GPHN**—Several variants of gephyrin were found in our GlyR β subunit YTH screen. That gephyrin exists in multiple isoforms has been established for some time (25, 28, 33); the most common variants have insertions in the “linker” region, where exon C3 and C4 exons, whereas no comparative data are available for chicken gephyrin. The MogA and MoeA regions are indicated by underlined segments of the alignment, and the position of a putative myristoylation site (*) is indicated (26).

![Phylogenetic alignment of GPHN.](image)

**Fig. 2.** Phylogenetic alignment of GPHN. Shown is amino acid alignment of human, rodent, and chicken GPHN, with shaded regions representing points of divergence from the human sequence. The arrows indicate the insertion points for the C3 and C4 cluster exons within the linker region of GPHN. The alignment does not include the C3/C4 cluster exons representing points of divergence from the human sequence. The MogA and MoeA regions are indicated by underlined segments of the alignment, and the position of a putative myristoylation site (*) is indicated (26).

The RT-PCR assays for human GPHN were designed to capture regions of splicing activity implicated from rodent gphn studies (Table I). Twelve different human RNA preparations from neurological (n = 4), and nonneurological (n = 8) tissues were tested (see Fig. 3A). The presence of rat gphn exons C1 (region 1) or exon C5* described by David-Watine (27) was not detected in any of our transcripts from human tissues. Exon C2 (region 1) was ubiquitously expressed in all transcripts with no suggestion of differential splicing. Furthermore, murine gphn exons C6 and C7 were not detected; nor was the exclusion of exon 18 in human GPHN transcripts (25, 51). The transcript pattern surrounding the C3 and C4 cluster revealed a clear difference between neurogenic tissues and nonneurogenic tissues. A solitary transcript was found in all nonneurogenic tissues, which included the C3 exon and all exons from the C4 cluster (Fig. 3A). In contrast, neurological tissue generated a ladder effect, indicating the presence of a range of fragments. The fragments were cloned into pGEM-easy vectors, and a total of 547 clones were PCR-screened for size differences, from which 86 clones were selected and sequenced. All of the clones sequenced contained perfectly preserved splicing insertion points and followed the linear 5' to 3' order of exons on the BAC contig. In total, sequencing confirmed the presence of 11 distinct neurological GPHN isoforms (Fig. 3B). The majority of neurological isoforms excluded exon C3 and contained a combination of the C4 exon cluster; however, two rare clones were detected that demonstrated the co-inclusion of exon C3 and one of the C4 cluster exons (Fig. 3B). One isoform corresponded to the nonneurogenic pattern by exclusively retaining the C3 exon, and this presumably reflects the metabolic transcript of gephyrin required in all tissues including the human brain.

To validate our capture of all transcript isoform combinations, further RT-PCRs were generated from an upstream invariant forward primer and nested reverse primers within each spliced exon of the C4 cluster (Table I). This approach captured all previously identified isoforms and did not reveal any additional isoform combinations. Finally, additional testing of the remaining GPHN invariant regions (Table I) found no evidence to suggest any exonic splicing outside the C3 and C4 exon cluster.

**Mutation Analysis and SNP Distribution in GPHN.—**Three highly informative mutation detection techniques (SSCP, dyeoxy fingerprinting, and automated DHPLC) were adopted to detect GPHN mutations and polymorphisms in a cohort of 32 hyperekplexia patients. Several SNPs were detected in the promoter, intronic, and exonic regions in both patient and control cohorts (Table II). In addition, a 4-bp deletion was detected within the GPHN candidate promoter region (del tatta at position −976 −972), which was also detected in normal controls and was not functionally assessed. Significantly, a heterozygous missense mutation (A28T) was detected in a hyperekplexia patient that causes an asparagine to tyrosine amino acid substitution (N10Y) in exon 1 of the GPHN gene (Fig. 5A). There were no restriction site changes for GPHN A28T, so consequently we analyzed 94 controls by DDF and confirmed the exclusion of patient-specific patterns from the control group (Fig. 5B). The N10Y substitution is positioned 5' to 3' of exons on the BAC contig. In total, sequencing confirmed the presence of 11 distinct neurological GPHN isoforms (Fig. 3B). The majority of neurological isoforms excluded exon C3 and contained a combination of the C4 exon cluster; however, two rare clones were detected that demonstrated the co-inclusion of exon C3 and one of the C4 cluster exons (Fig. 3B). One isoform corresponded to the nonneurogenic pattern by exclusively retaining the C3 exon, and this presumably reflects the metabolic transcript of gephyrin required in all tissues including the human brain.

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FIG. 3. RT-PCR assays in putative regions of splicing activity in GPHN. A, each of the four assays has primers anchored on invariant GPHN-P1 sequence (refer to Table I) and is based on prior knowledge of splicing activity (25, 28). Neurological tissues represent the first four samples in each gel (left to right: adult brain (lane 1); fetal brain (lane 2); spinal cord (lane 3); cerebellum (lane 4)), and samples 5–12 represent RT-PCR-derived nonneurological tissue (heart (lane 5); kidney (lane 6); pancreas (lane 7); lung (lane 8); liver (lane 9); fetal liver (lane 10); placenta (lane 11); and trachea (lane 12)), with lane 13 representing a negative control PCR. Fragments were sized by a 1-kb ladder (Invitrogen). Only the C3 and C4 assays indicated the presence of transcript heterogeneity.

B, size-ordered GPHN-pGEM clones derived from a C3/C4 cluster combination assay and digested with insert-releasing EcoRI restriction enzyme and resolved on a 10% polyacrylamide gel. In total, 11 neuronal GPHN isoforms were discovered, and each isoform corresponded to the expected sizes from the possible exonic combinations within the PCR assay. Each of the spliced exons are represented with distinct patterns, and amino acid translations are provided. Exons in the C4 cluster have been assigned a new nomenclature, and a conversion key relating to the old nomenclature is provided. Clone 4 was the sole transcript observed in nonneurological tissues \( (n = 8) \).
clustering and reduced attenuation of the startle response.

Functional Analysis of GPHN N10Y—To assess whether the mutation N10Y could affect interactions between GPHN with the GlyRs or with the GDP/GTP exchange factor collybistin, we carried out functional expression experiments in transfected human embryonic kidney cells (HEK293). Primer sets for each candidate region of alternative splicing were designed in regions of the gene that were invariant from tissue to tissue. In addition, primer sets were designed to analyze the complete reading frame in order to exclude any further regions of exon splicing (Invariant 1 and 2). The exonic origin of each primer is shown in superscripts. Four nested primers located within each spliced exon were used in validation experiments to ensure capture of all GPHN transcript isoforms. UTR, untranslated region.

**TABLE I**

RT-PCR primers for the spliced regions of GPHN

These primers were used in the generation and capture of GPHN transcript isoforms. A GPHN-specific primer located at the 3′-end of the gene was used to generate cDNA fragments from mRNA templates derived from neurological (n = 4) and nonneurological (n = 6) tissues (Clontech). Primer sets for each candidate region of alternative splicing were designed in regions of the gene that were invariant from tissue to tissue. In addition, primer sets were designed to analyze the complete reading frame in order to exclude any further regions of exon splicing (Invariant 1 and 2). The exonic origin of each primer is shown in superscripts. Four nested primers located within each spliced exon were used in validation experiments to ensure capture of all GPHN transcript isoforms. UTR, untranslated region.

**Table II**

Polymorphisms within GPHN

Sequence variations were initially discovered in the cohort of hyperekplexia patients. The approximate population frequency of each GPHN genotype was assessed in a variable number of unrelated Caucasian control samples using a variety of screening techniques as indicated by RFLP, DDF, or SSCP. UTR, untranslated region.

**Table 3**

Isoform Heterogeneity of the Human Gephyrin Gene

668Kb

Fig. 4. A revised genomic organization of human GPHN. The previous version of the human GPHN gene described the existence of 27 exons distributed over 760 kb of genomic DNA on chromosome 14q32 (Fig. 2A). This remains true as a summary; however, our transcript data have led to a change in the order of exons, the exclusion of human exon C5′, and exclusion of rat C1, C6, and C7 from human GPHN transcripts. Based on these data, we suggest a new genomic representation of GPHN and a numbering system for all constitutional and spliced exons (Fig. 3B). We further recommend co-retaining the "C" nomenclatures for the C3 region and the (C4b-C4new-C4-C5) cluster, with revision of the latter to the more logical labeling of C4A-C4B-C4C-C4D, reflecting the physical genomic organization of these exons within human GPHN.

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The previous version of the human GPHN gene described the existence of 27 exons distributed over 760 kb of genomic DNA on chromosome 14q32 (Fig. 2A). This remains true as a summary; however, our transcript data have led to a change in the order of exons, the exclusion of human exon C5′, and exclusion of rat C1, C6, and C7 from human GPHN transcripts. Based on these data, we suggest a new genomic representation of GPHN and a numbering system for all constitutional and spliced exons (Fig. 3B). We further recommend co-retaining the "C" nomenclatures for the C3 region and the (C4b-C4new-C4-C5) cluster, with revision of the latter to the more logical labeling of C4A-C4B-C4C-C4D, reflecting the physical genomic organization of these exons within human GPHN.
gene and YTH clone capture, we provide evidence for extensive alternative intense splicing activity in neuronal tissue, which was in contrast to the single transcript observed in all eight nonneuronal tissues. The single nonneurological transcript includes exon C3, an exon that is absent from the majority of neuronal-specific transcripts, although human brain contains a mosaic expression of metabolic nonneuronal and heterogeneous neuron-specific isoforms. We suggest that the nonneuronal gephyrin transcript is ubiquitous in human tissues and serves a metabolic housekeeping purpose as a cofactor in molybdenum enzyme synthesis.

The substantial combinatorial nature of the C4 exon cluster is created from four alternatively spliced exons, which generate 10 neuronal specific isoforms. The majority of these transcripts do not retain exon C3; however, two rarely detected isoforms have retained exon C3 and one of the C4 cluster exons. Three of the four exons observed in the C4 region have been reported in rodent exons (25, 28). One novel exon was detected in human transcripts and is physically located, in the correct linear order, within the chromosome 14 BAC contig. Collectively, with the specific exon to exon construction pattern within all sequenced isoforms and the BAC contig validation, we are confident that the 5' → 3' order of the C3 and C4 spliced exons is correct. There remains a possibility that not all GPHN isoforms have been captured because of the sheer number of combinatorial possibilities; however, any additional isoforms may be extremely rare and transcribed in specific areas of the human brain. In phylogenetic terms, our lack of detection for rodent exons C6 and C7 is remarkable because (i) the complexity of alternative splicing of gephyrin is capable of interacting with multiple gephyrin isoforms; and (ii) a revised genomic structure for human GPHN and a simplified nomenclature for the exons within the C4 cluster is required; (iii) the GlyR β subunit is capable of interacting with multiple gephyrin isoforms; and (iv) molecular mechanisms exist whereby gephyrin could regulate complex associations with different cytoskeletal or cytoplasmic proteins at glycinegic versus GABAergic synapses.

The speculative functional consequence of the heterogeneous GPHN isoforms is attributed to interactions with a complex repertoire of protein interactions. The present list includes...
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evidence for protein interactions with the β subunit of the glycine receptor, tubulin, GABARAP, profilin, collybistin, and the dynein light chains. Considering that this is probably not the complete cast of interacting proteins, it becomes apparent that the splicing activity of C3 and C4 regions of the GPHN gene may form the basis for differential properties of GPHN isoforms. The "linker" segment also contains several potential protein-protein interaction domains including proline-rich and acidic segments as well as a short motif (amino acids 289–299) that has strong similarity to the tubulin-binding domain signature of tau and MAP2/MAP4 proteins (25). This may be of significance, since the tubulin-binding properties of gephyrin closely resemble those of mitogen-activated proteins (52).

Given the proximity of the site of insertion for the C4 cassette to the putative tubulin-binding domain, it is possible that C4 inserts might influence interactions with cytoskeletal proteins.

Interestingly, cassettes C4B and C4D are homologous to each other and to the α-helical region 1B of human keratin 8 (53). This motif is known to mediate keratin dimerization by the formation of coiled-coil structures. It is possible that inclusion of cassettes C4B and or C4D creates a binding site for a new gephyrin interactor. Ramming et al. (25) concluded that gephyrin transcripts containing the C3 and C4 exons were of low abundance but highly expressed in some subpopulations of neurons in the adult mouse brain. For example, cassette C4D was found in the granular cell layer of the cerebellum and olfactory bulb. However, this study did not take other developmental stages into account (e.g., cassette C3 is highly expressed throughout the central nervous system early in development) and did not focus on spinal cord or brainstem. Generating spliced exon-specific antibodies and immunohistochemical analysis in rodent and human brain will greatly enhance our understanding of spatial and temporal distribution of gephyrin transcripts.

Despite the established association of hyperekplexia mutations with subunits from glycineric ion channels in both humans and animal models, it is apparent that mutations in gephyrin are not a major cause of the disorder. Considering the high degree of phylogenetic conservation and that GPHN is an essential metabolic agent, it is possible that mutations in many regions of GPHN are embryonic lethal. Our promoter and exon by the exon mutation detection approach revealed a novel mutation causing a N10Y substitution at the extreme N terminus of GPHN. This portion of gephyrin is not part of the MogA consensus sequence, which begins at amino acid 15, and this positioning may be the basis of functional tolerance in the mutation carrier. Recent crystal structure determination of the GPHN N-terminal domain indicates a trimeric structure that presumably is the structural conformation required to form a subsynaptic lattice (54, 55). The N10Y mutation was found in a young male infant who presented with neonatal hypertonia and excessive startle response in the first year of life. At 4 years young male infant who presented with neonatal hypertonia and excessive startle response in the first year of life. At 4 years of age, there has been a transient recovery of the phenotype with no features of molybdenum metabolism disruption. GPHN10Y may have a limited detrimental window during developmental glycineric switching from GlyR α2 homopentamers to GlyR α1β heteropentamers. From a glycineric perspective, GPHN10Y does not disrupt GlyR-gephyrin interactions in human embryonic kidney cells and does not interrupt collybistin-induced cell surface clustering. Since in vitro clustering is not affected by GPHN10Y, the functional effect of this mutation remains elusive, and an alternative mechanism is responsible for the hyperekplexia phenotype.

The fact remains, however, that ~60% of our sporadic hyperekplexia cohort is devoid of GLRA1, GLRB, and GPHN mutations, and this clearly implies further genetic heterogene-

casting a wider net for the mutation analysis of glycineric transporters and GABAergic candidates may yet yield further genetic heterogeneity in hyperekplexia. Indeed, recent descriptions of GABA mutations in epilepsy demonstrates the subtle functional overlap between the two neuroinhibitory systems with the resulting phenotypes a reflection of the differential localizations and function of glycineric and GABAergic receptor subunit expression.

In conclusion, we have demonstrated the existence of a large number of neuron-specific gephyrin isoforms that may form the basis for specialized protein interactions. By identifying all exon constituents of the gene, we were able to exclude the gephyrin coding sequence as a major determinant for hyperekplexia. Nevertheless, a solitary mutation may yet have a functional explanation in a transient form of the disorder despite the exclusion of our initial functional hypothesis. We describe a number of synonymous and flanking intronic SNPs of GPHN that may be informative for mapping purposes and other disorders linked to chromosome 14q24. Finally, as a consequence of a YTH search for glycineric interacting proteins, we can confirm that the physical location of the GPHN-GlyR β interacting domain in the GPHN molecule does not lie in the MogA region, linker segment, or C3 and C4 splicing cluster. This domain lies within the C-terminal portion of the conserved MoeA region, and the definitive identification of an interacting GPHN motif represents the next challenge toward characterizing the physical nature of GPHN-GlyR coupling.

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