Genetic architecture of dystonia
Groen, Justus

Citation for published version (APA): Groen, J. L. (2014). Genetic architecture of dystonia

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
PHENOTYPES AND GENETIC ARCHITECTURE OF FOCAL PRIMARY TORSION DYSTONIA


SUMMARY

Background: The focal primary torsion dystonias form a group of clinical heterogeneous syndromes and can be considered a genetic complex disease; it is thought to be primed by genetic variants with variable impact and triggered by non-genetic factors. Thorough clinical description of focal primary torsion dystonias cohort is sparse, however essential for further progress in genetic research. Objective: To establish suggested relations between age at onset, site and family history in a large focal dystonias cohort and gain more insight in familial clustering for genetic research. Patients and Methods: A prospective cohort study between March 2008 and March 2011, including 676 focal primary torsion dystonias patients attending the botulinum toxin outpatient clinics of 6 Dutch movement disorder centres. Results and Conclusions: Of all focal primary torsion dystonias patients, 25% have a familial predisposition; in 2.4% a Mendelian inheritance pattern is noted. With a stronger family history, a significantly lower age at onset is seen in all focal dystonias. In both the sporadic and familial focal dystonias groups, the age at onset has an effect on the distribution of dystonia in a caudal-to-cranial tendency. In all focal dystonias forms, women are more frequently affected, except in writer’s cramp. Careful clinical characterisation will allow the formation of phenotype subgroups. We suggest that genetic research in focal primary torsion dystonias will benefit from this approach and discuss genetic research strategies to decipher the complex background of focal dystonias.
1.2 Phenotypes and genetic architecture of focal primary torsion dystonia

INTRODUCTION

Primary dystonia is clinically categorized based on the age at onset (AaO), site of onset, distribution, and segmental progression.\textsuperscript{1} \textit{Focal primary torsion dystonia} (FPTD) is the most frequent form of dystonia (1 in 2,500)\textsuperscript{2} and includes cervical dystonia (CD), blepharospasm (BSP), spasmodic dysphonia (SD), oromandibular dystonia (OMD) and focal hand dystonia such as writer’s cramp (WC). FPTD can be considered a \textit{genetic complex disease}; it is thought to be primed by genetic variants with variable impact and triggered by non-genetic factors. No causal genes have been confirmed for FPTD as yet.

For the benefit of genetic research, we distinguish three genetic risk categories in FPTD: in a small fraction a Mendelian inheritance pattern is noted, representing a genetic variant with a major genetic impact (\textit{high-risk – rare alleles}); in an estimate of 25 – 30\% of FPTD cases, a first- or second-degree family member with dystonia is present, however, no clear Mendelian inheritance pattern can be recognized. This is compatible with the presence of a strong genetic factor, however this factor does not have a large enough effect on disease risk to cause a clear Mendelian pattern (\textit{moderate-risk – low-frequency alleles}); in the remaining ‘sporadic’ group, environmental factors are likely to be more substantial compared to genetic ones (\textit{low-risk – common variants}). These categories are a simplification of the real genetic spectrum, and groups are probably overlapping.\textsuperscript{3}

In FPTD, some large autosomal dominant families with high penetrance\textsuperscript{4} and autosomal recessive families have been described.\textsuperscript{5} Genetic heterogeneity for familial FPTD is seen\textsuperscript{4} and three DYT loci have been found by linkage analysis: DYT7\textsuperscript{6}, DYT13\textsuperscript{7} and DYT21.\textsuperscript{8} Low frequency and common genetic variants have been studied in selected genes in sporadic FPTD patients. Mutation screenings showed frequencies of around 1\% of coding variants in the DYT6 gene \textit{THAP1}.\textsuperscript{9,10} Common genetic variants have been studied in a number of case-control candidate gene association studies. Variants in \textit{DYT1/TOR1A} \textsuperscript{11}, \textit{DYT6/THAP1}\textsuperscript{9}, and \textit{DRD5}\textsuperscript{12} have been found to increase FPTD risk in some studies, but often lack replication. The studied cohorts are relatively small and lack a detailed description of the dystonia phenotypes. Possibly, studied variants even exert different effects in variable genetic backgrounds. Such a complex interaction of risk alleles has been described for the \textit{H} allele at residue 216 in \textit{TOR1A}.\textsuperscript{11,13}

Unravelling the underlying genetic structure of FPTD is complicated by the phenotypic heterogeneity in FPTD. Comprehensive analyses of these disease characteristics have not been performed in single large cohorts as yet. In the present paper, we use the detailed clinical information from a single, large cohort to endorse previously suggested relations between family history, age at onset and site of dystonia. Furthermore, we exclude known genes for dystonia in high risk groups and describe how genetic risk categories and phenotypic subgroups can guide future genetic research in FPTD.
METHODS

Patients
This study was approved by the Medical Ethical Committees of all contributing centres. Patients were included from the botulinum toxin clinics of 6 Dutch centres, both academic and non-academic, specialized in movement disorders. Patients were diagnosed with primary dystonia based on accepted criteria. Secondary causes were excluded by patient history, examination, and additional neuroimaging and laboratory tests when indicated. After giving informed consent all patients were scored and included in an Access-based database (JLG). DNA samples were obtained from all participants for genetic analysis. In the clinical database we registered the patients’ dystonia characteristics, age and localization of onset, duration and spread (also dystonia-associated tremor), complexity and mobility of dystonic movements, presence or absence of a sensory trick, psychiatric symptoms, and history. In addition, we investigated the occurrence of environmental factors possibly triggering the development of dystonia: preceding trauma or infection of the dystonic site, usage of medication and drugs, and influence of stress on symptoms. Family history was considered positive when the index patient described movement disorders in a first- or second-degree family member. Based on family history, three subgroups were created: (1) sporadic patients, who did not report any family members with movement disorders; (2) patients with a familial predisposition (FH+); (3) patients with autosomal dominant focal dystonia (FH-AD). In families, all available family members were examined and videotaped using a standard video protocol and reviewed by a movement disorder specialist (MAJT). A subject was defined as “affected” if he/she had clear signs of dystonia, and as “possibly affected” if there were very subtle signs or symptoms suggestive of dystonia. Possibly affected subjects were not included in further analyses.

Genetic Analysis
DNA was isolated from EDTA blood using standard procedures (Gentra Technology). All patients with segmental dystonia and those with an age at onset before 26 years were tested for the GAG deletion in the \textit{TOR1A} gene and screening of the entire coding region of \textit{THAP1}. Patients with jerky movements accompanying the dystonia were tested for mutations in the DYT11 (SGCE) gene. For genotyping, standard PCR amplification and sequence analyses was performed (ABI big dye v3.1 chemistry, ABI 3730 capillary system, Applied Biosystems). If affected family members showed a DYT7-like phenotype with neck involvement, linkage analysis for the DYT7 locus on chromosome 18p was performed making use of markers D18S481, D18S54, D18S976, D18S452, D18S843, and D18S1153 (LI-COR 4300 analyzer). Exome sequencing (NimbleGen SeqCap EZ v2.0, SOLiD v4 genome sequencer) was employed to screen all coding regions in the DYT7-linked area.
Evaluation and Statistics
Patient characteristics were summarized using descriptive statistics and analyzed with ANOVA (AaO, family history). If a significant effect emerged, post hoc Fisher’s least significant difference (LSD) tests were applied. Analyses were performed in SPSS (IBM SPSS, PASW Statistics 18).

RESULTS
The clinical characteristics of the total group of 676 primary focal dystonia patients included in this cohort study are summarized in Table 1. Overall, 447 females and 229 males were included. In all focal dystonia subgroups, except WC, women are more frequently affected than men.
Table 1. Characteristics of studied cohort. The four FPTD subgroups in this study with their distinct clinical profile are shown. Psychiatric complaints are self reported. *Describes the frequency of dystonia in the first- or second-degree family. §Pain associated with dystonia at moment of interview. NA: not applicable.

<table>
<thead>
<tr>
<th></th>
<th>CD</th>
<th>BSP</th>
<th>SD</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of patients (n=676)</td>
<td>432</td>
<td>97</td>
<td>79</td>
<td>68</td>
</tr>
<tr>
<td>female:male (447:229)</td>
<td>1.34</td>
<td>1.75</td>
<td>1.67</td>
<td>0.55</td>
</tr>
<tr>
<td>family history* (%)</td>
<td>22.6</td>
<td>10</td>
<td>16.4</td>
<td>17.6</td>
</tr>
<tr>
<td>age at exam (SD)</td>
<td>59.6</td>
<td>67.4</td>
<td>58.9</td>
<td>54.1</td>
</tr>
<tr>
<td>age at onset (SD)</td>
<td>41.9</td>
<td>54.0</td>
<td>46.4</td>
<td>39.2</td>
</tr>
<tr>
<td>early onset (%)</td>
<td>14.1</td>
<td>3</td>
<td>11.3</td>
<td>11.7</td>
</tr>
<tr>
<td>segmental spread (%)</td>
<td>8.7</td>
<td>8</td>
<td>8.8</td>
<td>13.2</td>
</tr>
<tr>
<td>arm tremor %</td>
<td>21.7</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>pain § (%)</td>
<td>45.1</td>
<td>0</td>
<td>0</td>
<td>5.6</td>
</tr>
<tr>
<td>sensory trick (%)</td>
<td>64</td>
<td>38</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>depression (%)</td>
<td>11.7</td>
<td>12.4</td>
<td>Unknown</td>
<td>4.1</td>
</tr>
<tr>
<td>panic/anxiety disorder (%)</td>
<td>4</td>
<td>8</td>
<td>Unknown</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Familial clustering

In the group of FPTD, three subgroups can be recognized based on family history: sporadic, with familial predisposition (FH+) and familial (FH-AD, Figure 1). In the total cohort 25.5% reported to have relatives with a movement disorder (FH+, n=170), of whom 62 patients (9.1% of original dataset) had at least one first-degree family member with dystonia. Other movement disorders reported in first-degree family members were essential tremor (reported in 20 cases), parkinsonism (14 cases), myoclonic jerks (2 cases) and relatives of one proband suffered from vocal and motor tics.

Sixteen patients (2.4%) had two or more family members with dystonia (FH-AD), all with possible AD mode of inheritance. However, in Fam-2 and 9, only female members are affected, and in Fam-2, 3 and 6 also mitochondrial inheritance is possible. Six of the families were not further analysed in this study because of death of the index patient (n=1), the family did not want or could not cooperate (n=3) or a mutation in DYT6 and DYT1 was found during mutation screening (n=2; see below). Among the remaining 10 available pure dystonia families, we identified 29 additional affected family members.

FPTD families

All 16 families had a possible AD mode of inheritance, however also mitochondrial (2 and 3) inheritance is possible. Ten families were used for further studies (Figure 2 and Textbox 1) In 6 families all affected family members had FPTD: four families with isolated CD (Fam-7, 8, 9, 10), one with WC (Fam-1) and in one family both cervical
and hand dystonia occurred (Fam-5). Four of the families were classified as ‘dystonia-plus’: in Fam-6 both motor tics and cervical dystonia were present in two family members. In three families, both cervical dystonia and myoclonic jerks of the upper limbs (Fam-2, 3) or axial myoclonus (Fam-4) were present in family members of the index patient with focal dystonia.
Textbox 1. Clinical description of ten focal primary torsion dystonia families.

**Family 1** – A family with writer’s cramp. Five subjects (II-1, III-3, III-5, IV-3, IV-7) are affected, one subject was possibly affected (I-2). All affected family members have WC without other neurological symptoms. No sensory tricks are present, no alcohol response, symptoms worsen with stress. Affected subjects have a mean AaO of 36.2 years (range 22 to 54 years). The mode of inheritance (MOI) is autosomal dominant (AD).

**Family 2** – A family with cervical dystonia and additional myoclonus in upper extremities and writer’s cramp. Five subjects (II-1, II-3, II-4, II-6, III-4) show a mobile cervical dystonia, one subject (II-2) is possibly affected. Subject I-2 suffers from a hypokinetic rigid syndrome, possibly induced by antipsychotic medication. Subject III-2 shows a myoclonus–dystonia phenotype with CD, myoclonic jerks of upper body and writer’s cramp. In all affected family members a reduction of symptoms is seen with alcohol use, symptoms worsen with stress, fatigue and action. Affected subjects have a median AaO of 26.3 years (range 15 to 34 years). The MOI is AD or X-linked.

**Family 3** – A family with cervical dystonia and writer’s cramp. Five subjects (II-1, II-5, III-1, III-2, IV-3) are affected, one subject (IV-1) is possibly affected. All of the affected subjects have CD, additionally one subject (IV-3) shows writer’s cramp and myoclonus of the right hand. One family member (II-5) developed parkinsonian features at age 67, two family members have adjacent migraine. Sensory tricks were present in all affected family members; alcohol responsiveness is present in some subjects. The four subjects with CD have a median AaO of 33.67 years (range 18 to 50 years), age at onset of WC in IV-3 is 6 years. An AD or mitochondrial MOI is present.

**Family 4** – A family with dystonia-plus syndrome: cervical dystonia and myoclonus. Six subjects of this family (II-1, II-5, III-3, III-5, III-6, IV-6) are affected with jerky, mobile dystonia of the neck. One subject (III-5) has definite myoclonus-dystonia. Several family members show myoclonic jerks, one subject has WC (IV-6). No benefit of sensory tricks exist, alcohol reduces the symptoms. One subject with CD (III-3) suffers from severe depression. Cardiovascular heart disease is prominent in this family, but does not cosegregate. Subjects with CD have a median AaO of 40 years (range 35 to 55 years), the subject with WC has an AaO of 45 years. The MOI is AD, possibly with reduced penetrance.

**Family 5** – A family with CD and writer’s cramp in one subject. Three subjects have CD. One subject (II-1) also shows WC, one subject (III-1) also shows blepharospasm and oromandibular dystonia. All of the affected subjects show an irregular tremor of the hands. One subject (II-3) is possibly affected. No sensory tricks and jerks, reduction of symptoms with alcohol use, symptoms worsen with stress and action. Two subjects with CD have a median AaO of 44 years (range 43 to 45 years), the subject with writer’s cramp (II-1) has an AaO of 20 years. Most likely the MOI is AD with reduced penetrance.

**Family 6** – A family with dystonia plus: cervical dystonia and tics. Three subjects (I-1, II-1, II-2) are affected. One affected subject has CD only (II-2), one has dystonic tics (I-1) and one shows a mixture of CD and dystonic tics (II-2). The two subjects with dystonic tics have an AaO before age 4 years. CD in II-2 started at age 35 years. Subject II-3 developed a marijuana addiction and psychiatric problems since the age of 17 years; he did not show any movement disorders at examination. No sensory tricks were present, no response to alcohol and stress. MOI is AD.

**Family 7** – A family with pure, cervical dystonia. Three subjects (I-1, III-2, III-8) are affected; one subject (IV-2) is possibly affected. Subject III-1 shows segmental spread to larynx,
causing dysphonia. No sensory tricks and jerks, no response to alcohol and stress. Patients have a median AaO of 39 years (range 37 to 41 years). MOI is AD.

**Family 8** – A family with pure, cervical dystonia. Three subjects (I-1, II-1, II-2) are affected with jerky, mobile cervical dystonia. There are no sensory tricks, no response to alcohol and stress. Mean AaO of 51.5 years (range 50 to 53 years). MOI is AD.

**Family 9** – A family with pure, cervical dystonia. Three subjects (II-4, III-1, III-2) suffer from CD. No jerks are present. Subjects III-1 and III-2 show ‘tremor associated with dystonia’. III-2 has a sensory tricks with light touch of the chin, there is no alcohol response, symptoms worsen with stress and action. Mean AaO is 42 years (range 39 to 45 years). MOI is AD or X-linked.

**Family 10** – A family with pure, cervical dystonia. Four subjects (II-7, III-1, III-2, IV-1) have CD. No jerks or tremor are present. Mean age at onset is 35.5 years (18 to 45 years). No alcohol responsiveness is noted, also no sensory trick is present. MOI is AD.

**Genetic testing**

**Cervical Dystonia** – All patients with segmental spread of CD (n=38) and those with an age at onset before 26 years (n=61) were tested for the GAG deletion in the DYT1/TOR1A and screened for the whole coding region of DYT6/THAP1. One TOR1A mutation carrier was identified: she had developed cervical dystonia at the age of 14 years and was the proband of an AD dystonia family. No non-synonymous changes in THAP1 were identified. Six patients with jerky neck dystonia, clinically suspect for myoclonic jerks were negative for DYT11/SGCE mutations.

**Spasmodic Dysphonia** – All SD patients (n=79) were tested for mutations in THAP1. We identified two mutation carriers. One patient had SD since age 14 years with cervical and oromandibular spread, and a strong family history for dystonia. She carried a heterozygous missense mutation in THAP1 (p.Asn136Lys) as previously described.\(^{10}\) The second patient was a 35-year old woman who had SD since age 14 years that spread to the oromandibular region at age 21 with tongue dystonia and with laryngeal dystonia of the adductor subtype. She carried a heterozygous nonsynonymous mutation, c.151A>G (p.Ser51Gly).\(^{15}\)

**Writer’s Cramp** – All WC patients (n=68) were previously screened for mutations in TOR1A, THAP1 and SGCE.\(^{16}\) One patient, a 32-year old woman suffering from WC of her dominant right hand since age 12 years, showed the GAGdel in the TOR1A gene. She had a negative family history for movement disorders.\(^{16}\)

**Families** – All index patients of the families (n=14) were screened for mutations in TOR1A, THAP1 and SGCE. No additional mutation carriers were found. Linkage analysis excluded the DYT7 locus\(^{6}\) as a cause for dystonia in Fam-2, Fam-3 and Fam-10 and was inconclusive for Fam-5 due to limited informative meioses. In Fam-4 affected individuals shared 3 marker alleles (D18S481, D18S54, D18S976: 11-2-4). This haplotype was absent in the unaffected. Analysis of all exons in this 2.183 Mbp region (chromosome 18: 3056133 to 5239199, hg19) did not reveal any protein alternating changes in this region.
**Age at Onset**

**AaO and affected site** – When we look at the mean age at onset (mAaO) for the different affected sites, BSP has the highest onset with 54.0 years (SD 12.1) and WC has the earliest onset at 39.2y (SD 10.8). The mAaO of dysphonia (46.4y SD 15.2) and CD (41.9y SD 14.1) are between those of BSP and WC. An early AaO (<26y) was noted in 14% of CD patients, in 11.7% of WC, 11.3% of dysphonia and in 3% of BSP patients (Chi², p=.027 [3df]). Next, we looked if this distribution is present in patients with and without positive family history. (Figure 3) In the sporadic group (n=506), the mAaO of BSP was 54.4y (SD 12.3); of dysphonia 46.8y (SD 14.6); CD 42.9y (SD 13.4) and WC 40.3y (SD 10.5). One-way ANOVA showed a global significant difference in AaO for the different sites of involvement (p=.74x10⁻¹²). In a post-hoc analysis with Bonferroni correction, all differences in AaO were significant, except between CD and WC. In the FH+ subgroup (n=131), the overall mAaO was 40.9y (SD 15.6), for BSP mAaO was 52.6y (SD 11.5); for dysphonia mAaO was 44.9y (SD 18.9); in CD AaO was 39.8y (SD 15.3) and for WC the mAaO was 34.9y (SD 12.0; p=.01, significant differences in post hoc analysis of BSP vs CD and BSP vs WC). In the small subgroup of FH-AD patients (n=39) the mAaO was 37.5y (11.3) for CD and 31.0y (17.0) for WC (t-test, p=.21). AaO for dysphonia and BSP in this group were not investigated as only one FH-AD patient with dysphonia and one BSP were identified. These results indicate that AaO is related to site at onset independently of family history.

**AaO and family history** – To assess the influence of family history on the AaO, we used all CD and WC patients. Here, we compared AaO of sporadic (n=358), with FH+ (n=128) and FH-AD (n=37) focal dystonia cases. As expected, a stronger family history results in a decrease in mAaO (sporadic 42.5y (13.1); FH+ 39.3y (15.0) and FH-AD 36.4y (13.6); ANOVA p=.004. Post hoc analysis shows a significant difference between FH-AD and sporadics p=.018). (Figure 4)
**Figure 3.** Influence of age at onset on the site at onset of FPTD. The mean AaO is a modifying factor of the site at onset in all genetic subgroups (sporadic, with familial predisposition [FH+] and Mendelian [FH-AD], as previously described in a review of cases by O’Riordan et al. [Neurology 2004;63:1423-1426].

**Figure 4.** The CD and WC dystonia patients from AD dystonia families have a significantly earlier AaO than the sporadic focal dystonia patients. Patients with a family history for dystonia, lacking a clear Mendelian pattern of inheritance show an intermediate mean AaO. *difference is significant p .018 (Bonferroni Multiple comparison). ◊ mean Age at Onset, Error bars represent 2 standard errors of the mean.
AaO was not significantly influenced by sex (Table 2). The distribution of AaO in male and female patients was comparable and normally distributed. (Figure 5).

**Table 2.** Sex distribution and mean age at onset. The age at onset in subtypes of FPTD is not significantly influenced by sex. *Students t-test. y = years; SD = standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>CD mean</th>
<th>SD</th>
<th>BSP mean</th>
<th>SD</th>
<th>SD mean</th>
<th>SD</th>
<th>WC mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>female</td>
<td>42.7y</td>
<td>13.68</td>
<td>53.7y</td>
<td>11.38</td>
<td>47.3y</td>
<td>17.32</td>
<td>37.4y</td>
<td>11.91</td>
</tr>
<tr>
<td>male</td>
<td>40.1y</td>
<td>14.89</td>
<td>55.1y</td>
<td>14.61</td>
<td>44.6y</td>
<td>9.58</td>
<td>40.2y</td>
<td>8.47</td>
</tr>
</tbody>
</table>

| p-value* | 0.079 | 0.625 | 0.477 | 0.311 |

**Figure 5.** Distribution of the age at onset of FPTD in males and females. Curve represents the normal distribution.
Phenotypic subgroups in FPTD

Some (additional) features are only present in subgroups of FPTD patients.

**Spread and tremor** – Segmental spread was most frequently present in WC (13.2%) and around 8% in BSP, CD and SD (Chi², p = .50). (Table 1) Postural arm tremor was found in 21.7% of all CD patients. Of all CD patients, 5% (n=20) reported a first-degree family members with arm tremor. No patients with a primary writing tremor, without dystonia, were included in this study.¹⁷

**Sensory tricks** – Sensory tricks were objectified in 64% of all CD patients, 38% in BSP and 16% in SD (Chi² test, p < .05). In CD, sensory tricks included light touch to mental, temporal or occipital region, or pressure in the neck. In BSP light touch to the orbital arch and the temporal region resulted in reduced blinking in some cases. In SD, described tricks included lowering of the voice and whispering, but also manual pressure on the larynx was seen. Some patients with WC guide their writing hand with the other hand but in WC no clear sensory tricks were reported or observed.

**Pain** – Almost half of all CD patients reported neck pain over 10% of the time (as measured by the TWSTRS part III, score 1 or 2). Of this pain group, 15% reported a history of depression. Of the CD group without pain, 14% reported a previous or current episode of depression (Chi², p = .454).

**Psychiatric disorders** – We screened for the occurrence of psychiatric pathology by history. In the CD group, 17.5% (had) sought psychological help; depression being the most common diagnosis in 11.7% and anxiety/panic disorders in 4.0%. In the BSP group, 21.6% visited a psychiatrist or psychotherapist. In 12.4% of patients depression was the main complaint, 8% suffered from anxiety/panic disorders. In the WC group, 5.6% had consulted a psychotherapist; 4.1% for depression and 1.5% for anxiety.

Environmental and aggravating factors

**Aggravating factors** – Psychological stress was mentioned as a factor worsening dystonia in 55% of all cases (CD 64%; SD 53%; BSP 51%; WC 33%). Frequently mentioned for BSP were exposure to bright light (79%) and wind (64%).

**Alcohol, Medication and Drugs** – A positive effect of alcohol was mentioned by 11% of all patients (CD 15%; SD 15%; BSP 6%; WC 8%). Some patients in all groups mentioned a beneficial effect of benzodiazepines and cannabis; however this was not systematically assessed. 2.5% of all patients reported to have used a centrally acting dopamine receptor-blocking agents prior to dystonia onset. Of these patients, 90% had a cervical dystonia.

**Trauma** – A history of neck trauma was present in 12.4% of the CD group. In the BSP group, 4.5% had a history of eye disease preceding the onset of dystonia. 7% of the WC patients reported to have experienced trauma (defined as fractures or surgery) of the affected arm. In the SD group, 32% recalled an episode of sore throat or infection preceding the start of laryngeal dystonia.
DISCUSSION

We have here described the clinical characteristics from the largest published FPTD cohort. To guide genetic research in this cohort, we focus on careful clinical characterisation, family history and identification of subgroups in focal dystonias.

In our cohort, over 25% reported a positive family history; 2.4% had FH-AD dystonia. Leube and colleagues show similar numbers with a frequency of 18% hereditary predisposition and Mendelian patterns in 3% focal dystonia patients.18 Dhaenens report a 30.7% frequency of (first-degree) familial cases in a group of 150 FPTD patients.19 In a BSP cohort 32% had a positive FH.20 In half of the pedigrees (5/10), an intrafamilial variability of the phenotype was noted. CD and WC within families was seen, but also CD and myoclonic jerks, CD and tremor and, in one family, CD and tics. This suggests shared genetic factors for CD and WC19,21 and interestingly also for CD with tremor, myoclonus and tics. Also FH+ patients reported other movement disorders, parkinsonism being the most frequent one. Fourteen out of 170 FH+ dystonia patients in the cohort (8,34%) report a family member with parkinsonism. The population prevalence of parkinsonism in The Netherlands is 1,26% in female (95%CI 0,91-1,78) and 1,41% in male (95% CI 1,02-2,00)22, possibly suggesting a higher prevalence in the dystonia FH+ population. Other screenings studies investigating family history of focal dystonia also report other movement disorders in the family.20 This observed intrafamilial heterogeneity points to a possible, shared genetic background for dystonia with tremor, myoclonus and possibly tics and parkinsonism. However, large family based epidemiological studies are needed to confirm this association.

Looking at the AaO and family history shows a lower AaO in FPTD with a FH-AD compared to sporadic patients (Figure 4). FH+ patients have an intermediate AaO, probably reflecting the lower impact of the underlying genetic variants than in AD FPTD. This ‘intermediate’ familial group could represent familial dystonia with reduced penetrance.23 However, moderate-risk – low-frequency alleles should be considered, as seen in glucocerebrosidase (GBA) gene variants in Parkinson’s disease.24 When assessing the relation between site of dystonia and AaO, in all groups (sporadic, FH+ and FH-AD), a caudal-to-rostral distribution of dystonia onset is seen with an increase in mean AaO. (Figure 3) This relation was also present in a cohort study of the Epidemiologic Study of Dystonia in Europe (ESDE) 25 and denoted in a review of O’Riordan and colleagues.21 This gradient is also seen in monogenetic dystonia forms: DYT1 dystonia typically starts at young age with leg involvement21,26, and also early onset DYT6 dystonia starts with dystonia in a limb with spread to neck and larynx.27 In contrast, late onset DYT6 dystonia preferably affects the neck and larynx.9,10 Taken together, this caudal-to-cranial gradient likely reflects a primary effect of the AaO on the clinical expression of FPTD. Somatotopy has been demonstrated in the putamen28, and alteration of this organization is seen in WC.29 Possibly, age-dependent
somatotopic changes in the putamen gives rise to this observed age-dependent focal dystonia distribution\textsuperscript{21}, however this remains speculative.

Overall, the clinical characteristics in this cohort contained in a screening setting, are similar to frequencies in literature.\textsuperscript{19,21,30} Some identified clinical subgroups here described, might be very useful in future genetic analysis of FPTD: psychiatric pathology accompanying focal dystonia; young onset CD; arm tremor in CD; and the presence or absence of sensory tricks. An increasing amount of attention is paid to the non-motor complaints in dystonia.\textsuperscript{31,32} Depression is the most frequent psychiatric complaint (5.6-12.4\%), followed by anxiety. Possibly, a group of FPTD patients with psychiatric co-morbidity has a more extensive and complex disease, involving both psychiatric symptoms and dystonia motor symptoms. Shared genetic factors for both dystonia and depression might cause this relation. Alternatively, depression is a secondary symptom caused by the disability, pain and stigmatization. No relation between CD related neck pain and depression risk was found in our group. The prevalence in Dutch population of depression is between 4.9 – 7.4 \%,\textsuperscript{33} suggesting a higher prevalence in the dystonia patients (in our cohort: CD 11.7\% and BSP 12.4\%). However, we only collected numbers by history taking, limiting definite conclusions. The prevalence of anxiety is 8-10 \% in the Dutch population\textsuperscript{33}, similar to the numbers we collected in the FPTD cohort by history.

An AaO before the age of 26 years was present in 14\% of our CD cohort, comparable to a previous CD cohort study.\textsuperscript{34} This frequency is significantly higher in CD than in other FPTDs. ‘Young onset cervical dystonia’ (YOCD) has been suggested to have a different genetic background than other CD patients, based on higher frequencies of males, family history and chances of remission.\textsuperscript{35} We did not find differences in family history or sex distribution in the YOCD patients compared to the full CD cohort. Young AaO can indicate a larger genetic risk and therefore this ‘extreme phenotype group’ is of interest for research in moderate and high-risk variants. Tremor associated with dystonia\textsuperscript{36} was found in 21.7\% of all CD patients, and appears to be a specific feature for CD. There is ongoing discussion on the combination of dystonia and essential or dystonic tremor. However, the clinical presentation with symmetrical bilateral postural and action tremor of the arms without dystonia of the arms supports the combined occurrence of dystonia and essential tremor. This is also illustrated by arm tremor in cervical dystonia families (Fam-5 and Fam-9) and aggregation of dystonia in some AD ET families.\textsuperscript{37} Probably this group has overlapping genetic risk factors for both essential tremor and FPTD. The group with sensory tricks, present in 64\% of all CD patients, but only in 38\% in BSP and 16\% of SD patients, can be used to study the mechanisms of distorted sensorimotor integration, one of the suggested pathways involved in dystonia etiology.\textsuperscript{38}
**Limitations**

Patients in this study cohort were ascertained from specialized movement disorder clinics. This could introduce a selection bias, including more severe and complex cases. Also, the present study is not an epidemiological study; therefore, information on environmental factors should be interpreted with caution. Also, information on psychiatric symptoms was gathered in a screening setting and no scales were used. As previous studies showed, the assessment of family history by asking the index case can lead to an underestimation. Also, the reported arm tremor in family members was not assessed. Genetic screening of DYT1, DYT6 and DYT11 was carried out in ‘high risk’ groups only, therefore the presence of mutations in patients with atypical phenotypes cannot be ruled out.

**CONCLUSION**

The genetic spectrum of FPTD ranges from rare familial syndromes to sporadic FPTD. Moreover, the phenotypic range is broad, from late onset BSP to young onset CD. Research in the genetics of complex diseases like FPTD, will benefit if appropriate patient subgroups (phenotypes or endophenotypes) are assessed. Additional symptoms in FPTD, like depression, tremor and presence of sensory tricks, can help to form phenotypic subgroups. High throughput sequence techniques like whole-exome and whole-genome sequencing platforms can be employed in familial FPTD to look for segregation of rare variants; and in FH+ and selected extreme patient groups (eg young CD) for moderate-risk low-frequency variant discovery. Additionally, cohorts of well-characterised sporadic patients from different research groups should be combined to make genome-wide common variant discovery in FPTD feasible. Combining these approaches in careful examined FPTD groups will lead to more insight in the missing heritability of focal dystonia.

**ACKNOWLEDGEMENTS**

We are grateful to all participants in this study. We thank Katja Lohmann for performing DYT7 linkage. This study was supported by the Prinses Beatrix Fund.
REFERENCES


(15) Groen JL, Yildirim E, Ritz K et al. THAP1 mutations are infrequent in spasmodic dysphonia. Mov Disord 2011.


1.2 Phenotypes and genetic architecture of focal primary torsion dystonia

(37) Hedera P, Phibbs FT, Fang JY, Cooper MK, Charles PD, Davis TL. Clustering of dystonia in some pedigrees with autosomal dominant essential tremor suggests the existence of a distinct subtype of essential tremor. BMC Neurol 2010;10:66.


