

A Quantitative Trait Locus Analysis of the Dopamine Transporter Gene in Adults with ADHD

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Numerous lines of evidence have shown that attention deficit hyperactivity disorder (ADHD) is a highly heritable disorder, whether it is considered as a category or a dimension. We tested for an association between the dopamine transporter gene (DAT1) and ADHD considering the disorder as categorical as well as a continuous trait. Genotypes for the DAT1 variable number of tandem repeat (VNTR) alleles, along with Brown Attention Deficit Disorder Scale (BADDS) and Wender Utah Rating Scale (WURS) scores were available for 152 adult ADHD patients. In 72 of these patients DNAs from at least one parent were accessible to perform a family-based analysis (FBAT). The mean quantitative trait values of the whole sample of singleton patients were compared among the specific genotype groups

using ANOVA. The family-based analysis did not reveal any association between DAT1 alleles and ADHD either when it was considered as a dichotomous trait ($Z = 0.16, p = .86$) or as a continuous trait (Wender Scale $Z = -1.67, p = .09$; Brown ADD Scale $Z = 0.28, p = .77$). No significant differences were detected in the mean symptom scores among the specific genotype groups. The results from our study do not support a major role for the DAT1 VNTR alleles in our sample of adult ADHD. In view of several positive reports in child ADHD, more work is required to elucidate the potential role of the DAT1 VNTR as a risk factor in ADHD.

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Attention deficit hyperactivity disorder (ADHD) is a highly prevalent disorder in childhood characterized by severe impairment in attention span and/or marked hyperactive and impulsive behavior not appropriate for

age (American Psychiatric Association 1994). Several follow-up studies that evaluated the persistence of the disorder in adolescence and adulthood have revealed that one third to two thirds of ADHD children do not recover from the disorder (Gittelman et al. 1985; Barkley et al. 1990; Mannuzza et al. 1991; Weiss et al. 2000). However, the percentage of retained diagnosis in adulthood varies across studies. The relative lack of specific validated criteria for adult ADHD and the different definitions used to evaluate the persistence of the disorder may have contributed to these discrepancies. In the fourth edition of the DSM the criteria for ADHD are applicable to both adults and children. However, because the phenomenology in ADHD varies across the life span in quality of symptoms as well as in degree of severity (Weiss et al. 1985; Biederman et al. 2000), specific criteria for adults appear to be necessary. Even if there is little agreement on the proportion of children that

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continue to suffer from the disorder in adulthood, several lines of evidence have shown that adult ADHD represents a mental disorder of significant clinical relevance. Adult ADHD patients have at least twofold increased prevalence for a number of co-morbid psychiatric disorders (Weiss et al. 1985; Mannuzza et al. 1991; Biederman et al. 1993). These include: an increased risk compared with the general population of around 10 times for antisocial personality disorder, four times for substance and alcohol abuse (Weiss et al. 1985; Biederman et al. 1993), and approximately five times for mood and anxiety disorder (Biederman et al. 1991, 1993).

The decline of ADHD with increasing age may be advantageous to genetic investigations because the persistent form of the disorder is less common and appears to have a greater genetic liability. Proband with the persistent form of the disorder have a higher rate of ADHD in their relatives compared with the rate in relatives of child ADHD probands (Faraone et al. 2000).

Compelling evidence from genetic epidemiology studies have shown that ADHD aggregates in families (Thapar et al. 1999). The concordance for ADHD in twins has indicated that the disorder has heritability between 0.64 and 0.98, suggesting a large genetic influence (Willerman 1973; Goodman and Stevenson 1989; Gillis et al. 1992; Levy et al. 1997; Hay et al. 2001). Twin studies have also determined a genetic component for attention and impulsivity-hyperactivity (Goodman and Stevenson 1989; Levy et al. 1997; Sherman et al. 1997; Hay et al. 2001). Adoption studies have excluded any major contribution from shared environmental factors in predisposition to ADHD, further emphasizing the role of the genetic component (Welner et al. 1977; Biederman et al. 1990; Sprich et al. 2000). In a recent segregation analysis of ADHD families, several genetic models were supported when the disorder was considered as a quantitative trait (Maher et al. 1999). This study confirmed the evidence from a previous report conducted in a large Australian twin sample (Levy et al. 1997) in which a genetic contribution for a continuous dimension of the disorder was shown. It is therefore likely that the ADHD diagnosis represents an extreme of a trait distributed continuously in the population. In these circumstances, the use of dimensional measures of ADHD symptoms represents a valid approach to the identification of quantitative trait loci (QTL) underlying the disorder or symptom clusters.

Numerous findings support the involvement of the catecholamine systems in ADHD (Pliszka et al. 1996; Biederman and Spencer 1999) and converging evidence supports a key role for meso-cortical dysfunction (Castellanos 1997). A pharmacodynamic property of methylphenidate is the inhibition of the monoamine transporters with the highest affinity for the dopamine transporter (Gatley et al. 1996). The efficacy of methylphenidate in improving ADHD symptoms together

with its principal mechanism of action have pointed to the dopamine transporter gene (*SLC6A3*, alias *DAT1*) as a primary candidate to study in ADHD. *DAT1* has been mapped to chromosome 5p15.3 (Giros et al. 1992; Vandenberg et al. 1992), and is comprised of 15 exons. It contains a 40 base pair variable number of tandem repeats in the 3'-untranslated region (3'UTR-VNTR) (Vandenberg et al. 1992). Alleles with 3 to 13 repeats have been described, but the alleles with 10 and 9 repeats are the most frequent across several populations studied (Kang et al. 1999; Mitchell et al. 2000).

Cook et al. (1995) reported the 10-repeat allele of the *DAT1* 3'UTR-VNTR to be associated with the disorder. The association was replicated in two samples, one from Ireland (Gill et al. 1997; Daly et al. 1999), and one from the U.K. (Curran et al. 2001). It was partially replicated in a sample from the U.S. (Waldman et al. 1998) and in one from Canada (Barr et al. 2001) where the association was present with a haplotype that contained the 10-repeat allele. Waldman et al. (1998) regressed the 10-repeat allele association with hyperactive-impulsive and inattentive symptom dimensions and found higher scores of hyperactive-impulsive symptoms in the patients with the 10-repeat allele. The *DAT1* association, however, was not replicated in five different data sets: (Palmer et al. 1999; Holmes et al. 2000; Swanson et al. 2000; Curran et al. 2001; Roman et al. 2001). These contrasting results across different data sets could be determined by the heterogeneous nature of the disorder as identified by the current diagnostic criteria. As suggested by Waldman et al. findings (Waldman et al. 1998), for example, the *DAT1* 10-repeat allele may be associated only with specific subtypes of the disorder or only with more severe variants of the disorder.

In the present study we performed a family-based linkage disequilibrium analysis between the *DAT1* 3'UTR-VNTR alleles and ADHD in a sample of families with adult ADHD probands. The adult ADHD phenotype was considered as a qualitative as well as a quantitative trait. The quantitative trait of ADHD was derived from the Brown Attention Deficit Disorder Scale (BADDS), adult version (Brown 1996) and the Wender Utah Rating Scale (WURS) (Ward et al. 1993). In addition, a quantitative trait locus analysis of *DAT1* VNTR alleles was performed in a partially independent sample of singleton adult ADHD patients. The use of the quantitative phenotype will test for association between the *DAT1* 3'UTR VNTR alleles and dimensions of ADHD or specific core symptoms.

SUBJECTS AND METHODS

Subjects

This study was approved by the Centre for Addiction and Mental Health (CAMH) human subjects ethics

committee. All the subjects were clinician-referred to the Adult and Adolescent ADHD Research Program of the CAMH, an affiliate teaching hospital of the University of Toronto, for assessment. All subjects that participated had at least one first-degree relative willing to participate and collaborate in providing the collateral childhood history of the patients. After signing the informed consent, the patients underwent an extensive evaluation which has been described previously in detail (Muglia et al. 2000). The diagnosis of ADHD was based on fulfilling the ADHD DSM-IV criteria both at the time of the interview and during childhood, as recalled by the patient and by at least one first-degree relative involved in the study. For broad assessment of psychiatric conditions, the Structured Clinical Interview for DSM-IV (axis I) was administered by a trained psychiatrist (U.J.) with extensive experience in adult ADHD. Patients diagnosed with other psychiatric conditions, with the exception of dysthymia, were not considered for this study. In the five years period of recruitment at our adult ADHD clinic, approximately 30 percent of referrals were not included in our sample because of the presence of comorbid conditions or because they did not meet the criteria for the disorder. The Brown Attention Deficit Disorder Scale and the Wender Utah Rating Scale were completed by all the patients. The Brown ADD Scale consists of a 40-item self report that measures core symptoms of ADHD. The scale has been normalized using 142 adult ADHD patients and 143 healthy subjects, showing a good internal consistency and a good test-retest reliability (Brown 1996). The total score can range from 0 to 120; patients that score >55 are highly probable to suffer from ADHD, those between 40 and 54 have 'probable' ADHD, and those <40 have 'possible' ADHD. Five different adult ADHD dimensions are present in the scale: organizing and activating to initiate work, sustaining attention and concentration, sustaining energy and effort, managing affective interference, and utilizing working memory and accessing recall. The Wender Utah Rating Scale is a 61-item self-report questionnaire that retrospectively assesses childhood ADHD symptoms, as well as frequently associated behavioral, medical and learning problems. The validity and specificity of the WURS has been tested in a group of adults with ADHD versus patients with major depression, and healthy controls (Ward et al. 1993). Among the 61 WURS items, the 25 items that have shown the greatest mean difference between ADHD and non-ADHD patients has been used in our study. A total score higher than 46 on the 25-item scale is able to identify 86% of the ADHD patients (Ward et al. 1993). For the present study genotypes for the *DAT1* VNTR along with BADDs and WURS 25 item scores were available for 152 adult ADHD patients (mean age: 38.24; S.D. \pm 11.70; range: 19 to 69; male/female = 99/53), of which 98% were mixed European Caucasian while

the remaining three subjects consisted of two African Americans and one Caribbean.

For the family-based part of the study 102 pedigrees were available with a total of 338 individuals of which 126 were affected by ADHD. The structure of families in our sample consisted mainly of nuclear families (45 triads and 36 parent-proband pairs), 16 families with multiple sibs and five three-generation families. In the total number of 126 affected individuals 30 were non adults, that were assessed because they had one of their parents referred to our clinic.

Laboratory Methods

Blood samples were drawn from the patients and the relatives that participated in the study and the DNA was extracted following standard procedures. The DNA region containing the 40 bp 3'UTR-VNTR of the *DAT1* was amplified using the polymerase chain reaction and then genotyped using methods previously published (Vandenberg et al. 1992). The genotypes were assigned blind to the family structure and to the affection status of the subjects.

Statistical Methods

A family-based association strategy was used to test for the presence of association between the *DAT1* 3'UTR-VNTR and adult ADHD in the nuclear families. The study was performed using both categorical and dimensional measures of the disorder. The family-based association strategy that makes use of nuclear families consisting of an affected proband and their biological parents represents a well established approach, and it is more powerful than case-control designs in the presence of population substructure (Bacanu et al. 2000). A number of approaches that incorporate quantitative phenotypes into the TDT analysis have been developed (Allison 1997; Rabinowitz 1997; Waldman et al. 1999) and have been implemented in available software programs (Abecasis et al. 2000; Lake et al. 2000). The Family-Based Association Test (FBAT) is a recently developed software that can analyze for association pedigrees with heterogeneous family structure. Furthermore, FBAT allows the analysis of the phenotype as a qualitative or as a quantitative trait (Horvath et al. 2001). In the present study, we made use of the FBAT program (version 1.0) to perform the qualitative and quantitative family-based analyses of our ADHD families. For the quantitative analysis we used the additive model and the phenotypes were the mean centered variable of the BADDs total and cluster scores and the WURS scores. The *DAT1* 3'UTR-VNTR was considered as a biallelic polymorphism because the low occurrence of the rare alleles would have not provided any meaningful information. With these parameters FBAT (v 1.0)

Table 1: Family-based Analysis of Both Qualitative and Quantitative Phenotypes of ADHD Families. The Statistics for the 10-Repeat Allele of the *DAT1* 3'UTR VNTR are Listed.

Phenotype ^a	S ^b	E ^c	Z	P
Qualitative: (Affection status)	41.0	40.5	0.16	.86
Wender Utah Rating Scale Score	-197.0	-127.5	-1.67	.09
Brown ADD Scale Total Score	-102.6	-115.83	0.28	.77
Brown ADD Scale Attention cluster	-15.8	-24.84	0.68	.49
Brown ADD Scale Activation cluster	-26.0	-29.0	0.27	.78
Brown ADD Scale Effort cluster	255.0	263.1	0.27	.78
Brown ADD Scale Working Memory cluster	132.8	142.74	-0.55	.57

Note:

^a The quantitative phenotype is derived from the Brown Attention Deficit Disorder Scale, adult version (Brown 1996) and from the Wender Utah Rating Scale (WURS) (Ward et al. 1993).

^b S represents the test statistic and expresses the count of 10 repeat alleles observed in the affected offspring depending on the parental genotypes. For the quantitative analysis, S is weighted to the phenotype measures.

^c E is the value expected for S under the null hypothesis of no association.

performs a quantitative analysis as described by Rabinowitz (1997). The DNA was not available for all parents of the probands thus the quantitative family-based strategy was not applicable to the entire sample. Therefore, to test the *DAT1* 3'UTR-VNTR as a QTL in all the probands we used the *measured-genotype test* (Boerwinkle et al. 1986). In

this strategy, the mean quantitative trait values of the patients in each specific genotype group are compared using ANOVA. We compared the BADDs and WURS mean scores across three *DAT1* 3'UTR-VNTR genotype groups (9-9, 9-10, 10-10). ANOVA was performed using the Statistical Package for the Social Science (SPSS), version 10.

Table 2: The Mean Quantitative Trait Values of the Patients in Each Specific Genotype Group

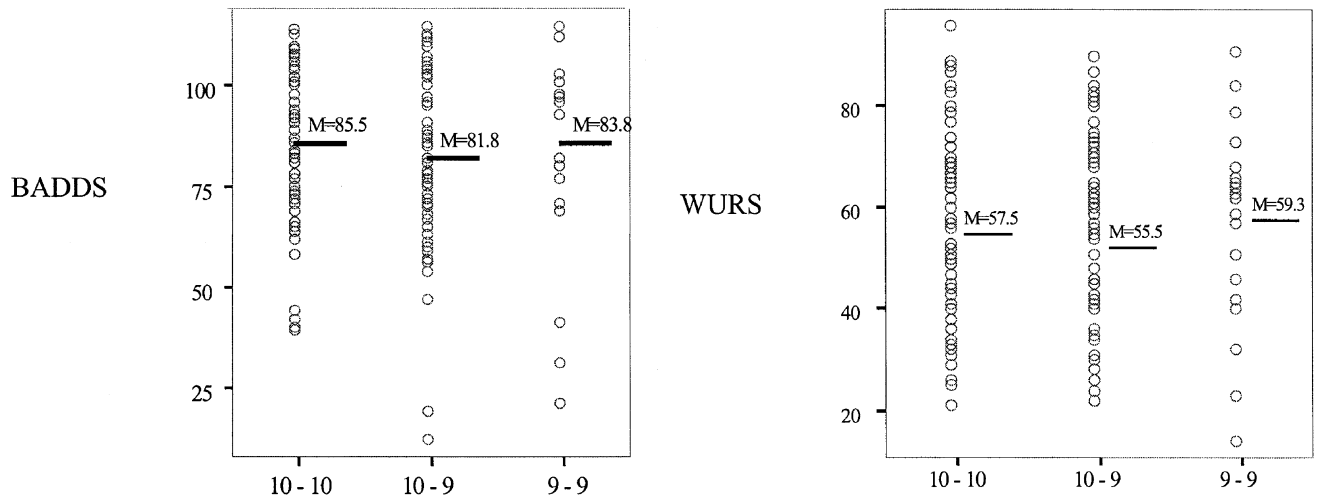
Brown and Wender Scores ^a Mean (S.D.)	Total sample ^b n = 152	Genotype			Statistic ^c F
		10-10	10-9	9-9	
Activation	19.86 (5.22)	20.17 (5.22)	19.41 (4.91)	20.31 (6.36)	0.43
Male	19.33 (5.68)	19.20 (5.80)	19.27 (5.27)	19.81 (6.77)	
Female	20.86 (4.10)	21.48 (4.06)	19.71 (4.17)	23.00 (2.64)	
Attention	20.48 (5.22)	21.20 (4.63)	19.75 (5.33)	20.42 (6.63)	1.29
Male	20.10 (5.64)	20.61 (4.77)	19.56 (5.84)	20.31 (7.14)	
Female	21.20 (4.28)	22.00 (4.39)	20.14 (4.17)	21.00 (3.60)	
Effort	18.10 (5.92)	18.23 (5.91)	17.96 (5.90)	18.01 (6.39)	0.48
Male	18.51 (5.93)	18.10 (6.19)	19.00 (5.37)	18.18 (6.99)	
Female	17.33 (5.88)	18.41 (5.60)	15.80 (6.47)	17.66 (0.57)	
Affect	12.90 (4.57)	12.89 (4.64)	12.63 (4.46)	13.89 (4.84)	0.55
Male	12.53 (4.81)	12.02 (5.01)	12.63 (4.53)	13.50 (5.18)	
Female	13.60 (4.04)	14.06 (3.87)	12.61 (4.39)	16.00 (1.00)	
Working Memory	12.36 (4.28)	12.97 (3.80)	12.10 (4.27)	11.10 (5.61)	1.65
Male	11.89 (4.59)	12.41 (4.06)	11.95 (4.52)	10.50 (5.89)	
Female	13.24 (3.48)	13.72 (3.35)	12.42 (3.76)	14.33 (2.08)	
BADDs Total	83.74 (21.35)	85.50 (20.02)	81.87 (21.01)	83.84 (27.19)	0.48
Male	82.39 (22.74)	82.38 (21.34)	82.43 (21.81)	82.31 (29.35)	
Female	86.26 (18.42)	89.68 (17.58)	80.71 (19.69)	92.00 (8.71)	
WURS	57.09 (17.52)	57.59 (18.35)	55.92 (16.87)	59.36 (17.39)	0.33
Male	56.28 (16.48)	53.65 (16.60)	57.29 (15.47)	59.75 (18.87)	
Female	58.61 (19.41)	62.92 (19.53)	53.04 (19.56)	57.33 (6.50)	

Table 2 Note:

^a Means are reported for the Brown ADD Scale Total Scores and Cluster symptom scores and the Wender Utah Rating Scale. Five different adult ADHD dimensions are present in the Brown scale (for more detail see text).

^b The total sample is composed of 53 females and 99 males.

^c ANOVA has been used to compare the mean scores. None of the analyses performed produced a significant difference of the means across the genotype groups at a p value < 0.05.



DAT1 3'UTR-VNTR Genotypes

Figure 1. Brown ADD Scale (BADDs) and Wender Utah Rating Scale (WURS) total scores and *DAT1* genotypes. M indicates the mean for each of the genotype groups. No statistical significant differences were detected in *DAT1* genotype groups for BADDs or WURS measures.

RESULTS

The family-based analysis of the *DAT1* VNTR alleles did not reveal the presence of association with ADHD when the phenotype was considered as a qualitative trait ($Z = 0.16$; $p = .86$). When the family-based analysis was conducted considering the ADHD phenotype as a quantitative trait no association was detected between *DAT1* alleles and the Brown ADD cluster, or total scores, or WURS score (see Table 1). In the analysis of the total sample of singleton probands, when the means of the BADDs symptom clusters, or total scores, or the WURS scores were compared among the subjects according to the different *DAT1* genotype groups, (as shown in Table 2, and Figure 1), no significant differences nor sex-specific effects were observed.

DISCUSSION

We were interested in testing for an association between the *DAT1* VNTR alleles and ADHD in adults with the persistent variant of the disorder. Faraone et al. (2000) have suggested that the adult version of ADHD is associated with a higher genetic contribution since the relative risk is higher in family members of the patients with the persistent form of the disorder than in the relatives of child ADHD patients. It has been dem-

onstrated that a higher relative risk confers stronger power of genetic association studies (Speer 1998), thus the persistent variant of ADHD arises as a promising phenotype for detection of susceptibility alleles. An example of successful use of a subset of high relative risk families to find an etiologic gene is represented in the case of early onset Alzheimer's Disease (Sherrington et al. 1995). Also, in the childhood ADHD study by Waldman and colleagues (Waldman et al. 1998) the association of the *DAT1* 10-repeat allele was reported to be stronger with the more severe form of the disorder. Therefore, it was important to test the *DAT1* association in a sample of adult ADHD patients since the variant of ADHD that persists into adulthood can be considered a more severe subtype of the disorder. Another interesting aspect of the persistent form of ADHD lies in the fact that non-genetic risk factors such as adverse family environment that have been shown to increase risk for child ADHD (Rutter and Quinton 1977; Biederman et al. 1995) may inflate the heterogeneity of the clinical samples. Adverse family environment is believed to play a reduced role as a risk factor in adults and it is arguable that children with ADHD largely determined by the adverse family environment will outgrow the disorder. It is therefore likely that adult ADHD samples are characterized by a reduced etiologic heterogeneity. In other words, adult samples would have a lower phenocopy rate (i.e. lower proportion of subjects with the same phenotype determined by non-genetic factors).

We were unable to replicate the association between the 10-repeat allele and ADHD. Furthermore, no correlation was found between the *DAT1* 3'UTR-VNTR alleles and ADHD severity or symptom scores as defined by the Brown ADD and the Wender Utah Rating Scale in our sample of 152 adults with ADHD. At least five studies in four independent samples (Cook et al. 1995; Gill et al. 1997; Waldman et al. 1998; Daly et al. 1999; Curran et al. 2001) have reported the presence of association between the 10-repeat allele of the 3'UTR-VNTR of the *DAT1* and ADHD in children. Four studies, however, did not replicate the association (Palmer et al. 1999; Holmes et al. 2000; Swanson et al. 2000; Roman et al. 2001). In a child study by Barr et al. (2001), initial analyses showed a non-significant increased number of transmissions for the 10-repeat allele. However, when haplotypes made up of the 10-repeat allele and specific alleles from two single nucleotide polymorphisms located in intron nine and in exon nine were examined, an association with ADHD was found (Barr et al. 2001). Cook et al. (1995) reported an odds ratio of 2.6 for ADHD determined by the presence of the *DAT1* 10-repeat allele. The power of our sample of families considering the 10-repeat allele as the risk allele with an additive model conferred a power > 0.8 for a significance level = 0.05 considering both qualitative as well as quantitative analysis as calculated by the program PBAT: Analytical Power Calculations for Family-Based Association Tests (PBAT, v 0.1 (Lange 2001)).

The apparently conflicting evidence for association across different data sets represents a common trend of association studies in complex diseases. Aside from lack of appropriate statistical power, a number of factors may contribute to the inability to reproduce genetic association findings. In the case of multiple predisposing genes, population substructure or clinical heterogeneity, a risk allele may (for stochastic reasons) play a larger or smaller role in a given sample of patients, thus producing contrasting results. The specific characteristic of our sample, comprised of adult ADHD probands, may have determined the lack of association with the 10-repeat allele. The association reported by Waldman et al. (1998) that indicates a prominent role for the 10-repeat allele in the hyperactive subtype in children is not, strictly speaking, testable in our sample. In fact, the symptoms of the hyperactive subtype largely disappear in approximately 80% of ADHD children as they grow up (Biederman et al. 2000). In our sample of adult patients only a small number of hyperactive ADHD patients were present and thus the testing of this specific hypothesis was not feasible. Another possible origin of the contrasting results in the *DAT1* and ADHD association studies is the presence of the recently described single nucleotide polymorphism located 135 bp downstream from the 3' end of the *DAT1* VNTR (Miller and Madras 2002); that none of the ADHD association studies to date have analyzed. If this SNP is common in the

population its analysis would be essential in the ADHD samples considering its proximity to the VNTR and its preliminary evidence of its function in the regulation of the gene expression (Miller and Madras 2002). The same study (Miller and Madras 2002) together with two concurrent publications (Fuke et al. 2001; Michelhaugh et al. 2001) are also suggesting a functional role for the *DAT1* VNTR. However, because the two studies that have assessed the influence of the repeat length on the control of gene expression are conflicting (Fuke et al. 2001; Miller and Madras 2002), additional work is necessary to clarify the exact functional role of the 3'UTR VNTR of *DAT1*.

In summary, the results from the present study together with the results from previous investigations on the role of the *DAT1* VNTR in ADHD are variable, making any conclusions speculative at the present time. While the use of haplotypes may increase the information at the DNA level, the increased degrees of freedom created by the use of haplotypes requires a larger sample size in order to have sufficient statistical power.

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