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FULL-LENGTH ORIGINAL RESEARCH

Genome-wide linkage meta-analysis identifies susceptibility loci at 2q34 and 13q31.3 for genetic generalized epilepsies

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Summary

Purpose: **Genetic generalized epilepsies (GGEs) have a lifetime prevalence of 0.3% with heritability estimates of 80%. A considerable proportion of families with siblings affected by GGEs presumably display an oligogenic inheritance. The present genome-wide linkage meta-analysis aimed to map: (1) susceptibility loci shared by a broad spectrum of GGEs, and (2) seizure type–related genetic factors preferentially predisposing to either typical absence or myoclonic seizures, respectively.**

Methods: **Meta-analysis of three genome-wide linkage datasets was carried out in 379 GGE-multiplex families of European ancestry including 982 relatives with GGEs. To dissect out seizure type–related susceptibility genes, two family subgroups were stratified comprising 235 families with predominantly genetic absence epilepsies (GAEs) and 118 families with an aggregation of juvenile myoclonic epilepsy (JME). To map shared and seizure type–related susceptibility loci, both nonparametric loci (NPL) and parametric linkage analyses were performed for a broad trait model (GGEs) in the entire set of GGE-multiplex families and a narrow trait model (typical absence or myoclonic seizures) in the subgroups of JME and GAE families.**

Key Findings: **For the entire set of 379 GGE-multiplex families, linkage analysis revealed six loci achieving suggestive evidence for linkage at 1p36.22, 3p14.2, 5q34, 13q12.12, 13q31.3, and 19q13.42. The linkage finding at 5q34 was consistently supported by both NPL and parametric linkage results across all three family groups. A genome-wide significant nonparametric logarithm of odds score of 3.43 was obtained at 2q34 in 118 JME families. Significant parametric linkage to 13q31.3 was found in 235 GAE families assuming recessive inheritance (heterogeneity logarithm of odds = 5.02).**

Significance: **Our linkage results support an oligogenic predisposition of familial GGE syndromes. The genetic risk factor at 5q34 confers risk to a broad spectrum of familial GGE syndromes, whereas susceptibility loci at 2q34 and 13q31.3 preferentially predispose to myoclonic seizures or absence seizures, respectively. Phenotype–genotype strategies applying narrow trait definitions in phenotypic homogeneous subgroups of families improve the prospects of disentangling the genetic basis of common familial GGE syndromes.**

KEY WORDS: **Genetic generalized epilepsy, Complex inheritance, Absence seizure, Myoclonic seizure, Linkage analysis.**

Genetic factors play a predominant role in about 40% of all epilepsies (ILAE Commission on Classification and Terminology, 1989). Genetic generalized epilepsies (GGEs, formerly called the idiopathic generalized epilepsies) represent the most common group of genetically determined epilepsies; they account for approximately 20–30% of all epilepsies (Jallon et al., 2001). The GGE syndromes are characterized by age-related recurrent unprovoked generalized seizures in the absence of detectable brain lesions or metabolic abnormalities (ILAE Commission on Classification and Terminology, 1989; Berg et al., 2010). The common classical GGE syndromes include childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and epilepsy with generalized tonic–clonic seizures (EGTCS) alone (Nordli, 2005). The electroencephalographic signature is generalized spike-wave discharges (GSW-EEG), which reflect a synchronized hyperexcitability state of thalamocortical circuits (Blumenfeld, 2005).

Despite heritability estimates of >80% obtained by twin studies (Berkovic et al., 1998; Kjeldsen et al., 2003), the genetic factors predisposing to common GGE syndromes remain elusive (Gardiner, 2005). The genetic architecture of GGEs most likely represents a biologic continuum, in which a small fraction (1–2%) follows monogenic inheritance, whereas the majority of GGE patients presumably display an oligogenic/polygenic predisposition. Moreover, twin and family studies provide evidence for genetic determinants shared across common GGE syndromes, but also suggest that heterogeneous configurations of genetic risk factors specify the phenotypic expression of absence and myoclonic seizures (Berkovic et al., 1987; Beck-Mannagetta & Janz, 1991; Berkovic et al., 1988; Schmitz et al., 2000; Winawer et al., 2003; Marini et al., 2004; Winawar et al., 2005; Kinirons et al., 2008).

Linkage mapping and positional candidate gene analysis provide a suitable approach to identify major susceptibility genes in families showing a clustering of GGE syndromes. Most of the currently known genes for rare monogenic forms of genetic epilepsies encode voltage-gated or ligand-gated ion channels (e.g., *SCN1A*, *GABRA1*, *KCNQ2*, and *CHRNA4*) (Reid et al., 2009; Poduri & Lowenstein, 2011). Although the known epilepsy genes identified in rare monogenic forms of epilepsy explain only a small proportion of the genetic liability, the casual gene mutations have allowed important insights into key mechanisms of epileptogenesis (Noebels, 2003; Reid et al., 2009). However, none of these epilepsy genes seems to play a substantial role in the genetic predisposition of common GGE syndromes.

Most of the linkage claims reported in genetically complex GGE syndromes (2q34-q36, 3p23-p14, 3q26, 5p15, 5q22, 6p12, 6p23.1, 7q14, 8p12, 8q24, 11q13, 13q31, 14q23, 15q14, 18q21, 19q13) remain controversial, because replication studies have often failed to confirm initial linkage hints in independent sets of families (Greenberg et al., 1988; Zara et al., 1995; Liu et al., 1996; Elmslie et al., 1997; Fong et al., 1998; Greenberg et al., 2000; Sander et al., 2000; Durner et al., 2001; Tauer et al., 2005;

Hempelmann et al., 2006; Chioza et al., 2009; Greenberg & Subaran, 2011). The failure to detect replicable susceptibility genes for common epilepsies most likely reflects the underestimated degree of genetic complexity and heterogeneity in human epilepsies. With regard to the drastic loss of power of a linkage scan by the extent of locus heterogeneity, >300 families would be necessary to achieve a reasonable power to map a genetic risk factor, which is present in at least 30% of the families (Table S5). Given that most of the published linkage studies on common GGE syndromes included relatively small samples of <100 GGE-multiplex families, these studies provided low power to detect a major susceptibility locus when <50% of the families were linked to the same locus. Therefore, it is not surprising that the linkage studies reported so far did not reveal replicable susceptibility loci.

To achieve adequate power, the present genome-wide linkage analysis combined three linkage datasets, including 379 clinically well-characterized GGE-multiplex families of European ancestry. Our linkage meta-analysis aimed: (1) to map susceptibility loci shared by a broad spectrum of common GGEs and (2) to dissect out seizure type–related susceptibility genes contributing to the familial clustering of the same seizure type in familial GGEs.

Subjects and Methods

Family ascertainment and clinical assessment

The family sample comprised 379 GGE-multiplex families of European descent, including at least two siblings with GGEs. All families were ascertained under the following inclusion criteria: (1) proband with either genetic absence epilepsy (GAE: CAE/JAE), juvenile myoclonic epilepsy (JME), or EGTCS alone starting before the age of 26 years and exhibiting a GSW-EEG during the course of the epilepsy; and (2) one or more siblings with GGE. In the presence of multigenerational inheritance, family ascertainment was extended toward additional affected first-degree relatives. Phenotyping and diagnostic classification of GGE syndromes was carried out according to standardized protocols available at: http://portal.ccg.uni-koeln.de/ccg/research/epilepsy-genetics/sampling-procedure (ILAE Commission on Classification and Terminology, 1989; Berg et al., 2010). The status of affectedness of patients with GGE and with a history of severe major psychiatric disorders (autism, schizophrenia, affective disorder: recurrent episodes requiring pharmacotherapy or treatment in a hospital), or severe and profound intellectual disability (no basic education, permanently requiring professional support in their daily life) was classified as ''unknown'' in the linkage analyses.

The entire sample of 379 European GGE-multiplex families comprised three sets of families collected since 1995 by EPICURE partners and collaborating international groups (Table S1) (Sander et al., 2000; Hempelmann et al., 2006; EPICURE Integrated Project). The 379 GGE-multiplex families included 1,920 family members, of whom 982 were affected by GGEs (Tables 1 and S2; 596 female/386 male; syndrome classification: CAE/JAE [n = 504] JME [n = 258], EGTCS alone (GSW-EEG, age of onset <26 years) n = 205, unclassified GGE syndromes [n = 15]; origins by country: Austria [n = 3], Australia/United Kingdom [n = 32], Belgium [n = 1], Bosnia [n = 1], Bulgaria [n = 4], Denmark [n = 10], Finland [n = 7], France [n = 45], Germany [n = 93], Greece [n = 8], Italy [n = 93], Poland [n = 2], Russia [n = 4], Spain [n = 6], Sweden [n = 1], The Netherlands [n = 23], Turkey [n = 21], United Kingdom [n = 25]).

Family groups and trait models

The genome-wide linkage scan for susceptibility loci shared by a wide spectrum of common GGE syndromes was carried out under the broad trait model in the entire group of 379 GGE-multiplex families. The broad trait model classified family members with any GGE as ''affected.''

To dissect out seizure type–related susceptibility loci, two family subgroups were ascertained through a family member affected either by JME-related myoclonic seizures (118 JME families) or typical absence seizures (235 GAE families) and the occurrence of at least two siblings affected by either typical absence seizures or JME-related myoclonic seizures (Tables 1, S3, and S4). Linkage analysis was performed under the narrow trait model, which considered individuals with either typical absence seizures or JME-related myoclonic seizures as ''affected.'' Together, our ascertainment scheme and the application of the narrow trait model

Table 1. Clinical characterization of the groups of GGE-multiplex families

Sample	Trait	Fam. N	Ind. N	Typed Ind.	Affected Ind.	GAE	GAE & JME	JME	EGTCS alone	Unclass GGE	GSW-EEG only	Other epilepsies	FS only
GGE	BM	379	1,920	1,728	982	504	60	198	205	15	21	6	18
GAE	NM	235	1,213	1,095	567	442	54	71	60	7	18	3	9
JME	NM	118	624	560	289	74	54	161	37	5	9	3	7

Fam., family; Ind., individual; GGE, genetic generalized epilepsy; GAE, genetic absence epilepsy; JME, juvenile myoclonic epilepsy; EGTCS, epilepsy with generalized tonic–clonic seizures; unclass GGE, unclassified GGE; GSW-EEG, generalized spike-wave EEG discharges; FS, febrile seizure; BM, broad trait model (all GGE syndromes); NM, narrow trait model (typical absence or myoclonic seizures).

result in a familial clustering of the target seizure type in the affected individuals of both family subgroups (Table 1): (1) 235 GAE families included 567 affected relatives, of whom 87.5% exhibited typical absence seizures; (2) 118 JME families comprised 289 affected relatives, of whom 74.4% exhibited JME-related myoclonic seizures.

The ''affection'' status of family members with forms of seizures or epilepsies (e.g., febrile seizures, focal epilepsies) other than those specified in the trait model, or known GSW-EEG discharges without seizures, or missing clinical information were classified as ''unknown.'' All remaining individuals were considered as ''unaffected.''

Genome scan marker panels

The present genome-wide linkage meta-analysis consists of three datasets (Table S1): (1) 107 GGE-multiplex families genotyped by a genome-wide panel including 383 autosomal short tandem repeat polymorphisms (STRs) (Sander et al., 2000); (2) 95 GGE-multiplex families genotyped by 639 STRs (Hempelmann et al., 2006); and (3) 177 GGE multiplex-families collected by the EPICURE Integrated Project and genotyped by the Illumina HumanLinkage-12 BeadChip consisting of 6,090 single nucleotide polymorphisms (SNPs). Rutgers sex-averaged combined linkage-physical map of the human genome (Rutgers map v.2) was used for integrating the genetic map positions of STR and SNP marker panels (Matise et al., 2007). The map positions of new STRs were obtained from Rutgers Map Interpolator software (http://compgen.rutgers.edu/old/map-interpolator). STR markers and SNPs had to achieve a genotyping call rate >95% and >98%, respectively. Mendelian errors were assessed by the program Pedcheck (O'Connell & Weeks, 1998) and in case of errors of a marker, the marker genotypes were set to missing for all family members. The pedigree relationship was validated by the graphical representation of relationship errors (GRR) program (http://bioinformatics.well.ox.ac.uk/GRR; Abecasis et al., 2001). The linkage program MERLIN (http://www.sph.umich.edu/csg/abecasis/merlin/index.html; Abecasis et al., 2002) was applied to detect unlikely recombination events and unlikely genotypes were set to missing.

Statistical linkage analyses

Multipoint linkage analysis was performed for nonparametric (NPL) and parametric inheritance models using the ALLEGRO v2 software program (Gudbjartsson et al., 2005). NPL analysis applied the linear model of the S_{all} scoring statistics, which measures identical-by-descent (IBD) allele sharing among all affected family members in a pedigree (Whittemore & Halpern, 1994). For any marker polymorphism linked to an epilepsy gene, we expect an excess in IBD allele-sharing by the affected family members, relative to expectations of a random Mendelian allele segregation. NPL analyses were chosen as screening method, because this linkage statistic does not require the specification of an inheritance model and allows the simultaneous detection of oligogenic linkage signals.

For parametric multipoint linkage analyses, we analyzed models of dominant (risk allele frequency 0.01) and recessive (risk allele frequency 0.1) inheritance with reduced penetrance (narrow trait model [NM]: 50%, broad trait model (BM): 70%; phenocopy rate: 0.5%) with allowance for locus heterogeneity. This approach covers a wide range of oligogenic inheritance models and can be more powerful than NPL statistics, as long as the parameters of the inheritance model are correctly specified (Hodge et al., 1997; Abreu et al., 2002). Notably, power simulations demonstrate that the entire sample of 379 GGE-multiplex families has a power of nearly 100% to achieve genome-wide significance (heterogeneity logarithm (base 10) of odds [HLOD] = 3.5) under the broad trait model and a recessive mode of inheritance, when we presume that 30% of the families are linked to the same susceptibility locus. For the dominant approximation model, the power is about 80% for this scenario. Power simulations for the family groups are shown in Table S5.

Thresholds for suggestive and significant linkage for each analysis were assessed empirically by simulation analyses implemented in the simulate option of the MERLIN software package (Abecasis et al., 2002). The gene-dropping approach allowed us to account for incomplete extraction of inheritance information and for the diversity of pedigree structure. This empiric approach is more appropriate than theoretically derived significance thresholds as proposed by Lander and Kruglyak (1995), which are often conservative, because of their assumption of fully informative inheritance (Wiltshire et al., 2002). The empirically derived threshold for suggestive linkage refers to the probability that a linkage score greater than this threshold occurs only once at random in a single genome scan, and once in 20 linkage scans for significant linkage. These critical significance thresholds based on 5,000 simulations of the real data sets were the following: (1) NPL analyses (Table S6); suggestive: nonparametric logarithm of odds (LOD_{npl}) >1.80, significant: LOD_{npl} >3.15; and (2) parametric linkage analysis (Table S7); suggestive: HLOD >2.13, significant: HLOD >3.49 including a correction of HLOD = 0.3 for testing two inheritance models (Hodge et al., 1997; Abreu et al., 2002). We did not include a correction for performing parametric as well as NPL analyses, because of the strong correlation of both linkage statistics, and we did not adjust for multiple testing of three family groups, because these tests evaluate specific phenotype–genotype relationships.

Results

To search for genetic risk factors shared by a wide spectrum of familial GGE syndromes, we performed a genome-wide NPL scan under the broad trait model (all GGEs). The genome-wide NPL results are presented in Fig. 1A. None of

the NPL results met genome-wide significance (LOD_{npl} >3.15), but multipoint NPL analysis revealed four loci achieving suggestive evidence for linkage (LOD_{npl} >1.80) at 3p14.2 (LOD_{npl} = 2.96 at rs624755, chromosomal position: chr3:61709002 according to NCBI build 36.3), 5q34 (LOD_{npl} = 1.95 at rs1432881, chr5:166865098), 13q12.12 (LOD_{npl} = 2.42 at rs1008812, chr13:22864145), and 19q13.42 (LOD_{npl} = 2.86 at rs9788, chr19:58411062) (Table 2; Fig. 1A). Consistent with the NPL results, parametric HLOD analyses revealed suggestive evidence for linkage at 3p14 for both inheritance models (dominant: HLOD = 2.84 for α = 0.20 at rs782728, chr3:66408992; recessive: HLOD = 3.21 for α = 0.13 at rs1374679, chr3:63050307) (Table 3; Fig. 1B,C). In addition, suggestive evidence for linkage was obtained at 1p36.22 assuming dominant inheritance (HLOD = 2.50 for α = 0.17 at rs1216213, chr1:9969047), and at 13q31.3 assuming recessive inheritance (HLOD = 2.67 for α = 0.11 at D13S1230, chr13:88834621) (Table 3; Fig. 1C).

To dissect out seizure type–related susceptibility genes, linkage analyses under the narrow trait model were carried out in two family subgroups exhibiting a clustering of the target seizure type. The genome-wide parametric and non-parametric linkage results in 235 GAE families are shown in Fig. 2; Tables 2 and 3. NPL analysis revealed suggestive evidence for linkage in the chromosomal region 5q34 (Fig. 2A; LOD_{npl} = 2.31 at rs244903, chr5:167846088). Corresponding to the NPL results, parametric HLOD score analysis showed suggestive evidence for linkage at 5q34, assuming dominant inheritance (Fig. 2B; HLOD = 3.23 for α = 0.31 at rs357608, chr5:150820573). Significant parametric linkage was found in the chromosomal region 13q31.3, assuming recessive inheritance (Fig. 2C; HLOD = 5.02 for α = 0.22 at rs1332470, chr13:90215191).

The genome-wide parametric and NPL results in 118 JME families are presented in Fig. 3; Tables 2 and 3. A genome-wide significant NPL score was obtained at 2q34 (LOD_{npl} = 3.43 at D2S143, chr2:214624639) and a suggestive NPL score of LOD_{npl} = 2.62 at 5q34 (rs1025482, chr5:166825835) (Fig. 3A). Consistently, both loci at 2q34 and 5q34 were supported by the parametric linkage results (Table 3; Fig. 3B,C). We observed suggestive evidence for linkage at 2q34 for both inheritance models (dominant: HLOD = 2.50 for α = 0.39 at D2S143; recessive: HLOD = 2.59, α = 0.25 at D2S143). Suggestive evidence for linkage was found at 5q34 (HLOD = 2.96 for α = 0.40 at rs2069347, chr5:162799773) and at 21q22.3 (HLOD =

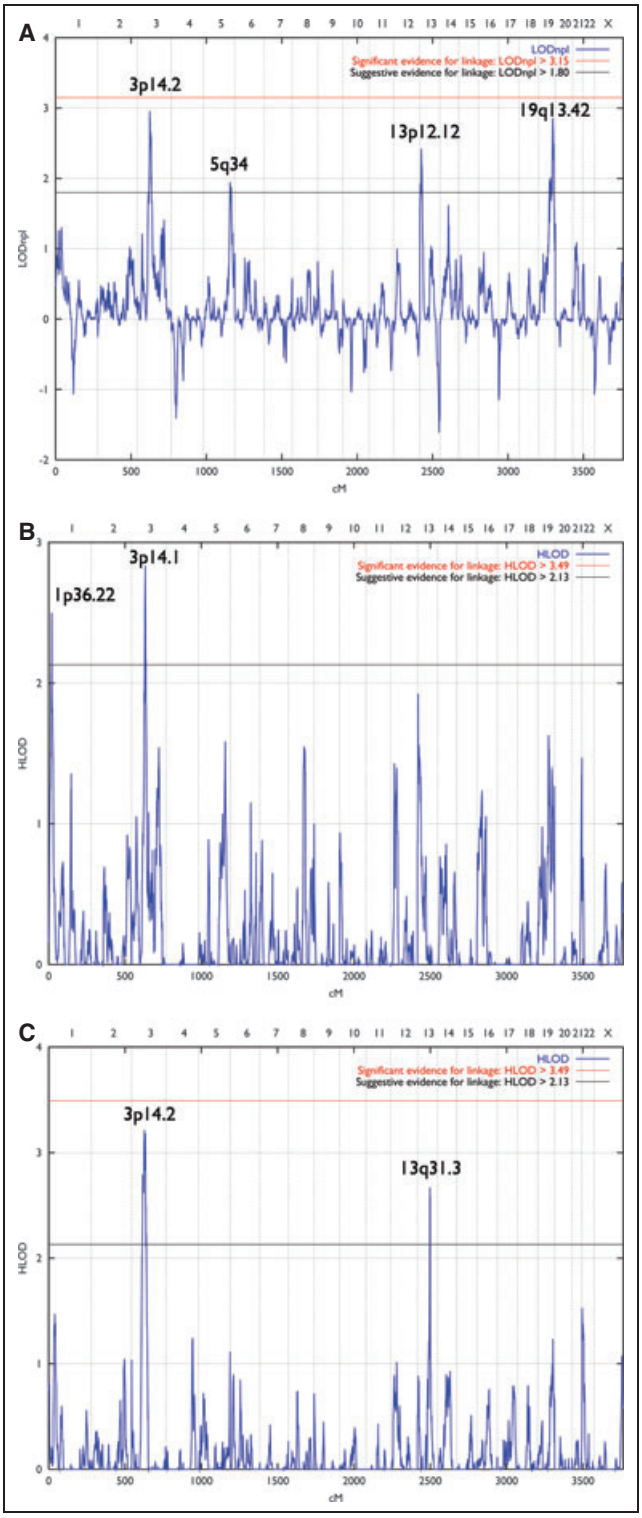

Figure 1.
Genome-wide linkage scan in 379 GGE families assuming a broad trait definition. Linkage results are shown for: (**A**) non-parametric linkage analysis, (**B**) parametric linkage analysis applying a dominant inheritance model with 70% penetrance, and (**C**) a recessive inheritance model with 70% penetrance. The chromosomes are arranged in linear scale from pter to qter on the upper x-axis. Empirically derived genome-wide significance thresholds are indicated in the plots as horizontal lines. Broad trait definition: all GGEs.

Table 2. Suggestive and significant nonparametric linkage results

Sample	Trait	LOD_{npl}	Chrom.	Marker	Chrom. pos.
GGE	BM	2.96	3p14.2	rs624755	3:61709002
GGE	BM	1.95	5q34	rs1432881	5:166865098
GGE	BM	2.42	13q12.12	rs1008812	13:22864145
GGE	BM	2.86	19q13.42	rs9788	19:58411062
GAE	NM	2.31	5q34	rs244903	5:167846088
JME	NM	**3.43**	2q34	D2S143	2:214624639
JME	NM	2.81	5q34	rs1025482	5:166825835

GGE, genetic generalized epilepsy; GAE, genetic absence epilepsy; JME, juvenile myoclonic epilepsy; BM, broad trait model (all GGE syndromes); NM, narrow trait model (typical absence or myoclonic seizures); Chrom., chromosome; Chrom. pos., physical chromosomal nucleotide position; significant linkage scores are highlighted in bold scores.

Table 3. Suggestive and significant parametric linkage results

Sample	Trait	MOI	HLOD	α	Chrom.	Marker	Chrom. pos.
GGE	BM	AD70	2.50	0.17	1p36.22	rs12136213	1:9969047
GGE	BM	AD70	2.84	0.20	3p14.1	rs782728	3:66408992
GGE	BM	AR70	3.21	0.13	3p14.2	rs1374679	3:63050307
GGE	BM	AR70	2.67	0.11	13q31.3	D13S1230	13:88834621
GAE	NM	AD50	3.23	0.31	5q33.1	rs357608	5:150820573
GAE	NM	AR50	**5.02**	0.22	13q31.3	rs1332470	13:90215191
JME	NM	AD50	2.50	0.39	2q34	D2S143	2:214624639
JME	NM	AD50	2.96	0.40	5q34	rs2069347	5:162799773
JME	NM	AD50	2.57	0.39	21q22.3	rs2839377	21:46902240
JME	NM	AR50	2.59	0.25	2q34	D2S143	2:214624639

GGE, genetic generalized epilepsy; GAE, genetic absence epilepsy; JME, juvenile myoclonic epilepsy; BM, broad trait model (all GGE syndromes); NM, narrow trait model (typical absence or myoclonic seizures); MOI, mode of inheritance; AD70, autosomal dominant inheritance with 70% penetrance; AR70, autosomal recessive inheritance with 70% penetrance; AD50, autosomal dominant inheritance with 50% penetrance; AR50, autosomal recessive inheritance with 50% penetrance; HLOD, heterogeneity LOD score; α, proportion of linked families; Chrom., chromosome; Chrom. pos., physical chromosomal nucleotide position; significant linkage scores are highlighted in bold scores.

2.57 for $\alpha = 0.39$ at rs2839377, chr21:46902240), assuming dominant inheritance (Table 3; Fig. 3B,C).

Discussion

The present linkage meta-analysis includes a sample of 379 European GGE-multiplex families, which is at least three times larger than any other study sample of GGE-multiplex families reported so far. This linkage study was designed to map genetic risk factors shared by a broad spectrum of common familial GGE syndromes and to dissect out seizure type–related susceptibility genes. To search for genetic risk factors shared by GGEs, we have carried out linkage analyses in the entire sample of 379 GGE-multiplex families under the broad trait model, considering all GGEs as ''affected.'' None of the linkage results for shared genetic risk factors met genome-wide significance. However, we found suggestive evidence for linkage to six chromosomal segments: 1p36.22, 3p14.2, 5q34, 13q12.12, 13q31.3, and 19q13.42. Given that a linkage finding that reaches the threshold of suggestive evidence for linkage is expected to occur once by chance in a single genome-wide linkage analysis, these linkage findings support an oligogenic predisposition of familial GGE syndromes. In particular, the linkage peak at 5q34 is consistently supported by both nonparametric and parametric linkage results across the entire set of families and both family subgroups. This linkage peak maps close to the gene cluster encoding the $GABA_A$ β2-, α6-, α1-, and γ2 subunits (gene symbols: *GABRB2*, *GABRA6*, *GABRA1*, *GABRG2*). With respect to the important role of an impaired GABAergic inhibition in epileptogenesis (Noebels, 2003; Macdonald et al., 2010), the $GABA_A$ subunit genes at the 5q34 gene cluster represent plausible candidate genes. Specifically, the *GABRA1* and *GABRG2* genes are strong candidates, because most known $GABA_A$-receptor mutations associated with GGEs have been found in the *GABRA1* and *GABRG2* genes (for review see Macdonald et al., 2010; Lachance-Touchette et al., 2011). Accordingly, mutations of the *GABRB2*, *GABRA6*, *GABRA1,* and *GABRG2* genes may also play a causative role in some of the GGE-multiplex families investigated in the present study.

To dissect out seizure type-related genetic factors preferentially predisposing to either absence or myoclonic seizures, we stratified two subgroups of families that showed a strong clustering of the target seizure type, when linkage analysis was performed under the narrow trait definition (typical absence or myoclonic seizures). This ascertainment scheme allowed a partial overlap among both family sub-

groups (78 of 275 GGE-multiplex families), which reflects the common individual and familial co-occurrence of absence seizures and JME-related myoclonic seizures, but also takes into account the familial clustering of these target seizure types in both family subgroups (Berkovic et al., 1987; Beck-Mannagetta & Janz, 1991; Wirrell et al., 1996; Schmitz et al., 2000; Winawer et al., 2003; Marini et al., 2004; Winawer et al., 2005; Kinirons et al., 2008). Thereby, we aimed to reduce the genetic heterogeneity of these oligogenic traits and to accumulate single susceptibility factors that contribute to the familial clustering of either absence or myoclonic seizures. It is noteworthy that this approach focused on the dissection of seizure type–related but not seizure type– or syndrome-specific susceptibility factors.

To search for susceptibility loci involved in the genetic predisposition of absence seizures, linkage analysis was performed in 235 GAE families, in which 87.5% of the affected family members exhibited typical absence seizures. NPL analysis showed suggestive evidence for linkage in the 5q34 region. Parametric linkage analysis for a recessive genetic model met genome-wide significance at 13q31.3, a region previously implicated as a susceptibility locus for GGE and specifically for photosensitive GGEs (Tauer et al., 2005; Hempelmann et al., 2006). Because of the large overlap of these previous studies with the GGE-multiplex families included in the present linkage meta-analysis, these findings are not independent and cannot be considered as replicated linkage finding. Notably, the linkage evidence at 13q31.3 for parametric HLOD scores is substantially higher than that of the regional nonparametric LOD_{npl} scores, suggesting that linkage evidence from unaffected individuals supports the linkage result. Post hoc exploratory analyses of inheritance parameters (e.g., affecteds-only analysis, trimming of large pedigrees to nuclear families, removal of markers with a pairwise linkage disequilibrium of $r^2 > 0.1$) did not indicate that one of these parameters might have led to a spurious linkage finding (HLOD > 4.2 for all tests). Therefore, the significant parametric linkage finding at 13q31.3 appears to be robust and reliable, despite the lack of adequate support by NPL analysis. Among the most interesting candidate genes located in this region is the gene encoding glypican proteoglycan 5 (*GPC5*), which is expressed at the external surface of neuronal plasma membranes and has been implicated in brain patterning, synapse formation, axon regeneration, and guidance (Lee & Chien, 2004; Van Vactor et al., 2006; Luxardi et al., 2007). Of interest, genome-wide

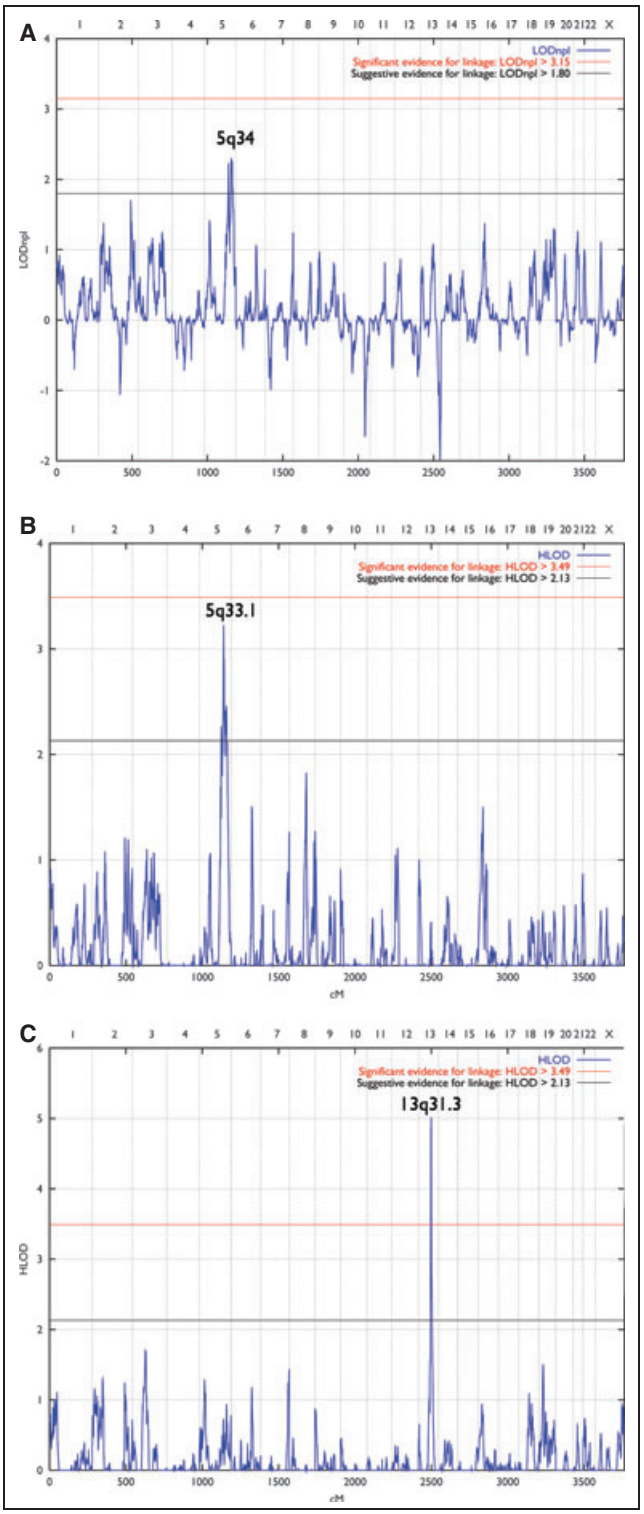

Figure 2.
Genome-wide linkage scan in 235 GAE families assuming a narrow trait definition. Linkage results are shown for: (**A**) nonparametric linkage analysis, (**B**) parametric linkage analysis applying a dominant inheritance model with 50% penetrance, and (**C**) a recessive inheritance model with 50% penetrance. The chromosomes are arranged in linear scale from pter to qter on the upper x-axis. Empirically derived genome-wide significance thresholds are indicated in the plots as horizontal lines. Narrow trait definition: typical absence or myoclonic seizures.

association analysis in multiple sclerosis (Baranzini et al., 2009; Lorentzen et al., 2010) and acquired nephrotic syndrome (Okamoto et al., 2011) revealed significant associations of SNPs in the genomic *GPC5* gene region.

Linkage mapping of susceptibility loci contributing to the expression of myoclonic seizures was carried out in 118 JME families, in which 74.4% of the affected family members exhibited JME-related myoclonic seizures under the narrow trait model. NPL analysis provided suggestive evidence for linkage in the chromosomal regions 2q34 and again in 5q34. The linkage peak at 5q34 is in direct vicinity of the *GABRB2/A6/A1/G2* gene cluster. This linkage finding is of particular interest, because a loss-of-function *Ala322Asp* mutation in the *GABRA1* gene causes JME in a large multigeneration family including eight members with JME (Cossette et al., 2002). Furthermore, the present JME locus at 2q34 is supported by a recent significant linkage finding that maps JME to the region 2q33-q36 in a multigeneration family with seven JME members (Ratnapriya et al., 2010). An interesting positional candidate gene is the sodium-independent electroneutral anion exchanger 3 gene (AE3, gene symbol: *SLC4A3*), which regulates extracellular and intracellular pH in neurons and thereby may influence seizure susceptibility. For the *SLC4A3* missense SNP rs635311 (c.2869A>C, p.A867D), we have previously demonstrated an allelic association of the p.867D allele with common GGE syndromes (Sander et al., 2002). Subsequently, it has been shown that the p.867D allele is a functional *SLC4A3* mutation that causes changes in cell volume and abnormal intracellular pH in the brain, potentially leading to neuronal hyperexcitability (Vilas et al., 2009). In addition, mice with a targeted disruption of *SLC4A3* display a reduced seizure threshold (Hentschke et al., 2006). These lines of evidence suggest that *SLC4A3* modulates seizure susceptibility and represents a high-ranking candidate gene for JME.

Despite a relatively large sample of 379 European GGE-multiplex families, we found significant linkage only in two subgroups of families stratified for a more homogenous phenotypic spectrum of seizure types. This finding suggests that the outcome of linkage mapping is not only a question of large numbers of families, but more critically depends on an accurate dissection of more homogeneous epilepsy traits. Our present linkage results demonstrate that phenotype–genotype strategies that apply narrow trait definitions in phenotypic homogeneous subsets of families may improve the prospects to disentangle the genetic basis of common

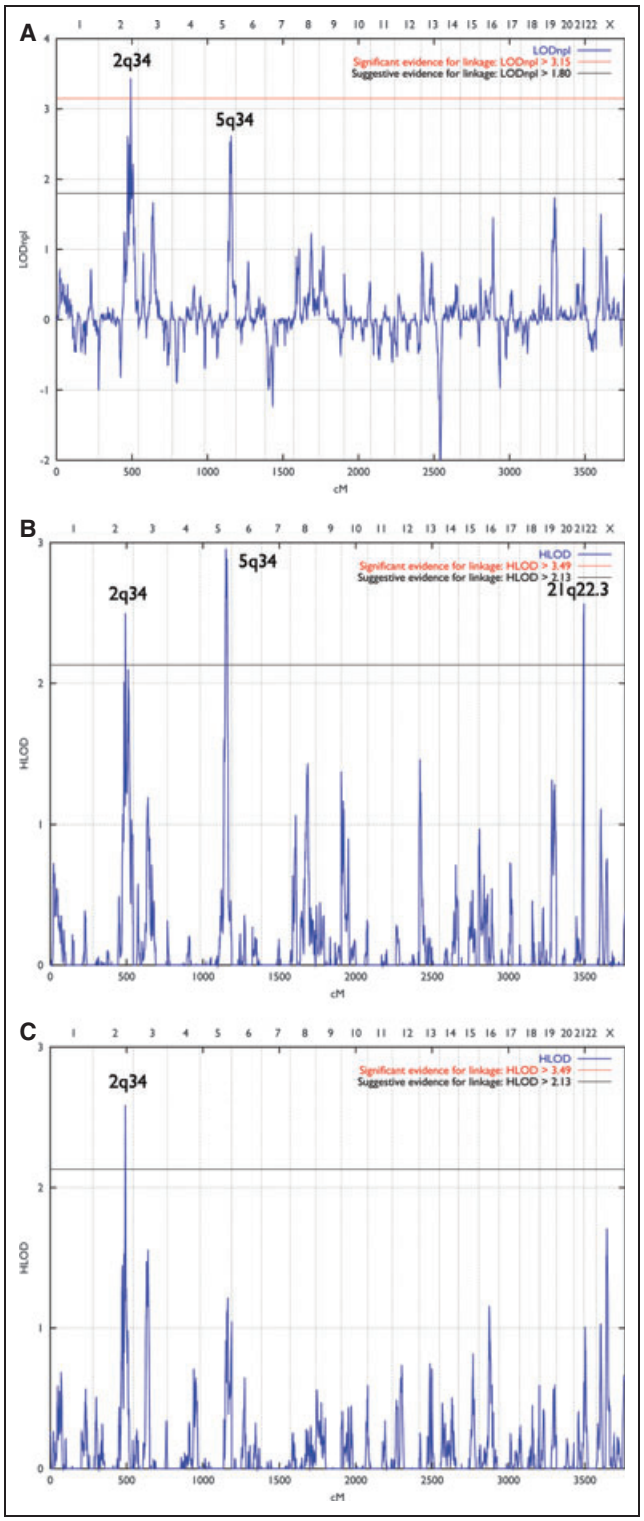

Figure 3.
Genome-wide linkage scan in 118 JME families assuming a narrow trait definition. Linkage results are shown for: (**A**) nonparametric linkage analysis, (**B**) parametric linkage analysis applying a dominant inheritance model with 50% penetrance, and (**C**) a recessive inheritance model with 50% penetrance. The chromosomes are arranged in linear scale from pter to qter on the upper x-axis. Empirically derived genome-wide significance thresholds are indicated in the plots as horizontal lines. Narrow trait definition: typical absence or myoclonic seizures.

familial GGE syndromes. With regard to the considerable effort to collect large samples of multiplex families and the rapid advances in next-generation sequencing technologies, large-scale linkage studies of genetically complex traits will probably not be continued in the traditional approach. Future research strategies will apply linkage mapping in combination with exome and genome sequencing of familial GGE syndromes. Thereby, linkage information in single families will provide an efficient tool to prioritize epilepsy genes and to filter out causal mutations from the nearly comprehensive assembly of individual sequence variations (Ku et al., 2011). This integrative approach together with advances in genomics, technologies, and bioinformatics has a great potential to accelerate progress in finding the numerous susceptibility variants conferring risk to common GGE syndromes (Cooper & Shendure, 2011).

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Appendix

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Sample composition of the linkage meta-analysis.

Table S2. Genome-wide linkage scan: GGE.

Table S3. Genome-wide linkage scan: genetic absence epilepsy.

Table S4. Genome-wide linkage scan: juvenile myoclonic epilepsy.

Table S5. Power calculations for parametric linkage analysis.

Table S6. Significance thresholds for NPL analysis.

Table S7. Significance thresholds for parametric HLOD analysis.

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