Getting 'Personal' with Rasagiline Therapy in Early Parkinson's Disease: A Retrospective Pharmacogenetic Study of the ADAGIO Trial

Mario Masellis*, Shannon Collinson, Natalie Freeman, Maria Tampakeras, Joseph Levy, Amir Tchelet, Eli Eyal, Elijahu Berkovich, Rom Eliaz, Victor Abler, Iris Grossman, Arun Tiwari, James L. Kennedy, Jo Knight, Anthony E. Lang on behalf of the ADAGIO investigators

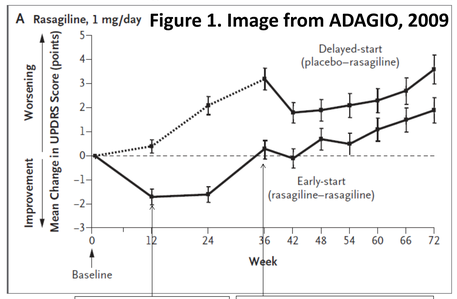
*Movement Disorders Clinic, Division of Neurology, Department of Medicine, Sunnybrook Health Sciences Centre, University of Toronto, 2075 Bayview Ave, Toronto, ON Canada M4N3M5

Background

- Rasagiline [N-propargyl-(1R)-aminoindan] (Azilect®) is a selective, irreversible inhibitor of monoamine oxidase B (MAO-B).
- It is approved for use in the symptomatic treatment of Parkinson's disease (PD) (TEMPO, 2002; PRESTO, 2005; LARGO, 2005).
- **Mechanism of Action:** Inhibition of MAO-B reduces the oxidative deamination of endogenous and exogenous (i.e., produced from levodopa) dopamine.
- This improves nigrostriatal dopaminergic function resulting in symptomatic benefit to PD patients.

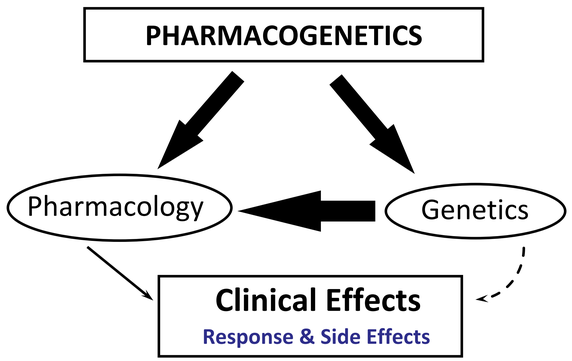
"Attenuation of Disease Progression with Azilect Given Once-daily (ADAGIO)"

Figure 1. Image from ADAGIO, 2009



- As with all PD drugs, there is variability among individuals in their clinical symptomatic benefit to rasagiline (observed in LARGO and TEMPO studies).

Figure 2: Principle of Pharmacogenetics



"To understand the hereditary basis underlying person-to-person differences in drug response and adverse drug reactions."
(Kalow, 1962; Motulsky, 1957)

Objectives & Hypotheses

Objective: To identify candidate gene polymorphisms associated with peak motor benefit to rasagiline at 12 weeks and associated with sustained benefit over a 36-week evaluation period.

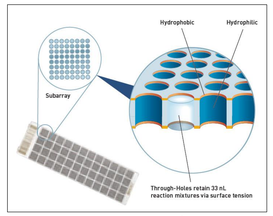
Hypotheses: Controlling for placebo effects:

1. Polymorphisms in one or more genes will be associated with the PEAK change in Unified PD Rating Scale (UPDRS) scores at 12 weeks.
2. Polymorphisms in one or more genes will be associated with change in UPDRS scores over entire 36 week observation period (i.e., PROLONGED).

Methods

- **Participants / Samples**: 805 PD patients (UK PD Society Brain Bank Criteria for probable disease) from ADAGIO provided consent for the pharmacogenetics substudy. See Table 1 for demographic and baseline characteristics. 753 DNA samples were available for genotyping.
- **Study Design:** Retrospective genetic association study using demographic and clinical data obtained from the ADAGIO trial.
- **Candidate Gene Polymorphisms:** Candidate genes were selected based on the role of the gene product in rasagiline's pharmacokinetics, pharmacodynamics or association with PD in prior Genome Wide Association Studies (GWAS). A total of 204 single nucleotide polymorphisms (SNPs) and 5 Variable Number Tandem Repeats (VNTRs) from 28 candidate genes were genotyped. Tag SNPs (r^2 = 0.8, mean allele frequency [MAF] = 10%, coverage > 80% per gene including 10 kb on either side of translated region) and known functional markers were assessed in the following genes:
  - ***Pharmacokinetic:*** *CYP1A2*
  - ***Pharmacodynamic:*** dopamine receptors, catecholamine synthetic and catabolic enzymes, catecholamine transporters, and GAPDH
  - ***PD-related genes:*** *BST1*, *GPNMB*, *LRRK2*, *MAPT*, *MMP16*, *NMD3*, *RAB7L1*, *RIT2*, *SCARB2*, *SNCA*, *SREBF1*, *STX1B*, *SYT11*, and *STK39*
- **Genotyping Platforms**:
  - SNPs: Life Technologies TaqMan OpenArray NT Genotyping System
  - VNTRs: Amplified using standard PCR cycling methods and electrophoresed on the Applied Biosystems 3130 Genetic Analyzer.

OpenArray® Plate



QuantStudio™ Real-Time PCR

- **Outcome Measures**: Change in UPDRS scores from baseline at 12, 24, and 36 weeks after administration of rasagiline or placebo.
- **Statistical Analysis**:
  - **Hypothesis 1 (PEAK response):** Determines the change in UPDRS based on genotype from baseline to 12 weeks in all individuals on rasagiline and placebo using a linear model:
    Change in UPDRS = (Treatment [rasagiline vs. placebo] x genotype) + time since diagnosis + baseline UPDRS + age + country
  - **Hypothesis 2 (PROLONGED response):** Determines the change in UPDRS based on genotype at 12, 24, and 36 weeks from baseline using a mixed effects linear model:
    Change in UPDRS = (Treatment [rasagiline vs. placebo] x UPDRS_week x genotype) + (1 + UPDRS_week per individual)

Results

Table 1: Demographic and Baseline Clinical Characteristics

		Informed Consent	Informed Non-Consent	Other
Total Sample (n = 1174)		805 (68.6%)	120 (10.2%)	249 (21.2%)
Sex		487 (60.5%) M	74 (61.7%) M	156 (62.7%) M
Smoking Status	Current smoker	29 (3.6%)	1 (0.8%)	4 (1.6%)
	Former smoker	186 (23.1%)	10 (8.3%)	19 (7.6%)
	Never smoked	305 (37.9%)	27 (22.5%)	41 (16.5%)
	Unknown	285 (35.4%)	82 (68.3%)	185 (74.3%)
Age at baseline (years)		62.5 ± 9.4	60.7 ± 11.2	62.0 ± 9.7
Time Since PD Diagnosis (months)		4.7 ± 4.7	3.8 ± 4.7	4.3 ± 4.3
Baseline UPDRS		20.4 ± 8.2	21.1 ± 9.6	20.2 ± 8.9
Treatment Randomization	1 mg	200 (24.8%)	30 (25.0%)	58 (23.3%)
	2 mg	204 (25.3%)	25 (20.8%)	64 (25.7%)
	Placebo	401 (49.8%)	65 (54.2%)	127 (51.0%)

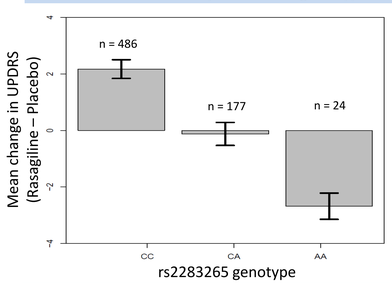
N.B. 'Consent' refers to that provided for pharamacogenetic substudy of ADAGIO. 'Other' refers to those who were never asked to provide consent for this substudy.

Table 2: SNP Markers Significantly Associated with Rasagiline Response based on 12 Week Change in UPDRS Model (i.e., PEAK response)

Gene	Chromosomal Location	SNP	Unadjusted p-value	False Discovery Rate-Corrected p-value
Dopamine D2 receptor gene (*DRD2*)	11q23	rs2283265	0.0006	0.0449
		rs1076560	0.0007	0.0449
Norepinephrine transporter gene (*SLC6A2*)	16q12.2	rs36023	0.0005	0.0449

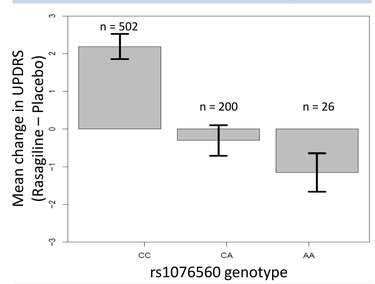
N.B. rs2283265 (A allele MAF = 0.16) and rs1076560 (A allele MAF = 0.17) were in strong linkage disequilibrium with each other (r^2 = 0.96). rs36023 has an A allele MAF = 0.37.

Figure 3: Placebo-adjusted mean change in UPDRS at 12 weeks by rs2283265 genotype



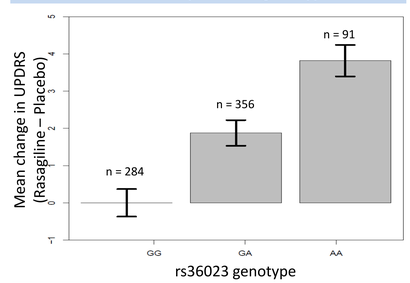
N.B. Positive values indicate clinical benefit.

Figure 4: Placebo-adjusted mean change in UPDRS at 12 weeks by rs1076560 genotype



N.B. Positive values indicate clinical benefit.

Figure 5: Placebo-adjusted mean change in UPDRS at 12 weeks by rs36023 genotype



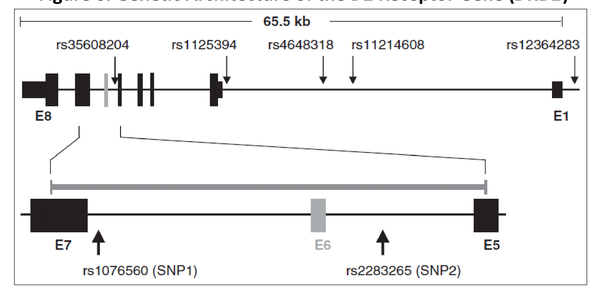
N.B. Positive values indicate clinical benefit.

N.B. The values on the y-axis of Figures 3, 4, and 5 were calculated as follows: $(\text{UPDRS mean change}_{\text{rasagiline}} - \text{UPDRS mean change}_{\text{placebo}}) \pm (V\,[SD^2_{\text{rasagiline}} + SD^2_{\text{placebo}}])$

Discussion / Conclusions

- rs2283265 and rs1076560 in the dopamine D2 receptor gene (*DRD2*) and rs36023 in the norepinephrine transporter gene (*SLC6A2*) were found to be significantly associated with clinical benefit to rasagiline.
- D2 receptors are a major target of endogenous dopamine, dopamine derived from exogenously administered levodopa, as well as dopamine agonists.
- rs2283265 and rs1076560 are located in introns 5 and 6, respectively, of *DRD2* and alter transcriptional processing of exon 6 (Bertolino et al., 2009).

Figure 6. Genetic Architecture of the D2 Receptor Gene (*DRD2*)



Adapted from Moyer et al. (2011). Neuropsychopharmacology 36, 753–762

- Specifically, both SNPs modulate putative splice factor binding sites leading to the expression of two distinct D2 receptor isoforms:
  - **D2 long (D2L):** Post-synaptic receptor; striatum and nucleus accumbens; insertion of 29 amino acids in the third cytoplasmic loop (Khan et al., 1998).
  - **D2 short (D2S):** Pre-synaptic autoreceptor; soma and axons of midbrain nigrostriatal dopaminergic neurons; deletion of 29 amino acids.
- Minor A alleles of these *DRD2* SNPs favour inclusion of exon 6 resulting in significant reductions of pre-synaptic D2S autoreceptors (Zhang et al., 2007).
- The relative increased expression of D2L over D2S is predicted to increase activity of striatal medium spiny neurons modulating cortico-striato-thalamo-cortical networks [i.e., 'direct' and 'indirect' pathways] (Zhang et al., 2007).
- fMRI studies have shown that the minor A alleles of rs2283265 and rs1076560 are associated with increased striatal and prefrontal activation in response to a working memory task in controls (Zhang et al., 2007).
- The minor A alleles of both SNPs are also associated with worse performance on working memory and attentional control tasks (Zhang et al., 2007).
- A *DRD2* haplotype containing these SNPs was also associated with an increased risk of levodopa-induced dyskinesias in PD (Rieck et al., 2012).
- With respect to the *SLC6A2* rs36023 association, little is known about the functional consequences of this SNP.
- The norepinephrine transporter encoded by *SLC6A2*, together with the dopamine transporter, is responsible for the pre-synaptic reuptake of catecholamines, and this SNP may alter nigrostriatal dopamine levels.
- **Study Limitations:**
  - Marginally significant results – potential power issue.
  - No replication sample.
  - Does not cover the entire genome.

Acknowledgements

This study was funded by Teva Pharmaceuticals Industries Ltd. (Israel). We thank the patients and their families for their participation.

References

TEMPO (2002). *Arch Neurol.*, 59:1937-1943
PRESTO (2002). *Arch Neurol.*, 62:241-248
LARGO (2005). *Lancet*, 365: 947-54
ADAGIO (2009). *N Engl J Med.*, 361:1268-78
Kalow (1962). Pharmacogenetics, Saunders
Motulsky (1957). *JAMA*, 165(7):835-837
Bertolino (2009). Brain , 132: 417–425
Khan et al. (1998). *PNAS*, 95: 7731–7736
Zhang et al. (2007). *PNAS*, 104(51): 20552–20557
Rieck et al. (2012). Pharmacogenomics.13:1701-10