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CAG Nucleotide Repeat Profiles in Persons With Schizophrenia or Schizoaffective Disorders With and Without Tardive Dyskinesia: Pilot Study

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Tardive dyskinesia (TD) is a drug-induced syndrome of involuntary movements often associated with neuroleptic treatment of psychiatric conditions. Huntington's disease (HD) and other neurological conditions are caused by CAG nucleotide repeat expansions in specific genes. We, therefore, explore the hypothesis that TD may be related to CAG repeat expansion by using the repeat expansion detection (RED) method as a measure of CAG content without knowledge of the location of the responsible gene. The number of CAG repeats ($[CAG]_n$) from persons with schizophrenia or schizoaffective disorders with ($n = 10$) and without ($n = 9$) TD are determined. A comparison of $[CAG]_n$ in persons with (56.90 ± 23.45 repeats) and without (57.00 ± 19.35 repeats) TD was not statistically different. The total $[CAG]_n$ was determined by combining $[CAG]_n$ for both groups. The median of 45 repeats was used to divide the total into two groups (SG1 and SG2 with smaller and larger $[CAG]_n$ fragments, respectively) and a means analysis of the two subgroups based on $[CAG]_n$ demonstrated that SG1 ($n = 10$ samples at 45 repeats per sample, mean $[CAG]_n = 45.00 \pm 0.00$) was significantly smaller than SG2 ($n = 9$, ranging from 48 to 120 repeats, mean $= 70.22 \pm 24.83$; $P < 0.005$). Thus, this lends support to the idea of CAG repeat expansions in the study population. Results are encouraging that a larger population and a more structured subject selection process may yield more meaningful information about the relationship between CAG repeat expansion and TD.

KEY WORDS: CAG repeat expansion; tardive dyskinesia; repeat expansion detection

Tardive dyskinesia (TD) is a syndrome of abnormal involuntary movements and is thought to be drug-induced and associated with neuroleptic treatment of psychiatric conditions. The currently accepted hypothesis of TD suggests that exposure to neuroleptic medication exacerbates the expression of excessive motor activity in some persons. There is some indication that the incidence of TD increases with cumulative exposure to these drugs. However, the majority of persons exposed to neuroleptics do not develop involuntary movements, even after substantial cumulative exposure. The prevalence of TD in the psychiatric population is on the order of 25–30%, a proportion consistent with an autosomal recessive phenomenon. This lends support to the idea that TD may be a genetic trait phenomenon as opposed to the state phenomenon previously considered.

The motoric manifestations of severe TD often bear a phenotypic resemblance to those of Huntington's disease (HD). Increased frequency of adventitious movements or other subtle neurological signs, cognitive dysfunction, and psychiatric symptoms are often noted at the time of onset of both clinical conditions. HD, six spinocerebellar ataxias (SCA), Friedreich's ataxia (FA), as well as other neurological conditions have been shown to be caused by the expansion of a CAG nucleotide repeat in specific genes [Zoghbi and Orr, 2000]. In addition, a number of studies have suggested an association between large CAG repeats and schizophrenia [Morris et al., 1995; O'Donovan et al., 1995; Burgess et al., 1998; Ayton et al., 2002]. However, a relationship between CAG repeat profiles, if any, and the involuntary movements associated with TD has not be been established. We hypothesize that as in other movement disorders, TD may be related to CAG nucleotide repeat expansion. We examined the number of CAG repeats in schizophrenic or schizoaffective disorders with and without TD using the repeat expansion detection (RED) method [Schalling et al., 1993].

Nineteen subjects with either schizophrenia or schizoaffective disorders were identified in an outpatient community mental health center. The study was approved by the Institutional Review Board of Robert Wood Johnson Medical School and informed consent was obtained from all subjects prior to their participation in the study. All patients had documented exposure to neuroleptic medication though cumulative dose was not determined. In addition, none of the test subjects reported a family history of HD, mental retardation, brain injury, or movement disorder other than TD. Historical data such as clinical diagnosis, exposure to antipsychotic medication, and a prior diagnosis of TD were otherwise confirmed by chart review. Each subject was then evaluated for the presence of involuntary movements using the Abnormal Involuntary Movement Scale (AIMS) [Guy, 1976]. The TD group consists of 10 subjects each meeting minimum AIMS criteria for a diagnosis of TD with a mean age of 48.4 ± 10.22 years. The group is comprised of 70% female, 30% male, 40% Caucasian, 40% African-American, and 20% Hispanic subjects. The control (non-TD) group includes nine subjects with no TD

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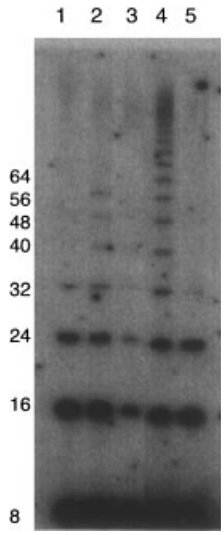

Fig. 1. An autoradiogram of five repeat expansion detection (RED) reactions. Using a $[CAG]_8$ oligonucleotide, the RED was performed on samples from patients with tardive dyskinesia (TD). The number of CAG repeats is indicated on the **left** by comparing to molecular weight markers.

according to AIMS criteria and has a mean age of 44.22 ± 11.48 years. The group consists of 67% male, 33% female, 89% Caucasian, and 11% African-American. The mean age compared between non-TD and TD groups was not significantly different ($P = 0.41$)

The RED method was chosen for use in this study to determine the $[CAG]_n$ because it can detect a repeat expansion without knowledge of the location of the responsible gene. The general procedure followed the published protocols [Schalling et al., 1993] with modifications to optimize the length of the oligonucleotide and the annealing and denaturing temperatures for each different oligonucleotide. Briefly, DNA from donated blood lymphocytes was isolated by the standard high salt procedure [Miller et al., 1988] using the Wizard genomic DNA purification system (Promega Co., Madison, WI). Two micrograms of genomic DNA from TD and non-TD individuals were annealed with oligonucleotides consisting of 8 or 15 copies of CAG repeat. Ligation reactions were then carried out and followed by a Southern blotting technique. The membrane was hybridized with ^{32}P-labelled complementary oligonucleotides and the multimers of the oligonucleotides $[CAG]_8$ or $[CAG]_{15}$ were detected by autoradiogram (Fig. 1). The maximum size of ligation product was determined for each sample and it represents the maximum CAG repeat length in the DNA of the subjects. For all samples, the analysis was performed four times.

The $[CAG]_n$ for each subject in both TD and non-TD (NTD) groups is listed in Table I along with the demographic data. The unpaired, 2-tailed Student's t-test was used for comparison of the means for all measures. Mean $[CAG]_n$ for NTD (range 45–104 repeats, mean $= 57.00 \pm 19.35$) and TD (range 45–120 repeats, mean $= 56.90 \pm 23.45$) were not statistically different ($P = 0.99$) when compared (Table I). $[CAG]_n$ from NTD and TD groups were combined. The median of 45 repeats was used to divide the total into two subgroups (SG1 and SG2 with smaller and larger $[CAG]_n$ fragments, respectively) and a means analysis of the two subgroups based on $[CAG]_n$ demonstrated that SG1 ($n = 10$ samples at 45 repeats per sample, mean $[CAG]_n = 45.00 \pm 0.00$) was significantly smaller than SG2 ($n = 9$, ranging from 48 to 120 repeats, mean $= 70.22 \pm 24.83$). According to this analysis, SG1 in comparison to SG2 was statistically different at $P < 0.005$ (Table II).

The relationship of age, gender, and ethnicity to the distribution of $[CAG]_n$ was characterized. In the TD group, the number of CAG repeats increases as the age of subjects increases. A reverse relationship has been observed in the non-TD group (Fig. 2). Gender differences were minimal in both

TABLE I. Summary of Data and Comparisons of CAG Trinucleotide Repeats for Patients With and Without Tardive Dyskinesia (TD)

	Non-TD				TD			
	Age	Gender	Ethnicity	$[CAG]_n$	Age	Gender	Ethnicity	$[CAG]_n$
	30	m	a	64	36	f	c	45
	35	m	c	45	39	f	c	64
	37	m	c	60	42	m	c	45
	40	m	c	104	42	f	h	48
	42	m	c	45	43	f	a	48
	43	f	c	45	47	m	a	45
	47	m	c	45	48	f	c	120
	57	f	c	60	61	m	a	45
	67	f	c	45	63	f	a	45
					63	f	h	64
Min	30	67% m	89% c	45	36	30% m	40% c	45
Max	67	33% f	11% a	104	63	70% f	40% a	120
Mean	44.22			57.00	48.40		20% h	56.90
SD	11.48			19.35	10.22			23.45

	P
Non-TD $[CAG]_n$ vs. TD $[CAG]_n$	0.99
Non-TD age vs. TD age	0.41
Non-TD m $[CAG]_n$ vs. non-TD f $[CAG]_n$	0.48
TD m $[CAG]_n$ vs. TD f $[CAG]_n$	0.32
Non-TD m $[CAG]_n$ vs. TD m $[CAG]_n$	0.29
Non-TD f $[CAG]_n$ vs. TD f $[CAG]_n$	0.48
TD c $[CAG]_n$ vs. TD nc $[CAG]_n$	0.22

Non-TD, persons with schizophrenia or schizoaffective disorder and no signs of TD; TD, persons with schizophrenia or schizoaffective disorder and signs of TD; P, probability as determined from an unpaired, two-tailed Student's t-test; m, male; f, female; c, Caucasian; nc, non-Caucasian; h, Hispanic; a, African-American.

TABLE II. CAG Trinucleotide Repeats for Subjects 1 Through 19 (Non-TD and TD Groups)

SG1		SG2	
Subject	$[CAG]_n$	Subject	$[CAG]_n$
1	45	11	48
2	45	12	48
3	45	13	60
4	45	14	60
5	45	15	64
6	45	16	64
7	45	17	64
8	45	18	104
9	45	19	120
10	45		
Mean	45		70.22
SD	0		24.83
		P	
SG1 vs. SG2		0.005	

All subjects were combined and segregated into two groups (SG1 and SG2) according to the median of the total sample (median $[CAG]_n = 45$). The means of the two subgroups were then compared against each other.

groups and when related to $[CAG]_n$, these differences were not statistically meaningful (mean $[CAG]_n$ male NTD = 60.50 ± 22.92 repeats and mean $[CAG]_n$ female NTD = 50.00 ± 8.66 repeats, $P = 0.48$; mean TD male = 45.00 ± 0.00 repeats and mean TD female = 62.00 ± 26.90 repeats, $P = 0.32$). Similarly, results did not approach statistical significance when compared between non-TD and TD groups (NTD male vs. TD male, $P = 0.29$ and NTD female vs. TD female, $P = 0.48$). Ethnic differences were characterized with respect to $[CAG]_n$ as well. While these differences are observable when compared in absolute terms, a small N precluded tests of significance within groups.

Several reports have addressed the issue of a familial basis for TD. Weinhold et al. [1981] described two male siblings with the triad of schizophrenia, involuntary movements, and cognitive impairment. Yassa and Ananth [1981] examined familial relationships to TD in 500 psychiatric inpatients. They were able to identify eight pairs of patients in this population who were first degree relatives, two pairs of whom had TD. Full concordance between siblings was found in those with as well as without TD. Similarly, Waddington and Youssef [1988] examined an extensive pedigree in which both parents and 9 of 14 offspring had chronic mental illness. Five of these siblings developed movement disorders and had lower performance on a test of basic cognitive functions than the siblings without involuntary movements. In comparison, Bartels et al. [1985] found no relationship between a family history of psychosis and the occurrence of TD in a population of schizophrenic patients. However, no mention was made of the concordance rates between the siblings who did not have TD. Moreover, "anticipation" phenomena have not been studied in the case of TD. Consequently, we have begun to explore the idea that TD may be related to CAG trinucleotide phenomena similar to neurological conditions such as HD, SCA, FA, and others.

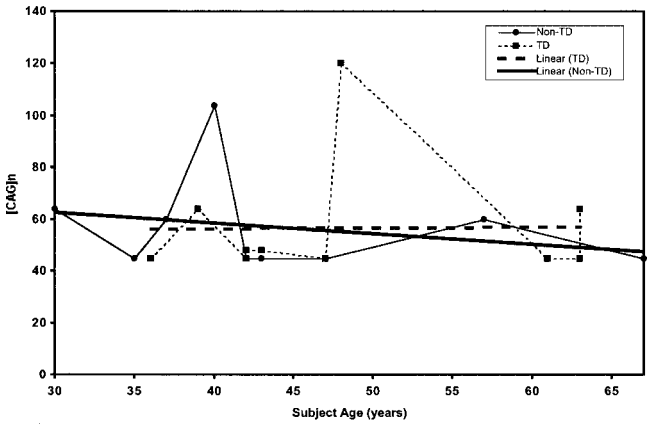

Fig. 2. Graphical representation comparing CAG trinucleotide repeats in schizophrenic patients with TD and schizophrenic patients without TD (non-TD). CAG trinucleotide repeats increase with age among TD patients, but decrease with age among non-TD patients.

Although the objective of this study was to characterize the length of the CAG repeats in subjects with and without TD, differences in gender and ethnicity were noted in the sample population. Given the average age of non-TD subjects in this study and a preponderance of males (67%) in the non-TD group and females (70%) in the TD group, our findings do not conform to current theory which holds that males are at greater risk for developing TD at an earlier age than females [Almeida et al., 1995]. However, the selection process employed for this study was unstructured and cumulative exposure to neuroleptic medication was not determined. These facts combined with a small sample size did not permit a segregation analysis, which might yield data to support the idea that TD may present earlier in persons genetically predisposed to developing it. A useful experimental design might segregate subjects according to age between the two subject groups based on the assumption that persons predisposed to developing TD will do so earlier in their cumulative exposures to neuroleptic medications than those without a similar predisposition.

As a pilot study, data were collected based on verbal report and clinical chart review. As such, many confounding variables were not controlled for such as exact diagnosis, variable cumulative exposure to neuroleptic medications, recent medications changes with associated withdrawal dyskinesias or pseudoparkinsonism, other historical information, or illicit substance use. These variables are known to influence phenotypic presentation of both psychiatric status and the presence or absence of involuntary movements and can obfuscate selection of a pure sample. Thus, it is expected that greater variability will exist in statistical measures based on this limited sample.

In conclusion, we find no statistically meaningful differences on any of the measures between subjects with and without TD using the RED method. However, it appears that a subgroup of the study population did exhibit larger than expected $[CAG]_n$ relative to the mean. It is possible and encouraging that under the conditions of a more structured selection process, these differences might sort preferentially with respect to TD. Thus, further exploration of trinucleotide profiles in TD is warranted.

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