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HLA and migraine genes

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Abstract Human leukocyte Antigens (HLA) are encoded by genes located on chromosome 6p21. Genes important in migraine are being recognized in two basic ways: association studies and linkage analysis. One of the strongest associations is with the HLA region. Actually, genome scan studies suggest that multiple genes are involved in both migraine without aura (MWoA) and migraine with aura (MWA). However, both MWoA and MWA are disorders in which multiple factors, including environmental and genetic factors, confer disease susceptibility. Linkage analysis is identifying new candidate genes that will help to explain the etiology of migraine. In this review previous studies regarding genetic susceptibility to migraine are analyzed, particularly those related to the HLA region. I discuss evidence that HLA shared-haplotypes in MWoA-affected pairs is different than that expected, that HLA-DR2 antigen provides additional basis for the proposed genetic heterogeneity between MWoA and MWA, and lastly that TNFB gene studies seem to play an important role in the susceptibility to MWoA. In the past years, major advances have been made in understanding the genetic foundation of MWoA and MWA. Our reported genome-scan studies support the concept that MWoA/MWA are co-inherited with a particular HLA region. However, the examination of candidate genes (Ca^{2+} channel, vascular, CNS, etc.) in a large migraine population seems to be the correct direction in which we have to move. More MWoA/MWa gene studies are needed to test this developing hypothesis and to further establish the complete genetic scenario of migraine.

Key words Migraine • Genetics • Human leukocyte antigens • Heredity • Susceptibility

Major histocompatibility complex and genetic susceptibility to migraine

Studies on genetic susceptibility or resistance to disease show that a large number of human and animal pathologies are related to particular genetic and immunological arrangements. Major histocompatibility system (MHC) is a genetic system of more than 70 known genes that occupy the mid-portion of the short arm of the sixth chromosome (C6p). MHC spans approximately 4 million base pairs.

In the early 1960s, an association between leukemia and some MHC antigens has been demonstrated in mouse. Such an observation led to the search for the same associations in man. The diseases related to human MHC, namely the human leukocyte antigens (HLA) complex, have unclear etiology, show familial recurrence and are supported by polygenic and environmental characters. HLA antigens are

namely as related to three different C6p regions (class I, II or III) or can also defined as "classic" or "non-classic" antigens.

The genetics of migraine is a complex interplay between multiple genes. As more is learned about the migraines, their polygenic nature becomes increasingly clear. In the past decade, increasing attention has been given to the study of molecular genetics of migraine on the basis of previous evidence of familial hereditary influence [1, 2]. More studies mainly focused on familial hemiplegic migraine (FHM), a rare autosomal dominant subtype of migraine with aura (MWA) associated with ictal transient hemiparesis and (often) progressive cerebellar atrophy, on both chromosome 19 and chromosome 1 [3–5]. However, in spite of the scientific resonance of these discoveries, the rarity of FHM and the widespread diffusion of both MWA and MWoA (0.5% vs. 95% of all migraine headaches) lead us to continue the HLA studies of genetic factors of MWA and MWoA.

Class I and class II regions of human leukocyte antigens and migraine

The first study of HLA class I antigens in migraine was published by Lee Kudrow more than twenty years ago [6]. In the case of MWoA, my colleagues and I previously demonstrated in 8 households an increase of shared HLA haplotypes, suggesting that migraine heredity was HLA-linked [7]. Sib-pair analysis performed in 20 sons demonstrated that 30% of the affected sons shared two haplotypes, 60% shared one haplotype, and 10% shared no haplotype (Table 1). This is clearly different from the expected distribution according to simple genetic mendelian models. Therefore, if HLA genes have no influence on the development of the disease, then the affected sib-pairs would have to share HLA haplotypes with a normal frequency, while the outcome supports the hypothesis that migraine inheritance is HLA-linked. Genes in the HLA system have a marked effect on suceptibility to a wide variety of diseases, both immunological and non-immunological. The HLA system governs the liability to certain immunological diseases by controlling either the immune recognition or self/non-self discrimination and the interaction of idiotypic T-cell receptor (TCR). To explain an association between HLA and non-immunological diseases, such as migraine, it has been hypothesized that HLA antigens may interfere with the interaction between ligands and receptors on central nervos system (CNS) or vascular cell membranes.

More recently, my colleagues and I reported for the first time the molecular analysis of DRB1 and DQB1 alleles in Italian subjects affected by migraine [8]. We demonstrated that HLA class II DR2 antigen shows a decreased frequency in MWA compared with both MWoA and controls. These results support the hypothesis of a protective role of DR2 antigen in MWA and provide additional basis for the proposed difference within MWoA and MWA (Table 2). It is also interesting to note that a decreased frequency of DR2 antigen is observed in many HLA-associated diseases, such as IDDM and celiac diseases [9]. In this light, I would like to stress recent functional studies dealing with lymphocyte trafficking in migraine and immunological mechanisms in pathogenesis of migraine. Emerging results from a sentinel study showed an expansion of normal T cells (CD3+) and of natural killer (NK) cell subsets (CD16+, CD56+) [10, 11]. Recent evidence indicates that NK cell activity is directly dependent on L-arginine [12], the main precursor of nitric oxide, and that nitric oxide (NO) in migraine [13] operates synergistically with both the serotonin (5HT) system [14] and the cyclooxygenase (COX) 1 and 2 systems [15] in the development of painful migraine attacks. These molecular interactions in migraine may represent an important step in understanding the genetic-biological basis of this multifactorial disease.

Table 1 Observed frequencies in 8 migraine families (Modified from [7] with permission)

	Frequency n (%)	
Clinically studied individuals	60 (100.0)	
Subjects entering the HLA family study	41 (68.3)	
Migraine-affected subjects	33 (55.0)	
HLA typed sons	24 (40.0)	(100.0)[a]
HLA typed migraine-affected sons	20 (33.3)	(83.3)[a]
Affected sib-pairs sharing 2 haplotypes	6 (30.0)[a]	
Expected sib-pairs sharing 2 haplotypes	5 (25.0)[a]	
Affected sib-pairs sharing 1 haplotype	12 (60.0)[a]	
Expected sib-pairs sharing 1 haplotype	10 (50.0)[a]	
Affected sib-pairs sharing 0 haplotype	2 (10.0)[a]	
Expected sib-pairs sharing 0 haplotype	1 (25.0)[a]	

[a] Percent of 20 sons with migraine

Table 2 DR2 allele frequencies in migraine without aura (MWoA), migraine with aura (MWA), and healthy controls (C) (Modified from [8] with permission)[a]

Allele	MwoA (n = 31) n (%)	MWA (n = 14) n (%)	C (n = 53) n (%)
DR15 (DR2)	2 (3.22)	4 (14.28)	8 (7.55)
DR16 (DR2)	0 –	0 –	5 (4.72)
DR2	2 (3.22)	6 (21.40)*	13 (12.26)**

[a] Fisher's exact test from 2x2 contingency tables. Corrected p (p_c) value was calculated as p x number of comparisons. Differences were considered statistically significant when p_c was less than 0.05

* $p = 0.01$, MWA vs. MWoA

** $p = 0.039$, MWA vs. C, RR = 0.21

Class III region human leukocyte antigens and migraine

Tumor necrosis factors (TNF) A and B have similar biologic activities and are 30% identical at the protein level. Each gene is about 3 kb long and contains 3 introns. The genes are closely linked and are situated on chromosome 6, according to the findings in human-mouse somatic cell hybrids [16]. A study of hybrid cells made with rearranged human chromosome 6 showed that both TNFA and TNFB map to the 6p23-q12 segment. Only the last exons of these genes, which code more than 80% of the secreted protein, are homologous (56% identical) [17].

TNFA is derived from activated monocytes, and TNFB from mitogen-activated T lymphocytes. Nedwin et al. speculated that the close situation of these 2 loci to HLA may be useful for a coordinate regulation of immune system gene products [16]. By Southern blot analysis of a panel of major histocompatibility complex (MHC) deletion mutants, Spies et al. established that TNFA and TNFB are closely linked and situated in the MHC either between HLA-DR and HLA-A or centromeric HLA-DP. By in situ hybridization, they assigned TNFA and TNFB to 6p21.3-p21.1 [17].

One of the most striking features of the list of polymorphisms observed to date is related to the TNF gene. It is significant and functional in the development of various diseases [18].

The associations between HLA genes and different diseases presenting their pathophysiological scenario as an inflammation-based route have been described. TNF gene polymorphism seems to be associated with susceptibility to Behçet's diseases [19], rheumatoid arthritis [20, 21], systemic lupus erythematosus [22], multiple sclerosis [23, 24], celiac disease [25], and narcolepsy [26].

The pathophysiology of migraine is still controversial, although sterile inflammation has been demonstrated to play a key role at the cranial vascular endothelial level [27]. The cytokines TNFA and TNFB are polypeptide effectors of inflammatory reactions and endothelial function [28]. TNFA has been described to act as crucial mediator in another form of unilateral headache, the cervicogenic headache [29, 30].

Thus, to better define the involvement of HLA region genes in migraine, we performed an association study of the TNF genes, located in the HLA class III region, with MWoA and MWA. The results indicate that the frequency of TNFB*2 allele is significantly increased in MWoA patients [31]. The frequency of TNFB*2 was significantly increased in MWoA patients (78.72%) as compared with that in the controls (61.4%) (p_c = 0.004), while no significant differences were found between patients with MWA and controls (Table 3). The TNFB genotypic frequencies are shown in Table 4. There was a significant decrease of TNFB 1/1 homozygotes in MWoA patients (p_c = 0.0201).

Table 3 TNFB allele frequencies in migraine without aura (MWoA), migraine with aura (MWA), patients and healthy controls (Modified from [31] with permission)[a]

Allele	Controls (n = 101) n (%)	MWA (n = 30) n (%)	MWoA (n = 47) n (%)
1	78 (38.6)	19 (31.67)	20 (21.28)
2	124 (61.4)	41 (68.33)	74 (78.72)*

[a] Fisher's exact test from 2x2 contingency tables. Corrected p (p_c) value was calculated as p x number of comparisons. Differences were considered statistically significant when p_c was less than 0.05
* p = 0.02, (p_c = 0.004); MWoA vs. controls

Table 4 Distribution of genotypes in migraine without aura (MWoA), migraine with aura (MWA), patients and healthy controls (Modified from [31] with permission)[a]

Genotype	Controls (n = 101) n (%)	MWA (n = 30) n (%)	MWoA (n = 47) n (%)
1-1	17 (16.8)	5 (16.67)*	1 (2.13)
1-2	44 (43.6)	9 (30.00)	18 (38.30)
2-2	40 (39.6)	16 (53.33)**	28 (59.57)

[a] Fisher's exact test from 2x2 contingency tables. Corrected p (p_c) value was calculated as p x number of comparisons. Differences were considered statistically significant when p_c was less than 0.05
* p = 0.0067 (p_c = 0.0201); MWoA vs. controls;
* p = 0.0182 (p_c = ns); MWA vs. controls

When the frequencies of TNFB genotypes were compared in MWA patients and controls, no differencies were found [32]. The observed increase of TNFB*2 in MWoA is distributed in TNFB 2,2 and TNFB 1,2 genotypes, meaning that the susceptibility allele acts as "dominant": people with TNFB 1,1 genotype are less predisposed to the disease.

Exploring migraine candidate genes as the key passage toward targeted disease management

TNF is a proinflammatory cytokine that provides a rapid form of host defense against infection but is fatal in excess. Because TNF is employed against a variety of pathogens, each involving a different pattern of risks and benefits, it is expected that this would favor diversity in the genetic elements that control TNF production [28].

The contribution of TNF and related genes to susceptibility to various human disease, and the possibility that the TNF gene within the MHC may potentially complicate the interpretation of studies in animal models in which the TNF

gene itself is experimentally manipulated, have been recently discussed by Ruuls and Sedgwick [33].

My colleagues and I recently reported for the first time the molecular analysis of TNFA and TNFB genes in Italian subjects affected by migraine [31, 34]. The observed significant increase of TNFB*2 in MWoA suggests that this gene may influence the strength, effectiveness and duration of local inflammation (perivascular brain plasma extravasation) namely "sterile inflammation" as *primum movens* of migraine pain [35]. The structural or regulatory defective TNFB genes in migraine may contribute to the reach of threshold brain excitability and to the subsequent propagation of the neuronal hyperexcitability that is now considered among the prevailing hypotheses for the mechanisms of this disorder, especially of MWA [36].

However, it should be stressed that our data may be in contrast with the notion that TNFB gene expression is involved in MWoA, since TNFB gene expression is increased only in the MWA population. In this light, the old-fashioned migraine hypothesis, based on endothelial permeability and leakage of dura mater vessels, may be rejected by considering the reported inhibition of this critical step by both the decrease of adhesion molecule ICAM-1 [37] and ELAM-1 [38] and by fluorescein-indocyanine angiography [39].

Lastly, emerging results from a sentinel open study in chronic cervicogenic headache patients showed that etanercept (soluble TNF p75 inhibitor) produces a substantial clinical amelioration of disease activity [40]. Similar studies in refractory MWoA and MWA patients may contribute to a better comprehension of the biological implications of the reported TNFB*2 expression increase in MWoA and may confirm the influence of TNF genes as susceptibility genes that act in combination with others as risk cofactors in migraine.

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