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Glutathione S-Transferase Ω 1 variation does not influence age at onset of Huntington's disease

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Abstract

Background: Huntington's 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. Circumstantial evidence suggests that additional features of the HD course are based on genetic traits. Therefore, it may be possible to investigate the genetic background of HD, *i.e.* to map the loci underlying the development and progression of the disease. Recently an association of Glutathione S-Transferase Ω 1 *(GSTO1)* and possibly of *GSTO2* with AO was demonstrated for, both, Alzheimer's (AD) and Parkinson's disease (PD).

Methods: We have genotyped the polymorphisms rs4925 *GSTO1* and rs2297235 *GSTO2* in 232 patients with HD and 228 controls.

Results: After genotyping *GSTO1* and *GSTO2* polymorphisms, firstly there was no statistically significant difference in AO for HD patients, as well as secondly for HD patients vs. controls concerning, both, genotype and allele frequencies, respectively.

Conclusion: The *GSTO1* and *GSTO2* genes flanked by the investigated polymorphisms are not comprised in a primary candidate region influencing AO in HD.

Background

HD is an autosomal dominantly transmitted, progressive, neurodegenerative disorder that is associated with an expanded block of CAG repeats in exon 1 of the *IT15* gene (chromosome 4p) which encodes the protein huntingtin. The CAG repeat mutation is translated into an abnormally long polyglutamine tract, which is believed to acquire a deleterious gain of function in the mutant protein. However, though variation in repeat length may explain some of the variation in AO and, together with the observed progression of expansion over generations, it provides a molecular basis for the phenomenon of anticipation, other variables play a role, particularly in those cases where pathological CAG repeat numbers range in the high 30s or low 40s [1]. This insight has prompted the search for additional genes influencing AO in HD. Defining such independent predisposition factors is of paramount

importance, since they may provide further clues pertaining to the pathology arising from the expanded repeats.

Recent association studies showed that single nucleotide polymorphisms (SNP) in the *GSTO1* and *GSTO2* gene region are associated with AO in AD and PD, rendering this region a likely candidate to affect AO in other neurodegenerative diseases, too [2]. The GSTs belong to a family of enzymes that utilize glutathione in reactions contributing to the transformation of a wide range of exogenous and endogenous compounds, including drugs, carcinogens, and the products of oxidative stress [OMIM 60548]. Therefore, the *GSTO1* and *GSTO2* genotypes were determined for HD patients that had previously been analysed with respect to CAG repeat length.

Methods

232 patients with clinical diagnosis of HD were recruited from the Huntington Center (HZ) NRW. Clinical assessment and determination of AO was performed exclusively by experienced neurologists/psychiatrists of the HZ NRW. The motor AO was ascertained by the clinical specialists for 143 patients. Controls (healthy blood donors from the German populations of Essen and Hamburg) were also genotyped for comparison. All patients and controls had given informed consent for genotyping.

CAG repeat sizes were determined after PCR amplification of genomic DNA from peripheral white blood cells. CAG repeats were amplified by established methods [3]. The polymorphism Ala140Asp (rs4925) of the *GSTO1* gene was demonstrated after PCR amplification using the following primers: 5'-AAAGTTGTTTCTTAAACGTGCC-3' and 5'-AAGTGACTTGGAAAGTGGGAA-3'. PCR was carried out in a final volume of 20 μl with 50 ng of DNA, 200 μM dNTP and 0.25 U Taq Polymerase. Thermal cycling was performed with an initial activation step at 95°C for 15 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min; and a final extension at 72°C for 10 min. PCR products were digested with the restriction enzyme Cac8 I at 37°C overnight and visualised on 2% agarose gels stained with ethidium bromide. This digestion reveals a bi-allelic polymorphism with altogether 4 bands of different sizes: a 388 bp fragment (together with a 67 bp band) corresponding to the A allele (restriction site absent) as well as a set of 243, 145 and 67 bp fragmenst corresponding to the C allele (restriction sites present).

The transition polymorphism A to G (rs2297235) in the *GSTO2* gene was demonstrated using the following primers: 5'-TTACTTACCAAAGAGTGGCCAG-3' and 5'-CCGCAGGCTCCAGAAAC-3' under the same conditions mentioned above. PCR products were digested with the restriction enzyme Tsp509 I at 65°C overnight. The digestion reveals a bi-allelic polymorphism with altogether 5 bands of different sizes: a 344 bp fragment specific for the G allele (one restriction site absent) *vs*. a set of 282, 62, 52 and 5 bp corresponding to the A allele (restriction sites present).

The variability in AO attributable to the CAG repeat number was calculated by linear regression. For the regression analysis, we used the AO as the dependent variable, and the size of the expanded CAG block, normal CAG repeat, their interaction, and the rs4925 *GSTO1* as well as the rs2297235 *GSTO2* genotypes as independent variables. SPSS, Ver.11.0 for Windows (SPSS Inc.), was used for all statistical analyses.

Results and discussion

In the cohort with defined AO, the mean AO (±SD) was 47.61 (±8.7) years (range 25–73). The expanded CAG repeat ranged from 41–45 (mean ± SD: 42.94 ± 1.2) and the unexpanded repeat size ranged from 13–29 (mean ± SD: 18.67 ± 2.8; one individual had a smaller allele carrying 37 trinucleotides). As detailed in an earlier study concerning the clinically thoroughly characterised HD cohort [4], AO is negatively correlated with repeat lengths also in the lower range of CAG expansions. The CAG repeat length on normal chromosomes and the interaction failed to show any association with the age of onset of HD (Table 1). The expanded CAG repeat explained 27.7% of the variance in AO. This relatively low value may be explained by the small range of CAG repeats (41–45) investigated. Adding the unexpanded CAG repeat and the interaction term in the model did not affect the variance of the AO variance explained (from 27.7 to 27.8%). With respect to the small CAG repeat range the results are in agreement with a recently published study which showed a positive association with delayed AO of HD only for expansions ≥ 47 CAG [5]. In order to increase statistical power, further DNA samples of HD patients were included in these *GSTO* polymorphism studies. Both SNPs in the *GSTO* region showed a virtually identical association pattern due to the LD between these two. Nevertheless, a rare haplotype (*GSTO1* – *GSTO2*, *ac-aa*) was more prevalent among HD patients compared to controls. This difference was statistically insignificant. Thus all in all, the distribution of the genotypes and allele frequencies between HD and control subjects was not statistically different (Table 2). Observed frequencies were in Hardy-Weinberg equilibrium. Furthermore, the rs4925 *GSTO1* and rs2297235 *GSTO2* genotypes failed to show any association with the AO of HD (Table 1, Fig. 1 and Fig. 2).

The presence of insoluble protein aggregates within neurons is a common hallmark of AD, PD and HD [6]. These aggregates associated with the three neurodegenerative disorders may represent a common final pathway by

Table 1: Regression coefficients (SE) of the AO in dependence of the size of expanded CAG, normal CAG repeat, their interaction, and the rs4925 *GSTO1* and rs2297235 *GSTO2* genotypes

	Regression coefficients (SE)	P
Expanded CAG size	-3.842 (0.522)	<0.0001
Unexpanded CAG size	-0.0228 (0.199)	0.909
Expanded* unexpanded CAG size	-0.000527 (0.005)	0.909
rs4925 *GSTO1*	-0.841 (1.042)	0.421
rs2297235 *GSTO2*	-1.720 (1.042)	0.095

Table 2: Allele and genotype frequencies of the *GSTO1* and *GSTO2* variations in HD patients and controls

	Patients (n = 232)	Controls (n = 228)
Alleles (%)	*GSTO1*/*GSTO2*	*GSTO1*/*GSTO2*
A/G	168 (36%)/151 (33%)	142 (31%)/134 (29%)
C/A	296 (64%)/313 (67%)	314 (69%)/322 (71%)
Genotypes		
aa/gg	28 (12%)/24 (10.3%)	22 (10%)/20 (9%)
ac/ga	112 (48%)/103 (44.4%)	98 (43%)/94 (41%)
cc/aa	92 (40%)/105 (45.3%)	108 (47%)/114 (50%)

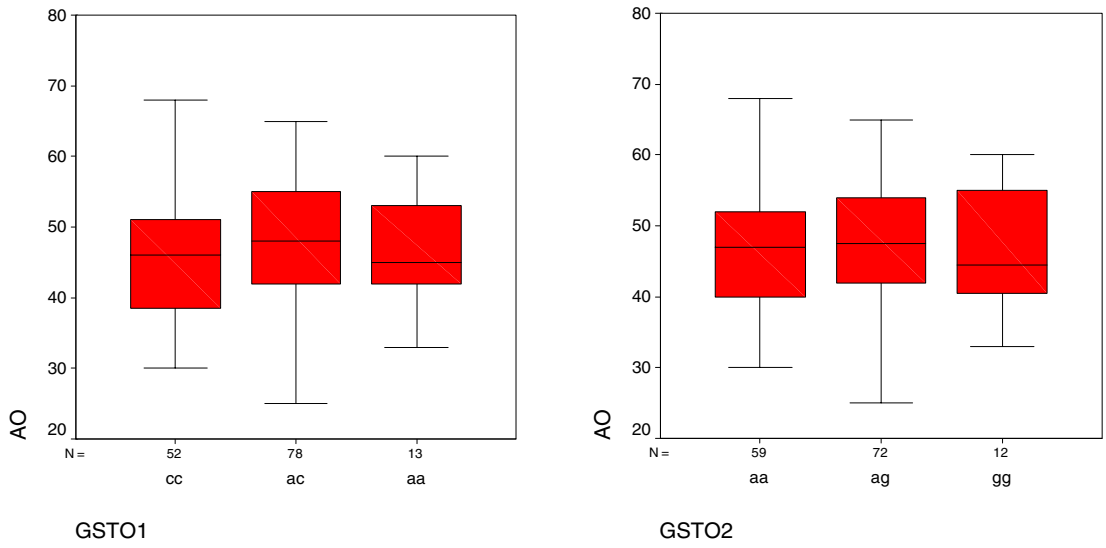

Figure 1
a and b. Relationship between *GSTO* genotypes and AO for 143 HD patients. For each genotype, the median AO is represented as black bar, the quartile is shown as solid box and the range is indicated by the margins. Differences are statistically insignificant.

compromising the axonal transport through trapping of molecules which are essential for cellular function such as chaperons and the proteasomes [*op. cit.*] The AD-causing mutations in the amyloid precursor protein (APP) render valuable clues as to the mechanisms of the disease and point to the relevance of amyloid β-protein (Aβ) toxicity in sporadic AD. In addition, the highly penetrant mutations of early-onset AD and PD implicate either Aβ or its precursor, APP, and α-synuclein as a cause of the toxicity and neuronal loss. The APOE*E4 as a risk factor for sporadic AD has contributed further to this understanding by adding cholesterol and lipid metabolisms to the model for Aβ toxicity [7]. As recently reported GSTO1 may be involved in the activation of interleukin 1 (IL-1β), and thus variation in GSTO1 could alter the efficacy of IL-1β post-translational processing, modulating any inflammatory response [8]. In particular, since IL-1β is induced by Aβ peptide, our results suggest different modifying mechanisms for AO in HD in comparison to AD and PD, the latter two entities being perhaps more closely related to each other in this aspect. This assumption is further substantiated by findings which could not confirm the AO-delaying effect of the ε*4* allele of the *ApoE* genotype in HD [9].

In conclusion, the rs4925 *GSTO1* and rs2297235 *GSTO2* genotypes failed to show any association with the AO of HD. Furthermore, we found no evidence that the expanded and unexpanded CAG repeats interact to influence the AO of HD in the range of 41–45 repeats.

Competing interests
None declared.

Authors' contributions
LA initiated the study, carried out the molecular genetic studies and drafted the manuscript. PJ and SW assisted in data assembly and analysis. JA and CS had ascertained the clinical status of the patients, and JTE participated in the study design, the coordination and finalized the analyses as well as the paper.

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

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