Failure to confirm influence of Methyltetrahydrofolate reductase (MTHFR) polymorphisms on age at onset of Huntington disease

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Abstract

Background: Huntington disease (HD) is a fully penetrant, autosomal dominantly inherited disorder associated with abnormal expansions of a stretch of perfect CAG repeats in the 5’ part of the IT15 gene. The number of repeat units is highly predictive for the age at onset (AO) of the disorder. But AO is only modestly correlated with repeat length when intermediate HD expansions are considered. Recently, suggestive association has been reported between a single nucleotide polymorphism (SNP; rs1801131, also known as A1298C) in the methyltetrahydrofolate reductase (MTHFR) gene and AO of HD. 5,10-MTHFR is a key enzyme in the folate metabolism, diverting metabolites toward methylation reactions or nucleotide synthesis. Using part of a previously established study cohort plus additional patients and appropriate statistical methods, we reinvestigated two polymorphisms in the MTHFR gene, C677T and A1298C, as well as their association with AO in 167 HD patients.

Results: There was no statistically significant impact on AO for HD patients, neither of MTHFR SNPs nor of the combinations thereof.

Conclusion: Contrary to previously described evidence the A1298C polymorphism in the MTHFR gene does not appear to modulate AO of HD patients.

Background

Huntington disease (HD) is caused by expansion of a cytosine-adenine-guanine (CAG) trinucleotide repeat in the 5'-translated region of the IT15 gene on chromosome 4, which encode the protein huntingtin [1]. The expansions result in the formation of elongated proteins with a variety of new properties. The extent of the expansion is inversely correlated with the age of onset (AO). Nevertheless, large part of the variance in AO remains unexplained [2]. The pathogenesis of HD has been implicated to relate to different aspects of the homocysteine metabolism: Cystathionine [beta]-synthase (CBS) appears to bind specifically to huntingtin (htt) [3]. CBS deficiency is associated with homocystinuria, which affects various physiological systems, including the central nervous system. Homocysteine, one of the substrates of CBS accumulates in homocystinuria and is metabolized to homocysteate and homocysteine sulphinate, both components of which are
Table 1: Number of different genotype combinations of the MTHFR 677C→T\(^a\) and 1298 A→C\(^b\) polymorphism in 167 HD patients

| MTHFR genotype | 677CC (%) | 677CT (%) | 677TT (%) |
|----------------|----------|----------|----------|
| 1298AA (n = 80) | 23 (13.7) | 37 (22.1) | 20 (12) |
| 1298AC (n = 74) | 35 (21) | 38 (22.8) | 1 (0.6) |
| 1298CC (n = 13) | 13 (7.8) | 0 | 0 |

\(^a\)Allele frequency of MTHFR 677T amounts to 0.35,
\(^b\)Allele frequency of MTHFR 1298C amounts to 0.29.

Amino acids with significant excitotoxic potential. In this context homocysteine was suggested to influence the pathogenesis of HD. Two common polymorphisms have been described in the MTHFR gene, both single nucleotide substitutions resulting in amino acid changes (C677T → Ala222Val and A1298C → Glu429Ala) [4,5]. Whereas C677T unequivocally affects enzyme function and has been associated with increased plasma homocysteine concentrations and an altered balance of folate metabolites [4], the functional relevance in vivo of the A1298C allele is less well defined. A1298C affects enzyme function in vitro to a lesser degree, and individuals carrying the variant have frequently normal homocysteine and plasma folate concentrations [6,7]. It is unclear whether the substitution affects folate metabolism under specific physiological conditions, e.g. under low nutrient intake. Apparently, the 677C polymorphism results in 167 HD patients.

Results and discussion

Analysis of the MTHFR 677C→T and the 1298A→C polymorphisms in 167 patients revealed allele frequencies of 0.35 for MTHFR 677T and 0.29 for MTHFR 1298C, respectively. Observed frequencies were in Hardy-Weinberg equilibrium.

The prevalences of the combined MTHFR genotypes for patients and controls are listed in Table 1. 23.3% of the subjects represented combined heterozygotes for the two SNPs (1298AC/677CT). We found no double homozygous individuals (1298CC/677TT) and no patient carrying the 1298CC/677CT genotype, a result to be expected based on genotype frequencies reported in other populations [11,12]. Thus our findings comply with the suspicion that these two polymorphisms occur rarely in cis [7]. Addition of the MTHFR genotype variations, alone and in combination (data only shown for the dominant model of the rare allele or the model for compound heterozygosity, respectively) to the effect of CAG repeat lengths resulted in no significant increase in the R\(^2\) value (Table 2). Hence, this study failed to replicate the association finding between the genotypes of the A1298C polymorphism in MTHFR with the AO of HD. Since our cohort comprises mostly the same individuals as investigated before (144/167), the initial description of association is due to weaker exclusion criteria concerning relatedness of patients as well as exclusive reference to motor AO. In addition different statistical principles were employed.

Table 2: Linear regression analysis concerning polymorphisms in the MTHFR gene

| Gene (polymorphism) | R\(^2\) | ΔR\(^2\) | % additional explained variance | P value |
|---------------------|--------|--------|-------------------------------|--------|
| HD CAG              | .308   | -      |                               | < .0005|
| HD CAG + MTHFR A1298C | .304  | .004  | -                             | .850   |
| HD CAG + MTHFR G677T | .305  | .003  | -                             | .590   |
| HD CAG + compound heterozygotes | .304  | .004  | -                             | .828   |

Variance in age at onset for the CAG repeats is indicated as such as well as in combination with the different polymorphisms examined. R\(^2\) illustrates the relative improvement of the regression model when the various genotypes are considered in addition to the HD CAG repeats, ΔR\(^2\) values quantify these differences. P values refer to R\(^2\).
Conclusion

We failed to replicate the association finding between the 1298CC genotype in the MTHFR gene and earlier AO in HD. In future studies in this context, also the folate levels of individual patients should be taken into account as well as environmental factors.

Methods

One hundred sixty-seven patients clinically diagnosed as suffering from HD were ascertained for their motor AO in the Huntington Center (HZ) NRW, Bochum (Germany) [10]. All patients gave informed consent for genotyping. The CAG repeat sizes and the MTHFR A1298C and C677T genotypes were determined as described before [9]. The dependence of the AO on CAG repeat number was determined by linear regression. Residuals from this model were verified, and there was no evidence of departure from normality and equality of variance assumptions. The possible genotypic effects of the two polymorphisms were assessed with multiple linear regressions, while allowing for the predictive effects of the CAG repeat size. We used the AO as dependent variable and the respective genotypes as independent variables. The CAG repeat number was considered as numerical variable. All of the other putatively modifying genotypes were considered as nominal variables by assigning the value "0, 1" or "0, 1, 2" according to the subject's number of variant alleles under a model of dominance or otherwise according to a model of generalized additive allelic effect. SPSS Ver.11.0 for Windows (SPSS Inc.) was used for all statistical analyses.

Competing interests

The author(s) declare that they have no competing interests.

Authors’ contributions

WH and LA initiated the study; WH carried out the molecular genetic studies and drafted the manuscript. JA and CS had ascertained the clinical status of the patients. SW and JTE participated in the study design and finalized the analyses as well as several versions of the paper.

All authors read and approved the final version of the manuscript.

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