

## Artigo de Revisão

# Towards a personalized risk assessment for exposure of humans to toxic substances

Em direção a uma avaliação de risco personalizado para exposição de seres humanos a substâncias tóxicas

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**Abstract**

Great response variability caused by genetic and/or environmental factors has been observed among organisms exposed to hazardous chemicals. This subject has been a topic of intense discussion in the USA since President Obama announced support for an “era of precision medicine”, which consists in the inclusion of genetic data of patients in the treatment design, imposing a new approach to risk assessment. Personalized evaluation must consider the phenotypic factors of an individual. Among the markers that have been developed to evaluate any alteration in the structure or function of organisms, biomarkers of susceptibility are of great importance because they indicate the natural characteristics of a given organism which make it more sensitive to a specific adverse effect or disease, or more responsive to exposure to a specific chemical/drug. The ‘-omics’ technologies provide an insight into the relationship between chemical effects and molecular mechanisms of action. These technologies are the pillars for a personalized toxicology and precision medicine. Predictive toxicology requires a more comprehensive knowledge on specific individual factors or susceptibilities predisposing to diseases, enabling personalized risk assessment and adequate medical treatment.

**Keywords:** personalized risk; susceptibility; predictive toxicology; human exposure; toxic substances.

**Resumo**

Há uma grande variabilidade nas respostas observadas entre os organismos expostos a uma substância química perigosa. Essa variabilidade é causada por causas genéticas e / ou ambientais. Esse assunto tem sido intensamente discutido, mesmo nos Estados Unidos, desde que o presidente Obama anunciou o apoio a uma “era da medicina de precisão”, a qual consiste na inclusão de dados genéticos do paciente no projeto do tratamento, impondo uma nova abordagem para avaliação de risco. A avaliação personalizada deve considerar fatores fenotípicos de um indivíduo. Entre os biomarcadores que foram desenvolvidos para avaliar qualquer alteração da estrutura ou função do organismo, os biomarcadores de susceptibilidade têm uma grande importância, uma vez que indicam as características naturais de um dado organismo, que o tornam mais sensíveis a um efeito ou doença adversa específica ou em resposta a uma determinada exposição. As tecnologias “ômicas” permitem a compreensão da relação entre os efeitos químicos e dos mecanismos moleculares de ação. Essas tecnologias “ômicas” são os pilares para a toxicologia personalizada e para a medicina de precisão. Toxicologia preditiva exige uma melhor compreensão dos fatores ou susceptibilidades individuais específicas predisponentes a doenças, permitindo uma avaliação de riscos personalizada e um tratamento médico adequado.

**Palavras-chave:** risco personalizado; susceptibilidade; toxicologia preditiva; exposição humana; substâncias tóxicas.

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## INTRODUCTION

Life is a complex and risky process; hence, it is necessary to understand and consider its complexity and risks to improve its quality.

The word 'risk' can have a number of meanings and interpretations, but in this paper, it is considered as 'the chance or probability of a person being harmed or experiencing an adverse health effect if exposed to a hazardous chemical'. To this end, a process called risk assessment (RA) was developed to decrease and/or eliminate these risks. It involves hazard identification, that is, an estimate of the probability of risk to individuals or populations associated with that hazard<sup>1</sup>.

In fact, risk can be expressed by the following equation:

$$\text{Risk} = \text{Hazard} \times \text{Exposure} \times \text{Susceptibility} \quad (1)$$

According to Equation 1, absence of risk (risk = 0) requires that all components be equal to zero, which is a difficult state to be achieved<sup>2</sup>. Among the three components of this equation, the third one, susceptibility, is the most difficult to evaluate, and it is usually abandoned.

In the case of hazardous chemicals, knowledge on exposure assessment and toxicological properties of the substance is essential for a RA process. Exposure assessment requires the understanding of pathways and patterns (such as air, water, food, soil, and workstation), frequency and duration of exposure to the hazard, and evaluation of the probability of any adverse effect. It is based on cause-effect and dose-response data<sup>1</sup>. However, workers or the population in general are seldom exposed to a single substance, rather, they are usually exposed to complex mixtures which may present additive, synergistic, or antagonistic actions, increasing the complexity of risk assessment even more<sup>1,3</sup>.

Risk characterization of exposure to a xenobiotic involves hazard identification, dose-response evaluation, and exposure assessment. This procedure results in qualitative and/or quantitative description, under specific exposure conditions. It is very important to develop risk management and risk communication procedures, thereby providing a scientific basis to support decision-making in risk management. This helps determine the means of eliminating or controlling exposure to a given chemical<sup>3</sup>.

However, even under risk management policies, unexpected toxicity and adverse events can be observed, most frequently in hypersensitive individuals or in a relatively small, undetected, susceptible population group<sup>4</sup>. Assuming that the main objective of any risk assessment study is the development of methodologies to prevent or avoid any adverse effect on the entire population, these susceptible groups must be considered in order to improve the effectiveness of any risk management policy.

Once the basis of our understanding of effective risk assessment is ascertained, an important question to be answered is: How can we identify these susceptible groups?

Responding to this question is not an easy task because several of these health effects are usually complex and may present multifactorial causes.

## METHODS

A literature search of these issues was conducted to improve knowledge for questioning and discussion on susceptibility and personalized medicine. In addition, observation and further discussion were directed to the applications of the '-omics' technologies for identification of susceptible groups and for personalized medical treatments.

A search in the PubMed<sup>TM</sup> database was conducted in the first trimester of this year to collect data. PubMed<sup>TM</sup> database is a research platform which primarily catalogs information on medical and biomedical themes. The survey was conducted using specific keywords from 1995 to 2016. The results were subsequently refined to provide information only from original scientific articles, reviews, and book chapters.

The number of publications related to personalized medicine and the '-omics' technologies has increased exponentially over the past decade, and the contribution of these sciences to personalized medical treatment according to individual susceptibility is remarkable.

## RESULTS AND DISCUSSION

There is great variability of responses between organisms exposed to hazardous chemicals. Similar doses of the same xenobiotic can present different responses even in organisms of the same species. Even twins (mono or dizygotic) and isogenic animals may respond differently after exposure to a xenobiotic. This difference has been attributed to genetic and environmental factors<sup>5</sup>. It is apparently clear that chromosomal DNA alone cannot completely determine the susceptibility of an individual. DNA interacts with the environment and this interaction can predispose or protect the organism from a disease or other health hazards<sup>6,7</sup>.

In fact, this susceptibility results from complex interactions between several factors. In addition to genetic factors, experiments in animals reveal that 20-30% of the observed individual differences are due to environmental influences and the remaining 70-80% are attributed to a third component<sup>6,8</sup>.

It is important to state that a particular cellular environment is determined by the physiology and metabolism of the entire organism and of the cells in its immediate neighborhood. Signals from this local environment influence cellular gene expression appropriate for replication, differentiation, quiescence, or apoptotic death, depending on the cell type and developmental context<sup>7</sup>.

In a multicellular organism, the cells are genetically homogeneous, but they can be structurally and functionally diverse owing to different gene expression. Therefore, identification of the proteins produced by these different tissues or group of cells may be an important tool to understand the complex cellular processes or even the impact of a toxicant on the organism exposed to it<sup>9</sup>.

To evaluate any alteration in the structure or function of an organism, several categories of biomarkers have been developed, such as those of exposure, internal dose and early biological effects, as well as that of susceptibility, as shown in Figure 1. Determination of biomarkers can help delineate the continuity of events, occurring from exposure to response time. Certain biomarkers can provide qualitative and quantitative indices of the status of individuals at different stages of the toxicological process, from exposure to disease, especially in diseases with a long latency period, e.g., tumorigenesis<sup>11</sup>. A new era for biomarker discovery may result from the application of ‘-omics’ technologies (genomics, proteomics, or metabolomics), and these technologies are completely modifying our understanding of medicine and biology.

To evaluate the susceptibility of an individual, it is necessary to establish biomarkers of susceptibility which are capable of indicating the natural characteristics of an organism that are more sensitive to a specific adverse effect or disease, or responsive to a specific chemical/drug exposure, e.g., polymorphisms in enzyme or carriers.

The biological action of a xenobiotic on any organism (susceptible or non-susceptible) requires the substance to reach its target. Hence, after absorption, the xenobiotic will

be distributed within the organism either as an unmetabolized species or as a metabolite and, in general, will cross biological membranes to reach its active site.

To prevent any deleterious effect, after absorption of an exogenous and toxic substance, the organism should excrete it. This substance can be excreted either in its original form or as a metabolite (biotransformed form), resulting from an enzymatic attack on the xenobiotic molecule by a wide array of metabolic enzymes present within the organism. In general, metabolism produces less toxic and more soluble products than the parent compound that can be easily excreted. Additionally, these metabolites may serve as substrates for other enzymes. Occasionally, this produces reactive metabolites, which can then react with cellular macromolecules such as DNA and proteins<sup>12</sup>.

Some important enzyme families involved in the biotransformation of xenobiotics are cytochrome P450 (CYP450), aldehyde dehydrogenase (ALDH), myeloperoxidase (MPO), epoxide hydrolase (EPH), N-acetyltransferase (NAT), sulfotransferase (SULT), glucuronosyltransferase (GLUT), and glutathione-S-transferase (GST). Polymorphisms in the expression of these enzymes are common and have a great impact on enzyme efficiency, because the extent of a metabolic reaction and the kinetics of the reaction are regulated by the chemical structure/spatial configuration of the involved enzyme<sup>12</sup>. For example, there are 57 encoding genes for the CYP superfamily and the enzymes are grouped in 17 families. Among these, the CYPs 2B6 (48 alleles), 2C9 (32 alleles), 2D6 (92 alleles), and 3A4 (34 alleles) are the most polymorphic ones<sup>13</sup>. As a whole, the majority of these polymorphisms present enzymatic activity

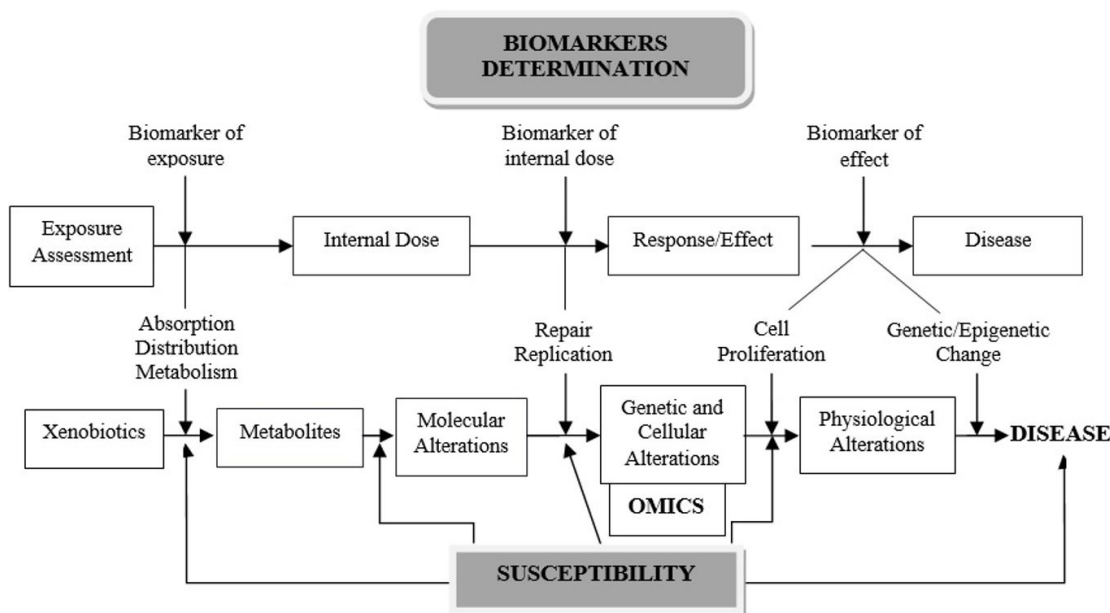


Figure 1. Important factors in the relationship between exposure and disease and its monitoring. Adapted from Vineis and Perera<sup>10</sup>

lower than their wild types<sup>14</sup>. Single nucleotide polymorphisms (SNPs) predominantly occur in three polymorphic CYPs<sup>15</sup>: 2D6 (114 SNPs), 2A6 (68 SNPs), and 2B6 (57 SNPs), with the former being the highest metabolizing CYP (Table 1).

The prevalence of certain polymorphisms varies according to ethnicity and even within the same ethnic group. For instance, approximately half of the Japanese population lacks aldehyde dehydrogenase 2 (ALDH2) activity, causing the accumulation of acetaldehyde in their body<sup>16</sup>. Thirty-four polymorphic alleles in four major CYPs (1A2, 2D6, 2C9, and 2C19) with significant impact on drug metabolism were identified in Caucasians<sup>15</sup>. CYP 2D6 was the greatest contributor of polymorphic alleles in this ethnic group. The reported frequencies of CYP2A6\*2 in Asian, African, and Caucasian populations were 28%, 62%, and 8%, respectively.

The phenotypic variability of xenobiotic metabolism occurs due to genetic polymorphisms in phase I enzymes (mainly cytochrome P450 dependent mixed function oxidases) that are involved in the bioactivation of carcinogens, and in phase II enzymes that are involved in detoxification by binding to glucuronic acid, glutathione, and sulfate<sup>17</sup>. Some polymorphic genes of phase I and phase II metabolic enzymes, their characteristics, and their related biological activities are shown in Table 2. The impact of genomic markers on cytochrome P450 enzymes follows the order: CYP2D6>CYP2C19>CYP2A6>CYP2B6>CYP2C9>CYP3A4/5<sup>42</sup>.

A study on 15 polymorphisms in the CYP superfamily in the south of Brazil involving African and European Brazilian descendants showed that the most influential polymorphic variants in these populations and their frequencies were in agreement with most descriptions of CYP allele frequencies in other populations of European and African origins. The most frequent alleles found were CYP3A5\*3, CYP1A2\*1F, CYP3A4\*1B, and CYP2C19\*2. Significant differences in allelic distribution between African and European descendants were observed for CYP3A4 and CYP3A5 genes. CYP3A4\*1B presented higher frequency in African descendants, whereas CYP3A5\*3 was observed in European descendants<sup>43</sup>.

**Table 1.** Number of SNPs and drugs metabolized per CYP, according to Preissner et al.<sup>15</sup>

Enzyme CYP	Number of single nucleotide polymorphisms	Number of drugs metabolized
2D6	114	223
2A6	68	51
2B6	57	74
3A4	46	434
1A2	41	165
2C9	39	163
2C19	29	140
1B1	28	17
3A5	26	128

In parallel, the distribution mechanism of the xenobiotic and/or its metabolites within the organism is regulated by a series of endocrine substances such as carriers (transporters) and receptors. In recent times, it has been widely shown that transporters are of great significance in the disposition of a xenobiotic to its active sites, where it ultimately shows its effect<sup>44</sup>.

Generally, simple diffusion is the most common pathway by which chemicals cross the cellular membranes. However, physicochemical properties such as hydrophobicity, size, and charge are also very important factors that govern the crossing of chemicals by both active and passive mechanisms<sup>45</sup>. In fact, to cross the biological membranes, exogenous substances can use a set of specialized proteins called transporters. These proteins are involved in both absorption and excretion of substrates by the cells.

Over 400 membrane transporters have been identified so far, and they are classified into two super families: ABC (ATP-binding cassette) and SLC (solute carrier)<sup>46</sup>. The SLC group of transporters is composed of proteins comprising 300 - 800 amino acids with molecular masses ranging from 40 to 90 kDa, whereas the ABC group of transporters is composed of proteins comprising 1200-1500 amino acids with molecular masses varying between 140 and 200 kDa<sup>47,48</sup>. The ABC superfamily, for example, has been associated with drug pharmacokinetics and individual susceptibility to side effects, interactions, and treatment efficacy of several drugs. Excellent reviews on this subject are available in the literature<sup>45,46,48</sup>.

An interesting example of such a relationship is the codeine-morphine effect. Both are weak bases and coexist in the body as protonated and non-protonated forms. The non-protonated form is able to permeate cellular membranes by passive diffusion. However, codeine is metabolized by CYP2D6 to morphine in the human body. CYP2D6 is a highly polymorphic enzyme with 5-10% of the Caucasian population carrying poor metabolizer allelic variant<sup>49</sup>. The activity of this enzyme is important in that in case of poor metabolizers, reduced, or absence of morphine after administration of codeine results in low or no analgesic effect. In contrast, the presence of ultra-rapid metabolizing variants results in a quicker analgesic effect and some undesirable effects, including death. Morphine independently permeates cellular membranes and its uptake, especially at low concentrations, is mediated by OCT1. Codeine, on the contrary, is also independently transported and its uptake is not dependent on this carrier. As OCT1 is highly polymorphic, the pharmacokinetics of morphine is greatly dependent on the activity of OCT1 variants. In humans possessing low or absent OCT1 activity, the administration of codeine will result in high plasma concentrations of morphine, and in case of ultra-rapid CYP2D6 metabolism, as seen in 1-10% of Caucasians, it may result in severe morphine toxicity<sup>50</sup>.

Additionally, it is known that single nucleotide enzyme polymorphisms in Hg-transporter genes can affect the urinary excretion of mercury in gold mining workers exposed to Hg(0)<sup>51</sup>.

As previously discussed, biological diversity between organisms can improve or decrease the effect of a xenobiotic on them. In fact, no two humans are equal; their DNA is 99.5% equal,

but studies conducted with identical twins indicate that DNA alone cannot be responsible for susceptibility<sup>6</sup>. Other equally complex factors are also involved in such phenomena.

Therefore, to improve the necessary understanding of this multifactorial problem, new analytical tools have been developed - the '-omics technologies. The development of these '-omics

**Table 2.** Polymorphic enzymes for susceptibility assessment in biomonitoring studies

Function	Polymorphic enzymes	Allele <sup>a</sup>	Enzymatic activity <sup>a</sup>	Endpoint	References
Phase I reactions	ADH1	*1	Null	Telomere	Pavanello et al. <sup>18</sup>
		*2 *3	Increased	Mean corpuscular volume of erythrocytes	
	ALDH2	*1	Normal	Sister chromatid exchanges	Wong et al. <sup>19</sup>
		*2	Decreased	DNA adducts	Yukawa et al. <sup>20</sup>
	CYP1A1	*1	Normal	1-hydroxypyrene	Ada et al. <sup>21</sup>
		*2	Increased		
		*3	Increased	DNA adducts	Rojas et al. <sup>22</sup>
		*4	Increased		
	CYP1A2	*1	Normal	Urinary metabolites	Gross-Steinmeyer et al. <sup>23</sup>
		*2	Inducible	Amino-dimethylimidazol	Stillwell et al. <sup>24</sup>
	CYP2A6	*1 *8	Normal	3-hydroxycotinine	Nagano et al. <sup>25</sup>
		*7 *9	Decreased		
	CYP2D6	*1	Normal	S-phenyl mercapturic acid	Rossi et al. <sup>26</sup>
		*3 o A	Null		
*4 o B		Null			
*5 o D		Null	Comet assay		
*6 o E		Null	Acetyl cholinesterase		
CYP2E1	*1	Normal	Sister chromatid exchanges	Wong et al. <sup>19</sup>	
	*3	Increased	Micronuclei	Jiy et al. <sup>28</sup>	
			Trans,trans-muconic acid	Chanvaivit et al. <sup>29</sup>	
Phase II reactions	EPHX	R o 1*	Normal	Steroidogenesis	Knag et al. <sup>30</sup>
		H	Decreased		
		Y	Increased		
	NAT2	*4	Rapid	N-acetylbenzidine-hemoglobin adduct	Beyerbach et al. <sup>32</sup>
		*5A	Slow		
		*5B	Slow		
		*5C	Slow		
*6A		Slow			
NQO1	*1	Normal	Plasma prolactin	Vinayagamoorthy et al. <sup>34</sup>	
	*2	Null			
PON1	A	Low	Enzyme activity levels	Sözmen et al. <sup>35</sup>	
	B	High			
GSTM1	*1	Normal	DNA adducts	Pavanello et al. <sup>36</sup>	
			Albumin adduct	Wu et al. <sup>37</sup>	
	*0	Null	Micronuclei	Angelini et al. <sup>38</sup>	
GSTT1	*1	Normal	Hydroxyethyl-valine	Yong et al. <sup>39</sup>	
	*0	Null	Micronuclei	Fennell et al. <sup>40</sup>	
GSTP1	A	Normal	Amino-methylimidazol	Koutros et al. <sup>41</sup>	
	B	Low	Amino-dimethylimidazol		
	C	Low	Amino-trimethylimidazol		

ADH1 – alcohol dehydrogenase 1 (class I); ALDH2 – aldehyde dehydrogenase 2 family; CYP1A1 – cytochrome P450, family 1, subfamily A, polypeptide 1; CYP1A2 – cytochrome P450, family 1, subfamily A, polypeptide 2; CYP2A6 – cytochrome P450, family 2, subfamily A, polypeptide 6; CYP2D6 – cytochrome P450, family 2, subfamily D, polypeptide 6; CYP2E1 – cytochrome P450, family 2, subfamily E, polypeptide 1; EPHX – epoxide hydrolase; NAT2 – arylamine N-acetyltransferase 2; NQO1 – NAD(P)H: quinone oxidoreductase; PON1 – paraoxonase; GSTM1 – glutathione S-transferase mu 1; GSTT1 – glutathione S-transferase theta 1; GSTP1 – glutathione S-transferase pi 1. \*variant allele; <sup>a</sup>From Pavanello et al.<sup>16</sup>

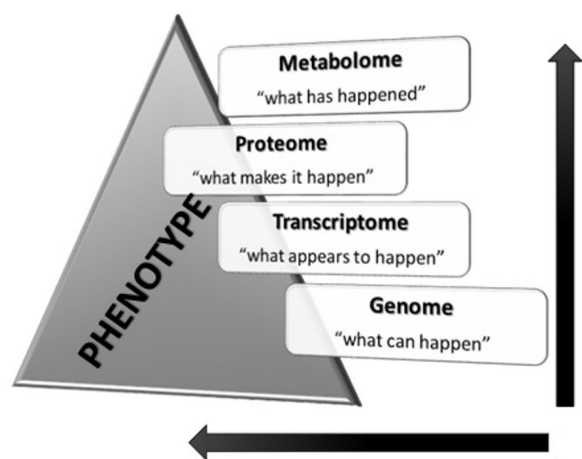
technologies has contributed greatly to our understanding of living organisms at a molecular level. These technologies are able to simultaneously deal with a great set of data in a short period of time, facilitating the knowledge of cellular molecular mechanisms, thus making it possible to study not only the genotypic behavior, but also and specially the phenotypic one<sup>52,53</sup>.

Thus, these technologies facilitate in-depth approaches in understanding the relationship between chemical structure-activity effects and molecular mechanisms of action. They also help in the comprehension of the differences between proteins and other cellular substances, between similar or different organisms, and the adverse effects of xenobiotics on exposed organisms<sup>54,55</sup>. Thus, these methods are generating very important sets of data which improve our understanding of cellular responses, tissue damage, and functional perturbations caused by exposure to a xenobiotic<sup>54</sup>. Moreover, these data are being used for constitution of databases containing a great variety of genetic sequence monitoring; epigenomic, proteomic, and metabolomic data; and clinical and toxicological information, thereby allowing the study of their interrelations.

It is well known that molecular profiles can be different when cells or tissues are exposed to the same toxicant, and this allows for the monitoring of biochemical homeostasis and toxic effects, simultaneously.

'Omics' datasets are fundamental to systems biology studies in order to integrate data, and thus understand the experimental system in question (disease, phenotype, therapeutic intervention)<sup>56</sup>. As shown in Figure 2, proteomics should be the technique of choice to identify susceptible groups prior to health effects.

In addition to genomics, two other '-omics' are very important for understanding the differences in cellular behavior under exposure to xenobiotics: proteomics and metabolomics.



**Figure 2.** Relationship between some '-omics' technologies and the type of answered questions related to effects resulting from exposure to xenobiotics that help in decoding phenotype. Modified from Pesce et al.<sup>53</sup>

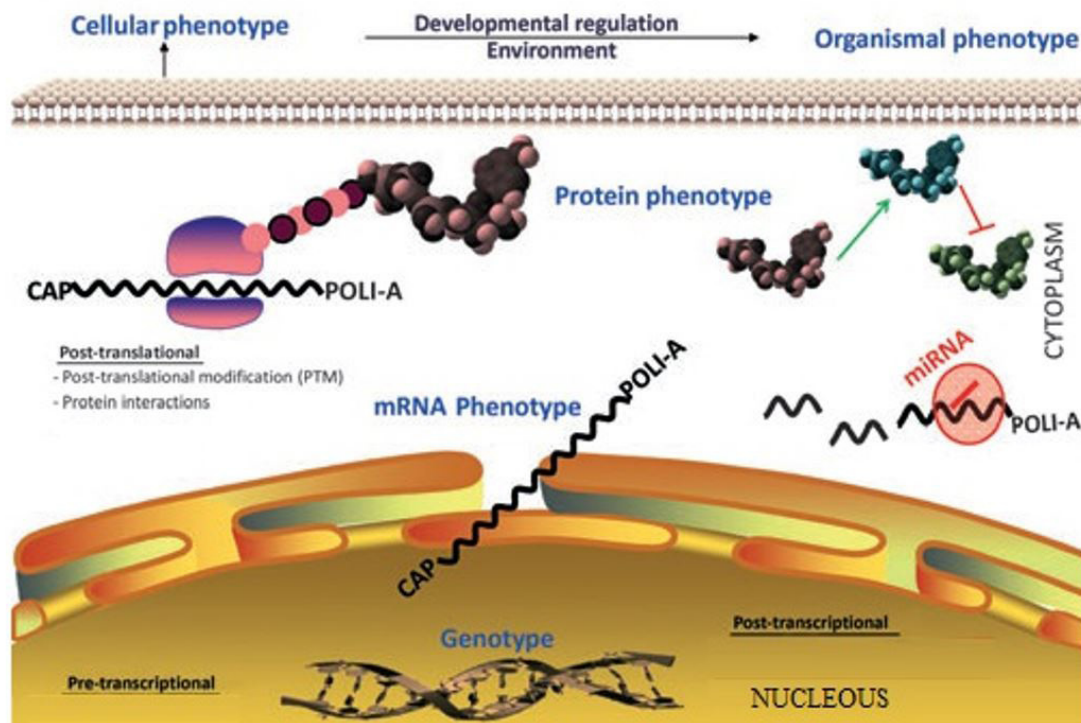
Metabolomics involves a comprehensive analysis of small molecule metabolites (< 1 kDa) of an organism. Hence, the differences between unperturbed and perturbed pathways could provide an insight into the underlying disease pathology and disease prognosis and diagnosis. In fact, this tool provides clinical biomarkers useful for identification of early-stage diseases<sup>57,58</sup>.

Proteomics is devoted to the study of the dynamics of protein expression, regulation, and interactions. Its function is closer to the phenotype and more directly responsive to natural selection or adaptation, making it easier to predict phenotype from genotype<sup>59</sup>. In a multicellular organism, cells are genetically homogeneous, but structurally and functionally diverse; making identification of the proteins that mediate these effects a very important tool to understand the complex processes of diseases<sup>1,9</sup>.

According to Wetmore and Merrick, proteomics is in a unique position to contribute to new protein discovery for the benefit of public health, and in linking toxicology and pathology to a systems biology view of protein dysfunction in toxicity and environmental disease<sup>60</sup>.

It is well known that, in higher organisms, there is no simple one-to-one relationship between genes and proteins; as a rule, this is a one-to-many relationship. Mechanisms such as alternative splicing of mRNA precursors, cleavage and processing of polypeptide chains, and post-translational modifications may generate multiple protein isoforms (Figure 3). In addition to these mechanisms, developmental processes and environmental factors may cause proteomic differentiation across tissues and organs, resulting in a distinct phenotype for each level. Therefore, it is not possible to collect information regarding protein properties from a static DNA alone<sup>59,61</sup>.

The large-scale analysis of proteins leads to a more comprehensive view of molecular and cellular pathways of the complex processes in living systems. The analysis of the proteome involves a variety of methods including gel-based techniques, protein microarrays, high and ultra-performance liquid chromatography coupled to mass spectrometry (MS) - a high-throughput technology with high sensitivity. Currently, mass spectrometric identification of gel-separated proteins and of fractionated peptides by multidimensional protein identification technology (MudPIT) is a widely used analytical tool for proteomic studies<sup>62</sup>. In general, the former has enough sensitivity to deal with protein mixtures, and it uses a digestion of gel-separated proteins into peptides by certain specific proteases such as trypsin. Peptides rather than proteins are easier to elute and to be identified by MS, whereas in the MudPIT approach, after in-solution digestion, peptides are fractionated by strong cation exchange (SCX) or hydrophilic interaction chromatography (HILIC), followed by reversed phase (RP) chromatography prior to their identification by MS<sup>62</sup>.



**Figure 3.** Phenotype formation according to the expression of mRNA and its corresponding protein, regulated by diverse mechanisms. Adapted from Diz et al.<sup>59</sup>

### Biomarkers of susceptibility

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic. These markers indicate individual or population differences which affect their response to that chemical substance. Such markers may include inborn differences in metabolism, variations in immunoglobulin levels, low organ-reserve capacity, or other identifiable genetically determined or environmentally induced variations in absorption, metabolism, and response to environmental agents<sup>63</sup>. Other factors that may affect individual susceptibilities include nutritional status of the organism, the role of the target site in overall body function, condition of the target tissue (present or prior to disease), and compensation by homeostatic mechanisms during and after exposure<sup>64</sup>. This means that variation in the response to xenobiotics by individuals is closely related to specific genotypes and phenotypes, which is the basis of individual clinical toxicology<sup>65</sup>.

Recently, several authors have been discussing the application of systems biology, or rather, systems toxicology to address risk assessment issues. Sauer et al.<sup>66</sup> report a need for more predictive and accurate approaches to risk assessment, requiring a mechanistic understanding of the process by which a xenobiotic perturbs biological systems. Wetmore and Merrick<sup>60</sup> defend the toxicoproteomics approach, which is positioned towards an

expanded understanding of protein expression during toxicity and environmental disease, for the advancement of public health. Titz et al.<sup>67</sup> highlight the fact that protein alterations could be a close reflection of biological effects, and Sturla et al.<sup>68</sup> discuss the identification of how biological networks are perturbed by exposure to xenobiotics and enable the development of predictive mathematical models of toxicological processes. Sauer et al.<sup>69</sup> recommend that the use of systems toxicology with advanced analytical and computational tools should be integrated with classical toxicology. They also suggest that quantitative analysis of large networks of molecular and functional changes occurring across multiple levels of biological organization should be conducted.

Clearly, the use of systems biology/toxicology will produce a great amount of data, facilitating a more complete understanding of the overall biological processes. However, the use of these data for risk assessment in a significant number of people will have a very high cost. Therefore, the identification of susceptibility biomarkers would provide the development of inexpensive and useful tools that could be applied worldwide.

The use of such susceptibility biomarkers will allow the identification of individual responses to chemical exposure. In fact, two types of susceptibility biomarkers are possible: those used for prognosis and those used for prediction purposes. Prognostic biomarkers are biological measurements capable of

indicating an individual's susceptibility to develop an adverse effect during a clinical treatment. Predictive biomarkers are used to indicate the possibility of an adverse effect on health when a xenobiotic is administered to an individual in a particular condition, indicating the need to avoid exposure to certain xenobiotics<sup>11</sup>. In both cases, susceptibility biomarkers are indicators of individual differences in the development of adverse effects in response to a specific chemical exposure, and are used to differentiate patients according to their degree of susceptibility<sup>70</sup>.

The recognition of such susceptible groups is very important in risk assessment studies for therapeutic or toxicological purposes.

### Personalized clinical toxicology

The human health status is always a great concern worldwide. Noticeably, '-omics' will contribute to improving the necessary knowledge in healthcare. The interaction of these techniques and medicine will certainly produce new diagnostic tools to determine individual risk factors and personalized medical treatments. It is clear that '-omics' are the pillars of personalized clinical toxicology, which is based on knowledge of individual characteristics, and requires an understanding of the factors that influence the organic variability<sup>71</sup>. Technological development has led us to an era of individualized therapy<sup>72</sup>.

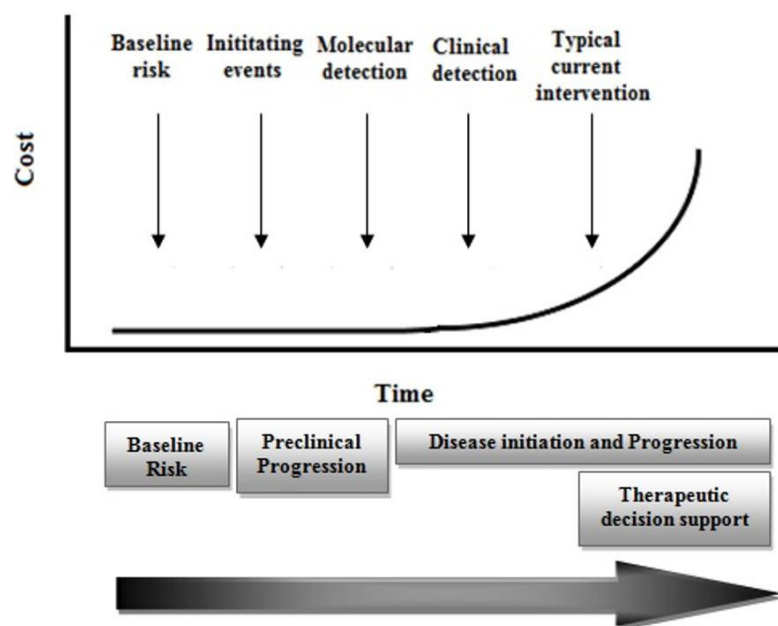
Nowadays, this subject has been a topic of intense discussion in the USA since President Obama announced support for an "era of precision medicine", where prevention and treatment

strategies will consider individual variability, with a great potential for improving health<sup>73-75</sup>.

Predictive medicine will develop due to a better comprehension of the specific factors that cause predisposition to diseases. According to Hocquette<sup>63</sup>, it will be possible to predict the probability of developing specific diseases through scientific results and applied biotechnologies that arise from proteomics, metabolomics, transcriptomics, epigenomics, and genomics. This requires associations of gene expression patterns with diagnoses, treatments, and clinical data. Thus, preventive medicine and medical therapy will be personalized.

The applications of metabolomics and especially of proteomics in personalized toxicology encompass the understanding of susceptibility, diagnosis and prognosis, toxicogenomics, and monitoring of any health effect related to xenobiotic intake. During the progression from a healthy to a disease state (Figure 4), there are important time points at which these '-omics' can be applied to personalize health care<sup>76,77</sup>. Disease susceptibility and risk can be estimated and predicted by assessing DNA and its interaction with the environment and external factors. The integration of these omics-based technologies in assessing the prevention of toxic effects can minimize the cost of disease treatment in the health system.

Nowadays, omics-based risk assessments of common and complex diseases, application of molecular signatures in diagnosis and prognosis, and dose-response relationships are important issues in personalized toxicology. In order to increase



**Figure 4.** Progression of a chronic disease and time points at which several molecular and clinical tools are essential to assess disease risk, screening, diagnosis, prognosis, and therapeutic strategy. Adapted from Ginsburg and Willard<sup>76</sup>



their effectiveness, these techniques must be standardized and integrated to health systems<sup>77,78</sup>.

A molecular signature is a set of biomolecular features such as a DNA sequence, mRNA, and protein expression. In conjunction with a computational procedure, it can help to predict a phenotype of clinical interest<sup>52</sup>, based on single or multiple data types, for instance, the prediction of any health risk or organic response to toxic xenobiotic exposure and its physiological actions. The identification of molecular signatures from ‘-omics’ data for diverse clinical applications consists of four stages: 1) definition of the scientific and clinical context for the molecular signature; 2) data acquisition; 3) feature selection and modeling; and 4) evaluation of the molecular signature from independent datasets<sup>52,76</sup>.

The identification of genetic variants associated with individual response may help predict the occurrence of health events in susceptible individuals. In toxicokinetics, polymorphisms in encoding genes for both drug metabolizing enzymes and transporters affect drug availability at the target site, whereas in toxicodynamics, target proteins such as receptors, enzymes, and intracellular signaling proteins affect sensitivity to chemicals<sup>79</sup>. Individual attributes such as resistance, regimens, and dosing interfere in the results of toxicological trials. Genetic, phenotypic, and environmental factors should be considered in order to provide biomarkers with analytical validity and clinical utility to optimize the effectiveness of specific treatment<sup>65,78</sup>.

## CONCLUSIONS

Human susceptibility is one of the most problematic components of risk assessment owing to its complexity and difficulties in determination. Additionally, it is not externally

controllable as exposure. For these reasons, in general, it is not scientifically considered in risk assessment calculations. However, it is as important as the other factors.

The expectancy brought by the ‘-omics’ technologies in relation to personalized toxicology and individual health conditions is still far away from its myriad of possibilities. Indubitably, the development of genomics and the new derived ‘-omics’ technologies represent a major advancement to the better understanding of human organisms and their similarities and individual differences, allowing for the study of individual susceptibilities.

As proteomic is the ‘-omic’ technology closer to phenotype, proteomic biomarkers will be of great value for risk assessment studies because they involve susceptible individuals or population groups. The analytical methodologies available for separation and quantification of proteins, such as HPLC/MS/MS, electrophoresis, and protein microarray still present some limitations, and require improvements to increase the number of molecular signatures for clinical applications and personalized prediction of disease outcomes.

The applications of these new analytical tools to identify biomarkers of susceptibility or individual susceptibility will help generate data to be used in any risk assessment procedure. Such a scenario will certainly contribute to improving public health.

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