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Familial generalized epilepsy in Bulgarian Roma

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ABSTRACT – *Aims.* Gypsy communities constitute cultural and frequently inbred genetic isolates. Several genetic neurological disorders have been identified in these communities. Epilepsy appears as a fairly frequent medical condition among Bulgarian Gypsies, and many patients can be related to large pedigrees that may then be studied by conventional genetic linkage analyses. *Patients and methods.* We identified two large Wallachian Gypsy families from the Plovdiv and Varna regions of Bulgaria, with detailed clinical questionnaires and examination, and EEG recordings for many. Genetic linkage analysis was performed using microsatellite markers spaced across the human genome. *Results.* Although phenotypes were not always easy to identify, epilepsy appears in both families as a dominant, or pseudo-dominant trait, with the characteristics of idiopathic generalized epilepsy with onset at various ages, with infrequent, generalized tonic-clonic seizures, some associated with fever in childhood, but without sensitivity to fever in later life. While few markers yielded LOD scores > 2, no locus showed significant linkage, assuming autosomal dominant or recessive modes of inheritance.
Conclusion. Idiopathic generalized epilepsy, with a marked familial character, has not been reported to date in Bulgarian Gypsies. Both pedigrees studied here present with an identifiable epilepsy type inherited as a Mendelian trait. Despite the current lack of significant linkage, these families may constitute interesting ground for further genetic studies, on condition that more patients and families can be recruited.
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Key words: epilepsy, genetics, Roma, idiopathic generalized epilepsy

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The Roma (Gypsies) are a transnational minority with an overall population size estimated between 10 to 15 million (Liégeois 1994). There are about eight million Roma in Europe, with close to 50% resident in Eastern Europe, mainly in the Balkans. Oppressive policies of persecution, exclusion, containment and forced assimilation practiced towards the Roma

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in most, if not all, European countries, together with the Roma's adherence to an ancient social tradition, have acted together to result in endogamy and isolation, making the Roma one of Europe's largest genetic isolates (Kalaydjieva *et al.* 2001). Founder populations have been an invaluable resource for understanding the molecular basis of Mendelian disorders (Motulsky 1995, Rish *et al.* 1995, de la Chapelle *et al.* 1998, Sheffield *et al.* 1998, Peltonen *et al.* 1999, Ostrer 2001, Arcos-Burgos and Muenke 2002), and their (still disputed) potential to contribute to research into genetically complex disorders is closely related to demographic history and population structure. The greatest diversity in Gypsies exists in the Balkans (Liégeois 1994), for example in Bulgaria, where the 700-800 000 Roma form three metagroups: Jerlii (with three major divisions: Dassikane, Xoroxane and Vlahichki), Kalderas and Rudari. Each comprises numerous small groups with different rules and endogamy (Tournev 2001).
During our fieldwork, we identified a large number of Gypsy patients with familial epilepsy. To our knowledge, familial epilepsy has never been reported among large pedigrees in the Bulgarian Gypsies. Given the specific cultural and genetic characteristics of this population, we report the clinical and demographic characteristics of non-progressive generalized epilepsy with slight variations in phenotypes.

Patients and methods

Clinical study

We identified a large number of patients in the Bulgarian Gypsy community presenting with non-progressive generalized epilepsy. Most individuals that could be studied accurately belonged to a single, large and multigenerational pedigree of the Varna region while additional patients belonged to another family from the Plovdiv area. Individual patients and key informants were interviewed and/or examined over a period of four years from 1999 on, by one or several of the authors. Informed consent was obtained from each individual according to the appropriate local ethics committee. A neurological and electroencephalographic assessment was performed in 29 patients (18 males and 11 females). A specifically-developed epilepsy questionnaire was used. General cognitive functioning and nonverbal intelligence were evaluated with Raven's Standard Progressive Matrices. Memory was tested with: 1) verbal learning - list of 10 words (immediate recall, delayed recall, recognition); 2) short-term memory - Digit Span, forward and backward, subtest of WAIS; 3) visuo-spatial and constructive abilities - Block Design, subtest of WAIS. Standard EEG recordings and cognitive assessment were performed in the field using a *Deltamed* portable polygraphic recorder. Magnetic resonance imaging of the brain and spinal cord was obtained in seven patients, using a Philips Gyroscan T5-NT.

Genetic analysis

High-molecular-weight genomic DNA was isolated from whole blood by standard procedures. Highly polymorphic microsatellites markers were analyzed by PCR amplification of 40 ng of genomic DNA as previously described (Roll *et al.* 2006). Forward primers were labeled at the 5′ terminus with a fluorescent dye (FAM, HEX or TET/NED). Fluorescent PCR products were analyzed on a MegaBACE™ 1000 Sequencer (Amersham Biosciences), using the appropriate software. Single nucleotide polymorphisms (SNPs) were analyzed by direct sequencing at GATC Biotech. Linkage analysis was performed under the assumption of two (autosomal dominant or autosomal recessive) modes of inheritance with penetrances at 0.85 or 0.95, frequency of the disease at 0.001, and phenocopy rates at 0.01 or 0.03, by use of MLINK in the LINKAGE computer package (Lathrop and Lalouel 1984).

Results

Family DW was studied in the Varna region and belonged to one endogamous Gypsy subgroup, which represents a genetic isolate: Kalderas Roma (Kelderari). This subgroup belonged to the Wallachian Gypsy group. These are former nomads and keep strongly to their group identity. A large number of subjects with epilepsy were reported in family DW *(figure 1A)*, and detailed study concerned a subset (18 patients) *(table 1)*. Other epileptic patients also belonging to the Wallachian Gypsy group but differentiating in the Romani dialect, the handicrafts and the cultural traditions, were identified in the Plovdiv region (*family GP*) *(figure 1B)* and belong to another Gypsy subgroup - Tracean tinsmiths (Kalaydzii). Eleven members of the GP family could be phenotyped accurately and repeatedly *(table 1)*.
Age at last examination varied between five and 64 years in family DW. None of the patients was neurologically remarkable, except one (DW11), who had mild mental retardation (MR), and another, (DW7) who had a history of head trauma. Whenever determined, the age at first seizure varied between 2-3 months and young childhood. Five patients had a definite history of febrile seizure (FS) and another five a possible FS. The last FS could not be determined with certainty. All patients in family DW had generalized tonic-clonic seizures (GTCS) and two also had complex partial seizures. The frequency of the seizures varied in family DW: four patients had > 1 seizure/month and 10 patients had < 1 seizure/month, among whom five patients had rare seizures (< 1 seizure/year). Seizure frequency appeared to be low but could not be properly assessed in the three remaining patients. Treatment was received by a minority of patients and consisted of low-dose carbamazepine or sodium valproate. It was given intermittently and resulted in apparent control of the epilepsy. Most of the patients were never exposed to anticon-

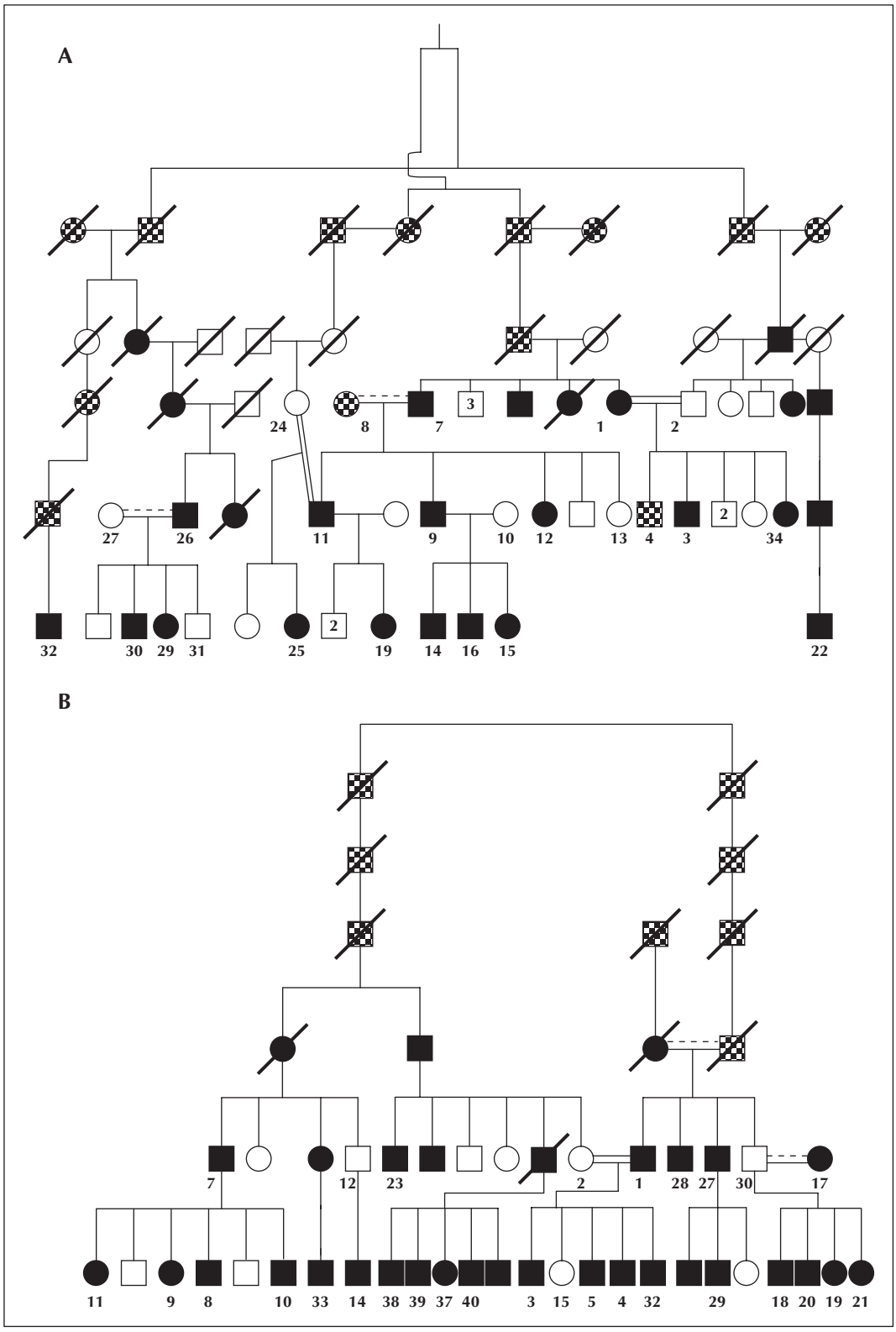

Figure 1. Inheritance pattern of the epileptic phenotype in families DW (1A) and GP (1B). Black symbols represent affected patients. White symbols represent unaffected individuals. Half-filled symbols represent individuals with unknown phenotype. Dotted lines indicate that consanguinity was reported, albeit without certainty.

Table 1. Clinical, EEG and neuroimaging data of the patients from both families.

Patient	Age* (years)	Gender	Associated problems	EEG	Age at first seizure	Febrile seizure?	Other seizure type	Frequency of seizures since onset	MRI	AEDs
Varna region										
DW1	66	F	no	normal	young child	yes	GTCS	< 1/month	ND	CBZ
DW11	37	M	MR	normal	unknown	unknown	GTCS	<5	normal	none
DW12	33	F	no	normal	unknown	unknown	GTCS	unknown	ND	none
DW14	18	M	no	normal	young child	yes	GTCS, CP	<5	ND	none
DW15	25	F	no	GS	young child	no	GTCS	> 1/month	ND	none
DW16	12	M	no	GSW	3 years	yes	GTCS	just one	ND	none
DW19	7	F	no	normal	4 years	unknown	GTCS	just one	ND	none
DW22	16	M	no	normal	1 years	yes	GTCS	> 1/year	ND	none
DW25	23	F	no	normal	10 months	yes	GTCS	> 5	ND	none
DW26	46	M	no	GSW	young child	unknown	GTCS, CP	rare	ND	none
DW29	28	F	no	normal	2-3 months	no	GTCS	> 1/month	normal	CBZ
DW3	43	M	no	normal	young child	no	GTCS	< 1/month	ND	none
DW30	26	M	no	normal	unknown	no	GTCS	unknown	normal	none
DW32	32	M	no	GS	2 years	no	GTCS	< 1/month	normal	VPA
DW34	36	F	no	normal	1 year	no	GTCS	> 1/month	ND	CBZ
DW7	58	M	head trauma	focal + GSW	5 years	unknown	GTCS	daily	PTE	VPA
DW8	56	F	no	normal	childhood?	no	GTCS?	unknown	ND	none
DW9	35	M	no	GS	young child	no	GTCS	<1/month	ND	CBZ
Plovdiv region										
GP1	43	M	head trauma	normal	1-2 months	yes	GTCS, SGTCS	< 1/month	normal	CBZ, PB
GP11	13	F	MR	normal	unknown	unknown	GTCS	> 1/month	ND	VPA
GP19	13	F	no	phot.	2 years	yes	GTCS	> 1/month	ND	none
GP3	18	M	no	normal	1 year	yes	GTCS	> 1/month	normal	CBZ, PB
GP32	9	M	asthma	phot.	2.5 years	yes	GTCS	1/month	ND	CBZ
GP37	28	F	MR dysmorphia	SBS	1 year	unknown	GTCS	< 1/month	ND	CBZ
GP38	23	M	MR dysmorphia	SBS	1 year	unknown	GTCS	< 1/month	ND	CBZ
GP39	19	M	MR dysmorphia	SBS	1 year	unknown	GTCS	> 1/month	ND	CBZ
GP4	15	M	slight MR	GS	2 years	no	GTCS	> 1/month	ND	VPA,CBZ
GP40	15	M	MR dysmorphia	SBS	2 year	yes	GTCS	< 1/month	ND	CBZ
GP8	18	M	MR	normal	1.2 years	unknown	GTCS	> 1/month	ND	CBZ

M, male; F, female; MR, mental retardation; GTCS, generalized tonic-clonic seizures; CP, complex partial seizures; GS: generalized spikes; GSW: generalized sharp waves; phot.: photosensitivity; SBS: slight background slowing; CT, computerized tomography; MRI, magnetic resonance imaging; PTE: post-traumatic encephalomalacy; AEDs: antiepileptic drugs; VPA, valproic acid; CBZ, carbamazepine; PB, phenobarbital. ND: not done.*: age at examination.

vulsant therapy and their history is one of spontaneous evolution without therapeutic interference. It was thus not possible to separate patients with, from patients without treatment. Those who received drugs were not covered for long periods, as drugs are expensive and difficult to access for these groups. In our opinion, our data broadly reflect the natural course of the condition.

The epileptic phenotypes in *family GP* looked quite similar to those observed in *family DW*. Mental retardation seemed to be present at a higher frequency (7/11) in the patients of the Plovdiv area. Moderate to severe mental retardation was associated with dysmorphism (microcephaly, low body weight) in four siblings (Patients GP37-40; *table 1*), while the three other cases had only slight to moderate mental retardation without distinctive dysmorphism. Consanguinity was highly probable, but there was no specific information about inbreeding in this particular branch. All patients in *family GP* had GTCS, and five had a history of FS, among whom one also had a history of head trauma. The frequency of the seizures varied from > 1 seizure/month to > 1 seizure/year. Treatment was received by all patients but one (GP19), and consisted in low-dose CBZ, VPA or PB. As in family DW, this too resulted in apparent control of epilepsy.

EEGs revealed generalized sharp waves and/or spike waves in five patients from *family DW (figure 2A)*, among whom one (DW7, with a history of head trauma) also had focal changes. In *family GP*, we found two children with

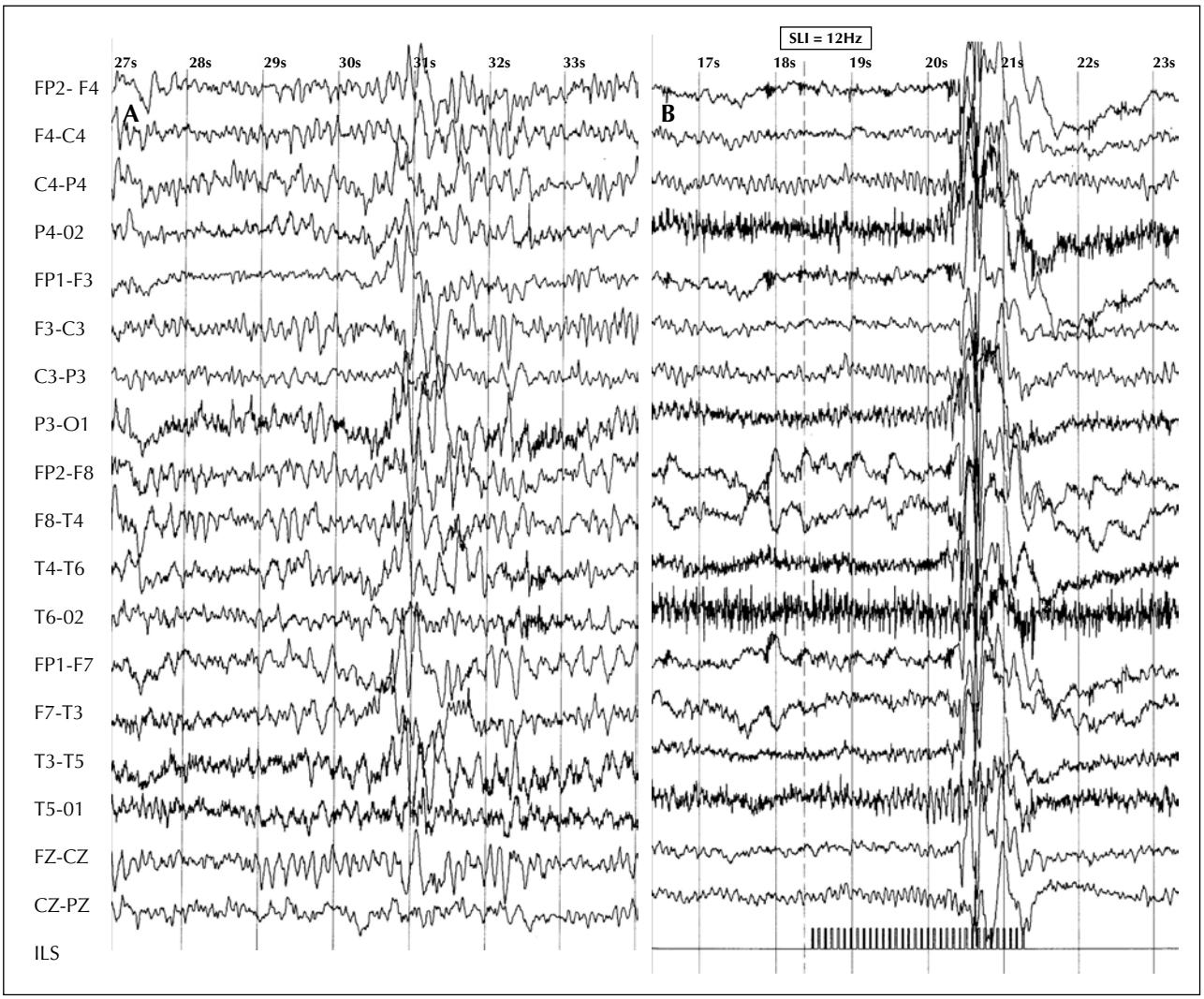

Figure 2. Paroxysmal EEG changes. 10-20 electrode placement system. **A**) Patient DW16. This 12-year-old boy had a single, apparently febrile, generalized tonic-clonic seizures at age three. He developed normally, and was without treatment. The EEG shows a spontaneous burst of irregular sharp waves. **B**) Patient GP19. This 13-year-old girl had apparently febrile generalized tonic-clonic seizures at age two, and GCTS persisted throughout childhood. There was no report of clinical photosensitivity. She did not receive anticonvulsant treatment. The EEG shows a clear photoparoxysmal response, which was associated with a feeling of uneasiness.

photosensitivity *(figure 2B)*, and generalized paroxysmal activity in one further patient. The four siblings with marked mental retardation and dysmorphia had slow background activity without paroxysmal changes. MRIs were normal in most patients. In one patient with head trauma (patient DW7), MRI showed diffuse cortical atrophy and post-traumatic focal encephalomalacia.

A whole genome screen was performed, using highly polymorphic markers regularly spaced across the genome, with an average interval of 10 centiMorgans (cM). Consanguinity was highly suspected and was confirmed in a few marriages *(figures 1A,B)*. Because the mode of inheritance in the two families appeared to be autosomal dominant or pseudo-dominant, two models were tested: one that assumed autosomal dominant (AD) inheritance, and one that assumed autosomal recessive (AR) inheritance. Penetrance was set up at 85 or 95% and phenocopy rate at 1% or 3%. No locus showed significant linkage (Supplementary *tables 1*-4 available on DVD), although a few markers were suggestive (LOD scores > 2). These were D3S1263 at chromosome 3p25 (maximal LOD score = 2.18 in family GP) and D4S1534 at chromosome 4q21 (maximal cumulative LOD score = 2.22 in families DW + GP). In both cases, a recessive model was assumed. However, allelic heterozygosity was detected for these two markers in the majority of the patients (data not shown). As consanguinity would very probably sustain a recessive mode of inheritance in these families, the LOD scores mentioned above may represent false positives. Using an autosomal dominant model, the LOD score nearly reached significance for another marker, D12S352 at chromosome 12p12 (maximal LOD score = 2.88 at theta = 0 in family DW). Additional microsatellites markers (Supplementary *figure 1*), as well as a few informative SNPs (rs10744561 and rs797765 at the SNP database: http://www.ncbi.nlm.nih.gov/projects/SNP/) at chromosome 12p12 were then used and indicated that this promising LOD score was obtained because of the lack of informativeness of D12S352 in a branch of the family. Consequently, LOD scores dropped down to non-significant values when these surrounding markers were analyzed.

Discussion

The identification of a growing number of novel Mendelian disorders and private mutations in the Roma (Gypsies) points to their unique genetic heritage (Kalaydjieva *et al.* 2001). In recent years, novel single-gene disorders (Morar *et al.* 2004), as well as private mutations causing known Mendelian disorders (Kalaydjieva *et al.* 2001), have been identified in Gypsies. The Gypsies may also offer unique opportunities for the mapping of genes involved in complex disorders. The strong primary founder effect raises expectations of a relatively homogeneous genetic basis of complex disorders, similar to all founder populations (Kalaydjieva *et al.* 2001). Large Romani families with psychiatric disorders are being studied in an effort to localize susceptibility genes (Kaneva *et al.* 1998), and epidemiological evidence suggests that there are differences in the prevalence of other complex disorders, such as Parkinson disease and multiple sclerosis, between the Roma and surrounding European populations (Kalman *et al.* 1991, Milanov *et al.* 2000).

Given the discovery of numerous cases of epilepsy in large pedigrees, the Roma are thus emerging as an interesting population for genetic research on the epilepsies. As much as 45% of the marriages in these groups might be consanguineous (Tournev 2001). In both the pedigrees reported here, segregation was consistent with autosomal dominant or pseudo-dominant inheritance *(figure 1)*. Most patients exhibited a fairly benign phenotype with infrequent GTCS, and very few had other seizure types; febrile seizures during childhood were found from history in at least ten of the 29 patients analyzed and may thus constitute a marker of this phenotype. Conversely, only nine had no history of febrile seizures. Focal seizures occurred as an exception, with two cases documented from the history. The GTCS occurred at random; no patient had had status epilepticus. Apart from fever, there were no reported triggering factors. The EEGs were not very informative, showing significant changes in five patients of the *DW family*, and in three patients of the *GP family*. These changes were mostly diffuse, but not typical of any classical generalized epilepsy syndrome. Clinical photosensitivity was not reported by any patient, and only two children in *family GP* had a photoparoxysmal response during the EEG recording. Neuroimaging investigations were normal in all patients except in patient DW 7 who had suffered from head trauma.

The epileptic phenotype in *families DW* and *GP* thus appears as fairly homogeneous with possible febrile seizures, infrequent GTCS, a spontaneously, benign course in most patients, and non-specific interictal EEG changes in only some. This epileptic phenotype is globally consistent with that found in some of the GEFS+ families reported (Scheffer *et al.* 2005), although we must underscore that febrile seizures beyond early childhood were not reported here. We would assume that these patients have an inherited predisposition to GTCS that is not limited to childhood or to febrile diseases. The phenotype in *family GP* also includes several patients with MR, and a sibship with severe non-progressive MR and dysmorphism, features not described in GEFS+ families. In these patients, the epilepsy had the same course as in the other patients and none exhibited characteristics of encephalopathic epilepsy. It is also noteworthy that none of the three loci containing the GEFS+ genes known to date (i.e. *SCN1A* at 2q24, *SCN1B* at 19q13, and *GABRG2* at 5q34) gave positive LOD scores. Although the three genes have not yet been firmly excluded by direct mutation screening, the

Supplementary tables and figures available online and on the DVD

Supplementary *figure 1*: Segregation of the microsatellite marker D12S352 in family DW. Black symbols represent affected patients. White symbols represent unaffected individuals. Half-filled symbols represent individuals with unknown phenotype. Dotted lines indicate that consanguinity was reported, albeit without certainty. Haplotypes squared in red represent the tentative disease haplotype assuming suggestive linkage to chromosome 12p12.
Supplementary *table 1*: two-point LOD scores for the microsatellite markers used for the genome-wide scan. Dominant mode of inheritance was assumed. Penetrance and phenocopy rates were set up at 95% and 1%, respectively.
Supplementary *table 2*: Two-point LOD scores for the microsatellite markers used for the genome-wide scan. Dominant mode of inheritance was assumed. Penetrance and phenocopy rates were set up at 85% and 3%, respectively.
Supplementary *table 3*: Two-point LOD scores for the microsatellite markers used for the genome-wide scan. Recessive mode of inheritance was assumed. Penetrance and phenocopy rates were set up at 95% and 1%, respectively.
Supplementary *table 4*: Two-point LOD scores for the microsatellite markers used for the genome-wide scan. Recessive mode of inheritance was assumed. Penetrance and phenocopy rates were set up at 85% and 3%, respectively.

linkage data do not argue in favor of their involvement. The DW and GP families stem from a clear cultural and genetic isolate in which epilepsy might possibly be related to an original genetic variation. Although the few genomic regions that yielded LOD scores > 2 may represent starting points for further studies, no linkage could be firmly demonstrated after the genome screen was performed. The difficulty in obtaining accurate and reliable clinical information in a subset of family members, especially when it is based on a historical evidence only, may explain the linkage data. Despite the current lack of significant linkage in the two families studied here, the Roma families may constitute interesting ground for further genetic studies on the epilepsies. This obviously will require more patients and families to be recruited. □

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