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## Pharmacogenetics of antipsychotic-induced Parkinsonism and tardive dyskinesia

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# **CHAPTER 1**

## **GENERAL INTRODUCTION, SCOPE AND OUTLINE OF THE THESIS**



### ***Pharmacogenetics, DNA, genetic variability, and ethnicity***

Many centuries ago (~500 B.C.), Pythagoras noted that some people cannot tolerate Fava beans (*Vicia faba* member of the Fabaceae family) [Meletis and Konstantopoulos 2004]. Nowadays, it is clear that Fava beans can induce haemolytic anemia in persons with glucose 6-phosphate dehydrogenase deficiency.

To describe such conditions, Garrod coined the term ‘inborn errors of metabolism’ in the beginning of the 20th century [Garrod 1902]. Initially, this term was reserved for metabolic disorders and diseases, although Garrod already speculated that certain inborn errors of metabolism might be causally related to the phenomenon that certain medicines can lead in some, but not all, patients to rare and unexplainable reactions (idiosyncratic drug reactions). The proposed explanation was initially simple and clear-cut; subjects that exhibit idiosyncratic drug reactions may have genetic defects (imperfections) that other subjects do not have. This view has evolved through the years to eventually give birth to a new scientific discipline – called pharmacogenetics – a term that is first introduced by Vogel in 1959 and subsequently in 1960 defined by Price-Evans and Clarke as “the study of genetically determined variations in animal species that are revealed by the effects of drugs” [Price-Evans and Clarke 1961]. Probably one of the first prominent pharmacogenetic examples relates to butyrylcholinesterase deficiency in anesthesia. Since the 1950s, numerous reports have confirmed the link between inherited butyrylcholinesterase deficiency and “scoline apnea”, in which paralysis persisted for 20-40 min, or more, after administration of succinylcholine, a depolarizing neuromuscular blocker with a rapid onset (45 seconds) and a short duration of action (2–6 minutes) in most patients [Gardiner and Begg 2006].

Currently, pharmacogenetics may be defined – according to the European Medicines Evaluation Agency – as the study of variations in DNA sequence as related to drug response [European Medicines Agency (EMA) 2007].

After its introduction, scientists and clinicians expected that the study of pharmacogenetics would provide the means of identifying individuals who are more likely to respond to a particular drug and also those individuals who are less likely to have an adverse effect from a specific drug. They also hoped that the application of pharmacogenetics would eventually lead to the realization of concepts as “personalized medicine” and “tailor-made pharmacotherapy” [The Royal Society 2005].

However, adoption of pharmacogenetics into routine clinical practice has been relatively slow and tedious [Grossman 2007; Nebert et al., 2003; Pirazzoli and Recchia 2004; The Royal Society 2005], with only few exceptions, such as testing for TPMT (thiopurine S-methyltransferase) genetic variation in azathioprine/6-mercaptopurine therapy [Al Hadithy et al., 2005].

Psychiatry is a striking and non-exclusive example of a field where implementation of pharmacogenetic testing into routine clinical practice has not been generally established, despite tremendous efforts, as witnessed by the multiple literature

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reviews, meta-analyses, and guidelines [Arranz et al., 2001b; Arranz et al., 2001a; Arranz et al., 1998; Arranz and de Leon J. 2007; Bakker et al., 2006; Bakker et al., 2008; Kirchheiner et al., 2004; Kirchheiner et al., 2005; Mancama et al., 2003; Reynolds et al., 2005; Swen et al., 2008; Wilffert et al., 2005]. However, pharmacogenetic research remains valuable to basic and clinical research, since it may enhance our understanding of the mechanisms of drug action and explain at least partly the variability in drug response.

### ***Human DNA***

The human nuclear genome consists of 23 pairs of chromosomes (22 autosomal chromosome pairs and 1 sex chromosome pair, XY or XX) with each chromosome containing a single deoxyribonucleic acid (DNA) molecule. Chemically, DNA is a long polymer (double helix) of 2 non-covalently bound strands of complementary nucleotide bases. There are two types of nucleotide bases (DNA building units), purines and pyrimidines. Purine bases are adenine (A) and guanine (G). Pyrimidine bases are cytosine (C) and thymine (T). Complementary DNA strands are linked through base-pairing of pyrimidine with purine nucleotides (A with T; G with C) [Court MH 2007; Guttmacher and Collins 2002].

The basic functional unit of the genome is the gene. A gene is a portion of the genetic information that codes for the synthesis of messenger ribonucleic acid (mRNA), which is then used as a secondary template by ribosomes to translate into one or more proteins.

Generally, a gene is comprised of the following regions; promoter, exons, introns, and 3'-untranslated regulatory regions (3'-UTR). The promoter region determines the expression of the protein. Exons dictate the actual amino acid sequence of the protein. Introns do not determine the amino-acid sequence, but are non-coding regions that are spliced-away following transcription. 3'-UTR may control protein translation and mRNA degradation [Court MH 2007; Guttmacher and Collins 2002].

The genetic information dictated by genes is represented by the sequence in which nucleotides occur (i.e., DNA sequence). DNA sequence determines which and how much proteins must be expressed (synthesized) at a given point of time. Such instructions are partially carried by codons, which are nucleotide triplets that define the type of amino acid to be incorporated in the nascent polypeptide chain. For example, whereas the CAT sequence may instruct the cell to incorporate the amino acid Histidine in a polypeptide, the GAT sequence may instruct the cell to incorporate the amino acid Aspartic acid instead. Genes may thus carry essential information for development and functioning of cells and tissues.

The entire human genome contains about 3 gigabases ( $3 \times 10^9$  nucleotide base pairs) with up to 30,000 protein-coding genes [Court MH 2007; Guttmacher and Collins 2002]. Interestingly, these protein-coding genes are estimated to account for less than 2% of the total DNA sequence in humans, whereas the remaining intergenic sequence (commonly referred to as 'Junk DNA') is thought to be important

for chromosomal structure, genetic integrity, and evolution [Court MH 2007; Guttmacher and Collins 2002].

It should be mentioned that, in addition to the nuclear genome, eukaryotic cells contain essential genetic information separate from the chromosomal DNA, stored in the mitochondria (mitochondrial genome). In humans, mitochondrial DNA is small, circular, and does not contain introns. Mitochondrial DNA codes for proteins of the respiratory chain, subunits of ATPase and NADH-dehydrogenase complexes, and a number of transfer and ribosomal RNAs.

### ***Genetic variability***

Generally, the forms and sources of genetic variability can be categorized as following:

#### ***Chromosomal variations***

Chromosomal variations (e.g., chromosomal trisomies and deletions) are larger scale genetic variations that involve an extensive number of genes and that mostly lead to a severe impact on the physiology. Well known examples of such variations are Down's syndrome (trisomy 21) and Klinefelter's syndrome (X chromosome duplication in males; XXY) [Tyler and Edman 2004]. Due to their severe impact, chromosomal variations have no place in pharmacogenetic testing, but are important for other medical disciplines (e.g., oncology and clinical genetics).

#### ***Alternative splicing***

As stated before, current knowledge suggests that the human genome includes approximately 30,000 protein-coding genes. However, this number is substantially smaller than what was previously thought and more than 100,000 proteins can be derived from these 30,000 genes [Court MH 2007; Guttmacher and Collins 2002]. This phenomenon may be ascribed to the mechanism of alternative splicing, in which one gene may give rise to more than one protein [Graveley 2001].

Splicing is a crucial step in the process of protein expression. During splicing, the introns are removed from the primary gene transcript (RNA) and the exons are joined to create messenger RNA (mRNA). Any variation in the splicing (e.g., removal of a particular exon) is called alternative splicing. Since it utilizes the same single gene to induce the synthesis of a greater variety of proteins, alternative splicing invalidates the classical one-gene-one-protein theory [Graveley 2001]. Relevant examples in psychiatry are alternative splicing of the genes encoding for serotonin 2C and histamine H1 receptors [Dracheva et al., 2003; Swan et al., 2006; Tohda et al., 2006].

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Although rarely acknowledged, alternative splicing may be of considerable pharmacogenetic importance, when it is controlled by genetic polymorphisms affecting the process of alternative splicing [Bracco and Kearsey 2003].

### *Copy number variation*

Copy number variation (CNV) involves entire gene deletions or duplications. The CYP2D6 gene is a good example of such genetic variation. This gene exhibits duplications ( $\leq 13$  copies) and deletions (0 copies) in various rates across various ethnicities [Court MH 2007; Guttmacher and Collins 2002]. Gene duplication and deletion may lead to subjects that are ultra-rapid and poor metabolizers of CYP2D6 substrates, respectively.

### *Insertions/Deletions*

An insertions/deletions (Indel) polymorphism involves the insertion or the deletion of one or more nucleotides [Court MH 2007; Guttmacher and Collins 2002]. Indels can be of substantial pharmacogenetic importance. For example, a simple indel polymorphism involving insertion or deletion of 1 or 2 nucleotides within the protein coding region of a gene can have severe impact on protein function, particularly when leading to a reading frameshift mutation; a case where protein sequence and length are significantly altered.

Special types of the indel variation are the so-called microsatellite and minisatellite polymorphisms. Microsatellites are repeats of sequences less than 5 base pairs in length and may be present as mononucleotide (e.g., “t t t t t”), dinucleotide (e.g., “ta ta ta ta”), trinucleotide (eg, “tta tta tta tta tta”), and polynucleotide repeats [Court MH 2007; Guttmacher and Collins 2002]. Minisatellites involve longer blocks ( $\geq 5$  base pairs). Microsatellite and minisatellite polymorphisms are called collectively: Variable Number of Tandem Repeats (VNTR).

Well-known examples of indel polymorphisms relevant for the field of psychiatric pharmacogenetics are the -141C Ins/Del polymorphism of dopamine D2 (DRD2) gene and a 44-base pair insertion/deletion polymorphism in the 5' regulatory region of the serotonin transporter (SLC6A4) gene.

### *Single Nucleotide Polymorphisms*

The majority of the observed sequence variations between people appears to result from single nucleotide polymorphisms (SNPs). SNPs are single base pair substitution mutations that occur at an average frequency of roughly 1 SNP for every 1000 base pairs of DNA sequence [Court MH 2007; Guttmacher and Collins 2002].

The location of the SNP in relation to a gene may determine the possible effects on the function of that particular gene.

SNPs localized inside the protein coding region of a gene (exonic SNPs), may or may not change protein structure. When the SNP results in the substitution of a single amino acid within the protein polypeptide, then it is called non-synonymous (missense) SNP. However, when the exonic SNP does not affect the amino acid sequence, it is called a synonymous (silent) SNP. Exonic SNPs may not only result in the substitution of a single amino acid, but may also result in the introduction of a stop codon that prematurely stops protein translation. Such SNPs result in a truncated and potentially non-functional protein and are called nonsense polymorphisms. About 1% of all human SNPs affect the protein coding portion of the DNA sequence and result in substitution of a single amino acid within the protein polypeptide chain. Well-known SNPs from the field of psychiatric pharmacogenetics are Ser9Gly (DRD3), His452Tyr (HTR2A), and Cys23Ser (HTR2C).

In addition to exons, SNPs may also be present in any place outside the protein coding regions. However, non-exonic SNPs that are localized in 5'-untranslated regulatory region (UTR), intron-exon boundaries, and 3'-UTR can be of possible pharmacogenetic relevance [Court MH 2007; Guttmacher and Collins 2002]. Non-exonic SNPs may result in a quantitative, but not in a qualitative, change in the expressed protein.

SNPs located in the promoter or the 5'-UTR region of a gene may increase or decrease gene transcription through changes in the DNA sequence essential for binding of transcription factors that are responsible for enhancement or repression of gene expression [Court MH 2007; Guttmacher and Collins 2002]. An example of a 5'-UTR SNP is the -1438G/A polymorphism in serotonin 2A receptor (HTR2A).

SNPs that occur in the introns (intronic SNPs) may affect splicing, which is – as stated previously- an important translational step. During splicing the introns (noncoding regions) are removed from the primary mRNA transcript to ultimately leave a sequential chain of joined exons that is used as a template for protein translation. The removal of intronic mRNA is mediated by enzymes. For these enzymes to act, specific sequence elements (e.g., splice donor and splice acceptor sites at the exon–intron boundaries as well as splice repressor and enhancer sites) are considered to be essential for mRNA splicing to occur properly [Court MH 2007; Guttmacher and Collins 2002] [Court MH 2007; Guttmacher and Collins 2002]. SNPs localized within any of the aforementioned splicing-regulatory sequence elements have the potential to alter the efficiency of splicing. Furthermore, such SNPs may result in more severe scenarios in which mRNA is produced that lacks one or more exons or that has retained (some of) the intron sequence. Furthermore, intronic splice SNPs may create novel splice donor, acceptor, or regulatory sites that compete with the original splice sites to create novel mRNA variants [Court MH 2007; Guttmacher and Collins 2002].



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Finally, there are SNPs that are located in the 3'-UTRs of the mRNA. Although 3'-UTR regions are not translated to protein, this region is an important regulatory region. Disruption of the structural integrity of the 3'-UTR (due to a SNP), may have effects on protein formation rate through an altered balance between mRNA degradation, stability, and protein translation efficiency.

### ***Ethnicity as a pharmacogenetic determinant***

Cumulative evidence suggests that a significant difference in drug response exists among different ethnicities [Frackiewicz et al., 1997; Swartz et al., 1997]. For instance, FDA has recently approved a drug (BiDil®, isosorbide dinitrate+hydralazine hydrochloride) for the treatment of heart failure exclusively in African-Americans [Anonymous 2005; Carmody and Anderson 2007]. Furthermore, enalapril (an angiotensin-converting enzyme inhibitor) has been found to be less effective in African-Americans, as compared to Caucasians [Wallace and Drazner 2005]. Not only the efficacy, but also side effects may exhibit racial dependency. African-Americans for instance have been found to be more sensitive to tardive dyskinesia, a movement disorder exhibited in some of the antipsychotic-treated subjects [Eastham et al., 1996; Morgenstern and Glazer 1993].

When the observed differences in the drugs response are due to genetic differences (and not due to environmental or socio-economic differences), then race and ethnicity are merely surrogates for certain genetic variations present in different frequencies in one, but not in another, racial group. CYP2D6 gene, which as mentioned earlier exhibits duplications (some subjects may have up to 13 functional copies), forms a good example. About 25% of Negroid subjects from Ethiopia (North-Eastern Africa) has more than 2 copies of CYP2D6 and hence has an increased activity of this enzyme (CYP2D6 ultra-rapid metabolizers), whereas less than 3% of the Dutch population has CYP2D6 gene duplication. If treated with for example tramadol, then Ethiopian Negroids would theoretically be far more often at risk of developing opioid-related respiratory depression (CYP2D6 catalyzes the conversion of the pro-drug tramadol to an active metabolite, O-desmethytramadol) [Stamer et al., 2008], whereas the underlying mechanism is genetic rather than racial.

Ethnicity may not only determine the frequency of discrete polymorphisms (SNPs and CNVs), but also determines how SNPs are inherited together and passed on to further generations. In fact, sets of SNPs may be collectively inherited and passed to next generations, if strong linkage disequilibrium exists between the SNPs. Linkage disequilibrium is the tendency for SNPs located in relative close proximity on a chromosome to be co-inherited as one block of SNPs (haplotype) [Court MH 2007]. Since linkage disequilibria patterns are ethnicity-sensitive, the biologic effects of a SNP may be expressed in one ethnic population but not in another.

### ***Antipsychotics in psychiatry***

Antipsychotics comprise a group of drugs applied in the symptomatic treatment of a variety of psychotic disorders: e.g., schizophrenia, delirium, delusional disorders (paranoia), psychotic depression, dementia-related psychotic symptoms, and mania. Various classification schemes for antipsychotics have been proposed. Some of these schemes classify antipsychotics according to their chemical structure (e.g., phenothiazines, thioxanthenes, butyrophenones, etc.), while other classification schemes are based on the potency, efficacy, and side-effect profiles of antipsychotics [Lidow 2000].

The most widely used classification of antipsychotics divides these agents into classical and atypical antipsychotic drugs. The parameters for distinguishing classical from atypical antipsychotics are unfortunately vague and ill-defined.

The strongest distinction relates to their capacity to induce movement disorders. These antipsychotic-induced movement disorders (AIMDs) have been collectively known as extrapyramidal syndromes (EPS). The earlier antipsychotics (e.g., chlorpromazine and haloperidol) have a relatively strong tendency to induce AIMDs and have historically been referred to as neuroleptics (“seize the neuron”, from the Greek word *lepsis*). In fact, the strong link between the early antipsychotics and the presence of AIMD has led some scientists, including the German psychiatrist Hans-Joachim Haase, to believe that these effects are an essential part (or “typical”) of the antipsychotic effectiveness [Lange et al., 1975]. However, this hypothesis appeared to be a misconception with the introduction of clozapine, an antipsychotic with proven effectiveness in 50% of patients refractory to treatment with conventional antipsychotics, but whose effectiveness is not coupled with any notable AIMDs. Clozapine was therefore called an “atypical” antipsychotic<sup>1</sup>. In fact, the introduction of clozapine introduced a concept of ‘old’ typical antipsychotics highly prone to generate AIMDs and ‘modern’ atypical antipsychotics with absent or reduced propensity to induce AIMDs [Lidow 2000]. According to this concept all of the antipsychotics introduced within the last two decades can be considered as atypical antipsychotics. In the Netherlands these currently include clozapine, olanzapine, sertindole, quetiapine, risperidone, paliperidone, and aripiprazol.

However, as mentioned above, the distinction between typical and atypical antipsychotics is vague for some of the atypical antipsychotics behave as typical at higher doses and lead to the emergence of AIMDs in patients (e.g. risperidone can be classified as a typical drug at daily doses above 6 mg) [Lidow 2000].

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<sup>1</sup> Although never confirmed by the manufacturer (Sandoz, Basel), it has been anecdotally suggested that clozapine refuted the theory of Haase (Hase is German for hare or rabbit) to such an impressive extent that clozapine’s trade-name (Leponex®) was derived from the term *lepus* (Latin for hare) and means accordingly something like “Ha(a)se exit” to imply the failure of Haase’s theory (<http://de.wikipedia.org/wiki/Clozapin>).

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Interestingly, the definition of atypicality also includes low capacity to elevate prolactin levels [Lidow 2000]. Hyperprolactinaemia may result in infertility, galactorrhea, gynecomastia, osteoporosis, and impotence. Again, although hyperprolactinaemia is usually seen in most of the typical antipsychotics, some of the atypical antipsychotics particularly risperidone, sulpiride, and amisulpiride may also induce dose-dependent elevation in prolactin secretion and may thus be classified as typical by this extended standard.

A third requirement for atypicality has also been proposed; the ability to ameliorate the negative and cognitive signs of schizophrenia. In fact the best way to describe schizophrenia, a disease for which antipsychotics are primarily used, is to dissect schizophrenia into five symptom dimensions; positive (delusions and hallucinations), negative (e.g., anhedonia, apathia, and blunted affect), cognitive (impaired speech fluency and impaired executive functioning), aggressive (e.g., impulsivity and automutilation), and depressive and anxious symptoms (e.g., feelings of grief, guilt, and anxiety) [Stahl 2000]. Since negative and cognitive symptoms are more predictive of social and occupational functioning than other clinical symptoms [Stahl 2000], adequate treatment of cognitive and negative symptoms may be considered as necessary as the treatment of the positive or aggressive symptoms. In comparison to typical antipsychotics, atypical antipsychotics are possibly more effective in treating both negative symptoms and cognitive deficits [Tandon et al., 2008]. However, atypical agents may induce side effects that are less seen with typical antipsychotics (e.g., weight gain, hyperlipidemia, and hyperglycemia).

### ***Extrapyramidal syndromes and antipsychotics***




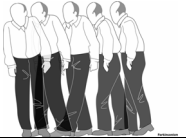

Extrapyramidal syndromes or symptoms (EPS) is a generic non-specific term [Trosch 2004] used to collectively refer to antipsychotic-induced movement disorders (AIMDs) [van Harten 1998]. Phenomenologically, AIMDs can be divided into dyskinesia, dystonia, akathisia, parkinsonism, and ataxia (Figure 1) [Loonen and van Praag 2007].

AIMDs can furthermore be classified as those with early onset (acute AIMD), those with late onset (tardive AIMD), and idiosyncratic events [Casey 2004; Gerlach 2002; Gerlach 1999; Trosch 2004; van Harten 1998]. Although acute and tardive AIMDs are phenomenologically similar, classification according to the onset of occurrence is of considerable importance, since the reaction to a change in antipsychotic dosage may differ between acute and tardive AIMDs. An increase in the dose of antipsychotic medication generally exacerbates acute AIMDs, but soothes tardive AIMDs.

Acute AIMDs appear in the first few days of treatment or follow an increase in the dose of an antipsychotic and can be subdivided into acute dystonia, parkinsonism, akathisia, and ataxia. Late-onset AIMDs usually appear after months or years of antipsychotic exposure and can be subdivided into tardive dyskinesia,

tardive akathisia, and tardive dystonia. Idiosyncratic events consist of neuroleptic malignant syndrome, which is a rare medication-induced reaction characterized by rigidity, dystonia, hyperthermia, and lowered consciousness among other symptoms.

With the exception of akathisia, all of the major AIMDs are abnormal involuntary movements that disappear during sleep. Furthermore, the severity of movement disorders may alter with the level of excitement or relaxation. In an anxious patient the disorder may be more severe whereas during relaxation it may decrease [van Harten 1998]. AIMDs may cause the patient considerable distress leading to therapeutic non-compliance and hence frequent psychotic relapses [Gerlach 2002; Strejilevich et al., 2005].

	<p><b>Dyskinesia</b></p>	<p>Rapid, irregular, repetitive contractions; motionless intervals between contractions; increases during activity and anxiety.</p>
	<p><b>Dystonia</b></p>	<p>Slow, irregular, continuous contractions (spasms); contractions result in slow movements or abnormal postures; contractions continue for more than 2 seconds.</p>
	<p><b>Akathisia</b></p>	<p>Increased frequency of regular movements; primarily, but not exclusively affects the legs; an aversivity to standing or sitting still.</p>
	<p><b>Parkinsonism</b></p>	<p>Reduction and/or slowing of all movements; equable plastic resistance to passive movement; hypersalivation and shiny skin; slow tremors that appear during rest and decrease during activity.</p>
	<p><b>Ataxia</b></p>	<p>Irregular movements while maintaining posture; inappropriate execution of voluntary movements; not a result of muscular weakness.</p>

**Figure 1:** Characteristics of the most significant antipsychotic-induced movement disorders.

Adopted from Loonen and van Praag [2007], with the permission of Journal of Clinical Psychopharmacology.

### ***Dyskinesia***

Dyskinesias are characterized by involuntary, repetitive, purposeless, and irregular movements that may or may not be continuous [van Harten 1998]. Tardive dyskinesia, a potentially disabling irreversible movement disorder, has a prevalence of around 30% in patients chronically exposed to antipsychotics [Glazer 2000; Kane et al., 1988]. It can be subdivided into orofaciolingual and limb-truncal dyskinesias [van Harten 1998]. Orofaciolingual dyskinesias involve movements of mouth and face muscles (e.g., chewing, lateral jaw movements, twisting, curling, and protrusion of the tongue). Severe orofacial dyskinesia may impair eating and swallowing. Limb-truncal dyskinesias involve purposeless choreiform movements of trunk and/or limbs (e.g., writhing movements of the fingers/toes and rotations of wrists, arms, legs, as well as pelvic thrusting). Limb and trunk dyskinesias may cause gait disturbances and falls.

The severity of tardive dyskinesia can vary over time and even during the day. Voluntary movements may provoke or aggravate the dyskinesia in other parts of the body. Walking is an example of an activating task that brings out dyskinetic movements in the upper limbs. Furthermore, lowering the dose of the antipsychotic medication may exacerbate or unmask tardive dyskinesia. Other known examples of risk factors for tardive dyskinesia are older age, female gender, Negroid ethnicity, and smoking [Glazer 2000; Kane et al., 1988; Morgenstern and Glazer 1993; van Harten et al., 1998; Wonodi et al., 2004].

### ***Dystonia***

Dystonia is characterized by slow or sustained involuntary muscle contractions, frequently causing twisting and repetitive movements or abnormal postures [Cardoso and Jankovic 1997; van Harten and Kahn 1999]. The muscles involved are usually localized in the head (mouth and eyes), neck, trunk and limbs. When dystonia becomes irreversible (which is often the case in tardive dystonia), it is one of the most disabling and untreatable adverse events in psychiatry [Gerlach 1999]. In contrast to tardive dyskinesia, tardive dystonia occurs more frequently at younger age, lacks the female predominance, and may be alleviated by anticholinergics [van Harten and Kahn 1999].

### ***Akathisia***

Akathisia, which literally means ‘not to sit’, is a syndrome characterized by unpleasant sensations of inner restlessness and irritability (subjective complaints) which are then expressed as inability to sit still or remain motionless (objective motor movements) [Barnes 2003; van Harten 1998]. The subjective complaints may have strong affective components such as fright, anger, and rage. The objective features are typically movements of the legs.

### ***Parkinsonism***

Parkinsonism is a hypokinetic rigid syndrome with bradykinesia, rigidity, postural instability, and tremor at rest as cardinal features.

Bradykinesia and akinesia are the most common and sometimes the only manifestations of parkinsonism. Bradykinesia and akinesia account for monotonous speech, hypomimia, slowing and poverty of movements, muscle fatigue, slow walking with short steps, and decreased arm swing. Sometimes sialorrhea appears when saliva is not swallowed as fast as it is produced, although increased saliva production may also occur as an autonomic symptom of Parkinsonism.

Rigidity is a fluently persistent increase in muscles' tone, which renders the muscles firm and tense during passive movements. Patients with rigidity may complain of generalized muscle stiffness, muscle or joint pain, body aching, or lack of coordination during sports.

Postural instability is reflected in a reduction or absence of postural response which can be judged with the pull test (a sudden posterior displacement produced by a pull on the shoulders).

Tremor at rest is a coarse, rhythmic tremor characteristic of pill-rolling tremor seen in Parkinson's disease, but is relatively less frequently present in drug-induced parkinsonism.

### ***Ataxia***

The word ataxia finds its roots in Greek language, in which ataxia means a "lack of order". Ataxia (cerebellar, sensory, vestibular, frontal, and extrapyramidal) is a movement disorder that is characterized by a gross lack of coordination of muscle movements and by an aspecific clinical manifestation. However, unsteady gait and staggering walk are the most prominent features of this disorder [Loonen and van Praag 2007].

### ***Candidate gene approach in studying pharmacogenetics of parkinsonism and tardive dyskinesia***

In contrast to pharmacogenomics and genome-wide association scans, pharmacogenetic research involves a hypothesis-driven approach where one or more candidate genes are selected on the basis of their relevance to the pharmacologic actions (pharmacokinetics and pharmacodynamics) of the drug in question and/or on the basis of their relevance to the etiology and pathogenesis of the disorder (phenotype) investigated. Alternatively, if a gene encodes for a protein that is neither involved in the pharmacology nor in the etiology, then it is plausible to expect no or spurious genetic association [Wilffert et al., 2005]. In the following section we

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provide examples for candidate genes that are of possible relevance to parkinsonism and tardive dyskinesia.

### ***Genes encoding for proteins involved in the pharmacokinetics (PK)***

Antipsychotics are metabolized and distributed by a variety of proteins. Perphenazine for example is extensively metabolized by cytochrome P450 (CYP) 2D6. Polymorphism of CYP2D6 may thus significantly impact the exposure in perphenazine-treated subjects. In contrast to perphenazine, pimozide is metabolized predominantly through CYP3A4 enzyme and its metabolism is thus much less sensitive to variations in CYP2D6 gene. Therefore, when studying for example antipsychotic-induced parkinsonism (the occurrence of parkinsonism is exposure-dependent), CYP2D6 is a suitable candidate gene when studying perphenazine-induced but not pimozide-induced parkinsonism.

### ***Genes encoding for proteins involved in the pharmacodynamics (PD)***

Parkinsonism may be caused by the antagonistic effects of antipsychotics on nigrostriatal dopamine D2 receptors (DRD2 gene) [Gerlach 1999; Lidow 2000; Reynolds 2004], which are modulated by serotonin 2A and 2C receptors (HTR2A and HTR2C genes, respectively) [Alex et al., 2005; Di et al., 2006; Haleem 2006; Lidow 2000]. DRD2, HTR2A, and HTR2C are therefore plausible candidate genes for the study of the pharmacogenetics of parkinsonism. For tardive dyskinesia the dopamine D3 receptor (DRD3 gene), HTR2A, and HTR2C are of relevance as well.

However, proteins involved in the PD of antipsychotics are not only limited to receptors, but also involve second-messenger proteins (e.g., Regulators of G-protein Signaling), pre-synaptic transporters (e.g., dopamine transporter), and enzymes involved in the biosynthesis and biotransformation of endogenous neurotransmitters (e.g., Catechol O-Methyltransferase). Furthermore, antipsychotics often display relevant affinities for multiple neurotransmitter receptors. Clozapine for example has affinities for several receptors (albeit with varying magnitude), with among others dopamine D1-D4, serotonin 2A, 2C, histamine H1, and muscarinic M1 and M4 receptors [Buckley 2007]. The list of possible candidate genes may therefore be extended to include many genes.

As with PK proteins, it is plausible that the biological effects of polymorphism in PD genes are only genuinely expressed in those situations where the antipsychotic drug interacts (directly or indirectly) with the PD proteins in question [Wilffert et al., 2005]. However, it is also hypothetically possible that the effects are expressed even in the absence of any relevant affinity, if these proteins are involved

in the pathophysiology of the clinical entity studied (e.g., constitutional over-activity of dopamine D2 genes).

### ***Genes encoding for proteins involved in the etiology and pathogenesis***

As mentioned above, proteins involved in the etiology and pathophysiology of a disorder may also be involved in the PD of the pharmacologically active substance intended to treat the disorder. This is however not always the case. For example, neuronal degeneration due to oxidative stress has been proposed as a mechanism for tardive dyskinesia pathogenesis. Cellular defense mechanisms against oxidative stress involve detoxification enzymes like manganese or superoxide dismutase-2 (SOD2) [Lohr et al., 2003]. Oxidative stress enzymes involved in oxidative stress form therefore plausible candidate genes for studying pharmacogenetics of tardive dyskinesia, even though antipsychotics are not a substrate for these enzymes.

### ***SCOPE AND OUTLINE OF THE THESIS***

The present thesis describes several pharmacogenetic aspects of two major antipsychotic-induced movement disorders (antipsychotic-induced parkinsonism and tardive dyskinesia) in two ethnic groups (African-Caribbeans from Curaçao and Slavonic Caucasians from Siberia). The pharmacogenetics of these ethnic groups has not been described previously in the scientific literature.

Consequently, this thesis is divided into two parts. Part I presents pharmacogenetic studies on tardive dyskinesia. Part II presents pharmacogenetic studies on parkinsonism.

The outline of the thesis is as follows:

### ***PART I***

In **chapter 2**, we examine and describe the pharmacogenetic associations between orofaciolingual and limb-truncal dyskinesias and polymorphisms of the dopamine D3, serotonin 2A, and 2C receptor genes in African-Caribbean psychiatric inpatients from Curaçao, Dutch Antilles.

In **chapter 3**, we examine the same associations as in chapter 2, however, in Slavonic Caucasians from Siberia, Russia.



## Chapter 1

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In **chapter 4**, we describe in Siberian Caucasians the pharmacogenetic associations between dyskinesias (orofaciolingual and limb-truncal) and three missense polymorphisms in three oxidative-stress genes (glutathione S-transferase P1, superoxide dismutase-2, and glutathione peroxidase-1).

### **PART II**

In **chapter 5**, we investigate in the African-Caribbean sample the association between several polymorphisms in dopamine D2, dopamine D3, serotonin 2A, and 2C receptor genes and antipsychotic-induced parkinsonism and three of its subsymptoms (rigidity, bradykinesia, and rest-tremor).

In **chapter 6**, we endeavor to reproduce previous findings from the literature that suggest a clinically and statistically significant association between a certain SNP in RGS2 gene (Regulators of G-protein Signaling-2) and antipsychotic-induced parkinsonism and its subsymptoms in our African-Caribbean sample.

Finally, the thesis is concluded with a summary and final remarks.

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## **PART I**

# **Pharmacogenetics of Tardive Dyskinesia**



