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The Genetic Makeup of Azoreans *Versus* Mainland Portugal Population

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1. Introduction

Since the first draft of the human genome we observed a boost in biomedical research. As consequence, nowadays, it is possible to know a person's predisposition to a genetic disease or even how its organism will metabolize a given drug. Although, there is some delay in translating this knowledge to the development and implementation of personalized medicine, there are currently available some successful pharmacogenetic based treatment decisions. One of such example is abacavir, a nucleoside analog reverse transcriptase inhibitor used in treatment of HIV-1 infection. Abacavir hypersensitivity is strongly associated with HLA-B*57:01 allele. Genetic testing before abacavir's prescription is now recommended in clinical guidelines and is practiced in most western countries (Chaponda & Pirmohamed 2011). In a near future, personalized medicine will, most certainly, bring considerable health gains to society.

The new approaches to analyze the human genome, – genome-wide association studies (GWAS; Orange et al., 2011), deep resequencing (1000 Genomes Project Consortium, 2010) and gene expression variability (Li et al., 2010) –, are producing massive data, which are already historic marks in the understanding of the genetic makeup of traits. A good example is the 9p21 genomic region association with coronary artery disease (McPherson et al., 2007; Helgadottir et al., 2007). However, only a small fraction of the heritable variation of complex diseases has been identified. One possible explanation may be that many rare variants, which are not included in the common genotyping platforms, may contribute substantially to the genetic variation of complex diseases. Therefore, researchers are becoming aware that common disease – common variant and common disease – rare variant models (Gorlov et al., 2011; Carvajal-Carmona, 2010; Zhu et al., 2011) will largely contribute to understand the genetic architecture of the populations with a diverse ancestry, such as the Azoreans. Hence, regional and local studies are good approaches to comprehend the genetic specificities of each population.

1.1 Mainland Portugal and Azores populations: historic and demographic data

Portugal, with a population of 10,637,713 inhabitants, is the most western country of the European continent and is bordered by the Atlantic Ocean to the west and south and by Spain to the north and east. The present Portuguese genetic landscape is the outcome of an

old and slow process of gene flow, admixture with many different populations and local differentiation. These include the expansion from isolated population nuclei in refuges following the Last Glacial Maximum, the movement of peoples related to the introduction of agriculture, and subsequent Roman and Germanic invaders, which may have influenced the distribution of genetic diversity in the territory (Amaral & Amaral, 1997). Roman settlers strongly influenced Portuguese culture, particularly the Portuguese language, which is derived from Latin. During the 8th century Muslim Moors occupied most of the Iberian Peninsula, contributing also to admixture events observed by the presence of north African paternal lineages in the Portuguese genetic background (Adams et al., 2008; Pereira et al., 2000a). With the establishment of the country in the year 1139, after several wars, Portugal is the oldest European nation-state. In the 15th and 16th centuries, as the result of maritime expeditions, the country established a global empire that included possessions in Africa, Asia, Oceania and South America, becoming the world's major economic, political and military power. Portugal's empire was the first and most long-lived global empire in the world, spanning almost six centuries (Russel-Wood, 1998; Jenkins & Sofos, 1996). During the Portuguese age of discovery, two archipelagos – Madeira and Azores (Figure 1) –, which are

Fig. 1. Portugal's regions map.

currently part of the country's territory, were discovered. The Azores is composed of nine volcanic islands unevenly distributed by three geographic groups: the Eastern group with two islands – São Miguel and Santa Maria –, the Central includes five islands – Terceira, Pico, Faial, São Jorge and Graciosa –, and the Western group with Flores and Corvo. This archipelago has a total area of 2332.74 km^2, unevenly distributed by the nine islands, varying from São Miguel, the largest, with 746.82 km^2 to Corvo, the smallest, with 17.13 km^2. The present-day population is composed of 241,763 inhabitants (National Institute of Statistics – Portugal, 2001 Census), derived from about 27 generations. The majority of the population lives on São Miguel (54.4%). The remainder is unevenly dispersed throughout

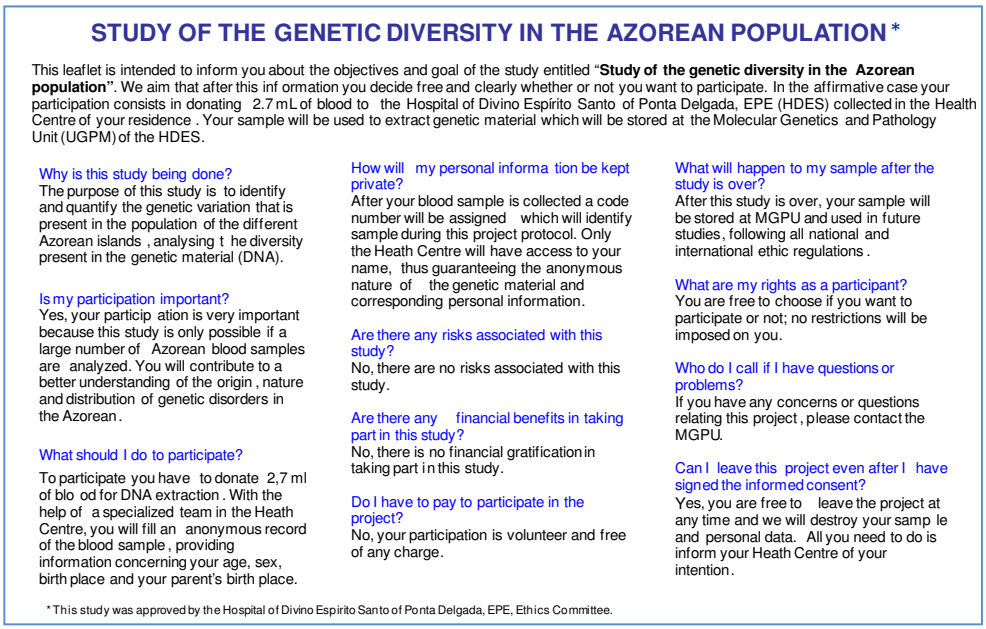
the other eight islands; for example, Corvo has only 425 individuals. From the total Azorean population, 41.4% are living in the Central group, where Terceira is the most populated of this group (24.9%; 55,833 inhabitants). The first settlers arrived in the mid 15th century and were mainly Portuguese, but the peopling was a slow and difficult process (Mendonça, 1996; Guill, 1993). Someone wrote "…The Azorean settlement was done with people from the interior of mainland Portugal, those who could not swim nor build boats, making impossible the abandonment of the islands…". Historical data report that the Portuguese crown was compelled to give out land and privileges in order to attract people to the islands (Guill, 1993). The first islands to be settled were Santa Maria and São Miguel in 1439 and the last were Flores and Corvo in the beginning of the 16th century. Some early settlers were of foreign origin, including Flemish, Jews, Moorish prisoners and black slaves from Guinea, Cape Verde and São Tomé. In the following centuries, contributions from Spanish, French, Italians, Germans and Scottish also occurred.

2. DNA banking of the healthy Azorean population

Biobanks have become an absolute requirement for biomedical research (Simon et al., 2007; Deplanque et al., 2009) and are defined as collections of samples of human bodily substances that are or can be associated with personal and clinical data. Depending on the purpose of a given biobank, both genetic and health information may be linked with the samples. The location of the Azorean population in the middle of the Atlantic, its geography, socio-cultural characteristics and, finally, the same environmental conditions, make a priori this population a good model to perform population genetic studies. Bering this in mind, the Molecular Genetics and Pathology Unit (UGPM, located at the main Azorean Hospital, Hospital of Divino Espírito Santo of Ponta Delgada, EPE - HDESPD) researchers adopted a strategy that began with the establishment of a DNA bank of the Azorean healthy population. Currently, it comprises a collection of 1558 representative genetic samples obtained from healthy non-family-related adult volunteers. The blood collection counted with the collaboration of the Department of Haematology of HDESPD for São Miguel samples, and municipalities Health Centres (Centro de Saúde de Vila do Porto, Centro de Saúde de Angra do Heroísmo, Centro de Saúde da Praia da Vitória, Centro de Saúde da Calheta, Centro de Saúde de Velas, Centro de Saúde de Santa Cruz da Graciosa, Centro de Saúde da Horta, Centro de Saúde das Lajes, Centro de Saúde da Madalena, Centro de Saúde de São Roque, Centro de Saúde de Santa Cruz das Flores) of volunteers resident in the other eight islands. As part of the research project entitled "Study of the genetic diversity in the Azorean population", the biobank was approved by the local Ethics Committee, and follows the international ethical guidelines, which include informed consent, confidentiality, anonymity of personal data and abandonment option in case of expressed will. To each volunteer was hand-out a leaflet (Figure 2) explaining thoroughly: (1) the purpose of the study; (2) the involvement in the blood donation process and of personal information sharing; (3) the risks and benefits of participation; and (4) the privacy protection measures.

A code number was assigned to each blood sample, identifying it during the whole project's protocol. Only authorized staff at the previously mentioned blood collection facilities had access to the participant's name. After individual's acceptance, written informed consent was signed and, with the help of the team involved, an anonymous blood sample record was filled, providing information regarding the participant's age, sex, birthplace and

parental birthplace. As far as São Miguel is concerned, the sample consisted of 7.5mL of blood, while in the other islands only 2.7mL were collected. In order to assure individual ancestral origin, the blood collection was preferably taken from individuals whose parents were born in the same island. A thorough analysis of the parental birthplace revealed a distribution of individuals by four different groups (Table 1).



STUDY OF THE GENETIC DIVERSITY IN THE AZOREAN POPULATION *

This leaflet is intended to inform you about the objectives and goal of the study entitled "**Study of the genetic diversity in the Azorean population**". We aim that after this inf ormation you decide free and clearly whether or not you want to participate. In the affirmative case your participation consists in donating 2.7 mL of blood to the Hospital of Divino Espirito Santo of Ponta Delgada, EPE (HDES) collected in the Health Centre of your residence . Your sample will be used to extract genetic material which will be stored at the Molecular Genetics and Pathology Unit (UGPM) of the HDES.

Why is this study being done?
The purpose of this study is to identify and quantify the genetic variation that is present in the population of the different Azorean islands , analysing t he diversity present in the genetic material (DNA).

Is my participation important?
Yes, your particip ation is very important because this study is only possible if a large number of Azorean blood samples are analyzed. You will contribute to a better understanding of the origin , nature and distribution of genetic disorders in the Azorean.

What should I do to participate?
To participate you have to donate 2,7 ml of blo od for DNA extraction . With the help of a specialized team in the Heath Centre, you will fill an anonymous record of the blood sample , providing information concerning your age, sex, birth place and your parent's birth place.

How will my personal informa tion be kept private?
After your blood sample is collected a code number will be assigned which will identify sample during this project protocol. Only the Heath Centre will have access to your name, thus guaranteeing the anonymous nature of the genetic material and corresponding personal information.

Are there any risks associated with this study?
No, there are no risks associated with this study.

Are there any financial benefits in taking part in this study?
No, there is no financial gratification in taking part in this study.

Do I have to pay to participate in the project?
No, your participation is volunteer and free of any charge.

What will happen to my sample after the study is over?
After this study is over, your sample will be stored at MGPU and used in future studies, following all national and international ethic regulations .

What are my rights as a participant?
You are free to choose if you want to participate or not; no restrictions will be imposed on you.

Who do I call if I have questions or problems?
If you have any concerns or questions relating this project , please contact the MGPU.

Can I leave this project even after I have signed the informed consent?
Yes, you are free to leave the project at any time and we will destroy your samp le and personal data. All you need to do is inform your Heath Centre of your intention.

* This study was approved by the Hospital of Divino Espirito Santo of Ponta Delgada, EPE, Ethics Committee.

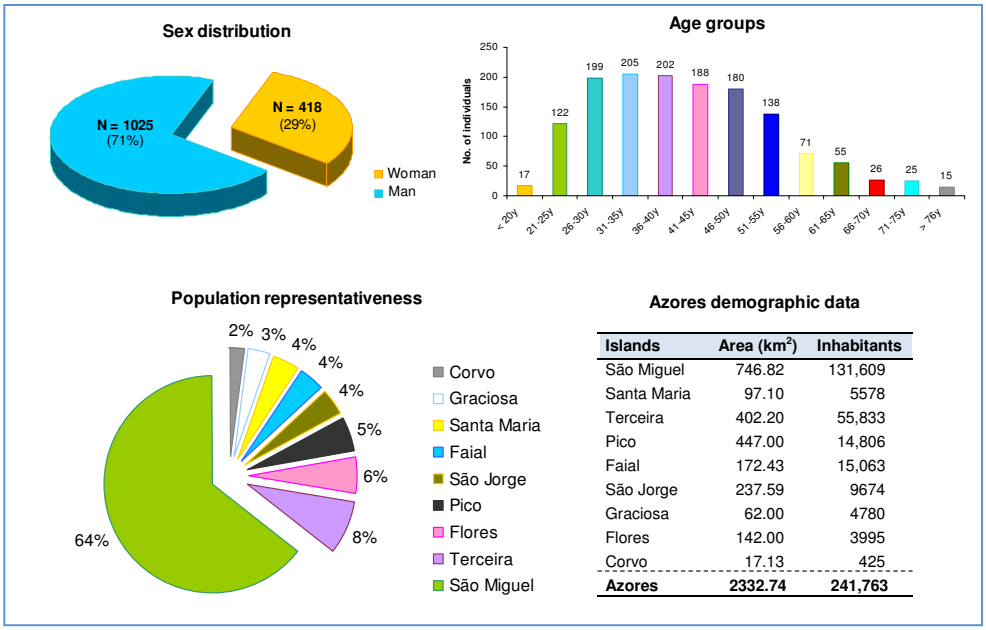
Fig. 2. Leaflet containing information for participation in the Azores DNA bank.

Parents origin	Samples	
	No.	%
Both parents were born in the same island	1373	88,13
Both parents were born in different Azores islands	70	4,49
Only one parent was born in the Azores islands	59	3,79
No parents born in the Azores islands	56	3,56
Individuals that inhabit the Azores islands	**1558**	**100,00**

Table 1. Parental birthplace analysis of the individuals that compose the Azorean DNA bank.

A total of 1443 individuals (92.6%) presented Azorean parents. Age and sex distribution show an average age of 42 years old, ranging from 18 to 88 years, and the majority of individuals are men (71%, Figure 3). Considering the individuals who both parents were born in the same island (N=1373), the population representativeness varies from 0.2% (Terceira) to 7% (Corvo). The largest sample representation is observed in São Miguel with 64% (Figure 3). The relation between the number of inhabitants and samples in each island

indicates a very high correlation coefficient (r=0.92; p<0.01), demonstrating the sample representativeness of the population distribution by all nine Azores islands.
Overall, Azoreans were willing to participate and understood its importance for long-term health gains. Until now, no requests to remove samples have been made. This biobank constitutes a very significant resource for biomedical research in Azoreans, some of them described below.



Islands	Area (km^2)	Inhabitants
São Miguel	746.82	131,609
Santa Maria	97.10	5578
Terceira	402.20	55,833
Pico	447.00	14,806
Faial	172.43	15,063
São Jorge	237.59	9674
Graciosa	62.00	4780
Flores	142.00	3995
Corvo	17.13	425
Azores	**2332.74**	**241,763**

Fig. 3. DNA bank samples distribution by sex, age and general Azores population representativeness. Calculations were based only in the individuals whose parents were born in the same Azorean island (N=1443).

3. Population studies: knowing the past to predict the future

The plethora of genomic research, produced since the first draft of the human genome (Venter et al., 2001; International Human Genome Sequencing Consortium, 2001), led to the acknowledgment that disease related rare variants with small effects are very difficult to identify (Figure 4) and that common variants implicated in complex diseases are frequently determined by GWAS. Additionally, the Human Genome Project (http://www.genome.gov/ 10001772) also contributed to the understanding of the structure and organization of the genome. Variability is observed through single nucleotide polymorphisms (SNPs), variable number of tandem repeats (VNTRs; e.g. mini and microsatellites), presence or absence of transposable elements (e.g. *Alu* elements) and structural alterations, which include insertions, deletions, duplications, inversions, translocations and copy number variants (CNVs).
The global analysis of population neutral variation is an essential part in the comprehension of the disease related variation, since it has also been subject to evolutionary forces, such as,

genetic drift, mutation, selection and migration. Altogether, the perception of our "roots" and genetic signature has several implications in society's own knowledge, in the design of future genetic studies, as well as, in the healthcare system.

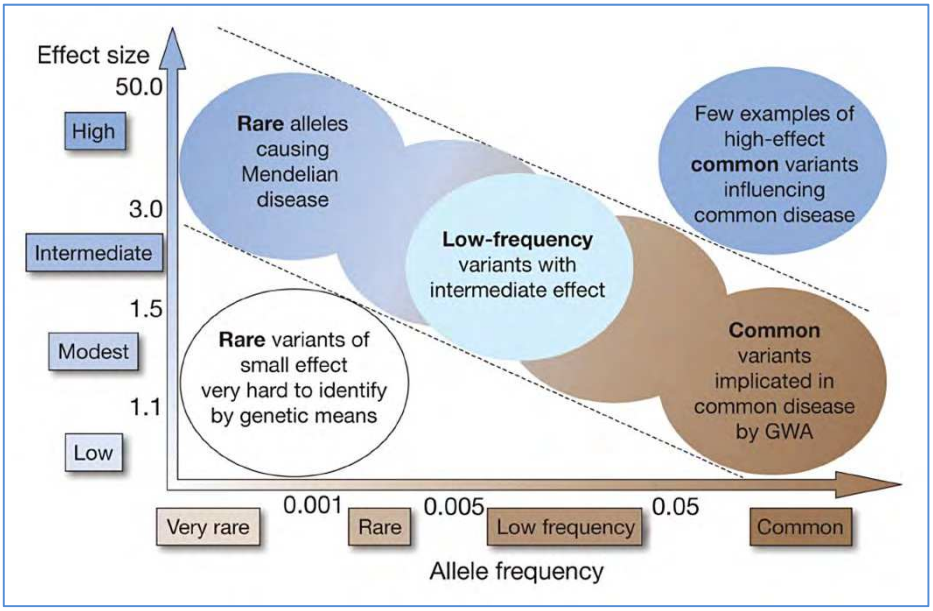


Fig. 4. The frequency spectrum of human disease risk alleles includes alleles at all frequencies from rare to common, with effect sizes from high to low (from Manolio et al., 2009).

3.1 Diversity, ancestry and linkage disequilibrium

The comprehension of how genetic diversity is structured in the human being is not only of anthropological importance, but also of medical relevance with significant implications for human evolution, forensics, genetic diseases and pharmacogenetics (1000 Genomes Project Consortium, 2010; The International HapMap Consortium, 2010; Heard et al., 2010). As more geographic populations are studied with high density genotype arrays it is also becoming apparent that allele frequencies for the relevant disease markers can vary widely. For instance, what is true for Europeans may not be for Africans, such is example the Type 2 Diabetes protective allele with increased frequency in non-Africans compared to Africans (Silander et al., 2009; Kral et al., 2011). These emerging data must be incorporated into a strategy that positions genomic medicine for a clinical role.

To grasp the genetic origins of mainland Portugal and Azorean populations studies on Y-chromosome lineages (Pacheco et al., 2005; Montiel et al., 2005; Beleza et al., 2006; Gonçalves et al., 2005; Fernando et al., 2005), mitochondrial DNA (Pereira et al., 2000b; Santos et al., 2003; Santos et al., 2005; Santos et al., 2008; Santos et al., 2010) and autosomal *Alu* insertion polymorphisms (Branco et al., 2006) were performed. The nonrecombining portion of the Y-chromosome retains a record of the mutational events that occurred along male lineages throughout evolution (Karafet et al., 2008). Presently, new binary polymorphisms reshaped

and increased resolution of the Y-haplogroup tree; however, for comparison purposes old nomenclature was maintained. According to Pacheco et al. (2005), the Azorean population presented nine different haplogroups, most of which are frequent in Europe (Figure 5). Hapogroup J* is the second most frequent in Azores (13.4%), but it is modestly represented in mainland Portugal (6.8%). The other non European haplogroups – N3 and E3a –, which are prevalent in Asia and subSahara, respectively, have been found in Azores (0.6% and 1.2%, respectively) but not in mainland Portugal (Neto et al., 2007). The absence of haplogroup E3a in western Iberia suggests that, despite the massive introductions of African slaves in historical times, there was little admixture between the African males and western Iberian populations (Pereira et al., 2000a). In general, four major haplogroups – P*(xR1b8,R1a,Q3), J*, BR*(xB2b,CE,F1,H,JK) and E*(xE3) – account for the majority of the male lineages in the Azores and mainland Portugal.

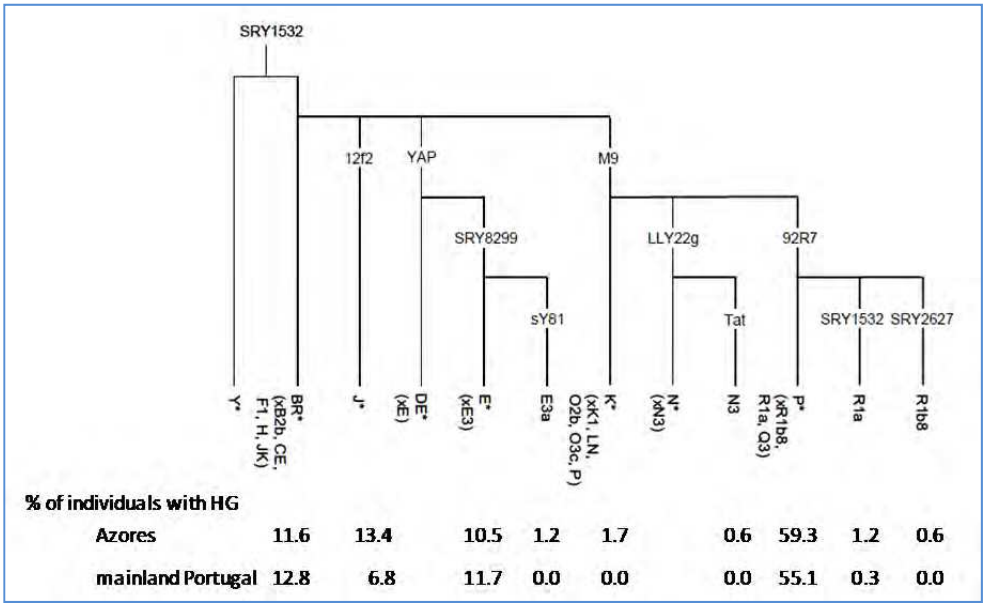


Fig. 5. Phylogenetic tree of the Y-chromosome haplogroups and their percent frequencies in the Azores and mainland Portugal. Numbers of % individuals with HG for mainland Portugal represent average values based on literature reports by Beleza et al. (2006) and Gonçalves et al. (2005).

All studies (Pacheco et al., 2005; Montiel et al., 2005; Beleza et al., 2006; Fernando et al., 2005) report that the main contributors to the genetic origin of the Azores are, as expected, the mainland Portuguese. Moreover, an important contribution of Middle eastern (HG J*) and north African (HG E*(xE3)) populations is observed in both populations. This observation is corroborated by mitochondrial DNA HVRI (hypervariable region I) analysis, where the majority of mtDNA lineages originated from the Iberian peninsula, mainly from mainland Portugal. The presence of Jews in the Azorean Central group is also supported by the mtDNA data (Santos et al., 2003).

Without any doubt, Y-chromosome and mtDNA studies are crucial to address the origin of the population; however, a population loses mtDNA when a woman has only sons and Y-chromosome DNA when a man has only daughters. As a result, these genetic markers may give less correct information on broad ancestry of most genes in a population. A full picture of the histories of populations requires studies of markers in the recombining parts of the nuclear DNA, namely the autosomes. Albeith several types of markers can be used to achieve this purpose, *Alu* insertion polymorphisms present some interesting advantages. These markers arose within the human population as a unique event in evolutionary history, making *Alu* repeats identical by descent from a common ancestor (Batzer & Deininger, 2002). Moreover, the ancestral state, which is absence of the *Alu* insertion, is always known. The allele frequencies for each *Alu* polymorphism in Azoreans and mainland Portugal (Table 2) are very similar to those obtained in other European populations.

		Autosomal *Alu* insertion polymorphisms					
Population	**N**	**TPA-25**	**ACE**	**APO**	**PV92**	**D1**	**B65**
Azores	65						
Frequency		0.592	0.385	0.946	0.208	0.254	0.585
Heterozygosity		0.424	0.485	0.106	0.257	0.348	0.409
HW (*p value*)		0.323	1.000	1.000	0.033	0.524	0.211
Locus diversity		0.493	0.481	0.117	0.343	0.392	0.493
Av. gene diversity		0.383 +/- 0.233					
F_{IS}		0.117					
Portugal	30						
Frequency		0.600	0.367	0.917	0.283	0.233	0.500
Heterozygosity		0.517	0.483	0.034	0.345	0.275	0.483
HW (*p value*)		0.665	1.000	0.073	1.000	0.453	1.000
Locus diversity		0.496	0.480	0.128	0.404	0.370	0.517
Av. gene diversity		0.392 +/- 0.240					
F_{IS}		0.094					

Table 2. *Alu* insertion frequencies, heterozygosity and gene diversity for Azores and mainland Portugal. F_{IS} represents inbreeding coefficient (from Branco et al., 2006).

The evolution of populations is dependent on several mechanisms, such as migration, genetic drift, selection and mutation, all affecting the patterns of diversity of neutral and disease variants. Consequently, the measure of diversity of neutral markers allows the inference of how these processes are shaping the overall signature of a population and has further implications in the general diseases apportionment. Genetic diversity values, based on autosomal STR markers, for Azores (0.788; Branco et al., 2008a; Santos et al., 2009) and mainland Portugal (0.782; Perez-Lezaun et al., 2000) indicate that both populations are very diverse. Studies of HLA markers in mainland (Spinola et al., 2005a; 3 *loci*) and in Azores

(Spinola et al., 2005b; 6 *loci*) demonstrate values of average diversity of 0.92. The results obtained for Azores (Pacheco et al., 2010), based in 7 *loci*, presented a smaller value (0.83). HLA haplotype analysis showed that A*01-B*08-DRB1*03 haplotype, known to be of Indo-European Celtic origin, is present in Centre and North Portugal regions at relatively low frequencies of 3% and 2.2%, respectively. However, this haplotype is the most frequent in São Miguel Island (8%). According to Spínola et al. (2005b), it's presence results from a colonizing event from people originating from the Centre of Portugal.

The patterns of genetic diversity of a population have a direct influence in the linkage disequilibrium (LD) extent, which has been found to improve the knowledge of human evolution and origin, and to identify genes causing disease. Table 3 describes the number of haplotypes, gene diversity and standardized multiallelic disequilibrium coefficient (D'), based on X-linked markers for Azores and mainland Portugal populations (Branco et al., 2008b; Branco et al., 2009).

Populations	HN	GD	D'
Azores			
Western group	93	0.718	0.328
Central group	150	0.690	0.189
Eastern group	207	0.686	0.176
Total	450	0.695	0.142
mainland Portugal	97	0.683	0.226

Table 3. Haplotype number (HN), gene diversity (GD) and standardized multiallelic disequilibrium coefficient (D') for Azorean and mainland Portugal populations (from Branco et al., 2008b).

The Azorean Western group shows a higher genetic diversity (0.718) when compared with the other two groups. Overall, Azoreans and mainland Portuguese do not show extensive LD, as direct consequence of the large genetic diversity of these populations. These data were also corroborated by Silva et al. (2010), based on pairwise linkage of 10 X-chromosome STRs, and Service et al. (2006), who compared levels of LD between several European populations. Finally, both mainland Portuguese and Azoreans constitute admixed and outbred populations.

4. Studies of rare and common genetic diseases

Population genetic architecture can also be revealed by distribution patterns of mutations causing Mendelian disorders. Bering this in mind, the UGPM developed three main research areas, based on the Azorean healthy population. The first encompassed the analysis of mutations causing Gilbert's syndrome (GS) and hereditary haemochromatosis (HH), the second constituted the evaluation of some genetic variants predisposing to complex diseases, namely, congenital heart diseases (CHD) and thrombosis, and, finally, the third included the characterization of variants involved on drug metabolism, particularly, irinotecan and warfarin. The results from these research projects are presented and discussed in this section.

4.1 Gilbert's syndrome

Gilbert syndrome is a mild hereditary unconjugated hyperbilirubinemia without liver dysfunction or hemolytic anemia (Matsui et al., 2010; Zhang et al., 2007). No treatment or long-term medical attention is necessary and it is usually one of the differential diagnoses of liver or hemolytic disease. The primary cause of this syndrome is the accumulation of bilirubin – hyperbilirubinemia –, due to decreased hepatic activity levels of glucoronosyl transferase, an enzyme responsible for glucoronidation reaction (Figure 6; Matsui et al., 2010).

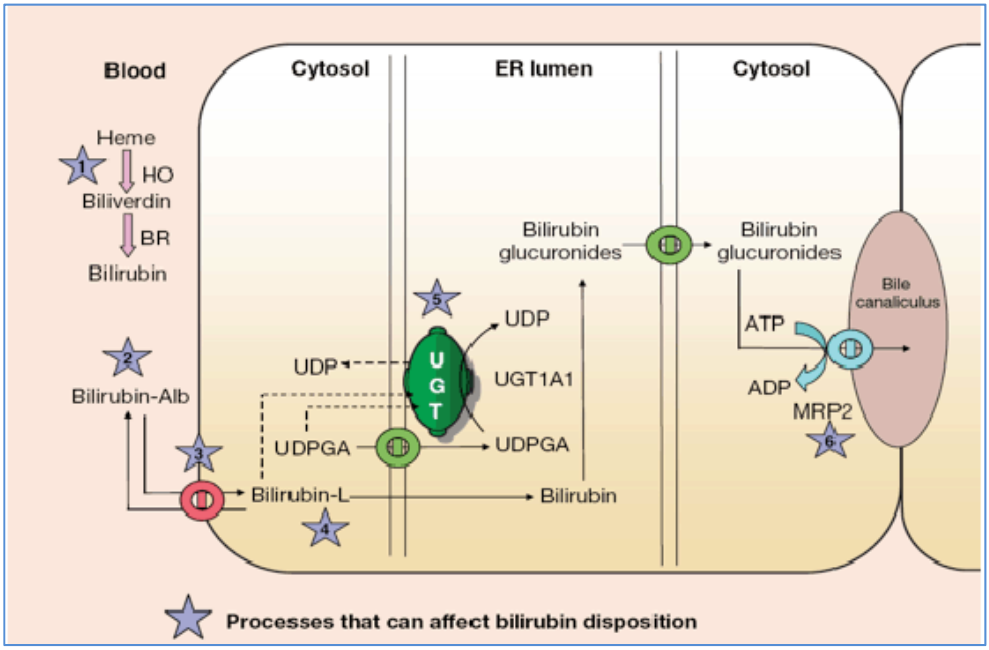


Fig. 6. Bilirubin uptake and processing in the liver cell. The heme initially breaks apart into biliverdin, which is rapidly reduced to bilirubin (1). Bound to albumin, bilirubin is transported from sites of production to hepatic sinusoids (2). At the sinusoidal surface of hepatocytes, bilirubin dissociates from albumin and enters hepatocytes by facilitated diffusion (3). Binding to cytosolic glutathione-*S*-transferases (GSTs) increases net uptake of bilirubin by inhibiting its efflux (4). Bilirubin is converted to mono- and diglucuronide by the action of UGT1A1, which catalyses the transfer of the glucuronic acid moiety from UDP-glucuronic acid (UDPGA) to bilirubin (5). Bilirubin glucuronides are actively transported into bile against a concentration gradient by the ATP-utilizing pump MRP2 (6; Figure from http://www.natap.org/2011/EASL/EASL_83.htm).

Bilirubin has been shown to inhibit DNA synthesis, uncouple oxidative phosphorylation, and inhibit ATPase activity in brain mitochondria (Strassburg, 2010). It also inhibits a variety of different classes of enzymes including dehydrogenases, electron transport proteins, hydrolases, enzymes of RNA synthesis, protein synthesis and carbohydrate metabolism. All toxic effects of bilirubin are reversed by binding to albumin. In fact,

albumin plays a vital role in the distribution of bilirubin in the body by keeping the compound in solution and transporting it from sites of production (primarily bone marrow and spleen) to the liver (Strassburg, 2010).

GS is associated with a dinucleotide polymorphism in the TATA box promoter of the UDP glucuronosyltransferase 1 (*UGT1A1*) gene, more precisely the UGT1A1*28 allele (Costa, 2006). In São Miguel Island a group of 67 suspected GS patients and 469 unrelated healthy blood donors were studied (Pacheco et al., 2006; Table 4). The results demonstrated that 65.9% of patients (45 out of 67) and 9.2% controls (43 out of 469) were homozygous for allele *28/*28. The genotype frequency in the control group showed a similar value to the reported to mainland Portugal (9.9%; Gonçalves et al., 2001). Additionally, Oliveira et al. (2007) reported, in a group of healthy individuals, an UGT1A1*28 allele frequency of 28.0%, a value very similar to the obtained in the Azorean control group (29.0%; Pacheco et al., 2009).

UGT1A1			Genotype frequency			
			Suspected GS patients (N=67)		Control group (N=469)	
Alleles		Genotypes	N	%	N	%
*1	A[TA]$_6$TAA	*1*1	4	6.0	237	50.5
*28	A[TA]$_7$TAA	*1*28	18	29.9	186	39.7
*36	A[TA]$_5$TAA	*1*36	-	-	2	0.4
*37	A[TA]$_8$TAA	*1*37	-	-	1	0.2
		*28*28	45	65.9	43	9.2

Table 4. Genotype frequencies of *UGT1A1* variants in suspected GS patients and control group in São Miguel islanders (adapted from Pacheco et al., 2006).

A molecular study based in a cohort of 120 Portuguese patients with the clinical diagnosis of Gilbert syndrome demonstrated that 110 individuals were homozygous for the UGT1A1*28 allele (*28/*28), and one patient was a compound heterozygote for two different insertions *28/*37. The remaining 9 patients were heterozygous, *1/*28 (Costa et al., 2006). The analysis of the control group allowed the identification of alleles characteristic of African populations, *36 (0.2%) and *37 (0.1%), which corroborates previous data on the Azorean and mainland Portuguese genetic background.

4.2 Hereditary hemachromatosis

Hereditary haemochromatosis (HH) is an inherited disorder characterized by accumulation of iron. The phenotypic condition was uncommon and typically diagnosed only when significant complications had ensued. The transmission mode is autosomal recessive and the disease manifests more commonly in males than in females, where natural iron losses are greater (Pietrangelo, 2004; Griffiths, 2007). In HH, gradual deposition of iron occurs in the liver and in a number of other tissues including the pancreas, joints, skin and heart. Disease manifestations include hepatic fibrosis, diabetes mellitus, arthropathy, pigmentation, cardiomyopathy and hypogonadotrophic hypogonadism. Fatigue and arthralgia are

common early symptoms and painful arthropathy is a considerable cause of morbidity. However, it is iron toxicity within the liver which increases mortality in patients with HH. Cirrhosis of the liver is associated with significantly reduced survival and there is a 200-fold increased risk of hepatocellular carcinoma (Pietrangelo, 2004; Griffiths, 2007; Swinkels et al., 2006).

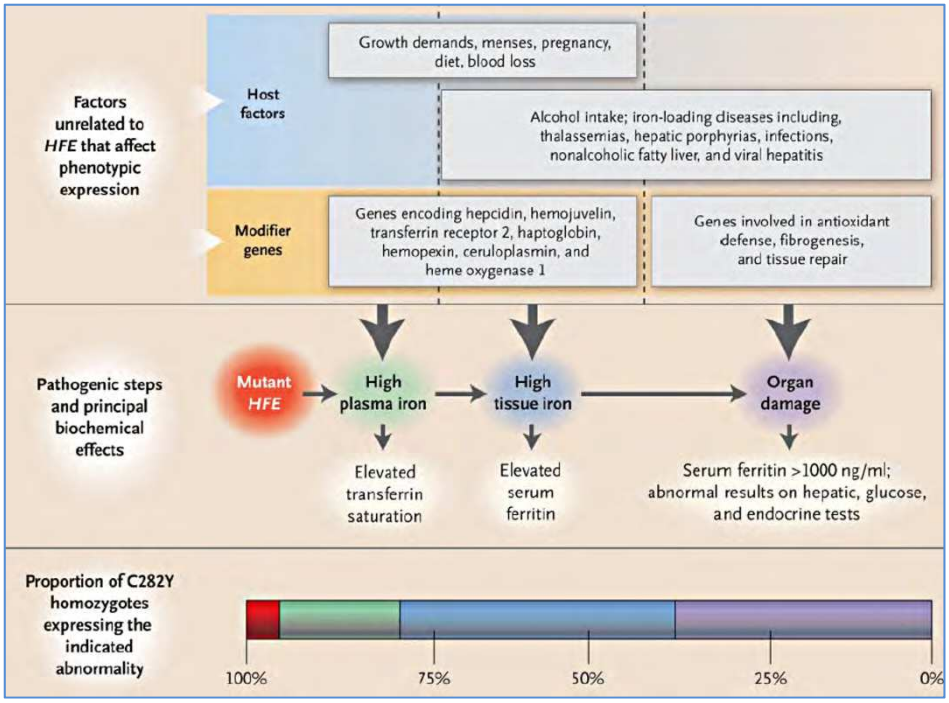


Fig. 8. *HFE* -related Hereditary Hemochromatosis (adapted from Pietrangelo, 2004).

The *HFE* gene encodes an HLA-A class 1-like protein and is located on 6p21.3, 4 megabases (Mb) telomeric to the human leukocyte antigen region (HLA). Two *HFE* mutations – C282Y (845 G>A) and H63D (187 C>G) – are significantly correlated with HH (Feder et al., 1996). The majority (60% to 90%) of clinically diagnosed probands are homozygous for C282Y, and 5% are compound heterozygous for C282Y and H63D.

The C282Y allele frequency in São Miguel population is 5.01%, a similar frequency (5.8%; de Fez et al., 2005; Gomes et al., 2007) to the obtained in the north of mainland Portugal, but statistically different from that observed in the south populations (0.9%; Cardoso et al., 2001). Contrary to this C282Y decreasing gradient, the H63D presents an even country distribution (Figure 10), with values of 17.88% and 20.36% for mainland and São Miguel Island, respectively.

Because C282Y mutation seems to have originated by chance on a HLA-A*03-B*07 haplotype in an individual from northwestern Europe and early spread, by Celts or Vikings (Milman & Pedersen, 2003; Distante et al., 2004), in many countries including Portugal, the background of this mutation was assessed in São Miguel islanders (Gomes et al., 2007). Four

non-ancestral HLA haplotypes were associated with C282Y: A*01-B*35, A*02-B*44, A*02-B*55 and A*24-B*15, contrary to mainland, where the C282Y occurred mainly on the ancestral haplotype HLA-A*03-B*07 (Cruz et al., 2006). In addition, the C282Y mutation was also identified in two HLA A*03 bearing: A*03-B*27 and A*03-B*50. These results evidence that C282Y mutation was introduced in the Azorean population by individuals with genetic background other than Celts or Vikings.

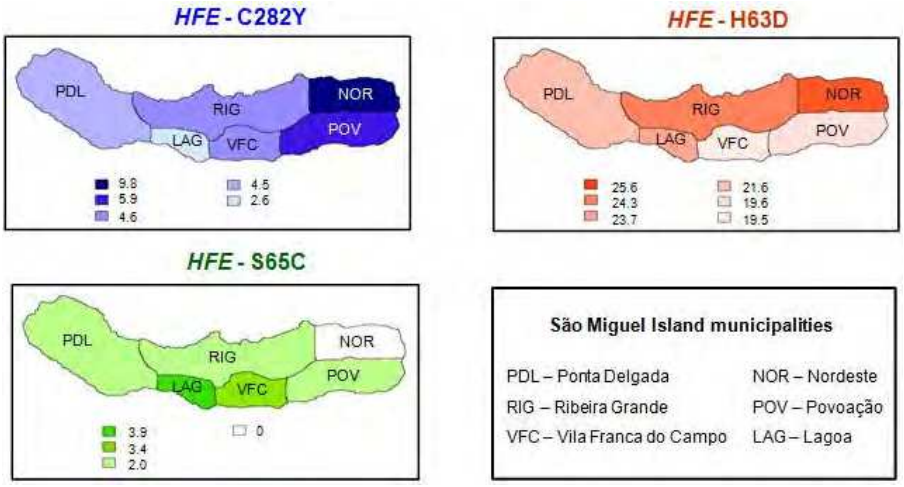


Fig. 9. Geographic distribution of *HFE* mutations in São Miguel Island. Frequency values are expressed in percentage.

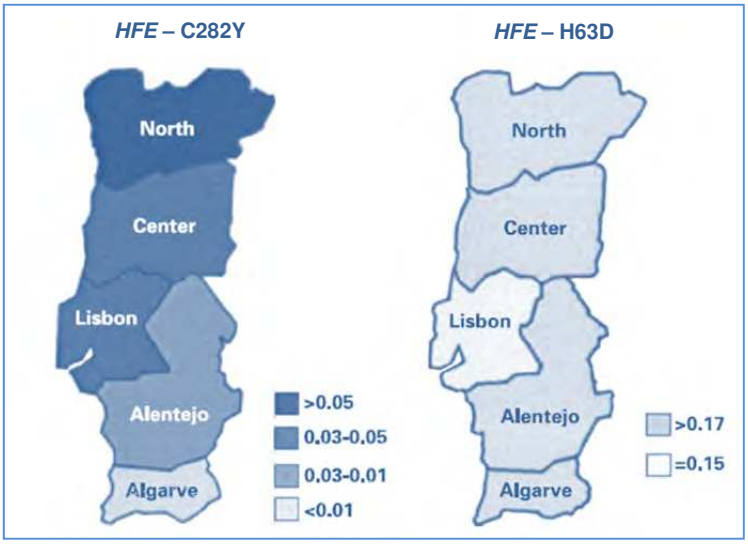


Fig. 10. Geographical distribution of the allelic frequencies of the C282Y and H63D *HFE* mutations in five Portuguese regions. (adapted from Cardoso et al., 2001).

4.3 Congenital heart disease

The evaluation of genetic variants predisposing to complex diseases in Azores began with the characterization congenital heart diseases (CHD). Congenital malformations of the heart and great vessels are among the most frequent of all clinically significant birth defects, with a major contribution to paediatric morbidity, mortality and healthcare costs.

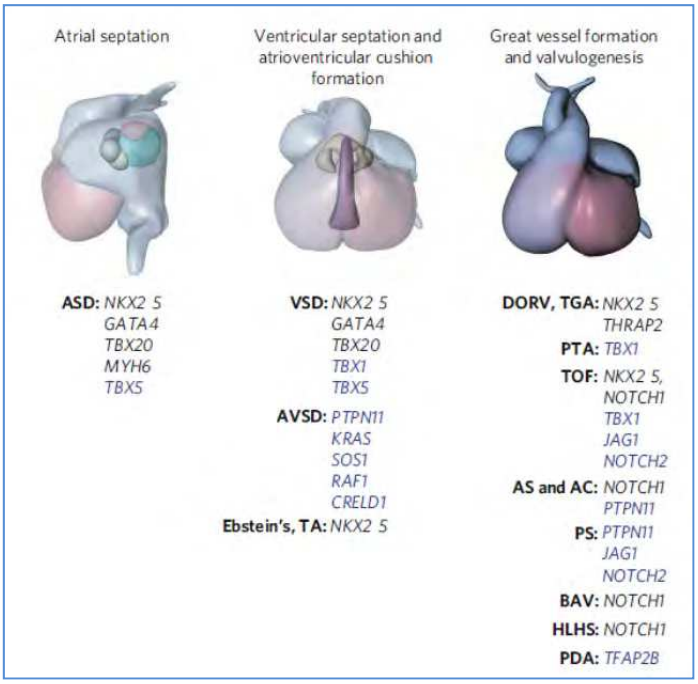


Fig. 11. Three major classes of developmental defects are indicated: defects in atrial septation, in ventricular or atrioventricular septation, and in the great vessels. The types of congenital heart disease that occur within each class are indicated, with the associated mutated genes listed. AC, aortic coarctation; AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; DORV, double outlet right ventricle; Ebstein's, Ebstein's anomaly of the tricuspid valve; HLHS, hypoplastic left heart syndrome; HRHS, hypoplastic right heart; IAA, interrupted aortic arch; MA, mitral atresia; MS, mitral stenosis; PDA, patent ductus arteriosus; PS, pulmonary artery stenosis; PTA, persistent truncus arteriosus; TA, tricuspid atresia; TAPVR, total anomalous pulmonary venous return; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect (From Bruneau, 2008).

Population based epidemiologic studies indicate a prevalence of CHD ranging from 3.23 to 12.23 *per* 1000 live births (Dolk et al., 2011; Bruneau, 2008; Engelfriet et al., 2005). This wide variation in the reported values is mainly due to the difference in the methodologies used, but a number of other factors, such as consanguinity, ethnic background, environmental pollutants and access to healthcare also contribute to this variation (Becker et al., 2001; Nabulsi et al. 2003; Botto et al., 2001). In 2006, the first study on the prevalence of CHD in

children born alive in São Miguel Island from January 1992 to December 2001 was carried out by Cymbron et al. (2006). Based on the Azorean Registry of CHD, which includes complete clinical and personal information, 189 patients were diagnosed. During this 10-year period, the average prevalence of CHD is 9.16 *per* 1000 live births placing Azores in the 10 European regions with higher prevalence of CHD (Table 5).

Countries	**Total births**	**N**	**Prevalence** (*per* 1000 births)
Austria	62,667	960	15.32
Malta	23,668	348	14.70
Switzerland	42,874	576	13.43
Germany	124,952	1457	11.66
Poland	206,170	2304	11.18
Norway	346,838	3538	10.20
São Miguel Island	**20,634**	**189**	**9.16**
Denmark	32,003	291	9.09
France	343,715	2853	8.30
Ukraine	25,835	201	7.78
UK	951,001	6497	6.83
Italy	431,727	2944	6.82
Belgium	182,467	1212	6.64
Ireland	215,021	1423	6.62
Netherlands	119,104	732	6.15
Spain	194,234	1080	5.56
Croatia	33,933	182	5.36
Total	3,356,843	26,787	7.97

Table 5. Prevalence of CHG in European countries and São Miguel Island (adapted from Dolk et al., 2011). The period of time analysed was from 2000 to 2005, with the exception of São Miguel Island (1992 to 2001). No data for mainland Portugal is available.

Considering that half of the São Miguel population lives in small rural localities (range from 309 – 7407 inhabitants for Lomba de São Pedro and Rabo de Peixe, respectively) and the internal migration is reduced, aspects that increase endogamy and inbreeding, a structured family questionnaire to the parents with CHD children was performed. The questionnaire included *i*) questions for CHD risk factors – maternal diabetes mellitus, alcohol and drug abuse by the mother during pregnancy, viral infections of the fetus and genetic conditions –, *ii*) queries concerning the number of family members affected with CHD and parental consanguinity, and *iii*) a detailed family history to construct the ascending genealogy until the 3th generation. Results revealed a relatively high number of multiplex families (44;

40.37%, Table 6) and a significantly high value of consanguinity (9.17%). Half of the consanguineous families (5 out of 109 total families) are multiplex. In addition, 36 out of 44 multiplex families are endogamous (81.8%; Table 7).
The data suggest that genetic factors may be responsible for the development of CHD in São Miguel Island. Familial aggregation, which is of great interest for understanding the genes involved in these complex pathologies, is evident.

Parameters	Families (N=109)	
	No.	%
Type of family:		
Simplex	65	59.63
Multiplex	44	40.37
Parental consanguinity:		
With	10	9.17
Without	99	90.83

Table 6. Parental consanguinity evaluation in CHD families from São Miguel Island.

No. affected individuals *per* multiplex family	No. families	Consanguinity		Distribution of families according to grandparental (GP) endogamy				
				Same locality			Different localities	Do not know
		With	Without	Four GP	Three GP	Two GP	All GP	
2	26	3	23	5	5	10	3	3
3	16	1	15	4	3	7	1	1
4	1	0	1	-	-	1	-	-
5	1	1	0	1	-	-	-	-
Total	**44**	**5**	**39**	**10**	**8**	**18**	**4**	**4**

Table 7. Analysis of consanguinity and grandparental endogamy in multiplex families from São Miguel Island.

Research has implicated both folate deficiency and genetic variation in folate pathway genes with birth defects, including CHD (Lee et al., 2005). Some studies suggest that polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR*) gene may be implicated in cardiac defects development. In mainland Portugal, Marinho et al. (2009) reported a higher prevalence of the 677T allele in tetralogy of Fallot (TF) compared to the control group (OR=1.675; 95% CI [1.022- 2.743]; p=0.05). The 677TT genotype increased by 4.856 the risk for this congenital disease (95% CI [1.308-12.448]; p=0.028), suggesting that *MTHFR* can be considered a susceptibility gene for TF. Cabral et al. (2008) genotyped two polymorphisms, C677T and A1298C, in *MTHFR* in CHD children (N=95) and respective mothers (N=89), as well as in the control group (N=469; Table 8). No significant differences

were obtained between all studied groups, indicating that in São Miguel Island *MTHFR* C677T and A1298C polymorphisms are not the main cause of CHD development. Currently, further genetic studies, based on functional candidate genes, in the Azorean population are on-going.

Variants	Allele frequency (%)		Genotype frequency (%)		
MTHFR - C677T	**C**	**T**	**CC**	**CT**	**TT**
Control group (N=469)	58.32	41.68	34.54	47.55	17.91
CHD children (N=95)	57.89	42.11	29.47	56.84	13.68
Mothers of CHD children (N=89)	62.92	37.08	39.33	47.19	13.48
MTHFR - A1298C	**A**	**C**	**AA**	**AC**	**CC**
Control group (N=469)	75.16	24.84	56.29	37.74	5.97
CHD children (N=95)	57.89	42.11	58.95	40.00	1.05
Mothers of CHD children (N=89)	62.92	37.08	49.44	44.94	5.62

Table 8. Allele and genotype frequencies for *MTHFR* C677T and A1298C polymorphisms in the São Miguel CHD study.

4.4 Thrombosis

In February 2007, the National Geographic Portugal magazine published a special issue dedicated to cardiovascular diseases. Based on the Euro Heart Survey 2006, the number of deaths *per* 100,000 inhabitants in 2004, due to CVD, places Portugal with higher values (390) compared with south-western European countries like Spain (290), France (262) and Belgium (364). In a similar analysis, performed within Portugal regions, the Azores archipelago clearly stands out with the highest value (116.9), followed by Lisbon (84.8). Family based investigation tends to have biased relative risk estimation, because oversampling of affected individuals is normally present. Therefore, the genetic characterization of disease variants in the general population is important to correct this potential bias. Since thrombosis is a common cause of CVD, Branco et al. (2009) analysed four polymorphisms in three thrombotic risk genes – *F5* (G1691A), *F2* (G20210A) and *MTHFR* (C677T, A1298C), in 469 healthy blood donors from São Miguel Island. Figure 12 shows allele frequencies in Caucasian populations, including São Miguel, for the above mentioned polymorphisms.

Comparison of allele frequencies for thrombotic risk factors between São Miguel and mainland Portugal (Mansilha et al., 2006) revealed statistically significant differences (χ^2, $p<0.001$; Table 9) for *F5* - 1691A, justifying the need to perform regional biomedical research to ultimately benefit the patient.

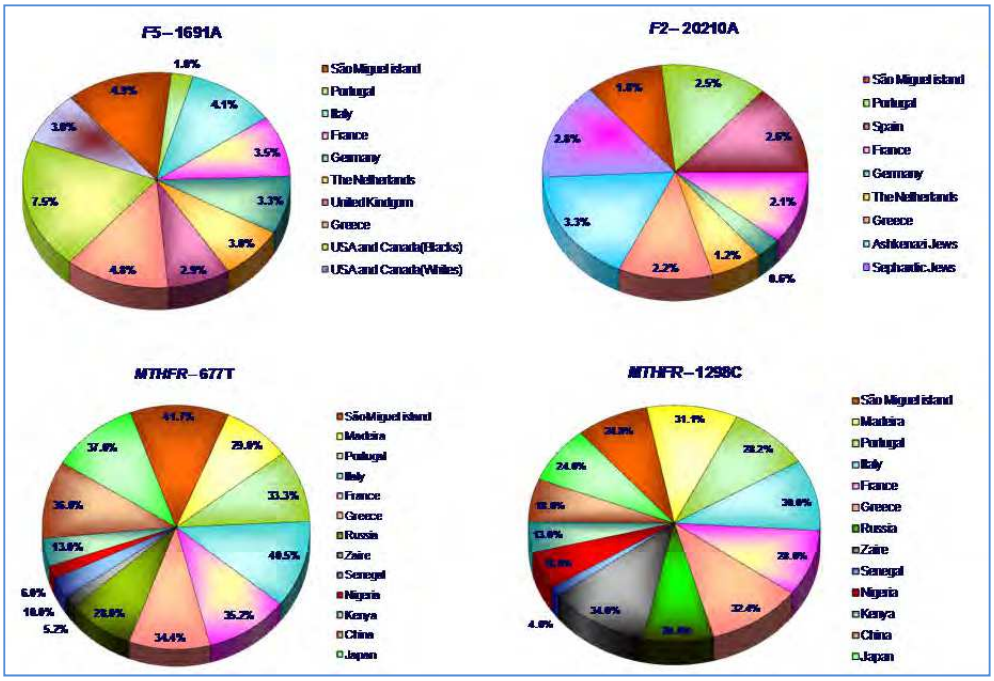


Fig. 12. Allele frequencies of the thrombotic risk factors – *F5* (1691A), *F2* (20210A) and *MTHFR* (677T, 1298C) – in different populations, including São Miguel (adapted from Branco et al., 2009).

	Allele frequency (%)			
Populations	***F5* (1691A)**	***F2* (20210A)**	***MTHFR* (677T)**	***MTHFR* (1298C)**
São Miguel Island	4.9	1.8	41.7	24.8
mainland Portugal	1.0	2.5	33.3	28.2

Table 9. Comparison between thrombotic genetic risk variants frequency between Azoreans and mainland Portugal.

Twenty-two different genetic profiles for *F5*, *F2* and *MTHFR* (order in genotype profile) were observed in São Miguel's population (Figure 13). The frequency of individuals who present a wild-type genotype for all polymorphisms (GG GG CC AA; 11.7%) was almost half of the major profile (GG GG CT AA; 22.4%), differing in heterozygosity for *MTHFR* 677CT. No heterozygous or homozygous profiles for all four variants were observed. Almawi et al. (2004) reported an OR of 10.5 (95% CI [4.3–25.3]) or 6.3 (95% CI [1.5–26.0]) for joint occurrence of the *F5*-G1691A or *F2*-G20210A with *MTHFR*-677TT genotype, respectively, enhancing the risk for deep vein thrombosis (DVT). Based on this criterion, fifteen (3.2%, *F5*/*MTHFR*) and two individuals (0.4%, *F2*/*MTHFR*) would have higher risk for DVT development (Figure 13 – asterisk character).

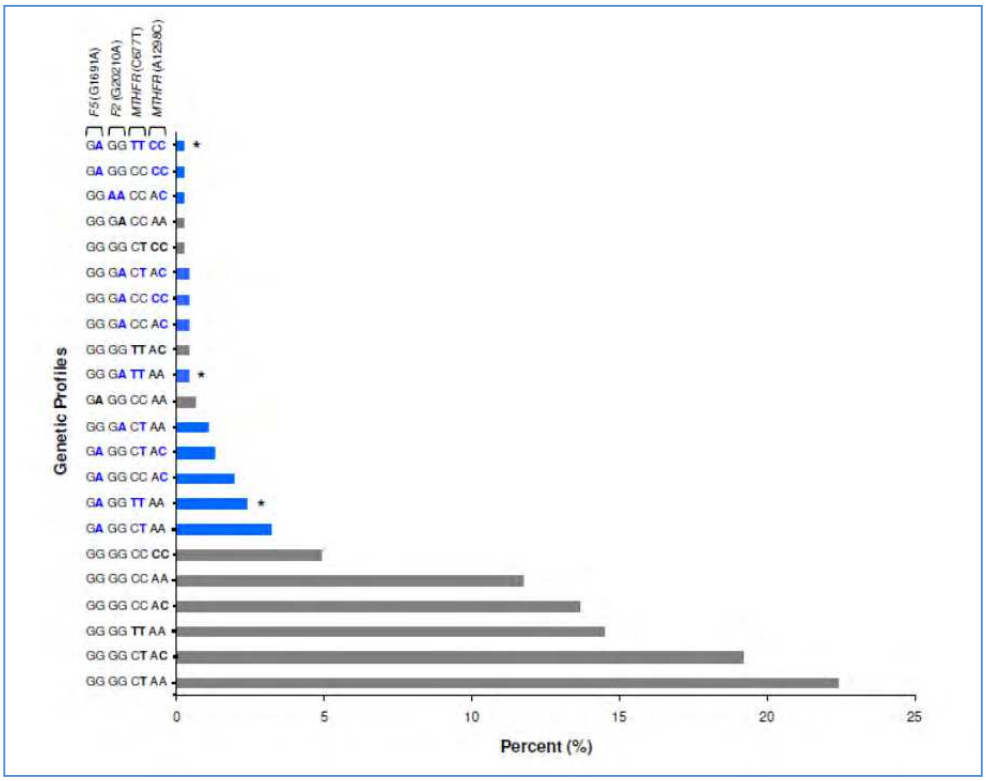


Fig. 13. Combined genotypes for thrombotic risk factors – *F5*, *F2* and *MTHFR* – in São Miguel population. Twenty-two different genetic profiles were obtained. Bold characters (blue and black) indicate nucleotide changes compared to the wild-type allele. Blue bars represent profiles with increase genetic predisposition to thrombosis. The asterisk (*) represents the combination of the *F5*-G1691A or *F2*-G20210A with *MTHFR*-677TT genotype (adapted from Branco et al., 2009).

Taken together, 12.4% (58 out of 469) of São Miguel islanders have increased genetic predisposition to thrombosis. No general population profile analysis for mainland Portugal is reported in the literature, and, consequently, no comparison was performed. These results are corroborated by further CVD studies, where 2 genomic regions associated with CVD risk, *USF1* and 9p21 were evaluated (Correia et al., 2010). Preliminary results, based on haplotype inference, showed that *USF1* "CCGCC" (0.288) and "CTGCT" (0.346) are the two most frequent haplotypes in Azoreans, which, according to Komulainen et al. (2006), correspond to the protective and high risk haplotype in females, respectively. In 9p21

region, the risk haplotype "TGGGCGCGC", associated with CHD in Europeans (Silander et al., 2009), is the most frequent (0.414). Analysis of homozygosity patterns for risk genotypes showed similar frequencies for *USF1* (12.4%) and 9p21 region (14.7%), indicating that, despite recombination, homozygosity must be taken in consideration when performing risk profile studies. Collectively, the data suggest that there is a considerable genetic risk for CVD in Azoreans, evidencing the need to carry out regional genetic studies with the aim to improve and complement health strategies adopted by the decision makers of each country. Further genetic studies on CVD are on-going.

4.5 Pharmacogenetics of irinotecan and warfarin

In the last 50 years, fundamental developments in pharmacology and genetics led to important improvements in personalized medicine based on pharmacogenetics and pharmacogenomics. Pharmacogenomics uses a whole genome approach to investigate or predict drug responses, or to relate the application of these technologies to drug discovery, while pharmacogenetics is the study of the clinically relevant inherited differences in drug response that can be in part explained by genetic variations. Pharmacogenetic analysis contributes to reduce adverse drug reactions (ADRs) or to maximize drug efficiency. Variants involved in drug metabolism of two commonly prescribed drugs, irinotecan and warfarin, were studied in Azoreans and mainland Portugal.

4.5.1 Pharmacogenetics of irinotecan

Irinotecan, an antineoplastic-prodrug, is widely used for the treatment of colorectal, lung and other cancers. The most clinically significant adverse events for patients receiving irinotecan-based therapy are diarrhea, neutropenia, nausea, vomiting and alopecia (Kehrer et al., 2001; Marques & Ikediobi, 2010; Lankisch et al., 2008; Fujiwara & Minami, 2010). Irinotecan can induce both early and late forms of diarrhea and requires dose adjustment based on severity of diarrhea. Also, sepsis related death following severe neutropenia has been reported in irinotecan treated patients. The active metabolite SN-38 is responsible for the pharmacological and toxic effect of irinotecan. SN-38, is a topoisomerase I inhibitor generated by hydrolysis of irinotecan by carboxylesterases (Figure 14). SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferase 1As (UGT1As) to form an inactive metabolite, SN-38G (Marques & Ikediobi, 2010; Fujiwara & Minami, 2010).

The human UDP-glucuronosyltransferase 1A gene *locus* is organized to generate enzymes, which share a carboxyterminal portion and are unique at their aminoterminal variable region. Expression is tissue-specific and overlapping substrate specificities include a broad spectrum of endogenous and xenobiotic compounds, as well as many therapeutic drugs targeted for detoxification and elimination by glucuronidation. The absence of glucuronidation leads to fatal hyperbilirubinemia (Marsh & Hoskins, 2010; Zhang et al., 2007). Genetic variants and haplotypes have been identified as risk factors for unwanted drug effects of cancer treatment with irinotecan. Since variants in *UGT1A1*, *UGT1A6* and *UGT1A7* are related to irinotecan toxicity, these were studied in 469 individuals from São Miguel Island by Pacheco et al. (2009). Haplotype analysis revealed that H3 (Table 10), which includes all low activity allelic variants of the three UGT isoforms, accounts for 23.5% of the population, suggesting that those individuals have reduced glucuronidation activity due to the combination of UGT1A1*28, UGT1A6*2 and UGT1A7*3 alleles.

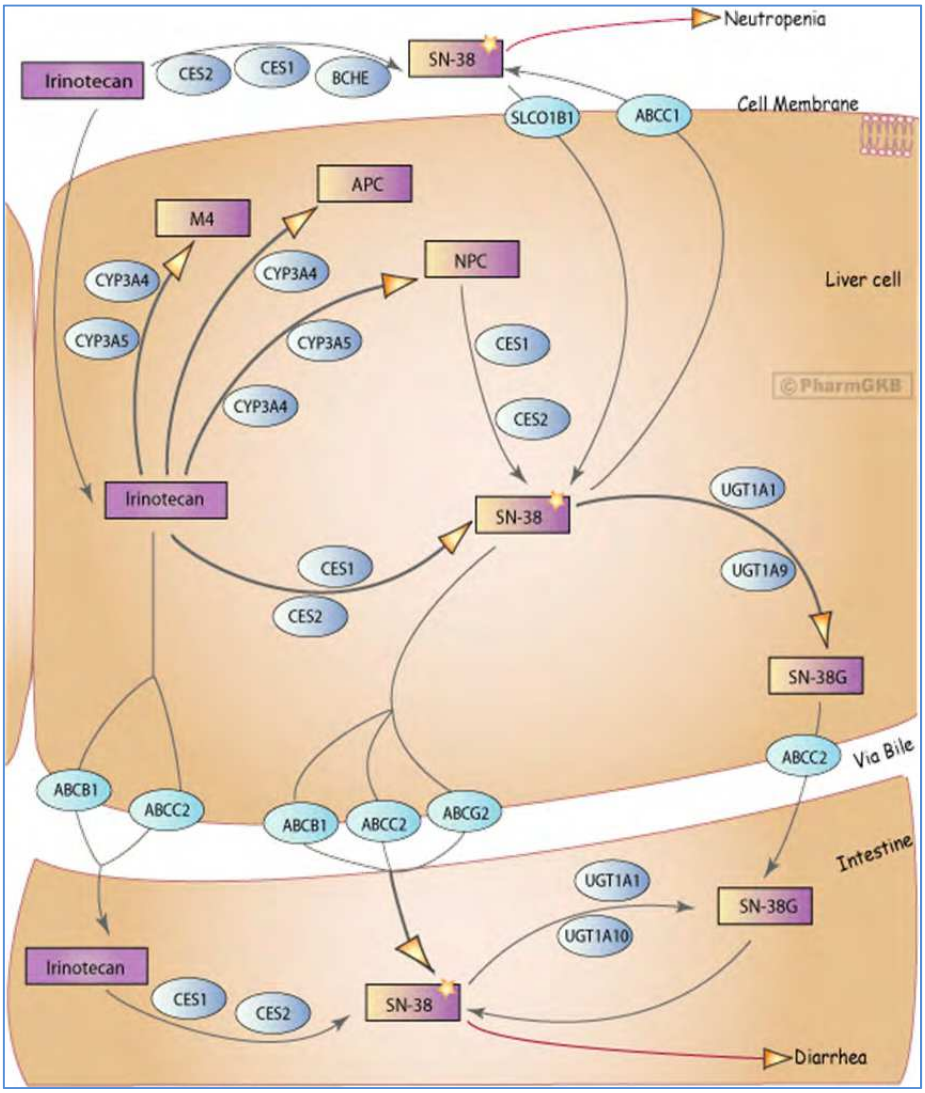


Fig. 14. Irinotecan pathway indicating tissue specific involvement of genes in the irinotecan metabolism. PharmGKB copyright. Available at http://www.pharmgkb.org/search/pathway/irinotecan/liver.jsp.

Haplotype (H)	*UGT1A* genes								Haplotype frequency	
	UGT1A1	*UGT1A6*			*UGT1A7*					
	A[TA]$_n$TAA	541	552	541/552	387	391/392	622	387/391/392/622	N	(2N=938)
01	TA_6 (*1)	A	A	*1	T	C-G	T	*1	296	0.316
02	TA_6 (*1)	A	A	*1	G	A-A	T	*2	225	0.240
03	TA_7 (*28)	G	C	*2	G	A-A	C	*3	220	0.235
04	TA_6 (*1)	A	A	*1	G	A-A	C	*3	65	0.069
05	TA_6 (*1)	G	C	*2	G	A-A	C	*3	31	0.033
06	TA_6 (*1)	A	C	*3	G	A-A	T	*2	26	0.028
07	TA_7 (*28)	A	A	*1	T	C-G	T	*1	23	0.024
08	TA_6 (*1)	A	A	*1	T	C-G	C	*4	17	0.018
09	TA_7 (*28)	G	C	*2	G	A-A	T	*2	7	0.007
10	TA_7 (*28)	A	C	*3	G	A-A	T	*2	6	0.006
11	TA_7 (*28)	A	A	*1	G	A-A	T	*2	5	0.005
12	TA_7 (*28)	A	A	*1	G	A-A	C	*3	5	0.005
13	TA_7 (*28)	A	C	*3	G	A-A	C	*3	3	0.003
14	TA_7 (*28)	A	C	*3	T	C-G	C	*4	3	0.003
15	TA_5 (*36)	A	A	*1	G	A-A	T	*2	2	0.002
16	TA_6 (*1)	A	C	*3	T	C-G	C	*4	2	0.002
17	TA_7 (*28)	A	A	*1	T	C-G	C	*4	2	0.002
18	TA_6 (*1)	A	C	*3	T	C-G	T	*1	1	0.001
19	TA_8 (*37)	G	C	*2	G	A-A	C	*3	1	0.001

Table 10. Haplotype frequencies of *UGT1A1, UGT1A6* and *UGT1A7* variants in São Miguel Island population. *UGT1A6* alleles are defined by permutations of two SNPs (nucleotide positions 541A>G and 552A>C) and *UGT1A7* by permutations of four SNPs (387T>G, 391C>A and 392G>A, and 622T>C; adapted from Pacheco et al., 2009).

4.5.2 Pharmacogenetics of warfarin

Warfarin is one of the most widely used anticoagulant drug, which requires a thorough risk-benefit analysis since the dose prescribed should avoid hemorrhagic complications and achieves suppression of thrombosis (Wadelius & Pirmohamed, 2007; You 2011; Limdi & Veenstra, 2008). The administrated drug is a racemic mixture of S- and R-enantiomers, having S- the majority of the therapeutic effect (Figure 15). Warfarin pharmacogenetic studies demonstrated that variants in the *CYP2C9* (Cytochrome P450 2C9) and *VKORC1* (Vitamin K epoxide reductase complex subunit 1) genes account for approximately 50–60% of drug dosing variability (Finkelman et al., 2011). The remaining variability can be explained by clinical and environmental factors (age, sex, diet, concomitant drugs, body mass index), which in conjunction with genetics are used, in warfarin dosing algorithms, as

an advice for the best dose prescription. Cytochrome P450 2C9 is the major enzyme responsible for metabolising the active S-enantiomer. Although there are many polymorphisms in *CYP2C9*, the most clinically relevant variants are: *CYP2C9**1 (Arg144/Ile359, wild-type), *CYP2C9**2 (Arg144Cys) and *CYP2C9**3 (Ile359Leu). These last two are associated with decreased metabolic efficiency of the CYP2C9 enzyme and increased risk of bleeding when administrated initial dosages of warfarin (Yasar et al., 1999). Considering *VKCORC1*, a G>A variation at position 1639 in the gene's promoter region results in decreased mRNA transcription and increased sensitivity to warfarin inhibition of hepatic synthesis of functional vitamin K-dependent coagulation factors. Rieder et al. (2005) studied *VKORC1* polymorphisms and classified individuals according to warfarin dose requirements into distinct groups: high (GG), intermediate (GA) and low (AA). Recently, the U.S. Food and Drug Administration (FDA) added to the warfarin product label dosing recommendations stratified by combined *CYP2C9* and *VKORC1* genotypes (http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm).

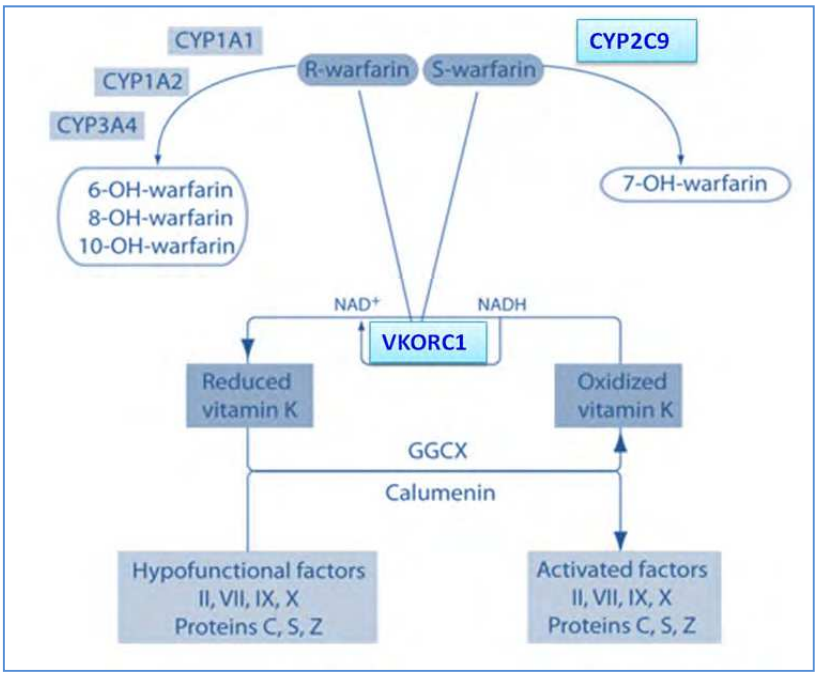


Fig. 15. Warfarin pharmacokinetic and pharmacodynamic pathway. Warfarin is administered as a racemic admixture of R- and S-enantiomers. The more potent S-enantiomer is metabolized principally by CYP2C9. The pharmacological effect of warfarin is mediated by the inhibition of vitamin K epoxide reductase complex 1 (VKORC1). This results in decreased concentrations of activated clotting factors (II, VII, IX and X) producing therapeutic anticoagulation (adapted from Božina 2010).

Comparison of separate genotypes for *CYP2C9* and *VKORC1* between mainland Portugal (Jorge et al., 2010) and the Azores (Pereirinha et al., 2009, personal communication) populations revealed no significant differences (Table 11). In the Azores population no individuals with *CYP2C9*-*3*3 genotype were identified, however a higher frequency of *CYP2C9*-*2*3 was observed when compared to mainland. The joint analysis of *CYP2C9* and *VKORC1,* in Azoreans, showed that around 12% of individuals need low doses of warfarin, if treatment is started (Table 11; blue characters).

CYP2C9			*VKORC1*			*CYP2C9/VKORC1*		
Genotype	**Frequency (%)**		**Genotype**	**Frequency (%)**		**Genotype**	**Frequency (%)**	
	Azores	**m. Portugal**		**Azores**	**m. Portugal**		**Azores**	**m. Portugal**
*1/*1	57.6	64.0	GG	30.0	33.0	*1*1-GG	17.1	NA
*1/*2	25.3	22.0	GA	56.5	50.5	*1*1-GA	34.1	NA
*1/*3	8.2	9.0	AA	13.5	16.5	*1*2-GG	7.0	NA
*2/*2	5.3	3.3				*1*3-GG	2.4	NA
*2/*3	3.5	1.1				*1*1-AA	6.4	NA
*3/*3	0	1.1				*1*2-GA	13.5	NA
						*1*3-GA	4.1	NA
						*2*2-GG	1.8	NA
						*2*3-GG	1.8	NA
						***1*2-AA**	**4.7**	NA
						***1*3-AA**	**1.8**	NA
						***2*2-AA**	**0.6**	NA
						***2*2-GA**	**2.9**	NA
						***2*3-GA**	**1.8**	NA

Table 11. Genotype frequencies of *CYP2C9* and *VKORC1* variants in Azores (N=170) and mainland Portugal (N=91) populations. **NA**- not applicable; no joint analysis of both genes has been described for mainland Portugal populations. Blue characters represent individuals who would require low doses of warfarin.

Overall, irinotecan and warfarin studies demonstrated that the Azores population shows significant differences on allele frequencies of pharmacogenetic variants, which must be taken in consideration when treating patients. These results have a high impact on the physicians' decisions in managing patient's treatment.

5. Conclusion

Genomic medicine, which uses the individual information to provide better healthcare, has been considerably developed since the Human Genome Project. One of its current challenges is the identification of risk alleles for multifactorial diseases and the study of their frequency in different populations. In summary, both the Azores and mainland Portugal populations are outbred with high genetic diversity, relative gene flow among its individuals, and without extensive LD. Nevertheless, the islanders have a particular genetic makeup evidenced by the African and Asian traits, and by the differences in terms of common diseases apportionment compared to mainland. Biomedical investigation has been

the driving force in improving practices in patient care, based on new drugs, diagnostic methods, medical instruments and services. The genetic research performed in the Azorean population allowed the implementation of molecular diagnosis in the main Hospital of the archipelago, as well as a faster and most costly effective response to clinicians, benefiting the patient and, ultimately, the Azorean Health System.

6. Aknowledgments

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7. Databases and Websites of interest

1000 Genomes Project	http://www.1000genomes.org/
ALFRED - Allele Frequency Database	http://alfred.med.yale.edu/alfred/index.asp
Copy Number Variation Project	http://www.sanger.ac.uk/humgen/cnv
Cytochrome P450 database	http://bioinformatics.charite.de/supercyp/
DrugBank database	http://www.drugbank.ca/
European Directory DNA Diagnostic Laboratories	http://www.eddnal.com/
Ensembl Database	http://www.ensembl.org/index.html
Food and Drug Administration - FDA	http://www.fda.gov/default.htm
Human Gene Mutation Database	http://www.hgmd.cf.ac.uk/ac/index.php
Human Genome Project	http://www.genome.gov/10001772
Human Genome Variation Database	http://hgvbase.cgb.ki.se
Human Variome Project	http://www.humanvariomeproject.org/
IMGT/HLA Database	http://www.ebi.ac.uk/imgt/hla
mtDB - Human Mitochondrial Genome Database	http://www.mtdb.igp.uu.se/
National Centre for Biotechnology Information	http://www.ncbi.nlm.nih.gov
National Human Genome Research	http://www.genome.gov/

Institute (NIHGRI)	
Online Mendelian Inheritance in Man	http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM
Orphanet	http://www.orphanet.pt/
Pharmacogenetics of Membrane Transporters Database	http://pharmacogenetics.ucsf.edu/
Rare diseases database	http://www.rarediseases.org/
Single Nucleotide Polymorphism Database	http://www.ncbi.nlm.nih.gov/projects/SNP
The International HapMap Project	http://www.hapmap.org
The Pharmacogenomics Knowledge Base	http://www.pharmgkb.org/
Y-Chromosome Consortium	http://ycc.biosci.arizona.edu

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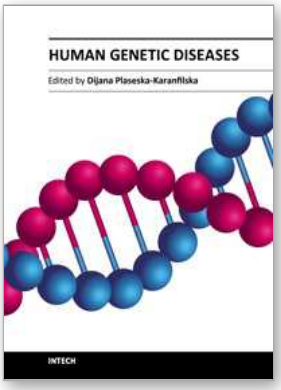

Human Genetic Diseases

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The genetics science is less than 150 years old, but its accomplishments have been astonishing. Genetics has become an indispensable component of almost all research in modern biology and medicine. Human genetic variation is associated with many, if not all, human diseases and disabilities. Nowadays, studies investigating any biological process, from the molecular level to the population level, use the â€œgenetic approachâ€ to gain understanding of that process. This book contains many diverse chapters, dealing with human genetic diseases, methods to diagnose them, novel approaches to treat them and molecular approaches and concepts to understand them. Although this book does not give a comprehensive overview of human genetic diseases, I believe that the sixteen book chapters will be a valuable resource for researchers and students in different life and medical sciences.

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