Genetic architecture of dystonia
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SUMMARY
AND GENERAL DISCUSSION

Dystonia is defined as a neurological movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both. Dystonic movements are typically patterned, twisting, and may be tremulous. Dystonia is often initiated or worsened by voluntary action and associated with overflow muscle activation.\(^1\) The dystonia syndromes are diverse in clinical presentation, enigmatic in electrophysiological measurements and show a complex genetic background. The aim of this thesis was to shed light on the genetic architecture of primary dystonias and identify causal and phenotype-modifying genetic variants.

**What we know / What we have**

Primary dystonia has no biomarkers, there are currently no pathognomonic clinical tests, nor does it show abnormalities on MR imaging. Therefore, careful clinical examination is key for diagnosis, characterization of syndromes and the formation of subgroups. In 2008, we have started the project ‘Genetic association studies in primary dystonia’. The main goals of this project were to form a prospective dystonia patient cohort with comprehensive clinical data, to collect DNA and search for genetic risk variants. The inclusion of patients took place at the botulinum toxin outpatient clinic of five major Dutch movement disorders centres (Academic Medical Centres in Amsterdam, Leiden, Nijmegen and Rotterdam and Medical Centre Alkmaar). Over a three-year period, an approximate of a thousand dystonia and Myoclonus-Dystonia patients were included, characterized and clinically categorized.

In Chapter 1, the collected cohort of focal primary torsion dystonia (FPTD) patients is described. We show the broad phenotypic range of FPTD, from late onset blepharospasm, on to young onset cervical dystonia. For the benefit of genetic research, three genetic risk categories were distinguished in this cohort: patients with a Mendelian inheritance pattern, carrying a genetic variant with a major genetic impact (high-risk rare alleles). In this cohort, 2.4% had such a familial dystonia with autosomal dominant inheritance. Second, the large group of ‘sporadic’ patients (low-risk common variants) was recognized. Third, there was a group not fitting in either of these two categories: two or more patients were present in one family, however no clear familial pattern was present (25%). This is compatible with the presence of a strong genetic factor, however this factor does not have a large enough impact on its own to cause disease (moderate-risk–low-frequency alleles). These categories are a simplification of the real genetic spectrum, however helpful in genetic research. We have studied different clinical characteristics of these three groups. As expected, a significantly lower age at onset is seen in all focal dystonia subgroups with a stronger family history, representing the genetic burden. In both the sporadic and familial FPTD groups, the age at onset has an
effect on the distribution (site) of dystonia in a caudal-to-cranial tendency (“from legs to eyes”). This distribution gradient possibly represents age-dependent somatotopic changes in the putamen.

We discussed the strategies for tackling the complex aetiology by proposing a breakdown in appropriate phenotype subgroups. Various subgroups were described and might prove very useful in gene discovery: patients with psychiatric pathology accompanying dystonia; a young onset cervical dystonia (CD) subgroup; a CD subgroup with arm tremor and the presence of sensory tricks in most, but not all CD patients.

**Rare variants**

Chapter 2 handles both the identification of new rare genetic variants in Myoclonus-Dystonia syndromes (M-D) and the screening of known dystonia genes in focal dystonia cohorts, in order to assess their phenotypic spectrum.

In the outpatient clinic we have encountered a three-generation family with a previously unknown dystonia phenotype. Family members had a progressive action-induced multifocal dystonia and generalized myoclonus, resembling M-D. However, a remarkable feature of the syndrome was an action myoclonus in the lower extremities triggered by upright posture, causing instability.\(§2.1A\) During dystonic posturing of the right foot, electrophysiological assessment registered a frequency peak of 8 Hz and 4 Hz with intermuscular and corticomuscular coherence peaks around 4 Hz with a cortical drive. While standing, without dystonic posturing of the foot, the peak in autospectra of leg muscles shifted to 12 Hz, with intermuscular and corticomuscular coherences in the same frequency band without a cortical drive. The posture-dependent myoclonus resembles the previously described ‘orthostatic myoclonus’ seen in 15 elderly patients with gait decline.\(^2\) This has never before been described as a symptom in a familial syndrome. Eye movement recordings were suggestive of cerebellar pathology.\(^3\) Recently, cerebellar abnormalities have been discovered to be involved in dystonia\(^4-6\) and abnormal functional activity in the cerebellum is associated with myoclonus, especially cortical myoclonus.\(^7\) The somatosensory evoked potential (SSEP) showed an intermediate enlarged cortical potential in two of the affected family members, indicating cortical involvement. Intermediate enlarged P25-N35 complexes have been described in dystonia and myoclonus\(^8\), possibly indicating secondary changes of the cortical areas, resulting from basal ganglia pathology. Together, the electrophysiological assessment of this hyperexcitability phenotype suggests a subcortical origin, with possible cerebellar involvement.

Whole exome sequencing combined with linkage analysis in this family have resulted in the identification of a missense mutation c.4166G>A in the CACNA1B gene (MIM 601012).\(§2.1B\) This mutation is located in exon 28 of CACNA1B and causes an amino acid change from Arginine to Histidine at residue 1389 (Arg1389His). The CACNA1B gene codes for Ca\(_{\nu2.2}\), a N-type calcium channel. N-type (‘N’ for “Neural-Type”) calcium channels are found primarily at presynaptic terminals. Residue 1389 of
CaV2.2 is part of the putative extracellular loop and is highly conserved between species. Together with CaV2.1, the activity of CaV2.2 controls the amount of transmitter released in response to a depolarization of the presynaptic terminal. To assess the functional consequences of the Arg1389His mutation on CaV channel activity, we have expressed wild-type and mutant human CaV2.2 channels in the mammalian cell line (tsA201) and compared calcium channel currents using whole cell patch recording. Interestingly, calcium currents in cells expressing Arg1389His mutant were on average twice greater, as compared to control currents recorded under identical conditions, predicting an increase in the amount of transmitter release at excitatory and/or inhibitory synapses at terminals with mutant His1389-CaV2.2 channels. Likewise, other identified genes for familial dystonia syndromes were found to be related to ion channelopathies, ATP1A3 in DYT12 and ANO3 mutations, a Ca2+ gated chloride channel in DYT24 dystonia.

In another 3-generation family with a DYT11-like M-D phenotype we combined homozygosity mapping and exome sequencing and found co-segregation of a rare missense variant (c.5711C>T/p.Thr1904Met) in the RELN gene (MIM 600514). By screening a cohort of 24 additional M-D patients, we have identified two families with rare missense variants in RELN (Thr1904Met, Ile1217Met) co-segregating with disease and two sporadic RELN mutation carriers (Pro1703Arg, Leu411Ile). Reelin is a large secreted glycoprotein which is essential for the cytoarchitecture of laminated brain structures and modulation of synaptic transmission and plasticity, a hallmark of physiological findings in dystonia. In neuronal development, reelin controls the migration and positioning of cortical neurons and the foliation of the cerebellum via phosphorylation of tau. The DYT1-associated torsinA also relates to microtubule dynamics and intracellular trafficking: torsinA binds to kinesin light chain 1 (KLC1, a subunit of the kinesin 1 motor complex) and is an important component of the cytoskeletal network, responsible for maintaining the shape of the nuclear envelope and endoplasmatic reticulum. Recently, mutations in beta-tubulin 4a (TUBB4), coding for a major constituent of microtubules, have been found to cause DYT4 dystonia.

After the identification of THAP1 as the causal gene for DYT6 dystonia, we have screened our patient cohort and refined the phenotype. We (and others) have shown that DYT6 includes in particular those with early-onset, generalized pure dystonia with laryngeal or oromandibular involvement. We have not found any non-synonymous mutations in almost 400 adult-onset dystonia patients, thus inclining us to surmise that isolated focal dystonia is not typical of the DYT6 dystonia phenotype. Genetic testing for DYT6 should, however, be considered in patients with adult-onset dystonia and a family history of generalized or segmental dystonia.

Furthermore, in the response of four patients with an early onset DYT6 dystonia on bilateral GPi stimulation was described. Deep brain stimulation (DBS) has been shown to be a safe, effective surgical treatment for medically refractory...
dystonia, with the globus pallidus internus (GPI) being the most favoured target site. In generalized dystonia, a 58% improvement in motor function was observed three years after bilateral GPI DBS. In our four patients GPI DBS gave a mean motor improvement ranging from 16% to 55%, as measured by dystonia rating scales. Overall, GPI DBS improved dystonic symptoms of limbs, neck and trunk with consequent improvement of disability. Unfortunately, the laryngeal and oromandibular dystonia were less influenced by DBS. Since dystonic dysarthria appears to be an important feature in DYT6 dystonia patients, the possible lack of speech improvement after surgery should be thoroughly discussed with the DYT6 dystonia patients before surgery.

To determine the contribution of genetic variants in known dystonia genes in focal dystonia, we sequenced candidate genes in cohorts of spasmodic dysphonia (SD) patients (DYT6) and writer’s cramp (WC) patients (DYT6, DYT1, DYT11 and DYT16). This screening showed very low mutation frequencies in these genes in the SD en WC patient groups, narrowing down the clinical spectrum of these known dystonia genes and providing arguments for targeted genetic testing in clinical practice.

**Common variants**

In Chapter 3 we studied common genetic variants in focal dystonia groups, employing case-control association studies in candidate genes and selected pathways. Common variants in and around the DYT1 gene TOR1A and common haplotypes spanning the TOR1A region have been associated with different forms of focal dystonia. The results of these association studies have been contradictory, however. Therefore, we took a closer look at TOR1A common variants, assessing the four most frequent TOR1A variants in 364 cervical dystonia patients compared to controls. Results were thus integrated with previously performed studies in a meta-analysis. This meta-analysis showed a small effect in a subgroup of familial focal dystonia patients: a functional SNP rs1801968 showed association with a borderline significant odds ratio of 1.43 (95%CI 1.01-2.02). Other variants and groups did not show an association. From this we concluded that TOR1A is not a major risk factor for adult-onset focal dystonia. The lack of replication in performed association studies may be explained by ethnic genetic differences and clinical variety in between the studied populations, false positive findings because of small sample sizes in previous studies and/or a complex interplay between risk variants in TOR1A and associated pathways. These are arguments for more concise subgroup formation, collection of larger homogeneous groups and assessing the structure of haplotypes or performing whole gene sequencing, rather than studying single variants in candidate genes.

Previous imaging and functional studies in primary dystonia pointed to aberrant basal ganglia dopamine handling. We therefore chose to assess the dopamine metabolism genes for the first pathway-based association study performed in dystonia. (§3.2) Haplotype-tagging single nucleotide variants complemented
with selected variants of functional importance in COMT, DAT, TH, MAO-A and -B, DDC and DBH were investigated in 363 cervical dystonia patients and compared to matched healthy controls. This screen of 143 variants showed that common variations in key genes of the dopamine pathway do not contribute to dystonia risk in cervical dystonia. Possibly, risk alleles in this pathway may be more rare than detectable in this study, or are located in downstream DA signalling pathway (e.g. dopamine receptors) or, alternatively, dopamine level changes observed in imaging studies are secondary to the dystonia disease processes.

As we have found in Chapter 1, in 21.7% of all cervical dystonias, a (bilateral) postural tremor of the arms was present. The mechanisms leading to dystonia and tremor are not clear; aberrant neuroplasticity may play a role. Therefore, we hypothesised that a functional polymorphism in the plasticity related gene Brain derived neurotrophic factor (BDNF) could contribute to dystonia risk. We have not found an association for dystonia, though a significantly higher frequency of arm tremor was present in cervical dystonia patients with the BDNF Met66Met (53%), compared to Val66Met (35%) and Val66Val (29%) carriers (p=0.02; OR 2.52; 95% CIs: 1.10-5.76). This finding contributes to the idea that common genetic variants could modulate the clinical presentation of dystonia.

**Bridging the gap**

In Chapter 4 we described a pilot study in the twilight between familial and sporadic disease. As concluded from above mentioned studies, only about 35% of familial dystonia patients and less than 2% of sporadic patients carry mutations in one of the known dystonia genes. Common variants have been studied in candidate gene case-control association studies, however effects are small and replication has been challenging. Therefore, we have performed extensive parallel sequencing in a phenotype extreme population, presumably enriched for genetic risk variants. We have selected patients with severe, early onset, limb onset dystonia. Genes selection involved key players in the synaptic vesicle processing pathway (STON2, SNAPIN, KLC1), involved in neurotransmitter excretion and vesicle re-uptake, subtype 1 dopamine receptors (DRD1, DRD5) and the DYTs THAP1 and TOR1A, together stretching 100kb. Two rare missense SNVs were found in THAP1, implying that these patients have a DYT6 dystonia. The vesicle pathway did not show enrichment of rare coding variants, compared to control groups. Interestingly, in DRD1 we have identified rare missense variants in two early onset segmental dystonia patients with neck, oromandibular and arm involvement. This presentation resembles tardive dystonia, a condition associated with the use of dopamine receptor D2 antagonists. Comparing the frequency of DRD1 rare missense SNVs in this patients group (2/41) to controls (EVS cohort: 31/4300), results in an increased OR of 5.35 (95% CI 1.29-23.1) for developing dystonia. Dysfunctional striatal dopamine signalling system can induce dystonic symptoms and a role for torsinA in dopamine transport and abnormal neurotransmission has been suggested. In addition, the recently identified GNAL (alpha subunit of a G-protein,
DYT25\textsuperscript{31} was found to be coupled to dopamine 1 receptors\textsuperscript{32} providing ground for future research in the downstream dopamine handling pathway.

**Considerations | Future perspectives**

Besides our findings presented in this thesis, exome sequencing in familial dystonia syndromes has resulted in the identification of four new genes (\textit{ANO3}\textsuperscript{31}, \textit{TUBB4A}\textsuperscript{16}, \textit{GNAL}\textsuperscript{31} and possibly \textit{CIZ1}\textsuperscript{33}). New dystonia genes, even when identified in very rare dystonia syndromes, can be a cornerstone of the dystonia puzzle and achieve mechanistic understanding. Tagging common pathways affected by genetic variation in dystonia syndromes is an auspicious approach in the further understanding of disease pathophysiology. From the identified genetic variants described in this thesis three common pathways in particular gain strength in their link to dystonia. First, the identification of rare missense variants in \textit{dopamine receptor D1} substantiate a role for \textit{dopamine processing} in pathophysiology of dystonia. Second, \textit{Reelin} is an important player in \textit{microtubule dynamics and intracellular trafficking}. Lastly, and possibly most interesting, is the disturbance of the function of \textit{ion pores} in dystonia syndromes, such as shown by the identification of \textit{Ca}_{2}\textsubscript{2} (\textit{CACNA1B}).

Next to these three pathways, the transcriptional regulation (\textit{THAP1/DYT6}, \textit{TAF1/DYT3}) and cell cycle control (\textit{CIZ1/DYT23}) are implicated various dystonia syndromes. (See Table 1 in Introduction)

**How to carry on dystonia research into the future?**

\textit{Clinical research} - There is at present still a large delay in the recognition of dystonia in general doctors and neurologists outpatient clinics. In cervical dystonia, the most common form, patients see a mean of 3 health providers over an average period of 14 months, from symptom onset to diagnosis.\textsuperscript{34} Potential reasons for this diagnosis delay are the relative rarity of dystonia and the poor awareness of the different motor manifestations of dystonia. Training of clinicians in description of movement disorders, implementation of diagnostic tools and formal diagnostic criteria seem important steps towards shortening time to diagnosis of dystonia.

Due to the complexity and the delicate differences in phenotype of dystonia, examination and classification by a movement disorders specialist and inclusion in a clinical database should be part of the work-up of every dystonia patient. This extensive clinical data on dystonia patients is most important when searching for the causal genetic background and is useful to more fully describe the associated clinical features of novel genetic variants.

Discovery of endophenotypes (like temporal discrimination threshold\textsuperscript{35}) and biomarkers should be a priority in the search for new genes. This will facilitate formation of homogeneous patient subgroups. Appropriate subgroups from different research groups could be combined to make genome-wide association studies and sequencing efforts in large cohorts (in search for low-frequency and rare variants) feasible.
Summary and General discussion

Genetics – Each personal genome combines common to rare inherited alleles, plus new variation introduced by de novo mutations. An estimate of the human germline mutation rate is 74 de novo point mutations per generation. The significance of de novo point mutations has not been studied in dystonia as of yet. Therefore, aside from focusing on familial dystonia syndromes alone, we ought to focus our efforts on sequencing studies in phenotypical extremes, such as those detailed in Chapter 4.

The genetic studies comprised in this thesis focus on disease risk contributed by single nucleotide variants (SNV): aberrations at single nucleotide level. These are certainly not the only genetic variants, however. The role of copy number variations (CNV, deviations from the normal diploid state in the human genome because of a deletion or duplication) has not been studied in dystonia. Mutation rates for CNVs are 100 to 10,000 times more frequent than SNVs. CNV may contribute in a significant way to dystonia risk, similar to findings in autism and schizophrenia research. Therefore, future screening studies should include CNV essays.

Integration of clinical description, imaging, electrophysiology and genetics – The last chapter title 'Bridging the Gap' does not only refer to the frequency spectrum of genetic variants (ranging between rare and common), but also to the need for integration of careful clinical and neurophysiological examination in genetic dystonia research. The modulation of synaptic transmission and plasticity are the hallmarks of electrophysiological findings in dystonia. Further insights into the ‘wiring of the brain’ (imaging) and ‘functional connectivity’ (electrophysiology) are essential in the understanding of the neuronal network disturbances in dystonia. Therefore, in genotyping studies clinical description and subgroup formation should be combined with imaging studies (especial functional MRI (BOLD) and magnetic resonance diffusion tensor imaging (DTI)) and electrophysiological characterization. Finally, generation of induced pluripotent stem cell-derived neurons could be helpful in connecting the dots on a functional level.

Genetic architecture of dystonia

Connecting genetic risk factors with the sharp divergence in clinical presentation of dystonia may be called challenging, to say the least. Making use of this clinical variability in dystonia, composing subgroups and perform in-depth studies in these subgroups is essential in gene discovery. As far as the genetic architecture of dystonia is concerned: whereas the first raw outlines of the underlying structures have been put forward in this thesis, more details, measurements and connections await discovery.

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REFERENCES


