Pharmacogenetics of Tardive Dyskinesia: a “Movement” Towards Individualized Therapy
Part II

Dr. Vincenzo S. Basile

Pharmacokinetics: The Cytochrome P450 1A2

Example of CYP2D6 Drug Metabolism

Bertilsson et al., 1992

Bradford, 2002

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Pharmacogenetics of Debrisoquine Metabolism (CYP2D6)

Theoretically, PMs have ↑ plasma drug concentrations resulting in ↑ TD, given that total neuroleptic exposure is an established risk factor for TD.

CYP2D6 has been studied as a potential risk factor for TD (Andreassen et al., 1997; Armstrong et al., 1997; Kaplanyan et al., 1998; Ohnori et al., 1998).

These studies show ↑ prob. of developing TD in PMs or heterozygous EMs.

Preliminary results in our sample of TD patients do not support these results: CYP2D6 variation (wt/wt, wt/mut, mut/mut) vs. AIMS score:

NEGATIVE: ANCOVA F[2,53] = 0.68, p = 0.60 (n = 55)

Why CYP1A2?

Contribution of 2D6 to antipsychotic disposition is comparatively ↓ during multiple dose (Bjoenn, 1989; Jeeling et al., 1996; Linnet, 1996; Ozdemir et al., 1999) vs. single dose (Bindrup et al., 1992; Dahi-Puustinen et al., 1989) drug administration.

At multiple doses, under steady-state conditions, it is likely that 2D6 gets saturated and typical antipsychotic metabolism is shunted to alternate P450 pathways that exhibit affinity for the antipsychotic.

Possibly CYP1A2 ????

CYP1A2 vs. CYP2D6: Multi-Dose vs. Single-Dose

Studies that implicate CYP2D6 as the major metabolizer of typicals were single dose studies conducted on healthy volunteers (non-smokers).

These subjects do not reach steady-state plasma concentrations. Schizophrenia patients have a higher incidence of smoking, which is known to induce CYP1A2 further increasing its abundance.

Schizophrenia patients are chronic, receive multiple doses of antipsychotics, and for the most part reach steady-state concentrations.

At steady-state levels, CYP2D6 may become saturated, at which point other P450’s with affinity for the antipsychotic may come into play (i.e. CYP1A2).

Although CYP1A2’s affinity for typicals is lower than CYP2D6, its abundance is greater.
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Why CYP1A2? (cont)

CYP1A2 is induced by the cyclic aromatic hydrocarbons in cigarette smoke.
Smoking has consistently been shown
to reduce plasma concentration of typicals by at least 40% (Pantuck et al., 1982; Ereshefsky et al., 1985; Jain et al., 1986; Miller et al., 1990; Perry, 1993; Shimoda et al., 1999).
Smokers are prescribed higher doses of typicals (Hughes, 1986; Glassman, 1993).
Cessation of smoking results in ↑ of plasma concentration of typicals
as well as an ↑ in rate of EPS (Stimmel & Falloon, 1983).
CYP1A2 accounts for 12% of total P450 liver content (6x > 2D6)
and provides a “low affinity-high capacity” alternate pathway.
Schizophrenia patients are chronic, receive multiple doses of neuroleptics,
reach steady-state plasma concentrations and have a much higher
incidence of smoking (80% vs. 20% in general population) (Hughes et al., 1986; Lohr et al., 1992).
In these patients it is likely that 1A2 also plays a significant role
in typical neuroleptic metabolism.

Functional Polymorphism in CYP1A2 (Sachse et al., 1999)

<table>
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<th>n = 32</th>
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<td>Mean 6.6</td>
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<tr>
<td>C/C</td>
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</table>

Mean AIMS Scores for CYP1A2 Bsp I Polymorphism
After Typical Neuroleptic Treatment

F[2.82] = 7.41, p = 0.0007 (n = 85), Power = 0.69, r-square = 0.31
(Bonferroni p = 0.0035)
(Basile et al., 2000)
Ethnically Stratified Means

Caucasian Means (N = 63)

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<tr>
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Afro-American Means (N = 22)

<table>
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</thead>
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<tr>
<td>A/C</td>
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</tr>
<tr>
<td>C/C</td>
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</table>

F[2,60] = 5.96, p = 0.0004
Bonferroni p = 0.002
(Basile et al., 2000)

Results for CYP1A2:

Genetic association analysis of functional polymorphisms in the cytochrome P450 1A2 (CYP1A2) gene with tardive dyskinesia in Japanese patients with schizophrenia.

Genetic susceptibility to tardive dyskinesia in chronic schizophrenia subjects: I. Association of CYP1A2 gene polymorphism

Smoking and tardive dyskinesia: lack of involvement of the CYP1A2 gene

Lack of association between a functional polymorphism of the cytochrome P450 1A2 (CYP1A2) gene and tardive dyskinesia in schizophrenia:

Cytochrome P-450 2D6*10 C188T polymorphism is associated with antipsychotic-induced persistent tardive dyskinesia in Chinese schizophrenic patients

CYP2D6 polymorphism and tardive dyskinesia in schizophrenic patients
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CYP2D6 Meta-analysis Patsopoulos et al. 2005

CYP2D6 polymorphisms and the risk of tardive dyskinesia in schizophrenia: a meta-analysis
Patsopoulos NA, Ntzani EE, Zintzaras E, Ioannidis JP.

Results for Other Candidate Genes

a) DRD2 variation (TaqI RFLP) vs. AIMS score:
NEGATIVE: ANCOVA $F[2,91] = 0.103$, $p = 0.361$ (n = 93)

b) DRD4 variation (48 bp repeat) vs. AIMS score:
NEGATIVE: ANCOVA $F[3,99] = 2.17$, $p = 0.100$ (n = 101)

c) CYP2D6 variation (wt/wt, wt/mut, mut/mut) vs. AIMS score:
(wt = CYP2D6*1, mut = CYP2D6*3, CYP2D6*4, CYP2D6*5)
NEGATIVE: ANCOVA $F[2,53] = 0.68$, $p = 0.60$ (n = 55)

Oxidative Stress:
The Manganese Superoxide Dismutase Gene (MnSOD)

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Free Radical Hypotheses of TD

Superoxide radicals produced by ↑ in DA turnover caused by long term typical antipsychotic Rx. (Matsumoto et al., 1983)

TD may result from ↑ in free radical (superoxide) activity in the basal ganglia ⇒ apoptotic neuronal death

↑ in radical activity in patients with TD vs. no TD (Lohr et al., 1990)

↓ SOD activity in patients with TD vs. no TD (Yamada et al., 1997; Tsai et al., 1998)

Anti-oxidant Rx ↓ TD symptoms. (Placebo trials of α-tocopherol or Vitamin E show efficacy)

↓ SOD activity in patients with TD vs. no TD (Reddy et al., 1991; Makherjee et al., 1996)

↑ in lipid peroxidation in TD (in CSF + plasma), marker of free radical damage of membranes (Mahadik et al., 1995)

MnSOD / Oxidative Stress Hypothesis TD

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Why MnSOD and TD?

In rats,↑↑ expression of MnSOD in the basal ganglia and striatum with haloperidol R
(Chan et al., 1995)

In human post mortem brains, MnSOD was distributed in the striatum, nucleus basalis, substantia nigra, as well as in certain regions of the cerebellum (in matrix) (Zhang et al., 1994)

↓ MnSOD activity in patients with schizophrenia vs. controls (Yamada et al., 1996)

MnSOD knockout mice ⇒ neonatal lethality. Lethality is rescued by Rx with a MnSOD mimetic (MnTBAP). When ↓ this Rx, mice develop an irreversible movement disorder, with “gait abnormality, dystonic-like posturing of the hindlimbs, and tremor” (Melov et al., 1998, Nature Genetics)

Recently, Hori and colleagues (2000) reported an association between an MnSOD genetic variant and TD (n = 39 with TD)

In vitro studies have shown that there is differential transport of MnSOD into the mitochondria:

Ala variant normal vs. Val variant not transported

Helical structure is necessary for amphiphilic enzyme and transport through mitochondrial membranes

Val substitution leads to misdirected intracellular trafficking and therefore causes ↓ MnSOD activity in the mitochondria

∴ Expect ↑↑ Val in TD patients as found by Hori et al., 2000

Functional Polymorphism in MnSOD (Shimoda-Matsubayashi et al., 1998)

C>T base change results in substitution of Valine for Alanine AA in MTS of MnSOD

Mean AIMS Scores for MnSOD Ala9Val Polymorphism

After Typical Neuroleptic Treatment

Mean AIMS score

MnSOD Genotype

F[2,91] = 0.34, p < 0.70 ( n = 93)
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Other Studies of MnSOD

Manganese superoxide dismutase gene polymorphism and schizophrenia: relation to tardive dyskinesia
Hori H, Ohmori O, Shinkai T, Akijima H, Ohno C, Suzuki T, Nakamura J
Neuropsychopharmacology. 2000 Aug;22(2):170-7 (POSITIVE)

The increased activity of plasma manganese superoxide dismutase in tardive dyskinesia is unrelated to the Ala-9Val polymorphism

If a trait is determined by the joint effect of more than one gene, then an issue is how to analyze the interaction between the genes
This interaction can be due to:

- **heterogeneity** (having either one or the other or both of the risk genotypes produces the same phenotype)
- **additive effect** (each contributes to phenotype, having both risk genotypes gives the most severe phenotype)
- **epistatic effect** (both risk genotypes needed to cause the effect)

Model for Specific Interaction Contrasts:
Assuming a dominant model both for locus A and locus B with the allele A dominating over B, we can model the interaction by:

- **HETEROGENEITY** (Dominant A causative over B)

<table>
<thead>
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<th>A/A</th>
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Mean AIMS Scores for DRD3 Msc I Polymorphism
After Typical Neuroleptic Treatment

Mean AIMS Scores for CYP1A2 Bsp I Polymorphism
After Typical Neuroleptic Treatment

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Interaction Between DRD3 & MnSOD

Interaction between polymorphisms of the dopamine D3 receptor and manganese superoxide dismutase genes in susceptibility to tardive dyskinesia


OBJECTIVES: Interaction between DRD3 gene and MnSOD genes

RESULTS: Combination of the MnSOD -9val and DRD3 9ser alleles was associated with tardive dyskinesia

CONCLUSIONS: There is a possible synergistic effect of genetic factors influencing mitochondrial free radical scavenging and dopamine receptor function on the susceptibility to tardive dyskinesia

Interaction Between DRD3 & BDNF

Association analysis of the dopamine D3 receptor gene ser9gly and brain-derived neurotrophic factor gene val66met polymorphisms with antipsychotic-induced persistent tardive dyskinesia and clinical expression in Chinese schizophrenic patients

Interaction Between DRD3 & 17α-hydroxylase

Interactive effect of cytochrome P450 17α-hydroxylase and dopamine D3 receptor gene polymorphisms on abnormal involuntary movements in chronic schizophrenia
Biol Psychiatry. 2002 Feb 1; 51(3):261-3

Interaction Between DRD3 & HTR2C

Association between the serotonin 2C receptor gene and tardive dyskinesia in chronic schizophrenia: additive contribution of 5-HT2Cser and DRD3gly alleles to susceptibility

Summary:
Although negative, our data supports a minor contribution for the HTR2A gene in TD.
Our studies do not support a role for MnSOD in TD.

Given the numerous replications of the DRD3-TD finding, it appears that the dopamine D3 receptor may be involved in TD susceptibility.
Our functional FDG PET/MRI studies support this.

Although preliminary, further replication of our CYP1A2-TD finding may suggest a role for this drug metabolizing enzyme in TD susceptibility.
At this point a majority of studies are negative.

The interaction between DRD3 and CYP1A2 fits a recessive-recessive model with each gene interacting additively.
The DRD3 and CYP1A2 results account for ~55% of the variance in TD; other genes and environment may account for the rest...?
Limitations

- gene translation & transcription
- sample size
- multiple testing
- definition of TD phenotype

Possibilities for the Future

- A full model DNA test to identify response status & high side effect risk of patients
- Subgrouping of patients for research and clinical trials
- Aid in elucidating mechanism of action of antipsychotics

This may help in:
- screening for new compounds
- designing new, more specific therapeutic agents
- may lead to the elucidation of disease causing mechanisms

Recommendations for Psychiatric Pharmacogenetic Studies

- Employment of clinical designs suited for pharmacogenetic studies
  - appropriate sample sizes should be determined a priori
  - prospective, randomized control trials should be the standard
  - dose and/or plasma levels should be controlled for when looking at pharmacodynamic hypotheses
- Inclusion/exclusion criteria and response definition should be determined by consensus so that results of independent studies can be better compared
  - definition of response should include multiple criteria (psychopathology ratings, quality of life ratings, etc.)
  - continuous data with parametric analysis is preferred over categorical data with non-parametric analysis
- Any positive results should be replicated in independent samples
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Pharmacogenetics: the Genetics of Drug Response and Side Effects
- Full Model
- Complex Interactions
- Artificial Neural Networks

How Do We Find These GENES?

Vincenzo S. Basile M.D. B.Ed.
vincebasile@ica.net
Division of Neurology, Department of Medicine, Sunnybrook & Women’s College Health Sciences Centre, University of Toronto
Department of Psychiatry, Neurogenetics Section, Centre for Addiction & Mental Health, University of Toronto

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