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The Role of Genetic Polymorphisms in the Occupational Exposure

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Abstract

In the last years, genetic polymorphisms have raised interest for their role on the environmental and occupational exposures. They not only are studied at population level to identify genetic diversity among ethnicities but have been recognized also as biomarkers of genetic susceptibility in many fields including medicine, health prevention, epidemiology and pharmacology. In the occupational context, the investigation of gene polymorphisms is part of the biomonitoring of workers exposed to occupational toxicants and carcinogens. However the majority of workers coming from foreign countries may be not familiar with the standard procedures used in the biomonitoring campaigns, which include human biosample harvesting for genetic, metabolic and genotoxic studies. Here we describe the importance of gene polymorphism association with dose and genotoxicity biomarkers and propose a statistical model predicting ethnic-specific susceptibilities based on the genotypes available in public databases when the access to blood genotyping test is not always feasible.

Keywords: biological monitoring, biomarker, biosample, environmental, ethnicity, gene polymorphism, occupational, susceptibility risk, worker

1. Introduction

The genetic variability is something common to all the living organisms and has important implications for the evolution and conservation of every species. It is widely known the differences in the phenotype among individuals are strictly dependent on two factors: the genotype, which is inherited from our ancestors, and the surrounding environmental exposure that contributes to shape the phenotype. The interindividual and intraindividual genetic differences make humans special and unique determining for each a single identity. Although genetics influences our physical appearance, behaviour, thoughts and habits, we recognize also the environment plays a crucial role in modifying many other parameters of our body. This depends on several factors: the country where we live, the ethnicity to which we belong to, the historical heritage, tradition and religion, the interaction with other people as well as the influence of the climate change on our life. If, on the one hand, our genetic heritage makes us unique, we acknowledge that classic genetics cannot explain the wide diversity of phenotypes within the population; nor classic genetics can explain the different phenotypes and disease susceptibility found in identical twins as well as in organisms obtained by cloning technology despite the DNA being exactly the same [1]. That means we cannot forget the strong influence

of the surrounding environment on our genotype and on our physiological, physical and behavioral habits. Such influence, namely, epigenetics, means that the heritable changes in gene expression are not due to alteration in the DNA sequence but to some modifications occurring upon the DNA without changing the genetic code. That said, although we are aware the genetic variability is something peculiar to every human being, regardless of the inheritance of favorable or unfavorable genes, the transmission to the offspring will be modulated by epigenetics contributing to increase further the genetic variation in the population.

2. Variability among individuals: why it is important to study gene polymorphisms

Gene polymorphisms are the most common type of genetic variations in humans. They are present in the human population at frequency higher than 1% and differ from DNA mutations which are generally observed at extremely low frequency and in a restricted number of individuals. While gene polymorphisms are not necessarily associated to a specific disease, the gene mutation is generally known to cause a genetic disease. In humans the simplest type of polymorphism is the single nucleotide polymorphisms (SNPs), representing the most common type of nucleotide variation where a single base is substituted by another one (**Figure 1**). A SNP in the coding region of a gene may have four different effects on the resulting protein: (1) *synonymous substitution*, also known as silent mutation, causing no amino acid change in the protein, (2) *non-synonymous substitution* where a nucleotide mutation alters the amino acid sequence of a protein, (3) *missense substitution* consisting of an amino acid change with another and (4) *nonsense substitution* resulting in end of protein translation by a termination codon. About half of all the coding sequences of SNPs end up in non-synonymous codon changes. However SNPs may occur also in the regulatory region of the gene, affecting various properties of the protein. These SNPs may influence the protein in terms of quantity and quality, activity, processing and trafficking [2]. In the last 20 years, SNPs have raised a lot of
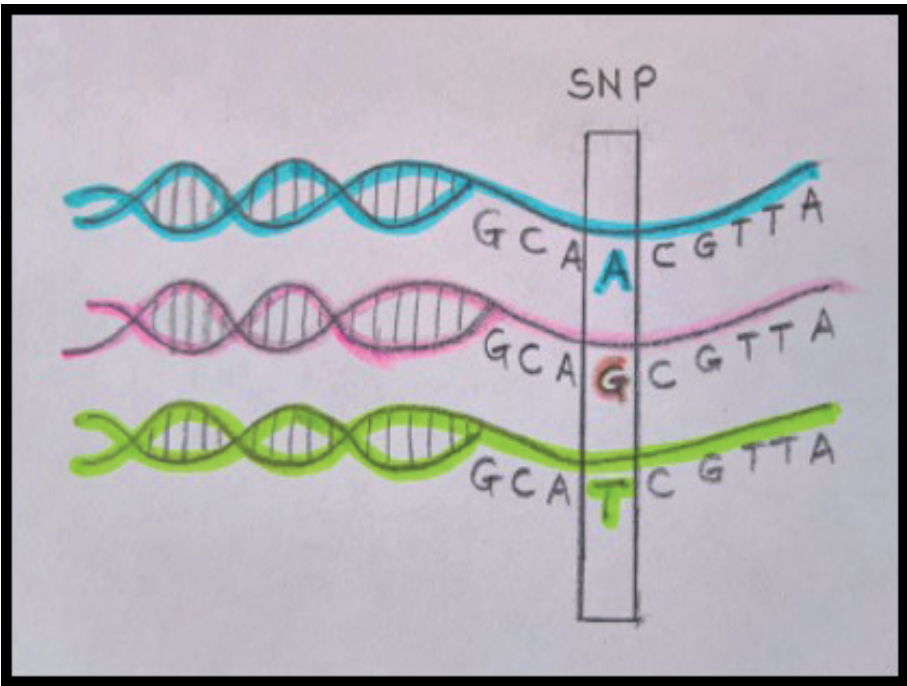

Figure 1.
Variation of single nucleotide in the human DNA coding sequence among individuals.

interest in many scientific fields related to public health and disease. They are also investigated in many scientific research areas, ranging from the human ethnicity study to the genetics of populations, personalized medicine, pathology, epidemiology, pharmacology, immunology and nutrition. More recently they have been considered relevant also for their role in the exposure science, a novel scientific branch linked to toxicology which characterizes and elucidates the contact of humans with dangerous chemical, physical and biological agents which represent a potential risk for the human health [3]. Actually the environmental exposure, in which also the occupational exposure is comprised, is acknowledged to play an important role in common chronic diseases, representing a major health concern in the economically developed countries. In this context the study of SNPs has a fundamental role to detect the human response to toxic and dangerous substances. Although the majority of SNPs of the human genome are of low prevalence [4], including the genes implicated in metabolism of environmental chemicals, these may substantially contribute to increase the population disease burden [5]. Since the exposure factors are numerous and several chronic diseases remain uncertain, it is fundamental to study the human genetic variation to understand the exposure-disease associations within the global population [6]. On the whole the genetic diversity associated to the variability of polymorphisms and to the genetic recombination is a valuable resource for humans and other living organisms. The interactions between the genetics of human beings and the surrounding environment reciprocally shape one with each other in order to reach an equilibrium. However such equilibrium might be perturbed by several exogenous factors such as the influence of natural selection, the global climate change, the re-emergence of extinct diseases and the admixing of different ethnicities due to massive migrations from one country to another. All these factors may contribute to change the variation of the gene polymorphism pool in the worldwide population. Nowadays the characterization of gene polymorphisms of the human population is carried out on individuals belonging to the same ethnicity so as to identify ethnic-specific gene/allele frequencies. This strategy should help geneticists to identify the genotype frequencies typical of members of a community sharing the same language, culture, religion, tradition, nationality, ancestry, nutrition, habits and lifestyle which influence the genetic background of the group [7]. For this reason the investigational studies of polymorphic genes refer more to the concept of ethnicity than to the race. The genetic characterization of separate ethnic groups provides useful information to evaluate the difference in the susceptibility risk of each cluster. Several papers have been published to report the typical genotype frequency of different gene polymorphisms in selected ethnicities [8–11]. However, due to the abundance of variation in gene polymorphisms, the characterization of the gene pool of a specific group of individuals is incomplete, and the genotype frequencies are known only for polymorphisms which have been investigated for their role or association to a specific susceptibility or disease. To our knowledge the commonest public databases collecting the gene polymorphisms available to the scientific community are the following: (1) the single nucleotide polymorphism database (dbSNP), a public archive for genetic variation hosted by the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/snp), and (2) the Ensembl project of genome databases for vertebrates and other eukaryotic species (http://grch37.ensembl.org/Homo_sapiens/Variation), which is the one we are using in the study of the human SNPs. These two archives represent a fundamental resource for our investigational studies on human genetic diversity when the laboratory genotyping is not feasible. In this chapter we describe some of our studies carried out during the biomonitoring campaigns of exposed workers where the biomarkers of dose, effect and susceptibility have been assessed in the occupational exposure. In addition we show also a statistical model we

previously elaborated to identify ethnic-specific differences in the susceptibility risk to the typical exposure found in the workplace. In such model a statistical analysis has been done using the publicly available genotype frequency of four ethnic groups (Africans, East Asians, South Asians and Europeans) downloaded from the Ensembl project of genome databases.

3. Gene polymorphisms as susceptibility biomarkers in the occupational setting

It is widely known the exposure to dangerous substances and carcinogens is commonly associated to the individual capability of metabolizing such compounds which may vary between different individuals and among ethnicities, each characterized by its own specific genetic ancestry. In the occupational setting, the exposure risk is traditionally evaluated by the biological monitoring of workers manipulating hazardous agents. Common work-related substances are polycyclic aromatic hydrocarbons (PAH), a large class of organic compounds with carcinogenic properties (IARC group 1, 2B) used in the manufacture of chemicals, bitumen, and rubber and in shoe factories, while in fibreglass industries and in ship constructions, the most common dangerous substances are volatile organic compounds (VOCs) such as styrene (IARC group 2A), toluene (IARC group 3), xylene (IARC group 3), ethylbenzene (IARC group 2B) and benzene (IARC group I). The majority of these hazardous chemicals are highly toxic and carcinogenic to humans, and in each company, the officially approved occupational exposure limits (OELs) must be respected to ensure a safe environment for the workers' health. The procedure of workplace monitoring is generally standardized. It basically consists first of the environmental monitoring which measures the workers' airborne exposure to dangerous compounds and second of the biomonitoring which detects metabolites excreted in the biological specimens (urine, blood, saliva) and analyses the genotoxicity and gene polymorphisms in the subjects [12]. More specifically the biomonitoring allows to detect the following specific indicators: (i) the dose biomarkers, i.e. metabolites excreted in urine or in other bio-fluids; (ii) the early effect biomarkers, i.e. genotoxicity in peripheral blood lymphocytes and/or in buccal exfoliated cells; and (iii) the susceptibility biomarkers, i.e. the gene polymorphisms encoding for biotransformation enzymes during the exposure. This practice is carried out on exposed workers and nonexposed controls with the goal to assess the presence and quantify the potential health risks. While the dose biomarkers and the genotoxic damage may be immediately analyzed and quantified, the susceptibility biomarkers, being a qualitative parameter, are not quantifiable in terms of single polymorphism belonging to a subject. In such case the results obtained by the genetic analysis on groups of exposed workers have to be considered all together to quantify the relative susceptibility risk with respect to a control group. Based on our experience, the polymorphic genes of the worker population are identified following extraction of the genomic DNA of the subject to identify the genotype encoding the enzyme involved in the detoxification process. Once the worker genotype has been identified, it gives an important indication of the specific susceptibility to that substance by associating the genetic information with the dose and/or the early effect biomarker. In general the homozygous variant genotype (minor allele) is considered a risk allele with respect to the homozygous wild-type genotype (major allele) although there might be a few exceptions [13]. The workers exposed to potential hazardous substances are enrolled in the biomonitoring study only on a voluntary basis to allow the occupational health risk assessment. The results of the gene polymorphism assay together with the data gained by environmental and personal biomonitoring are analysed at the epidemiological level (1) to quantify the

indoor exposure and the individual absorbed dose according to the company OELs for the specific hazardous agent, (2) to assess the potential genotoxic effect and (3) to evaluate the influence of the investigated polymorphic genes on the toxicant absorbed dose. Although workers make use of personal protection equipment (PPE), it is possible, in some cases, the high volatility of organic compounds might spread indoor, increasing the toxic substance threshold within the work environment. The finding of an association between the exposure to specific dangerous substances, the presence of cell genotoxicity and the variable functionality of gene polymorphisms are useful to identify specific or common susceptibilities in the exposed groups.

3.1 Identification of gene polymorphisms relevant to the occupational exposure

In our experience several biomonitoring studies have been promoted and carried out in industrial companies to assess the exposure risk of workers manipulating toxic and carcinogenic agents. Here we report the most recent results obtained during four different campaigns carried out in the last 6 years. In 2012, our research group analyzed the exposure of 315 workers to benzene in a petrochemical plant in Italy to evaluate the dose biomarkers S-phenylmercapturic acid (SPMA) and trans,trans-muconic acid (t,t-MA) in the worker urine samples and the susceptibility biomarkers in the blood specimens [14]. Since the GST enzymes may influence the metabolite excretion, the research project analyzed the association between metabolite excretion and contribution of the glutathione S transferase T1 (GST-T1) and M1 (GST-M1) polymorphisms to the detoxification. The results confirmed the modulating effect on the excretion of SPMA metabolite in urine by the genetic polymorphism of GST-T1 after exposure to low benzene doses. The same modulating effect was caused by the GST-M1 polymorphism but only at higher benzene doses, like those produced by cigarette smoking. The genotype of the 315 workers has been characterized to identify groups with the highest susceptibility; the workers' gene frequency of GST-T1 positive/null genotype was 0.78 vs. 0.22, while the frequency of GST-M1 positive/null was 0.47 vs. 0.53. Taking into account the genotype frequency of both enzymes, it looks that the GST-M1 polymorphism positive/null is less efficient in the detoxification process than GST-T1. A second study on benzene exposure has been carried out on 301 employees in the oil refinery. The effect of polymorphic genes GST-T1, GST-M1, glutathione S transferase A1 (GST-A1), epoxide hydrolase 1 (EHPX1), NAD(P)H quinone dehydrogenase 1 (NQO1), cytochrome P450 2E1 (CYP2E1), cytochrome P450 1A1 (CYP1A1*2A) and myeloperoxidase (MPO) involved in the detoxification of benzene has been analyzed. Here the dose biomarker was worked out as the ratio (R) between t,t-MA and S-PMA metabolites excreted in the urinary samples. The effect of smoking as confounding factor contributed to increase the relative production of S-PMA with respect to t,t-MA reducing the R value. This result was attributed to the higher levels of glutathione (GSH) in the red blood cells of smokers than in non-smokers. The analysis of susceptibility biomarkers showed a strong influence of GST-T1 positive polymorphism on the excretion of urinary S-PMA, reducing the conjugation rate of benzene epoxide with GSH in the GST-T1 null subjects. To a lesser extent, a similar effect was observed in individuals with GST-M1 null, GST-A1 and NQO1 mutant genotypes. It has been observed that in subjects with the double null GST-T1 and GST-M1 genotypes, an amplification of the t,t-MA biochemical pathway occurred with respect to the SPMA pathway [15]. However the activity of one GST is compensated by another in GST-M1 and GST-A1 defective subjects, but not in GST-T1 null genotypes, whose S-PMA average excretion is about 50% with respect to the positive genotypes for the same benzene exposure [16]. A further biomonitoring campaign has been conducted in two different manufacturing sites of central Italy on 30 styrene-exposed workers and 26 unexposed controls. In this work the authors

investigated the effects of polymorphic genes CYP2E1, EPHX1, GST-T1 and GST-M1 on the urinary concentrations of the styrene metabolites, i.e. mandelic acid (MA) and phenylglyoxylic acid (PGA), and on the ratio between MA and PGA. Here the concentrations of urinary styrene and of airborne styrene have been determined. In the exposed workers, a lower excretion of PGA and MA metabolites was detected in subjects with cytochrome P450 2E1*5B (CYP2E1*5B) and cytochrome P450 2E1*6 (CYP2E1*6) heterozygous genotype with respect to the homozygous wild type indicating the influence of SNPs on the dose biomarkers. Furthermore a reduced value of metabolite ratio (MA + PGA/urinary styrene) was observed in exposed workers with the EPHX1 Tyr[113]His slow/mutant allele in comparison to those with the wild-type allele. The results indicate a reduced excretion of MA + PGA, evidenced also by other authors only in association with other genotypes. This study confirmed the variability in the excretion of urinary styrene metabolites, strictly related to the individual gene polymorphisms, can significantly impact on the biological monitoring of styrene exposure. In the CYP2E1*5B and CYP2E1*6 heterozygous genotypes as well as in the EPHX1 slow mutant genotypes, the average excretion of MA + PGA is, respectively, reduced 20 and 35% in comparison to the wild-type population [17].

3.2 Operative procedure and ethical use of workers' biosamples for occupational risk assessment

The availability of human biological samples is fundamental for the biomonitoring since it allows to estimate the exposure risk of the workers. The mostly used biosamples are whole blood with its separated fractions (plasma, serum, buffy coat, peripheral mononuclear cells, blood clot), urine, saliva, oral cell mucosa and cell components such as DNA and mRNA. In the occupational studies, biosamples are kindly donated from the workers agreeing to the biomonitoring project, provided that they give the informed consent. In Italy the biomonitoring procedure is sub-conditioned to the availability of the host manufacture company to agree the investigational study. The final purpose of the research has to be approved by the local health unit, by the research institute and, only in case of experimental clinical protocols, by the ethical committee. As volunteers the workers might join but not necessarily have to participate to the study related to the occupational health risk exposure, and they are not forced to undergo genetic screening. Workers participating to the programme agree by informed consent and are invited to fill a questionnaire to exclude all the potential confounding factors interfering with the analysis (drug assumption, alcohol consumption, smoking habit, chronic or acute diseases) before donating their biological samples. Those wishing to support the observational study are aware their specimens will be used only for the scientific purpose and will be collected, stored and used according to the ethical guidance of the Declaration of Helsinki [18]. The collection, storage and processing of the biosamples must be performed under strictly controlled procedures, in order to preserve their integrity and quality. In particular, the storage of genetic data collected in the workplace is fundamental since they contain sensitive health and non-health-related information about individuals which must be adequately protected in research as well as in clinical studies. This principle has been formalized in Europe on 27 April 2016 with the new General Data Protection Regulation (EU GDPR 2016/679), effective from 25 May 2018, which has entered into force and repealed the previous Directive 95/46/EC for personal data protection. The aim of the regulation is to promote and harmonize the personal data protection in the EU [19]. In the occupational setting, three types of biosamples are mainly requested to the workers, i.e. whole blood, urine and buccal cells. To perform genetic analysis on the biospecimen, the workers who

agree to donate the sample must be informed of the purpose of the research project in all the aspects; they also should understand the utility, the scope and the limit of the analysis. Workers and their supervisors or representatives are informed on the modality, procedure and scheduling related to the donation of the biosamples. For instance, in the case of urine and buccal cells, workers can themselves self-collect their specimens provided that they are informed in advance on the day of collection. The collaborating institute ensures analysis of all the data, and for the genetic results, it ensures anonymity and confidentiality, un-disclosure in public databases, exclusivity and availability to medical staff of the company if required for internal use. Most importantly the genetic data are analysed as groups and not individually in order to avoid any misinterpretation or discrimination. As stated before, the information on the susceptibility risk is not relevant at the individual level, while it becomes significant once a large number of subjects belonging to the same ethnicity are available. The genetic analysis should be scientifically validated and must not involve any kind of discrimination for the individual.

3.3 Our method for gene polymorphism analysis

The workers' blood samples are harvested in tubes containing either heparin or K_2-EDTA from the medical staff of the company following workers' informed consent and according to the ethical guidelines of our research institute. All procedures performed in this study involving human participants [18] are in accordance with the ethical standards of our institutional committee and with the local health unit. Urine samples may be immediately frozen at −20°C after harvesting, and saliva and buccal cells may be stored at RT, while the blood samples may be stored up to 24 hours at a temperature between 4 and 10°C or immediately frozen for the genetic analysis. Blood genomic DNA is isolated by using a DNA blood kit, checked for the quality by agarose gel electrophoresis, quantified by a nanophotometer and stored at 4°C or −20°C. Although there are many novel and reliable techniques used to assess the gene polymorphisms such as Taqman assay, amplification refractory mutation system mass spectrometry (PCR-ARMS), confronting two-primer pair (CTPP-PCR), high-resolution melting and different types of mini-sequencing, our choice is oriented towards the traditional method based on polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) (**Figure 2**). We have tried also the fast and less expensive CTPP-PCR protocol developed previously in which the resulting genotype is obtained by a traditional PCR carried out with two couples of primers, avoiding to proceed for the enzymatic digestion [11]. However in our hands such protocol turned out
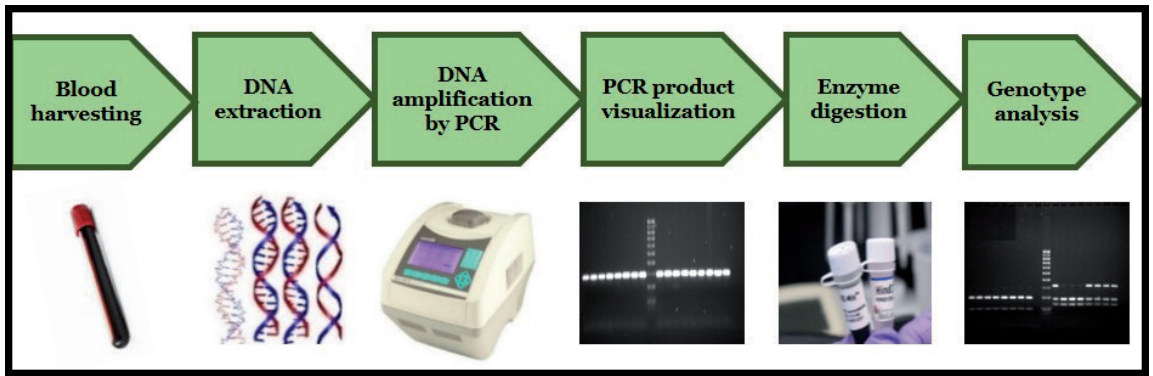
Figure 2.
Methodological procedure for gene polymorphism analysis by PCR-RFLP.

to be unreliable. In particular we applied this alternative method to detect two polymorphisms of one gene involved in response to oxidative stress and ototoxicity (NRF2 -617C/A and -653A/G), but the data were not completely satisfactory since CTPP-PCR produced contradictory results, particularly for the heterozygosis classification, requiring another orthogonal technique for confirming the data [20]. In the following section, we propose a list of the gene polymorphisms that we usually evaluate in the biological monitoring of the occupational exposure. They have been grouped on the basis of the enzyme function, i.e. detoxification, oxidative stress and DNA repair. The majority of them has been analyzed and reported in our previous published papers as biomarkers of susceptibility to the exposure of several organic compounds including styrene, toluene, ethylbenzene, benzene as well as biomarkers of genotoxic damage and of oxidative stress [14–17, 21]. **Table 1** shows a list of the analyzed susceptibility biomarkers together with the PCR-RFLP protocols which have been used by our group with some modifications.

3.4 Use of an alternative method to detect human gene polymorphisms without genotyping

In the context of occupational and environmental exposure, the role of biotransformation enzymes is to ensure efficient detoxification of endogenous and exogenous compounds by specific biochemical pathways. These modify the dangerous substances into inactive compounds which once excreted into urine will avoid metabolite accumulation and harm the human organism. Although the screening of individual gene polymorphisms by the molecular biology laboratory is the ideal procedure to assess the susceptibility of each individual participating to the study, the availability of workers to donate the biosample is fundamental to proceed with the genetic analysis. One difficulty may be represented by the lack of workers' consent to venipuncture or in general to the biosample harvesting, either because they are simply not used to this procedure or because the venipuncture is perceived as too invasive and painful technique or due to the worker fear and insecurity of the potential analysis result. However the gene polymorphism assessment has no diagnostic value in terms of predisposition to develop a particular disease. In our experience we noted that workers of mixed ethnicities are employed in many industrial companies, and in this context, it might be difficult, if not impossible, to collect particularly the blood samples in comparison to urine. This may depend on several factors: the poor knowledge of the language, the difficulty of communication and the difference in culture, habit, diet and belief among workers. To bypass such critical issue and achieve the ethnic-specific genotype information without making use of the laboratory analysis, we took advantage of a public and online available database (http://grch37.ensembl.org/Homo_sapiens/Variation) containing a provisional collection of the majority of genotype and allele frequencies of several ethnic groups. This resource is freely accessible and allowed us to obtain the genetic profile of different ethnicities which helped to predict and identify in silico specific susceptibilities within the population. This model, based on the statistical method of principal component analysis (PCA), has been designed to assess the relative risk of the homozygous variant and heterozygous genotype in four macro-groups, i.e. Africans, Eastern Asians, South Asians and Europeans, with respect to the worldwide population [34]. It has been conceived to identify the critical susceptibilities in the polymorphisms of genes involved in three main functional biochemical pathways, i.e. detoxification, oxidative stress and repair of damaged DNA, following exposure to the hazardous compounds. The SNPs have been selected on the basis of the exposure to toxic and carcinogenic substances commonly found in manufacturing factories and shipyards. Below we list the gene polymorphisms

SNPs	rs number	Primer sequences	Annealing temperature (°C)	Restriction enzyme	Restriction pattern (bp)	References
Detoxification						
GST-T1	17856199	F: 5′ -TTC CTT ACT GGT CCT CAC ATC TC-3′ R: 5′ -TCA CCG GAT CAT GGC CAG CA-3′	62	None	None	Teixeira et al. [22]
GST-M1	366631	F: 5′-GAA CTC CCT GAA AAG CTA AAG C-3′ R: 5′-GTT GGG CTC AAA TAT ACG GTG G-3′	62	None	None	
GST-P1	1695	F: 5′-ACC CCA GGG CTC TAT GGG AA-3′ R: 5′-TGA GGG CAC AAG AAG CCC CT-3′	62	BsmAI	Wt: 176 Het: 176, 91, 85 Mut: 91, 85	
GST-A1	3957357	F: 5′-GCA TCA GCT TGC CCT TCA-3′ R: 5′-AAA CGC TGT CAC CGT CCTG-3′	64	EarI	Wt: 400 Het: 400, 308, 92 Mut: 308, 92	Ping et al. [23]
CYP1A1*2A	4646903	F: 5′-CAGTGAAGAGGTGTAGCCGCT3′ R: 5′-TAGGAGTCTTGTCTCATGCCT3′	65	MspI	Wt: 340 Het: 340, 200, 140 Mut: 200, 140	Nie et al. [24]
CYP2E1*5B	3813867	F: 5′-CCA GTC GAG TCT ACA TTG TCA-3′ R: 5′-TTC ATT CTG TCT TCT AAC TGG-3′	55	RsaI	Wt: 360, 50 Het: 410, 360, 50 Mut: 410	Le Marchand et al. [25]
CYP2E1*intron6	6413432	F: 5′-TCG TCA GTT CCT GAA AGC AGG-3′ R: 5′-GAG CTC TGA TGC AAG TAT CGC A-3′	62	DraI	Wt: 572, 302, 121 Het: 874, 572, 302, 121, Mut: 874, 121	Liu et al. [26]
EPHX1 Ex_3	1051740	F: 5′-GAT CGA TAA GTT CCG TTT CAC C-3′ R: 5′-ATC CTT AGT CTT GAA GTG AGG AT-3′	52.6	EcoRV	Wt: 160 Het: 160, 140, 20 Mut: 140, 20	Erkisi et al. [27]
EPHX1 Ex_4	2234922	F: 5′-GGG GTA CCA GAG CCT GAC CGT-3′ R: 5′-AAC ACC GGG CCC ACC CTT GGC-3′	58	RsaI	Wt: 295, 62 Het: 295, 174, 62 Mut: 174, 121, 62	Hassett et al. [28]
MPO	2333227	F: 5′-CGG TAT AGG CAC ACA ATG GTG AG-3′ R: 5′-GCA ATG GTT CAA GCG ATT CTT C 3′	56	AciI	Wt: 168, 121, 61 Het: 289, 168, 121, 61 Mut: 289, 61	Cascorbi et al. [29]

SNPs	rs number	Primer sequences	Annealing temperature (°C)	Restriction enzyme	Restriction pattern (bp)	References
Oxidative stress						
NRF2 – 617C/A	6721961	F1 5′- CCC TGA TTT GGA GTT GCA GAA CC-3′ R2 5′- CTC CGT TTG CCT TTG ACG AC-3′	62	NgoMIV	Wt: 191, 91 Het: 281, 191, 91 Mut: 282	Chiarella et al. [20]
NRF2 – 653A/G	35652124	F1 5′-CTT TTA TCT CAC TTT ACC GCC CGA G-3' R2 5'-GGG GTT CCC GTT TTT CTC CC-3′	62	BseRI	Wt: 180, 138 Het: 318, 280,180, Mut: 318	
NQO1	1800566	F: 5′-TCC TCA GAG TGG CAT TCT GC-3′ R: 5′-TCT CCT CAT CCT GTA CCT CT-3′	65	HinfI	Wt: 195 Het: 195, 151, Mut: 151	Chen et al. [30]
HO-1	2071746	5′-GTT CCT GAT GTT GCC CAC CAA GC-3′ 5′-CTG CAG GCT CTG GGT GTG ATT TTG-3′	60	HindIII	Wt: 131 Het: 20, 131 Mut: 20	Song et al. [31]
DNA repair						
XRCC1 399 G/A	25487	F: 5′-TTG TGC TTT CTC TGT GTC CA-3′ R: 5′-TCC TCC AGC CTT TTC TGA TA-3′	61	MspI	Wt: 374, 221 Het: 615, 374, 221 Mut: 615	Kowalski et al. [32]
XRCC1 194	1799782	F: 5′-GCC CCG TCC CAG GTA-3′ R: 5'-AGC CCC AAG ACC CTT TCA TC-3'	61	MspI	Wt: 292, 174 Het: 313, 292, 174 Mut: 313, 174	
hOGG1 326	1,052,133	F: 5′-GGA AGG TGC TTG GGG AAT-3′ R: 5′-ACT GTC ACT AGT CTC ACC AG-3′	58	Fnu4HI	Wt: 200 Het: 200, 100 Mut: 100	Le Marchand et al. [33]

Wt, Homozygous wild type; Het, heterozygous; Mut, homozygous mutant.

Table 1.
List of susceptibility biomarkers analysed in our biomonitoring campaigns.

reported in **Table 1** and commonly assessed in the laboratory genotyping during the biomonitoring campaigns that in our predictive model we integrated with other relevant polymorphic genes [34].

1. Detoxification pathway genes: Glutathione S transferase (GST-A1) rs3957357, (GST-M1) rs366631, (GST-T1) rs17856199, (GST-P1) rs1695, epoxide hydrolase 1 (EPHX1 Ex_3) rs1051740, (EPHX1 Ex_4) rs2234922, cytochrome P450 1A1 (CYP1A1_2A) rs4646903, cytochrome P450 2E1 (CYP2E1*6) rs6413432, (CYP2E1*5B) rs3813867, myeloperoxidase (MPO) rs2333227.

2. Oxidative stress genes: Nuclear factor (erythroid-derived 2)-like 2 (NRF2) rs6721961, NRF2 rs35652124, heme oxygenase (HO-1) rs2071746, NAD(P)H quinone dehydrogenase 1 (NQO1) rs1800566.

3. DNA repair pathway genes: X-ray repair cross-complementing 1 (XRCC1) rs25487, X-ray repair cross-complementing 3 (XRCC3) rs1799782, 8-oxoguanine glycosylase (hOGG1) rs1052133.

The model provides the relative risk (RR) for each ethnic group. RR has been calculated as the ratio between the variations of the gene frequency of the specific ethnic group with respect to the variation of the gene frequency of the worldwide population. If the variation of the gene frequency is >1, it means the susceptibility risk is higher in the ethnic group than in the general population. The most disadvantageous condition, unless specified, is generally associated with the frequency of the homozygous variant, namely, the mutant genotype, although in very few exceptions it might associate to the homozygous wild-type genotype. The details of the rationale, method and elaboration of the susceptibility relative risk of the model are available in our previous paper [34]. Briefly, the predictive model allows to identify (1) ethnicity similarity in the susceptibility risk, (2) correlation of the ethnicity with specific metabolic genes and (3) estimation of the RR indicator in the four ethnic macro-populations. In **Figure 3** we report a quantification of the RR for all the four ethnic groups which has been worked out for the three gene polymorphism clusters (i.e. detoxification, oxidative stress and DNA repair). As seen in the graph, the
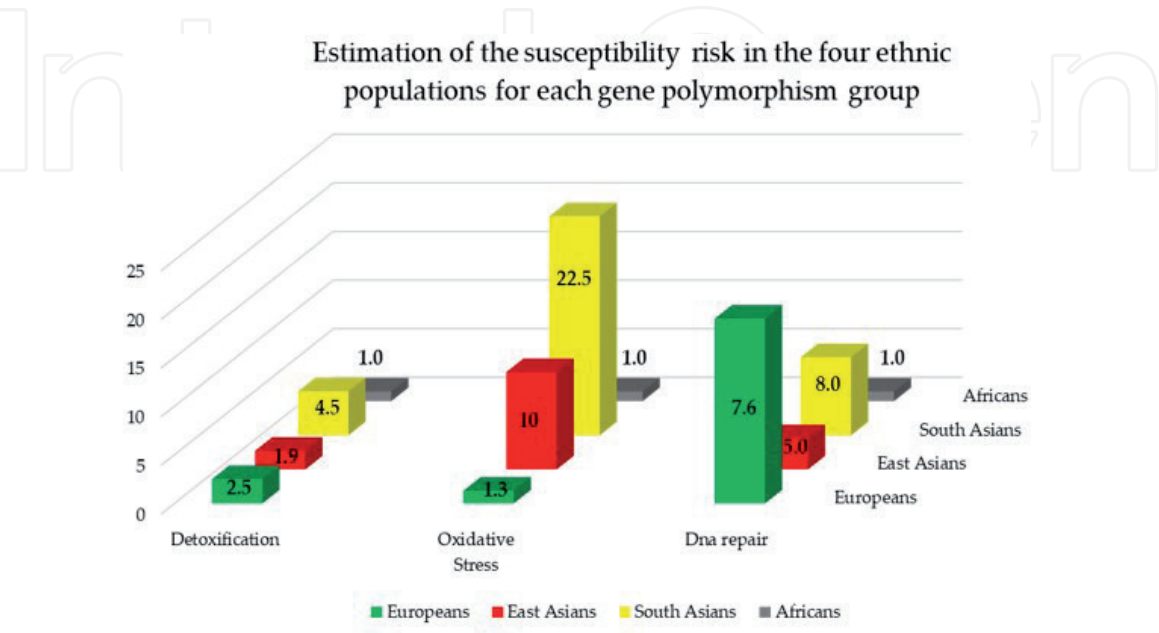

Figure 3.
Calculation of the indicator risk in the four ethnic populations.

minimum risk for the three categories of gene polymorphisms is observed in the case of Africans, while South Asians are associated to the highest risk for detoxification and oxidative stress; Europeans show the highest risk in the DNA repair; and East Asians show a high risk in the oxidative stress. According to the following results, it seems that South Asians are associated with a cumulative highest susceptibility risk in comparison to the other populations, while Africans are associated to the lowest risk. The model cited here may represent a useful tool to predict the susceptibility risk associated to the occupational exposure and a potential alternative substituting the genotype screening of workers. However, it is still provisional, and it could be improved considering other ethnicities and including in the analysis a higher number of polymorphic genes involved in the biotransformation of the toxic and carcinogenic substances found in the workplace.

4. Conclusions

The recent and massive migration of populations of various ethnicities in the European countries and the global climate changes not only are affecting the human social life and behaviors but represent also a serious health and safety concern for the population. Any individual response to the environmental and occupational exposure is dependent on different factors; one of the most important and obvious is related to the personal genetic background characterizing different population groups. However many other exogenous factors such as the individual lifestyle, the smoking habit, the use of drugs, the type of diet and the ubiquitous presence of toxicants are factors affecting the individual genetic heritage. A novel and effective approach to the management of the public health risk is urgently needed. Although the genetic information is correctly used in the occupational risk assessment models, various ethical and social issues may arise when dealing with gene polymorphisms at workplace, particularly when the labour force is heterogeneous. The possible reluctance of immigrants and of local workers to give the consent to the biosample donation, the expenses sustained by the laboratory for the analysis as well as the necessity of approvals by the health unit and the manufacturing company to allow the investigational study do not facilitate the success of the biomonitoring campaign which, if not mandatory as the health surveillance, is still important to assess and quantify the exposure to hazardous substances and the susceptibility risk at workplace. Nonetheless the worker misinterpretation or the miscommunication of genetic susceptibility and vulnerability concepts might be misunderstood and considered as personal weakness or inability to perform a specific job task. This erroneous interpretation of susceptibility should be transferred to the workers in a clear and correct form so as they know the individual variability, regardless of the difference in genetic heritage, is not a negative aspect but should be conceived as a personal trait. The role of genotyping in the occupational exposure, no matter if it is carried out in a laboratory or by a statistical analysis, is to define a susceptibility risk for the investigated ethnic group. This information will be useful to take into account the difference between ethnicities so as to verify the company OELs are safe or need to be adjusted taking into account different susceptibilities. In the absence of blood sample or consent from the worker to donation, the availability of a statistical predictive model, based on the genotype publicly available on web databases, should be necessarily regarded as useful indicator of the probable subject's genotype of the ethnic group to which the individual belongs to. Even though the access to genotyping is not possible, a certain number of advantages might be achieved: reduction of the laboratory costs for the research institute, no need of informed consent from workers and no need of trained staff and

laboratory equipment for blood collection and therefore no venipuncture, which means no harm to the worker. The predictive model mentioned here may represent a potential but also amendable alternative to the laboratory genotyping of workers and might involve the study of other ethnic groups. However keeping updated these databases will be useful not only to better characterize all the ethnic groups' genotypes but also to manage the novel susceptibility risks that might compromise the individual health protection and safety in the workplace [35].

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Conflict of interests

The authors declare no conflict of interest.

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