Idiopathic epilepsy with generalized tonic clonic seizures in Antioquia, Colombia: Is the joint Amerindian and Negroid racial admixture the cause of its high prevalence?

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Most Colombian populations stem from the admixture of Caucasians, Amerindians and Negroids. In the world, these two latter ethnical groups show a significantly higher prevalence of epilepsy than the former one. We tested the hypothesis that the high prevalence of idiopathic epilepsy with generalized tonic clonic seizures found in the Antioquian population (Paisas), from Colombia, is due to their possible joint Negroid and Amerindian ethnic components. We have previously demonstrated that inheritance is the principal factor for developing epilepsy in this community. Analyses of racial admixture, heterogeneity between populations, genetic distance, and phyletic relationships were performed among epileptic and non epileptic samples from the Antioquian community. Also Caucasians, Spaniards, Basques, Jews, Chileans, Negroids, Amerindians and Mongoloids were included in the analysis. Four highly polymorphic blood systems were used as genetic markers: RH, MNS, ABO and FY. They were chosen because of their high discriminant power in these ethnic groups. In the population affected with idiopathic epilepsy, the estimated Negroid and Amerindian rates of admixture were low (3% and 14%, respectively). Although, these degrees of admixture can be explained due to common ancestral origins, the estimated proportion of Amerindian admixture in the epileptic affected population, was significantly higher than the estimated for the Non affected Antioquian population. The latter finding is consistent with the analysis of heterogeneity between populations that discriminated epileptic population from non epileptic Antioquian population ($p<0.05$). Epileptic and non epileptic Paisas clustered in topology with Caucasians, very close to Spaniards and Basques and highly distant from Negroids and Amerindians. Thus far, the origin of the high prevalence of idiopathic epilepsy in the Antioquian (Paisa) population cannot be explained by the hypothetical joint Negroid and Amerindian ethnical admixture, but using additional genetic markers and other methods of racial estimation of admixture it is necessary to corroborate if the Amerindian admixture component is significantly higher in the epileptic population than in the non epileptic Paisa population.

Key terms: *etiology of epilepsy, genetic epidemiology, genetics of idiopathic epilepsies, racial admixture.*

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INTRODUCTION

The population of Colombia was originated from Caucasians (mostly Spaniards), Amerindians and Negroids. Most people from Antioquia, a political department of Colombia, belong to the self-designed Paisa community. The Paisa population speaks Spanish and is geographically located between the Central and Western branches of the Andes Mountains (Fig 1). The Paisas have based their economy on the exploitation of coffee, one of the greatest economical resource of the country. Studies about their genetic structure and phyletic relationships confirm that they represent a very homogeneous group with a predominantly Caucasoid ancestry (Gomez and Bravo, 1985; Arcos and Bravo, 1992). Anthropological and historical studies describe this population as the most clearly defined in Colombia. They are predominantly catholic, endogamic and socially very conservative with a strong matriarchal familial structure and considerable big sib-ships with sizes ranging between five to twenty children and occasionally even more. Their ethno-historical origin stems from the Spaniards, Jews (Christianised Sephardim or *Marranos*), and Basques (Parsons, 1949; Agudelo, 1986; Mesa, 1988).

The prevalence of epilepsy in Medellín, the capital city of Antioquia, have been reported to be near 2%, and the main risk for developing epilepsy has been attributed to genetic factors (Jiménez *et al*, 1984; Jiménez

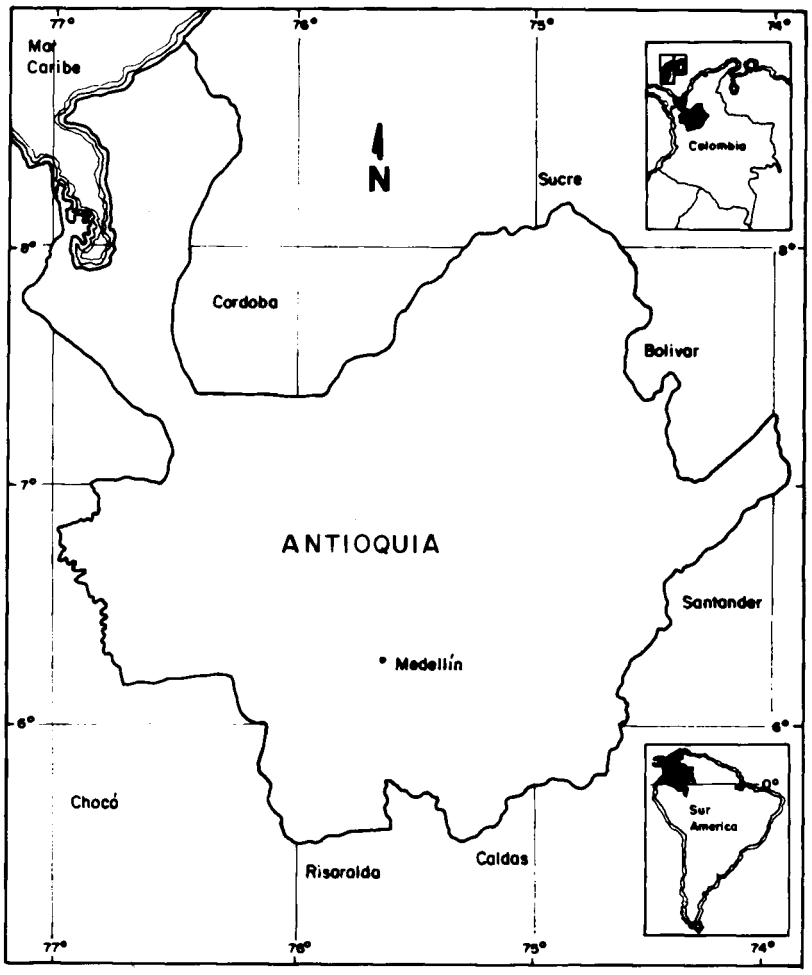

Fig 1. Map of the Department of Antioquía, Colombia. Lower inset, location of Colombia within South America. Upper inset, location of Antioquía within Colombia.

et al, 1991; Zuluaga *et al*, 1986). This figure is much higher than the 0.5 % reported for Caucasian populations (Sander and Shorvon, 1987), one of the assumed main ethnic component of the Antioquians. The Negroid and Amerindian populations have been reported to have the highest prevalence of epilepsy: 3,7% in Nigeria (Osuntokun and Schoemberg, 1982), 5.0% in Panamá (Gracia and de Lao, 1990). It has been shown that the prevalence of diseases with complex etiology varies among populations with varying degree of admixture, even when part of their gene pool stems from the same ancestral population (Chakraborty and Weiss, 1986; Valenzuela and Herrera, 1993). Thus, we hypothesize that the higher prevalence of epilepsy in Paisas than in Caucasians could be due to an important component of Negroid and Amerindian admixture which is present in Paisas. This hypothesis needs, to be true, that Paisas (particularly epileptic Paisas) have, at least, over 30% of Negroid or Amerindian mixture.

In this study we intend to estimate the Caucasian, Negroid and Amerindian admixture, build the ethnophylogenetic relationships between populations affected with idiopathic epilepsy with generalized tonic clonic seizures and non affected populations obtained from samples of the major races and from several different ethnic groups. Also, we are going to test the genetic heterogeneity between populations and whether the estimate of the joint Amerindian and Negroid admixture is compatible with the hypothesis.

METHODS

In a two-year period, 40 nuclear families ascertained through affected probands with diagnosis of idiopathic epilepsy with generalized tonic clonic seizures were randomly selected from among individuals seeking for medical care at the Antioquian League Against Epilepsy (ALAE) in Medellín, Colombia. ALAE is a non governmental institution where approximately ninety percent of the epileptic affected population from Antioquia is ascertained (ascertainment probability p=0.92). Idiopathic epilepsy with generalized tonic clonic seizures was operatively defined by age-related onset, and clinical and electroencephalographic (EEG) characteristics (Commission, 1989). Each proband was carefully examined by trained neurologists. Pedigree data and a complete family history of epilepsy were collected using a specially devised questionnaire that was filled out either at a home visit or when interviews were carried out at ALAE. Clinical and EEG studies were performed in probands and in all first and second degree relatives with epilepsy or suspected symptoms of epilepsy. Affected relatives were classified according to their previous medical records and to clinical analysis done with the same criteria as the cases by the same group of researchers. A Computerised Axial Tomography (CAT) scan was carried out in all probands and affected relatives with symptoms or with suggestive signs of focalisation.

The criteria to exclude an individual from the present study were the following: *i-* medical record with obstetric antecedents such as prolonged childbirth, abnormal presentations at delivery, multiple childbirth, ovulatory membrane-premature rupture, perinatal anoxia, amniotic liquid bronco aspiration, incubator usage after childbirth, and premature delivery; *ii-* clinical matching to Juvenile Myoclonic Epilepsy (JME) or Childhood Myoclonic Epilepsy (CME); *iii-* history of severe cerebral trauma; *iv-* probands with relatives affected with JME; *v-* cases with residence outside Antioquia; *vi-* probands with non-Antioquian parents. Segregational analyses on this sample have given strong support for a genetic transmission of the disease (Jiménez *et al*, 1996).

Individuals from the nuclear families were typed for ABO, RH, MNS and FY blood groups under standard international procedures. Only 31 families had complete records that allowed a complete rebuild of the haplotypes and a non biased analysis. Some individuals from the extended families were also included to better ascertain the genotypes of the parents of epileptic probands. Thus a nuclear family gives four independent genes (ABO,FY) or four independent haplotypes (RH, MNS). In a few cases, and depending on the particular

genetic system, evident technical errors or exclusions of paternity did not allow us to reach the 124 maximal number of independent genes or haplotypes. Although, this number (124) seems to be small, it is the result of the direct count of haplotypes and therefore is more reliable. These blood systems were chosen because of their ethnic discriminant power. For example, Amerindians lacked the A, B (ABO) and d (RH) alleles; Negroids have a high frequency of the cDe (Ro) RH (60% Vs 2-5% of Amerindians and Caucasians) and FY- alleles (90%, while it is almost absent in Amerindians and Caucasians).

Gene frequencies for Caucasians, Amerindians and Negroids were obtained from the literature (Roychoudhury and Nei, 1988). The gene frequencies used as input for the major races were the result of weighted gene frequencies from populations with a major number of individuals and belonging to these major races. Moreover, among Caucasians we also included samples from Basques and Spaniards (Campillo, 1976) and from the Jews (Roychoudhury and Nei, 1988), the main Caucasian ancestors of Antioquians (Paisas) according to anthropological and historical studies. Data from a Chilean population were also included (Cifuentes *et al*, 1988); given this population has been demonstrated to stem from a Caucasian-Amerindian admixture (Valenzuela, 1984; Valenzuela, 1988), so it can be considered as a control of the admixture process. A sample of Antioquian university students was also included as non epileptic Paisa controls. This population has showed to be a good sample of Antioquian population (Alarcon, 1992).

Qualitative analysis of racial admixture proportions were performed using the method based on gene identity (Chakraborty, 1975). Pearson heterogeneity test between populations, Prevosti distance and the Wagner tree were obtained using BIOSYS-1 software (Swofford, 1989).

RESULTS AND DISCUSSION

Table 1 shows the allele frequencies for the four systems and the ten populations studied. The number of epileptic individuals was calculated as half of the number of independent genes obtained from direct and manual counting from nuclear families. Samples over 10000 individuals were replaced by 9999 to fit the input format of BIOSYS.

The cDe RH haplotype showed clear clusters. Negroids with a high frequency, 0.739; Caucasians samples (Caucasians, Spaniards, Basques) had frequencies between 0.073 and 0.019; Jews, 0.110; Chileans, 0.026; and Mongoloids and Amerindians, 0.017 and 0.012, respectively; non epileptic Paisas and epileptics showed frequencies close to those of Basques (both 0.060). The frequency of the d allele showed a similar pattern. Caucasians, non epileptic Paisas, Spaniards, Basques and epileptics showed frequencies over 0.360. Jews, Chileans and Negroids had frequencies between 0.340 and 0.190. Mongoloids and Amerindians had under 0.040. The MNS system did not show a clear pattern. In the ABO system a pattern could be observed in relation to the O allele. Amerindians were all O. Frequencies between 0.800 and 0.730 were non epileptic Paisas, Basques, Chileans and epileptics. Negroids presented a O frequency equal to 0.709. A frequency lower than 0.660 was found in Caucasians (0.650), Spaniards (0.648), Jews (0.577) and Mongoloids (0.559). The Duffy system did not show an evident pattern, with the exception of the FY- allele mostly present in Negroids. Only Jews showed a frequency different from 0.00. The two FY- alleles found in epileptics were deduced indirectly from two FYb mothers with FYa children.

The estimated proportions of racial admixture, and their standard errors, for epileptic and non epileptic Paisa populations are presented in Table II. Three models of racial hybridization (Amerindian-Caucasoid-Negroid, Spaniard-Basque-Negroid, and Spaniard-Basque-Amerindian) were tested and sums of squares and residual for each model are given for fitting purposes. The first model that established Amerindians, Caucasoids and Negroids, as the parental populations of epileptics, showed the best fit to the data. At this point, Amerindian and Negroid admixture are very low (14.70% and

Table I

Populations gene frequencies

	CAUC	PAIS	JEWS	SPAN	BASQ	CHIL	EPIL	NEGR	MONG	AMER
						RH				
(N)	8297	500	200	2500	626	1429	58	644	2455	168
Allele										
CDE	.001	.010	.010	.002	.014	.013	.034	.001	.003	.000
CDe	.412	.396	.458	.422	.403	.500	.405	.020	.645	.756
cDE	.170	.166	.093	.127	.043	.226	.138	.042	.262	.235
cDe	.019	.060	.110	.043	.073	.026	.060	.739	.017	.012
CdE	.001	.001	.000	.001	.000	.000	.000	.001	.000	.000
Cde	.005	.021	.055	.016	.027	.006	.017	.030	.011	.000
cdE	.003	.014	.010	.007	.004	.010	.000	.006	.029	.000
cde	.389	.332	.265	.382	.436	.219	.345	.161	.033	.000
						MNS				
(N)	1000	251	168	1547	75	258	54	205	230	168
Allele										
MS	.237	.273	.351	.243	.320	.138	.167	.093	.063	.149
Ms	.305	.289	.467	.284	.313	.388	.259	.488	.467	.545
NS	.070	.122	.039	.081	.107	.093	.176	.044	.026	.021
Ns	.388	.317	.143	.392	.253	.382	.398	.376	.443	.283
						ABO				
(N)	9999	500	306	9999	146	9999	62	858	9999	2516
Allele										
A	.273	.190	.302	.286	.226	.172	.153	.177	.271	.000
B	.077	.042	.123	.066	.031	.058	.065	.114	.170	.000
O	.650	.768	.577	.648	.743	.770	.782	.709	.559	1.000
						FY				
(N)	1944	449	936	1988	483	684	60	365	103	1789
Allele										
a	.420	.539	.436	.397	.365	.521	.458	.062	.908	.575
b	.549	.460	.531	.602	.635	.479	.525	.001	.092	.425
(-)	.001	.001	.033	.001	.000	.000	.017	.938	.000	.000

CAUC, Caucasoids; PAIS, Paisas; JEWS, Jews; SPAN, Spaniards; BASQ, Basques; CHIL, Chileans; EPIL, epileptics; NEGR, Negroids; MONG, Mongoloids; AMER, Amerindians.

3.19%). The proportion of racial admixture for non epileptic Paisas is similar to those calculated for the epileptic sample. However, the estimated Amerindian proportion was significantly higher in the epileptic population ($p<0.05$).

The Prevosti distances are presented in Table III. Four groups were directly observed. Negroids, Amerindians and Mongoloids, and Caucasoids. This latter group included Caucasians, Spaniards, Basques, Jews, non epileptic Paisas, epileptics and Chileans, which showed a small distance from Amerindians (0.179). Epileptics clustered within the Caucasoid group, very close to Paisas (0.075) and far from Negroids (0.480), Mongoloids (0.330) and Amerindians (0.267). The biggest distance was found between Negroids and Amerindians (0.568) and the smallest one between Caucasians and Spaniards (0.147).

The optimized Wagner tree rooted at the midpoint of the longest path constructed with the Prevosti distances is shown in Figure 2.

Table II

Racial admixture proportions in a sample affected with idiopathic epilepsy with generalized tonic clonic seizures (epileptics) from Antioquía population, Colombia, and sample from non epileptic Antioquia population, Colombia (non epileptic Paisas)

	M	SE	SST	SSRes	R^2
		Epileptics			
		Model (1)			
Amerindian	.1470	.0004			
Caucasoid	.8211	.0010	.852722	.000002	.999998
Negroid	.0319	.0010			
		Model (2)			
Spaniard	.7197	.0122			
Basque	.2353	.0096	.863818	.000124	.999856
Negroid	.0450	.0082			
		Model (3)			
Spaniard	.1047	.2584			
Basque	.7164	.3952	10.3678	.115634	.988847
Amerindian	.1789	.1469			
		Non Epileptic Paisas			
		Model (1)			
Amerindian	.1899	.0137			
Caucasoid	.8007	.0323	.786841	.002327	.997042
Negroid	.0095	.0382			
		Model (2)			
Spaniard	.6782	.0421			
Basque	.3025	.0322	.934064	.001225	.998688
Negroid	.0193	.0261			
		Model (3)			
Spaniard	-.2964	.2589			
Basque	1.0431	.4902	113.6508	.237861	.997907
Negroid	.2534	.2315			

M = Mean
SE = Standard Error
SST = Total Sum of Squares
SSRes = Residual Sum of Squares

Samples clustered in four groups: A) Negroids; B) Mongoloids and Amerindians; C) Chileans; D) Caucasians, Spaniards, Jews, Basques, epileptics and non epileptic Paisas.

All populations were significantly and highly different from one another ($P < 0.0001$). However, epileptics and non epileptic Paisas differed less than the other pairs of samples ($p<0.05$)

Four different analyses, namely: *i*- qualitative estimation of racial admixture proportions, *ii*- heterogeneity test between

Table III

Matrix of Prevosti distance coefficients

Population	1	2	3	4	5	6	7	8	9	10
1 Caucasians	***	.097	.147	.031	.108	.121	.094	.487	.286	.285
2 Paisas		***	.174	.103	.105	.092	.075	.489	.311	.238
3 Jews			***	.147	.157	.188	.193	.481	.299	.305
4 Spaniards				***	.092	.138	.094	.490	.308	.308
5 Basques					***	.174	.128	.497	.388	.317
6 Chileans						***	.101	.459	.236	.179
7 Epileptics							***	.480	.330	.267
8 Negroids								***	.506	.568
9 Mongoloids									***	.262
10 Amerindians										***

Paisas = non epileptic Paisas.

populations, *iii-* Prevosti distances, *iv-* phyletic relations, show that this sample of Antioquian individuals affected with idiopathic epilepsy have a small proportion of Amerindian (14.7%) and Negroid (3.2%) races. This small ethnic contribution cannot be responsible for the high frequency of epilepsy found among Paisas. The high frequency of this disease seems to be, most probably, the result of both, a Caucasoid founder effect and a low Amerindian contribution, as far as this sample of individuals affected with idiopathic epilepsy shows. Paisas Caucasoid founders could carry a higher frequency of genes for this kind of epilepsy than Caucasoids who remained in their countries. Amerindians contributed with their own high gene frequency for the disease. This may be the reason because the Amerindian ethnic component was higher in epileptics than in the non epileptic Paisa population. With the exception of the two FY- genes, the epileptic sample clustered completely with Caucasians, as well as Paisas did. However, these alleles could be introduced by Spaniards or Jews (Sephardim) who have a low frequency of them. Thus, the proposed joint Negroid and Amerindian origin of the high frequency of epilepsy in Antioquians (Paisas) does not have a factual support. Although, the Amerindian con-

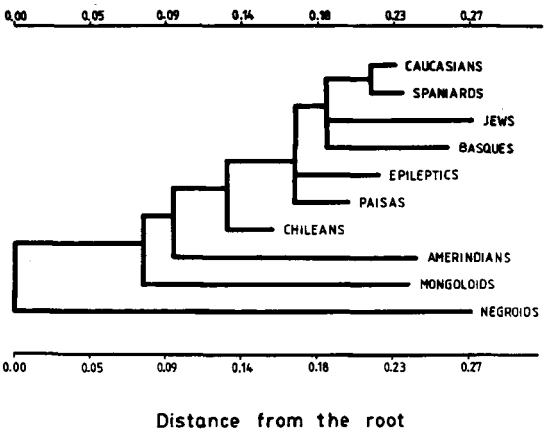

Fig 2. Wagner tree produced by rooting at midpoint of longest path. Paisas = non epileptic Paisas.

tribution in the sample of patients must be evaluated using a major number of marker systems, and other methods, to estimate the racial admixture, are needed to elucidate its neuroepidemiologic and genetic importance.

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