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ORIGINAL ARTICLE

Human *CHIT1* gene distribution: new data from Mediterranean and European populations

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Abstract A 24 bp duplication in the *CHIT1* gene (H allele) is associated with a deficiency in the activity of chitotriosidase, an enzyme with the capability to hydrolyse chitin. A recent study in European and two sub-Saharan populations suggested a relationship between the presence of the mutation, improved environmental conditions, and the disappearance of parasitic diseases, including *Plasmodium falciparum* malaria. This result was not supported by the high frequency of the 24 bp duplication in a sample from Taiwan, an area with high malaria endemicity until 40 years ago. In this study, we analysed the frequency variability of the H allele in Mediterranean populations and its internal variability in Sardinia (Italy) with respect to malaria, which had been endemic on the island until its eradication during 1946–1950. The pattern of H frequency distributions is not consistent with the hypothesis of selective pressures acting on *CHIT1* gene. The Moran's index coefficient and correlogram seem to indicate, indeed, that allele distribution was determined by random factors. The pattern of frequency distribution suggests a possible Asiatic origin of the H allele, but it could be possible also that the mutant allele had diffused out of Africa, and was subsequently lost from African populations.

Keywords *CHIT1* · H allele · Spatial autocorrelation · Malaria · Mediterranean populations

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Introduction

Human chitotriosidase is an enzyme synthesized by activated macrophages and has the capability to hydrolyse chitin, a structural component present in the coatings of many living species, including fungi, parasitic nematodes, and insects. Chitotriosidase is secreted mainly as an active 50 kDa enzyme containing a C-terminal chitin-binding domain. It is proteolytically processed to a C-terminally truncated 39 kDa isoform characterized by hydrolase activity, which accumulates in lysosomes (Renkema et al. 1997). The 50 kDa form is synthesized by neutrophilic granulocyte progenitors and stored in their granules (Boot et al. 1995). It is considered as a component of the innate immunity that may play a role in defence against chitin-containing pathogens. Chitotriosidase exerts activity towards chitin-containing pathogens, such as *C. neoformans*, *M. rouxi* and *C. albicans* both in vitro and in vivo (van Eijk et al. 2005; Boot et al. 2001). Additional evidence of a role for chitotriosidase during immunological responses is the observation that the enzyme is shortly and acutely up-regulated, both at the level of RNA and

in activity following stimulation with prolactin, IFN-γ, TNFα, LPS, and IL-4 (Di Rosa et al. 2005; Malaguarnera et al. 2004; van Eijk et al. 2005). Moreover, *CHIT* activity has been used in the screening of lysosomal storage diseases (Grosso et al. 2004) and in the monitoring of the treatment of Gaucher disease (Cabrera-Salazar et al. 2004).

The 24 bp duplication has been associated with susceptibility to infection by *Wuchereria bancrofti* in south India (Choi et al. 2001) but not in Papua New Guinea (Hise et al. 2003). The discovery of the existence of a second chitinase, named acidic mammalian chitinase (AMCase), in humans, has opened the possibility that a deficiency in chitotriosidase might be partly compensated for by the presence of the latter enzyme (Boot et al. 2001).

A significant increase in plasma chitotriosidase levels, with respect to healthy subjects, in African children infected with acute *Plasmodium falciparum* malaria has been reported. Moreover, chitotriosidase levels are higher in healthy African samples than in those of Caucasians (Barone et al. 2003). Increased levels of plasma chitotriosidase have also been reported in patients with Gaucher disease (Hollak et al. 1994), in various lysosomal storage disorders, in several haematological and infectious diseases involving activated macrophages (Guo et al. 1995; Den Tandt and Van Hoof 1996), and in patients with β-thalassaemia (Altarescu et al. 2002; Barone et al. 1999, 2002, 2001).

The *CHIT1* gene is located on chromosome 1q31–32 and consists of 12 exons, spanning approximately 20 kb (Boot et al. 1998). A 24 bp duplication in exon 10 of the gene causes the deletion of amino acids 344–372, resulting in a deficiency in enzyme activity. The enzyme is totally inactive in individuals homozygous for the duplication (Boot et al. 1998; Canudas et al. 2001). This mutation is not found in anthropomorphic primates, suggesting that it originated during human evolution (Gianfrancesco and Musumeci 2004).

A recent study (Malaguarnera et al. 2003) of the 24 bp duplication in the *CHIT1* gene in some European populations suggested a relationship between the presence of the mutation, improved environmental conditions, and the disappearance of parasitic diseases, including *P. falciparum* malaria. Furthermore, widespread parasitic diseases and the poor social status of the area may have contributed to the maintenance of the wild-type (wt) *CHIT1* gene in sub-Saharan populations (Malaguarnera et al. 2003). This result was not supported by the recent study by Chien et al. (2005), in which a frequency of 58% was reported for the 24 bp duplication, together with high frequencies of both thalassaemia and glucose 6-phosphate dehydrogenase (G6PD) deficiency, in a sample of Chinese Han individuals from Taiwan, an island characterized by high malaria endemicity until 40 years ago (Lin et al. 1991).

In the present study the *CHIT1* gene distribution in eight Mediterranean and European populations was analysed with the aim of increasing the knowledge of the frequency distribution of the *CHIT* gene from a population point of view, since six of the populations under scrutiny have not been examined for this marker until now.

The relationship between *CHIT1* and malaria was particularly investigated in the island of Sardinia, where malaria was endemic until its eradication during 1946–1950 (Logan 1957).

The aim of this study was to increase our knowledge of the distribution of the *CHIT1* alleles in the Mediterranean area and to interpret its variability.

Materials and methods

Samples from 1,104 individuals from continental Italy (n = 99), Sardinia (Italy) (n = 335), Spain (n = 103), Basque country (n = 60), continental France (n = 128), Corsica (France) (n = 194), Turkey (n = 95), and Morocco (n = 90) were genotyped. The samples were from unrelated individuals of both genders, born and resident in their countries of origin, as their relatives had been for at least three generations. Basque, Corsican, and Sardinian samples were analysed separately from Spanish, French, and Italian samples, because of their genetic peculiarities (Calafell and Bertranpetit 1994; Varesi et al. 2000; Vona 1997).

The Sardinian samples, taken from several historical–geographical areas of the island (Sulcis, Trexenta, Campidano of Oristano, Nuorese, and Gallura), were subdivided into three altimetric zones, characterized by different past malarial endemy (Fermi 1934, 1938; Brown 1981).

The protocols and procedures used in this research were undertaken in compliance with the declaration of Helsinki. DNA was extracted with the standard phenol–chloroform technique, and polymerase chain reaction (PCR) was performed using the primers described by Hise et al. (2003) under the following conditions: 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, with a final extension of 5 min at 72°C. The two fragments of 99 bp and 75 bp were separated on 3% agarose gel stained with ethidium bromide.

Allele frequencies were determined by direct gene counting. Hardy–Weinberg equilibrium was tested with GENEPOP version 3.4 software (Raymond and Rousset 1995). Genic and genotypic differentiation

among samples was tested with the chi-square test. We also tested the associations between allele frequencies, latitude, and longitude with the Pearson's correlation. The analysis was performed with SPSS version 8.0 software.

The effects of natural selection on the *CHIT1* gene were evaluated with the Ewens–Watterson neutrality test (Ewens 1972; Watterson 1978), implemented by Slatkin (1994), and was carried out using Pypop software (version 0.6.0) (Lancaster et al. 2003).

To test the spatial distribution of allele frequencies, we estimated Moran's index (*I*), a product–moment correlation coefficient (Cliff and Ord 1973), and one-dimensional correlograms (Sokal and Oden 1978; Sokal et al. 1989), using SAAP version 4.3 software (Wartenberg 1989). The plot of autocorrelation coefficient *I* against distance is referred to as a correlogram, the overall significance of which was assessed with the Bonferroni test. To infer evolutionary patterns from correlograms, we applied the classification suggested by Barbujani (2000).

Results

Genotype and allele frequencies of the studied Mediterranean populations are shown in Table 1. All samples are in Hardy–Weinberg equilibrium. The frequencies of the H allele ranged from 10.5% (Morocco) to 25.0% (France). The genotype frequencies (Table 1) indicated an absence of H/H homozygotes in two of the populations studied (Basque and Morocco). In the other populations, the frequencies of H/H homozygotes ranged from 1% (Corsica) to 9.3% (continental France). The frequencies of wt/H heterozygotes ranged from 20.0% (Morocco) to 34.3% (continental Italy). The chi-square test showed a high variability in the *CHIT1* allele distribution among the eight populations ($P = 0.001$) and in pairwise comparisons. The French sample had the greatest number of significant pairwise comparisons, with Basque country, Corsica, Sardinia, and Morocco, followed by the Spanish sample with Basque country, Corsica, Morocco and Morocco with Italy, France and Spain.

The frequencies of the H allele in these populations were very different from those in both the sub-Saharan African populations, characterized by an absence of the 24 bp duplication, and the Asiatic population, characterized by high frequencies of the duplication (58% in Taiwan island) (Chien et al. 2005) (Table 2).

To analyse the geographic differentiation of *CHIT1* within the Mediterranean area, we performed a spatial autocorrelation analysis with all our samples and samples from Portugal (Rodrigues et al. 2004), Sicily (Malaguarnera et al. 2003), and Israeli (Boot et al. 1998) (Table 2). The resulting correlogram (not shown) indicated a random spatial distribution of variation ($P = 0.173$) with a lack of clinal variation and with no significant Moran's *I* coefficients for any distance classes. Pearson's correlation did not suggest any association between *CHIT1* and either longitude or latitude ($P_{long} = 0.356$; $P_{lat} = 0.900$).

When we considered all the 18 populations of Table 2 (Boot et al. 1998; Malaguarnera et al. 2003;

Table 1 Genotype and allele frequencies for the *CHIT1* locus in the populations studied and *P* values of the chi-square test. Significant values are in italics

Populations	Number	Genotype frequencies			Allele frequencies		Hardy–Weinberg
		wt/wt	wt/H	H/H	wt	H	*P* value
Morocco(1)	90	75.6	20.0	0.0	81.0	10.5	0.589
Spain(2)	103	61.2	32.0	6.8	77.2	22.8	0.399
Basque country(3)	60	76.7	23.3	0.0	88.3	11.7	1.000
Continental France(4)	128	59.4	31.3	9.3	75.0	25.0	0.061
Sardinia(5)	340	68.6	27.9	3.5	82.5	17.5	0.572
Corsica(6)	194	74.8	24.2	1.0	86.9	13.1	0.540
Continental Italy(7)	99	63.7	34.3	2.0	80.8	19.2	0.514
Turkey(8)	95	66.3	29.5	4.2	81.1	18.9	0.731
	1	2	3	4	5	6	7
2	*0.008*	–	–	–	–	–	–
3	0.730	*0.040*	–	–	–	–	–
4	*0.002*	0.778	*0.015*	–	–	–	–
5	0.068	0.216	0.223	*0.021*	–	–	–
6	0.433	*0.005*	0.720	*0.001*	0.119	–	–
7	*0.024*	0.252	0.174	0.071	0.413	0.149	–
8	0.055	0.636	0.164	0.286	0.901	0.110	0.569

Table 2 Allele frequencies of *CHIT1* in populations from different continents

Populations	Number	Allele frequencies		References
		wt	H	
Morocco	90	89.5	10.5	Present study
Benin	100	100.0	0.0	Malaguarnera et al. (2003)
Burkina Faso	99	98.0	2.0	Malaguarnera et al. (2003)
Portugal	295	79.9	21.0	Rodrigues et al. (2004)
Spain	103	77.2	22.8	Present study
Basque country	60	88.3	11.7	Present study
Continental France	128	75.0	25.0	Present study
Netherlands	171	76.0	24.0	Boot et al. (1998)
Sardinia	340	82.5	17.5	Present study
Corsica	194	86.9	13.1	Present study
Continental Italy	99	80.8	19.2	Present study
Sicily	100	73.0	27.0	Malaguarnera et al. (2003)
Finland	50	80.0	20.0	Choi et al. (2005)
Turkey	95	81.1	18.9	Present study
Israel	68	77.0	23.0	Boot et al. (1998)
South India	67	56.0	44.0	Choi et al. (2001)
Taiwan	82	42.0	58.0	Chien et al. (2005)
Papua New Guinea	906	88.0	12.0	Hise et al. (2003)

Choi et al. 2005, 2001; Hise et al. 2003), we obtained a significant P value of Pearson's coefficient for longitude ($P = 0.038$) but not for latitude ($P = 0.992$).

A spatial autocorrelation analysis was performed using the frequencies in Table 2. A correlogram of Moran's index for seven distance classes showed positive but not significant values until the 6,500 km class, beyond which Moran's index values became negative and significant (Fig. 1). Such a pattern is partly similar to that for long-distance differentiation (LDD) (Barbujani 2000). In fact, LDD is characterized by positive and significant values in the smallest distance classes and by negative and significant values in the largest distance classes. The pattern is marginally significant with the Bonferroni test ($P = 0.039$), and this may be the result of the discontinuous distribution of the population samples.

The hypothesis of selective neutrality on *CHIT1* gene was tested with the Ewens–Watterson neutrality test (Ewens 1972; Watterson 1978; Slatkin 1994). Results suggest that all the populations do not significantly shift from the infinite allele model.

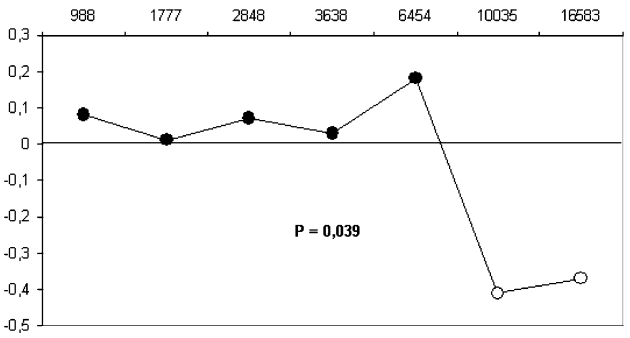

Fig. 1 Patterns of spatial autocorrelation analysis observed for the *CHIT1* alleles in the populations given in Table 2

A particular analysis was performed for the island of Sardinia, which was characterized until 1945 by endemic malaria. In the Sardinian population, the average frequency of the H allele was 17.5%, whereas the genotype frequencies were 3.5, 27.9, and 68.6 for H/H, wt/H, and wt/wt, respectively (Table 1). A comparison of our results with previous data for the north Sardinian population (Malaguarnera et al. 2003) showed no significant difference ($\chi^2 = 0.738$; $df = 1$; $P = 0.390$).

The Sardinian sample was subdivided according to the municipality of origin, into three different altimetric zones: 0–200 m, 201–400 m, and >400 m. The genotype and allele frequencies are summarized in Table 3. The frequency of the H allele decreases with altitude from 24.6% to 11.4%, as do the H/H and wt/H genotypes (from 6.3% to 0.7% and from 36.7% to 21.3%, respectively). The frequency of the wt/wt homozygote increases with altitude, ranging from 57.0% to 77.9%. The chi-square test showed a significant degree of global differentiation among the altimetric zones ($P = 0.0037$), particularly at 0–200 m and >400 m ($P < 0.001$).

We recalculated the frequencies quoted by Sanna et al. (1997) for G6PD deficiency in 103 municipalities and by Siniscalco et al. (1966) for thalassaemia in 52 municipalities, grouping the data in the altimetric zones used in our study (Table 4). The allele frequencies for G6PD deficiency decrease regularly with increasing altitude, and thalassaemia frequencies are lower at altitudes above 400 m, whereas they are

Table 3 Genotype and allele frequencies in altimetric zones of Sardinia together with *P* values of the chi-square test. Significant values are in italics

Altitude	Number	Genotype frequencies			Allele frequencies		Hardy–Weinberg
		wt/wt	wt/H	H/H	wt	*H*	*P* value
0–200 m	128	57.0	36.7	6.3	75.4	24.6	1.000
201–400 m	71	69.0	26.8	4.2	82.4	17.6	0.435
>400m	136	77.9	21.3	0.7	88.6	11.4	1.000

Pairwise comparison: 0–200 m and 201–400 m, $P = 0.250$

0–200 m and >200 m, $P < 0.001$

201–400 m and >400 m, $P = 0.133$

Table 4 Allele frequencies for *CHIT1*, G6PD deficiency and Thalassaemia in altimetric zones of Sardinia

Altimetric zones (m)	Allele frequencies		
	CHIT1 (wt)	G6PD deficiency	Thalassaemia
0–200	75.4	22.9	22.9
201–400	82.4	18.6	23.7
>400	88.6	8.7	14.4

similar in the other two altimetric zones. A χ^2 test showed significant differences among altitude zones for both traits. The wt/H and H/H genotype frequencies for the *CHIT1* locus and the frequency of the H allele decrease with altitude, as do the frequencies of the thalassaemia and G6PD deficiency alleles. The contrary trend was followed by wt/wt genotype and wt allele frequencies.

Discussion

A recent study of African and Mediterranean populations (Malaguarnera et al. 2003) suggested that subjects carrying the mutant H allele at the *CHIT1* locus have increased susceptibility to parasitic diseases. The persistence of parasitic diseases in sub-Saharan Africa would be favoured by the maintenance of the wild-type allele, as demonstrated by the low incidence of heterozygotes wt/H and by the absence of homozygotes H/H in these regions. Therefore, Malaguarnera et al. (2003) suggested that low frequencies of the H allele represent a protective factor in populations living under environmental conditions favourable to parasitic diseases, such as malaria, since the individuals bearing the H allele might exhibit elevated susceptibility to infective diseases.

Our analysis of samples from the island of Sardinia does not confirm the suggestion made by these authors. In fact, the highest frequency of the H allele appeared in the lowlands, where the incidence of malaria was highest. On the other hand, we found the highest frequency of the wt allele at altitudes over 400 m, in villages with the lowest or negligible endemic malaria (Fermi 1934, 1938). In Sardinia, the frequency pattern of the wt allele varies with altitude in a direction opposite to that of G6PD deficiency (Gd^{Med}) and thalassaemia (Th), which are correlated with malaria (Siniscalco et al. 1966). Analysis of sample $\beta^0 39$ carriers from Sardinia and Corsica does not show significant differences for genotype and allele frequencies with respect to healthy individuals (Piras et al. 2006). Our results seem to confirm the data reported by Chien et al. (2005) for Taiwan, where the frequency of the H allele is very high (58%), even though malaria was eradicated only 40 years ago, and those reported by Choi et al. (2001) for south India, where malaria is still present, and the frequency of the mutant allele is 44%.

The distribution of the *CHIT1* gene was also analysed in eight Mediterranean populations. The results highlight significant heterogeneity among the populations studied. Moreover, the frequencies appear remarkably different from those of African and Asian populations. The variability of the *CHIT1* gene frequencies in the Mediterranean area does not appear to be linked to its spatial distribution. In fact, the correlation of gene frequencies with latitude and longitude is not statistically significant, and the Moran index and Bonferroni's test for the correlogram support this result. The correlogram is evidence for the random distribution of the *CHIT1* allele frequencies in these Mediterranean populations. The spatial distribution of *CHIT1* allele frequencies at the microgeographic and macrogeographic levels does not provide evidence of spatial patterns that can be interpreted as selection effects, which in many populations have produced the high H allele frequencies associated with thalassaemia, haemoglobin variants, and G6PD deficiency. The Moran's index coefficients and correlogram suggest that these allele distributions were determined by random factors.

This result is confirmed by the Ewens–Watterson neutrality test, which suggests the absence of natural selection on the *CHIT1* gene.

This analysis of populations in the Mediterranean region does not support the notion of a progressive variation in the *CHIT1* allele frequencies, as suggested by Malaguarnera et al. (2003). It is unlikely that the frequencies found in some Mediterranean populations are linked to the disappearance of malaria. This parasitic disease has recently been eradicated in some areas, little more than two generations ago (Hay et al. 2004), which is too limited a period of time to cause allele differences among the populations studied.

These results seem to suggest that the H allele originated among East Asian populations, where the highest frequencies are observed, and then spread to the West. The absence of this allele in sub-Saharan Africa seems to confirm this hypothesis, but another scenario is possible. The mutant allele (if considered neutral with respect to natural selection) could have diffused out of Africa and been subsequently lost from African populations due to genetic drift, while its frequency increased in Europe and Asia.

However, for a definitive conclusion to be drawn, it would be necessary to clarify the roles of the H allele and the selective pressures acting on *CHIT1* or on flanking genes. The extent of linkage disequilibrium in this genic region should be investigated. The collection of data about other Asiatic populations, for which there is an overwhelming lack of information from Turkey to India, may clarify the significance of the correlation between the allele frequencies and longitude observed for all populations, and between Moran's index and distance classes over 6,500 km.

Moreover, a larger number of populations sampled would help to verify the validity of the hypothesis that the H allele originated in Asia and the effects of natural selection or genetic drift, with a stronger spatial autocorrelation analysis.

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