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ASSOCIATION BETWEEN THREE POLYMORPHISMS IN RP1 HOTSPOT REGION AND RISK OF RETINITIS PIGMENTOSA IN ITALIAN PATIENTS: A PILOT STUDY

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ABSTRACT

Retinitis pigmentosa (RP) represents a heterogeneous inherited ocular disorder characterized by progressive retinal degeneration. Individuals affected by RP show night blindness, tunnel vision and progressive visual field reduction which usually culminates in complete blindness. Histologically, accumulation of lipofuscin granules represents the most frequent sign. Today, more than 80 genes are associated with RP, and among them *RP1* is one of the most frequently mutated. Variants in this gene may be inherited as autosomal recessive, dominant, X-linked or sporadic patterns. In Italian individuals affected by RP we detected three polymorphisms within *RP1* exon 4 (rs446227, rs414352, rs441800), falling in its exon 4 hotspot polymorphic region, and without a certain association with RP disease. Therefore, we studied the frequencies of the previously cited polymorphisms in the Sardinian population and verified a possible association with RP. The analyses showed a significant association, although we cannot exclude the role of the three polymorphisms in other related disorders.

1. Introduction

Retinitis pigmentosa (RP) is a rare genetic disease involving the retina; the back portion of the eye. The retina is photosensitive and its role is to focus light signals first towards the optical nerve and then towards brain, after their transduction into electrical stimuli [1]. RP is an uncommon condition affecting about 1 in 4,000 people in the United States, and 1-5/10.000 in Italy [2]. The term "pigmentosa" refers to the typical presence of abnormal areas of lipofuscin pigment in the retina during advanced states of the disease. Degeneration affects photoreceptors, in particular rods during the first stages of pathology, and retinal pigment epithelium, inducing a slow and progressive death in these cells [3]. This scenario leads to loss of ability to transmit visual information to the brain. Symptomatology consists of night blindness (decrease of crepuscular nocturnal visual acuity), decline of peripheral visual field (e.g. difficulty in perceiving objects placed laterally) and, in the final stage of the disease, in some cases, the loss of central vision and blindness [4]. Diagnosis consists of examination of the fundus and of the field of view, followed by visus, electroretinogram and fluorescein angiography [5].

RP progression rate and age of onset depend on numerous factors, the principle factor being genetic transmission pattern [6]. Today, more than 80 genes and several non – coding RNAs are associated to RP syndromic and non – syndromic forms [7, 8]. Such genes are involved in the canonical retinoid cycle in rods (twilight vision) and cones (daylight vision) [9], regulation of the phototransduction cascade, cargo trafficking to the periciliary membrane, signal transduction, oxidative stress response [10] and many other areas [11].

Mutations in these genes, predominantly non – sense, may be inherited in autosomal recessive, dominant, X-linked or sporadic pattern [12]. Additionally, regulative variants could also be involved in RP and the etiopathogenesis of other hereditary macular dystrophies [13], as already established for other rare pathologies like CCMs [14].

Among the genes known to cause RP , *RP1* is one of the most frequently mutated [15]. Initially named *ORP1* (oxygen-regulated protein-1) and subsequently renamed *RP1* when it was found to be mutated in autosomal dominant RP, is localized to chromosome 8q and consists of four exons encoding for a ~2,200 amino acid protein [16]. The RP1 protein localizes to the connecting cilia of both photoreceptor cells and is required for correct stacking of the outer segment disc [17].

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Variants in this gene are generally inherited as autosomal recessive (50-60%) or dominant (30-40%) pattern [18]. In 41 Italian RP affected individuals we detected three polymorphisms (SNPs) in *RP1* exon 4 "hot – spot" region [19]: c.5008 G>A p.A1670T (rs446227), c.5071 T>C p.S1691P (rs414352) and c.5175 A>G p.Gln1725 (rs441800). There is no data available with regard to their frequency in the Italian population, and little information exists about occurrence in the world population. Therefore, in this study we aimed to assess the frequency of these polymorphisms in the Sardinian population and to verify their possible association with RP. The choice of Sardinia is based on the assumption that an island population is considered a genetic isolate, which differs significantly from more promiscuous populations such as the Sicilian one [20], due to rather limited contacts with other Mediterranean communities during prehistoric and historical times [21].

2. Methods

Study Sample collection

We collected and analyzed samples from 210 unrelated healthy donors born and living in Sardinia 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: Arbus, Cabras, Guspini, San Gavino, Sant'Antioco, Villacidro. Subsequently, 41 Italian RP patients were recruited and underwent full ophthalmological examination. 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.

DNA extraction and 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 overlapping set of primers designed according to RP1 exon 4 published nucleotide sequence of GenBank (accession no. AF152242.1). Conditions and sequences of primer set are available upon request. Examined variants are reported in the dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/).

Sanger 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

SPSS statistical software version 25 (SPSS, Inc., Chicago, Illinois) was used for statistical analysis. For each population allelic frequencies were calculated using direct gene counting. Deviations from Hardy-Weinberg equilibrium were tested using the chi-square test on a 2 X 3 contingency table with 2 degrees of freedom. The significance level was set at $p < 0.05$.

3. Results

A detailed analysis of the distribution of allelic frequencies for each Sardinian subpopulation is indicated in Table 1 and Figure 1.

rs446227								
	N°	Genotype (n)			Allelic Frequency			
		+/+	+/-	-/-	*+ allele freq*	*- allele freq*	*Dev st*	*HW (χ2)*
SARDINIA	210	125	74	11	0.771	0.229	0.020	0.00012
San Gavino	35	22	10	3	0.771	0.229	0.050	126.103
Villacidro	35	20	12	3	0.743	0.257	0.052	0.36817
Guspini	35	14	19	2	0.671	0.329	0.056	185.701
Cabras	35	19	15	1	0.757	0.243	0.051	0.95717
Arbus	35	24	10	1	0.829	0.171	0.045	0.00115
Sant'Antioco	35	26	8	1	0.857	0.143	0.042	0.15555
rs414352								
	N°	Genotype (n)			Allelic Frequency			
		+/+	+/-	-/-	*+ allele freq*	*- allele freq*	*Dev st*	*HW (χ2)*
SARDINIA	210	112	86	12	0.739	0.261	0.021	0.73691
San Gavino	35	19	13	3	0.729	0.271	0.053	0.12975
Villacidro	35	17	13	5	0.671	0.329	0.056	0.87580
Guspini	35	13	21	1	0.671	0.329	0.056	453.227
Cabras	35	15	19	1	0.700	0.300	0.055	299.481
Arbus	35	23	11	1	0.814	0.186	0.046	0.05360
Sant'Antioco	35	25	9	1	0.843	0.157	0.043	0.02999
rs441800								
	N°	Genotype (n)			Allelic Frequency			
		+/+	+/-	-/-	*+ allele freq*	*- allele freq*	*Dev st*	*HW (χ2)*
SARDINIA	210	123	73	14	0.760	0.240	0.021	0.49168
San Gavino	35	14	13	8	0.586	0.414	0.059	192.713
Villacidro	35	21	11	3	0.757	0.243	0.051	0.73987
Guspini	35	19	16	0	0.771	0.229	0.050	307.270
Cabras	35	19	15	1	0.757	0.243	0.051	0.95717
Arbus	35	24	10	1	0.829	0.171	0.045	0.00115
Sant'Antioco	35	26	8	1	0.857	0.143	0.042	0.15555

Table 1. Mutated (-) and wild-type (+) allelic frequencies of rs446227, rs414352 and rs441800 polymorphisms at 6 locations in Sardinia. Deviation from Hardy–Weinberg equilibrium (HWE) of genotypic frequencies was determined using the χ2 test.

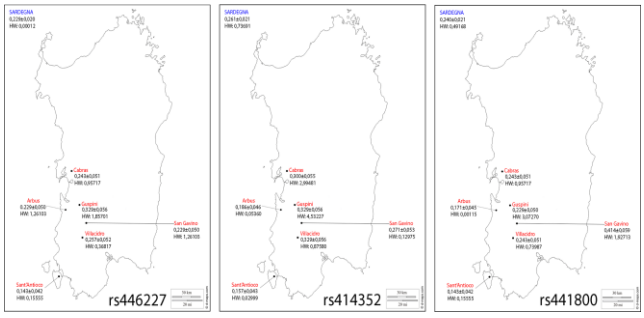


Figure 1. Map of Sardinia. The allelic frequencies of the three examined RP1 polymorphisms are shown together with the geographic locations of the individual towns. Each frequency value with standard deviations is located at the approximate geographic site from which the corresponding subjects originated.

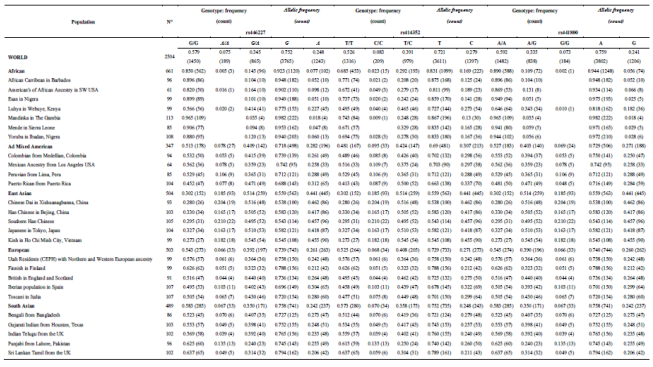
The frequency of rs446227 mutated allele was heterogeneous and less than 0.50 across the different Sardinian subpopulations (0.226), ranging from 0.171 (Arbus) to 0.329 (Guspini). A similar scenario is evident in rs414352, for which we could see a value of 0.261 for the whole island, with the lowest and highest peaks, respectively, in Sant'Antioco (0.157) and Villacidro and Guspini (both 0.329). The last analyzed polymorphism, the rs441800, revealed an analogous trend, with the highest frequency peak in San Gavino (0.414), while the mutated allele frequency for the whole of Sardinia was of 0.240.

Furthermore, the lowest values for all analyzed SNPs were seen in the Sant'Antioco subpopulation (rs446227 = 0.143, rs414352 = 0.157, and rs441800 = 0.143), and values increase through the regions in the following order: Arbus, San Gavino, Cabras, Villacidro, the highest values were found in Guspini for the first two polymorphisms, (rs446227=0.329, rs414352=0.329) and in San Gavino for the last one (rs441800=0.414), without any distinct pattern (Figure 1).

4. Discussion

No statistics exist on rs446227, rs414352 and rs441800 polymorphisms in the Italian population, and only a few data about their allelic frequencies in the world's population is available (Supplementary Table 1). In this work, we determined genotypic and allelic frequencies of three previously cited polymorphisms in 210 unrelated healthy individuals living in 6 different geographic locations on the Mediterranean's second biggest island, Sardinia.

The trend revealed through the data could be explained by considering that the Sardinian population, unlike the Mediterranean basin transition point represented by the Sicilian one, has remained unchanged for about 2,000 years because the island never witnessed permanent settlements [21]. Therefore, when tested, the population of Sardinia is consistent with the Hardy–Weinberg equilibrium.



Supplementary Table 1. Mutated allelic frequencies of RP1 polymorphisms from world's populations.

Finally, we wanted to verify the possible association of three selected polymorphisms with Retinitis pigmentosa although we do not have clinical data for the Sardinian population. We investigated the mutated allelic frequencies of the three SNPs in 41 Italian individuals affected with RP. The observed frequencies in these patients (Table 2) were about twice (fr=0.549±0.055 for all polymorphisms) those observed in the Sardinian control population. Finally, the case – control chi-squared test performed showed a Pearson's chi-squared of 35.347 (p – value ~ 0) for all 3 SNPs, also highlighted by bar graphs of their genotype distributions in affected members versus controls (Figure 2).

Concluding, we performed a small population screening of three *RP1* SNPs, in the Sardinian population. Then, assuming that such a population is a reliable representative sample of the Italian population because of its history, a case – control study was carried out. The results suggest a possible association of *RP1* rs446227, rs414352 and rs441800 polymorphisms with RP in the Italian population, although we cannot exclude their role in other related disorders.

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. Therefore, the genetic information obtained could be of noticeable importance in the future of RP treatment, as decisions could depend on genetic profiles, leading to personalized therapy.

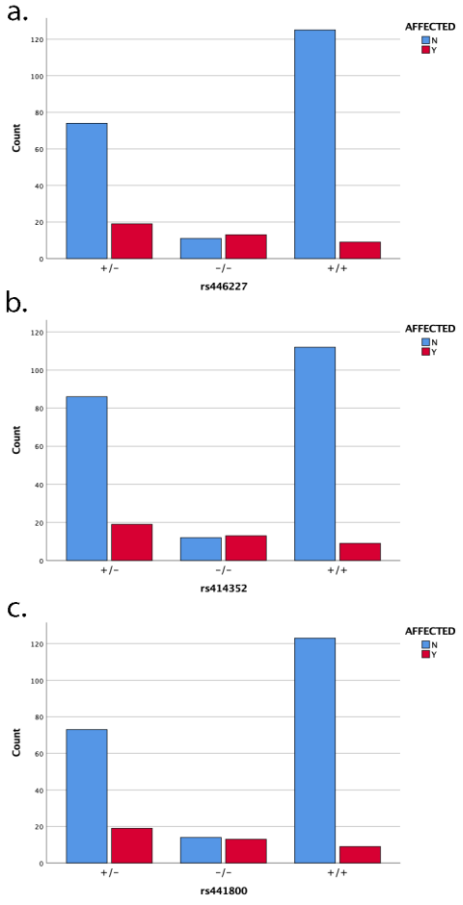


Figure 2. Bar graph of genotype distribution of the 3 analyzed SNPs' in case/control study. The most important resulting elements concern the homozygous condition of all SNPs, more prevalent in patients than in controls. a) rs446227; b) rs414352; c) rs441800. N = Controls, Y = Cases.

				rs446227			
	N°	Genotypes			Allele Frequency		
		+/+	+/-	-/-	+ allele freq	- allele freq	Dev st	HW (χ2)
AFFECTED GROUP	41	9	19	13	0.451	0.549	0.055	0.1693
				rs414352			
	N°	Genotypes			Allele Frequency		
		+/+	+/-	-/-	+ allele freq	- allele freq	Dev st	HW (χ2)
AFFECTED GROUP	41	9	19	13	0.451	0.549	0.055	0.1693
				rs441800			
	N°	Genotypes			Allele Frequency		
		+/+	+/-	-/-	+ allele freq	- allele freq	Dev st	HW (χ2)
AFFECTED GROUP	41	9	19	13	0.451	0.549	0.055	0.1693

Table 2. Genotype and Allelic frequencies of three examined RP1 SNPs in affected group of 41 Italian patients.

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