Domain-dependent clustering and genotype-phenotype analysis of *LGI1* mutations in ADPEAF

Yuan-Yuan Ho, PhD
Iuliana Ionita-Laza, PhD
Ruth Ottman, PhD

Correspondence & reprint requests to Dr. Ottman: ro6@columbia.edu

ABSTRACT

Objective: In families with autosomal dominant partial epilepsy with auditory features (ADPEAF) with mutations in the *LGI1* gene, we evaluated clustering of mutations within the gene and associations of penetrance and phenotypic features with mutation location and predicted effect (truncation or missense).

Methods: We abstracted clinical and molecular information from the literature for all 36 previously published ADPEAF families with *LGI1* mutations. We used a sliding window approach to analyze mutation clustering within the gene. Each mutation was mapped to one of the gene's 2 major functional domains, N-terminal leucine-rich repeats (LRRs) and C-terminal epitempin (EPTP) repeats, and classified according to predicted effect on the encoded protein (truncation vs missense). Analyses of phenotypic features (age at onset and occurrence of auditory symptoms) in relation to mutation site and predicted effect included 160 patients with idiopathic focal unprovoked seizures from the 36 families.

Results: ADPEAF-causing mutations clustered significantly in the LRR domain (exons 3–5) of *LGI1* (p = 0.026). Auditory symptoms were less frequent in individuals with truncation mutations in the EPTP domain than in those with other mutation type/domain combinations (58% vs 80%, p = 0.018).

Conclusion: The LRR region of the *LGI1* gene is likely to play a major role in pathogenesis of ADPEAF. *Neurology*® 2012;78:563–568

GLOSSARY

ADPEAF = autosomal dominant partial epilepsy with auditory features; **EPTP** = epitempin; **LRR** = leucine-rich repeat; **nt** = nucleotides.

Autosomal dominant partial epilepsy with auditory features (ADPEAF) (OMIM 600512) is an idiopathic focal epilepsy syndrome with auditory symptoms or receptive aphasia as a prominent ictal manifestation.[1–3] These symptoms strongly suggest localization to the lateral temporal lobe; hence, the syndrome is also called autosomal dominant lateral temporal lobe epilepsy. A substantial proportion (approximately 50%) of affected families have mutations in the leucine-rich, glioma inactivated 1 (*LGI1*) gene,[4–6] with an average penetrance of 67%.[7]

LGI1 encodes a secreted protein, Lgi1, with 2 major domains: an N-terminal leucine-rich repeat (LRR) domain containing 4 LRRs flanked by 2 conserved cysteine-rich regions, and a C-terminal epitempin (EPTP) domain containing 7 EPTP repeats.[8,9] To date, 33 unique *LGI1* mutations have been reported in ADPEAF families (n = 36) and sporadic patients with idiopathic focal epilepsy with auditory symptoms (n = 2).[7,8,10–15] Missense and truncation mutations have been found in both LRR and EPTP domains. Although previous studies have reported that pathogenic *LGI1* mutations are uniformly distributed across the gene,[8] none has used a quantitative approach to assess mutation clustering or investigated genotype-phenotype correlations in detail. Establishment of genotype-phenotype associations has the potential to elucidate the biologic pathways involving *LGI1*, including the mechanisms leading to ADPEAF symptoms.

In this study, we tested 2 hypotheses: 1) the distribution of ADPEAF-causing mutations is not uniform across the *LGI1* gene and 2) penetrance and phenotypic features in ADPEAF-affected individuals differ, depending on the predicted effect (truncation or missense) or domain location (LRR or EPTP) of mutations.

METHODS ADPEAF families. Molecular and clinical information from all 36 previously reported ADPEAF families with *LGI1* mutations (11 reported by our group and 25 reported by others) was collected and assembled into a database. For our previously reported families,[2–4,13,16] we obtained this information directly from our existing database. For families reported by others, we abstracted the clinical information from published reports.[6,10–12,14,15,17–26]

Mutation density and clustering analysis. To visualize the distribution of previously reported mutations in the gene, we first computed mutation density by counting the number of mutations in each exon and dividing the count by the nucleotide length of the exon (excluding intronic regions). For statistical analysis of mutation clustering across the coding sequence of *LGI1*, we used a sliding window approach. We considered all overlapping windows of a fixed length L, and for each such window W we calculated a statistic S_W defined as the maximum number of mutations over all windows of length L, i.e., $S_L = \max_W S_W$. Statistical significance was assessed using a classic approach from scan statistic theory that efficiently corrects for the multiple correlated windows that are considered.[27] S_W is sensitive to L and the total length of the nucleotide sequence scanned.

Genotype-phenotype correlation analysis. We estimated penetrance through a method similar to that in our previous article, which was based on the proportion affected among obligate carriers (i.e., parents of affected individuals who were not married into the family).[7] To increase precision, we modified our previous approach by including offspring of affected individuals in addition to obligate carriers. In each family, we estimated the number of mutation carriers (denominator of the penetrance estimate) from the published pedigree, assuming that all obligate carriers and half of the offspring of affected individuals were carriers. The number of these individuals who were affected served as the numerator of the penetrance estimate. To increase the likelihood that the offspring included in these calculations were old enough to have passed through the risk period for ADPEAF, we included only offspring of affected individuals aged >40 years at the time of the study or death, as reported in the original articles. (Age of the affected individuals served as a proxy for age of their offspring, because age of the offspring was seldom reported.) Then we divided the families into 2 groups based on the median penetrance estimate among all families and used χ^2 tests to assess differences in the proportion with high vs low penetrance in relation to the mutations' domain localization (LRR or EPTP) and predicted effect (missense or truncation).

For genotype-phenotype association analyses, we examined age at onset of unprovoked seizures and occurrence of auditory symptoms in 160 patients from 32 families with idiopathic focal unprovoked seizures in *LGI1* mutation-positive ADPEAF families. (For the remaining 4 families, information from published reports was not sufficiently detailed for analysis.) These 2 features were selected because they were the only ones systematically documented in the families reported in the literature. As described above, analyses were performed by stratifying the mutations by their domain location (LRR or EPTP) and predicted effect (missense or truncation). Genotype-phenotype association was assessed by linear regression (for age at onset) and logistic regression (for auditory symptoms) using generalized estimating equations to account for the clustering of individuals within families. All analyses were performed using SPSS (IBM, Chicago IL).

Standard protocol approvals, registrations, and patient consents. This study was approved by the Columbia University Medical Center Institutional Review Board, and all participants from our research group gave written informed consent. Data from individuals studied by other research groups were taken from published reports and were anonymous.

RESULTS Mutation density and clustering analyses. Figure 1A summarizes the genomic structure of *LGI1* with all 33 reported pathogenic mutations labeled. As shown in the figure, 5 mutations have been observed more than once, either in 2 families (n = 3) or in a family and a sporadic case (n = 2). Mutation density within each exon is represented by the color intensity of the exon; higher color intensity represents higher density. Exons 1–6 encode the protein's LRR domain, and exons 7–8 encode the EPTP domain. Figure 1B is a schematic diagram of the Lgi1 protein with predicted effects of the mutations. With the exception of one in-frame deletion mutation (377–379delACA) resulting in the deletion of asparagine in the encoded protein,[11] all other mutations are predicted to produce either amino acid substitution (missense mutations, n = 21, 66% of all mutations) or truncation of the Lgi1 protein (n = 11, 34% of all mutations).

Figure 2A shows that mutation density is the highest in exon 4, followed by exon 5 and exon 3. Stratification of mutations by their predicted effects on the protein revealed that the high density of mutations in exon 4 is primarily attributed to missense mutations, which are more common than truncation mutations.

To assess the significance of mutation clustering by the sliding window analysis, we chose a window length $L = 118$ bp, the median size of the *LGI1* exons, to scan the mRNA length of the gene. Because the scanning statistic is sensitive to the length of mRNA analyzed, we chose 2 lengths (1,774 and 1,823 nucleotides [nt]) to capture the sequencing range covered in the literature.[28,29] Among the total of 38 mutational events, 35 were included in this scanning analysis. The remaining 3 mutations were intronic and were excluded because intronic sequences have not been systematically sequenced. As shown in figure 2B, the maximum number of mutational events observed in any window is 9, i.e., $S_L = 9$. The resulting p values for 2 scanning lengths are 0.030 and 0.026 for nt = 1,774 and nt = 1,823, respectively. Moreover, 2 of the 3 previously reported intronic mutations are located within the genomic sequence spanning exons 3–5 of *LGI1*, corresponding to the region where the exonic mutations

Figure 1 *LGI1* mutations cluster in leucine-rich repeat (LRR) domain
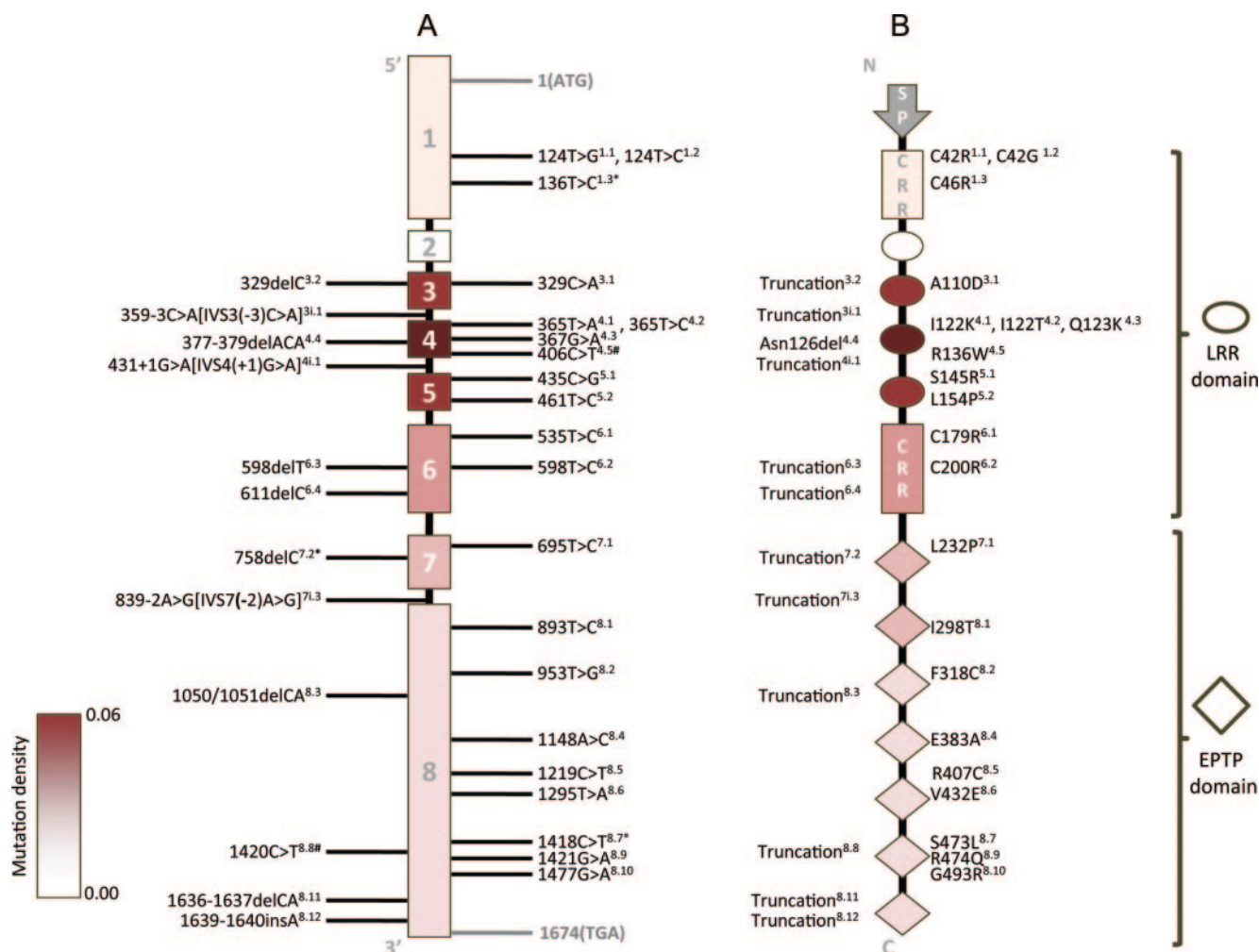
(A) *LGI1* gene structure with the reported mutation sites indicated. For superscripts next to mutations, the first digit indicates the exon localization of the mutation, and the second digit (after the decimal point) indicates the order of the mutation within the exon (mutations at the same nucleotide numbered arbitrarily). i = intronic mutation; * = mutation found in 2 families (n = 3); # = mutation found in a family and a sporadic case (n = 2). (B) Lgi1 protein with effect of mutations on protein summarized. The 4 LRRs in the LRR domain are represented by ovals and the 7 epitempin (EPTP) repeats in the EPTP domain are represented by diamonds. Different intensities of the red shade correspond to the density of mutations in each exon. The superscripts next to protein variants are cross-indexed to the nucleotide changes listed in the gene structure (A). CRR = cysteine-rich region; SP = signal peptide.

cluster. The number of mutations was too small for formal statistical analysis of missense and truncation mutations separately.

To confirm that the clustering of *LGI1* variants observed in the ADPEAF families is specific to pathogenic mutations, we searched the UCSC Genome Browser (db build 131) for *LGI1* variants.[30] The search returned 266 variants in the *LGI1* gene, of which 170 were validated by HapMap (270 individuals) or the 1000 Genomes Project (629 individuals). Only 2 of 170 validated variants were in the coding region, one encoding a synonymous change in exon 6 and the other encoding a change in the 3′ untranslated region of exon 8. Neither of these has been associated with ADPEAF. The relative invariability of the protein coding sequence of *LGI1* in reference genomes indicates that the gene is highly conserved, and the clustering of ADPEAF-

causing mutations in the LRR region is probably specific to pathogenic mutations.

Genotype-phenotype correlation analyses. The overall penetrance estimate was 66% (SE 3.6%), with a median of 75% among the 36 families. The proportion of families with penetrance ≥75% was similar among those with missense (52%) vs truncation (58%) mutations. The proportion with penetrance ≥75% was higher among families with mutations in the LRR (58%) vs EPTP (47%) domain, but the difference was not significant.

The prevalence of auditory symptoms was significantly lower among individuals with truncation mutations than among those with missense mutations (65% vs 81%, $p = 0.031$) but did not differ according to domain location of mutations (table). Further

Figure 2 Significant clustering of pathogenic *LGI1* mutations in the leucine-rich repeat (LRR) domain
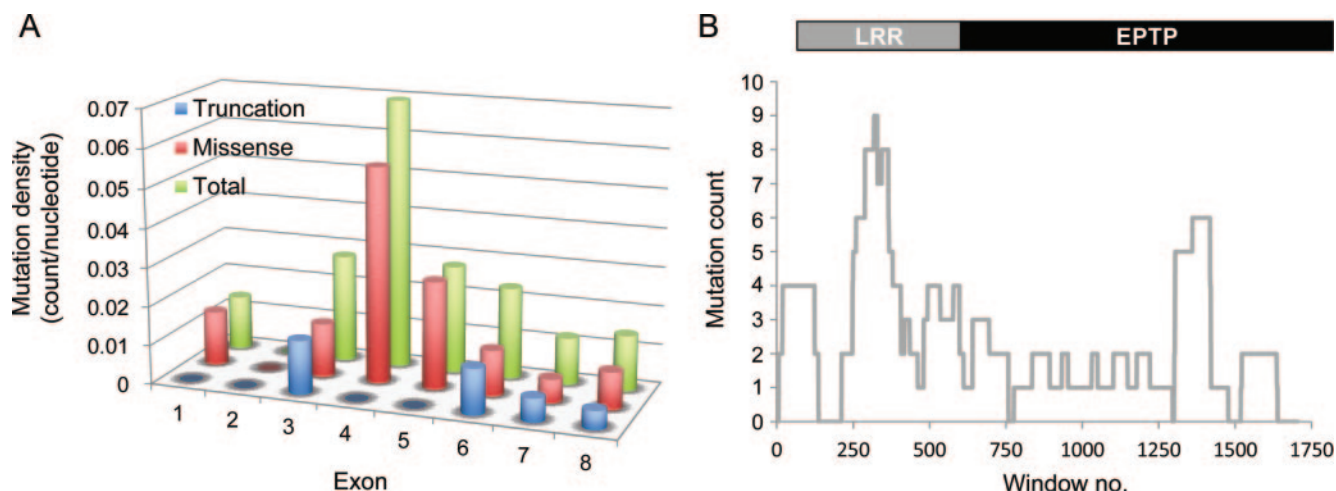
(A) Mutation density per nucleotide within each exon. Blue bars represent truncation mutations, red bars represent missense mutations, and green bars represent total mutations. (B) A sliding window analysis was performed on the sequence length of *LGI1* mRNA amplified by the reported sequencing primers (1,823 nucleotides). A window size of 118 bp (median size of exons in *LGI1*) was used and the maximum number of mutations observed per window was 9 ($p = 0.026$). EPTP = epitempin.

stratification by both mutation effect and domain showed that the lower prevalence of auditory symptoms in individuals with truncation mutations was attributed to a lower frequency in those with truncation mutations in the EPTP domain (58% vs 80% in those with other mutation types/localizations, $p = 0.018$). Age at onset was not associated with either domain location or predicted effect of mutations (table). These results were not changed after adjustment for the current age of the patients.

DISCUSSION Establishment of genotype-phenotype relationships is fundamental to elucidating pathogenic effects of human mutations. In addition to influencing susceptibility to ADPEAF, recent studies have also shown that *LGI1* plays an important role in

limbic encephalitis, a disorder in which autoimmunity against the Lgi1 protein is the underlying cause.[31] These observations support the importance of *LGI1* in a spectrum of neurologic functions.

We found that ADPEAF-causing mutations cluster significantly within the LRR domain of *LGI1*. This finding was primarily attributed to missense mutations. The most likely explanation for this finding is that mutations in the LRR domain confer a greater susceptibility to ADPEAF than those in the EPTP domain, although mutations in both domains are clearly pathogenic. In addition, given the very strong conservation of this gene, an alternative explanation is that mutations in the LRR domain are more compatible with life and hence are observed more frequently than those in the EPTP domain. Either or both possibilities could underlie the mutation clustering in the LRR domain.

We observed a significantly lower frequency of auditory symptoms among individuals with truncation mutations in the EPTP domain than among those with other mutation types/locations. This finding is difficult to interpret with our current state of understanding of the biological function of the *LGI1* gene products. Analysis of disease severity (e.g., medication response or seizure frequency) might have been more revealing but was not possible with the data abstracted from the literature. One possible explanation for the lower frequency of auditory symptoms among patients with truncation mutations in the EPTP domain is that they had a milder form of epilepsy, with fewer seizures overall and less recognition of associated symptoms.

Table	Age at onset and occurrence of auditory symptoms in 32 ADPEAF families by predicted effect and domain localization of *LGI1* mutations					
Mutation effect and domain	**Age at onset**			**Auditory symptoms**		
	No. individuals	Mean (SEM)	p Value	No. individuals	No. (%)	p Value
Truncation	54	15.7 (1.37)	0.116	71	46 (64.8)	0.031
Missense	78	18.7 (1.34)		82	66 (80.5)	
LRR	70	16.1 (0.99)	0.203	74	58 (78.4)	0.176
EPTP	65	18.6 (1.75)		84	58 (69.0)	
Truncation-LRR	25	14.8 (1.32)		26	20 (76.9)	
Truncation-EPTP	29	16.4 (2.19)	0.196	45	26 (57.8)	0.018
Missense-LRR	42	17.1 (1.27)		43	34 (79.1)	
Missense-EPTP	36	20.4 (2.35)		39	32 (82.1)	

Abbreviations: ADPEAF = autosomal dominant partial epilepsy with auditory features; EPTP = epitempin; LRR = leucine-rich repeat.

Further elucidation of the function of *LGI1* and its products is essential for better understanding of the possible phenotypic consequences of particular mutations. The pathogenicity of *LGI1* mutations may involve both haploinsufficiency and dominant-negative mechanisms. In *Lgi1* mouse knockout models, homozygous ablation of the gene leads to spontaneous seizures and early death,[32,33] whereas heterozygous *Lgi1*+/− mice showed a lowered threshold for auditory stimuli–induced seizures.[32] These findings are consistent with a mechanism involving haploinsufficiency, because the phenotype is more extreme in homozygotes than in heterozygotes. Although in many genes, truncation mutations lead to nonsense-mediated decay of the abnormal mRNA,[34] evidence from animal and human studies indicates that this process does not always occur with truncation mutations in *LGI1*.[35,36] For example, in transgenic mice with an extra copy of the *LGI1* gene containing a truncation mutation in exon 6, maturation of glutamatergic synapses was arrested, clearly indicating a dominant-negative effect of the mutation.[36] These results imply that the abnormal mRNA transcript resulting from the truncation mutation was not simply degraded by nonsense-mediated decay.

Protein-protein interactions involving Lgi1's EPTP domain have been studied more extensively than those involving the LRR domain. Several studies have used EPTP truncation mutations to investigate interactions of Lgi1 with Kv1 channels in presynaptic neurons[35] or with ADAM proteins at transsynaptic sites.[37,38] However, a recent model proposed that the formation of Lgi1 dimers through the LRR domain may influence pre- and postsynaptic transport or communication.[39] These interactions may underlie the maturation and activity of glutamatergic synapses and thus modify the manifestation of ADPEAF.[36,37] Given our finding of mutation clustering in the LRR domain, the reported dominant-negative effects of the EPTP truncation mutants, and the observation of naturally occurring Lgi1 isoforms that resemble EPTP truncation mutants,[40] further investigation of protein-protein interactions or other molecular mechanisms involving the LRR domain that might influence epileptogenesis is warranted.

Our inclusion of all known *LGI1* mutation-positive ADPEAF families in these genotype-phenotype association analyses provided an opportunity to gain insights into the structure-function relationships of *LGI1* in a physiologic context. The sliding window analysis used here may be applicable to the analysis of mutation clustering in other disease genes.

Conversely, the lack of consistent reporting of phenotypic characteristics in the literature restricted our genotype-phenotype association analysis to penetrance, age at onset, and auditory symptoms. Statistical power was also limited by the relatively few previously reported ADPEAF families with *LGI1* mutations. We are in the process of developing a more structured and standardized platform that can be used across different research centers for collection of clinical information on ADPEAF. Future analyses using a larger collection of ADPEAF families and more comprehensive phenotypic information will facilitate further elucidation of genotype-phenotype relationships of *LGI1* mutations.

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