De novo mutations (GAG deletion) in the DYT1 gene in two non-Jewish patients with early-onset dystonia

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The DYT1 gene recently has been cloned and shown to contain a three nucleotide (GAG) deletion responsible for most cases of autosomal dominant early-onset torsion dystonia. This deletion results in the loss of one of a pair of glutamic acids in a conserved region of a novel ATP-binding protein (torsinA). Previous haplotype analysis revealed that this same deletion had arisen at least two different times in history, suggesting independent mutational events. This deletion is the only sequence change found thus far to be associated uniquely with the disease status, regardless of ethnic origin. Here we describe two patients with typical early-onset torsion dystonia of Swiss–Mennonite and non-Jewish Russian origin, respectively, that both carry this same mutation as a de novo GAG deletion. This finding proves that this 3 bp deletion in the DYT1 gene is indeed a mutation that causes early-onset dystonia. The DYT1 mutation is one of the rare examples of the same recurrent mutation causing a dominantly inherited condition. The sequence surrounding the GAG deletion contains an imperfect 24 bp tandem repeat, suggesting a possible mechanism for the high frequency of this mutation.

INTRODUCTION

Idiopathic torsion dystonia (ITD) is characterized by involuntary twisting, repetitive movements and abnormal postures in the absence of other neurological symptoms or secondary causes (1). Early-onset ITD usually begins in childhood, most commonly starts in a limb and frequently generalizes to other body parts as the disease progresses (2). It follows a pattern of autosomal dominant inheritance with low penetrance of ~30%, including both mild and severe cases (3,4). Early-onset ITD has been estimated to occur at a frequency of 1/160 000 in the general population (5) but is ~5–10 times more prevalent among Ashkenazi Jews (6,7). This may, however, still be an underestimate; based on a recent study in the New York Metropolitan area, the frequency of the GAG deletion in the early-onset ITD gene, DYT1, in Ashkenazim has been calculated to be as high as 1/6000 to 1/2000, or up to 1/6000 affected (8). In a large proportion of Ashkenazi Jewish patients with early-onset dystonia, linkage disequilibrium between the DYT1 gene and a particular haplotype of alleles on chromosome 9q34 implied a founder mutation in this population (9) which probably arose ~350 years ago in Byelorussia or Lithuania (8). Linkage analysis in non-Jewish families with a similar syndrome placed the gene in the same region on chromosome 9q34 as in the Ashkenazi Jewish patients, but all of these families had different haplotypes from the Ashkenazi Jews and from each other (10,11). However, the recent cloning of the DYT1 gene revealed that a single mutation is responsible for the majority of cases of early-onset ITD, regardless of ethnic origin (12). Gene carriers are heterozygous for a unique 3 bp (GAG) deletion in the DYT1 gene which results in the loss of one of a pair of glutamic acid residues in the C-terminal domain of a novel ATP-binding protein (torsinA) (12). Previously, we showed that families with the GAG deletion possessed two different haplotypes within 5 kb of this deletion, suggesting that multiple independent events have given rise to the deletion (12). In support of this notion (i.e. the same mutation arose independently), we here present haplotype analysis and mutation status in two families of non-Jewish origin in which affected individuals manifested a de novo GAG deletion in the DYT1 gene.

RESULTS

Clinical evaluation

Patient 1 was the first to be affected with dystonia in her family. She was a 43-year-old, non-Jewish female patient of Russian
Molecular analysis

Haplotype analysis in family 1 shows that individuals II-2 and III-1 have inherited the disease-bearing chromosome from their unaffected mother/grandmother (I-1). The sister (II-3) of the index patient (II-2) has also inherited the same chromosome from her mother (I-1) without being affected. Both individuals I-1 and II-3 do not carry the GAG deletion which has occurred spontaneously in the index patient (II-2) and has been passed on to her daughter (III-1). Markers typed in a 1 Mb region of chromosome 9q34 and order on the chromosome are: cen–D9S62a, D9S62b, D9S2158, D9S2159, D9S2160, D9S2161, D9S863, D9S2162, D9S2163, ASS–tel (for individual distances see ref. 15).

Figure 1. Russian family with de novo GAG deletion. All four individuals share the same boxed chromosome but only the two affected family members (II-2, III-1) carry the GAG deletion (in these individuals the two markers flanking the GAG deletion are indicated by *). The deletion has occurred spontaneously in individual II-2 and has been passed on to her daughter (III-1). Markers typed in a 1 Mb region of chromosome 9q34 and order on the chromosome are: cen–D9S62a, D9S62b, D9S2158, D9S2159, D9S2160, D9S2161, D9S863, D9S2162, D9S2163, ASS–tel (for individual distances see ref. 15).

Figure 2. Mennonite family with de novo GAG deletion. (a) Pedigree of a family with typical early-onset dystonia. Individual II-1 is affected; both parents (I-1 and I-2) are unaffected. (b) Analysis of PCR products in the DYT1 region of individual II-1 reveals two distinct bands (247 and 250 bp, respectively), characteristic of the GAG-deleted and normal alleles. Both parents (I-1 and I-2) show the normal, single 250 bp fragment. (c) Autoradiograph of a sequencing gel demonstrating the GAG deletion on one allele in the patient (II-1) and the normal sequence on both alleles in his parents (I-1 and I-2).

DISCUSSION

Both index patients as well as the daughter of patient 1 presented with typical early-onset ITD (childhood-onset of dystonia in a limb with subsequent generalization), thus displaying the phenotype of the DYT1 founder mutation in the Ashkenazi Jewish population (2). Prior to the detection of the GAG deletion in their DYT1 genes, however, patient 2 and, initially (before manifestation of the disease in her daughter), also patient 1 were considered to have sporadic and ‘non-genetic’ dystonia, although secondary causes could be excluded in both. This supports our prior assertion that apparently non-genetic cases of typical early-onset ITD can indeed be genetic (9), due to the GAG deletion in the DYT1 gene. However, presumed non-genetic cases could be the result of either de novo mutations or the low penetrance of the mutant gene. In both of our patients, it is clear that their sporadic dystonia is due to a de novo GAG deletion in the DYT1 gene.

Although the haplotype presented for family 1 (Fig. 1) is the most likely, it could be argued that the mutation is inherited from the deceased (untyped) grandfather (individual I-2) rather than from the grandmother (I-1). Based on the haplotype data, however, patient III-1 definitely inherited her maternal grandmother’s allele at the DYT1 gene-flanking loci D9S62b and D9S863. Therefore, if the mutation had been inherited from the grandfather (individual I-2), a double crossover or gene conversion event would have had to occur between these markers. As the physical distance between D9S62b and D9S863 is ~350 kb and we have not observed recombinations between these markers (> 800 meioses) (8,14,15), this explanation seems improbable. Moreover, individual II-2 (sister of the index patient) is genetically identical to her affected sister at all nine loci from D9S62b parents (I-1 and I-2) showed the normal, single 250 bp fragment (Fig. 2). Sequence analysis demonstrated the GAG deletion on one allele in the patient (II-1), whereas his parents showed the normal sequence on both alleles (Fig. 2). Marker analysis of the patient, his parents and his two unaffected siblings using 11 highly polymorphic markers (average heterozygosity 0.83) on seven different chromosomes was consistent with appropriate inheritance (data not shown).
phenotype associated with the DYT6 relative (seventh degree) of one of the two families carrying the patient 2 is also of Mennonite origin and, in fact, even a distant origin, is thought to be inherited from a common ancestor (13). Our been mapped to chromosome 8 in two families of Mennonite background is not necessarily always due to the same mutation. 

The surprising finding of the same mutation causing dystonia in most cases with typical early onset of the disease, no matter what the ethnic origin, could be explained in two ways: either identical mutations have arisen repeatedly or all cases have inherited the exact same mutation from a common ancestor of ancient lineage (12). Based on epidemiological and haplotype data, the former possibility appears most likely as polymorphisms immediately surrounding the GAG deletion are different in affected individuals of different ethnic origin (12) (N.J. Risch, personal communication and unpublished data). The detection of de novo mutations in two patients of different ethnic origins (non-Jewish Russian and non-Jewish Mennonite Swiss) also clearly proves that the GAG deletion can arise repeatedly and independently in various populations and causes early-onset dystonia.

In order to determine the accurate frequency of new mutations in the DYT1 gene, parents of apparently sporadic patients need to be assessed for the GAG deletion. It is interesting to note that a gene for a mixed dystonia phenotype (DYT6), which recently has been mapped to chromosome 8 in two families of Mennonite origin, is thought to be inherited from a common ancestor (13). Our patient 2 is also of Mennonite origin and, in fact, even a distant relative (seventh degree) of one of the two families carrying the DYT6 gene. His dystonia, however, more closely resembled the phenotype associated with the DYT1 mutation rather than the mixed form of dystonia characteristic for the DYT6 gene, and thus he was tested for the GAG deletion in DYT1. This illustrates that dystonia in patients sharing the same ethnic or even familial background is not necessarily always due to the same mutation.

DYT1 is one of the few genes in which the same recurrent mutation causes a dominantly inherited disease (12). Other examples include hypokalemic periodic paralysis (16), achondroplasia (17) and hypertrophic cardiomyopathy (18). The occurrence of similar or identical mutations is remarkable and most likely due to predilection for mutagenesis at certain ‘hotspots’ of the DNA sequence and/or a distinct phenotype caused only by particular mutations (18). The mutation in the achondroplasia gene and most mutations in the hypertrophic cardiomyopathy gene are single base pair changes that take place at CpG dinucleotides (17,18) which are known to be associated with increased mutation rates (19). However, the reason for the extremely high new mutation rate at the FGF3 1138 nucleotide in the achondroplasia gene—which renders it the most mutable single nucleotide reported in the entire human genome—remains unclear (17). In all these diseases, it appears that only one or a few types of mutations in the target gene produce a distinct clinical phenotype. Other mutations in these genes are either silent, result in a different phenotypes or are lethal (12,16–18).

Thus far, the GAG deletion in the DYT1 gene is the only mutation we have found in early-onset dystonia patients. This suggests that there is an increased occurrence of this mutation as compared with other types of mutations in this gene. A possible explanation for the high frequency of this particular mutation in the DYT1 gene is suggested by the unusual sequence adjacent to the GAG deletion. The GAG dimer occurs in an imperfect 24 bp tandem repeat bearing a (T)5-(C)4-(A)3 sequence. Direct alignment of these repeats shows 54% identity, whereas deletion of one of the pair of GAGs gives 65% identity (Fig. 3). Therefore, it seems possible that this increased homology occasionally may cause slippage of RNA primers or Okazaki fragments during DNA replication of the lagging strand, resulting in a deletion, as has been reported during replication of trinucelotide repeats (20).

MATERIALS AND METHODS

Patient ascertainment and clinical evaluation

Patient I was one of 37 patients with classical early-onset ITD (17 of Jewish and 20 of non-Jewish origin) identified through the Neurogenetic Department of the Institute of Neurology in Moscow, Russia, as part of the project ‘Dystonia in Russia’ by the Russian–American NeuroGenetics (RANG) Group. Patient 2 of Mennonite USA background was acquired as part of our genetics research program for evaluation of the GAG deletion in the DYT1 gene.

After giving informed consent, all participants underwent a standardized neurological examination. The diagnosis of early-onset ITD was established according to current criteria (1) and previously published protocols (3,21), including videotaped examinations reviewed by neurologists trained in movement disorders who were blinded to patient status or relationship.
Identification of the GAG deletion

DNA from available family members was extracted from whole blood following standard protocols. We used published primers, 6418 and 6419 (12), for PCR amplification across the critical region of the DYT1 gene. PCR products were resolved in a denaturing 6% polyacrylamide gel and visualized by silver staining (22).

Sequence analysis

Dideoxy cycle sequencing was performed with the Perkin-Elmer AmpliSequence Sequencing Kit (Perkin-Elmer) using the specific primer 6419 (12) labeled with [α-33P]dATP (2000 Ci/mmol; NEN). Direct cycle sequencing (step 1, 95°C 2 min; step 2, 60°C 1 min; step 3, 60°C 1 min; step 4, 72°C 1 min; cycle steps 2–4 x25; step 5, 4°C 5 min) was performed after enzymatic clean-up with exonuclease I and shrimp alkaline phosphatase (USB) for 15 min at 37°C and 15 min at 85°C.

Haplotype analysis

To establish haplotypes in family 1, several chromosome 9 markers surrounding the GAG deletion were tested (methods according to Research Genetics—http://www.resgen.com and ref. 15; marker heterozygosities given in parentheses): D9S62a (0.44), D9S926 (0.85), D9S2158 (0.78), D9S2159 (0.67), D9S2160 (0.75), D9S2161 (0.79), D9S63 (0.89), D9S2162 (0.76), D9S2163 (0.81) and ASS (0.85). In family 2, a panel of highly polymorphic DNA markers (average heterozygosity 0.83) representing seven different chromosomes was used to verify appropriate inheritance: D1S158 (0.84), D4S1627 (0.81), D7S1808 (0.78), D8S1113 (0.81), D8S1119 (0.80), D9S2161 (0.79), D9S63 (0.89), ASS (0.92), D11S1985 (0.87), D11S4464 (0.78) and D15S817 (0.79). PCR products were analyzed on a LICOR automated sequencer (LICOR) or detected by silver staining (22).

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ABBREVIATIONS

DYT1 gene, gene for early-onset torsion dystonia; DYT6 gene, gene for a mixed dystonia phenotype; ITD, idiopathic torsion dystonia.

REFERENCES