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Primary dystonia: In search of new genes...

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ABSTRACT
Primary dystonia are movement disorders, genetically heterogeneous. The only gene identified for primary dystonia is the DYT1 gene (or TOR1A) implicated in generalized forms. Three loci have been implicated in focal dystonia but no genes have been identified. Many families excluded these known loci suggesting larger heterogeneity and existence of other genes. In this article, we will review the strategies useful for identifying disease gene in primary dystonia.

INTRODUCTION
The dystonias are a group of movement disorders with neurological origin, characterized by “involuntary, sustained muscle contractions affecting one or more sites of the body, frequently causing twisting and repetitive movements or abnormal postures” [1]. Dystonia is the result of involuntary concurrent contractions of agonist and antagonist muscles, with overflow of unwanted muscle contractions into adjacent muscles. Dystonic movement can vary in speed and intensity, some are defined as “task-specific dystonia”, when it appears only with specific action (e.g., writer’s or musician’s cramp).

CLASSIFICATION OF DYSTONIA
Dystonia can be classified in different ways, based on the description of clinical features, etiology and genetics. The descriptive classification according to either age at onset or distribution of symptoms is important since evolution of the disease is correlated to these factors. When the disease begins in childhood or young adulthood, it usually progresses from focal limb dystonia to the severe generalized form, whereas dystonia that begins after the age of 25 years usually involves craniocervical muscles, nearly always remains localized or segmental, and usually does not progress. The etiologic classification distinguishes primary and secondary forms (table 1). In primary dystonia (also known as “Idiopathic Torsion Dystonia” ITD), dystonia is the sole symptom (with exception of tremor) and the cause is mainly genetic. In secondary (symptomatic) forms, dystonia is usually caused by environmental insults such as lesion, drug/toxin, or metabolic disorder. Other subgroups have been proposed to list dystonia (table 1), as Dystonia-plus that refers to disorders in which dystonia is associated with other types of movement disorders like Parkinsonism or Myoclonus and the heredodegenerative dystonia in which dystonia is part of a widespread neurodegenerative syndrome. Paroxysmal dyskinesia constitute also a subgroup that is clinically distinct from others because they are characterized by episodic dystonia and patients may have also other neurological features such as epilepsy or chorea. More recently genetics has been used to classify dystonia according to their loci. This molecular classification distinguishes 15 genetically different types of dystonia designated dystonia types (DYT) 1-15 (table 1).
### Table 1: Classification of dystonia

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primary Dystonia</th>
<th>Dystonia plus syndromes</th>
<th>Heredodegenerative dystonia</th>
<th>Paroxysmal dyskinesias</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DYT1</strong></td>
<td>Dystonia 1: Early-onset torsion dystonia</td>
<td>GTP CycloHydrolase 1, GCH1</td>
<td>Dystonia 3: X-linked dystonia parkinsonism; “Lubag”</td>
<td>Dystonia 8: Paroxysmal non-kinesigenic dyskinesia</td>
</tr>
<tr>
<td><strong>DYT2</strong></td>
<td>Dystonia 2</td>
<td>TH Tyrosine hydroxylase</td>
<td>Dystonia 9: Choreoathetosis</td>
<td>Dystonia 9: Choreoathetosis</td>
</tr>
<tr>
<td><strong>DYT4</strong></td>
<td>Dystonia 4: Whispering dystonia</td>
<td>Dystonia 11: Myoclonus dystonia</td>
<td>Dystonia 14: Dystonia 14; Dystonia 14</td>
<td>Dystonia 10: Paroxysmal kinesigenic choreoathetosis</td>
</tr>
<tr>
<td><strong>DYT6</strong></td>
<td>Dystonia 6: Adult-onset ITD of mixed type</td>
<td>Dystonia 12: Rapid-onset dystonia parkinsonism</td>
<td>Dystonia 15: Dystonia 15; Myoclonic dystonia</td>
<td>Dystonia 10: Paroxysmal kinesigenic choreoathetosis</td>
</tr>
<tr>
<td><strong>DYT13</strong></td>
<td>Dystonia 13: Multifocal/segmental dystonia</td>
<td>Dystonia 13: Dystonia 13; Multifocal/segmental dystonia</td>
<td>Dystonia 3: X-linked dystonia parkinsonism; “Lubag”</td>
<td>Dystonia 8: Paroxysmal non-kinesigenic dyskinesia</td>
</tr>
</tbody>
</table>

**AD**: autosomal dominant; **AR**: autosomal recessive

**References**

[2]; [3]; [4]; [5]; [6]; [7]; [8]; [9]; [10]; [11]; [12]; [13]; [14]; [15]; [16]; [17]; [18]; [19]; [20-22]; [23]; [24]
GENETIC OF PRIMARY DYSTONIA
Currently, the only known gene for primary dystonia is the *TOR1A (DYT1)* gene identified by Ozelius *et al* in 1997 [2]. In the majority of cases, a three base pairs deletion (c.907delGAG) is responsible for early onset dystonia, typically with symptoms starting in the limbs and a tendency to generalize. Two other mutations have been reported in this gene: a 18-bp deletion in a family presenting with a form of dystonia mixed with myoclonia [25], and an out-of-frame 4-bp deletion in an asymptomatic blood donor [26]. There is strong clinical variability in *DYTI* linked dystonia, ranging from children profoundly handicapped to obligate carriers of the disease gene showing few or no sign of the disorder [27-29] due to low penetrance (30-40% regardless of ethnic origin) [30; 31].

Primary dystonia include also adult onset forms not linked to *DYTI*, which usually remain focal, affect one body part and occasionally spread to adjacent regions (segmental dystonia). Commonly described forms of focal dystonia include cervical dystonia (or spasmodic torticollis), blepharospasm, oromandibular dystonia, laryngeal dystonia (or spasmodic dysphonia), and limb dystonia (including task-specific dystonia such as writer’s cramp).

![Diagram of body parts affected by dystonia](image)

*Figure 1: body parts affected by dystonia*

Little is known about the genetics of focal dystonia that represent the most common forms of the disorder. In rare cases a hereditary component has been demonstrated in patients with focal dystonia such as the 3-bp deletion in the *DYTI* gene [32]. Up to 25% of patients with focal dystonia have an affected relative [33], suggesting the existence of one or more inherited genes. Currently, three loci associated to primary focal dystonia have been mapped:
DYT6 on chromosome 8 in Mennonites families [5], DYT7 on chromosome 18 in German families [6; 34; 35] and DYT13 on chromosome 1 in Italian families [7; 36]. No gene has been yet identified. It seems likely that DYT6, DYT7 and DYT13 account for only a small proportion of focal adult primary torsion dystonia as several families with clinically similar phenotype have been excluded by linkage analysis from these three loci, indicating larger genetic heterogeneity and demonstrating that the search for new genes is not over [37-43].

Linking phenotype with genotype is of major interest in genetics. Identifying the genomic variations that cause human disease remains difficult since investigations like mutagenesis or crosses are unavailable. In addition, many disease phenotypes may be heterogeneous, difficult to ascertain or influenced by environmental factors. In this article we will discuss about the strategies that can be used to identify new genes involved in diseases. We will take primary dystonia as an example and will approach the strategic difficulties raised by these disorders.

POSSIBLE STRATEGIES FOR IDENTIFYING DISEASE GENES IN PRIMARY FOCAL DYSTONIA

I) Identifying disease gene without knowledge on its genomic location...

I.a) ...when the gene product is known

Before 1980, few genes have been identified as disease loci, and they were generally the result of adequate knowledge about the biochemical basis of the disorder. Indeed, when the biochemical basis of an inherited disease is established and purification of the gene product can be achieved, the determination of the sequence of the protein product can lead to the identification of the gene and its chromosomal localization. This strategy is useful in few mendelian traits for which biological basis is known.

Some movement disorders are generally attributed to dysfunction of the basal ganglia, a region located at the base of the brain, which consists of specialized nerve cell clusters involved in the control of movement. Components of the basal ganglia, which are part of the extra-pyramidal system, include the caudate nucleus, the putamen, the globus pallidus (GP), the lentiform nucleus, the substantia nigra and others (Figure 2). Basal ganglia facilitate voluntary movements and inhibit competing movements that might interfere with the desired movement [44]. Dystonia are associated to abnormalities in this brain structure because patients with secondary dystonia exhibit lesions in basal ganglia structures [45]. In addition, for patients refractory to medication, Deep Brain Stimulation (DBS) can treat severe dystonia [46]. In DBS, a surgically implanted, battery-operated medical device (neurostimulator) is used to deliver electrical stimulation to GP internus (GPI), a highly irregular activity within the GPI with grouped discharges separated by periods of pauses being recorded intraoperatively [47]. GPI DBS decreases the overactivation in the contralateral thalamus, putamen, DLPFC and fronto-orbital cortex, thus restoring a more physiological activation pattern [48]. In spite of the success of DBS, relatively little is known about basal ganglia activity in dystonia and pathophysiology of primary dystonia. Biochemical or neurophysiologic abnormalities remain to be identified.
I.b) ...when comparison of gene expression is possible

Generally, genes implicated in a disease may be expressed differently in affected and non-affected people. A way to hunting disease gene is the use of differential gene expression techniques such as differential display [49], subtractive hybridization [50] or DNA microarray [51]. These techniques allow comparison of expression profiles between normal and disease states.

- In differential display (see figure 3), anchored primers are designed to bind to the 5' boundary of the poly-A tails. After reverse transcription, PCR amplification with additional upstream primers of arbitrary sequences is performed. Then, mRNA sub-populations are visualized by denaturing polyacrylamide electrophoresis and can be directly compared by side-by-side between or among different states. PCR bands with specific differences in intensity are isolated from the gel and DNA is submitted to further PCR cycles in order to be analyzed.

Figure 2: The basal ganglia components

Figure 3: Differential display
• In **subtractive hybridization (see figure 4)**, specific nucleic acid sequences are removed from a sample of nucleic acid sequences by specifically hybridizing the sequences to a complementary nucleic acid sequence bound to a target molecule such as biotin. The target molecule is then contacted with a binding partner such as avidin and separated from the sample of nucleic acid sequences. As the target is separated from the sample, the hybridized nucleic acid sequences are also removed from the sample. Subtracted nucleic acid sequences correspond to overrepresented or specifically transcribed species in the studied sample.

![Figure 4: subtractive hybridization](image)

• **DNA microarray**. Comparative hybridization experiments involve isolation of mRNA from the two samples to compare. After reverse transcription, mRNA are labeled with distinct fluorescent tag in each sample. The two pools of labeled RNA are then mixed, hybridized to the DNA microarray containing a full set of thousands or tens of thousands of DNA sequences based on genomic or cDNA sequences, and washed. The microarray is scanned using a specialized fluoimager, and the color of each spot is determined. Genes expressed only in sample 1 would have red color, genes expressed only in sample 2 would be green and genes expressed equally in both samples would be yellow. This allows
determining genes that are differently expressed in disease.

**Figure 5: DNA microarray**

Generally, these techniques do not allow the isolation of the disease gene directly, but lead to produce collections of sequences that may be candidate for a disease because of the modification of their pattern of expression. These techniques can be very useful when the tissue of interest is available. In focal dystonia, this type of techniques could give relevant results but the unavailability of brain tissues from patients and from healthy individuals is the major limitation in these investigations.

**1.c) ... when its function is known: the candidate gene approach**

The candidate gene approach relies on prior molecular knowledge about the disease pathogenesis. Genes with a known or proposed function with the potential to influence the disease appearance are investigated for a direct role in the disease. Genes identified in similar disease can be clues in the choice of candidate gene. Involvement in same pathways or interactions between these genes can be suggested. Furthermore, one important criterion for selecting candidate genes is their expression pattern that should be consistent with the disease phenotype. Indeed, these genes should be expressed at least at the time and in organs where the pathology is observed. Data accumulated about the transcriptome using SAGE or DNA microarrays are generally integrated in databases and, thus, available to the scientific community (see table 2).
Table 2: Different data sources

<table>
<thead>
<tr>
<th>Names</th>
<th>URL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniprot: the Universal Protein Knowledgebase</td>
<td><a href="http://www.ebi.uniprot.org">http://www.ebi.uniprot.org</a></td>
</tr>
<tr>
<td>Ensembl GeneView</td>
<td><a href="http://www.ensembl.org">http://www.ensembl.org</a></td>
</tr>
<tr>
<td>UCSC Genome Bioinformatics</td>
<td><a href="http://genome.cse.ucsc.edu/cgi-bin/hgGateway">http://genome.cse.ucsc.edu/cgi-bin/hgGateway</a></td>
</tr>
<tr>
<td>GENATLAS</td>
<td><a href="http://www.genatlas.org/">http://www.genatlas.org/</a></td>
</tr>
<tr>
<td>KEGG PATHWAY Database</td>
<td><a href="http://www.genome.jp/kegg/pathway.html">http://www.genome.jp/kegg/pathway.html</a></td>
</tr>
<tr>
<td>BIOCARTA</td>
<td><a href="http://www.biocarta.com/genes/index.asp">http://www.biocarta.com/genes/index.asp</a></td>
</tr>
<tr>
<td>Metabolic Pathways of Biochemistry</td>
<td><a href="http://www.gwu.edu/~mpb/">http://www.gwu.edu/~mpb/</a></td>
</tr>
</tbody>
</table>

To date, little is known about pathogenesis of dystonia, while causative genetic alteration of several genes has been demonstrated in different dystonia types (see Table 3).

Table 3: Genes identified in dystonia

<table>
<thead>
<tr>
<th>Dystonia type</th>
<th>Gene implicated</th>
<th>Potential functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>DYTI</td>
<td>TORIA</td>
<td>Chaperone protein, neuroprotection activity against oxidative stress, prevents the protein aggregation formation, protein folding.</td>
</tr>
<tr>
<td>DYTS</td>
<td>GCHI</td>
<td>Dopamine biosynthesis pathway</td>
</tr>
<tr>
<td>DYTS</td>
<td>TH</td>
<td>Dopamine biosynthesis pathway</td>
</tr>
<tr>
<td>DYTI11</td>
<td>SGCE</td>
<td>Dystrophin-glycoprotein complex, neuronal architecture, cytoskeleton</td>
</tr>
<tr>
<td>DYTS2</td>
<td>ATPA3</td>
<td>Ion pumps, ATPases, ion channels or carriers</td>
</tr>
<tr>
<td>DYTS3</td>
<td>MR-1</td>
<td>Stress response pathway, detoxification</td>
</tr>
</tbody>
</table>

The TORIA gene implicated in generalized dystonia (DYTI), encodes the TorsinA protein, which shares close sequence homology to members of the Clp protease/heat shock protein family, which belong to the AAA+ (ATPases Associated with a variety of cellular Activities) superfamily. Members of this family are known to have chaperone activities [52; 53]. TorsinA has been demonstrated to have chaperone functions, indeed it shows a neuroprotection activity against oxidative stress [54-56] and also prevents the protein aggregation formation [57; 58]. Although, the molecular mechanism underlying dystonia remains poorly known, the results obtained by investigations on TorsinA give clues to select candidate genes implicated in this pathology. Thus, genes implicated in protein folding, and those that participate to stress response pathways are potential candidates.

Other genes have been identified but in non-pure dystonia. Two genes have been implicated in Dopa Responsive Dystonia (DRD). The GCHI gene is mutated in dominant forms [8] while the TH gene is mutated in recessive forms [10]. These two genes encode respectively GTP cyclohydrolase and Tyrosine Hydroxylase, two enzymes involved in the Dopamine biosynthesis pathway. Depletion of dopamine caused by alteration in these genes is thought to be responsible of the symptoms, since treatment with L-dopa improved the clinical features. Even if other dystonia are not responsive to dopamine, disturbance of dopamine release has been suggested as a pathogenic mechanism in generalized dystonia [59]. A mutation in the dopamine D2 receptor has been found in a family with Myoclonic dystonia syndrome but not in others [60], and finally an association with a polymorphism in the D5 receptor gene has been reported in patients with focal dystonia. [61-63]. These results suggest that genes that are implicated in Dopamine biosynthesis pathway can be good candidate to screen.
In Myoclonic Dystonia Syndrome (MDS, DYTI1), the SGCE encoding the e-sarcoglycan protein is mutated [13]. SGCE is a member of the sarcoglycan family, transmembrane components of the dystrophin-glycoprotein complex, which links the cytoskeleton to the extracellular matrix [64]. Alterations of other members of this gene family, expressed mainly in muscles, cause limb-girdle muscular dystrophies [65]. Pathogenesis of MDS is unknown, SGCE is also expressed in brain, and it can be hypothesized that the loss of this gene results in changes in the neuronal architecture that give rise to the abnormal movements and psychiatric disturbances that are observed [13]. Thus factors implicated in neuronal architecture or in cytoskeleton can be candidates to found new genes.

Ion pumps have been also implicated in dystonia, indeed, mutations in the ATP1A3 gene has been reported in Rapid-Onset Dystonia Parkinsonism (DYTI2) [14]. This gene encodes the ATPase α3 subunit, the Na+K+ ATPases (sodium pumps) catalyzing active transport of cations across cell membranes and maintaining ionic gradients through hydrolysis of ATP. ATPase α3 is one of the 3 isoforms of the catalytic subunit α expressed in nervous system [66]. Sodium pumps participate in vital cellular function such as the control of cell volume, the resting membrane potential or the electrical excitability of nerve and muscle. In addition they provide the energy for active transport of various elements like ions, nutrients or neurotransmitters. The connection between sodium pump defect and dystonia remains unclear, but this gene introduces other types of factors to be taken into account like ATPases, ion channels or carriers in the disorder.

Mutations in Myofibrillogenesis regulator-1 (MR-1) cause Paroxysmal Non-Kinesigenic Dyskinesias (PNKD) [22]. The MR-1 is homologous to HydroxyAcylGlutathione Hydrolase (HAGH), a glyoxalase protein involving in a stress response pathway, detoxifying methylglyoxal a normal product of oxidative metabolism [67]. The function of MR-1 is unknown, but its homology with enzyme effective in stress response demonstrates that these pathways could be important for elucidation of dystonia pathogenesis [22; 68]. Considering all pathways that can be investigated to identify dystonia genes (protein folding, stress response pathways, Dopamine biosynthesis pathway, neuronal architecture, cytoskeleton, ATPases, ion channels or carriers, detoxification), the candidate gene approach can be hazardous without localization of the disease gene.

1.d) ... when animal models are available

Disease genes can be successfully identified using animal models. When the animal gene-invalidation resulting phenotype is comparable with a human disease phenotype, the human orthologous of the animal gene may represent a good candidate. Rodent models of dystonia have been reported but the homologous genes, identified in humans, have not been implicated in dystonia or in related disorders [69]. Several animal models, obtained by spontaneous mutations, exhibit movement disorder resembling dystonia in humans.

- The dystonia musculorum mice present a motor disorder parallel to generalized dystonia in humans, however, these mice exhibit numerous neurologic lesions that have not been observed in human dystonic patients. The disorder results from mutations in the Bpaq1 gene of which human orthologous gene is the dystonin gene. No human disease associated with alterations in the dystonin gene has been reported so far [70; 71].
- The mutant dystonic (dt) is a mouse model of generalized dystonia. The mutation follows an autosomal recessive pattern of inheritance. Clinical signs begin to appear 10 days after birth with twisting movement of the neck, paddling motions of the limbs and postural instability [72]. No gene has yet been associated with this recessively inherited
disorder.

- The di^th^ hamster presents a phenotype with close resemblance with paroxysmal dystonia in human, with attacks of twisting movements of the limbs and trunk. No gene has yet been associated with this recessively inherited disorder [73; 74].

- Mice bearing mutations within the calcium channel gene Cacna1a are also models of dystonia, indeed, dystonia is a prominent feature of the tottering and leaner mice motor phenotype. This gene has been associated with several human neurological diseases including ataxia or familial hemiplegic migraine [75; 76].

- The med(J) mouse with mutations in the Senko gene has been proposed as a model of kinesigenic dystonia. Mutation in this gene, induce cerebellar atrophy, ataxia, and mental retardation in humans [77].

For the search of new genes in dystonia, these models may guide in the selection of the kind of gene that may be tested. Nevertheless, animal model data have to be taken carefully since the experiment models are not always representative of the gene function. For TOR1A, the gene implicated in generalized dystonia, several experiments had been performed to obtain a model that presents human disease phenotype. Indeed, knock-out and homozygote knock-in of this gene in mouse cause death 48 hours after birth while a first torsinA knock-down mice do not display any abnormal involuntary movements [78; 79]. Other experiments show that an heterozygote knock-in and a knock-down mice exhibit alterations in their motor behavior like dystonic patients [80; 81].

Several knockout project or RNAi experiments are more and more available in the public domain, via for example the “Jackson laboratory” for mouse, “Wormbase” for Caenorhabditis elegans or “Flybase” for Drosophila melanogaster.

<table>
<thead>
<tr>
<th>Table 4: Knockout and RNAi experiment Databases</th>
<th></th>
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<tr>
<td>Species</td>
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</tr>
<tr>
<td>Caenorhabditis elegans</td>
<td>Wormbase</td>
<td><a href="http://www.wormbase.org/">http://www.wormbase.org/</a></td>
</tr>
<tr>
<td>Drosophila melanogaster</td>
<td>Flybase</td>
<td><a href="http://flybase.bio.indiana.edu/">http://flybase.bio.indiana.edu/</a></td>
</tr>
</tbody>
</table>

Wormbase, for example, presents for each gene extended data of RNAi investigations. The results are given with grossly observed phenotypes like “growth abnormal” (Gro), “larval arrest” (Lya), “organism morphology abnormal” (Bmd), “life span abnormal” (Age), “locomotion abnormal” (Unc) etc… This last phenotype ‘uncoordinated’ is relevant for the search of gene implicated in dystonia but corresponds to 1,371 mutants.

Another interest of animal models is to use results of specific genome-wide RNAi screen. For example, a genome-wide RNAi screen has been performed to identify genes that, when suppressed, resulted in the premature appearance of protein aggregates. This study has identified 186 genes that protect cells against the formation of protein aggregates [82]. These results are relevant taking into account the chaperon activity of torsinA that has been demonstrated in part with a C. elegans model.

II) Identify disease gene starting from its genome location

In the 1980s, the recombinant DNA technology allowed the development of tools supporting a new approach in identifying disease genes called the positional cloning. With the
availability of genetic maps constructed with polymorphic DNA markers, (RFLP, microsatellites and SNPs), the advent of the PCR and more recently the sequence of the entire human genome, the number of identified genes in disease increased much.

II.a) Searching for the chromosomal region

The starting point in positional cloning is to define the candidate region on the genome implicated as tightly as possible. Traditionally, this approach is performed via linkage studies in large families. The method consists in scanning the entire genome of multiple affected members using regularly spaced DNA polymorphisms, commonly microsatellites markers. A very important prerequisite for successful linkage mapping is a secure assessment of the phenotype. Linkage analysis methods rely on the biological phenomenon of recombination of homologous chromosomes. During meiosis homologous chromosomes pair up and exchange material. The probability of a recombination event occurring between loci far apart on a single chromosome is larger than for loci closer together. Hence, alleles at loci near each other are generally inherited (or co-segregate) together, making it possible to follow transmission from generation to generation at unknown loci by using alleles at neighboring loci (i.e. the neighboring locus alleles act as surrogates for the presence of alleles at other loci). Linkage analysis can be divided in two broad statistical tests: parametric (or model-based) and nonparametric (or model-free) methods.

Parametric methods
In parametric methods, a disease model has to be specified with parameters, such as mode of inheritance (recessive, dominant...), degree of penetrance (probability to be symptomatic when carrying the mutation), degree of phenocopies (probability to be symptomatic in the absence of the gene mutation) and disease frequency in the population. The results of a parametric test are often expressed as logarithm of the odds (LOD) score. The odds are the likelihood that linkage exists relative to the likelihood that it does not.

Models used in literature to localize DYT6 [5], DYT7 [35] and DYT13 [7] loci in adult-focal dystonia are described in table 5.

| Table 5: Models used for parametric methods in DYT6, DYT7 and DYT13 |
|---------------------------------|----------------------|----------------------|----------------------|
| Mode of inheritance | Penetrance | Gene frequency | Phenocopy rate |
| DYT6 autosomal dominant | Affected only method* | 0,0001 | 0 |
| DYT7 autosomal dominant | Definitely affected: 1 Possibly affected: 0.8 Unaffected descendants: 0.45 | 0,0003 | 0,001 |
| DYT13 autosomal dominant | 0,40 | 0,0001 | 0 |

* "The affected only method" has been used to localize the DYT6 gene. In this case, only definitely affected members of families are classified as "affected" for linkage and classification into unaffected or possible affected family members ("unknown") is not taken into account. This method is usually used to avoid bias from the inclusion of possibly affected
members or incorrect estimation of penetrance or age at onset. However, this approach weakens the power of analysis to detect linkage.

"Homzygosity mapping": A way to localize gene implicated in autosomal recessive diseases is homzygosity mapping [83]. Inbred pedigrees are highly informative for these studies because affected children of consanguineous parents are likely to have inherited the rare disease gene from the same ancestor. Then, they are likely to be homozygous at the markers tightly linked to the disease locus. In these studies, affected individuals are examined for regions inherited in common from the ancestors that they share.

Nonparametric methods
Conversely, for many common genetic diseases especially complex diseases, the mode of inheritance is unknown. For these cases, a variety of nonparametric methods have been described for linkage analyses that do not require the specification of a particular genetic model. These methods focus only on allele-sharing distribution between relatives that share the phenotype of interest. They determine whether the extent of sharing is greater than the sharing that would be expected. The most common allele-sharing design is the Affected-Sib-Pair (ASP) design. Alleles transmitted are compared with that which expected under no linkage in nuclear families with two or more affected children.

Whereas this nonparametric method has been explored in other dystonia, like Paroxysmal dyskinesias, and given that families large enough for a wide genome search are difficult to find, no model free method has been tested in primary adult-onset dystonia. In a recent study, Defazio et al examined the first-degree relatives of 76 primary adult-onset dystonia patients to assess the feasibility of model-free nonparametric methods [84]. They concluded that ASP methods necessitate the screening of a very large number of probands and their families. Thus, this approach may only be performed with the recruitment of families in large collaborative studies necessitating considerable time and money.

II.b) Refining the chromosomal region

New families linked to a locus previously identified can help to refine the region identifying new recombinant haplotypes and providing new subjects to be tested for candidate genes. Moreover, Linkage disequilibrium (LD), association at the population level of a particular marker allele with a disease can be used to narrow down a candidate region that is initially defined by standard parametric linkage analysis. LD is based on allele sharing from a common (typically older) founder ancestor. In practice, alleles of affected individuals and controls of the same population are compared. The regions of shared DNA among mutation carriers have been shortened by a series of historic recombination events, which lead to significant narrowing of the region in which the disease mutation must lie. DYT1, the gene causing early onset torsion dystonia, has been localized finely with LD [85].

II.c) Informations from pathological chromosomal rearrangements

Karyotype analysis of unusual patients can also help to identify the implicated genes. Chromosomal abnormalities can help to identify the potential chromosomal regions that are implicated. Further analyses are necessary to define the precise localization of the breakpoints and identify the genes spanning this region. It is possible also to have an access to a catalogue of chromosome aberrations in man which gives the phenotypic summaries for every specific reported imbalance [86]. The abnormalities are ordered first by chromosome number, secondly by the type of unbalanced rearrangement
(monosomies, deletions, duplications, trisomies), and thirdly according to the chromosomal segment ordered from centromere to telomere.

II.d) Defining “positional” candidate genes

Theoretically, in positional cloning, all genes localized in the region have to be tested to identify a mutation. However, regions highlighted are often so large that a sorting of genes localized in these regions is performed. If it is true that data are rarely enough precise to identify a gene only on its function as in candidate gene strategy aforementioned (I. c), they can be very useful for the selection of candidate gene after fine chromosomal localization. All genes expressed in the brain that can be related with genes already identified in this type of disorder are relevant candidate genes. Nevertheless, the little knowledge about the pathogenesis of the disease makes the selection difficult.

REFERENCES