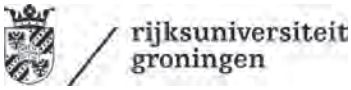
The genetics of spinocerebellar ataxia and dystonia

Nibbeling, Esther

The genetics of spinocerebellar ataxia and dystonia

Esther Anne Rieky Nibbeling



The genetics of spinocerebellar ataxia and dystonia

E.A.R. Nibbeling

The research presented in this thesis was performed at the Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands. The work described in this thesis was financially supported by a Rosalind Franklin Fellowship of the University of Groningen and grants from the Prinses Beatrix Muscle Funds, Funds NutsOhra, and the Dutch Brain Foundation.

Printing of this thesis was financially supported by the Research institute Groningen University Institute for Drug Exploration (GUIDE) of the University of Groningen, University Medical Center Groningen, Groningen, the Netherlands.

Lay-out by Ridderprint BV | www.ridderprint.nl

Printed by Ridderprint BV | www.ridderprint.nl

Cover image by InnaInna

ISBN: 978-94-6299-555-0

rijksuniversiteit
groningen

The genetics of spinocerebellar ataxia and dystonia

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. E. Sterken
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 29 maart 2017 om 12.45 uur

door

Esther Anne Rieky Nibbeling

geboren op 20 april 1987
te Zevenaar

Promotores
Prof. dr. D.S. Verbeek
Prof. dr. M.A.J. de Koning-Tijssen
Prof. dr. R.J. Sinke

Beoordelingscommissie
Prof. dr. F. Baas
Prof. dr. H.P.H. Kremer
Prof. dr. C.M.A. van Ravenswaaij-Arts

Propositions

I. Spinocerebellar ataxia and dystonia should not be seen as separate entities, but rather as a disease continuum (this thesis)

II. Alterations in synaptic transmission and transcriptional regulation are shared mechanisms underlying different spinocerebellar ataxia types (this thesis)

III. Combining whole exome sequencing, targeted re-sequencing, and gene network analysis is a successful approach to identify novel disease genes (this thesis)

IV. A promising new mechanism in dystonia pathogenesis is disturbed calcium signaling (this thesis)

V. Functional analyses are essential to confirm mutations as definitely pathogenic (this thesis)

VI. Glutamate signaling may be the molecular link between spinocerebellar ataxia and intellectual disability (this thesis)

VII. Genetic research becomes easier with the help of family members (this thesis)

VIII. The list of genetic variant prediction tools will soon be longer than the list of variants per patient obtained by whole exome sequencing

IX. There are no perfect human specimens – we are all genetically flawed in some way *Francis Collins*

X. The saddest aspect of life right now is that science gathers knowledge faster than society gathers wisdom *Isaac Asimov*

List of abbreviations

ADCA	Autosomal dominant cerbellar ataxia
ADDS	Arm dystonia dysability scale
AMPA-R	α-amino-3-hydroxy-5-methylisoxazole-4-proprionate receptor
AO	Age at onset
AON	Antisense oligonucleotide
CB	Cerebellum
CMT	Charcot-Marie-Tooth disease
CNS	Central nervous system
CNV	Copy number variation
DCN	Deep cerebellar nuclei
DYT	Dystonia
EBCC	Eye blink classical conditioning
EVS	Exome variant server
GABA	Gamma-aminobutyric acid
GWAS	Genome-wide association study
MAF	Minor allele frequency
MuD	Musician's dystonia
NGS	Next generation sequencing
NMDA-R	*N*-methyl-D-aspartate receptor
PC	Purkinje cell
polyQ	Polyglutamine
SCA	Spinocerebellar ataxia
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variant
TRS	Targeted resequencing
WC	Writer's cramp
WES	Whole exome sequencing
WGS	Whole genome sequencing
WT	Wild type

Table of contents

Preface

Preface

Movement disorders are a large group of neurological disorders affecting movement patterns of patients. They arise from disturbances in neuronal signaling within the brain and their effect is seen on diverse body parts. This thesis focuses on two of these disorders, namely spinocerebellar ataxia (SCA) and dystonia, both of which are genetically very heterogeneous.

Spinocerebellar ataxias comprise a large group of dominantly inherited, progressive, neurodegenerative disorders with an estimated prevalence of 1-3 in 100,000.[1] In general, SCAs are characterized by an ataxic gait, loss of balance, poor coordination of the hands, speech problems (dysarthria), and disturbances in oculomotor control. Other neurological signs such as pyramidal or extrapyramidal signs, ophthalmoplegia, and cognitive impairment may also be present, specifically in certain SCA subtypes.[2,3] The cerebellar symptoms are caused by atrophy of Purkinje cells (PCs) in the cerebellum and subsequent neuronal loss in spinocerebellar tracts.[4] Despite the clinical homogeneity among the 45 SCA types that are currently recognized, the genetic background in SCA is very heterogeneous. The majority of SCA patients (65%) can be explained by polyglutamine repeat expansions (SCA1, 2, 3, 6, 7)[1] and an additional 10% of patients harbor missense mutations in less frequent SCA genes (SCA13, 14, 17, 19, 23, and 27), although these genes are not commonly screened for mutations during diagnostic work. This implies that approximately 25% of SCA patients do not have a genetic diagnose.

Dystonia is defined as a hyperkinetic movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, or postures, or both. This is often initiated or worsened by voluntary action and associated with overflow muscle activation.[5] Symptoms can occur in only one body region (focal), in two or more contiguous body parts (segmental), or within the trunk and at least two other body parts (general). Furthermore, the symptoms can be continuous, follow a daily pattern, or are limited to a specific task. Similar to SCA, other features may also be present in the patients, such as tremor, myoclonus, parkinsonism, cognitive decline, and psychiatric traits, forming the so-called combined dystonias.[5] The worldwide prevalence of dystonia is estimated to be 16 in 100,000.[6] Currently, 29 separate dystonia subtypes are recognized and mutations have been identified in 21 genes.[7] The molecular pathology arises from basal ganglia dysfunctions and the cerebellar functions can also be affected.[8]

Despite the many genes that are identified in both SCA and dystonia, the molecular mechanisms underlying the disease etiology remain unknown. This results in a lack of therapies for both disorders, illustrating the need to elucidate the molecular mechanism. Therefore, we aimed to identify novel disease genes and unravel the molecular

mechanisms to improve genetic counseling and the development of possible therapeutic strategies.

In the past five years, the introduction of next generation sequencing (NGS) techniques has highly enhanced the success rate of identifying novel causal mutations in multiple Mendelian disorders. Therefore, nowadays, gene panels and whole exome sequencing (WES), in which the entire coding region of the genome is sequenced, are common practice when it comes to gene identification of Mendelian forms of dystonia and SCA; up to date 18 ataxia and dystonia genes have been identified using WES. In this thesis, WES was used to assess all coding variants within multiple affected family members per family to identify novel disease genes. Furthermore, targeted resequencing (TRS) was performed to screen a cohort of 96 independent cases for variants in our potential disease genes. The main challenge in this process is to distinguish the disease-causing variant from all the rare benign variants that are present in each person,[9] which is especially difficult in small families and single cases. However, bioinformatics approaches such as gene co-expression and gene co-functionality networks show great informative potential in gene prioritization. Moreover, we have used cell models to elucidate the functional effects of the candidate variants: we showed altered protein expression and localization for some, while other variants affected the functioning of the novel genes.

Outline of this thesis

In **chapter 1**, we describe the overlap in the symptoms and molecular background of SCA and dystonia. A gene co-expression network was used to identify genes and molecular pathways that might be involved in both disorders. This approach showed that synaptic transmission plays a major role in both etiologies, but also highlighted a role for genes that are involved in the development of the nervous system. Using this method we identified a group of 99 genes that are likely to play a role in the pathophysiology of both disorders and that may be an ideal starting point for targeted resequencing studies in cases with an unexplained genetic etiology.

Chapter 2 focusses on the identification of novel disease genes in twenty Dutch SCA families. Two or three most distantly related patients from each family were used for WES and a range of filtering steps was applied to pinpoint the disease-causing variant. To designate a candidate gene as disease causing, we aimed to identify at least one additional independent case carrying a variant in one of our potential candidate genes. Therefore, we designed a TRS gene panel containing all our candidate genes, and screened a cohort of 96 independent SCA singletons. This strategy revealed several additional variants in three candidate genes. To further confirm the involvement of the novel genes containing candidate variants for SCA, we made use of gene co-expression

and co-functionality networks based on known SCA genes. By using these strategies, in combination with accompanying functional work, we have identified five new SCA disease genes.

Chapter 3 describes the identification of *CACNA1H*, which encodes a voltage-gated calcium channel, as the first reported disease gene for writer's cramp, a task-induced form of focal dystonia. The variant p.Arg481Cys in this calcium channel was found by whole exome sequencing of two affected members of a Dutch family and validated by co-segregation analysis. Whole-cell patch-clamp experiments showed a hyperpolarizing shift in the half-activation voltage, implying the mutant channel is open in a larger window. Therefore, the p.Arg481Cys variant leads to overactivity of the channel, which is very likely the underlying cause of writer's cramp in this family.

In **chapter 4** we describe using a TRS approach to identify potential ataxia genes in the glutamatergic signaling system, as this might be a common theme in the development of ataxias. We identified predicted damaging variants in three genes, of which *GRIA3* shows a stronger link to intellectual disability than to SCA. In the NR3B subunit of the NMDA receptor, encoded by *GRIN3B*, we identified the novel p.Arg1003Trp variant that did not affect the channel characteristics but reduced surface expression of receptor complexes containing this subunit. Furthermore, in a single patient, we found two variants in the ionotropic glutamate receptor GluK1 (*GRIK1*), including the rare nonsense variant p.Leu411* and a novel frameshift variant p.Glu841fs29*. The unlikely combination of both variants may lead to critically altered GluK1 complexes and thereby induce the patient's trunk ataxia.

In **chapter 5** we describe the value of functional analysis in variant interpretation. Upon screening two cohorts of 316 and 532 cerebellar ataxia patients using conventional Sanger sequencing for mutations in *KCNC3*, the SCA13 gene,[10] we identified five patients carrying the previously known mutations p.Arg420His or p.Arg423His. Furthermore, we found 12 novel variants, of which only one was computationally predicted to be damaging. For all the missense variants we performed functional tests that included thorough electrophysiological analysis of the mutant potassium channels. Of the 12 novel variants, only p.Val535Met, p.Asp129Asn, and p.Ser391Gly were designated as potentially disease-causing variants.

Chapter 6 describes the identification of rare variants in the arylsulfatase G (*ARSG*) gene in task-specific dystonia. *ARSG* was pinpointed as a putative dystonia gene using GWAS, which showed that a single intronic SNV in this gene was associated to musician's dystonia and writer's cramp.[11] To identify the putative causal variant in *ARSG*, we used Sanger sequencing to investigate the gene's entire coding region. This led to the identification of various rare variants, but only rs61999318 was found to be associated

with writer's cramp, although not to musician's dystonia. However, we were unable to identify the causal variant in *ARSG* underlying both types of dystonia.

In **chapter 7**, I discuss the findings described in this thesis and elaborate on the consequences and clinical relevance of this work. Recommendations for future research and implications in genetic diagnostics are also made.

References

1. Durr, A. (2010). Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet. Neurol. *9*, 885–894.
2. Matilla-Dueñas, A. (2012). The ever expanding spinocerebellar ataxias. Editorial. The Cerebellum *11*, 821–827.
3. Hersheson, J., Haworth, A., and Houlden, H. (2012). The inherited ataxias: Genetic heterogeneity, mutation databases, and future directions in research and clinical diagnostics. Hum. Mutat. *33*, 1324–1332.
4. Chopra, R., and Shakkottai, V.G. (2014). Translating cerebellar Purkinje neuron physiology to progress in dominantly inherited ataxia. Future Neurol. *9*, 187–196.
5. Albanese, A., Bhatia, K., Bressman, S.B., Delong, M.R., Fahn, S., Fung, V.S.C., Hallett, M., Jankovic, J., Jinnah, H.A., Klein, C., et al. (2013). Phenomenology and classification of dystonia: a consensus update. Mov. Disord. *28*, 863–873.
6. Steeves, T.D., Day, L., Dykeman, J., Jette, N., and Pringsheim, T. (2012). The prevalence of primary dystonia: a systematic review and meta-analysis. Mov. Disord. *27*, 1789–1796.
7. Xiao, J., Vemula, S.R., and LeDoux, M.S. (2014). Recent advances in the genetics of dystonia. Curr. Neurol. Neurosci. Rep. *14*, 462.
8. Malone, A., Manto, M., and Hass, C. (2014). Dissecting the links between cerebellum and dystonia. The Cerebellum *13*, 666–668.
9. MacArthur, D.G., Manolio, T. A, Dimmock, D.P., Rehm, H.L., Shendure, J., Abecasis, G.R., Adams, D.R., Altman, R.B., Antonarakis, S.E., Ashley, E. a, et al. (2014). Guidelines for investigating causality of sequence variants in human disease. Nature *508*, 469–476.
10. Waters, M.F., Minassian, N. A, Stevanin, G., Figueroa, K.P., Bannister, J.P. A, Nolte, D., Mock, A.F., Evidente, V.G.H., Fee, D.B., Müller, U., et al. (2006). Mutations in voltage-gated potassium channel KCNC3 cause degenerative and developmental central nervous system phenotypes. Nat. Genet. *38*, 447–451.
11. Lohmann, K., Schmidt, A., Schillert, A., Winkler, S., Albanese, A., Baas, F., Bentivoglio, A.R., Borngräber, F., Brüggemann, N., Defazio, G., et al. (2014). Genome-wide association study in musician's dystonia: A risk variant at the arylsulfatase G locus? Mov. Disord. *29*, 921–927.