

Commentary

Genetic Polymorphisms and Mechanisms of Neurotoxicity: Overview

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Received 10 December 2004; accepted 31 May 2005

Available online 18 July 2005

Keywords: Single nucleotide polymorphisms; Environmental Genome Project; δ -Aminolevulinic acid dehydratase; Lead; Organophosphorus compounds; Paraoxonase

INTRODUCTION

Genetic polymorphisms may affect the susceptibility of individuals or populations to toxic agents that cause developmental defects or disease. This commentary outlines basic concepts of genetic polymorphisms and their relevance to environmentally-induced diseases. First, we provide a basic overview of genetic polymorphisms, with emphasis on single nucleotide polymorphisms (SNPs). Next, we summarize the approach of the NIEHS-sponsored Environmental Genome Project, which focuses on SNPs as an efficacious approach to understand gene–environmental interactions. Genes that have possible relevance to neurotoxicity are presented in a table with annotations regarding their known interactions with neurotoxins. Research on three of these genes, δ -aminolevulinic acid dehydratase (ALAD), human serum paraoxonase (PON1), and monoamine oxidase B (MAO-B) is briefly reviewed. We also comment on the high number of genes and gene products in the nervous system that increase the complexity of the search for environmentally relevant genes in neurotoxicity. Finally, we list several prospects for further research.

WHAT ARE GENETIC POLYMORPHISMS?

A variation in allelic DNA sequences between individuals is called a polymorphism if the most common allele is observed in a population no more than 99% of the time. Polymorphisms can occur in any region of a gene, including in its open reading frame, introns, intron/exon junctions, proximal regulatory sequences such as the promoter, or distal regulatory sequences such as an enhancer. Single nucleotide polymorphisms (SNPs) form about 90% of these variations (Collins et al., 1998). The typical frequency with which one observes single base differences in genomic DNA from two random chromosomes is of the order of 1/1000 bp (Li and Sadler, 1991; Wang et al., 1998; Taillon-Miller et al., 2004). These SNPs that occur in the genome are passed on to the next generation after recombination and are randomly distributed to the offspring, thus maintaining equilibrium in the population. However, some of these SNPs occur together nonrandomly (over several generations) and are said to be in linkage disequilibrium (LD). The probability of LD occurring is much greater within ethnic populations than between populations and in mixed populations and is a key consideration in association studies. In principle, association studies compare SNPs (marker frequencies) in unrelated cases and controls, and test for the co-occurrence of a marker and the disease at the population level. Classical linkage studies focus on disease genes transmitted through Mendelian inheritance, mainly through microsatellite or short tandem repeats (STR) analysis whereas association studies focus on

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multifactorial diseases, mainly through SNP analysis. A significant association between a marker and a disease may implicate a candidate gene in the etiology of a disease.

There are about 10 million common SNPs that may have to be scored in order to obtain statistical power sufficient for meaningful for whole genome association studies. In order to make association studies practical and affordable, Haplotype maps (HapMap) have been constructed by an International consortium (<http://www.hapmap.org/>). A set of closely linked SNPs present on one chromosome (mostly in LD) that are inherited together is called a haplotype. The International HapMap Project is identifying these common (i.e., frequency $\geq 1\%$) haplotypes in four populations from different parts of the world. It also is identifying “tag” SNPs that uniquely identify these haplotypes. This concept is similar to the use of marker genes to establish genetic linkage, except that tag SNPs involve single nucleotide variations rather than DNA nucleotide repeats (mostly bi, sometimes tri, tetra, or pentanucleotide) variations. By testing an individual’s tag SNPs, a process known as genotyping, researchers will be able to identify the collection of haplotypes in a person’s DNA. The number of tag SNPs that contain most of the information about the patterns of almost all genetic variation is estimated to be about 300,000. If an investigator knows the chromosomal location, which is true for vast majority of disease phenotype, the number of SNPs to be analyzed comes down dramatically. These haplotype maps in specific populations have been the basis of the HapMap, that enables geneticists to take advantage of SNPs and other genetic variants organized on chromosomes. There are several other private and public endeavors to discover SNPs and/or construct haplotype maps. The largest of which is the US National Institutes of Health funded program (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). Another example is the Genomic Disorders Research Centre in Melbourne, Australia (<http://www.genomic.unimelb.edu.au/mdi/dblist/ccent.html>).

Before SNP-based association studies became popular, genetic variation based on polymorphisms of short tandemly repeated DNA (i.e., STR or microsatellites) was important for genetic linkage studies (Bowcock et al., 1994), and this approach remains valuable. These loci are numerous, highly polymorphic in terms of numbers of repeats (usually of binucleotides), and are densely distributed across genome, facilitating inference of a disease phenotype to a genetic locus (Kruglyak et al., 1996). Although they have been used to map Mendelian genes, STRs they are

applicable to dissect complex genetic traits into their components (Lander and Kruglyak, 1995). Linkage analysis of STRs in families will be complementary to the use of SNPs for discovering environmentally responsive genes.

An important SNP resource specifically designed for toxicology research is the Environmental Genome Project (EGP) sponsored by the National Institute of Environmental Health Sciences (<http://www.niehs.nih.gov/envgenom/home.htm>) and developed by University of Utah Genome Center (<http://www.genome.utah.edu/genesnps/>). This web resource integrates gene annotation sequence and polymorphism data on specific genes that play role in susceptibility to environmental exposure. The human genes include DNA repair, cell cycle control, cell signaling, cell division, homeostasis and metabolism.

SCREENING FOR RELEVANT SNPS

The EGP was created in the expectation that information about human genetic susceptibility to environmental exposures would aid in protecting susceptible individuals from environmental hazards. Its major research activities include human DNA polymorphism discovery and characterization, comparative mouse genomics, the development of a SNPs database of environmentally responsive genes, and molecular epidemiology of environmentally induced diseases (Ladiges et al., 2004). Research approaches that make use of SNPs are diagrammed Fig. 1. The essential outcome of the search for environmentally responsive genes is that they are functionally relevant (Lopachin et al., 2003; Mohrenweiser et al., 2003; Waters and Fostel, 2004). Some of the questions one can ask to gauge functional relevance are as follows. First, does the gene code for a toxicant-sensitive protein? For example, have changes in protein conformation from amino acid substitutions occurred that render the protein more susceptible to direct oxidative damage or covalent modifications by a toxic agent? Second, does the sensitive protein alter phenotype (cell/tissue function, behavior) when exposed? This question must be considered within the matrix of age or developmental stage at the time of exposure to the toxicant, duration and dose of exposure, and possible co-exposure to other toxicants. Third, is the effect masked by genetic, biochemical, or cellular redundancy? It has been of great interest to observe the outcomes of gene knock-outs in mice and note that many appear to have little or no effect on phenotype because other gene products or

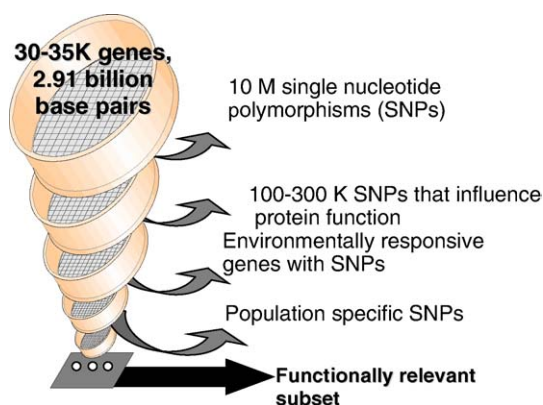


Fig. 1. Sifting through the genome for relevant genes. Research approaches that focus on SNPs are diagrammed as sieves of decreasing pore size. A SNP consists of an allele with one base substitution, insertion, or deletion in a codon. Within the human genome of 2.91 bp, there are up to 10,000 SNPs, an estimated 1% of which alter amino acid sequence or affect gene function. SNPs that occur within an ethnic population at a frequency of at least 1% are of genetic and toxicologic interest because they are amenable for the study of their effects on health. An unknown percentage of these population-relevant SNPs are responsive to environmental exposures. A further subset of these genes is likely to be functionally important in that altered function of the gene product is not compensated for by biochemical redundancy in the cell or by other higher level functional redundancy that masks the effects of the polymorphism. Thus, functionally relevant SNPs make up a very small portion of the total genes in the human genome.

alternative mechanistic pathways compensate for the loss. This is very striking proof of biological redundancy in gene families that will make more challenging the discovery of environmentally responsive genes.

The comprehensive approach of the EGP allows a systematic examination of genes with functional relevance. The EGP will sequence and identify SNPs of genes for enzymes such as cytochrome P450 (CYP), glutathione-S-transferase (GST) DNA repair enzymes, and other proteins involved in detoxifying environmental toxicants to identify biomarkers and genetic targets for association studies. The net effect of multiple enzyme activities determines the susceptibility of an individual to toxicant exposure. The strength of a multiple gene approach to association studies has not yet been demonstrated with genes suspected of altering susceptibility to neurotoxicants. However, its strength is exemplified by a recent report on “Anxiety Trait” based on the activity of serum acetylcholinesterase (AChE), a key component of anxiety related syndromes, and the availability of acetylcholine in patient and control cohorts. SNP markers for PON1 and AChE enzymes show a positive association between predictors of anxiety scores and polymorphisms of these enzymes (Sklan et al., 2004).

As of this writing, the EGP has generated an unfinished database of 554 environmentally responsive genes that are potential targets for further study. The

annotated database can be viewed on the University of Utah Genome Center website previously mentioned. Of the hundreds of SNPs identified thus far that are environmentally responsive, some stand out because of their tissue-specific functional relevance to the nervous system. In Table 1, a subset of these SNPs has been extracted from the list for their potential relevance to nervous system disease. We have further annotated the genes for function and have noted those SNPs for which an allelic variant has been associated with a toxicant interaction. Ubiquitous genes largely have been omitted from this table, though they may also be candidates for environmentally responsive genes related to nervous system diseases. It should be noted that the two best understood polymorphisms that alter susceptibility to environmental toxicants are blood proteins, rather than nervous system-specific proteins: ALAD and PON1.

There are two starting places to look for SNPs that interact with neurotoxicants and influence disease susceptibility. First, one might look for polymorphisms coding for protein products that are critical to a known toxic mechanism. The target molecules within affected cell types that have been discovered by toxicological studies are of obvious interest, such as receptors, ion pumps and channels, and enzymes. In addition, other potential candidates can be envisioned for exploration, such as those involved in the uptake of a neurotoxicant into the body, and its systemic transport, systemic detoxification, and distribution to target cells. Second, one might look for SNPs that have functional relevance to the nervous system and correlate their allelic variants with disease occurrence and severity and toxic exposure. Both approaches are being used. Three examples will be briefly discussed. These examples demonstrate that SNPs for proteins with widely disparate functions can alter susceptibility to a neurotoxicant. ALAD is an erythrocyte protein, PON1 is a serum protein, and MAO-B is a neurotransmitter enzyme.

Lead (Pb) and OPs have been sufficiently well studied to understand many aspects of their toxic mechanisms. Pb is known to interact with ALAD in red blood cells, deposit in bone as a calcium mimetic, and enter the CNS, where it interacts with all cell types (Deng and Poretz, 2003; Lasley and Gilbert, 2004; Tiffany-Castiglioni and Qian, 2001, 2004). Therefore, several research groups have studied populations of workers exposed to Pb in order to compare ALAD genotype, tissue burden of Pb, early biologic effects, and neurologic disease incidence. Pb binds to and inhibits ALAD, the second enzyme in the heme

Table 1

Single nucleotide polymorphisms with functional implications for nervous system disease

Symbol	EGP class	Name	Function	Variant-specific toxicant interaction
AD2	Metabolism	Alzheimer disease 2 (APOE*E4-associated late onset)	Cholesterol homeostasis	Lead (Stewart et al., 2002)
ALAD ^a	Metabolism	δ-Aminolevulinate dehydratase	Chaperone for hemoglobin	Lead (Ziemsen et al., 1986; Wetmur et al., 1991; Chia et al., 2004)
AREG ^a	Cell signaling	Amphiregulin (schwannoma-derived growth factor)	Anti-apoptosis	Unknown
CASP2 ^a	Cell division	Caspase 2 apoptosis-related cysteine protease (neural precursor cell expressed developmentally down-regulated 2)	Caspase cascade in apoptosis	Unknown
CKB	Cell signaling	Creatine kinase brain	ATP regeneration	Unknown
COMT ^a	Metabolism	Catechol- <i>O</i> -methyltransferase	Catecholamine metabolism	Unknown
CTNND2	Cell structure	Catenin (cadherin-associated protein) delta 2 (neural plakophilin-related arm-repeat protein)	Cell motility; mental development	Unknown
DDC ^a	Cell cycle	Dopa decarboxylase (aromatic L-amino acid decarboxylase)	Dopamine and serotonin production	Unknown
DRD2	Cell signaling	Dopamine receptor D2	Pain response	Alcohol (Konishi et al., 2004); bupropion (David et al., 2003); smoking (Noble et al., 1994; Comings et al., 1996)
ERBB2 ^a	Cell cycle	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 neuro/glioblastoma derived oncogene homolog (avian)	Upregulation of cyclin D1 level	Unknown
FGF9 ^a	Cell cycle	Fibroblast growth factor 9 (glia-activating factor)	Upregulation of cell proliferation and GFAP expression	Unknown
GAD1 ^a	Metabolism	Glutamate decarboxylase 1 (brain 67 kDa)	Biosynthesis of neurotransmitters	Unknown
GAD2 ^a	Metabolism	Glutamate decarboxylase 2 (pancreatic islets and brain 65 kDa)	GABA formation; glucose metabolism	Unknown
GGT1	Metabolism	γ-Glutamyltransferase 1	Glutathione metabolism	Unknown
GGT2	Metabolism	γ-Glutamyltransferase 2	Glutathione metabolism	Unknown
GSTM3	Metabolism	Glutathione-S-transferase M3 (brain)	Detoxification of toxicants	Smoking (Sikdar et al., 2004)
GSTP1	Metabolism	Glutathione-S-transferase Pi	Detoxification of toxicants	Styrene (Laffon et al., 2003; Teixeira et al., 2004); Smoking (Miller et al., 2003); Benzidine (Ma et al., 2003)
HSPA5	Homeostasis	Heat shock 70 kDa protein 5 (glucose-regulated protein 78 kDa)	Protein folding and assembly in endoplasmic reticulum	Unknown
IGF1 ^a	Cell signaling	Insulin-like growth factor 1 (somatomedin C)	Cell growth; glucose metabolism	Unknown
MT3 ^a	Metabolism	Metallothionein 3 (growth inhibitory factor (neurotrophic))	Inhibition of neurotrophin expression; zinc homeostasis	Unknown
NF1	Cell structure	Neurofibromin 1 (neurofibromatosis von Recklinghausen disease Watson disease)	Cognition; inactivation of Ras; activation of adenylyl cyclase; association with microtubules	Unknown
NOS1 ^a	Metabolism	Nitric oxide synthase 1 (neuronal)	Nitric oxide signaling pathway	Unknown

Table 1 (Continued)

Symbol	EGP class	Name	Function	Variant-specific toxicant interaction
NRAS ^a	Cell signaling	Neuroblastoma RAS viral (v-ras) oncogene homolog	Tumorigenesis	Unknown
NRG1	Cell cycle	Neuregulin 1	Neuroregulin receptor degradation protein-1 controls ErbB3 receptor recycling	Unknown
ODC1	Cell division	Ornithine decarboxylase 1	Biosynthesis of spermidine and spermine	Unknown
PON1	Metabolism	Paraoxonase 1	Detoxification of organophosphate	Paraoxon, phenylacetate and diazoxon (Costa et al., 2003; Hernandez et al., 2003)
SLC6A3 ^a	Cell signaling	Solute carrier family 6 (neurotransmitter transporter dopamine) member 3	Dopamine transport; target of cocaine	Smoking (Lerman et al., 1999; Sabol et al., 1999)
VDR ^a	Metabolism	Vitamin D (1,25-dihydroxyvitamin D3) receptor	Calcium regulation; bone growth	Lead (Schwartz et al., 2000)

^a Submitted to the NIEHS Environmental Genome Center.

synthetic pathway (Secchi et al., 1974). Polymorphisms of the *ALAD* gene affect the accumulation and distribution of lead in blood and bone of humans and laboratory mice (reviewed by Onalaja and Claudio, 2000; Kelada et al., 2001). The *ALAD* gene exists as two co-dominant alleles that code for proteins differing at amino acid residue 59: in the *ALAD-2* allele asparagine is substituted for lysine. These two alleles determine three isozymes, designated 1-1, 1-2, and 2-2, which are listed in increasing order of electronegativity, as lysine is positively charged and asparagine is neutral. Epidemiologic studies in humans support the conclusion that the *ALAD* polymorphism modifies the exchange of Pb between blood and bone and may therefore modify susceptibility to Pb toxicity, though the advantages of the various genotypes for health are not clear-cut (Schwartz et al., 1995; Hu et al., 2001). Two studies have linked neurobehavioral outcomes with Pb body burden and *ALAD* genotype. First, Belinger et al. (1994) compared 72 adolescents with high (>24 mg/g) and low (<8.7 mg/g) dentin Pb levels and found that body burden of Pb and its effects on neurobehavioral functions tended to be worse in *ALAD-1* homozygotes. Chia et al. (2004) showed that in a group of 150 male Chinese, Malays and Indians workers exposed to low to medium Pb levels, *ALAD-1* homozygotes had significantly higher urinary ALA (a positive indicator of Pb exposure) and significantly poorer neurobehavioral scores involving motor dexterity than heterozygotes or *ALAD-2* homozygotes, though blood Pb levels were similar in all genotypes. Thus, it is difficult to conclude the relationship among

ALAD polymorphisms, blood lead levels, and Pb neurotoxicity. Interpretation of such data is challenging because the metabolic outcome, in this case urinary ALA, may be a good marker for Pb exposure but a poor correlate for neuronal damage. Another possibility is that ALA urinary output may be modulated at the organ level, such as the health or toxic filtering capacity of the kidney of the individuals studied. However, these studies raise critical questions that are relevant to formulate testable hypothesis, design further studies that will shed light into mechanisms, and provide potential routes towards amelioration of symptoms of Pb-induced neurotoxicity.

A second enzyme of interest in the search for neurotoxicant-responsive SNPs is PON1 (Costa et al., 2003). PON1, a key OP hydrolyzing enzyme in serum, is hypothesized to confer susceptibility or resistance to OP exposure. Catalytic efficiency of PON1 is determined in part by a Q/R polymorphism at codon 192. The Q form of PON1 is more efficient at hydrolyzing sarin and soman, whereas the R form more efficiently hydrolyzes the smaller molecule paraoxon (Josse et al., 2001). The level of stable PON1 activity is dependent on “PON1 status.” PON1 status is dependent on genotype and phenotype: genotype is polymorphisms of the *PON1* gene that affect catalytic efficiency of the enzyme, and phenotype is serum activity of the PON1 enzyme, which is a function of the abundance of PON1 and age onset of catalytic activity in serum. There is wide inter-individual variability in plasma levels PON1 levels within a genetic class; therefore, SNP analysis alone would

be misleading in an epidemiologic study. *PON1*₁₉₂ polymorphisms and PON status recently have been studied in children, who are more sensitive than adults to most neurotoxicants. The role of PON1 as a genetic and temporal determinant of pesticide sensitivity in children is further discussed by Furlong et al. (2005).

The gene for MAO-B provides an example of a SNP having functional relevance to nervous system disease and an apparent interaction with toxicants. MAO-B is a mitochondrial enzyme localized primarily in serotonergic neurons and astrocytes in brain (Kitahama et al., 1991; Ekblom et al., 1993) that catabolizes the neurotransmitter dopamine. An A to G substitution in intron 13, 36 bases upstream of the exon 14 boundary, is associated with an increased risk for idiopathic PD (Costa et al., 1997). Checkoway, Costa, and colleagues (Kelada et al., 2002) have reported a protective effect of smoking against the risk for idiopathic PD in men with genotype G but not A. However, these genotypes have no effect on women. In contrast, Hernán et al. (2002) reported no protective effect of genotype G in either men or women. Unfortunately, this inability to replicate positively or reliably is a common difficulty with epidemiologic studies. Problems may include type I error (false positives) due to chance observation compounded by small sample size with insufficient power. In addition, molecular complexity arising from epistasis and epigenetic factors and population specific genetic differences further confound genetic association studies of complex diseases such as PD.

HOW MANY GENES ARE RELEVANT TO NEUROTOXICOLOGY?

Both genetics and genomics study the transmission of traits across generations. Genetics pertains to study of single genes, whereas genomics is the study of all genes in the genome including their function, their interaction, and their role in a variety of common disorders that are not due to single genes (Guttmacher and Collins, 2002). The first draft of the genome (McPherson et al., 2001; Venter et al., 2001) has been published. The estimate of protein coding genes is in the range of 30,000–40,000. Based on research in the mouse brain (the mouse is estimated to have the same number of genes as humans), at least 55% of the genes or approximately 16,500 genes, are expressed in the brain (Sandberg et al., 2000). At present the prediction of the number of genes is no more than an estimate, as the accuracy of computer programs for direct gene prediction is limited by the high signal to noise ratio

between the exons and the introns. Also, it is difficult to use species conservation methodologies for gene prediction on rapidly evolving genes.

The relatively low estimate of the number of genes in the human genome is misleading, as a large number of human genes code for multiple proteins through alternative splicing (Graveley, 2001; Kreahling and Graveley, 2004). If on average, for example, a gene expresses approximately three alternative forms of coding and there are 35,000 genes in the genome, there is a potential to code more than 100,000 proteins. Additional post-translational modification, such as phosphorylation, glycosylation, and proteolysis may ultimately yield higher number of human proteins by several fold. For example, during development the brain is formed through a complex stepwise process that includes migration and synaptogenesis of neurons. The implications of two examples of alternative splicing in neurodevelopment have recently been reviewed (Graveley, 2001; Kreahling and Graveley, 2004). In *Drosophila melanogaster*, the gene coding for the Down syndrome cell adhesion molecule (Dscam) participates in neuronal guidance for synapsis formation between neurites. The outstanding characteristic of Dscam is that its pre-mRNA can be spliced into more than 38,000 different isoforms, two to three times more than the total predicted genes in the entire organism. Another example is the gene coding for neurexins, which are receptors for neuropeptides. It is estimated that in rats, three neurexin genes code for more than 1000 different neurexin mRNA. Considering the above information it becomes clear that prediction of the number of genes or proteins in the brain is purely speculative at present.

Annotation of the human genome, which is in progress, will soon provide a better estimate. However, based on its extended developmental time period and wide-ranging cellular heterogeneity, the brain may ultimately prove exceed other organs in the number of genes and isoforms expressed. The brain contains more than 30,000 distinct mRNAs (Furey et al., 2004). Genetic regulation requires the timed, specific expression of members of gene families and extensive alternative gene splicing (Goldstrohm et al., 2001). Thus, the brain presents a surfeit of potentially environmentally responsive genes.

NEW DIRECTIONS

Other factors that influence the interactions between polymorphisms and environmental neurotoxicants must

be considered, such as fetal environment and intrauterine growth, as well as fetal plasticity based on this environment and its genome architecture. New directions for research include concerted efforts to constantly improve the scale and quality of association studies. Technical advancements both at the wet laboratory level, through improved high-throughput genotyping systems, and at the computational level through the development of algorithms for haplotype inference to deal with large scale data are vital to dissect the complex layers inherent in multifactorial diseases that have genetic and environmental components.

Only a few SNPs related to genetic susceptibility to environmental neurotoxicants have been described, the most studied of which are ALAD and human serum PON1. That these two genes are ubiquitously expressed reflects the interdependence of the nervous system with systemic organs and tissues. This finding can be exploited to explore easily accessible tissues such as peripheral blood, saliva, buccal cells and root of hair follicles for bio-markers that reflect damages in the nervous system, for large scale epidemiological studies. Possibilities abound for relevant SNPs not yet known, such as genes governing cell adhesion, cell–cell communication, transport, cytoskeletal structure, intracellular signaling, protein folding and degradation, growth factors, oxidative stress responses, and ion homeostasis. The studies of genomic contributions through polymorphisms and haplotype analysis and the study of environmental impacts through toxicologic methods are well established as distinct endeavors. However, in order to achieve more precise risk assessment and develop individualized medicine, we must focus the tools of both research approaches upon the interface between polymorphisms and toxicants.

Recently another type of variation is emerging from the shadow of SNPs that may become a key player in understanding genetics of human health and disease. Large, submicroscopic genomic imbalances in copies of DNA regions, about 100 kb or greater, comprise 5–10% of the human genome. Several reports have documented gene duplications in these regions and elsewhere in the genome. The polymorphism due to this variation in copy numbers has been called copy number polymorphism, or CNP (Sebat et al., 2004; Iafrate et al., 2004; Fredman et al., 2004; Boehm et al., 2004; Hollox et al., 2003). In one study, among the 70 genes associated with the newly-identified CNPs are genes involved in Cohen syndrome and neurological development, as well as genes implicated in leukemia and drug resistant forms of breast cancer (Sebat et al., 2004). Potentially, CNP may be another powerful

basis of individual variation to infectious disease and toxicants toxicant susceptibility of individuals in a population.

The identification of genetic polymorphisms is critical to understanding genetic susceptibility to neurotoxic agents, but is only a beginning. The functional significance of such polymorphisms in cells and tissues will be a major biological challenge. Though nearly 4000 genetic diseases in humans have been identified in which a mutation in a single gene has a major phenotypic effect, most of these disease alleles occur rarely in populations. The more common disease traits are multifactorial, requiring the interaction of multiple genes and the environment. New research tools, such as those provided by the International HapMap Project, will provide crucial frameworks for analyzing genetic polymorphisms suspected of affecting disease susceptibility.

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