# REPORT

# Intragenic *Cis* and *Trans* Modification of Genetic Susceptibility in *DYT1* Torsion Dystonia

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A GAG deletion in the *DYT1* gene is a major cause of early-onset dystonia, but clinical disease expression occurs in only 30% of mutation carriers. To gain insight into genetic factors that may influence penetrance, we evaluated three *DYT1* single-nucleotide polymorphisms, including D216H, a coding-sequence variation that moderates the effects of the *DYT1* GAG deletion in cellular models. We tested *DYT1* GAG-deletion carriers with ($n$ = 119) and without ($n$ = 113) clinical signs of dystonia and control individuals ($n$ = 197) and found the frequency of the 216H allele to be increased in GAG-deletion carriers without dystonia and to be decreased in carriers with dystonia, compared with the control individuals. Analysis of haplotypes demonstrated a highly protective effect of the H allele in *trans* with the GAG deletion; there was also suggestive evidence that the D216 allele in *cis* is required for the disease to be penetrant. Our findings establish, for the first time, a clinically relevant gene modifier of DYT1.

Primary torsion dystonia (PTD [MIM #128100]) is a genetically and clinically heterogeneous disorder, encompassing several clinical subtypes and six designated gene loci (*DYT1, DYT2, DYT4, DYT6, DYT7,* and *DYT13*).[1] Only one of these genes, *DYT1,* mapping to chromosome 9q34, has been identified.[2] *DYT1* is a major cause of childhood- and adolescent-onset PTD, especially among Ashkenazi Jews (AJs), in whom it accounts for 80%–90% of early-onset cases.[3,4] A single heterozygous and recurring mutation in the encoded protein, torsinA, accounts for all reported *DYT1*-associated PTD.[5] The mutation is an in-frame deletion of three nucleotides (GAG) in exon 5, which results in the loss of a glutamic acid residue at position 302 or 303 in the 332-aa protein. This same mutation has arisen independently in different families, although among AJs, there is an associated haplotype of alleles implicating a founder mutation.[2,6]

The clinical manifestations of the *DYT1* GAG deletion are broad, ranging from mild focal dystonia, usually affecting an arm, to generalized dystonia involving limbs, axial, and even cranial muscles.[4,7] Penetrance is estimated at only 30%; thus, most mutation carriers are clinically normal, or at least not affected with overt signs of dystonia.[8,9] To date, no studies have shed light on factors that may contribute to the markedly reduced penetrance and variable expression of the *DYT1* GAG deletion. More progress has been made in the understanding of torsinA and the effects of the *DYT1* GAG deletion. Several lines of evidence derived from both cellular and animal models suggest roles for torsinA in regulating nuclear envelope and endoplasmic reticulum (ER) organization.[10,11,12] It is postulated that the GAG deletion results in a distortion in torsinA structure[13]; this structural change may then underlie the mutant's tendency to produce ER-derived inclusions in cell culture[11,14] and its proposed loss of function.[12,15]

Aside from the GAG deletion, three other variations in torsinA have been found that change the amino acid sequence: an 18-bp deletion,[16] a 4-bp deletion,[5] and a SNP in the coding sequence for residue 216 that encodes aspartic acid (D) in 88% and histidine (H) in 12% of control-population alleles.[2,16] In addition, a synonymous polymorphic coding-sequence change (A82A) and several SNPs in the 5′ and 3′ UTR regions, as well as a single-base-pair deletion in the 3′ UTR (G-del), have been noted. None has been unequivocally associated with disease, although several SNPs in the 3′ UTR have been associated with focal dystonias.[17,18] A recent study, however, has demonstrated functional significance of the D216H SNP. Cells overexpressing torsinA with the H allele developed inclusions similar to those observed in cells overexpressing GAG-deleted torsinA. Further, coexpression of both 216H- and GAG-deleted torsinA *reduced* its tendency to form inclusions, implying the two changes together have a canceling effect.[13] These findings suggest that the D216H polymorphism may have a role in human dystonia, possibly influencing susceptibility to non-*DYT1* dystonia and modifying penetrance of *DYT1* dystonia.

To assess the possibility that the D216H as well as other SNPs in the *DYT1* gene influence penetrance, we investigated families with the *DYT1* gene, reported elsewhere, comparing family members with the GAG deletion who have dystonia (i.e., "manifesting" carriers, or MC) with family members with the deletion but without dystonia (i.e., nonmanifesting carriers, or NMC). For example, if the 216H variant reduces the pathogenicity of the GAG

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deletion, we would expect the frequency of the 216H allele to be decreased in the MC and increased in the NMC.

In our analyses, we specifically looked for both *trans* and *cis* effects. For a *cis* effect, we examined the chromosomes carrying the GAG deletion in each family. Thus, each independent family contributed one chromosome (and hence one allele for each SNP) to the analysis. To assure independence of the GAG deletion–bearing chromosomes across families, we examined haplotypes on the basis of microsatellite markers in each family, to identify GAG-bearing chromosomes with a unique origin. To search for *trans* effects, we examined alleles for each polymorphism on the chromosomes without the GAG deletion in mutation carriers. These alleles are inherited from the parent not carrying the GAG deletion.

DYT1 *and control subjects*.—Subjects were recruited from families participating in previous genetic studies of dystonia and were included in the present study if they were found to harbor the *DYT1* GAG deletion.[9,19] Control samples included unrelated noncarriers from DYT1-affected families, as well as AJ parents from dysautonomic families and CEPH controls. The study was approved by institutional review boards, and all subjects gave informed consent to participate. The methods for recruitment, ethnic determination, and diagnosis have been described elsewhere.[9,19,20] For analysis of *trans* effects, we included 148 AJ subjects with the GAG deletion (75 MC and 73 NMC) from 100 nuclear families and 104 AJ controls, as well as 84 non-Jewish (NJ) subjects with the GAG deletion (44 MC and 40 NMC) from 41 nuclear families and 93 NJ controls. For both AJ and NJ, controls were unrelated individuals with no personal or family history of dystonia.

*Molecular and statistical analyses*.—DNA was extracted from white-blood or buccal cells by use of the Purgene procedure (Gentra Systems). Three SNPs in the *DYT1* gene were selected on the basis of functional significance and heterozygosity. These included a synonymous alanine→alanine change at position 82 (A82A, *rs2296793*), a substitution of aspartic acid (D) for histidine (H) at position 216 (D216H, *rs1801968*), and a deletion of a single nucleotide G in the 3′ UTR ($G^-$ versus $G^+$ for the normal allele, *rs3842225*) (fig. 1). The SNPs were amplified by PCR with primers and conditions listed in table 1. The primers used for amplification and sequencing were designed using PSQ version 1.0.6 software (Biotage). Each amplification set contained a biotin-labeled primer. SNP genotyping was performed using a Pyrosequencing PSQ HS 96A system 1.2 (Biotage).

Five microsatellite markers spanning the *DYT1* gene—*D9S159, D9S2160, D9S2161, D9S63,* and *D9S2162*—were amplified using primers and conditions reported elsewhere.[21] All primer sets contain a fluorescent tag at the 5′ end of the sense primer. The amplified PCR products were resolved on the ABI 3730 automated DNA sequencer, and the results were analyzed using the GeneMapper 3.5 software (Applied Biosystems).

For the statistical analysis of *trans* effects, we compared

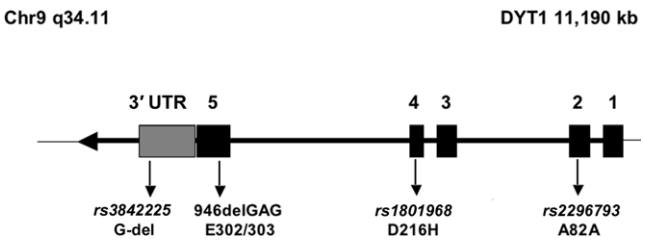


**Figure 1.** Schematic representation of the *DYT1* gene showing the position of the GAG-deletion mutation and the SNPs examined in the study.

allele frequencies or haplotype frequencies between MC and NMC on chromosomes inherited from the parent without the GAG deletion. Haplotypes were determined by family inheritance. We included all carriers in our analyses, including multiple siblings from the same nuclear family. The allele frequencies in such individuals are not independent, because of shared parentage. We adjusted for this relatedness in our analyses, as described below. Specifically, among the AJ families, there were 62 singleton carriers, 30 sibships with 2 carriers, 6 sibships with 3 carriers, and 2 sibships with 4 carriers. Among NJ families, there were 19 singleton carriers, 10 sibships with 2 carriers, 7 sibships with 3 carriers, 3 sibships with 4 carriers, and 2 sibships with 6 carriers.

Siblings are correlated in their allele frequencies because of shared inheritance from the non-GAG parent, whereas individuals from different sibships within the same pedigree are uncorrelated because they have inherited their non-GAG chromosome from unrelated individuals. To assess statistical significance, allele frequencies were compared between the MC and NMC. However, instead of doing a standard $\chi^2$ test treating everyone as independent, we allowed for the correlation of siblings in the analysis and adjusted the variance of the difference accordingly.

Specifically, let $p'$ be the observed allele (or haplotype) frequency in $n$ MCs and $q'$ be the frequency in $m$ NMCs. Let $T = p' - q'$. Our statistic is based on $T^2/\mathrm{Var}(T)$, where $\mathrm{Var}(T)$ is the estimated variance of $T$. Because, in the calculation of $T$, affected siblings from the same family are positively correlated and unaffected siblings from the same family are also positively correlated, we need to add additional covariance terms in the variance formula for $T$ because of these pairs. By contrast, affected-unaffected carrier sib pairs from the same family induce a negative correlation contributing to $\mathrm{Var}(T)$; thus, we need to subtract additional covariance terms for the variance of $T$ for these pairs. Specifically, for the variance of T, we employ the formula

$$\mathrm{Var}(T) = p'q'\left(\frac{1}{m}+\frac{1}{n}+\frac{I}{2n^2}+\frac{J}{2m^2}-\frac{K}{2mn}\right),$$

where $I$ is the number of concordant affected carrier sib

**Table 1. SNPs Genotyped in the *DYT1* Gene**

| SNP | Gene Position | DNA Change | Amino Acid Change | Primer Sequence (5′→3′) Forward | Reverse | Pyrosequencing | Fragment Size (bp) | PCR Temperature (C°) | $Mg^{+}2$ (mM) |
|---|---|---|---|---|---|---|---|---|---|
| *rs2296793* | Exon 2 | A→G | A82A | CGAAATTTTTGCCGGTGCC | GACGGCTTTGCTGGCTTTCTTAT[a] | TTGTTTATGAAACCAAACAC | 244 | 62 | 1.5 |
| *rs1801968* | Exon 4 | C→G | D216H | AACCCTGTCCTTACCCACTG[a] | TCTGCTTTCCACTCCTCCAG | GAAATCCAAAGCCACA | 97 | 57 | 2.5 |
| *rs3842225* | 3′ UTR | G-del | | AACTTGTCACTTGCCAGTCACTCC[a] | GAATCCAGCAGAGAGCACGTGT | AGAGAGCACGTGTGG | 89 | 62 | 1.5 |

[a] 5′ Biotinylated primer.

pairs, *J* is the number of concordant unaffected carrier sib pairs, and *K* is the number of discordant affected-unaffected carrier pairs.

For all analyses, there were no differences between the AJ and NJ samples in terms of allele or haplotype frequencies; thus, all results are presented for these two groups combined. We assumed an asymptotic $\chi^2$ distribution for $T^2/\mathrm{Var}(T)$.

Cis *and* Trans *Frequencies of* DYT1 *SNPs in affected and unaffected carriers of the GAG deletion.*—We observed complete linkage disequilibrium (LD) of the A82A SNP with the G deletion polymorphism ($G^+/G^-$), so we considered further only the deletion polymorphism, reducing the analyses to two SNPs: the G-del and the D216H. These two were also in LD but not complete LD. There were three haplotypes that occurred with these two SNPs: D216/$G^+$, D216/$G^-$, and 216H/$G^+$; the 216H/$G^-$ haplotype did not occur.

As can be observed in the analyses of non-GAG chromosomes (table 2), the 216H allele was increased in frequency in the NMC and was decreased in frequency in the MC compared with the controls. The allele-frequency difference between the NMC and MC is highly significant ($\chi^2 = 22.55$; $P < .000002$). By contrast, there was no difference in allele frequency for the $G^-$ allele among the MC, NMC, and NC groups.

In the analysis of haplotypes, the 216H/$G^+$ haplotype was increased in frequency, whereas the D216/$G^+$ haplotype was decreased in frequency in the NMC versus MC. The D216/$G^-$ haplotype had similar frequency in the two groups. This might suggest that the D216 allele in *cis* with $G^+$ is more predisposing than when the D216 allele is in *cis* with $G^-$. If the 3′ G deletion or another SNP that is in LD with the G deletion leads to a reduction in expression levels of the protein encoded in *cis,* then it could be that having the $G^-$ allele in *cis* with D216 is associated with reduced expression levels of the D216 allele, leading to a lower level of risk. However, there was not a statistically significant difference between the D216/$G^+$ and D216/$G^-$ haplotype frequencies ($\chi^2 = 1.02$; $P > .3$). It is clear that the principal effect is the large and opposite trend for the D216/$G^+$ and 216H/$G^+$ haplotypes, both of which carry the normal allele at G-del, suggesting that the primary impact on risk is due to decreased susceptibility associated with the 216H allele.

Table 3 provides the results of analyses of the GAG-deletion chromosomes that were deemed to be independently inherited on the basis of microsatellite haplotypes and ethnic origins (i.e., those with distinct original founders). One chromosome was unphased and was heterozygous and therefore could be 216H or D216. Of the remaining 21, all were D216. Five of these chromosomes came from subjects whose ethnicity is other than white. The D216 allele is the predominant allele in all populations, including East Asians (frequency = .95), West Africans (frequency = 1.0), and Europeans (frequency = .81) (Gene Cards for TOR1A). However, using the frequency of the D216 allele, in whites (.856), we found that, of 16 chromosomes, we would have expected .856 × 16 = 13.7 to carry the D216 allele and 2.3 to carry the 216H allele. The probability of actually observing no 216H allele is .083. This result is highly suggestive that, in addition to the *trans* effect we observed above with the D216 allele, there may also be a *cis* effect, in which the GAG deletion needs to be carried in conjunction with the D allele to be penetrant.

*The 216H allele in* trans *protects against disease expression.*—We found strong evidence indicating that the D216H polymorphism modifies clinical manifestation of the *DYT1* GAG deletion. We identified the H allele as highly protective; only 2 of 119 affected carriers had the 216H allele, compared with 24 of 113 unaffected carriers. This protective effect occurred in *trans*—that is, on chromosomes inherited from the non-GAG parent. There was also evidence, albeit less compelling, that the D allele in *cis* with the GAG deletion is required for disease to be penetrant.

The protective effect of the 216H allele is powerful. However, its overall contribution to explaining reduced penetrance is modest, because the H allele is uncommon (maximum frequency of 19% in whites). To estimate the allele-specific penetrances for the D216H polymorphism in *trans,* we employ two approaches. Let A denote the status of being affected and N denote unaffected. Let D denote the state of carrying the D216 allele in *trans,* whereas H denotes carrying the 216H allele in *trans*. Our

**Table 2. *DYT1* SNP and Haplotype Frequencies on Non-GAG Chromosomes in MCs, NMCs, and Controls**

| Status | No. of Chromosomes | SNP Frequency 216H | SNP Frequency $G^-$ | Haplotype Frequency D216/$G^+$ | Haplotype Frequency D216/$G^-$ | Haplotype Frequency 216H/$G^+$ |
|---|---|---|---|---|---|---|
| MC | 119 | .017 | .227 | .756 | .227 | .017 |
| NMC | 113 | .212 | .230 | .558 | .230 | .212 |
| NC | 394 | .144 | .236 | .619 | .236 | .144 |

**Table 3. *DYT1* D216H Allele Frequencies on GAG-Deletion Chromosomes from Independent Patients with PTD**

| Race/Ethnicity | No. of Chromosomes | Allele Distribution |
|---|---|---|
| White | 17 | 16 D; 1 H or D |
| Asian | 3 | 3 D |
| African American | 1 | 1 D |
| Mexican | 1 | 1 D |
| All | 22 | 21 D; 1 H or D |

first estimates are based on the allele frequencies observed in the MC and NMC groups (table 2). The penetrance associated with carrying the D216 allele is determined by

$$\frac{P(\mathrm{D}|\mathrm{A})P(\mathrm{A})}{P(\mathrm{D}|\mathrm{A})P(\mathrm{A}) + P(\mathrm{D}|\mathrm{N})P(\mathrm{N})} = \frac{(.983)(.30)}{(.983)(.30) + (.788)(.70)} = .348 ,$$

where $P$ denotes probability and the assumption is that the overall penetrance $P(\mathrm{A})$ for GAG-deletion carriers is 30%,[8,19] $P(\mathrm{D}|\mathrm{A})$ is the frequency of the D216 allele in MC, and $P(\mathrm{D}|\mathrm{N})$ is the frequency of the D216 allele in NMC. Similarly, the penetrance associated with carrying the 216H allele is determined by

$$\frac{P(\mathrm{H}|\mathrm{A})P(\mathrm{A})}{P(\mathrm{H}|\mathrm{A})P(\mathrm{A}) + P(\mathrm{H}|\mathrm{N})P(\mathrm{N})} = \frac{(.017)(.30)}{(.017)(.30) + (.212)(.70)} = .033 .$$

Our second estimates are based on the allele frequencies observed in the MC and NC groups (table 2). Here, the formula for the penetrance associated with carrying the D216 allele is

$$\frac{P(\mathrm{D}|\mathrm{A})P(\mathrm{A})}{P(\mathrm{D})} = \frac{(.983)(.30)}{.856} = .345 ,$$

where $P(\mathrm{D})$ is the frequency of the D216 allele in NC. Similarly, the penetrance associated with carrying the 216H allele is

$$\frac{P(\mathrm{H}|\mathrm{A})P(\mathrm{A})}{P(\mathrm{H})} = \frac{(.017)(.30)}{.144} = .035 ,$$

where $P(\mathrm{H})$ is the frequency of the 216H allele in NC. Thus, the penetrance associated with carrying the D216 allele in *trans* is ~35%, and, for the 216H allele, it is ~3%.

These estimates contrast with 30% penetrance when there is no knowledge of this SNP. Thus, clinical application of our finding in genetic counseling is important but will have a significant impact for only a minority of subjects. It allows assessment of a much-reduced risk in individuals who are 216H carriers and predicts a slightly increased risk for the D216 carriers.

Although originally controversial, systematic family studies have demonstrated early-onset PTD to have a dominant mode of inheritance with reduced penetrance.[8,19] Our finding of an intragenic SNP modifier now alters that conclusion, at least to a minor extent. There is an intragenic interaction between the GAG deletion and the D216H polymorphism in creating risk of disease manifestation. Mode of inheritance still appears dominant, because the permissive D216 allele has high frequency (>.80). If, in fact, the D216 allele were rare, the mode of inheritance would likely appear more recessive, since both the GAG deletion and the D216 allele would need to be inherited for disease expression.

Reports of intragenic *trans* modification in other dominant human diseases are rare. One example is provided by hereditary spastic paraplegia, which is due to spastin gene mutations. In that disorder, although the major effect is due to mutations in the AAA domain of spastin, other amino acid substitutions in the same gene impact the severity of the disease, including age at onset.[22]

The results of the present clinical study are consistent with overexpressing cellular models of GAG-deleted torsinA, where a high frequency (80% of cells) of inclusions is observed with coexpression of the D216 and lower frequency (60%) is observed when combined with the 216H allele.[13] Further, like the GAG deletion, cells overexpressing torsinA with the 216H allele form inclusions, although to a lesser extent than those with the GAG deletion. The reason for these effects is unclear, but the D216H polymorphism is predicted to be exposed on the surface of assembled hexamers. Thus, it may disrupt or alter hexamer subunit-subunit interactions.[13] Further delineation of these interactions, including studies examining *cis* and *trans* effects, should help refine understanding of this change.

We also present suggestive evidence that a deletion SNP in the 3′ UTR may have an influence on *DYT1* penetrance, although statistical significance was lacking. However, it is tempting to speculate that regulatory SNPs that occur in *cis* with either the GAG deletion or the D216 allele have an influence on disease expression; variants decreasing the expression of these alleles would be expected to reduce penetrance, and such was the direction of our results. We also note that, if this is the case, the 3′ UTR G deletion may not be the functional variant, since this allele was in complete LD with another synonymous SNP in the coding region (A82A). Whereas the latter has no effect on the amino acid sequence, it may still influence expression levels of the protein in cells. Or, since the region containing the *DYT1* and *TOR1B* genes shows strong LD, another SNP in this region could be functional.

Our study demonstrates the importance of studying *trans* and *cis* effects of polymorphisms on disease genes in the modification of their penetrance or expression. Although the 216H allele has a potent effect, it explains only a small proportion of the reduced penetrance associated

with carrying the *DYT1* GAG-deletion mutation. Also, almost all manifesting carriers, regardless of severity, were homozygous for the D216 allele, so factors moderating the extent of disease expression in these individuals remain unknown. Additionally, the 216H polymorphism and other *DYT1* variants require additional study in dystonias not due to the *DYT1* GAG deletion. Indeed, there is support for this approach; several studies have implicated common *DYT1* haplotypes in adult focal (non-GAG) dystonias,[17,18,23] although two other reports failed to replicate the findings.[24,25] The D216H SNP was specifically examined in two of the studies, and, in both cases, no associations were identified,[17,23] suggesting that other SNPs in the *DYT1* gene may play a role in focal dystonias.


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### Web Resources

The URLs for data presented herein are as follows:

Gene Cards for TOR1A, http://www.genecards.org/cgi-bin/carddisp.pl?gene=TOR1A

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for PTD)

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