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ASSOCIATION OF THREE POLYMORPHISMS IN RP1 HOTSPOT REGION IN SICILIAN PATIENTS WITH RETINITIS PIGMENTOSA: PRELIMINARY DATA.

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ABSTRACT

Rheumatoid Retinitis pigmentosa (RP) is the most common inherited retinal dystrophy affecting peripheral visual field and usually culminates in complete blindness. Among mutations in 73 genes implicated in RP pathogenesis, those in *RP1* gene are inherited in autosomal recessive or dominant fashion. In RP Sicilian patients, we detected 3 polymorphisms in *RP1* exon 4 hotspot region, not known to be associated with RP disease. Here, their frequency in Sicilian populations was assayed in order to detect possible association with RP. Samples from 220 unrelated healthy donors born and living in Sicily for at least two generations and 50 RP patients from Messina were screened. Frequencies of all three polymorphisms in RP patients from Messina were about twice those observed in the healthy controls from the same town and the overall Sicilian population. An association between 3 polymorphisms and RP was suggested, although their role in other related disorders cannot be excluded.

1. Introduction

Retinitis pigmentosa (RP) is a heterogeneous inherited ocular disease that results in a progressive retinal degeneration [1]. It is an uncommon condition affecting about 1 in 4,000 people in the United States [2] and 1-5 in 10,000 in Italy. The term "pigmentosa" deals with the characteristic appearance of abnormal areas of pigment into the retina, during the advanced states of the disease. Degeneration involves both eyes and affects retinal pigment epithelium [3] and the photoreceptors, inducing a slow and progressive death in these cells, leading to loss of ability to transmit the visual information to the brain. Oxidative stress has been shown to be a key contributor to the progression of RP and lack of the antioxidative proteins may influence the oxidative balance in retina as well as in other tissues [4,5]. Furthermore, age-related decrease in the expression of antioxidative genes in retina, generally regulated by estrogen, was observed [4,6]. A most frequent symptom of RP is a decrease of crepuscular nocturnal visual acuity that progresses in a narrowing of peripheral visual field and, in some cases, loss of central vision and blindness in the final stage of the disease. Retinitis pigmentosa can also be accompanied by cataract or deafness [7]. The rate of progression of the disease and the age of onset of the first symptoms vary according to many factors, including the pattern of genetic transmission [8,9].

More than fifty genes are known to be associated to this disease [10]. Among these, *RP1*, initially designated *ORP1* for 'oxygen-regulated protein-1' and subsequently designated *RP1* when it was found to be mutated in autosomal dominant RP (adRP). The encoded protein by *RP1* is involved in the vesicular trafficking of the connecting cilium [11], but the exact role in photoreceptor biology is still unknown.

Mutations in this gene, inherited in an autosomal recessive (50-60%) or dominant (30-40%) pattern [12-17], are predominantly non-sense mutations leading to elsewhere reported nonsense-mediated mRNA decay (NMD) [18,19].

In 13 RP affected individuals from Messina (Sicily), we detected three polymorphisms in RP1 exon 4: c.5008 G>A p. Ala1670Thr (rs446227), c.5071 T>C p. Ser1691Pro (rs414352) and c.5175 A>G p. Gln1725= (rs441800). The three polymorphisms fall in a hotspot region of gene [20]. While some information is available about the world-wide distribution of those polymorphisms, there is no evidence in the literature about their frequency in the Italian population. Therefore, in this study we aimed to assess the frequency of the three polymorphisms in Sicilian populations and to verify their possible association with RP. Sicily presents a relevant population heterogeneity, due to a long history of invasions and settlements by Neolithic farmers from Anatolia and Italic people [21] as well as by Phoenicians, Greeks, Romans, Byzantines, Arabs and Normans [22].

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2. Methods

Samples

We collected and analyzed samples from 220 unrelated healthy donors born and living in Sicily (n = 220) for at least two generations, constituting a heterogeneous group for age and sex. In detail, 35 samples were recruited from each of the following locations: Agrigento, Siracusa, Sperlinga and Pantelleria Island, while 80 from Messina. Likewise, we collected samples from 50 patients affected by RP. All patients are from Messina and surrounding areas. The RP patients underwent full ophthalmological examination, consisting of fundus analysis, autofluorescence (FAF), Optical Coherence Tomography (OCT), visual field (VF), ISCEV Electroretinography (ERG), pattern electroretinogram (PERG), flash electroretinogram (FERG), pattern evoked potential (PEP) and flash evoked potential (FEP), whose response confirms the typical clinical picture of retinitis pigmentosa. This study was approved by the Ethics Committee of "Azienda Policlinico Universitario of Messina" and conformed to the tenets of the Declaration of Helsinki. All subjects had given written informed consent prior to participation in the study.

Genotyping

Peripheral blood samples were obtained and genomic DNA was isolated from white blood cells using standard methods. The RP1 region of interest was amplified using one set of 9 overlapping primers designed according to RP1 exon 4 published nucleotide sequence of GenBank (accession no. AF152242.1). PCR protocol and primer sequences are available upon request. The variants examined are reported in the SNP database http://www.ncb.nlm.org.

Sequencing

Mutation screening was performed by direct nucleotide sequence analysis by the dideoxynucleotide method with the BigDye Terminator v1.1 Cycle Sequencing kit on the 310 ABI PRISM Sequencer Analyzer (Applied Biosystems, Foster City, CA).

Statistical analysis

For each population, allelic frequencies were calculated using direct gene counting. Deviations from Hardy-Weinberg equilibrium were tested using the chi-squared test on a 2 X 3 contingency table with 1 degree of freedom. Finally, a case – control association study, using chi-squared test, was performed for Messina population. Data were analyzed using the SPSS statistical software, version 24.

3. Results and Discussion

Table S1 shows the frequencies of rs446227, rs414352 and rs441800 polymorphisms in several populations around the world. Unlike polymorphisms in genes linked to other diseases [23,24], there are no data in the literature concerning the three polymorphisms in Italian populations. Therefore, in this study, we determined the genotype and allelic frequencies of these polymorphisms in 220 unrelated healthy individuals from the Mediterranean biggest island, living in 5 different geographic locations. A detailed breakdown of the allelic frequencies distribution for each studied is shown in Table 1 and Figure 1. The frequency of rs446227 mutated allele is heterogeneous and less than 0.50 across the different population living in Sicily (0.211), with a range from 0.171 (Pantelleria) to 0.300 (Siracusa). The situation is very similar for rs414352, in which we can see a value of 0.239 for the whole Sicily, with the lowest and highest peaks, respectively, in Messina (0.200) and Siracusa (0.300). The third examined polymorphism, the rs441800, also shows a similar trend, with the highest value of Siracusa (0.300) in contrast with the lowest one of Messina (0.137), while the whole Sicily shows a mutated allele frequency of 0.193. Our data clearly indicate a remarkable degree of fluctuation, with the same trend for all three polymorphisms. A distribution pattern is discernible, starting with the lowest values of Pantelleria (rs446227 = 0.171, rs414352 = 0.214, and rs441800 = 0.157) and Agrigento (rs446227 = 0.186, rs414352 = 0.257, and rs441800 = 0.186) to get to the highest values of Siracusa (rs446227 = 0.300, rs414352 = 0.300, and rs441800 = 0.300) and Sperlinga (rs446227 = 0.257, rs414352 = 0.271, and rs441800 = 0.257). Messina (rs446227 = 0.181, rs414352 = 0.200, and rs441800 = 0.137) represents an exception to the vertical stratification observable along the distribution pattern depicted before. This trend can be explained by considering that Sicily, due to its geographical location, is a transition point in the Mediterranean basin. In particular, Messina could represent the junction between Sicily and the Italian peninsula, determining a reduction of mutated alleles frequencies due to migration flows, absent or low in the other analyzed cities [25]. In order to verify a possible association of those three polymorphisms with retinitis pigmentosa, we analyzed 50 RP affected individuals from Messina. The observed frequencies in these patients were more than twice (0.550±0.049 for all three polymorphisms) those observed in the healthy controls from Messina, as well as in the controls from the overall Sicilian population (Table 2). Finally, the case – control chi-squared test performed on Messina population highlighted a Pearson's chi –squared of 16.019 (p – value near 0) for all three variants, also evidenced by bar graphs of their genotypes distribution in patients and healthy donors (Figure 2).

	N°	rs446227 Genotype (n)			Allelic Frequency				rs414352 Genotype (n)			Allelic Frequency				rs441800 Genotype (n)			Allelic Frequency			
		+/+	+/-	-/-	+ allele freq	- allele freq	Dev st	HW (χ2)	+/+	+/-	-/-	+ allele freq	- allele freq	Dev st	HW (χ2)	+/+	+/-	-/-	+ allele freq	- allele freq	Dev st	HW (χ2)
SICILY	220	146	55	19	0.789	0.211	0.019	1.376.108	134	67	19	0.761	0.239	0.020	576.689	151	53	16	0.807	0.193	0.019	1.135.384
Messina	80	57	17	6	0.819	0.181	0.030	645.348	52	24	4	0.800	0.200	0.032	0.31250	61	16	3	0.863	0.137	0.028	196.653
Agrigento	35	25	7	3	0.814	0.186	0.046	401.586	21	10	4	0.743	0.257	0.052	222.505	25	7	3	0.814	0.186	0.046	401.586
Pantelleria	35	25	8	2	0.829	0.171	0.045	133.637	22	11	2	0.786	0.214	0.049	0.15555	26	7	2	0.843	0.157	0.043	210.074
Siracusa	35	18	13	4	0.700	0.300	0.055	0.46809	18	13	4	0.700	0.300	0.055	0.46809	18	13	4	0.700	0.300	0.055	0.46809
Sperlinga	35	21	10	4	0.743	0.257	0.052	222.505	21	9	5	0.729	0.271	0.053	428.370	21	10	4	0.743	0.257	0.052	222.505

Table 1 - Mutated (-) and wild-type (+) allelic frequencies of rs446227, rs414352 and rs441800 polymorphisms at 5 locations in Sicily. Deviation from Hardy–Weinberg equilibrium (HWE) of genotypic frequencies was determined using the χ2 test.

	N°	rs446227 Genotypes			Allele Frequency				rs414352 Genotypes			Allele Frequency				rs441800 Genotypes			Allele Frequency			
		+/+	+/-	-/-	+ allele freq	- allele freq	Dev st	HW (χ2)	+/+	+/-	-/-	+ allele freq	- allele freq	Dev st	HW (χ2)	+/+	+/-	-/-	+ allele freq	- allele freq	Dev st	HW (χ2)
AFFECTED GROUP	50	19	17	14	0.55	0.45	0.05	4.90	19	17	14	0.55	0.45	0.05	4.90	19	17	14	0.55	0.45	0.05	4.90

Table 2 - Genotype and Allelic frequencies of three examined RP1 SNPs in affected group of 50 patients.

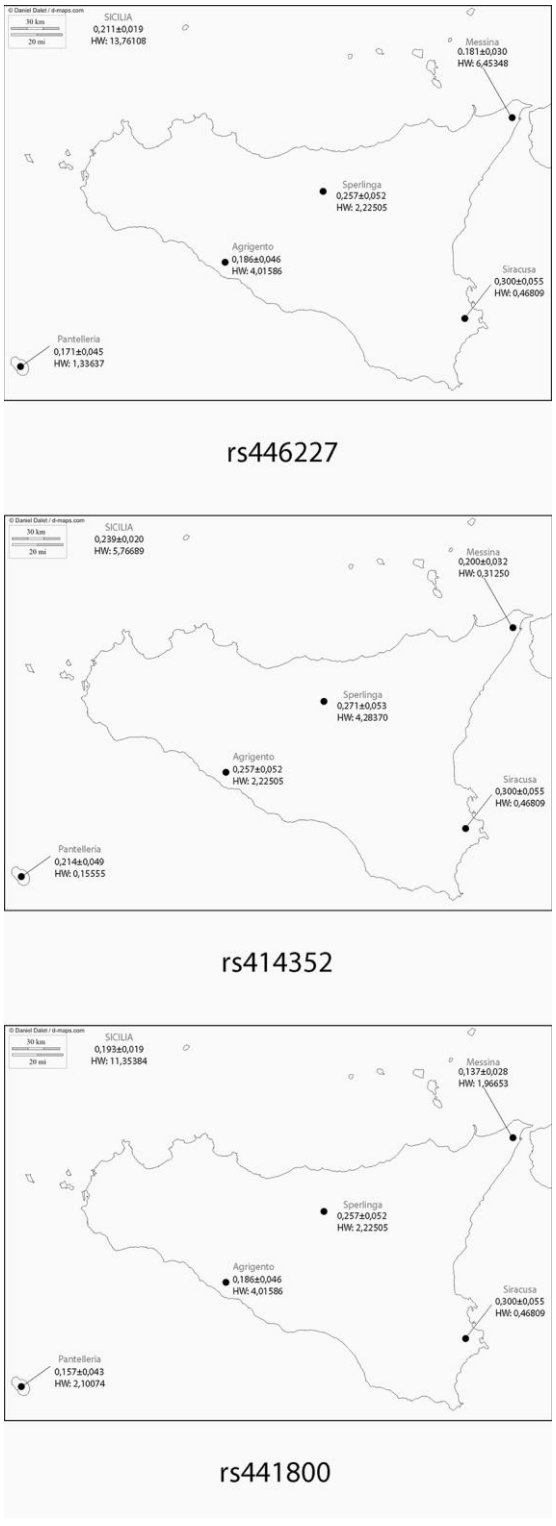

Figure 1 - Map of Sicily. Allelic frequencies and standard deviations of the three examined RP1 polymorphisms are shown together with the geographic locations of each town.

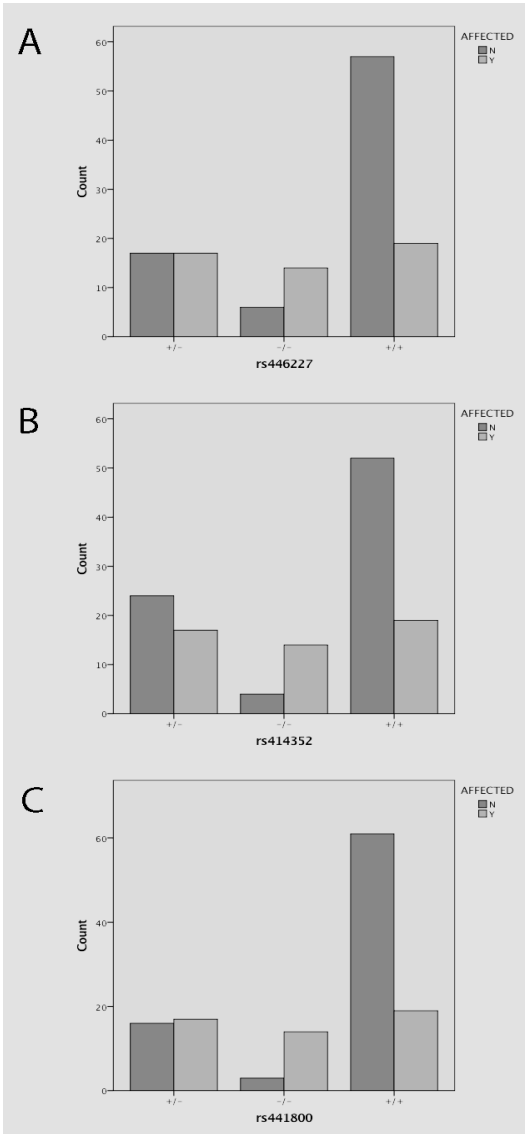

Figure 2 - Bar graph of 3 variants genotypes distribution in case/control study of Messina samples. A) rs446227 B) rs414352 C) rs441800. N = Controls, Y= Cases.

4. Conclusions

Our data suggest a possible association of RP1 rs446227, rs414352 and rs441800 polymorphisms with RP in Messina's population, and probably in Sicilian population too, although we cannot exclude their role in other related disorders. However, further studies in different populations are necessary to confirm our conclusion. Diagnosis of RP and its different forms is usually difficult to obtain. Genetic testing plays a fundamental role, especially in uncertain cases, acting as a support for clinical instrumental investigation.

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