Parkinsonism and Related Disorders 19 (2013) 346–349

Contents lists available at SciVerse ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Short communication

# Cervical dystonia and genetic common variation in the dopamine pathway

Justus L. Groen [a,b,*], Javier Simón-Sánchez [c], Katja Ritz [b], Zoltán Bochdanovits [c], Yue Fang [c], Jacobus J. van Hilten [d], Majid Aramideh [e], Bart P. van de Warrenburg [f], Agnita J.W. Boon [g], Frank Baas [b], Peter Heutink [c], Marina A.J. Tijssen [a,h]

[a] Department of Neurology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[b] Department of Genome Analysis, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[c] Department of Clinical Genetics, VU University Medical Center Amsterdam, The Netherlands
[d] Department of Neurology, Leiden University Medical Center, Leiden, The Netherlands
[e] Department of Neurology, Medical Centre Alkmaar, Alkmaar, The Netherlands
[f] Department of Neurology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
[g] Department of Neurology, Erasmus MC, Rotterdam, The Netherlands
[h] Department of Neurology, University Medical Centre Groningen, The Netherlands

ARTICLE INFO

*Article history:*
Received 17 March 2012
Received in revised form
26 July 2012
Accepted 30 August 2012

*Keywords:*
Cervical dystonia
Dopamine pathway
Common variants

ABSTRACT

Cervical dystonia, a late onset focal dystonia, has a complex genetic background. Multiple lines of evidence point to a role for aberrant dopamine levels in dystonia. We assessed whether common variation within genes that regulate brain dopamine levels and in key genes of the dopamine metabolic pathway, modulate the risk for cervical dystonia. DNA was collected from 363 Dutch CD patients and a cohort of Dutch control individuals. Haplotype-tagging single nucleotide polymorphisms (SNPs) complemented with selected variants of functional importance in *COMT, DAT, TH, MAO-A and -B, DDC* and *DBH* were investigated. We tested the 143 markers in single-SNP, haplotype and epistasis analyses. We did not find an association with any of the selected 143 SNPs in these key dopamine genes. Our data shows that common variations in key genes of the dopamine pathway do not contribute to dystonia risk in the Dutch population. Possibly, risk alleles in this pathway may be rarer than detectable in this study, or might be located in downstream dopamine signaling pathway. Alternatively, found dopamine level changes are secondary to the dystonia disease processes.



## 1. Introduction

Cervical dystonia (CD), a primary adult-onset focal dystonia, is the most frequent form of dystonia in Caucasians (prevalence 1 in 4500) [1] with an assumed complex genetic background. Changes in dopamine (DA) signaling have been implicated in the pathogenesis of dystonia: mutations in GTP cyclohydrolase I and Thyrosine Hydroxylase, both essential in DA production, cause dystonia (*DYT5*, MIM128230); imaging studies suggest a hyperdopaminergic state in focal [2] and familial dystonias [3]; post-mortem measurement of tissue in the striatum showed a significant increase in the striatal DOPAC/DA ratio in dystonia patients, suggestive of increased DA turnover [4]; DA changes are found in striatum of dystonia animal models (see for a review Tanabe and colleagues [5]).

The presynaptic DA transporter (*DAT*, *SLC6A3*, OMIM: 126455) and catechol-*O*-methyltransferase (*COMT*, OMIM: 116790) are crucial for the regulation of synaptic DA levels in the brain. *DAT* is the main regulator of dopamine signaling; it recycles DA back into the presynaptic terminal, thus terminating synaptic firing. In the 3′-UTR of *DAT* a 40-bp variable number of tandem repeat (VNTR) polymorphism is present, which acts as a modulator of gene transcription. The 9-repeat allele is associated with increased *DAT* availability in striatum, compared to the 10-repeat allele [6].

In *COMT*, four SNPs located in the promoter region (rs6269) and coding region His62His (rs4633), Leu136Leu (rs4818), and Val158Met (rs4680) form three functional haplotypes. These variants modulate protein translation efficiency by altering the mRNA secondary structure, influencing enzymatic activity [7]. *DAT* and *COMT* genes interact non-additively to modulate cortical function during executive processing [8]. Other enzymes involved in production and degradation of DA, and thus control the synaptic DA

* Corresponding author. Department of Neurology H2-237, Academic Medical Centre, University of Amsterdam, PO Box 22660, 1100 DD Amsterdam, The Netherlands. Tel.: +31 (0)20 5663842; fax: +31 (0)20 5669374.
*E-mail address:* justus.groen@gmail.com (J.L. Groen).

1353-8020/$ – see front matter © 2012 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.parkreldis.2012.08.016

availability, are the Tyrosine Hydroxylase (*TH*, OMIM: 191290), DOPA decarboxylase (*DDC*, OMIM: 107930), dopamine β-hydroxylase (*DBH*, OMIM: 609312), and monoamine oxidase A and B (*MAOA*, OMIM: 309850; *MAOB*, OMIM: 309860) genes(Fig. 1).

As disturbance of DA levels in the striatum has been observed in dystonia, genetic factors influencing the synaptic DA availability may be associated with CD. In this study we assessed whether common variation within genes that regulate DA levels and in key genes of the DA metabolic pathway, modulate the risk for CD. Three hundred sixty three Dutch CD patients and a large cohort of Dutch control individuals were studied for common variation in *COMT, DAT, TH, MAO-A and -B, DDC* and *DBH* by using a haplotype-tagging approach complemented with selected SNPs of functional importance.

## 2. Materials and methods

### 2.1. Study populations

All studied patients and controls were Dutch Caucasians. All procedures were carried out with written consent of the subjects involved and with the ethical approval of the Academic Medical Center Amsterdam institutional review board (www.amc.nl). *CD patients* – A group of 363 patients (66.9% female) with idiopathic non-familial CD were genotyped. All were diagnosed with primary dystonia based on the accepted criteria and included in a clinical database. Mean age at examination was 56.7 years (SD 13.0) and mean age at dystonia onset was 41.9 years (SD 13.6).

Three control groups were used (Table 1): *(1) Controls for DAT and COMT*: 720 sex-matched controls from the Dutch National Blood Bank; *(2) Controls for DA metabolism:* as genome-wide data from the Rotterdam study III [9] (ERGO Young) was available, we used these as control data for the dopamine pathway. This included genome-wide genotyping data from 2082 control participants (55.6% female) genotyped with Human610K Beadchips from Illumina (http://www.illumina.com). The mean age was 53.75 years with a range of 45–95 years; and *(3) Controls for MAO-A and MAO-B:* because the X chromosome was not covered in this genome-wide array, we genotyped (SNPlex Genotyping system, Life Technology) the informative SNPs in *MAO-A* and *MAO-B* in 1308 controls of The Longitudinal Aging Study Amsterdam (LASA control cohort). LASA is an ongoing cohort study of people aged 55–85 years [10].

### 2.2. SNP selection and genotyping

#### 2.2.1. COMT and DAT

rs6269, rs4818 and rs4680 tag the common functional haplotype blocks in *COMT* [7]. SNPs were genotyped with a TaqMan discrimination assay from Applied Biosystems (http://www.appliedbiosystems.com). The 40-bp VNTR in the *DAT* gene was amplified following a standard protocol with previously described primers [6]. PCR products were separated by electrophoresis in an ethidium bromide-stained 3% agarose gel, effectively separating different VNTR numbers.

#### 2.2.2. DA metabolic pathway SNP screen

SNPs within Tyrosine Hydroxylase (*TH*, OMIM: 191290), DOPA decarboxylase (*DDC*, OMIM: 107930), dopamine β-hydroxylase (*DBH*, OMIM: 609312) monoamine oxidase A (*MAOA*, OMIM: 309850) and *MAOB* (OMIM: 309860) (Fig. 1) were assessed using a haplotype-tagging approach, complemented with variants selected for possible functional importance. Haplotype-tagging SNPs were selected using the Haploview program with r2 threshold set on 0.8. We identified 1918 SNPs in the six candidate genes (NCBI dbSNP database, build 125) including 5 kb of flanking sequence on 5′- and 3′-side. From this, 139 SNPs were selected following our selection criteria (see Appendix A for SNP selection). In brief, SNPs were distributed with a maximum of 5 kb distance, had a minimum MAF of 5%, were haplotype tagging SNPs (haplotypes with a frequency of 0.05 and higher) or were in potential functional regions. The SNPs were genotyped with three assays of the SNPlex Genotyping system 48-plex (Life Technology), following the standard protocol. Electrophoresis and data collection was performed with an ABI3730 DNA analyzer from Applied Biosystems. Genotypes were called with GeneMapper® Software v4.0 provided by ABI. The Human610K Beadchip from Illumina includes 620,901 SNPs, 30 of our selected "dopa" SNPs were included in this panel. Using Markov Chain based haplotyper (MACH; version 1.0.16) the remaining 71 "dopa" SNPs were imputed (See Appendix A and Table S1 for quality of imputation).

### 2.3. Data analysis

Single variant association was performed using PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/), using additive, dominant and recessive genetic models and allele association. The cutoff *p*-value of Hardy–Weinberg equilibrium was 0.001. Haplotype construct in *COMT*, haplotype association and gene–gene interaction analysis was performed using Haplostats (http://cran.r-project.org/web/packages/haplo.stats/index.html). The rare haplotypes were pooled into a single category. The most frequent haplotype is the default and chosen as the baseline category for the design matrix. Logistic regression analysis using an additive mode of association and sex as covariate was performed. Power calculations were

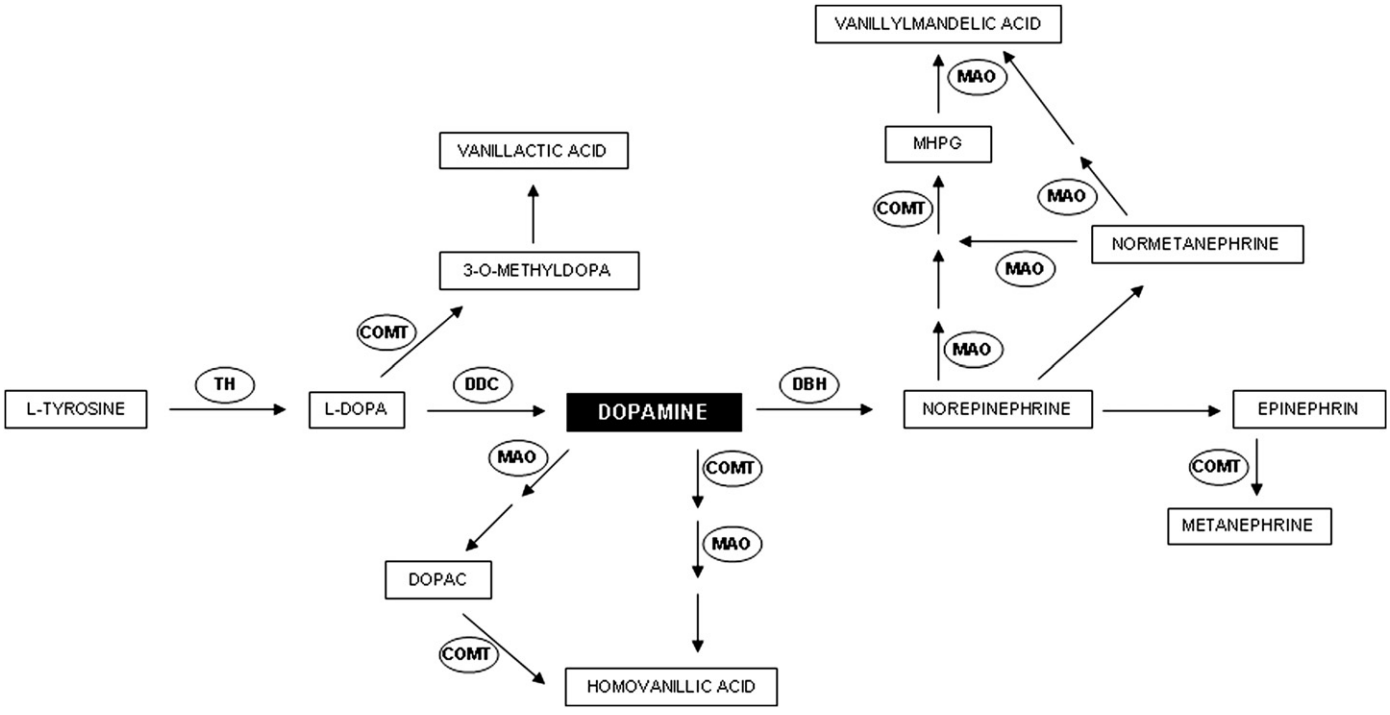


**Fig. 1.** Components and enzymes in the DA metabolic pathway. *TH* = tyrosine hydroxylase, *DDC* = DOPA decarboxylase, *DBH* = dopamine β-hydroxylase, PNMT = phenylethanolamine N-methyltransferase, *COMT* = catecholamine-O-methyltransferase, *MAO* = monoamine oxidase, DOPAC = dihydroxyphenylacetic acid, MHPG = 3-methoxy-4-hydroxyphenylglycol (adapted from: http://www.genome.jp/kegg/pathway/map/map00350.html).