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ORIGINAL ARTICLE

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Evidence for a modifier of onset age in Huntington disease linked to the HD gene in 4p16

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Abstract Huntington disease (HD) is a neurodegenerative disorder caused by the abnormal expansion of CAG repeats in the *HD* gene on chromosome 4p16.3. A recent genome scan for genetic modifiers of age at onset of motor symptoms (AO) in HD suggests that one modifier may reside in the region close to the *HD* gene itself. We used data from 535 HD participants of the New England Huntington cohort and the HD MAPS cohort to assess whether AO was influenced by any of the three markers in the 4p16 region: *MSX1* (*Drosophila* homeo box homologue 1, formerly known as homeo box 7, *HOX7*), *Δ2642* (within the *HD* coding sequence), and *BJ56* (*D4S127*). Suggestive evidence for an association was seen between *MSX1* alleles and AO, after adjustment for normal CAG repeat, expanded repeat, and their product term (model *P* value 0.079). Of the variance of AO that was not accounted for by HD and normal CAG repeats, 0.8% could be attributed to the *MSX1* genotype. Individuals with *MSX1* genotype 3/3 tended to have younger AO. No association was found between Δ2642 (P=0.44) and *BJ56* (P=0.73) and AO. This study supports previous studies suggesting that there may be a significant genetic modifier for AO in HD in the 4p16 region. Furthermore, the modifier may be present on both HD and normal chromosomes bearing the 3 allele of the *MSX1* marker.

Keywords Huntington disease · Modifier · Onset age · Genetics · Trinucleotide repeat · HD gene

Introduction

Huntington disease (HD) is a neurodegenerative disorder with complex clinical features [1, 2, 3, 4]. The genetic mutation associated with HD is located on 4p16.3 [5] and is characterized by CAG trinucleotide expansion [6].

It has been demonstrated that age at motor onset (AO) is inversely related to the size of expanded CAG repeat [7, 8, 9, 10], but little is known about other genetic modifiers (subsequently referred to as modifiers) of AO among HD-affected persons. Farrer et al. [11] were the first to suggest that HD expression may be modulated by a gene close to the *HD* locus. A recent genome scan for AO modifiers reported evidence for linkage to the *HD* locus [12]. *Δ2462* is an intragenic deletion of a codon in the *HD* gene area [13] and *BJ56* (start to stop 3,084,110 to 3,084,274 bp)—also known as *D4S127* [14]—is a dinucleotide repeat located 23.4 kb 5′ of the *HD* gene. The *MSX1* gene [15] (start to stop 4,925,948 to 4,930,000) is located 1.6 Mb proximal and 3′ to the *HD* gene. Given the proximity of these markers to the *HD* gene and a recent genome scan for AO modifiers showing linkage to the *HD* locus, we used information from 535 HD affected persons to test whether polymorphisms in the *MSX1* gene, *Δ2642,* or *BJ56* markers may explain variation in AO, after adjustment for normal, expanded CAG repeats and the interaction of these two as defined by their product term [16].

Patients and methods

Patients

Data were collected on 221 participants from the New England Huntington Disease Center (parent-offspring pairs referred to as HD PAIRS) and 533 participants of the Huntington Disease, Modifiers in Age at onset in Pairs of Sibs (HD MAPS study). Over 95% of these participants were Caucasian. Of these subjects, 535 had complete data on AO and CAG repeat sizes. However, limited subjects had genotype data on *MSX1* (n=192), *Δ2642* (n=518), or *BJ56* (n=471). Of the 535 subjects, 480 were from the HD MAPS study and 55 were from unaffected parent-affected offspring HD PAIRS described earlier [17]. DNA samples in the HD MAPS were collected mainly for the purpose of genome scan. Thus, the sample size of subjects genotyped for *MSX1*, *BJ56*, and Δ2642 was mainly driven by the amount of available DNA. We did not have enough DNA to analyze all three genes/markers in each subject. The fewer genotypes for *MSX1* were not a consequence of increased error rate, but that fewer samples could be typed.

For the HD PAIRS, the normal chromosomes were determined by the alleles transmitted by the unaffected parent and HD chromosomes as those not transmitted by the unaffected parent. For the HD MAPS, the HD and normal chromosomes were determined by the allele sharing of siblings, assuming that all affected siblings shared their HD chromosomes. In this sample this assumption is very likely true in all cases as the gene frequency for HD is very low. AO was defined as the onset of motor impairment [18, 19, 20]. For *MSX1, BJ56*, and *Δ2642,* there were no differences in allele frequencies for the New England versus all other samples.

CAG repeat size determination

CAG repeat sizes were determined by polymerase chain reaction of the number of CAG trinucleotide repeats responsible for the *HD* gene [6], using a modified protocol that eliminated an adjacent proline (CCG) repeat [21, 22]. Cases with 36 or more repeats were designated HD gene carriers, in accordance with published associations with disease expression [23].

Marker determination: *MSX1, Δ2642, and BJ56*

The *MSX1* dinucleotide repeat was typed using a forward primer: TTAGATTGTCTCAGTCCTC, and a reverse primer: GGGCATGTTGATGTCTGCTGAC. This dinucleotide repeat polymorphism has four alleles of 169, 171, 173, and 175 bp length. In the subsequent text, these alleles are referred to as 1 through 4, respectively.

The *Δ2642* polymorphism is a deletion of one of four consecutive GAG codons (Glu) at positions 2642–2645 [13]. The deletion (B allele) is found in about 7% of the general population and 38% of HD chromosomes. The *Δ2642* was assayed as previously described [13].

The *BJ56* (*D4S127*) polymorphism is located at 40 kb 5′ to the transcription initiation site for the *HD* gene. The polymorphism is a dinucleotide repeat with five alleles of 157, 155, 153, 151, and 149 bp length (referred to in text simply as 2, 4, 6, 8, and 10, respectively). The assay for *D4S127* was performed as previously described [14].

Of the 535 affected people with genotype data on at least one of the three genes/markers, the phase for *MSX1* could be established in 139 subjects. For *Δ2642* and *BJ56,* the phase was established in 286 and 260 subjects, respectively.

Statistical analysis

We initially conducted analyses within each cohort, HD MAPS and the HD PAIRS sample, separately. Since the results were similar within each cohort, we pooled the data. To evaluate the association between each marker and AO, we created residuals of AO by regressing AO on normal repeats, expanded repeats, and their product term. We used the natural logarithm to transform AO as this has been shown to provide the best linear relation of repeat size to onset age [7, 9, 10]. For each marker, we determined individual genotypes (referred to as “genotype only”). In addition, we determined genotypes along with the location of a particular allele on the normal or affected chromosome (referred to as “genotype and phase”). We were not able to set phase for all subjects. For example, when the parent and offspring were both heterozygous for the same alleles at a locus, we could not tell which alleles were transmitted from the parent. We used a generalized linear model (*Proc Mixed* in SAS) to compare mean residuals of AO among different genotypes (genotype only, genotype and phase). This method accommodates the familial correlation between subjects. The significance level was set at 0.05 and all analyses were performed using SAS for windows, release 8.02, and version 5.1.

Results

Among the 535 HD subjects, the mean AO was 38.3±12.1 years (range 8–78 years). The expanded CAG repeat ranged from 36 to 80 (mean±SD 46.7±6.0) and the unexpanded repeat (normal allele) size ranged from 6 to 33 (mean±SD 18.8±3.5). Complete data were available on 192 subjects genotyped for *MSX1*. For Δ2642 and BJ56, corresponding numbers were 518 and 471, respectively. We observed suggestive evidence for an association between *MSX1* and AO after controlling for normal, expanded CAG repeats, and their interaction (global *P* value for the model 0.079 for all subjects with genotype data on *MSX1* and 0.06 when restricted to subjects with known phase, Table 1). Only subjects with 3/3 genotype tended to have less than expected AO ($P<0.05$). We also noted that those with the 3/4 genotypes had the same mean level as the 3/3 group, but this effect was not statistically significant, perhaps due to the smaller sample size. A post hoc analysis assessing the presence of allele 3 (versus absence of allele 3) showed that the presence of allele 3 was associated with a significant younger than expected AO [beta coefficient −0.4424 (SE 0.1464), $P=0.013$]. The adjusted mean residuals of AO were −0.2743 (SE 0.116) for subjects with allele 3 and 0.1681 (SE 0.089) for people without allele 3. This means that subjects with allele 3 have an AO that is ~5.4 years earlier than subjects without allele 3.

Table 1 Relation between *MSX1* and unexplained variance of age at onset (AO) of Huntington disease (HD) among HD subjects from the HD MAPS and HD PAIRS data sets[a]

		n	Mean residual of AO±SE
By genotype only (*n*=192)			
1/1		70	0.12±0.12
1/2		13	0.48±0.27
1/3		46	−0.17±0.14
1/4		28	0.07±0.19
2/3		5	0.26±0.44
2/4		5	0.64±0.44
3/3		15	−0.63±0.25†
3/4		6	−0.63±0.40
4/4		4	−0.21±0.49
Model *P* value			0.079
By genotype and phase (*n*=139)[b]			
Normal chromosome	Affected chromosome		
1	1	67	0.10±0.12
1	2	4	0.32±0.48
1	3	9	−0.27±0.32
1	4	4	1.10±0.48*
2	1	9	0.50±0.32
3	1	11	−0.13±0.29
3	3	15	−0.61±0.25*
3	4	2	−1.62±0.68*
4	1	10	0.18±0.31
4	3	4	−0.26±0.48
4	4	4	−0.17±0.48
Model *P* value	0.06		

* Statistically different from zero at 0.05 level
[a] Residuals of AO adjusted for unexpanded and expanded CAG repeats and their product term
[b] Among 139 subjects with known phase

In the analysis restricted to subjects with established phase, there was a borderline significant association between *MSX1* and AO (model $P=0.06$, Table 1). About 0.8% of the residual variance of AO (after adjustment for normal CAG repeat, expanded CAG repeat, and their product term) could be explained by *MSX1* polymorphism. Neither *Δ2642* nor *BJ56* was associated with AO (Tables 2 and 3).

Discussion

HD is an autosomal dominant disease characterized by neurological and psychiatric symptoms. The *HD* gene is a trinucleotide repeat expansion and the size of the repeat is

Table 2 Relationship between Δ*2642* and unexplained variance of AO of HD in HD subjects from the HD MAPS and HD PAIRS data sets[a]

		n	Mean residual of AO±SE
By genotype only (*n*=518)			
A/A		275	−0.05±0.06
A/B		230	0.06±0.07
B/B		13	−0.14±0.28
Model *P* value			0.44
By genotype and phase (*n*=286)[b]			
Normal chromosome	Affected chromosome		
A	A	251	0.02±0.06
A	B	10	−0.08±0.32
B	A	13	−0.19±0.28
B	B	12	−0.15±0.29
Model *P* value			0.82

[a] Residuals of AO adjusted for unexpanded and expanded CAG repeats and their product term
[b] Among 286 subjects with known phase

Table 3 Relationship between *BJ56* and unexplained variance of AO of HD in HD subjects from the HD MAPS and HD PAIRS data sets[a]

		n	Mean residual of AO±SE
By genotype (*n*=471)			
2/2		31	−0.14±0.18
2/4		27	0.12±0.19
2/6		54	−0.27±0.14
2/8		128	0.008±0.09
2/10		4	0.003±0.50
4/4		3	0.43±0.58
4/6		28	0.07±0.19
4/8		60	−0.05±0.13
4/10		3	0.006±0.58
6/6		12	0.29±0.29
6/8		64	0.18±0.13
6/10		3	−0.25±0.58
8/8		50	0.04±0.14
8/10		4	−0.28±0.50
Model *P* value			0.73
By genotype and phase (*n*=260)[b]			
Normal chromosome	Affected chromosome		
2	2	31	−0.09±0.18
2	4	4	−0.45±0.50
2	6	21	−0.22±0.22
2	8	46	−0.09±0.15
2	10	2	0.27±0.71
4	2	3	−0.73±0.58
4	4	2	0.59±0.71
4	6	15	0.02±0.26
4	8	25	0.08±0.20
6	2	4	−0.84±0.50
6	4	2	−0.55±0.71
6	6	11	0.37±0.30
6	8	21	0.21±0.22
8	2	10	0.89±0.32
8	6	13	−0.15±0.28
8	8	45	0.06±0.15
10	2	2	−0.12±0.71
10	8	3	−0.37±0.58
Model *P* value			0.33

[a] Residuals of AO adjusted for unexpanded and expanded CAG repeats and their product term
[b] Among 260 subjects with known phase

known to be associated with AO for HD. Several studies have shown that AO is inversely related to the size of CAG repeat. In addition, the *GRIK2* (ionotropic glutamate receptor, kainite 2 subunit; formerly *GLUR6*) gene has been found to explain about 13% of the remaining variance of AO after adjustment for CAG repeats [24], with a particular allele being associated with a younger AO [25]. However, a large portion of the variation of AO remains unexplained after controlling for repeat sizes and their interaction [16]. It has been suggested that other genes on chromosome 4 might interact with the *HD* gene to modify the AO of HD [11]. A recent genome scan for AO modifiers in HD suggests that there may be an AO modifier in the region of the *HD* gene [12].

In the present study, we assessed whether *MSX1*, Δ*2642*, and *BJ56* —since these markers are close to *HD* gene—were associated with the AO of HD. Our findings are suggestive of an association between *MSX1* and AO. Individuals with *MSX1* genotype 3/3 appear to have earlier AO than expected from their CAG repeat lengths, with a mean residual of AO of −0.61; this means that their HD age at onset is about 7.5 years earlier than expected. Neither Δ*2642* nor *BJ56* was associated with AO in this population.

This is the first study to examine whether a gene in the *MSX1* region modifies AO in HD. Although a distance of ~1.5 Mb separates the *HD* and *MSX1* genes, these genes may interact through their end products to influence AO, although the biological mechanism remains unclear at present. Data from mouse/human hybrid cell lines indicate that *MSX1* maps to human chromosome 4p16.1 [15]. Genes containing the homeo box 183-bp DNA sequence [26] are thought to control segmentation in *Drosophila* [27, 28]. However, the role of *MSX1* in humans is not well established [29]. *MSX1* mutations have been associated with tooth and nail disorders in humans [30, 31]. It is perhaps more likely that the observed effect on AO might be attributable to another gene in disequilibrium with the *MSX1* polymorphism. These results are supported by evidence of linkage in the *HD* region in a recent genome scan using the same data set [12].

While Δ*2642* is intragenic and *BJ56* is very close to the *HD* gene, we did not find evidence for modification of

AO by either of these markers. The B allele of Δ2642 has been associated with a higher CAG repeat size on the normal chromosome [32]. Our null findings for *Δ2642* are consistent with the report of Novelletto et al. [33], who did not find an association between *Δ2642* and AO. In contrast, among French families, subjects with *Δ2642* deletion (allele B) had an increased CAG size and had a younger AO (about 4 years earlier) than HD subjects without *Δ2642* deletion [34]. We do not have a reasonable explanation for the discrepancy among these reports. Contrary to our study, both earlier studies did not adjust for the CAG repeat sizes when assessing the effects of *Δ2642* on AO. We do not know whether after correcting for CAG lengths in the French study [34], *Δ2642* deletion would have been associated with younger AO.

In conclusion, our study supports the hypothesis that there is a modifier of AO in the general region of *HD* and this modifier may be close to the *MSX1* gene. Study of genes close to the *MSX1* polymorphism may be important to identify the specific modifier of AO among HD patients.

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