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Simplicity and complexity of genetic susceptibility in the occupational environment

by

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Abstract

The individual response to physical or chemical stress may vary as a function of the particular gene combination regarding metabolism of chemical mutagens, DNA repair, cell death and cell cycle control. Nowadays, methods for genotyping have become easy to perform and in vitro phenotyping approaches are in development. It is therefore interesting to consider whether these methods assessing genetic susceptibility can be implemented for occupational biomonitoring. A major question is whether genotyping or phenotyping or both has the best predictive value for cancer risk and should be applied. To fully understand the relationship between genotype and phenotype, knowledge about the different factors influencing the expression of a genotype into a phenotype is still missing.

In this review we compare advantages and disadvantages of genotyping and phenotyping to assess individual susceptibility and discuss the different parameters modifying the genotype-phenotype relationship. The

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importance of both approaches is illustrated by a study conducted in our laboratory in workers exposed to low dose ionising radiation. Genotyping for hOGG1, XRCC1 and XRCC3, enzymes involved in base excision and double strand DNA repair was performed; the DNA strand break repair phenotype was assessed by in vitro challenging with γ-rays. The results indicate that hOGG1 and XRCC3 may be predictive for induced mutations after exposure to ionising radiation, and that the in vitro repair phenotype assay might also be a valuable approach to assess individual susceptibility.

Additional studies on larger population samples are needed before advising these genetic tests for susceptibility in daily practice.

Keywords: *Occupational exposure, genotype, phenotype, genetic susceptibility.*

Introduction

Biomarkers of exposure and early genetic effects are available and well validated methodologies to perform an adequate medical surveillance of workers exposed to mutagens/carcinogens. The individual response to a physical or chemical stress may vary as a function of the particular gene combination that a worker has regarding metabolism of chemical mutagens, DNA repair, cell death (apoptosis/necrosis) and cell cycle control. Nowadays methods for genotyping have become easy to perform and *in vitro* phenotyping approaches are in development. It is therefore interesting to consider whether these methods assessing genetic susceptibility can be implemented in the daily practice of occupational biomonitoring.

In this manuscript the following questions will be addressed: How different are the reactions of an individual to physical or chemical stress? How important is the follow up of this genetic susceptibility for occupational exposure? Can determination of the genotype and/or phenotype help to define individual susceptibility?

In a first part we describe the simplicity and complexity of the human genome. We then discuss the genotype-phenotype relation and compare advantages and disadvantages of genotyping and phenotyping. The genotype represents the genetic characteristics of an individual; the phenotype is the physical appearance of a trait. Finally we present a study of our laboratory, in which the DNA-repair genotype and phenotype were investigated in workers occupationally exposed to γ-rays, to illustrate the importance of applying both genotyping and phenotyping.

Simplicity and complexity of the human genome

The DNA contains several hierarchies of organization and is packaged in a complex way around protein cores to produce the chromosomes. The arrangement of the base pairs in the DNA, called a sequence, is the basis of the genetic code and will be translated into amino acids, the building blocks of proteins. This principle is called the "central dogma": DNA gives rise to mRNA via transcription, from this mRNA proteins will be formed by translation. It explains how the genotype gets translated into the phenotype. A given allele will give rise to a certain protein or enzyme. In the case of monogenic inheritance in an eukaryotic diploid cell a combination of two alleles will define the phenotype. Depending on the activity of these proteins or enzymes a certain phenotype will be defined. Different variants of alleles encoding enzymes can exist (genetic polymorphisms) which will result in polymorphic enzymes. This means that a population may contain two or more variants of an enzyme resulting in varying enzyme activity. While these polymorphisms may not directly cause a disease, some polymorphisms, either in combination with other factors, such as environmental factors, or with other polymorphisms, can affect the chance that an individual may develop a condition or possibly affect how they would react to certain exposures. Polymorphisms are considered to be relevant if the frequence of the polymorphic allele represents more than 1 % and if it codes for a significantly different functional protein activity. Polygenic conditions are features, health conditions, and diseases that are considered to be the result of the interaction of two or more genes.

The complexity is determined by many factors: the length of a DNA molecule (2.2 m), the large number of genes (ca 30,000) in the human genome coding for ca 100,000 different proteins, of which 80 % are not known until know, gene structure, the role of the tertiary and quaternary structure and dimerisation of proteins, monogenic or polygenic inheritance, different processes controlling one disease and one gene or gene combination leading to different syndromes (pleiotropy). Moreover a complex interaction between genes and environment (including development) is responsible for the phenotype figure 1 and influences the individual susceptibility for occupational diseases (1). If one gene determines a given phenotype (monogenic inheritance), it is considered that the influence of the environment on the genotype-phenotype is limited. However, if more genes are involved (polygenic inheritance) the role of the environment is increasing. One may consider that the genome defines a reaction norm modulated by the environment. The genotype-phenotype relation can therefore be influenced by the occupational and /or environmental exposure. As Figure 2 shows, the interaction with exposure can influence a biomarker (2).

Considering our limited understanding and the complexity of the genotype-phenotype relation one can wonder whether rather the genotype and/or the phenotype should be investigated for the study of occupational diseases.

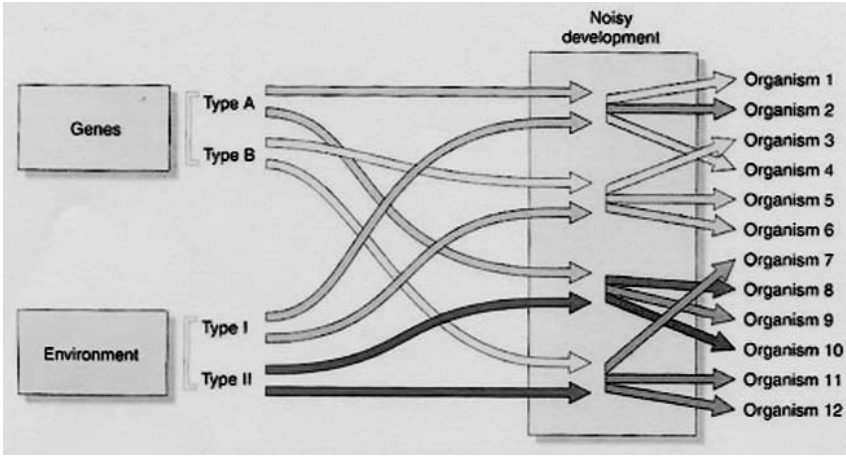
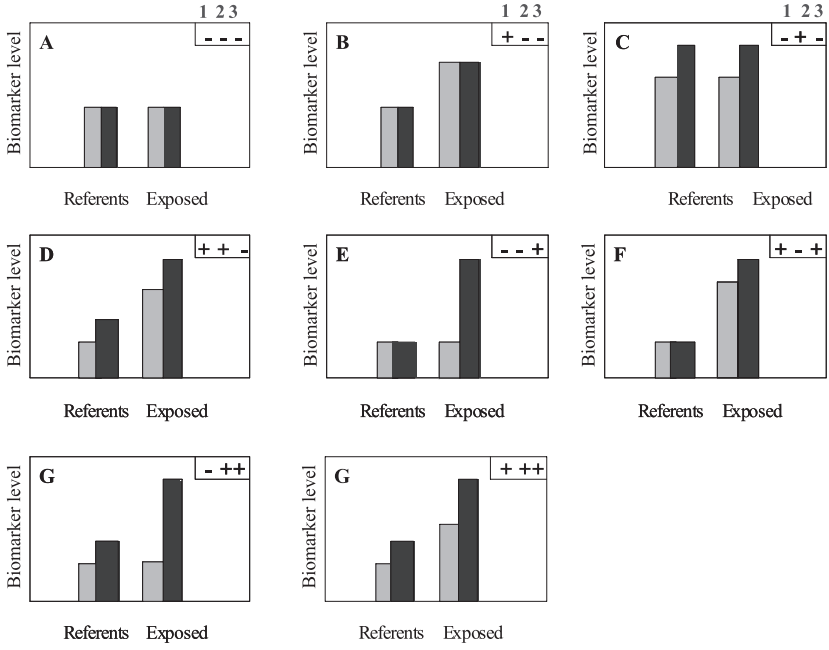


Fig. 1: A model to illustrate the complex genotype-environment-phenotype relation (1)



The boxed signs show the presence (+) or absence (-) of 1 = exposure,2 = genotype, 3 = genotype – exposure interaction.

Fig. 2: Results (group means) from imaginary biomarker studies with black columns representing expected "risk genotype" (2)

Why genotyping and/or phenotyping

Genetic polymorphisms provide us with the ability to study inter-individual differences in susceptibility to exposure and diseases and, to analyse whether the risk of cancer associated with particular environmental exposure differs with respect to functionally different polymorphisms of genes. Biomarkers of susceptibility include polymorphisms in drug/carcinogen metabolism, in DNA repair capacity, and in genes that control cell cycle, cell death and immune response. The identification of susceptibility genes could therefore lead to possible prevention programmes directed to high-risk individuals. In particular from the theoretical point of view Figure 3, it is expected that an individual with a less efficient genotype for DNA repair, as an example, will show a higher induction of mutations than an individual with an efficient genotype exposed to a relative low dose of a mutagen. At higher exposure level the extent of DNA damage overrules the repair capacity, even in the efficient genotype, and would lead to "similar" frequencies of mutations. Therefore the assessment of genetic susceptibility may be quite important to define exposure limits (safety factor) at concentrations encountered in occupational exposure.

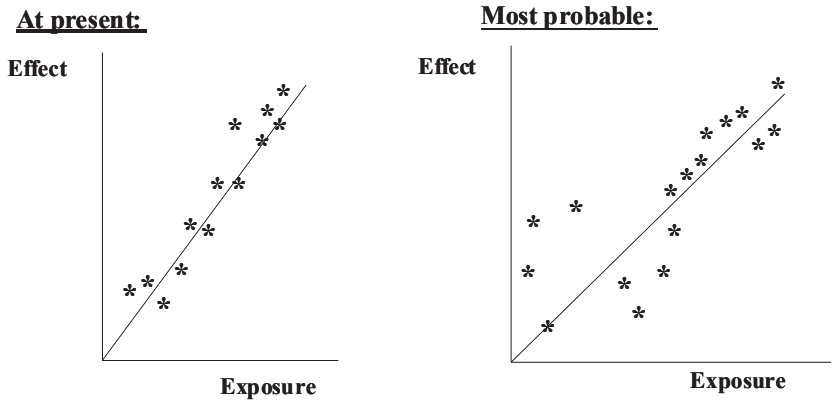


Fig. 3: DNA repair capacity in function of mutagen exposure

In Table 1 an overview of the advantages and disadvantages of genotyping versus phenotyping is given (3). Genotyping has the advantage of being technically easy and inexpensive, not influenced by the environment, and giving definite answers, but is more distal to the disease. The functional variation of genes presented in Table 2 is likely to have a subtle effect on cancer risk for an individual, but may have a large

population impact because the relevant polymorphism may be highly prevalent (4). Until now gene-environment interactions studies have been based on a relatively limited sample size and have analysed only one or several genes. Much larger studies based on many more genes would provide clearer answers.

Phenotyping is more proximal to the disease and integrates effects of several genes, including environmental factors, however the methodologies are not yet fully validated and tend to be expensive. Examples of the latter include the in *vitro* challenging assays and microarrays (3).

From this comparison one could conclude that both genotyping and phenotyping are important.

TABLE 1
Some advantages and disadvantages of genotyping versus phenotyping (adapted from (3))

	Genetic polymorphisms	Functional/phenotype measures
- Proximity to disease on causal chain	More distal	More proximal
- Expected relative strength of association	Weaker	Stronger
- Inductive or inhibitory effects of exposures	Not integrated into the measure	Integrated into the measure
- Epigenetic processes	Not integrated into the measure	Integrated into the measure
- Effects of post-transcriptional/ translational alterations	Not integrated into the measure	Integrated into the measure
- Relative degree of measurement error	Lower	Higher
- Measures single gene processes	Yes	Yes
- Measures multi-gene processes	Yes, but with difficulty	Yes
- Temporal stability of measure	Stable	Less stable
- Modifiability and applicability for prevention trials	Not modifiable and not applicable	Modifiable and applicable
- Person-to-person transmission within family and applicabality fot family-based analysis	Transmission is determined by Mendel's law and is applicable	Less deterministic transmission and not applicable
- Ethical considerations	Higher	Lower
- Array-based simultaneous measurement of many genes/gene products	Challenging, because amplification of each gene is involved	More feasible, because individual amplification is not involved

TABLE 2
Population sample size requirements for various combinations of OR and prevalence of polymorphisms (adapted from (4))

Frequency	OR (odds ratio)				
	1,25	1,5	2,0	5,0	25
50%	1267	387	136	30	13
20%	1850	535	172	28	8
5%	6020	1689	516	69	11

Comparison of DNA strand break repair genotype and phenotype to predict genotoxicity in workers exposed to gamma rays

The cellular response to chemical/physical stress is dependent on the bio-availability of the mutagen, its *in vivo* metabolism, its specific interaction with DNA and /or proteins, the repair capacity of the DNA lesions and the cell survival. To study the relationship between genotype and phenotype in a more simple occupational exposure type, we chose to study ionising radiation (IR) which does not require metabolism and whose DNA damaging and repair processes are well understood. γ-Rays are known to induce oxidative damage and DNA strand breaks. We have therefore selected three polymorphic genes to study: hOGG1 and XRCC1 in the base excision repair (BER) pathway, and XRCC3 in the double strand break repair process (DSBR) (6). Oxidative damage in the DNA results in the accumulation of 8-oxoguanine. hOGG1 encodes 8-oxo-guanine-DNA glycosylase in humans, which removes the 8-oxoguanine from DNA as part of the BER pathway. The DNA repair protein XRCC1 also involved in BER, forms complexes with DNA polymerase beta, DNA ligase III and poly-ADP-ribose polymerase (PARP) in the repair of DNA single strand breaks. The XRCC3 protein functions in the homologous DNA double strand break repair (DSBR) pathway and directly interacts with and stabilises Rad51, one of the key components of the pathway.

A study was conducted in 32 male seasonal cleaners of nuclear plants and 31 control workers in which the DNA-repair genotype and phenotype were investigated. We aimed at assessing the predictivity of the hOGG1^{326}, XRCC1^{194}, XRCC1^{280}, XRCC1^{399}, XRCC3 ^{241} genotypes and the single strand break *in vitro* repair phenotype for the induction of genotoxic effects (DNA damage and micronuclei) (5). The Ser326Cys polymorphic site was determined for hOGG1 with the possible genotypes being Ser/Ser, Ser/Cys and Cys/Cys. Codon 194, 399 and 280

polymorphisms and codon 241 polymorphism were determined for XRCC1 and XRCC3 respectively.

The repair phenotype was assessed by an *in vitro* challenge assay where the repair of alkali labile sites and DNA strand breaks were quantified by the comet assay (7). It has the potential to be considered as a general assay to estimate the repair capacity in workers exposed to mutagens.

The obtained data showed a significant contribution of the hOGG1^{326} and XRCC1^{399} genotypes to the *in vitro* DNA strand break repair capacity at the population level. At the individual level, the hOGG1 variants Ser/Cys and Cys/Cys showed a slower in *vitro* DNA repair than the Ser/Ser hOGG1^{326} wildtype genotype; genetic polymorphisms for XRCC1 did not influence repair capacity. A multivariate analysis performed with genotypes, age, cumulative dose, exposure status and smoking as independent variables indicated that in the control population, repair capacity is influenced by XRCC1^{280}polymorphism Table 3. In the exposed population, DNA damage is greater in individuals having polymorphisms in hOGG1^{326} or XRCC1^{280} genotypes Individuals with XRCC3^{241} variants have higher frequency of MNCB and MNMC than those with the wildtype. In addition MNMC are influenced the by XRCC1^{280} polymorphism with the variants having higher MNMC frequencies than the wildtype. The analysis confirms that MN frequencies are reliable biomarkers for the assessment of genetic effects in workers exposed to IR. A combined analysis of the three genotypes, hOGG1^{326}, XRCC1^{399} and XRCC3^{241} polymorphisms is advised in order to assess individual susceptibility to IR. As an alternative or complement, the *in vitro* DNA strand break phenotype, which integrates several repair pathways, is recommended. No statistically significant increase in genotoxic effects was observed in workers exposed to IR; on the contrary, a more efficient repair capacity was observed in the exposed workers, suggestive of adaptive response. However, the workers with hOGG1^{326} or XRCC3^{241} polymorphisms who smoke and who are exposed to IR represent a specific population requiring a closer medical surveillance because of their increased mutagenic/carcinogenic risk.

This study indicates that hOGG1 and XRCC3 may be predictive for exposure to ionising radiation and that the *in vitro* repair phenotype assay, covering different DNA repair enzymes, might be a valuable approach to assess individual susceptibility. To conclude whether this approach has a better predictive value than genotyping, or whether we need both genotyping and phenotyping, an analysis covering a larger number of samples is required.

TABLE 3
Determinants of genotoxicity in workers (5)

Population	Genotoxicity parameter	Independent variable	Partial r^2	slope	r^2	p-value
Control (n=28)	**DNA damage**	1. Age	0,448	0,019	0,448	**0.000**
	Repair phenotype at 120'	1. $XRCC1^{280}$	0,250	0,102	0,2	**0.041**
		2. $XRCC3^{241}$	0,327	-1.968	0,293	**0.016**
		3. hOGG1*smoking	0.202	0.048	0.171	0.063
	MNCB	1. Age	0.287	0.543	0.257	**0.027**
		2. $hOGG1^{326}$	0.227	20.81	0.191	**0.010**
	MNMC	1. Age	0.107	4.892	0.078	0.88
		3. $XRCC1^{399}$	0.133	14.89	0.100	**0.056**
Exposed (n=30)	**DNA damage**	1, Age	0.784	0,021	0.784	**0.000**
		2, $hOGG1^{326}$*$XRCC1^{280}$	0.213	0.292	0.058	**0,027**
	Repair phenotype at 60'	1. Age	0.342	0.631	0.226	**0.003**
		2. $XRCC1^{194}$	0.354	17.64	0.238	**0.002**
		3. hOGG1*$XRCC^{3241}$	0.411	17.48	0.304	**0.001**
	Repair phenotype at 120'	1, $hOGG1^{326}$	0.616	25.040	0.608	**0.000**
		2, hOGG1*smoking	0.551	-22.950	0.465	**0.000**
	MNCB	1.Age	0.704	0.139	0.679	**0.000**
		2. $XRCC3^{241}$	0.272	1.153	0.107	**0.022**
	MNMC	1. $XRCC1^{280}$	0.308	-1.600	0.299	**0.014**
		2. $XRCC3^{241}$*smoking	0.158	0.493	0.125	0.093
Total (n=58)	**DNA damage**	1. Age	0.555	0.020	0.531	**0.000**
		2. exposure	0.190	0.246	0.097	**0.001**
		3. exposure*smoking	0.054	0.115	0.024	0.088
	Repair phenotype at 5'	1, hOGG1	0,05	7.194	0,05	0,085
	Repair phenotype at 60'	1, Age	0.107	0.440	0.096	**0.013**
		2, hOGG1	0.113	9.423	0.103	**0.010**
		3. $XRCC3^{241}$*Smoking	0.067	8.027	0.058	**0.051**
	Repair phenotype at 120'	1, hOGG1	0.149	7.026	0.142	**0.003**
		2, exposure*smoking	0.100	-5.631	0.092	**0.015**
	MNCB	1, Age	0.241	0.109	0.081	**0.000**
		2, $XRCC3^{241}$*exposure	0.082	1.685	0.082	**0.002**
	MNMC	1.$XRCC1^{280}$	0.082	-0.804	0.082	0.078

The relative influence of the various independent variables, exposure categorised as controls or exposed, hOGG1326 categorised as Ser/Ser, Ser/Cys or Cys/Cys, XRCC1194 categorised as Arg/Arg and Arg/Trp, XRCC1280 categorised as Arg/Arg or Arg/His, XRCC1399 categorised as Arg/Arg, Arg/Gln or Gln/Gln, XRCC3241 categorised as Thr/Thr, Thr/Met or Met/Met, the interactions between hOGG1326 and smoking, hOGG1326 and XRCC3241,Exposure and smoking, XRCC3241and smoking, XRCC3241 and Exposure and age on the levels of TD (Tail DNA), MNCB (micronucleated binucleates) MNMC (micronucleated mononucleates), RD60 and RD120 was tested by stepwise multivariate linear regression (SPSS 11.0 statistical package). The significance level for entry into the model was 0.25; the significance for staying in the model was 0.10.

Conclusion

From all the observations and considerations described above, we can conclude that genotyping and sometimes phenotyping could be very useful in the occupational environment at individual level when an unexpected percentage of genetic aberrations is observed during follow-up. Yet the interaction between polymorphisms and the relationship between genotype and the functionality of proteins needs further exploration.

Therefore, not only additional research but also larger numbers of samples and simultaneous analysis of large numbers of candidate genes are required before considering genotype-phenotype analysis as an appropriate methodology to improve primary cancer prevention at the work place.

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