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Title: Genomic contextualization of single nucleotide polymorphisms used in forensic genetics for human identification

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Abstract:

Single nucleotide polymorphisms (SNPs) are an interesting option to facilitate the analysis of highly degraded DNA by allowing the reduction of the size of the DNA amplicons. The SNPforID 52-plex panel is a clear example of the use of non-coding SNPs in Forensic Genetics. However, nonstop advances in studies of genetic polymorphisms are leading to the discovery of new associations between SNPs and diseases.

The aim of this study was to perform a comprehensive review of the state of association between the 52 SNPs in the 52 plex-panel and diseases or other traits related to their treatment, such as drug response characters. In order to achieve this goal, we have conducted a bioinformatic search for each SNP included in the panel and the SNPs in linkage disequilibrium (LD) with them. A total of 424 SNPs (52 in the panel and 372 in LD) were investigated in PubMed, Scopus and dbSNPs databases. Our results show that three SNPs in the SNPforID 52-plex panel (rs2107612, rs1979255, rs1463729) have been associated with diseases such as hypertension or macular degeneration, as well as drug response. Similarly, three out of the 372 SNPs in LD (rs2107614, rs765250, rs11064560) are also associated with various pathologies. In view of these results, we propose the need for a periodic review of the SNPs used in forensic genetics in order to keep their associations with diseases or related phenotypes updated and to evaluate the interest of their continuity in the panels of forensic utility.

Keywords:

SNPs, linkage disequilibrium, forensic genetics, genetic susceptibility to disease.

Introduction

The increasing knowledge of the genome has represented a major impact on forensic genetics [1]. Genetic characterization of individuals through DNA analysis allows identification processes from small amounts of biological remains [2, 3]. Microsatellites (STRs) of autosomes and X and Y chromosomes, as well as mitochondrial DNA, are the polymorphisms most frequently used [3]. However, the analysis of STR loci becomes problematic when applied to degraded DNA samples, common in genetic casework, since the multiplex PCR amplification of the STR loci requires lengths of template DNA fragments between 150 and 400 bp [4, 5, 6].

Two approaches have been proposed in order to solve this problem, the shortening of the amplicons of the STR loci (miniSTRs) or the use of single nucleotide polymorphisms (SNPs). However, reducing the size of the amplified product is not always enough to solve the numerous cases in which DNA is highly degraded [5]. In those cases, the use of SNPs, which are highly common genetic variations with a distribution of approximately one SNP every 100-300 nucleotides, has been proposed [7].

SNPs allow the reduction of the amplified product to less than 100 bp and, in addition, they have a low mutation rate in the order of 10^{-8}/locus/generation [8], which makes them really stable genetic markers. Another advantage of SNPs analysis is the wide range of available typing methods, which are fast and amenable to automation [9]. In particular, the latest massive sequencing platforms allow the simultaneous study of a large number SNPs in a short time [10]. Because of all these advantages, it is expected that these markers become frequently used in forensic laboratories in the near future.

Sanchez et al. [11] developed a panel of 52 biallelic SNPs selected by the SNPforID consortium. This panel was successfully validated for use in forensic genetic investigations by the SNPforID 52-plex assay performed by Musgrave-Brown et al. [12].

The selection criteria for these polymorphisms included that the size of the amplicon were less than 120 bp, that they presented a minimum 30% heterozygosity in at least one of the studied populations, African, European and Asian, and a minimum 20% heterozygosity in the three populations. SNPs were selected from the distal portions of the p and q arms of each autosome and they checked that there was a minimum distance of 100 kb between candidate SNPs and close genes. In addition, they checked that there were not a probable association with the STRs loci most commonly used in forensic analysis. The DNA sequences flanking the candidate SNPs had to be reliably reported and should be free of polymorphisms that could interfere with primer binding.

In addition, SNPs are commonly used markers in determining susceptibility to various diseases [13, 14] because some SNPs may play an important biological role as they cause changes in the DNA sequence and, sometimes, they are able to affect the expression of genes or the encoded proteins [15]. However, it is well established that the selected markers for human identification testing have to be in sequences that do not affect the expression of the genome or the encoded proteins, nor should they be associated with the risk or progression of any disease [16].

Therefore, if such a condition were demonstrated, it would be advisable not to include those markers in forensic tests [17, 16]. Additionally, we must take into consideration that the SNPs may be part of linkage disequilibrium (LD) blocks, so that an SNP is in correspondence with all the SNPs from that block. As a result, non-coding SNPs may be in LD with other SNPs located in coding or regulatory regions [7]. Therefore, it should be taken into consideration that those 52 SNPs or any of the SNPs in LD with them might provide information about the risk or progression of a disease and, if so, it would be necessary to evaluate their stay in the current panel.

Subsequently, the objective of this study is to determine whether there is any reported association between any of the SNPs proposed by the SNPforID consortium and any disease or other endophenotypic trait, taking into account both the 52 SNPs included in the panel and the SNPs in LD with them.

Materials and methods

Search of SNPs in likage disequilibrium

As a criterion to determine the size of the region where the SNPs in LD were searched, among the genes harboring any of the SNPs included in the work, the larger was selected (*RBFOX1*). As a result, a region of 2,662 Mbp was defined.

In order to identify SNPs in LD with the reference SNP in that area, the International HapMap Project databases (www.hapmap.org) and Haploview V.4.2 software were used. A r^2 LD threshold of 0.8 was established using the existing data for individuals with European ancestry (CEU). SNPs with a minor allele frequency greater than 1% (MAF $\geq$ 0.01) were selected.

Data sources

To determine if the selected SNPs were associated with any disease, we conducted a search in the bibliographic databases PubMed (www.ncbi.nlm.nih.gov/pubmed) and Scopus (http://www.scopus.com/), introducing the rs ID from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) for each SNP and their clinical relevance was checked.

Results

When we analyzed the 52 SNPs selected by the SNPforID consortium, we found that 39 of them were part of Hapmap haplotypes (Figure 1). In addition to the 52 SNPs included in the panel proposed by the SNPforID consortium, other 372 SNPs, in LD with them according to the different haplotypes found, were added making a total of 424 SNPs (Additional data are given in Online Resources 1 and 2).

In order to determine possible relationships between those SNPs and diseases, we performed a search for each of the 424 SNPs in the three databases (Fig. 1).

The search of the 424 SNPs in PubMed referred to 16 SNPs out of the 52 SNPs in the panel and to 9 out of the 372 SNPs in LD. In Scopus, we obtained information for 9 out of the 52 SNPs in the panel and 5 out of the 372 SNPs in LD. The search for clinical relevance in dbSNP of each of the 424 polymorphisms included in this study provided no matches.

After removing duplicates between databases, reviews and papers which referred to the optimization of a technique or to the analysis of SNPs in different ethnic groups, 13 articles were included for further analysis (Table 1). Those articles referred to 16 SNPs, 7 out of the 52 SNPs in the panel and 9 out of the 372 SNPs in LD. Among them, 8 were excluded due to lack of association with the diseases and phenotypes in relation to which they had been studied (Table 1).

Finally seven studies were considered, referring to 8 SNPs, 5 out of the 52 SNPs proposed by the SNPforID consortium and 3 out of the 372 SNPs in LD. Six studies related 7 SNPs with several diseases or phenotypes (rs876724, rs2076848, rs2107614, rs765250, rs11064560, rs2107612, rs1463729). The remaining SNP, rs1979255, was studied in relation to the trans- (or distal) effects over region 22q11, where *COMT* gene is located (Table 1).

Discussion

We have found eight SNPs associated with several diseases among the 52 SNPs in the panel routinely used in forensic genetics and the SNPs in LD with them.

SNPs rs2107614, rs765250, rs2107612, rs11064560

Three studies found association between four polymorphisms (rs765250, rs2107612, rs2107614, rs11064560) in the gene *WNK1* (WNK lysine deficient protein kinase 1) and variation in blood pressure and hypertension [18, 19, 20]. This gene is a member of the WNK subfamily [21]. The WNK serine/threonine kinases are key regulators of blood pressure through the control of the transport of sodium and chloride ions [22].

Essential hypertension is a heterogeneous and multicausal disease, related to hereditary or genetic factors but, above all, to high-sodium diet, environmental stress and obesity [23]. Most genes in which mutations have been linked to hypertension encode molecules that control the ability of the kidney to maintain the balance of salt. This reiterates the relevance of this physiological pathway in the regulation of blood pressure [24].

The SNP rs2107612 in the *WNK1* gene, included in the panel, as well as the SNP rs765250, in LD with the former, contribute to variation in blood pressure and hypertension. Newhouse et al. [18] demonstrated the association of rs2107612 A allele with changes in systolic blood pressure (95% CI = 0.2-2, p = 0.008). The rs765250 A allele showed the strongest association with variation in blood pressure (Systolic Blood Pressure, 95% CI = 1.3-4.9, $p = 5x10^{-4}$, Diastolic Blood Pressure: 95% CI = 0.7-3.2, p = 0.002) and hypertension (95% CI = 1.0-1.7, p = 0.01) [18]. Furthermore, the association between the rs765250 SNP and hypertension and blood pressure variation was confirmed in a meta-analysis, which combined data from other populations with data from BRIGHT (British Genetics of Hypertension Study) ($p = 2x10^{-4}$, n = 17851) resulting from the work by Newhouse et al. [18]. The results from this meta-analysis ruled out the possibility that the association were a false positive.

On the other hand, Turner et al. [19] investigated SNPs in genes that encoded or were involved in renal sodium transport systems as possible predictors of blood pressure in response to a thiazide diuretic. Three SNPs located in the *WNK1* gene, rs2107614, rs2277869 and rs1159744, were associated with differences in ambulatory blood pressure in response to hydrochlorothiazide, a diuretic commonly used to treat high blood pressure (p = 0.039, p = 0.007 and p = 0.034, respectively).

In particular, the SNP rs2107614, in LD with rs2107612 in the panel, is located in intron 1 of *WNK1* gene. Wilson et al. [21] showed that the deletion of intron 1 of *WNK1* gene resulted in an increase in the expression levels of the gene. Moreover, this deletion is one of the causes of a type of inherited hypertension known as Pseudohypoaldosteronism Type II (PHAII, also known as Gordon Syndrome or Syndrome of Family Hypertension and Hyperkalemia) [21, 22].

Another polymorphism in the *WNK1* gene, SNPs rs11064560, in LD with rs2107612 in the panel, was associated with bevacizumab-induced hypertension, the first anti-angiogenic agent approved for use in the clinic (p = 0.028, OR: 1.41, 95 %: 1.04-1.92) [20]. In patients treated with bevacizumab, hypertension was reported in 46 of the 305 (15%) individuals with TT genotype, in 51 of the 313 (16%) individuals with TG genotype and in 20 of 71 (28%) individuals with GG genotype. Bevacizumab is used in combination with chemotherapy in the treatment of various tumours [25, 26, 27, 28]. Hypertension is one of the most common side effects of treatment with this drug. The same relationship Lambrechts' team found had already been described by Frey et al. [29] in a smaller cohort (p = 0.0026). Furthermore, the G allele of SNP rs11064560, which correlated with hypertension induced by bevacizumab in the study by Lambrechts et al. [20] is in LD with the A allele of the SNP rs765250, in LD with rs2107612 in the panel, which has been associated with increased blood pressure. Therefore, the observed association between the SNP and the hypertension induced by the drug is biologically plausible. Therefore, the SNP rs11064560 is a potential predictor of the response profile to bevacizumab.

It would be advisable to consider these evidences to evaluate the permanence of these polymorphisms in the current panel used in forensic genetics. Also, caution should be taken not to include in the future, SNPs in LD with rs2107612, given its relationship with hypertension and drug response.

SNP rs1463729

Strunnikova et al. [30] demonstrated the association of SNP rs1463729 and age-related macular degeneration (AMD). AMD is a leading cause of irreversible loss of central vision in elderly populations, with a higher prevalence in women than in men [31]. AMD affects all layers of the macula, the structure responsible for central vision, encompassing in different degenerative patterns photoreceptors, retinal pigment epithelium (RPE) and Bruch's membrane [32]. Aging is the most important risk factor for this disease [33] but, in addition, other risk factors have been identified to date showing a strong association with the disease, such as smoking, history of cataract surgery, and family history of AMD [34].

AMD susceptibility is influenced by genetic factors [35, 36]. It has been shown that there are several genomic regions and candidate genes affecting the susceptibility to this disease [37, 38, 39]. Using an expression study, Strunnikova et al. [30] identified 154 genes that make up the genetic signature of the retinal pigment epithelium (RPE). Subsequently, they conducted a comparative analysis of these genes with the results of a genome-wide association study (GWAS) for AMD [40]. The SNP rs1463729, near the *LHX2* gene, one of the genes included in the signature of the RPE, is associated with AMD in that GWAS ($p = 0.001846$).

Given the relationship between the rs1463729 polymorphism and AMD, its continuity in the panel of SNPs selected by the SNPforID consortium of interest in forensic genetics should be evaluated.

SNP rs1979255

The gene *COMT* encodes the enzyme catechol-O-methyl transferase, involved in the catabolism of monoamines, which are influenced by the psychotropic drugs, including antidepressants and neuroleptics. Linkage studies have provided evidences of one or more loci in the 22q11 region, where *COMT* is located, which influence the susceptibility to various psychiatric phenotypes as schizophrenia [42, 43], bipolar disorder [43], schizoaffective disorder [44] and others. Thus, *COMT* is a candidate gene for a number of neurological and psychiatric disorders [45].

Xing et al. [41] studied the effect of different transcriptional regulatory regions on region 22q11, where *COMT* is located. Ten regions showed additive trans-effects on the region 22q11. One of them was the 4q35 region, where the SNP rs1979255, included in the panel, is located ($p = 3.4x10^{-4}$). Variants that act in trans-, also known as distal regulators, can be anywhere in the genome relative to the target gene [46].

The results of the genetic maps suggest that the effects of these variations on gene expression are smaller than the effects of cis-acting variants. This is due to the fact that the genes are generally influenced by various trans-acting regulators and, therefore, the effect of each one on the target gene expression is moderate [46].

It would be advisable to take into account the relationship between the rs1979255 polymorphism and the *COMT* gene to evaluate the continuity of this SNP in the processes of human genetic identification.

SNPs rs876724, rs2076848

It has been shown that there is a link between SNP rs876724 and parental imprinting in alcohol dependence [47].

The Diagnostic and Statistical Manual of Mental Disorders classifies excessive drinking as an addictive disorder [48]. Alcohol dependence is a complex disease caused by genetic, epigenetic and etiological factors [49]. Alcohol consumption is the third leading cause of death worldwide, after hypertension and tobacco consumption, with 5.5 million deaths per year, representing 3.2% of all deaths worldwide [50].

Early work conducted to investigate the genetic basis of alcohol consumption and addiction were based on candidate genes studies [51, 52, 53]. Recently, loci involved in dependency and alcohol consumption have been proposed by GWAS [54, 55, 56, 57, 58, 59].

It is known that genetic imprinting plays a role in susceptibility to alcohol dependence [60]. Genetic imprinting is the best known cause of parent-of-origin effects (POE), due to which a gene is expressed differently depending on the parental origin of the chromosome [61]. The alleles have different levels of transcript and, therefore, may produce a different effect on the phenotype when they are transmitted by the father or the mother [62]. In addition, genetic imprinting is related to several diseases, including diabetes, breast cancer, and obesity, in addition to alcohol dependence [63, 64, 65].

Liu et al. [47] conducted a search throughout the genome of places associated with parental imprinting in alcohol dependence. They used data from 112 Caucasian families collected in the Collaborative Studies on Genetics of Alcoholism (COGA). The SNP rs876724, included in the panel showed excess maternal inheritance (maternally greater contribution) (LOD = 2.73, p = 0.0002).

In relation to alcohol dependence, another study showed the association of SNP rs2076848, belonging to the panel, with the electroencephalogram beta 2 phenotype (EEG), a quantitative measure related to alcoholism [66]. These waves are associated with mental alertness [67].

Porjesz et al. [68] showed that the GABAergic system and human EEG measures were significantly associated. Subsequently, they reported the association between the EEG phenotype and a group of GABA receptors (A), whose genes are located on chromosome 4. Specifically, *GABRA2* gene showed a strong association with alcohol dependence and the beta frequency of the EEG. Therefore, the researchers suggested that this gene may influence susceptibility to alcohol dependence by modulating the level of neuronal excitation [69].

Roy-Gagnon et al. [66] estimated the specific heritability of EEG phenotype for a locus through ROMP regression (Regression of Offspring on Mid-Parent). They used data from COGA. SNP rs2076848 was one of the SNPs associated with the EEG phenotype (ROMP: HL2 ± SE = 0.005 ± 0.001).

The studies of Liu et al. [47] and Roy-Gagnon et al. [66] have shown, respectively, the preferential transmission of SNP rs876724 and the heritability of EEG phenotype associated with SNP rs2076848 in relation to alcohol dependence. However, the results of both studies have not been replicated. Therefore, future studies would be needed to confirm these results and clarify the role of these SNPs in alcohol dependence.

Other SNPs studied in relation to genetic susceptibility to various conditions

We found 8 additional SNPs in six studies which concluded that there was no association with the diseases or characteristic phenotypes of different traits.

Cai et al. [70] studied the association between rs1990021 and rs3858703 polymorphisms, located in the *WNK1* gene and in LD with rs2107612 in the panel, and ischemic stroke in Chinese Han population, finding no association for these SNPs.

Suarez et al. [71] obtained the same result for SNPs rs826472 and rs964681, both included in the panel of interest in forensic genetics, after conducting a GWAS in a sample of 409 families with schizophrenia.

Similarly, two studies evaluated the association between rs717227 and rs6688537 polymorphisms, in LD with rs891700 in the panel and located at the *CHRM3* gene, with atopic dermatitis and bipolar disorder respectively, finding no association in any of the cases [72, 73].

The SNPs rs1493232, in the panel, and rs6657343 in LD with rs891700, were analyzed in two GWAS performed with the descendants of the original Framingham cohort (Framingham Heart Study Offspring) [74, 75]. Kathiresan et al. [74] analyzed the levels of blood lipids, including high and low density lipoprotein cholesterol (HDL-C, LDL-C) and triglycerides (TG), as they are highly heritable traits. Benjamin et al. [75] analyzed the concentration of 22 systemic biomarkers in four biological

functions: inflammation / oxidative stress, natriuretic peptides, liver function and vitamins. Systemic biomarkers provide insights into the pathogenesis, diagnosis and risk stratification of a disease. As in the previous case, the concentrations of many of these biomarkers are heritable phenotypes. In both studies, no association between SNPs and the analyzed variables was found.

Therefore, these polymorphisms are suitable markers for use in human identification because they have not been associated with diseases or phenotypic traits.

Conclusion

Our study shows that 3 out of the 52 SNPs in the panel proposed by the SNPforID consortium show association with various diseases and drug response (rs2107612, rs1979255, rs1463729). Similarly, 3 out of the 372 SNPs in LD (rs2107614, rs765250, rs11064560) were associated with various pathologies. It would be advisable to consider these associations in order to evaluate their permanence in the current panel of interest in forensic genetics.

This work highlights the need for a periodic review of the possible relationships of the SNPs used in forensic genetics processes with various diseases, in order to eliminate the risk of using SNPs associated with diseases or other phenotypic traits.

The increasing number of studies demonstrating such associations makes essential the inclussion of a specific monitoring criterion for those polymorphisms in order to avoid the use of markers associated with human features that exceed the scope of Forensic Genetics.

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Conflict of Interest

The authors reported no potential conflicts of interest.

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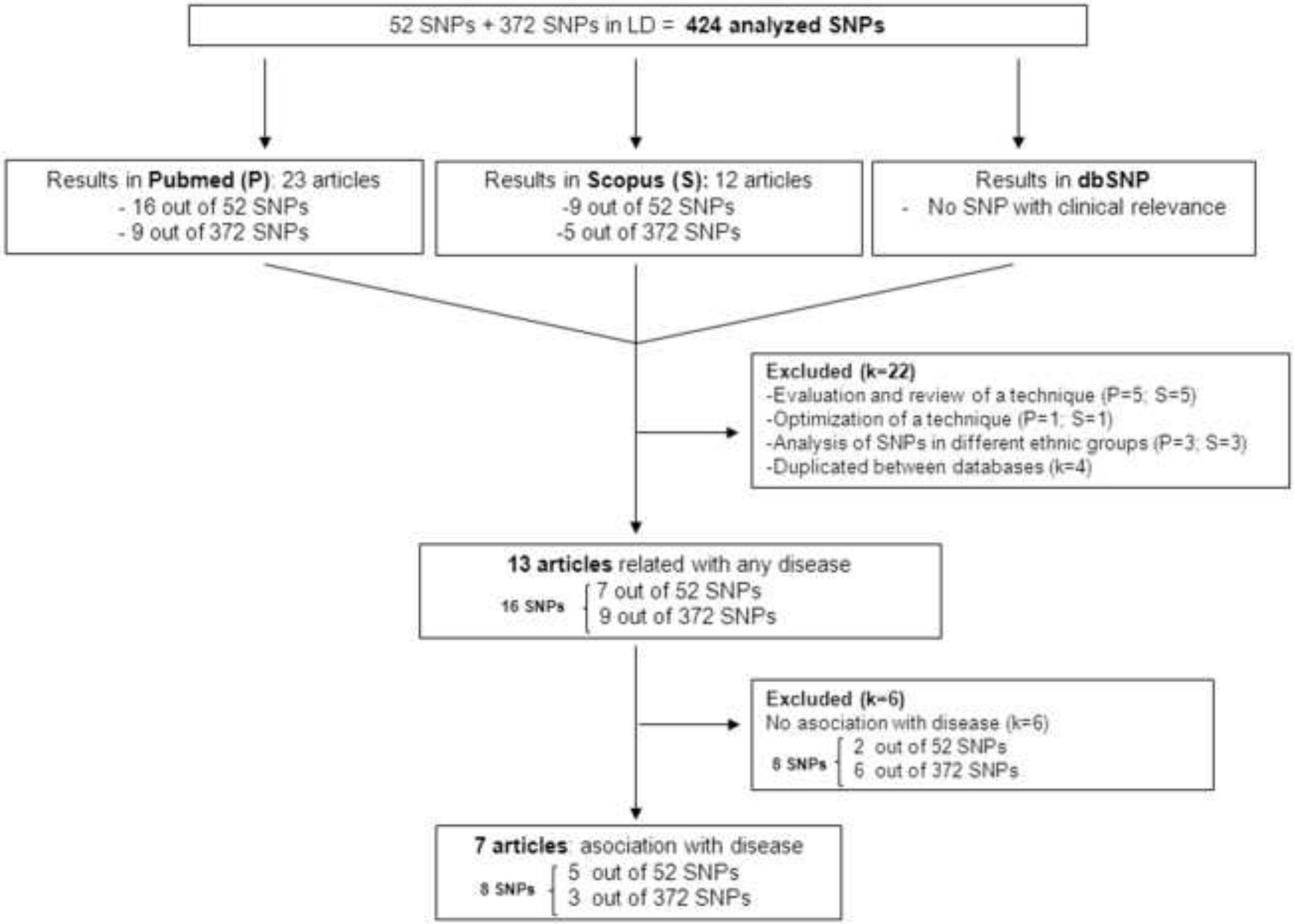
Table 1. List of the 16 SNPs studied in association with any disease or phenotype

Fig. 1 Flow diagram of the study


52 SNPs + 372 SNPs in LD = **424 analyzed SNPs**
Results in **Pubmed (P)**: 23 articles
- 16 out of 52 SNPs
- 9 out of 372 SNPs
Results in **Scopus (S)**: 12 articles
-9 out of 52 SNPs
-5 out of 372 SNPs
Results in **dbSNP**
- No SNP with clinical relevance
Excluded (k=22)
-Evaluation and review of a technique (P=5; S=5)
-Optimization of a technique (P=1; S=1)
-Analysis of SNPs in different ethnic groups (P=3; S=3)
-Duplicated between databases (k=4)
13 articles related with any disease
16 SNPs
7 out of 52 SNPs
9 out of 372 SNPs
Excluded (k=6)
No association with disease (k=6)
6 SNPs
2 out of 52 SNPs
6 out of 372 SNPs
7 articles: asociation with disease
8 SNPs
5 out of 52 SNPs
3 out of 372 SNPs

Table

Table 1. List of the 16 SNPs studied in association with any disease or phenotype

SNP	SNPs in LD	r^2	Chr	Alleles	Position[1]	Gene	Evaluation of the SNP	Disease or phenotipe	Reference
rs2107612	rs1990021	0,851	12	C/G	951775	*WNK1*	No related	Ischemic stroke	Cai et al, 2014
	rs2107614	0,859		C/T	903079		Related	Hypertension	Turner et al, 2005
	rs765250	0,858		A/G	908283		Related	Hypertension	Newhouse et al, 2009
	rs3858703	0,926		A/G	863517		No related	Ischemic stroke	Cai et al, 2014
	rs11064560	0,887		G/T	943953		Related	Hypertension	Lambrechts et al, 2014
	rs2107612	1		A/G	888320		Related	Hypertension	Newhouse et al, 2009
rs1493232	rs6505623	0,965	18	C/T	1125057	-	No related	Lipid levels	Kathiresan et al, 2007
rs826472	-	-	10	C/T	2406631	-	No related	Schizophrenia	Suarez et al, 2006
rs1979255	-	-	4	C/G	190318080	-	Related	*COMT* gene	Xing et al, 2007
rs1463729	-	-	9	A/G	126881448	-	Related	Age-related macular degeneration (AMD)	Strunnikova et al, 2010
rs2076848	-	-	11	A/T	33154927	-	Related	Alcoholism	Roy-Gagnon et al, 2005
rs876724	-	-	2	C/T	114974	-	Related	Alcoholism	Liu et al, 2005
rs964681	-	-	10	C/T	132698419	-	No related	Schizophrenia	Suarez et al, 2006
rs891700	rs717227	1	1	C/T	239882599	*CHRM3*	No related	Atopic dermatitis	Enomoto et al, 2007
	rs6657343	1		A/T	239891511		No related	Systemic biomarkers	Benjamin et al, 2007
	rs6688537	0,934		A/C	239850588		No related	Bipolar disorder	Shi et al, 2007

[1] Position in bp based on the GRCh37/hg19 version of the genome. Abbreviations: Chr, chromosome.