Huntington disease and other polyglutamine diseases : using CAG repeat variations to explain missing heritability

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Chapter 2

AGE OF ONSET IN HUNTINGTON DISEASE

Part 2.1

AGE OF ONSET IN HUNTINGTON'S DISEASE IS INFLUENCED BY CAG REPEAT VARIATIONS IN OTHER POLYGLUTAMINE DISEASE-ASSOCIATED GENES

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Sir,

We read with great interest the recent article by Tezenas du Montcel et al. (2014), who showed that the age of onset in several spinocerebellar ataxias (SCAs) is modulated by CAG repeat sizes in the normal range in other polyglutamine disease-associated genes. Interestingly, the age of onset in patients with SCA3 was also influenced by the CAG repeat size in the *HTT* gene: long normal *HTT* CAG repeat size was associated with a delayed age of onset in SCA3 patients.[1] Similarly, in a subsequent study in patients with SCA3 from mainland China, it was shown that the difference in CAG repeat size between the two *HTT* alleles interacted with the *ATXN3* expansion and affected age of onset in these patients.[2] A CAG repeat expansion in the *HTT* gene is the cause of Huntington's disease, the most common polyglutamine disease worldwide. Like other polyglutamine diseases, the age of onset in Huntington's disease is inversely associated with the CAG repeat expansion size in the mutant allele, which accounts for between 47 and 72% of the variance in age of onset in different Huntington's disease populations.[3] However, there is a wide distribution of age of onset in individuals carrying a mutation with an identical number of CAG repeats, suggesting the existence of other important (epi)genetic and/or environmental factors.[4,5] Given that the age of onset in SCA3 patients was recently found to be influenced by the *HTT* CAG repeat size, we wondered whether the age of onset in patients with Huntington's disease could also be influenced by the CAG repeat size variations in other polyglutamine disease-associated genes (PDAGs), particularly *ATXN3*. Therefore, we assessed the association between the number of CAG repeats in all known PDAGs and age of onset in a large cohort of patients with Huntington's disease.

We obtained clinical data and DNA samples from a subset (n = 1000) of manifest Huntington's disease patients participating in the European Huntington Disease Network REGISTRY study (http://www.euro-hd.net/html/registry). All these participants had entered the study before 21 July 2015. All participants for whom data on age of onset were available and in whom CAG repeat numbers in both alleles of each PDAG could be determined were included in the analyses. Using 10 ng of genomic DNA, two multiplex PCRs were performed in a TProfessional thermocycler (Biometra) with labelled primers flanking the CAG stretch for *ATN1*, *ATXN1*, *ATXN7*, *CACNA1A* and *HTT* in one mix and *AR*, *ATXN2*, *ATXN3*, and *TBP* in a second mix (Biolegio) (primers and PCR conditions are available upon request). Every PCR included a negative control without genomic DNA, a reference sample of CEPH 1347-02 genomic DNA and two positive control samples with predetermined 40 and 47 *HTT* CAG repeats (Applied Biosystems). Repeat size determination was performed by running the PCR products on an ABI 3730/3130 automatic DNA sequencer (Applied Biosystems) and analysing the results with GeneMarker software (version 2.4.0).

To assess whether CAG repeat lengths in PDAGs were associated with age of onset in Huntington's disease, we applied multiple linear regression. Given the known exponential association between age of onset and mutant *HTT* CAG repeat size, the natural logarithmic transformation of age of onset was used as the dependent variable.[6] We modelled the effect of each PDAG on age of onset separately by including its two alleles (with both linear and quadratic terms to account for potential nonlinear effects) as well as their interaction as predictor variables while also adjusting for the effects of sex and CAG repeat sizes in both *HTT* alleles and their interaction.[7] For the *AR* gene only, CAG repeat size in the longer allele was used as males carry only one allele of this X-linked gene. Next, to assess whether the effect of mutant *HTT* CAG repeat size on age of onset was modified by CAG repeat lengths in other PDAG, the interaction between CAG repeat size in the mutant *HTT* allele and CAG repeat size in each of the two alleles of the other PDAG was additionally included. To reduce multicollinearity, particularly with respect to the interaction terms, all continuous predictors were centred around their respective means. To account for the effects of heteroscedasticity and influential points all statistical significance tests were based on robust estimators of standard errors. Moreover, to assure that the results were not unduly affected in case of deviations from model assumptions we also applied a nonparametric method by dividing the group based on median values of each PDAG and comparing differences in age of onset by the non-parametric Mann-Whitney U-test. Given the exploratory nature of this study, no specific correction for multiple comparisons was applied. All tests were two-sided and significance level was set at $p<0.05$. All analyses were performed in SPSS version 23.0 (IBM SPSS Statistics for Windows, IBM Corp).

The mean age of onset was 48.8 with a standard deviation of 12.2 years. The number of assessed samples per gene is summarized in **Supplementary Table 1**. The distribution of CAG repeat lengths followed a unique pattern for each gene and in some cases had a strong preference for a particular range of repeat lengths (**Supplementary Table 1** and **Supplementary Figure 1**). As expected, age of onset was inversely associated with CAG repeat length in the expanded *HTT* allele ($\beta = -0.060$, $p < 0.001$), which accounted for 66.1% of the variation in age of onset in this cohort. Longer CAG repeat size in the larger *ATXN3* allele was associated with a later age of onset in Huntington's disease patients ($\beta = 0.003$, $p = 0.048$). Nonparametric comparison of age of onset between participants with CAG repeat sizes below the median versus those with CAG repeat sizes above the median in the larger *ATXN3* allele confirmed this association (median age of onset: 47.6 versus 50.0 years, $p = 0.025$; **Figure 1**). There was no significant interaction between either of the *ATXN3* alleles and the expanded *HTT* allele ($p \geq 0.20$). However, there was a significant interaction between the CAG repeat size in the expanded *HTT* allele and the larger *CACNA1A* allele ($\beta = -3.87 * 10^{-3}$ and $p = 0.011$ for the interaction effect). Further scrutiny of this interaction revealed that for patients with a below median number of CAG repeats in the expanded *HTT* allele more repeats in the longer *CACNA1A*

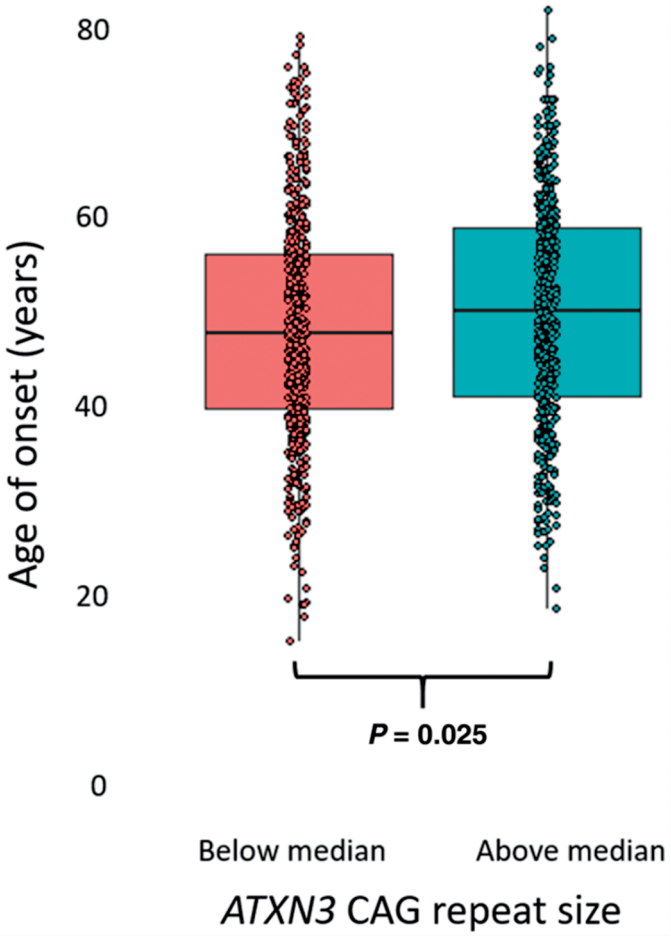

Figure 1. Association between *ATXN3* CAG repeat size and age of onset. Boxplots comparing the age of onset between participants with a below or above median number of CAG repeats in their larger *ATXN3* allele (Mann-Whiney U-test P = 0.025). Black horizontal lines represent medians, boxes display interquartile ranges and whiskers are 1.5 x interquartile range. Circles represent individual patient data with horizontally added jitter.

allele resulted in a later age of onset (median age of onset: 56.1 versus 61.1 years, p = 0.003), while for patients with an above median expansion the *CACNA1A* CAG repeat had little influence on the age of onset (**Figure 2**). There was also a significant interaction between the CAG repeat size in the expanded *HTT* allele and the larger *AR* allele, with a model including a quadratic term for the *AR* CAG repeat size providing the best fit ($\beta = -2.54 * 10^{-4}$ and p = 0.035 for the interaction effect). Comparison of the medians in the total group showed that for patients with a below median number of CAG repeats in the expanded *HTT* allele, more repeats on the longer *AR* allele tended to delay age of onset, while for patients with an above median expansion the longer AR CAG repeats

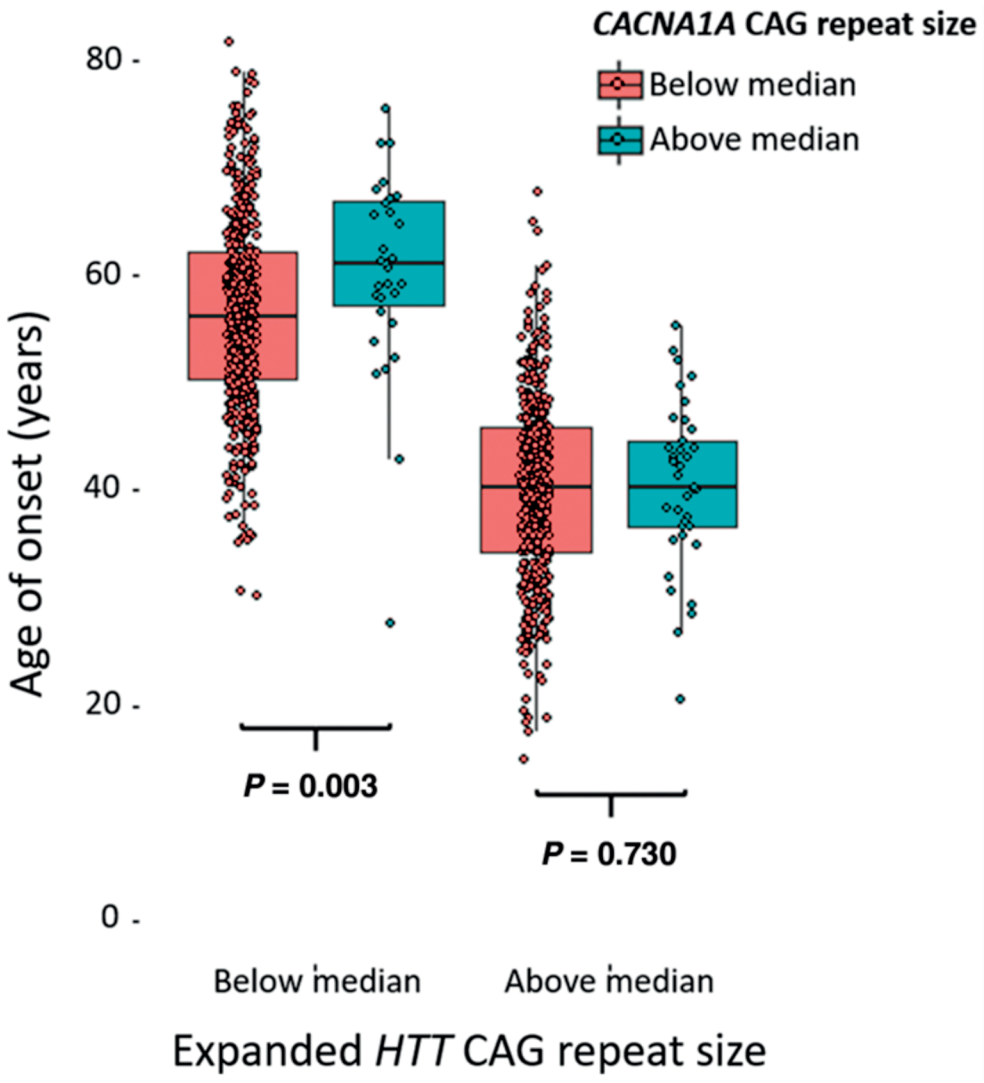

Figure 2. Interaction between the larger *CACNA1A* allele and mutant *HTT* CAG repeat size. Only in patients with mutant *HTT* CAG repeat size below median higher *CACNA1A* CAG repeat size was associated with a higher age-of-onset (Mann-Whiney U-test P = 0.003). Black horizontal lines represent medians, boxes display interquartile ranges and whiskers are 1.5 x interquartile range. Circles represent individual patient data with horizontally added jitter.

tended to advance age of onset (**Supplementary Figure 2**). However, given that *AR* encodes for the androgen receptor, we also performed additional analyses stratified by sex, which demonstrated that the actual effect differed between males and females. In males a longer *AR* allele tended to delay age of onset in subjects with a relatively low expanded *HTT* CAG repeat size (median age of onset: 58.5 versus 55.3 years, p = 0.004), while in females a longer *AR* allele resulted in an earlier age of onset in subjects with

a relatively larger expanded *HTT* CAG repeat size (median age of onset: 39.2 versus 42.1 years, p = 0.009) (**Supplementary Figure 3**). Although regression analysis suggested an effect of CAG repeat size in the smaller alleles of *ATXN7* and *TBP* and a non-linear effect of the longer *ATXN1* CAG repeat size, these effects were statistically non-significant when tested non-parametrically (data not shown).

In conclusion, we found that age of onset in patients with Huntington's disease is modulated by CAG repeat sizes in the normal range of *ATXN3*, *CACNA1A* and *AR*. Our findings extend those of recent reports in SCAs,[1,2,8] and provide further support for the notion that there may be a biological interaction between different PDAGs.[1,9] However, given the exploratory nature of this study, larger studies are needed to confirm these preliminary findings in other cohorts of patients with Huntington's disease.

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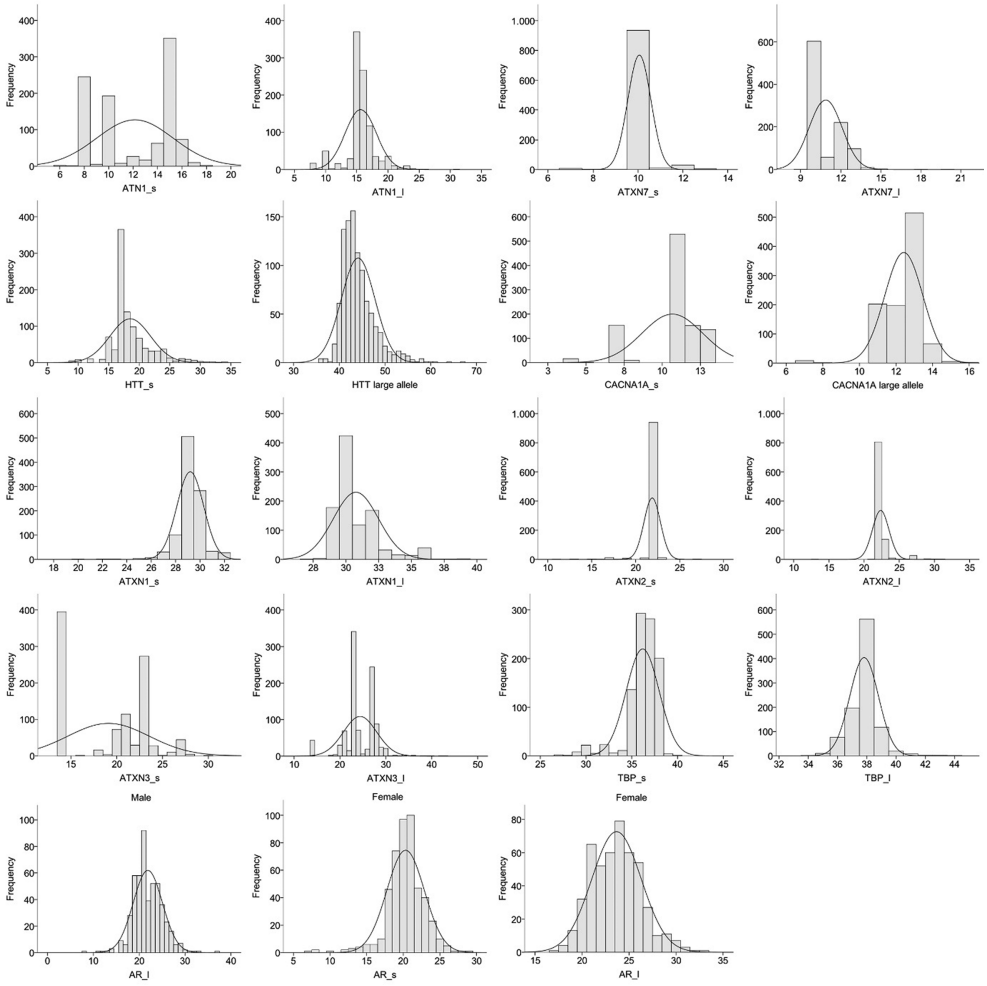
SUPPLEMENTARY MATERIAL

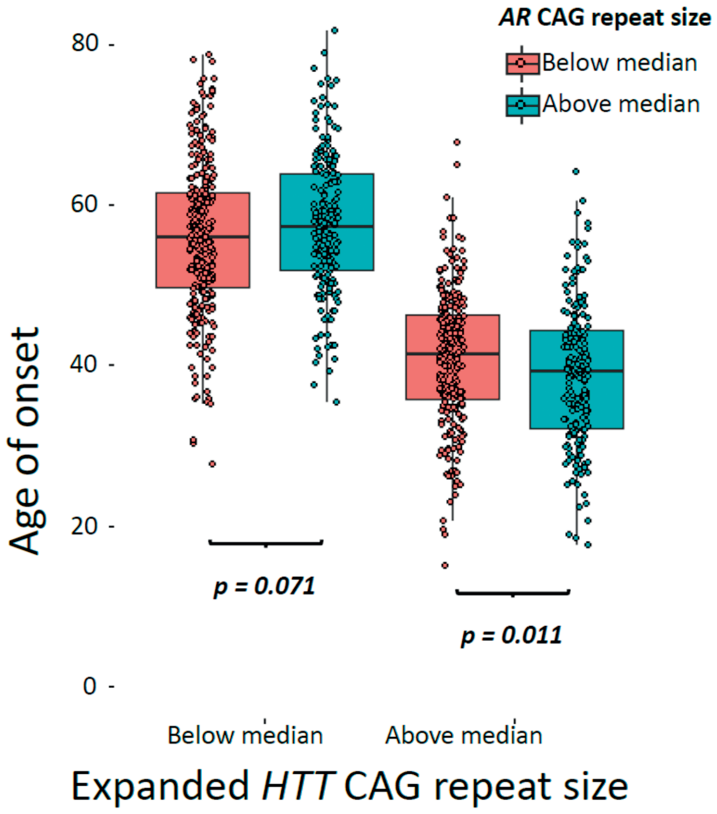
Supplementary Table 1. Distributions of the CAG repeat tracts in polyglutamine disease-associated genes.

PDAG	Allele	n	Mean	Median	Mode	Range
HTT	long	996	44.1 ± 3.7	43	43	36-67
	short	998	18.5 ± 3.3	17	17	9-34
ATN1	long	997	15.6 ± 2.5	16	15	8-31
	short	997	12.1 ± 3.1	13	15	6-18
ATXN7	long	995	10.9 ± 1.2	10	10	9-20
	short	995	10.1 ± 0.5	10	10	7-13
CACNA1A	long	996	12.4 ± 1.0	13	13	7-16
	short	996	10.7 ± 2.0	11	11	4-13
ATXN1	long	996	30.8 ± 1.7	30	30	27-39
	short	996	29.2 ± 1.1	29	29	20-32
ATXN2	long	991	22.4 ± 1.2	22	22	13-31
	short	991	21.9 ± 0.9	22	22	11-27
ATXN3	long	991	24.4 ± 3.6	23	14	14-43
	short	991	19.1 ± 4.4	21	23	14-30
AR	long	990	22.8 ± 3.0	23	21	8-37
	short	990	21.2 ± 3.0	21	21	7-37
TBP	long	995	37.8 ± 1.0	38	38	34-44
	short	995	36.2 ± 1.8	36	36	27-40

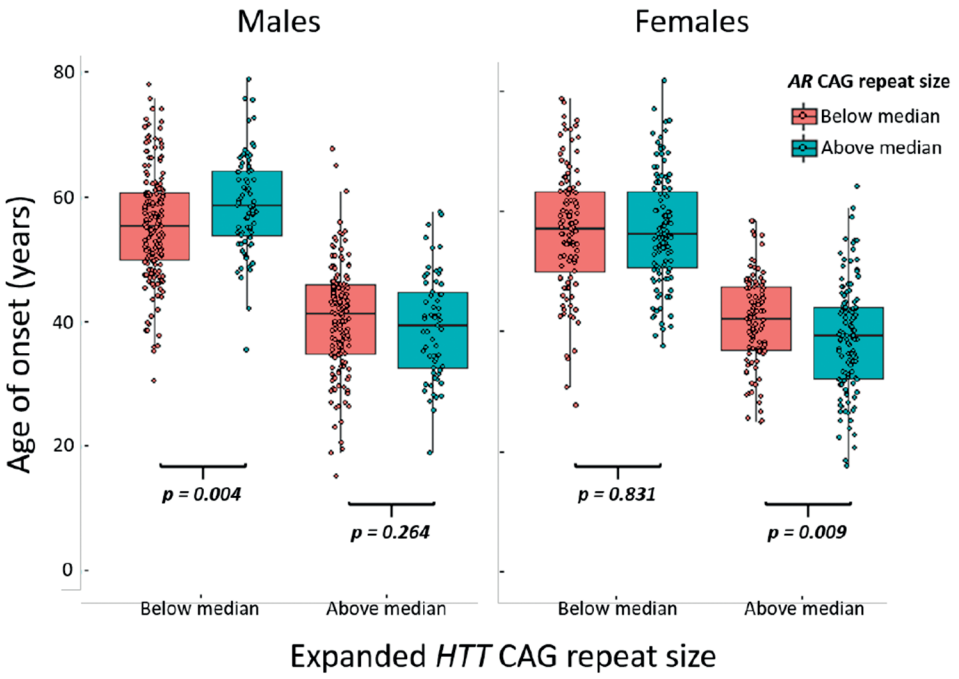
PDAG=polyglutamine disease-associated gene.



Supplementary Figure 1. Distribution patterns of CAG repeat tracts in various polyglutamine disease-associated genes. Bars represent the frequency of a particular CAG repeat size in either the shorter (s) or the longer (l) allele of each gene. Curves represent the hypothetical normal distribution. For the *AR* gene histograms were produced for men and woman separately.



Supplementary Figure 2. Interaction between *AR* and mutant *HTT CAG* repeat size. Longer *AR* CAG repeat size tended to delay age of onset in HD patients with a below median number of CAG repeats in the expanded *HTT* allele (Mann-Whitney U-test p=0.071), while for patients with an above median expansion longer *AR* CAG repeats tended to advance age of onset (Mann-Whitney U-test p=0.011). Black horizontal lines represent medians, boxes display interquartile ranges and whiskers are 1.5 × interquartile range. Circles represent individual patient data with horizontally added jitter.



Supplementary Figure 3. Interaction between *AR* and mutant *HTT CAG* repeat size is sex-specific. In males a longer *AR* CAG repeat size delayed age of onset in subjects with a relatively low expanded *HTT* CAG repeat size (Mann-Whitney U-test p=0.004), while in females a longer *AR* allele resulted in an earlier age of onset in subjects with a relatively larger expanded *HTT* CAG repeat size (Mann-Whitney U-test p=0.009). Black horizontal lines represent medians, boxes display interquartile ranges and whiskers are 1.5 × interquartile range. Circles represent individual patient data with horizontally added jitter.

ADDITIONAL SUPPLEMENTARY MATERIAL

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