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Pharmacogenomic research in Portugal

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Abstract

Aim: To present the efforts made towards pharmacogenomic research carried out in the last two decades in Portugal.

Materials and methods: A search for Portuguese-language and English-language works reporting pharmacogenomic studies in Portugal published between 2000 and 2019 was conducted. It was performed a systematic review using the databases PubMed, Semantic Scholar and "Repositório Comum". A list of exclusions and inclusion criteria was considered, and the selection of articles involved a two-step screening process.

Results: The search strategy included 34 studies for data extraction. Most of the published data were original articles (19) about 44% of the studies were Cohorts. Half of the studies performed only genotyping testing to characterize the population and 26,5% examined drug responses to a panel of genes and/or polymorphisms. The national surveys focused on non-communicable diseases such as Rheumatoid arthritis and Breast cancer. The genes and/or polymorphisms screened in the Portuguese population were mainly associated with drug metabolism and rheumatoid arthritis phenotypes. Of 16 diseases subjected to pharmacogenomic studies, only 5 conditions belong to the top leading causes of premature death in Portugal. On the other hand, the leading causes of disability are only partially subjected to pharmacogenomic studies.

Conclusions: The published outcomes are significant and can contribute for the improvement of personalized medicine in the country. Research should focus on the most prevailing diseases that affect the Portuguese population. The already established research consortia and networks should also make the organization and translation of pharmacogenomics studies more streamlined.

Introduction

Pharmacogenomics aims to study how genetic profile influences drugs responses with the goal to improve clinical outcomes and personalizing drug therapy. As different patients don't have the same response to the same treatment, for instance, a given drug can be beneficial for some individuals but ineffective for others or provoke adverse drug effects [1].

In the past many non-genetic factors were considered responsible for the differences in the risk-benefit ratio between patients taking the same drug. Currently, an important role in individual response to therapies is attributed to the differences in patient genetic framework. In fact, response to drug therapy is the result of the variability and synergy of all these factors. It has become clear that therapeutic and adverse effects depend on various molecular mechanisms and inter-individual genetic differences [1,2]. Such interindividual variability in therapeutic drug response can result in adverse drug reactions or lack of efficacy and represents a key challenge for health care systems. There are also evidences that genetic polymorphisms in drug metabolizing enzymes, transporters or drug targets contributing to 20–30% of these interindividual differences [3].

The treatment of common diseases often involves a series of therapeutic trials with different drugs or classes of drugs and the health-care burden imposed by inefficacy during those periods of trial and error can be considerable. The inability of a selected drug therapy to target the underlying disease mechanism, drug interactions, disease-related changes in drug concentrations or responsiveness, poor compliance, system errors, failure to deliver the correct drug or dose to the patient are common reasons for the above mentioned variability in drug response [4].

Advances in genomic science have led to the identification by regulatory agencies of a growing list of clinically important biomarkers for drug response and toxicity and created mechanisms for considering such biomarkers for targeted therapy and drug safety warnings. Clinically validated pharmacogenomic biomarkers can help physicians optimize drug selection, dose and treatment duration while averting adverse drug reactions. However, the drive to position pharmacogenomics as a core element in personalized medicine is still hindered by limited data. For example, it's estimated that over 90% of drugs currently used in clinical practice lack valid and predictive biomarkers for therapeutic effects and/or avoiding severe side effects [5].

With the emergence of new drugs designed towards specific targets, the use of biomarkers shall increasingly allow patients to receive the drug most likely to be effective for their disease. In addition, using biomarkers to target therapies is regarded as a way towards a more efficient and cost-effective healthcare system [6].

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As technology advances it is becoming clear that the broad screening of multiple pharmacogenes needs to done pre-emptively and that data should be stored in the electronic health records and drug prescription systems as well as recommendations for dosing. The pre-emptive translation of pharmacogenomic discoveries remains a challenge but implementation efforts have brought and will continue to bring more informed knowledge to constantly improve solutions [7].

In Portugal, pharmacogenomics and other "omic" information to individualize drug selection and use has some degree of weakness given it's not routine clinical practice yet nor well known among professionals and genetic tests are rarely used for therapeutic purposes. Nevertheless, pharmacogenomics has shown a potential use in the fields of oncology and infectious diseases [8].

Considering the importance and the relevance of the subject, the present review aims to demonstrate the efforts made in pharmacogenomic until the present moment in Portugal.

Aim of the review

The present review aims at presenting the efforts made towards pharmacogenomics research carried out in the last two decades in Portugal.

Ethics approval

No approval was necessary as no patients or professionals have been the direct subject of this study.

Method

A literature search for pharmacogenetic and pharmacogenomic studies in Portugal published between 2000 and 2019 was conducted. The review followed the "Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P)". The included databases were PubMed, Semantic Scholar and "Repositório Comum" [9] in which the following keywords where searched: pharmacogenetics, pharmacogenomics, polymorphisms, personalized medicine, Portuguese population.

A list of exclusions comprised: (1) full text unavailability, (2) conference proceedings, (3) non human studies. Duplicated studies were excluded from the final selection (Figure 1).

The selection of articles and monographies involved two screening steps: In the first step, it was screened the title, keywords with relevancy to the area of pharmacogenomics, abstract and evidence that the

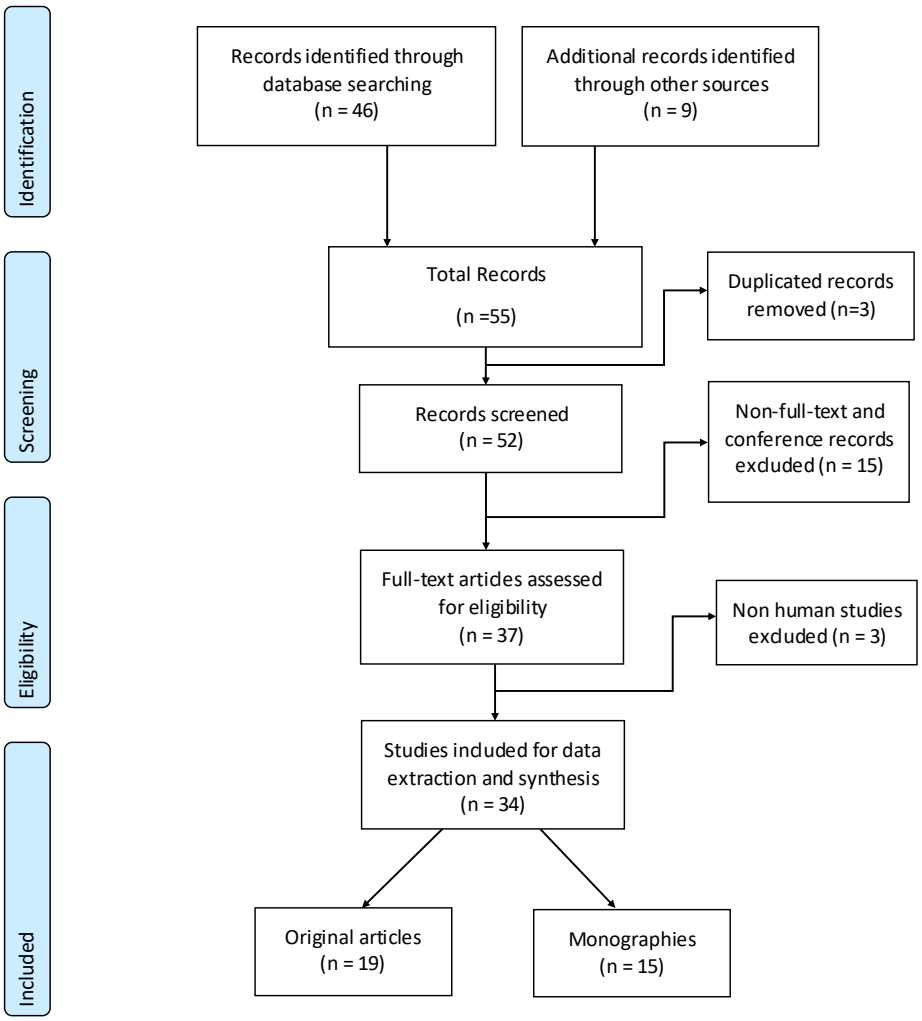

Figure 1. Flow diagram of the study selection procedure

research was conducted in Portugal. In the second step, the full texts were checked in order to assess the last exclusion criteria.

Data extraction focused on two main topics: strategies and study characteristics. The study strategies observed were the following: (1) study type, (2) time horizon, (3) patient age range (4) pharmacogenomics approach. The study characteristics comprised the following items: (1) year of the study, (2) Portuguese region, (3) disease indication or condition, (4) drug type and category, (5) genes / polymorphisms screened and associated metabolism. Drug categorization was done according to the Anatomical Therapeutic Chemical (ATC) classification system [10].

Results

The search strategy retrieved 55 studies during the screening process (Figure 1). We excluded 3 duplicated studies, 15 non full text studies or conference proceedings and 3 non human studies. 34 studies were selected for data extraction.

For a quick overview, data was compiled in Table 1 which shows the relevant studies in the pharmacogenomics field performed in Portugal between 2000 and 2019.

Summary of the study strategies

Most of the published data were original articles (19) and the remainder were monographies (15). About 44% of the studies were Cohorts and a minority randomized controlled trials (15%) (Table 2). The majority did not specify a time horizon (79,4%) nor had indication of the patients age range (70,6%). Half of the studies performed only genotyping testing to characterize the population and 9 (26,5%) examined drug responses to a panel of genes and/or polymorphisms (Tables 1 and 3).

Summary of the study characteristics

Studies published between 2011 and 2014 were 61,8% of the total whereas 26,5% were published prior to 2010. Most of the research

Table 1. Pharmacogenetic studies performed in Portugal (2000-2019) (references are presented by chronological order)

Population /Region (Type of work/ approach)	Aims	Drug tested/ Class	Genes / SNPs / allelic variants studied or identified		Associated metabolism / disease	Main findings/conclusions	Ref.
80 patients 75 (control group) Original article Genotyping	To screen for the presence of alterations in the phenobarbital-responsive enhancer sequence of the UGT1A1 gene. To investigate a possible association of these alterations with Gilbert syndrome (GS).	not applied	UGT1A1	c.-3279T_G	Gilbert syndrome	The c.-3279T_G polymorphism was a common finding in both GS and control individuals. The c.-3279T_G polymorphism of the UGT1A1 gene could be an additional, if only cumulative, risk factor for the development of GS, thus justifying the inclusion of this polymorphism in routine molecular screening protocols.	[47]
135 healthy individuals Original article Only genotyping	To characterize 15 genetic polymorphisms in a population sample from Portugal.	not specified	CYP2C9 *2 CYP2C9 *3 CYP2C19 CYP3A4*1B CYP3A5*3C GSTP1 313A>G GSTM1*0 ITPA 94 A>C UGT1A1*28 UGT1A1 - 3156 G>A ABCB1 1236 C>T ABCB1 2677 G>A ABCB1 2677 G>T ABCG2 421C>A ERCC2 2251>C TYMS1 1494del		Drug metabolism	Higher frequency of CYP2D6*10, and lower frequency of CYP2D6*6, and duplication of *1 and *2 compared to European population. The allele frequencies in the Portuguese were very similar to other Europeans. There is evidence of some African influence.	[8]
469 patients / Azores region Original article Only Genotyping	To assess 4 polymorphisms in 3 thrombotic risk genes in 469 healthy blood donors from Azores To analyze the CYP2C9 (C430T, A1075C) and VKORC1 (G1639A) variants in individuals with predisposition to thrombosis to evaluate their warfarin drug response genetic profiles.	**Antithrombotic Agents** Warfarin	F5 F2 MTHFR	G1691A G20210 C677T A1298C	Cardiovascular diseases	Azores population shows significant differences on allele frequencies of thrombotic risk factors when compared to mainland Portugal. Thrombotic risk allele frequencies: 1691A (4.9%), 20210A (1.8%), 677T (41.7%) and 1298C (24.8%) were similar to other Caucasians, but significantly different from mainland Portuguese.	[30]
Master's Thesis	Two distinct sub-studies were carried out: a descriptive cross-sectional study, whose information was collected from previously defined hospital services; and sub-study 2, which was based on a consensus technique - Delphi Panel.				Oncology	In sub-study 1 information was obtained from 4 oncologic research services, and in these services the use of genetics in therapeutic decisions, when it happens, it is not in a pharmacogenomics perspective, but from a perspective of characterizing the disease or its stage. In sub-study 2, the institutions identified the indication of the experts, the few responses received were mostly via telephone. Data with potential in cancer prevention and chemotherapy.	[48]

45 patients / Centre region Original article Only Genotyping	To analyze the effects of multiple candidate genes on clinical improvement and occurrence of adverse drug reactions, in 45 autistic patients who received monotherapy with risperidone up to 1 year. Candidate genes involved in the pharmacokinetics and pharmacodynamics of the drug, and the brain-derived neurotrophic factor (BDNF) gene, were analysed.	Risperidone	CYP2D6 ABCB1 HTR2A HTR2C DRD2 DRD3 HTR6 BDNF		Autism	Risperidone therapy is effective in reducing some autism symptoms and caused few serious adverse effects. The HTR2A c.-1438G4A, DRD3 Ser9Gly, HTR2C c.995G4A and ABCB1 1236C4T polymorphisms were predictors for clinical improvement with risperidone therapy. The HTR2A c.-1438G4A, HTR2C c.68G4C (p.C33S), HTR6 c.7154–2542C4T and BDNF c.196G4A (p.V66M) polymorphisms influenced prolactin elevation. HTR2C c.68G4C and CYP2D6 polymorphisms were associated with risperidone-induced increase in BMI or waist circumference. It was identified several genes implicated in risperidone efficacy and safety in autism patients.	[31]
92 Portuguese 151 Mozambican 91 Colombian subjects Original article Only Genotyping	To study selected genetic polymorphisms in drug metabolizing enzymes in 3 different ethnic groups - Portugal, Mozambique and Colombia. PCR-RFLP genotyping methods were developed for drug metabolizing enzymes, namely, cholesterol 7α-hydroxylase, sterol 27-hydroxylase and oxysterol 7α-hydroxylase to characterize the allelic distribution of these polymorphisms among 3 different ethnic/geographic origins.	not specified	CYP7A1	rs3808607 rs3808608 rs3824260 rs8192874 rs58192875 rs61733615	Cardiovascular diseases	A total of 12 CYP7A1, CYP27A1 and CYP7B1 genetic variants were genotyped. The variants N233S in CYP7A1 and 1774C>T in CYP7B1 were not detected in any population studied. The promoter polymorphisms in CYP7A1 (−203A>C, −346C>T, −496C>T) had high frequency in the 3 ethnic groups. G347S (CYP7A1), R164W, A169V and V400A (CYP27A1) were present in a low frequency but with a similar distribution in the 3 groups. Significant differences were observed for D273N (CYP27A1), −346C>T (CYP7A1), −116C>G and R324H (CYP7B1). There is a high variability of drug metabolizing enzymes between the populations, indicating that at least some of these polymorphisms are ethnic specific.	[32]
			CYP27A1	rs59443548 rs11559242 rs6994547			
			CYP7B1	rs5935258 rs8192907			
170 asthmatic individuals and 174 healthy individuals/ Center region Master's Thesis Only Genotyping	Determine the genotypic and allele frequencies of the repeat polymorphism in intron 4 of eNOS and of nucleotide substitution polymorphisms in amino acid 16 and 27 of ADRß2 in individuals suffering from asthma. Predict a possible association between the studied genotypes and the development of the disease.	not specified	ADRß2	Arg16Gly Gln27Glu	Asthma	It was found an association between the 27bp repeat polymorphism of the eNOS gene and susceptibility to asthma. The prevalence of the Arg and Gly alleles of the ADRß2 Arg16Gly polymorphism in asthmatics is 68.3% and 31.7%, respectively. In the ADRß2 Gln27Glu polymorphism, the prevalence in mutated allele (Glu) in asthma patients is higher than in the wild allele (Gln). No association was found between eNOS or ADRβ2 polymorphisms and response to therapeutics.	[49]
			eNOS				
Master's Thesis	Revision work about the importance of pharmacogenetics in rheumatoid arthritis. Identified the main polymorphisms that may influence the efficacy, safety and applicability of this knowledge in clinical practice.	**Immunosuppressants** antirheumatic agents anti-TNF azathioprine methotrexate sulfasalazine			Rheumatoid arthritis	Clinical pharmacogenetic testing is used only with AZA. The SSZ genotyping has the potential to identify patients with greater susceptibility to toxicity. More studies are needed for the methotrexate and biological agents in order to clarify the mixed and contradictory results of the current studies.	[50]
300 healthy individuals / Center region Original article	To characterize CYP2D6 polymorphisms and predict metabolic profiles in the Portuguese population.	not specified	CYP2D6*1 CYP2D6*2 CYP2D6*4 CYP2D6*10		Drug metabolism	Higher frequency of CYP2D6*10, and lower frequency of CYP2D6*6, and duplication of *1 and *2 compared to European population. Important data in terms of effectiveness and safety in the exposure to xenobiotics, and in personalized pharmacotherapy.	[33]

Master's Thesis	Revision work about the studies that demonstrate the influence of polymorphisms in the treatment of tuberculosis, aimed at the Caucasian / Portuguese population.	not applied	CYP2E1 GSTM1 GSTT1	Tuberculosis	The genotyping of certain enzymes may bring benefits not only in reducing complications and side effects, but in reducing appointments and hospitalizations.	[51]
101 patients Madeira region Doctoral Thesis	Analysis of multiple environmental factors assessed by questionnaire and the genotyping of SNPs IL131c.144 G/A, IL41590 C/T, IL41RP2 253183, ADRB21c.16 A/G, ADAM33-V4 C/G, ADAM33-S1 c.710 G/A, GSDML1236 C/T and STAT6121 C/T in a sample of Madeiran asthmatic patients and their families, and their association to asthma susceptibility and severity was assessed.	not applied	IL131-c.144 G/A IL4-590 C/T IL4RP2 253183 ADRB2-c.16 A/G ADAM33-V4 C/G ADAM33-S1 c.710 G/A GSDML-236 C/T STAT6-21 C/T	Asthma	IL41590*T, IL41RP2*183 as well as the combined genotypes IL41590*CT/ IL41590*TT and IL41RP2*253183/ IL41RP2*253183 were associated to both asthma susceptibility and severity. GSDML1236*TT was found associated only to asthma severity. ADAM331-V4*C was significantly over transmitted to asthmatic offspring being linked with the disease. The findings suggest that in addition to environmental influences, IL41590 C/T, IL41RP2 253183, ADAM331V4 C/G and GSDML1236 C/T SNPs may constitute important genetic factor contributing to asthma susceptibility and/or severity in Madeira population.	[52]
25 patients / not specified Original article Only Genotyping	To study the association of HLA-B*58:01 with allopurinol-induced sCADR in a Portuguese population.	**Antigout preparations** Allopurinol	HLA-B*58:01	DRESS SJS / TEN	HLA-B*58:01 was present in 16 patients with sCADR (64%) [12 DRESS (63%), four SJS/TEN (67%)], one allopurinol-tolerant individual (4%) and 63 normal controls. When compared with the normal population, HLA-B*58:01 was associated with a higher risk of sCADR, both DRESS and SJS/TEN. There was no statistically different risk between the 2 types of CADR. Portuguese patients with sCADR from allopurinol, both DRESS and SJS/TEN, have a high frequency of HLA-B*58:01, with an OR similar to European patients with SJS/TEN.	[34]
Master's Thesis	A bibliographic review focus on pharmacogenetics and HIV therapy, looking closely at several types of antiretroviral drugs: the nucleotide reverse transcriptase inhibitors, the non-nucleotide reverse transcriptase inhibitors and the protease inhibitors and the way that several genes change the therapeutical response.	**Antiretrovirals** CCR5 antagonists Fusion inhibitors Integrase inhibitors		HIV therapy	There is still a large amount of information that needs to be investigated and understood in relation to the possible interactions between genotypes, pharmacokinetics and pharmacodynamics of antiretrovirals. Genetic variants are responsible for inter-individual variability, regarding the effectiveness of antiretroviral treatment and in the emergence of adverse reactions.	[62]
116 patients 70 Portuguese non-Gypsy (control group) / not specified Master's Thesis Only Genotyping	To characterise a sample of Portuguese Gypsies for a selected panel of SNPs in genes with pharmacogenetic relevance, and to compare the derived pharmacogenetic profile with that of the Portuguese host population.	not specified	CYP2C9: rs1799853 rs1057910 CYP2C19: rs4244285 NAT2: rs1041983 rs1801280 TPMT: rs1800462 rs1800460 rs1142345 rs56161402 VKORC1: rs9923231	Drug metabolism	For the screened variations no departures from Hardy-Weinberg equilibrium were detected in the Portuguese Gypsy and Portuguese non-Gypsy populations. No major differences were detected in the results of the 2 populations.	[55]

51 patients / Centre Master's Thesis Only Genotyping	To characterize a sample of Portuguese diabetic individuals, through survey of a single nucleotide polymorphisms set identified in the literature and databases (related to pharmacogenetics of DM2 in coding region and for the European population (EUR)), followed by a correlation of the results with the drugs administered to individuals in the sample. To propose a set of SNPs candidates for pharmacogenetics of DM2.	**Sulphonylureas** Glibenclamida Gliclazida Glimepirida Sulphonylureas Tolbutamida Tolbutamida	ABCC8	rs1799859 rs1801261	Diabetes Mellitus type 2 (DM2)	There were identified SNPs associated with pharmacogenetics of DM2 in scientific articles and databases, where a total of 98 SNPs were identified. The SNPs were filtered for those in the coding region and associated with the EUR population. Sixteen SNPs were observed in 10 genes in the 51 patients. Correlation of drugs administered in the sample in study with the results from the survey of these SNPs, indicated that individuals carrying the SNPs rs12208357, rs34130495 and rs3405950 (SLC22A1) needed a higher dose of metformin, the carriers of SNP rs1801282 (PPAR) needed tighter control treatment metformin (given the increased risk of failure for this treatment) and carriers of SNP rs5219 (KCNJ11) needed a higher dose of gliclazide. It was proposed a set of potential SNPs candidates for future studies concerning the pharmacogenetics of DM2 in the Portuguese population 32 SNPs located in the coding region and not yet referenced in the EUR population were selected from the set of 98 SNPs. Additional 15 SNPs not yet related to the DM2 were also identified in the 51 patients.	[53]
			CYP2C9	rs1799853 rs1057910			
			IRS1	rs1801278			
			KCNJ11	rs5219			
		Meglitinides					
		Repaglinida Nateglinida	CYP2C8	rs10509681 rs11572080			
			CYP2C9	rs1057910			
			SLCO1B1	rs4149056 rs2306283			
		Thiazolidinediones					
		Rosiglitazona Pioglitazona	CYP2C8	rs10509681 rs11572080			
		Biguanides					
		Metformina	SLC22A1	rs72552763 rs35167514 rs12208357 rs34130495 rs34059508 rs1867351			
			PPARγ	rs1801282			
		Inibitors of α glicosidase					
		Acarbose	PGC-1α (PPARGC1A)	rs8192678			
		GLP-1 and analogues					
		GLP-1	THADA	rs7578597			
Master's Thesis	A review work that aimed at present an overview on the state of the art of pharmacogenomics, with the introduction of its basic concepts, how genetic polymorphisms may modulate individual's susceptibility to certain diseases or influences the therapeutic efficacy and safety of drugs.	not applied				It was concluded that Pharmacogenomics has reached a state in which it makes possible, if not an individual tuning of the pharmaceutical regímen, at leats the definition of groupings of individuals with similar genetic profiles and a corresponding more homogeneous response to drugs.	[54]
242 patients /Center Original article Only Genotyping	To identify clinical and/or genetic predictors of response to several therapies in Crohn's disease (CD) patients.	**Immunosuppressants** Azathioprine Biologicals Corticosteroids	ABCB1 C3435T, G2677T/A IL23R G1142A C2370A G9T CASP9 C93T Fas G670A LgC844T ATG16L1 A898G		Crohn's disease	Older patients responded better to 5-aminosalicylic acid (5-ASA) and to azathioprine while younger ones responded better to biologicals. Previous surgery negatively influenced response to 5-ASA compounds but favoured response to azathioprine. Heterozygotes for ATGL16L1 SNP had a significantly higher chance of responding to corticosteroids, while homozygotes for Casp9 C93T SNP had a lower chance of responding both to corticosteroids and to azathioprine. TT carriers of ABCB1 C3435T SNP had a higher chance of responding to azathioprine, while carriers of ABCB1 G2677T/A SNP, as well as responding better to azathioprine, had a lower chance of responding to biologicals, which became significant after adjusting for gender.	[35]

Cohort Original article	To elucidate the role of methylenetetrahydrofolate reductase (MTHFR) C677T and aminoimidazole carboxamide adenosine ribonucleotide transformylase (ATIC) T675C polymorphisms and clinicopathological variables in clinical response to MTX in Portuguese rheumatoid arthritis (RA) patients.	Methotrexate	MTHFR C677T ATIC T675C		Rheumatoid Arthritis	MTHFR 677TT and ATIC 675T carriers were associated with over 4-fold increased risk for nonresponse. For clinicopathological variables, noncurrent smokers, patients positive to anti-cyclic citrullinated peptide and antinuclear antibodies, with higher health assessment questionnaire score, and nonsteroidal anti-inflammatory drug users were also associated with nonresponse. Subcutaneous administration route was associated with response. MTHFR C677T and ATIC T675C genotyping combined with clinicopathological data may help to identify patients who will not benefit from MTX treatment and, therefore, assist clinicians in personalizing RA treatment.	[36]
233 Caucasian / North region Original article	To elucidate the influence of TYMS polymorphisms in MTX therapeutic outcome (regarding clinical response and toxicity) in Portuguese RA patients.	Methotrexate	TYMS	rs34743033 rs2853542 rs34489327	Rheumatoid Arthritis	TYMS polymorphisms could be important to help predicting clinical response to MTX in RA patients. Translation into clinical practice needs larger studies to confirm the evidences.	[37]
233 patients North region Original article	To evaluate the influence of single nucleotide polymorphisms (SNPs) in genes encoding for MTX transporters with the occurrence of MTX-related toxicity (overall and gastrointestinal).	Methotrexate	SLC16A7	rs3763980 rs10877333	Rheumatoid Arthritis	SLC19A1, SLC46A1 and SLCO1B1 genotypes may help to identify patients with increased risk of MTX-related overall toxicity and that SLC19A1 and SLCO1B1 genotypes, and SLC19A1 haplotypes may help to identify patients with increased risk of MTX-related gastrointestinal toxicity.	[38]
			SLC19A1	rs7499 rs1051266 rs2838956 rs3788200			
			SLC22A6	rs11568626			
			SLC22A11	rs11231809			
			SLC46A1	rs2239907			
			SLCO1B1	rs4149056			
			ABCB1	rs1045642 rs1128503 rs2032582			
			ABCC1	rs35592 rs246240 rs2074087 rs2230671 rs3784864			
			ABCC2	rs717620 rs4148396			
			ABCG2	rs2231142 rs13120400 rs17731538			
Master's Thesis	A review work about Pharmacogenomics of addiction	not applied	SNP VNTR		Drug addiction	Drug addiction remains an unsolved health issue and has limited treatment options currently available. The existing medications were not developed having a thorough knowledge of genetic and neurobiological causes of the disease. More replication data is needed concerning some genetic variants to allow the identification of functional variants, but also the need for larger population samples has become clear for detecting small effect variants from the many genes accountable for addiction.	[56]

Master's Thesis	A bibliographic review on pharmacogenetics in the treatment of breast cancer. To study of the influence that genetic polymorphisms of metabolic enzymes, efflux transporters and estrogen receptors, have on response to tamoxifen therapy for breast cancer	not applied	CYP2C19*2 CYP2C19*17 CYP3A4 CYP3A4*22 CYP3A4*1B CYP3A5 SULT1A1 SULT1A1*2		Breast cancer	Studies that analysed the role of CYP2D6 genotype in response to tamoxifen are controversial, most of these argue that poor metabolizers and intermediate have worse results than extensive metabolizers, although in only one of the studies with higher impact the same association was observed. In the CYP3A4, the T allele for the CYP3A4*22 polymorphism was associated with higher concentration of tamoxifen and its metabolites and CYP3A4*1B polymorphism was associated with increased risk of developing endometrial cancer. The polymorphisms at the estrogen receptors have demonstrated an important role in modulating the response to tamoxifen treatment on regards secondary and adverse effects. Larger population and greater control over variables are needed to reach a personalized medicine.	[57]
Lisbon Porto Coimbra Master's Thesis	Evaluation of pharmacogenomics in Portuguese clinical practice, identification of the skills and techniques for the application of pharmacogenomics and the potential areas of pharmacogenomics in clinical practice.	Azathioprine	not specified		Oncology and infectious diseases	Pharmacogenomics/genetics in Portugal has some degree of weakness; is not a routine in clinical practice, not well known among professionals, genetics is not mainly use for therapeutic purposes. The service of IPO Lisbon uses de detection of Human Epidermal growth factor Receptor-type 1 (HER1) for specific therapeutics of breast cancer.	[58]
Original article	To revise the major definitions in the pharmacogenetics as well as some classic examples of its application (related to cytochrome P450 genes, NAT2 gene and the Cholinesterase gene; the multitude of existing drug targets, like in case of G6PD gene, and the VKORC1 gene).	not applied	P450 genes NAT2 G6PD VKORC1		not applied	Pharmacogenetics contribute to the development of new drugs, since it gives an important data, namely the knowledge of the potential variability associated with metabolization and/or action of the drug. In addition, pharmacogenetics also plays an important role in reducing the number of patients who must participate in clinical trials, and potentially reduce the risk of failure in the lead-up stages in the market.	[39]
52 individuals with a suggestive phenotype of trimethylaminuria and 100 healthy individuals/ not specified Original article Only Genotyping	Portuguese patients with phenotype suggestive of TMAu were evaluated for molecular screening of the FMO3 gene.	not specified	FMO3		TMAu	Identification of 32 variants in the FMO3 coding region. P.Glu158Lys and p.Glu308Gly polymorphisms, in combination with other variants, originate different phenotypic patterns, which may lead to an abnormal drug metabolism in the liver and other organs and tissues.	[40]
208 patients / South region Original article Genotyping and drug testing	To assess the pharmacogenetic profile of a South Portuguese population according to established dosing guidelines for commonly prescribed drugs and to compare it with that of previously genotyped populations.	Thiopurines Clopidogrel Warfarin Fluoropyrimidines Irinotecan Codeine Tricyclics	ABCB1	rs1045642 rs1128503 rs2032582	Drug metabolism	It was found a significant small differentiation between the Portuguese regional populations regarding CYP2C9 rs1057910, CYP2D6 rs3892097, MTHFR rs1801133 and F5 rs6025. When considering 4 HapMap populations, ADH1B rs2066702, ADH1B rs1229984, NAT2 rs1799931 and VKORC1 rs9923231 displayed a significant population differentiation. 18.9% of the participants are intermediate or poor metabolizers for at least 3 drugs simultaneously and that 84.6% of the participants have at least one therapeutic failure or ADR risk allele for the considered drugs. There is a high prevalence of risk alleles associated with an altered drug metabolism regarding drugs largely used by the South Portuguese population.	[41]
			ADH1B	rs1229984			
			ADH1C	rs698			
			ADRB2	rs1042714			
			COMT	rs4680			
			CYP2C19	rs4244285 rs4986893			
			CYP2C8	rs11572080			
			CYP3A5	rs776746			
			CYP2D6	rs3892097			
			DPYD	rs67376798 rs1801265 rs55886062			
			F5	rs6025			
			GSTP1	rs1695			
			KCNJ11	rs5219			
			TPMT	rs1800462 rs1800460 rs1800584 rs1142345			

233 patients / North Original article Genotyping and drug testing	Clinicopathological data from rheumatoid arthritis patients treated with methotrexate were collected, clinical response defined, and patients genotyped for 23 single nucleotide polymorphisms. Genotype and haplotype analyses were performed using multivariate methods and a genetic risk index for non-response was created	**Immunosuppressants Antimetabolites**	SLC16A7	rs3763980 rs10877333	Rheumatoid Arthritis	Genetic polymorphisms in SLC22A11 and ABCC1 could be predictors of clinical response to methotrexate in Portuguese rheumatoid arthritis patients. Genotyping patients according to these genetic markers may be helpful to identify which patients will not benefit from methotrexate treatment, highlighting the relevance of developing the field of personalized medicine.	[42]
		Methotrexate; Methotrexate + drug panel	SLC19A1	rs7499 rs1051266 rs2838956 rs3788200			
			SLC22A11	rs11231809			
			SLC46A1	rs2239907			
			SLCO1B1	rs4149056			
167 patients Original article Genotyping and drug testing	To evaluate the repercussion of personalization of INH dosing by NAT2 genotyping in the management of tuberculosis patients. To assess the role of other candidate genes like CYP2E1, GSTM1 and GSTT1 encoding detoxifying enzymes, and ABCB11, encoding a protein involved in bile salt transport.	**Antimycobacterials**			Tuberculosis	Clinical variables such as gender and age were not associated with the occurrence of INH-induced hepatitis. Slow acetylators (52.3%), identified by *NAT2* genotyping, were significantly more prone to develop, as well as homozygous for variant Ala of *ABCB11* polymorphism (rs2287622). The presence of both risk genotypes was also significantly associated with increased susceptibility to hepatotoxicity. Risk genotypes were frequent among patients: 52% of SA (*NAT2*), 32% of Ala/Ala (*ABCB11*) and 21% with both risk genotypes.	[43]
		Isoniazid	NAT2 CYP2E1 GSTM1 GSTT1 ABCB11				
Master's Thesis	To develop a Sanger sequencing methodology for CYP2D6 gene to identify genetic variants that cause absence of enzyme activity and its application to post-mortem cases with tramadol	Tramadol	CYP2D6			The methods were successfully applied to post mortem blood samples. Alleles and genotype frequencies were in accordance with the expected for European population. Tramadol metabolism, expressed as its metabolites concentration ratio (N-desmethyltramadol/O-desmethyltramadol), has been shown to be correlated with the predicted phenotypes based on genetic characterization. This is presumably the first time that a CYP2D6 sequencing methodology is validated and applied to post-mortem samples, in Portugal.	[59]
56 Portuguese gypsies / not specified Master's Thesis Only Genotyping	To characterize the Portuguese Roma for Single Nucleotide Polymorphisms (SNPs), relevant from the Pharmacogenetics point of view. To evaluate if the Roma show any peculiarity regarding drug response, in comparison with the host population.	not specified	CYP2D6	rs1065852 rs28371706 rs61736512 rs3892097 s35742686 rs5030656 rs16947 rs28371725 rs59421388 rs1135840	Drug metabolism	For the variations studied, only one SNP revealed significant deviation from the Hardy – Weinberg expectation, even after the Bonferroni correction, which was the 4180 G>C. Comparatively to the Portuguese host population, the Roma showed some differences, especially an increased frequency of the CYP2D6*4, an allele implying null enzymatic activity. Regarding the theoretical metabolic profiles, differences were found, especially the IM and PM profiles.	[60]
1688 patients / North, Centre, South regions Original article Genotyping and drug testing	To determine the prevalence of genotypes associated with a lower efficacy or a higher risk of adverse side effects in the treatment with statins in the Portuguese population. Several SNPs involved in the metabolism, absorption, transport and/or excretion of the various types of statins were genotyped.	Statins	SLCO1B1	rs4149056	Dyslipidaemia	The SLCO1B1*5 variant, associated with an increased risk of developing myopathy on simvastatin treatment, has a frequency 2 times higher in the sample than described in the population databases. This fact, coupled with the large increase in national consumption of statins, mainly simvastatin, is an important factor that should be considered in the decision-making of the prescription of antidislipidemics.	[44]
			APOE	rs7412			
			ABCB1	rs2032582			
			KIF6	rs20455			
			HMGCR	rs17238540			
			POR	rs1057868			
			ABCB1	rs1045642			
			CYP3A5	rs776746			
			CYP2C9	rs1057910			

Master's Thesis	To relate the knowledge underlying the field of Pharmacogenetics with pharmaceutical activity in the hospital context, reviewing the most important topics in this area for hospital pharmaceutical practice.	not specified		Oncology	The pharmacist assumes a central role in the implementation of Pharmacogenetics in clinical practice, since is a health professional with basic knowledge important for understanding this area, having the duty to specialize and help to instruct the remaining members of the research team health about the correct interpretation of the results of pharmacogenetic tests and the best way to apply them.	[61]
Original article	To investigate the impact of CYP2D6 genotyping of the tamoxifen metabolizing enzymes in the clinical management of breast cancer patients.	Tamoxifen	CYP2D6	Breast cancer	Portugal presents an interesting case for comparison in international pharmacogenomics context. This is especially the case of CYP2D6 testing prior to tamoxifen therapeutics. This is because there are no implemented measures based on pharmacogenomics analysis prior to therapy. Changing clinical paradigms involves assessment of several factors and a country with a clinical context as that of Portugal might function as a sample control in such analysis.	[46]
Original article Genotyping and drug testing	To report on the real-life experience of 2 Portuguese dermatology departments with ustekinumab in patients with moderate to severe psoriasis. To identify the clinical characteristics associated with a weaker clinical response.	**Immunosuppressants** Ustekinumab	HLACw*0602	Psoriasis	A PASI75 therapeutic response was observed in 67.2%, 85.3%, 89.6% and 88.7% of patients at weeks 4, 12, 24 and 52, respectively. Neither cardiovascular events nor cases of reactivation of previous infections (tuberculosis, hepatitis B) were observed during follow-up. The therapeutic response was higher in patients naïve to biologic therapies as compared to non-naïve patients. A trend towards lower clinical response was observed in patients weighing between 90-100 kg, and dosage optimization in these patients may be of value prior to considering biologic switch.	[45]

Table 2. Summary of study strategies (N=34)

Type of study	n	%
Cohort	15	44
Review	8	24
Genotype-based screening	6	18
Randomized controlled trial	5	15
Time horizon		
Lifetime	0	0,0
≥ 20 years	0	0,0
3-10 years	4	11,8
≤ 3 years	3	8,8
No statement	27	79,4
Patient age range		
≥ 60 years	1	2,9
< 60 years	9	26,5
Not stated or not applicable	24	70,6
Pharmacogenomics approach		
Study focused only on genetic/genomic testing	17	50,0
Genotyping and drug testing	9	26,5
None of the above	8	23,5

Table 3. Summary of study strategies (N=34)

Drug	Category	n	%
Methotrexate; Methotrexate + drug panel	Immunosuppressants; Antimetabolites	7	20,6
Clopidogrel, Warfarin	Antithrombotic agents	2	5,9
Azathioprine, Biologicals, Corticosteroids	Corticosteroids, Immunosuppressants; biologics	1	2,9
Allopurinol	Antigout preparations	1	2,9
Azathioprine, Sulfasalazine, Methotrexate, Biological agents	Immunosuppressants; Intestinal anti-inflammatory agents; Antimetabolites; Biologics	1	2,9
Biguanides, Meglitinides, Sulphonylureas, Thiazolidinediones	Blood glucose lowering drugs	1	2,9
Codeine	Anti-infectives	1	2,9
Fusion inhibitors, CCR5 antagonists, Integrase inhibitors	Antiretrovirals	1	2,9
Isoniazid	Antimycobacterials	1	2,9
Risperidone	Psycholeptics, Antipsychotics	1	2,9
Statins	Lipid modifying agents	1	2,9
Tamoxifen, Irinotecan	Antineoplastic and Immunomodulating agents	1	2,9
Tramadol	Analgesics	1	2,9
Ustekinumab	Immunosuppressants	1	2,9
not specified		13	38,2

Table 4. Summary of study characteristics (N=34)

Year	n	%
2007-2010	4	11,8
2011-2014	21	61,8
2015-2018	9	26,5
Portuguese region		
North	3	8,8
Centre	4	11,8
South	1	2,9
Azores	1	2,9
Madeira	1	2,9
Not specified or not applicable	24	70,6
Disease indication or condition		
Rheumatoid Arthritis	5	14,7
Breast Cancer	3	8,8
Asthma	2	5,9
Tuberculosis	2	5,9
Cardiovascular diseases	2	5,9
Autism	1	2,9
Crohn's disease	1	2,9
Diabetes mellitus type 2	1	2,9
Drug addiction	1	2,9
DRESS; Stevens–Johnson Syndrome (S-JS) / Toxic Epidermal Necrolysis (TEN)	1	2,9
HIV	1	2,9
Leukemia	1	2,9
Lung cancer and Metastatic colorectal cancer	1	2,9
Psoriasis	1	2,9
Severe dyslipidemia	1	2,9
Trimethylaminuria	1	2,9
not specified	9	26,5

omitted the place of origin of the patients (70,6%) whilst the populations from North and Centre of Portugal were the most represented in the studies (20,6%) (Table 4).

The illness kind that deserved more attention from the local scientific community was heterogeneous (Table 4), namely the non-communicable diseases such as Rheumatoid arthritis and Breast cancer.

Similarly, the set of genes and/or polymorphisms screened or tested in the Portuguese population were mainly associated with drug metabolism and rheumatoid arthritis phenotypes (11.8%) (Table 1). About 35,3% of the studies presented a diverse set of analysed genes. The studied drug types were very diverse (Tables 1, 3, 4), being the immunosuppressants and antimetabolites the most tested categories (20,6%).

Research trends on pharmacogenomics in portugal

An overview of the research trends in pharmacogenomics and the leading causes of disability and premature death in the country is illustrated in Figure 2. From 16 diseases or conditions subjected to pharmacogenomic studies, only 5 (Breast cancer, Cardiovascular diseases, Diabetes, Lung cancer and Metastatic colorectal cancer) belong to the list of top leading causes of premature death in Portugal. In addition, the leading causes of disability (Cardiovascular diseases, Diabetes, Lung cancer and Metastatic colorectal cancer) are only partially subjected to pharmacogenomic studies. Most of the disabling diseases reported in the country are non-communicable diseases, namely those related with low back and neck pain and the cardiovascular system [11,12].

Discussion

Pharmacogenomic research represents an important tool in drug development and pharmacovigilance, which helps the improvement of healthcare [13]. Several options are available for the design of pharmacogenomic studies, among them the case-control study, cohort and randomised controlled clinical trials (RCTs) [14]. The study types performed in the pharmacogenomic area in Portugal were mostly cohorts (44%). In such cases, participants are enrolled based on their phenotype or drug response and information regarding their exposures are collected retro- or prospectively. This covers any number of exposures (medical, environmental, comorbidities, etc.) as well as a DNA sample for genotyping [15,16].

As the research progresses we should expect an increase of cohort and of RCTs studies in order to assess potential drug-gene interactions [14]. RCTs are considered the gold standard of study designs in pharmacogenomic-guided treatment approach. The lack of such research impairs the clinical utility and validity of pharmacogenomic testing [17].

Our findings showed that 50% of the studies in Portugal performed only genotyping testing to characterize the population, whereas 26.5% did drug testing for a disease or condition besides genotyping. The reported sample sizes are small, in line with many pharmacogenomics trials or drug response studies which had very limited sample sizes [16].

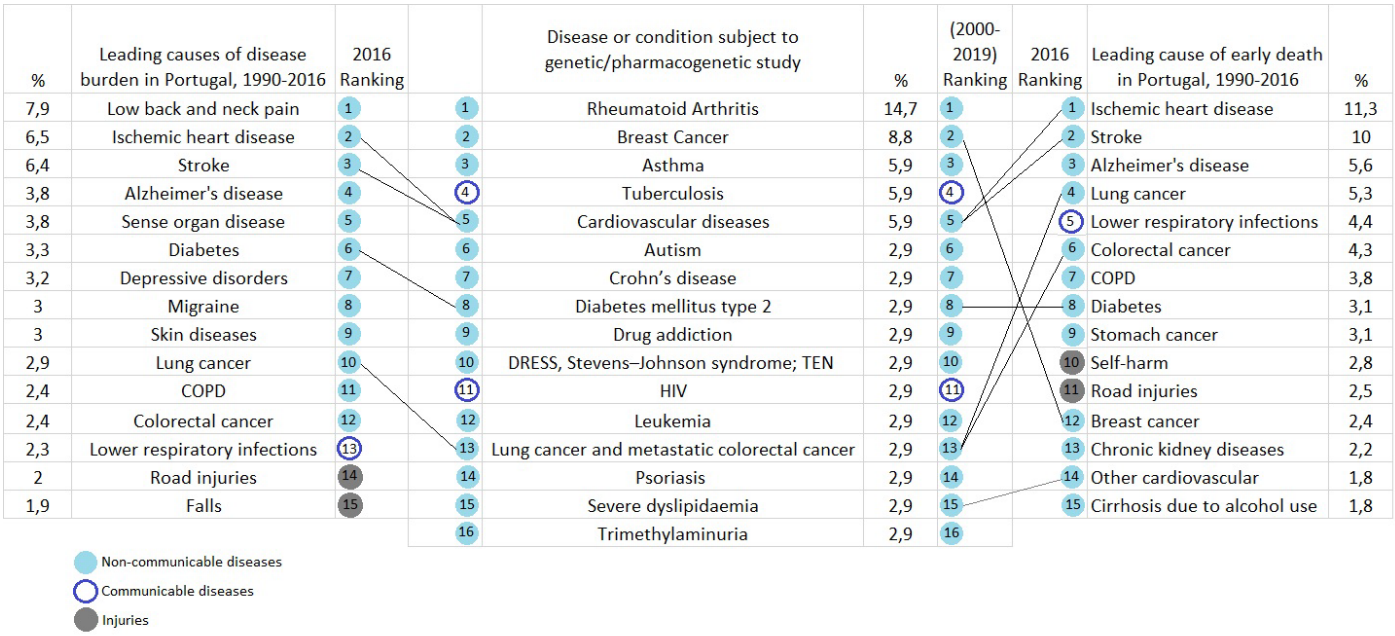

Figure 2. Correspondence between investigated diseases (present review) and the leading causes of disability and premature death in portugal (DGS, 2018)

Globally, laboratories which perform pharmacogenomic tests are still few, reflecting low clinical uptake, and our country is not an exception. Known reasons for the so far rather limited clinical implementation of pharmacogenomic testing include the following common barriers: scientific, educational, legal, ethical, social issue (ELSI), lack of information technology, reimbursement besides other barriers have been identified worldwide [17,18]. Regulations regarding Direct to Consumer (DTC) genetic testing including pharmacogenomic testing differ in scope and strictness from country to country. Laws in Portugal, France, Germany and Switzerland restrict the prescription of genetic tests by physicians after counselling and proper informed consenting process, which makes DTC genetic tests illegal in these countries [19].

Apart from the legal issues, each clinical practice setting has its own requirements for implementation and few physicians and health systems use pharmacogenomic in clinical practice [17]. As in other European countries, the majority of the pharmacogenomic surveys that took place in Portugal were developed in collaboration with medical institutions, Universities and research laboratories, a strategy that favors the integration of pharmacogenomics in the healthcare system.

We have found that over half (61.8%) of the studies were published between 2011 and 2014. In fact, pharmacogenomic testing worldwide has been performed since the last decade [20]. Also, the number of published economic evaluation analyses in the field of genomic and personalized medicine was particularly evident since 2011 [21]. As such, it is expected that more cost-effective analyses should cover as many populations and/or ethnic groups as possible in various countries [21], including Portugal.

It was shown that the diseases or conditions that gain most of the attention of the Portuguese scientific community, from a pharmacogenomic perspective, were Rheumatoid Arthritis, Breast cancer, Asthma, Cardiovascular diseases, and the communicable disease Tuberculosis. On the other hand, our survey revealed that the leading causes of disability in Portugal were only partially subjected to pharmacogenomic studies, namely those related with Cardiovascular diseases (stroke and ischemic heart disease), Lung cancer and Metastatic colorectal cancer. Most of the listed diseases (Figure 2) are complex disorders which, according to Warnich *et al.* [22], demand high standard genomics studies so that they can be integrated into a holistic approach adapted to the individual profile and preferences.

Regarding the Portuguese situation, there are strict laws on genetic testing. Firstly, a national law imposes that diagnostic or pharmacogenetic testing should follow the general principles of all other health care interventions. Secondly, when a hereditary disease is identified in a patient, the physician should inform the patient about the mechanism of transmission and refer him/her to a genetics team. Nevertheless, the advances in the implementation of personalized medicine at a national level, as in most of the countries currently involved in pharmacogenomic research, have been made essentially in the area of oncology and rare diseases [23,24]. On regards to rare diseases, genomic tests and pre-emptive genotyping have been directed to those specific populations which cannot be easily treated with conventional interventions or based on drugs with narrow therapeutic windows. Pre-emptive genotyping is often crucial for a specific population, in particular in carriers of the HLA-B*1502 allele which have a high risk of developing Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN). Studies in different populations, including in Portugal, reported a prevalence of the HLA-B*5701 gene of 3.7-6.1%, which justifies the importance to pursuit with the pharmacogenomic research in the Portuguese population.

It's expected that the introduction of genomic testing in clinical practice will contribute to the rationalization of established drug-prescription regimens and lead to the design of new individualized interventions with maximized efficacy and minimized adverse drug reactions [21]. Currently, all countries are at different stages of clinical

implementation or undergoing implementation projects to improve their strategy. In addition, the development of pharmacogenomics has not been as fast as expect and the Portuguese reality is similar that of other countries. According to Warnich *et al.* [22], to ensure sustainable research in pharmacogenomics in a country, particular attention should be given to key areas with regards to genomic study design: (i) national and international multi-disciplinary collaborations, consortia and research networks, (ii) the collection and storage of biological samples in biorepositories. On this respect, several ongoing initiatives of pharmacogenomic implementation have been launched in Europe, United States and Asia [7,25]. Two Portuguese entities (Ricardo Jorge Institute and Foundation for Science and Technology) along with more than 35 European and international institutions, are part of the ICPerMed Consortium that aims to identify and implement priority actions in personalised medicine research [26]. Ultimately, the set up of such consortia and research networks benefits the organization and extension of pharmacogenomic studies.

This review has shown that Portugal has capabilities to increase research in the field of genomic medicine, using its know-how on genotyping and genome sequencing for disease prediction, diagnosis, prevention and treatment. The *CYP2D6* gene and others CYP genes and its polymorphisms have been the most screened in the Portuguese population. This evidence is not surprising, considering that *CYP2D6* is one of the most extensively studied drug-metabolizing enzymes and pharmacogenes [27]. Further, the CYP genes are ubiquitous in nature, when expressed they function on many substrates and show an overlap of substrate specificities [28].

As in other similar countries, the national pharmacogenomic research has focused on a few common gene variants, although with large size effects. This spectrum of effects has complicated the design and lead to large clinical trials that often focus on individual drugs [4,22], a fact also reported in our work. It's known that variation in drug response may be due to multiple genes, each with small effects. In these cases, large sample sizes will be needed to identify the effects [16].

Lastly, the most studied drug types nationwide from a pharmacogenomic perspective were the ones associated with the treatment of the respective illness or condition (Table 4). However, pharmacogenomic studies should focus on drugs used in the treatment of the diseases that contribute to the highest disease burden faced by the population [22].

Important steps to determine the prevalence of the pharmacogenomic biomarkers in our population were taken. On this respect, Mizzi *et al.* [29], stressed that the frequency of a high-risk pharmacogenomic biomarker alone is not adequate for developing national medication guidelines, but rather in conjunction with other factors, such as the previously mentioned disease burden.

Conclusions

Most research on pharmacogenomics performed in Portugal occurred in the early 2010's and half of the studies performed only genotyping testing to characterize the population. The core investigation focused on non-communicable diseases like Rheumatoid arthritis, Breast cancer and Asthma.

As in other countries, regular pharmacogenomic testing is still scarce in the country and the leading causes of disability are only partially subjected to pharmacogenomic studies. However, there is a growing interest in research in pharmacogenomic area. The published results are significant and can contribute for the improvement of personalized medicine. The already established research consortia and networks should also make the organization and translation of pharmacogenomics studies more streamlined.

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Conflicts of interest

None.

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