Searching for migraine genes: exclusion of 290 cM out of the whole human genome

Monari L.[1], Mochi M.[1], Valentino M.L.[1], Arnaldi C.[3], Cortelli P.[2], De Monte A.[2], Pierangeli G.[2], Prologo G.[2], Scapoli C.[4], Soriani S.[3], Montagna P.[2]

[1]Laboratorio di Neurogenetica e [2]Centro per lo studio delle cefalee, Clinica Neurologica, Università di Bologna; [3]Istituto di Pediatria, Università di Ferrara; [4]Dipartimento di Biologia Evolutiva, Università di Ferrara, Italy

A linkage and association analysis was made on 14 Italian families with recurrent migraine. We analyzed five chromosomal regions surrounding the candidate genes $5HT_{1D}$ (1p36.3-34.3), $5HT_{1B}$ (6q13), $5HT_{2A}$ (13q14-21), 5HT transporter (17q11.2-12), CACNLB1 (17q11.2-22) and FHM (19p13), using 29 DNA polymorphic markers.
All two-point lod scores were negative, and the $\chi 2$ sib-pair analyses were not significant, thus indicating the probable exclusion of these regions as sites of migraine genes in our population.

Key Words: Migraine — Lod score — Sib-pair analysis — Serotonin — Calcium channel.

Introduction

Migraine is a common disease affecting about 12% of individuals in Caucasian populations [9].
It has a known genetic component, but the causative gene(s) and transmission model are as yet undefined, probably because of the involvement of many genes [12].
Familial Hemiplegic Migraine (FHM) is a rare migraine subtype which has been mapped to a region of chromosome 19 containing the calcium channel $\alpha 1$-subunit [7, 17]. The involvement of the same region in common forms of migraine is still controversial: a positive linkage has been reported by May et al.[10] in migraine with and without aura, whereas negative results have been obtained in both Finnish [5] and Italian families [14].
As serotonin is thought to be involved in the pathogenesis of migraine[18], some authors have analysed some 5HT receptor genes in families of Caucasian origin in Australia[15] and families of unidentified origin in the USA [1]; an association between migraine and the $5HT_{2A}$ and $5HT_{2C}$ genes was excluded in both papers.
These findings may relate not only to the genetic heterogeneity of migraine, but also to the different genetic features of the populations investigated so far. In other words, it is likely that different genes (or gene sets) play a role in migraine in genetically different populations.
This consideration prompted us to investigate a sample of Italian families in order to analyse the chromosomal regions around the following candidate genes: serotonin receptor genes ($5HT_{1D}$, $5HT_{1B}$, $5HT_{2A}$, 5HT transporter), and the $\alpha 1$ (FHM gene) and $\beta 1$ subunit of the calcium channel.

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Material and Methods

We analysed the DNA of 100 affected and 62 unaffected consenting individuals belonging to seven families with recurrent migraine with aura (MwA) and seven families with recurrent migraine without aura (MwoA). Two of the pedigrees analysed are shown in *Figure 1*. The DNA was extracted from blood samples by standard methods [11]. All of the individuals were examined by a neurologist working at the Headache Center of our Institute and diagnosed according to the Headache Classification Committee criteria [4]. We analysed DNA polymorphic markers in the following chromosomal regions :
five markers in 37cM around HTR_{1D} (1p36.3-p34.3)
four markers in 31cM around $5HT_{1B}$ (6q13)
five markers in 22cM around $5HT_{2A}$ (13q14-q21) *(Figure 2)*
seven markers in 97 cM around the 5HT transporter (17q11.2-12) and CACNLB1 (17q11.2-q22)
eight markers in 50 cM around the FHM gene (19p13) *(Figure 3)*
Detailed technical descriptions of the markers have been published elsewhere [3, 21, 22]; the PCR amplifications were performed as previously described [13].
Statistical evaluation of linkage or association was made using both a parametric (Linkage) and a non parametric analysis (sib-pair analysis). In the first case, we considered both an autosomal dominant and a recessive

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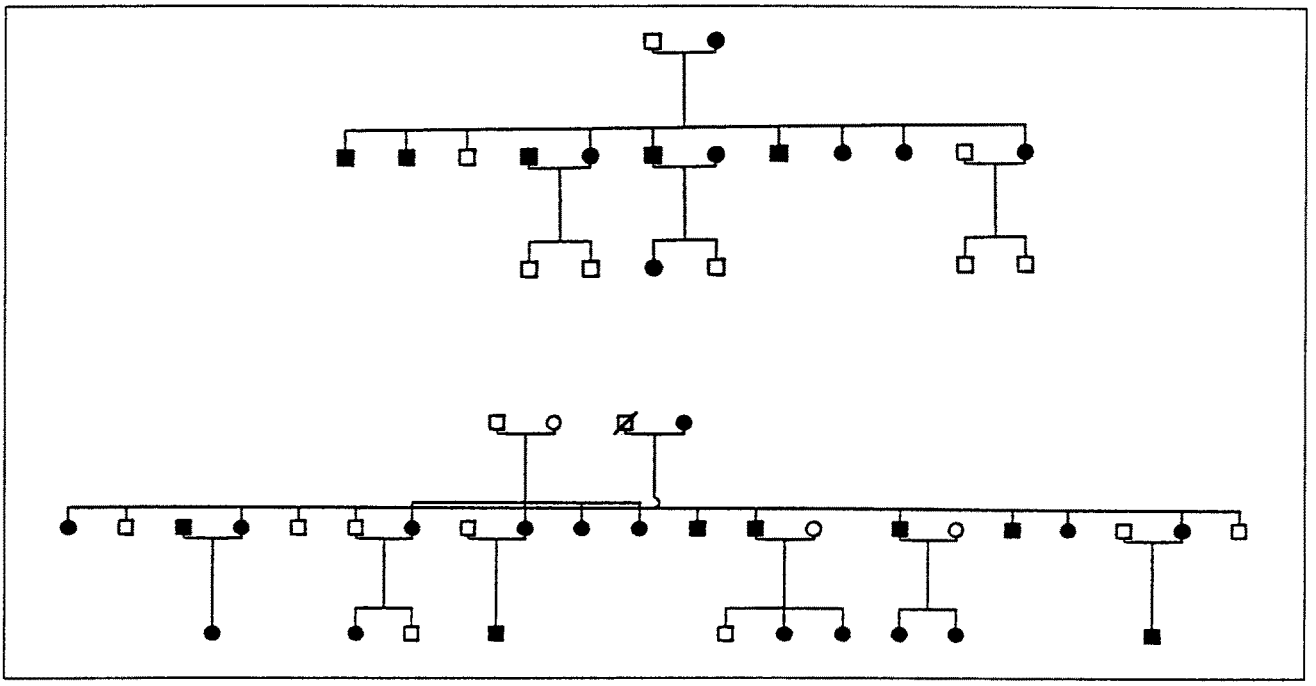

Fig.1. *Two of the pedigrees with recurrent migraine without aura analysed in this study.*

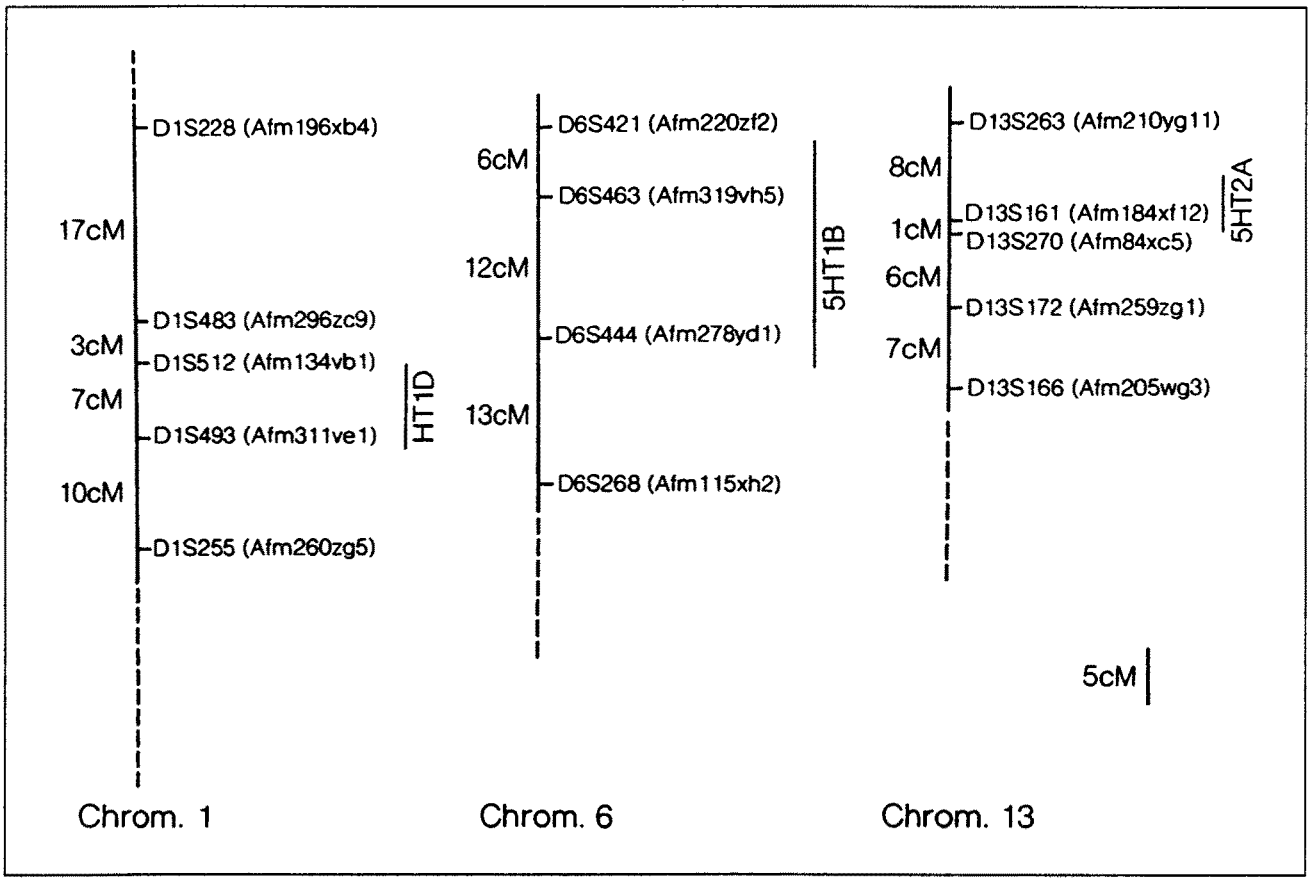

Fig.2. *Genetic map of the analysed regions of chromosomes 1, 6, 13. Sex-averaged distances are given in centimorgans.*

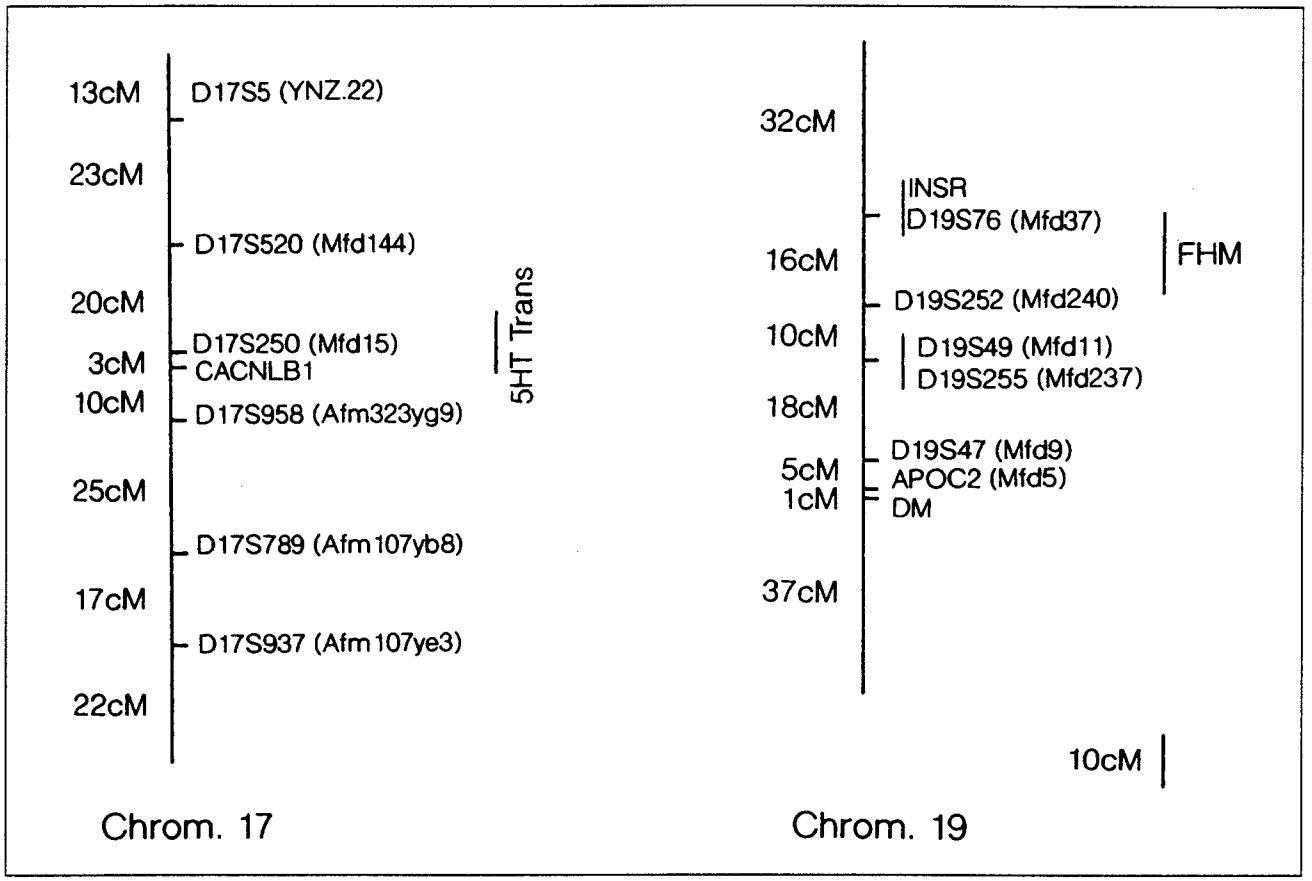

Fig.3. *Genetic map of the analysed regions of chromosomes 17, 19. Sex-averaged distances are given in centimorgans.*

model. The penetrance and phenocopy frequency were the same as previously described [12]. Migraine frequency was fixed at 0.12, as described in different populations [9]. Two-point lod scores were calculated using the MLINK option of the LINKAGE program package; heterogeneity was tested on the same lod scores using the HOMOG program. The sib-pair analysis was made using the ESPA (Extended Sib-Pair Analysis) program [20].

This method estimates how many alleles in a given sib-pair are identical by descent (IBD), but does not require information on penetrance, frequency or and mode of inheritance of a disease gene, phenocopy frequencies or genetic heterogeneity. It is used together with a lod score analysis and can remove errors due to an incorrect transmission model of a disease.

Families with recurrent MwA generally also include individuals with MwoA. These are considered unaffected if MwA and MwoA are considered to be two distinct entities casually associated in the same family (or even in the same individual), in which case MwA and MwoA families must be analysed separately. On the contrary, if MwA and MwoA are considered to be different expressions of the same alteration, all individuals with "migraine"(wA or woA) are considered affected, and all of the families examined together.

Results

We first analysed MwA and MwoA separately under the hypothesis that they were two distinct diseases, but found no differences in the lod scores of the two sets of families (data not shown). We therefore analyzed all of the families together and report here the results of this analysis.

The two-point lod scores were negative with each marker in all of the families under both transmission assumptions, *(Tables I and II)*. A small cluster of families had positive lod scores in all of the considered chromosomes: the values were not significant and the HOMOG $\chi 2$ test confirmed the lack of heterogeneity.

The $\chi 2$ and corresponding p values from the sib-pair analysis are shown in Table 3. A mean of 85 nuclear families derived from our group of 14 families were analyzed by ESPA: some of them proved to be not informative or without complete information. We only report the data concerning the completely known sibships because this leads to no loss of information and better results can be obtained. The sib-pair test is significant at a p value of < 0.001, but none of the analysed markers reached this value; we thus have no evidence of any association between them and migraine with any of the considered markers.

TABLE I *Two point lod-scores between migraine and the markers of chromosomes 1, 6, 13, 17, 19. Autosomal recessive genetic model.*

	Recombination fraction Φ				
Locus	**0.00**	**0.01**	**0.10**	**0.20**	**0.30**
D1S228	-5.43	-4.69	-1.79	-0.60	-0.10
D1S483	-1.75	-1.19	-0.25	-0.06	0.00
D1S512	-6.87	-5.92	-2.43	-0.92	-0.23
D1S493	-1.31	-1.07	-0.05	0.24	0.20
D1S255	-2.84	-2.48	-0.89	-0.19	0.06
D6S421	-3.37	-2.91	-1.24	-0.56	-0.21
D6S463	-5.87	-5.24	-2.46	-1.15	-0.46
D6S444	-2.88	-2.37	-0.56	0.00	0.13
D6S268	-6.60	-5.42	-2.11	-0.90	-0.32
D13S263	-3.49	-2.71	-0.15	0.45	0.42
D13S161	-3.30	-2.44	-0.48	0.09	0.15
D13S270	-1.73	-1.47	-0.36	0.01	0.07
D13S172	-2.59	-2.01	-0.50	-0.06	0.07
D13S166	-4.63	-3.49	-0.97	-0.18	0.04
D17S5	-1.17	-0.83	0.18	0.34	0.25
D17S520	-2.02	-1.74	-0.51	-0.07	0.07
D17S250	-1.50	-0.98	0.27	0.41	0.29
CACNLB1	-3.58	-3.02	-1.11	-0.43	-0.14
D17S958	-3.72	-3.22	-1.38	-0.59	-0.21
D17S789	-4.88	-4.21	-1.96	-1.00	-0.46
D17S937	-3.03	-2.56	-0.91	-0.27	-0.07
INSR	-1.96	-1.70	-0.81	-0.40	-0.15
D19S76	-1.21	-1.05	-0.41	-0.14	-0.03
D19S252	-5.16	-4.58	-2.34	-1.25	-0.57
D19S49	-1.78	-1.58	-0.85	-0.49	-0.25
D19S255	-2.82	-2.45	-1.10	-0.54	-0.23
D19S47	-3.15	-2.78	-1.34	-0.67	-0.29
APOC2	-1.77	-1.53	-0.66	-0.32	-0.16
DM	-2.99	-2.58	-1.08	-0.44	-0.13

Discussion

There are some peculiar features in the genetics of migraine, both MwA and MwoA: a high degree of familial involvement, a high level of frequency in all of the examined populations, and an as yet undetermined kind of transmission.

These aspects may indicate either allelic heterogeneity (different mutations in the same gene) or genetic heterogeneity (mutations in different genes) in the determination of migraine. Furthermore, this heterogeneity may indicate the presence of different mutations in different populations, but also different mutations within a single population. Genetic heterogeneity has been reported for FHM, the only kind of migraine that is genetically well understood: linkage to chromosome 19 is documented in only 50% of families [8, 16], and a new locus on chromosome 1q31 has recently been found to be associated with FHM in a large German pedigree [2]. Four mutations in CACNL1A4 have been discovered in five FHM families from Italy, the Netherlands, the United Kingdom and the USA, also indicating allele heterogeneity [17].

Besides FHM [5, 10, 14], some of the genes that may be relevant to the pathogenesis of migraine, such as the serotonin receptor genes [1, 15], have already been studied in some populations. In the case of genetic heterogeneity, we cannot exclude "a priori" the possibility of different associations in families with a different genetic background. Assuming a recombination fraction of 0.1, we analysed a distance of about 290 cM, which represents only 8% of the whole human genome, but which contains some of the candidate genes.

Our results from both linkage and sib-pair analysis exclude the localization of one or more migraine genes in the regions considered. This exclusion is total in our families, because no heterogeneity was detected: there were very few positive lod scores and these were very far from being significant. Our results confirm Hovatta's data [5] on the exclusion of the FHM region, as well as Nyholt's [15] and Buchwalder's [1] data on the 5HT receptor on chromosome 13. The exclusion of all of the 5HT receptors analysed so far ($5HT_{2A}$, 1D and 1B), as well as the 5HT transporter, indicates that these are not primary factors in the pathogenesis of migraine; however, they could conceivably be secondarily involved

TABLE II *Two point lod-scores between migraine and the markers of chromosomes 1, 6, 13, 17, 19. Autosomal dominant genetic model.*

	Recombination fraction Φ				
Locus	**0.00**	**0.01**	**0.10**	**0.20**	**0.30**
D1S228	-1.71	-1.56	-0.58	0.00	0.24
D1S483	0.12	0.15	0.28	0.27	0.19
D1S512	-1.97	-1.88	-1.20	-0.67	-0.30
D1S493	-1.78	-1.66	-0.87	-0.40	-0.15
D1S255	-4.34	-4.05	-2.34	-1.28	-0.63
D6S421	-1.69	-1.52	-0.70	-0.37	-0.23
D6S463	-1.91	-1.80	-1.08	-0.61	-0.31
D6S444	-2.40	-2.26	-1.30	-0.65	-0.30
D6S268	-0.01	0.24	0.87	0.82	0.56
D13S263	-4.24	-3.90	-2.14	-1.15	-0.56
D13S161	-1.60	-1.35	-0.28	0.06	0.11
D13S270	-2.62	-2.42	-1.24	-0.59	-0.26
D13S172	-0.86	-0.72	-0.21	-0.02	0.06
D13S166	-2.37	-2.13	-0.83	-0.16	0.08
D17S5	0.06	0.07	0.15	0.16	0.13
D17S520	-4.56	-4.28	-2.51	-1.37	-0.68
D17S250	-1.04	-0.91	-0.17	0.14	0.20
CACNLB1	-2.46	-2.21	-1.03	-0.44	-0.14
D17S958	-2.44	-2.25	-1.14	-0.49	-0.14
D17S789	-1.15	-1.08	-0.60	-0.27	-0.10
D17S937	-1.20	-1.09	-0.70	-0.51	-0.34
INSR	-2.79	-2.62	-1.56	-0.86	-0.42
D19S76	-1.96	-1.84	-1.08	-0.55	-0.24
D19S252	-3.11	-2.85	-1.50	-0.73	-0.31
D19S49	-0.47	-0.40	-0.04	0.08	0.09
D19S255	-1.21	-1.12	-0.57	-0.22	-0.02
D19S47	-2.50	-2.37	-1.48	-0.84	-0.41
APOC2	-1.29	-1.22	-0.78	-0.49	-0.30
DM	-1.22	-1.17	-0.80	-0.47	-0.25

TABLE III χ_2 values and corresponding *p*-values for sib-pair analysis, for all polymorphic markers. The sib-pair test is considered significant at a *p*-value<0.001.

Locus	χ_2	***p***	**Locus**	χ_2	***p***
D1S228	0.12	0.637	D17S5	6.13	0.993
D1S483	0.40	0.736	D17S520	1.81	0.911
D1S512	0.03	0.436	D17S250	3.08	0.960
D1S493	0.00	0.500	CACNLB1	0.35	0.723
D1S255	0.13	0.639	D17S958	5.55	0.991
			D17S789	0.15	0.649
D6S421	0.06	0.598	D17S937	0.56	0.649
D6S463	0.14	0.647			
D6S444	4.57	0.016	INSR	0.50	0.760
D6S268	0.10	0.624	D19S76	0.50	0.760
			D19S252	0.07	0.398
D13S263	0.20	0.673	D19S49	0.14	0.647
D13S161	2.31	0.064	D19S255	3.00	0.958
D13S270	0.06	0.594	D19S47	0.13	0.638
D13S172	0.27	0.699	APOC2	0.00	0.500
D13S166	0.91	0.831	DM	1.10	0.853

in disease expression. One of the DNA polymorphic markers we investigated on chromosome 17 consists of a dinucleotide repeat within the β1 subunit of the L-Type voltage-dependent Ca^{++} channel [6].
The same gene (CACNLB1) codes for the muscle and brain isoforms of the β subunit of the Ca^{++} channel [19]. This microsatellite could be a direct marker of the association of migraine with this Ca^{++} channel subunit, just as FHM is associated with the α1 subunit of the Ca^{++} channel on chromosome 19 [17].

Unfortunately, we found no association between migraine and CACNLB1. As a final consideration, we would like to underline the fact that migraine is not a simple Mendelian disease, and that our results apply only to these families and with these genetic models. Despite of these negative results, we think that the mutations in CACNLA1 could be indicative of the involvement of other ion channels, not only Ca^{++} channels, in migraine pathogenesis, and that some work should be done in this direction.

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Sommario

È stata condotta un'analisi di linkage ed associazione su 14 famiglie italiane con ricorrenza di emicrania. Abbiamo analizzato cinque regioni cromosomiche attorno ai geni candidati $5HT_{1D}$ (1p36.3-34.3), $5HT_{1B}$ (6q13), $5HT_{2A}$ (13q14-21), 5HT transporter (17q11.2-12), CACNLB1 (17q11.2-22) e FHM (19p13), usando 29 marcatori polimorfici del DNA.
Tutti i valori dei lod-score a due punti erano negativi, i $\chi 2$ della sib-pair analysis non erano significativi, escludendo così la presenza di geni per l'emicrania nella popolazione in esame nelle regioni analizzate.

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Address reprint requests to: Dr. Monari Lucia, Laboratorio di Neurogenetica, Clinica Neurologica, via Ugo Foscolo 7, 40123 Bologna, Italy.