Clinical and genetic aspect of 30 Tunisian families with febrile seizures

Etude Clinique et génétique de 30 familles tunisiennes avec crises fébriles

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RÉSUMÉ

Introduction : Les crises fébriles (CF) sont les crises occasionnelles de l'enfant les plus bénignes. Peu est connu sur le suivi à long terme.
But: Décrire à long terme le suivi des CF dans des familles Tunisiennes.
Méthodes: étude menée sur terrain pour 30 patients atteints de CF. Nous avons analysé le phénotype clinique des CF et non fébriles associés avec une étude génétique.
Résultats: Nous avons recueilli 107 sujets atteints de CF et / ou non fébriles. Les CF ont été trouvés chez 28,3% des patients. Le phénotype «CF isolée» a été trouvé dans 18 familles (60%), le phénotype «GEFS +» dans 7 familles (23,33%), et une épilepsie généralisée idiopathique chez 5 autres familles (16,66%). Le séquençage des gènes SCN1A, SCN1B et GABRG2 a révélé une mutation du gène SCN1B dans une famille avec « CF » et une mutation du gène SCN1A dans une famille « GEFS + ».
Conclusion: Si les CF sont apparemment isolées et rares, elles se produisent le plus souvent dans un contexte familial. Les études génétiques restent difficiles vu l'absence de corrélation phénotype-génotype.

Mots-clés
CF, GEFS +, phénotype clinique, études génétiques, Tunisie

SUMMARY

Background: FS are the most benign occasional seizures in childhood. Little is known about the long term follow up.
Aim: To describe a long term follow-up of FS in Tunisian families.
Methods: Field study was conducted for 30 patients with FS. We analyzed clinical phenotype of FS and associated afebrile seizures with genetic study.
Results: We collected 107 individuals with febrile and / or afebrile seizures. Afebrile seizures were found in 28.3% of patients. The «FS» phenotype was found in 18 families (60%), «GEFS +» in 7 (23.33%), and idiopathic generalized epilepsy in 5 (16.66%). Sequencing analyses of SCN1A, SCN1B and GABRG2 genes revealed a novel SCN1B gene mutation in one family with FS and a known SCN1A mutation in GEFS+ family.
Conclusion: If FS are apparently isolated and infrequent, they occur most often in a family setting. The genetic studies remain difficult mainly because of the lack of phenotype-genotype correlation

Key-words
FS, GEFS+, clinical phenotype, genetic studies, Tunisia

INTRODUCTION

Febrile seizures (FS) are the most frequently occasional seizures in children. Fever triggering and age of onset characterize this epileptic syndrome [1]. For this benign phenomenon, there is clear evidence for genetic basis: existence of familial FS, frequent association with later epilepsy, frequency of FS history in some epileptic syndromes [2]. However, the pattern of inheritance is not clear; it can be autosomal dominant, autosomal recessive or polygenic [3]. GEFS+ syndrome reflect this association between FS and epilepsy [4]. Few studies have reported long-term follow-up of patients with FS in large pedigree.

In Tunisia, few studies have analyzed the epidemiology and the genetics aspects of FS above. This is the first large study conducted to describe long-term clinical follow-up and genetic aspect of families with FS in Tunisia in order to identified pure FS from other epileptic syndromes with FS

METHODS

We conducted a prospective study for all children referred to the neuropeditaric consultation for FS. During the inclusion period of two years (2002-2004), we collected 30 children belonging to 30 families. Clinical study was conducted from 2004 to 2007 and genetic study is continued.

Clinical study

A collection of clinical data was established to determine the FS and eventually afebrile seizures history in children and their parents, the FS onset/offset age, detailed description and associated symptoms of FS and short-term and long-term treatment. Afebrile seizures were classified according to the International Classification of epileptic seizures and epileptic syndromes [5].

All children and parents (when they accompanied their children) had a clinical examination. A pedigree has been established for each family regardless of the number of affected individuals. A field study was subsequently conducted whenever parent interview revealed an affected member and when families cannot move to the consultation.

Genetic study

Genetic study was carried out for 16 families (A to P). A genome-wide scan, using 380 fluorescent microsatellite markers covering the entire human genome, was performed to identify the responsible loci in the large affected Tunisian family. To fine map these loci, additional markers were analyzed. Indeed, 42 microsatellite markers distributed on five GEFS+ loci (GEFS +1, GEFS +2, GEFS +3, GEFS +4, GEFS +5) and nine FS loci (FEB1, BEF2, FEB3, FEB4, FEB5, FEB6, FEB7, FEB8 and FEB9) were tested for the others studied families.

Haplotyping, parametric and nonparametric multilocus linkage analyses were carried out using Merlin program.

Mutational analyses were performed by sequencing the candidate genes SCN1A, SCN1B and GABRG2.

RESULTS

Study population

We collected 30 patients with FS, which represents 13.45% of all children seen in the pediatric neurology consultation at the same period. After field study and contact by telephone, we could collect 107 affected individuals with febrile and/or afebrile seizures. Among these individuals, 91 individuals had FS isolated or associated with afebrile seizures and 16 individuals had only afebrile seizures without a previously history of FS. At the time of the study, 3 individuals were dead (figure 1).

Consanguinity was found in 11 families. A family history of FS and / or afebrile seizures was found in 19 patients (63.33%) and FS history in siblings of a child with FS was found in 29%.

Neurological examination was evaluated in 58 patients. It was normal in 38 individuals (64%), showed learning disabilities in 10 children (17%) and intellectual disability in 8 individuals (13%) of which one patient had Dravet syndrome.

The clinical characteristics of FS and afebrile seizures could be studied in detail in only 67 individuals (62.61%).

FS characteristics

Among the 67 studied individuals, we found 60 patients with FS. The mean age of patients at the time of the study was 17 years [4- 65 years] and the sex-ratio was 3.28. The mean age of FS onset was 2.1 ± 1.5 years [6 months-6 years] and the average age of their disappearance was 3.3 ± 1.9 years [6 months-7 years]. FS were simple in 68.3% and complicated in 31.7%. The persistence of FS beyond the age of 5 years was found in 6 patients (10%).

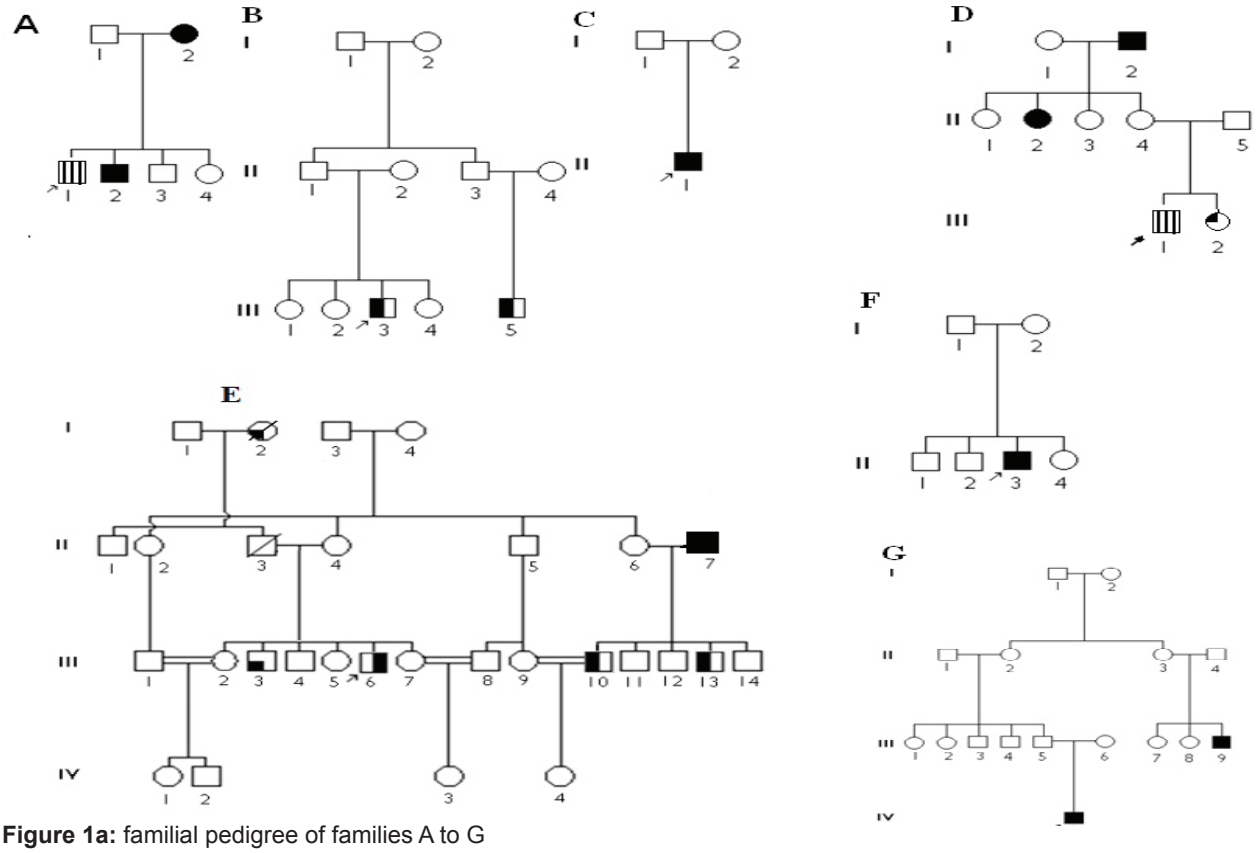

Figure 1a: familial pedigree of families A to G

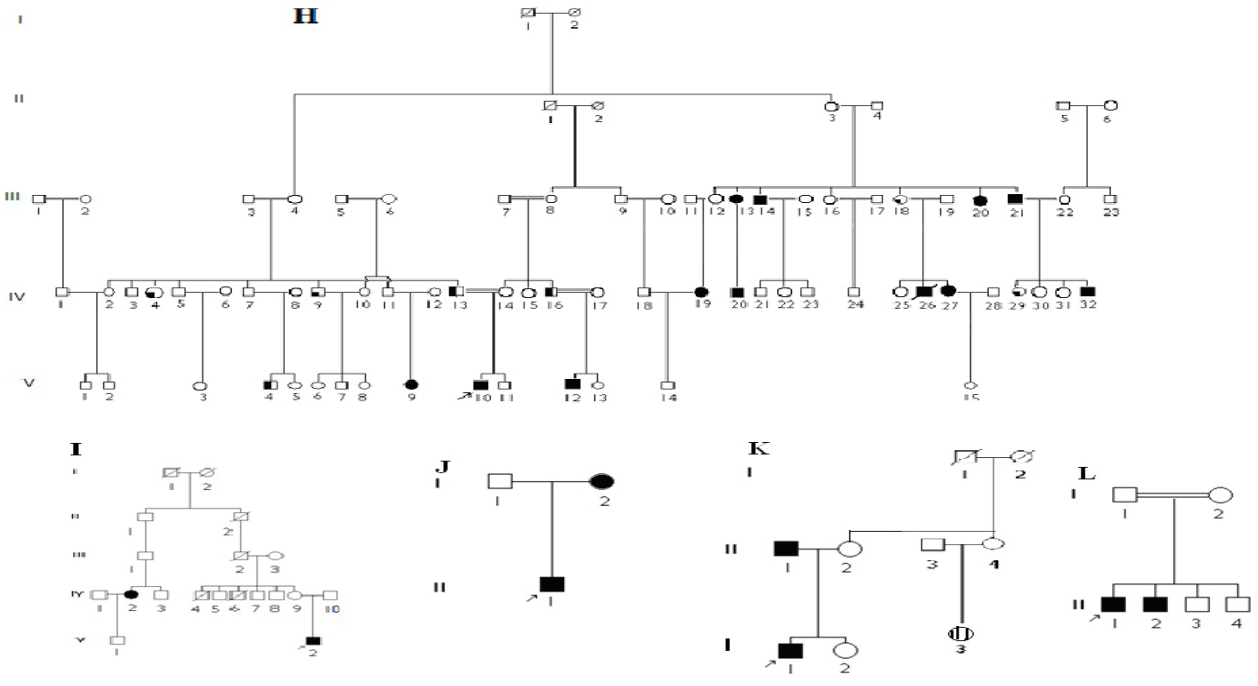

Figure 1b: familial pedigree of families H to L

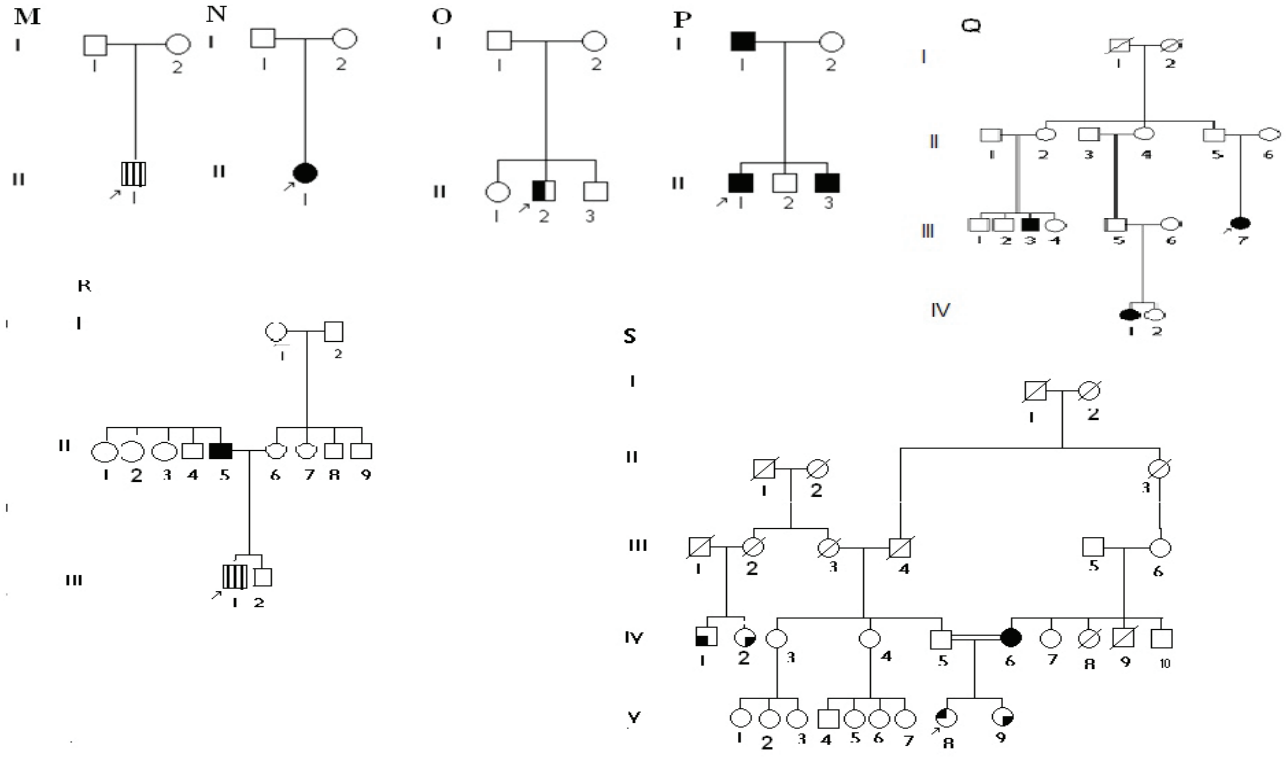

Figure 1c: familial pedigree of families M to S

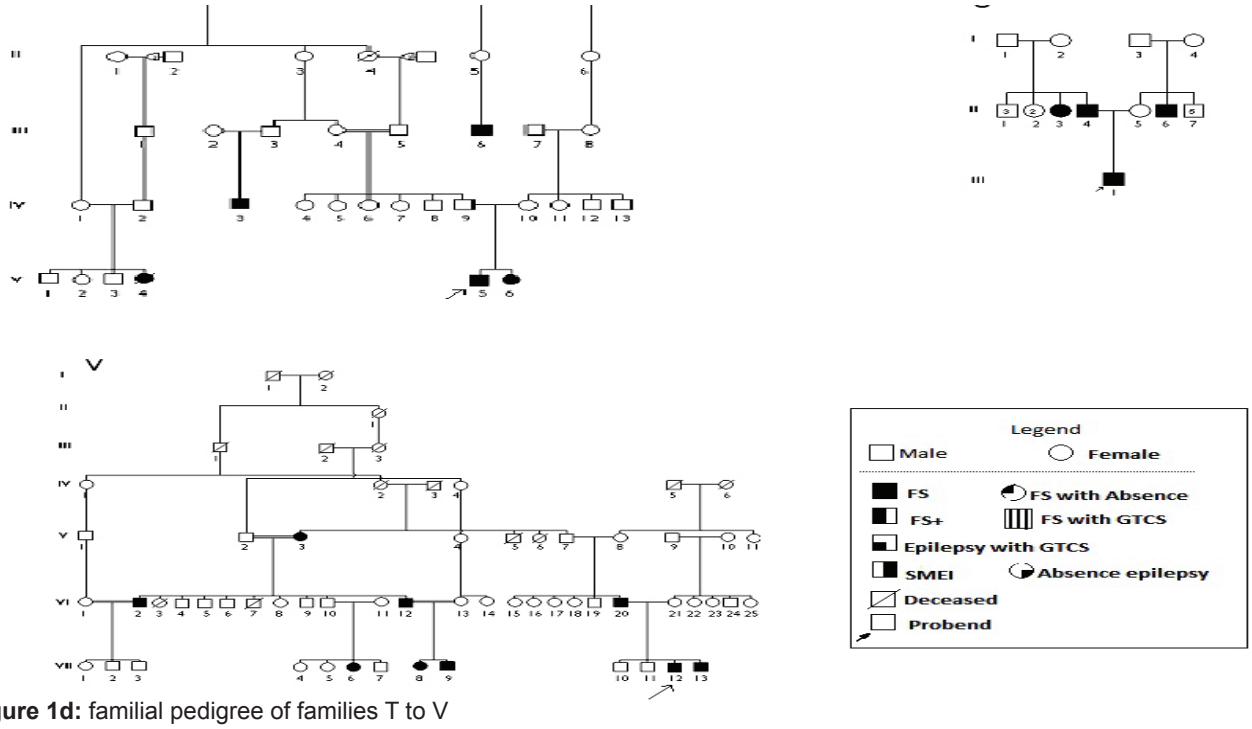

Figure 1d: familial pedigree of families T to V

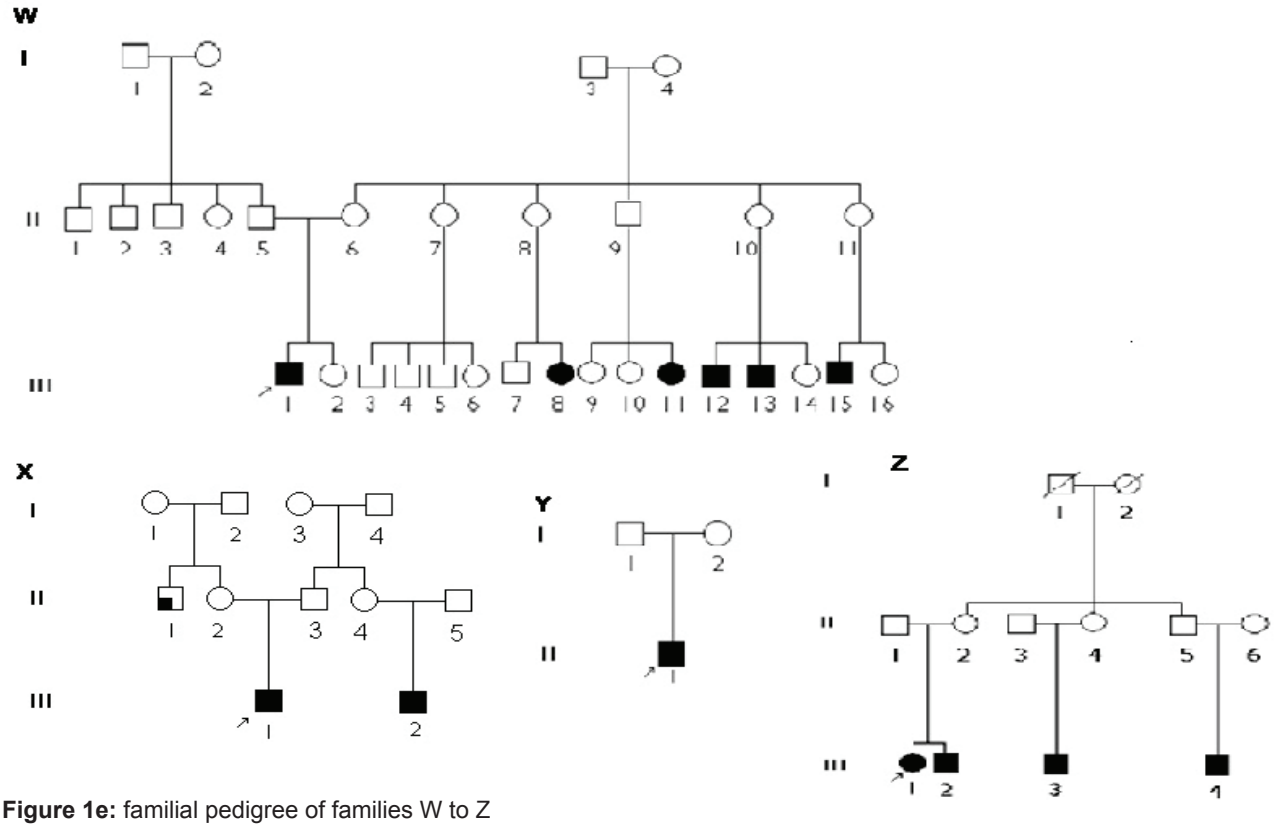

Figure 1e: familial pedigree of families W to Z

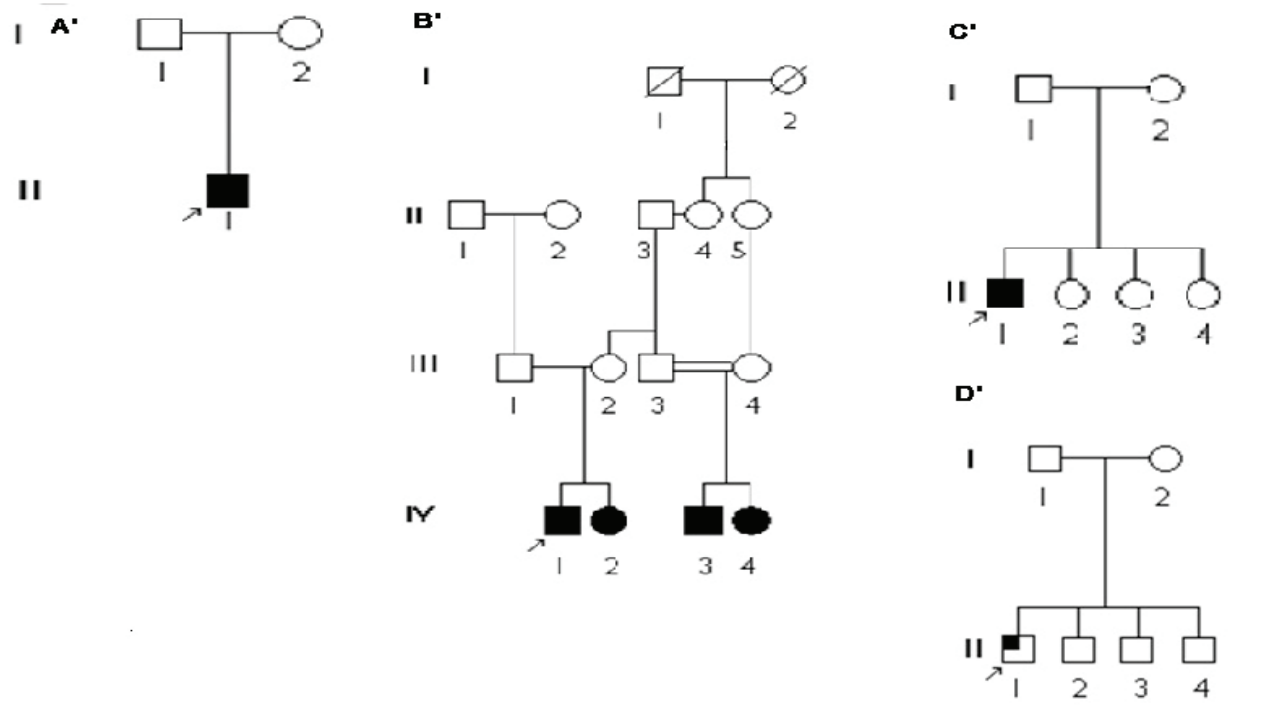

Figure 1f: familial pedigree of families A' to D'

Afebrile seizures characteristics

Afebrile seizures were found in 19 individuals (28.3%) of which 12 had FS in young age (63.2%) (Table 1).

In patients with FS history, the mean age of afebrile seizures onset was 8.33 years [3-23 years]. Seizures were generalized tonic-clonic (GTC) in 8 cases (66.66%), absence in 3 cases and and myoclonic type in 1 case.

In patients without FS history, the onset age of seizures ranged from 14 to 25 years. Six patients had epilepsy with GTC seizures and one patient had absence epilepsy.

Familial phenotype

«FS» phenotype was found in 18 families (60%) and «GEFS +» in 7 families (23.33%). For the other five families (16.66%), the phenotype is in favor of idiopathic generalized epilepsy with FS history. The pattern of inheritance remains difficult to precise. Some pedegres are apparently sporadic and cannot formally exclude the presence of other cases in the family (families C, F, L, M, N, O). For other families, the presence of several cases with consanguinity would be in favor of an autosomal recessive inheritance. Finally, others families without consanguinity

Table 1: characteristics of afebrile seizures

Families	Patients	Sex	Age (years)	Age onset /offset of FS (years)	Type and onset age of AS-/+ (years)	Clinical evaluation
A	II.1	M*	15	3/3	GTC† (7)	ADHD‡‡
	III.1	M*	13	1.5/ 5	GTC† (8)	LD
D	III.2	F**	16	5/5	Ab § (11)	Normal
E	III.3	M*	32	No	GTC† (25)	IDµ
	III.6	M*	20	0.75/ unknown	My- At†† (2)	IDµ
H	III. 18	F**	53	No	GTC† (16)	Normal
	IV. 4	F**	50	No	GTC† (14)	Normal
	IV. 9	M*	49	No	GTC† (14)	Normal
	IV. 13	M*	38	5/5	GTC† (23)	Normal
	IV. 16	M*	42	6/6	GTC† (20)	IDµ
	IV. 29	F**	20	No	GTC† (14)	Normal
	V.4	M*	18	1.16/1.16	GTC† (12)	LD
K		F**	11	0.5/ unknown	GTC† (3)	Normal
M	II.1	M*	11	3/3	GTC† (7)	Normal
R	IV.1	M*	10	3/7	GTC† (8)	ADHD‡‡
	V.8	F**	11	1.25/1.25	Ab § (6)	IDµ
S	V.9	F**	17	No	Ab § (17)	IDµ
	IV.1	M*	?	No	GTC † (12)	Normal
	IV.2	F**	17	2/ 5	Ab § (6)	

Abbreviations: *: male; **: female; ‡: afebrile seizure; †: generalized tonic clonic seizure; §: absence seizure; ††: myoclonic and atonic seizure; ‡‡: attention deficiency with hyperactivity disorder; : learning disabilities; µ: intellectual disability.

could present an autosomal dominant inheritance probably with incomplete or variable penetrance (families A, D, G, J, K, P, R, U, W, X, Z).

Genetic study

We performed a 10cM density genome-wide scan in the informative family (H) with GEFS+ and we obtained two regions with positive LOD scores. We analyzed additional markers for fine mapping and we obtained positive LOD scores for the two loci [6].

The sequencing analysis of the SCN1A gene in family E with GEFS+ phenotype revealed the presence of a pathogenic mutation c.1811G>A (p.R604H) and a putative disease-associated haplotype [7]. This mutation was present in a heterozygote state in two tested patients with FS+ and their fathers with FS. We did not find this mutation in 11 patients of the same family and 14 controls.

This transition (c.1811G>A) in exon 11 of SCN1A gene substitute an arginine to a histidine residue (p.R604H) in the cytoplasmic loop between domains 1 and 2 of Na+ channel alpha Isubunit (Nav1.1) protein. This mutation p.R604H disrupts a protein kinase A (PKA) consensus site that is conserved in the four major sodium channels of the central nervous system. We also identified two new and 11 known SNPs in SCN1A gene. The haplotypes construction and an analysis of recombination events were performed for all variants in SCN1A gene. The most notable aspect was the presence of putative disease-associated haplotype in a homozygous state only in a patient with Dravet syndrome. The other four patients in this family presented the same haplotype but in a heterozygous state. So we can suggest that there is an association of this haplotype with severity of epilepsy in this family [7].

In addition, the sequencing analyses of the GABRG2 gene and the association analysis suggest a positive association of an intronic SNP in the GABRG2 gene for the two Tunisian families (H and E) [7]. The sequencing analyses of the SCN1B gene revealed the presence of a new pathogenic variation c.374C>T (p.R125L) in family A and a putative disease-associated haplotype in five families (C, J, K, L and P). This mutation was present in a heterozygote state in two patients with FS (the mother and his sun) and one with FS+ (the second sun). This variant segregated with FS and FS+ phenotypes because it was absent in the father, in the other patients, and in 100 control chromosomes by direct sequencing. Using multiple alignments, the c.374G>T changed a highly conserved arginine to a leucine p.R125L located in the extracellular domain of the protein.

To investigate the eventual effect of this mutation, we modeled and compared both variants 125R and 125L. We consider that the variation R125L might affect the structure and stability of the protein by the loss of hydrogen bonding. Also, a change of a hydrophilic amino acid (R) by another hydrophobic (L) could change the protein electrostatic and intrinsic characteristics. We noticed that the transition of Arg to Leu in position 125 affected the electrostatic potential of this protein region. These results suggest that SCN1B gene was the responsible gene in one Tunisian family and might contribute to the FS susceptibility for the five others [8]. For the remaining families, genetic study is in progress.

DISCUSSION

This is a long-term follow up study of patients with FS phenotype belonging to different families conducted in Sfax-Tunisia. This study has methodological limitations particularly the advanced age of patients at the study moment, why it did not allow us to analyze FS phenotype and it explain the unavailability of some additional tests already made such as EEG.

From the 30 index patients, we collected 107 patients with febrile/afebrile seizures and a family history of FS and / or afebrile seizures was found in 63.33% of patients. The persistence of FS beyond the age of 5 years were found in 10% of patients thereby defining the phenotype “FS plus”. Afebrile seizures were GTC in the majority of cases. In our study, we found three major familial phenotypes: FS in 60%, GEFS+ in23.33% and idiopathic generalized epilepsy in the remaining cases. Genetic study showed involvement of SCN1B gene in only one family with FS and SCN1A in one family with GEFS+.

If FS is a common phenomenon, affecting 2-5% of children aged from 6 months to 5 years in populations of North America and Europe [9], in our study, we found a higher frequency (13.5%). It is explained probably by the systematic sending of patients with FS to our department for genetic study. One study conducted in pediatric consultation in Tunisia had found a frequency of 55.2% of FS; this high frequency is explained by the presence of emergency consultations [10]. However, the incidence among hospitalized children varies from 1.4 to 5.34% depending on whether the children with FS were hospitalized in a systematic manner or not [non published data].

A family history of FS or afebrile seizure was found in 25-40% in most series [3, 11, 12, 13]. Also, the frequency of FS in sibling of a child with FS is 9-22% [3]. The higher frequencies in our study can be explained by the direct contact of parents and other family members.

The majority of FS occur between the ages of 6 and 36 months with a peak at 18 months [3]. Our data are also consistent with the literature data. Their occurrence after the age of 4 years is observed in 6-15%. The occurrence or the persistence after the age of 5 years is exceptional as in our study, defining «FS plus» that are often multiple and may be with or without afebrile seizures [4]. They are particularly common in the GEFS + syndrome where they most often associate with afebrile generalized seizures [4]. In our study, afebrile seizures were significantly more frequent than in the literature (0.5 to 17 %) [11]. This is probably explained by the advanced age of our patients at the time of the study and also by the long-term follow-up. However, the mechanism underlying the effect of febrile seizures on the incidence of afebrile seizures is not clear.

Although there is a clear genetic basis of FS, the pattern of inheritance is still not clear; autosomal dominant transmission, recessive and polygenic have been reported [12, 14, 15, 16, 17, 18] like in this study. In addition, we did not find any statistical association with one of the 12 studied FEB loci in families particularly those involving only patients with isolated FS phenotype. Indeed, the presence of only patients with FS phenotype in «small» families does not exclude the possibility of a GEFS + phenotype [14]. Most FEB loci that were initially described in families with only FS phenotype were subsequently found in families with GEFS + phenotype. Only FEB5 locus described by Nabbout et al. in 2002 is regarded as a locus for pure FS [15].

If the phenotype of GEFS+ syndrome among patients with FS was rare in the previous studies, it was frequent in our study (23.33%). This is explained probably by the collection of large families and by the long follow-up of our study.

In this large study of familial FS, although the majority of clinical phenotypes are supportive of “FS phenotype”, the lack of involvement of FEB loci would be in favor of a “GEFS plus” phenotype. This confirms clinical and genetic heterogeneities reported in syndromes associated with FS.

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