PGC-1alpha as modifier of onset age in Huntington disease

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Abstract

Although there is a strong correlation between CAG repeat length and age at onset (AO) of motor symptoms, individual Huntington disease (HD) patients may differ dramatically in onset age and disease manifestations despite similar CAG repeat lengths. This has led to a search for genetic factors that influence AO. In order to identify such a genetic modifier, we analysed polymorphisms in the PGC-1alpha gene. Recent data indicate inhibition of PGC-1alpha function by mutant Htt supporting a link between transcriptional deregulation and mitochondrial dysfunction in HD. In > 400 HD patients, a polymorphism located within intron 2, a potential recombination hot spot, explains a small, but statistically significant, amount of the variability in AO. Our data suggest that PGC-1alpha has modifying effects on the pathogenic process in HD.
link between transcriptional deregulation and mitochondrial dysfunction in HD [17-19]. Altered PGC-1alpha function may, therefore, contribute to HD pathogenesis.

A total of 15 single nucleotide polymorphisms (SNPs) in the peroxisome proliferators-activated receptor γ coactivator 1 α (PPARγC1A) gene (rs2970865, rs2970866, rs4383605, rs2946386, rs2970869, rs17576121, rs2970870, rs7695542, rs2970873, rs2946385, rs12374310, rs7665116, rs2970855, rs2970848, rs8192678) were selected for genotyping in a German HD cohort of more than 400 unrelated patients recruited from the Huntington Center NRW in Bochum. Clinical assessment and determination of the motor AO was performed exclusively by experienced neurologists of the Center. The expanded CAG repeats ranged from 40 to 66 trinucleotide units and AO ranged from 16 to 76 years of age, with a mean of 45 years. HD CAG repeat sizes were determined by polymerase chain reaction using an assay counting the perfectly repeated (CAG)n units. Informed consent was obtained from all patients and controls. The studies were performed in a manner that fully complies with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the relevant university review board.

The polymorphisms were selected from NCBI SNP database due to their potential functional relevance, e.g. polymorphisms in the 5'-UTR were included due to their potential to influence gene expression, and their relative frequency. The genotype distributions of all the chosen polymorphisms were consistent with Hardy-Weinberg equilibrium (HWE).

Controlling for the effect of CAG repeat length on AO revealed an $R^2$ value of 0.729 indicating that nearly 73% of the variation in AO could be explained by the mutation itself (Table 1). In addition to the number of the expanded CAG repeats, the modifying effects of the polymorphisms in PGC-1alpha on the AO were examined.

Here, we saw evidence of association of the rs7665116 SNP. The $R^2$ statistic rose modestly but significantly (from 0.729 to 0.732, $p = 0.025$ in the additive model, TT vs TC vs CC, and from 0.729 to 0.733, $p = 0.012$, in the dominant model, TT vs TC+CC) when rs7665116 genotypes were added to the regression model (Table 1). The mean AO in patients homozygous for the wildtype allele T is 45.08 years of age, while the mean AO for patients homozygous for the C allele is 47.3 years of age. SNP rs2970848 in intron 7 shows a trend towards association, for all other polymorphism no impact on the $R^2$ statistic could be observed.

Figure 1 shows the HapMap $r^2$ values among these SNPs in the HD cohort and reflects the previously reported rough subdivision into 2 main haplotype blocks [20]. The first one includes the polymorphisms in the promoter

### Table 1: Variability in AO attributable to the CAG repeat length was assessed by linear regression using the logarithmically transformed AO as the dependent variable and SNP genotypes as independent variables.

| Model | $R^2$ | $\Delta R^2$ | % additionally explained variance | $P$ value |
|-------|-------|--------------|----------------------------------|-----------|
| HD CAG 40–66 (n = 401) | 0.729 | - | - | $< 0.0005$ |
| **PGC-1alpha Polymorphisms** | | | | |
| rs2970865 | promoter region | - | - | - |
| rs2970866 | promoter region | - | - | - |
| rs4383605 | promoter region | - | - | - |
| rs2946386 | promoter region | - | - | - |
| rs2970869 | promoter region | - | - | - |
| rs17576121 | promoter region | - | - | - |
| rs2970870 | promoter region | - | - | - |
| rs7695542 | promoter region | - | - | - |
| rs2970873 | intron1 | - | - | - |
| rs2946385 | intron2 | - | - | - |
| rs12374310 | intron2 | - | - | - |
| rs7665116 | intron2 | 0.732 | 0.003 | 1.1 | 0.025 |
| rs2970855 | intron5 | 0.733 | 0.004 | 1.5 | 0.012 |
| rs2970848 | intron7 | - | - | - |
| rs8192678 | exon 8 | 0.731 | - | - | 0.054 |

$R^2$ illustrates the relative improvement of the regression model when the genotypes are considered in addition to the CAG repeats; $\Delta R^2$ values quantify these differences. (-) indicates no increase in $R^2$. 

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Figure 1
Schematic representation of the PGC-1alpha gene and linkage disequilibrium (r2) in the analysed HD cohort. Black boxes and a horizontal line represent exons and introns respectively. The numbers in the cells denote the r² between the two SNPs corresponding to the cell. Cell shading indicates strength of r² as shown by the number.

region and intron 1 (rs2970865, rs2970866, rs4383605, rs2946386, rs2970869, rs17576121, rs2970870, rs7695542, rs2970873), whereas the second includes the SNPs located 3' downstream of rs7665116 in intron 2. The two haplotype blocks are separated by a region of high recombination frequency. These results comply with the HapMap database.

The rs7665116 T > C polymorphism is located within intron 2, a potential recombination hot spot. Sequence alignments of multiple species show that this SNP is located at the beginning of a 233 bp highly conserved sequence. Yet, the rs7665116 polymorphism itself is not conserved to any significant extent across species. No other SNPs are described in this conserved sequence.

In silico-analysis of rs7665116 using MatInspector [21] revealed loss of potential binding sites for cAMP-responsive element binding proteins (V$CHOP.01) in case of the C allele as compared to the wildtype T allele. On the other hand, in case of the C allele a new binding site is generated for v-Myb (V$VMYB.02) and X-box binding protein RFX1 (V$RFX1.01). Yet, it can only be speculated that the conserved region around rs7665116 represents a regulatory region controlling constitutive functions of PGC-1alpha. Functional studies are needed to assess whether PGC-1alpha is a true modifier gene and to identify the causal genetic variations contributing in the pathogenesis of HD in this region.

While this manuscript was under review, an article by Weydt et al. was published in this journal showing a modifying effect of PGC-1alpha haploblock 2 variations upon AO in an Italian cohort of 447 unrelated HD patients [22]. Our independent confirmation of their findings in a German cohort strengthens the conclusion that the PGC-1alpha gene appears to have modifying effects on the pathogenic process in HD and that it may be a therapeutically useful target for development of a treatment. Yet, it will be necessary to delineate of the precise basis for the PGC-1alpha modifier effect in order to effectively undertake a search for chemical compounds that delay HD onset.

Abbreviations
PGC-1alpha: peroxisome proliferator-activated receptor gamma co-activator

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
ETF carried out the molecular genetic studies and helped to design the study and draft the manuscript. CS and JA had ascertained the clinical status of the patients. SW interpreted the data and reviewed the manuscript. LA designed the study including statistical analysis and drafted the manuscript.

References
1. The Huntington’s Disease Collaborative Research Group: A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. Cell 1993, 72:971-983.
2. Snell RG, MacMillan JC, Cheadle JP, Fenton I, Lazarou LP, Davies P, MacDonald ME, Gusella JF, Harper PS, Shaw DJ: Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington’s disease. Nat Genet 1993, 4:393-397.
3. Andrew SE, Goldberg YP, Kremer B, Telenius H, Theilmann J, Adam S, Starr E, Squitieri F, Lin B, Kalchman MA, et al.: The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington’s disease. Nat Genet 1993, 4:398-403.
4. Duyao M, Ambrose C, Myers R, Novellotto A, Persichetti F, Frontali M, Falstein S, Ross C, Franz M, Abbott M, et al.: Trinucleotide repeat length instability and age of onset in Huntington’s disease. Nat Genet 1993, 4:387-392.
5. Kehoe P, Krawczak M, Harper PS, Owen MJ, Jones AL: Age of onset in Huntington disease: sex specific influence of apolipoprotein E genotype and normal CAG repeat length. J Med Genet 1999, 36:108-111.
6. The U.S.-Venezuela Collaborative Research Project and Wexler NS: Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington’s disease age of onset. Proc Natl Acad Sci USA 2004, 101:3498-3503.
7. Metzger S, Bauer P, Tomiuk J, Laccone F, Didonato S, Gellera C, Soliverti P, Lange HW, Weirich-Schwaiger H, Wenning GK, et al.: The S18Y polymorphism in the UCHL1 gene is a genetic modifier in Huntington’s disease. Neurogenetics 2006, 7:27-30.
8. Arning L, Saft C, Wieczorek S, Andrich J, Kraus PH, Epplen JT: NR2A and NR2B receptor gene variations modify age at onset in Huntington disease in a sex-specific manner. Hum Genet 2007, 122:175-82.
9. Andresen JM, Gayan J, Cherry SS, Brocklebank D, Alkorta-Aranburu G, Addis EA, Cardon LR, Housman DE, Wexler NS. Replication of twelve association studies for Huntington’s disease residual age of onset in large Venezuelan kindreds. J Med Genet 2007, 44:44-50.

10. Arning L, Monté D, Hansen W, Wieczorek S, jagiello P, Akkad DA, Andrich J, Kraus PH, Saft C, Epplen JT. ASK1 and MAPK6 as modifiers of age at onset in Huntington’s disease. J Mol Med 2008, 86:485-490.

11. Metzger S, Rong J, Nguyen HP, Cape A, Tomiuk J, Soehn AS, Propping P, Freudenberg-Hua Y, Freudenberg J, Tong L, Li SH, Li XJ. Riess O. Huntingtin-associated protein-1 is a modifier of the age-at-onset of Huntington’s disease. Hum Mol Genet 2008, 17:1137-1146.

12. Li JL, Hayden MR, Almqvist EW, Brinkman RR, Durr A, Dode C, Morrison PJ, Suchowiersky O, Ross CA, Margolis RL, et al. A genome scan for modifiers of age at onset in Huntington disease: The HD MAPS study. Am J Hum Genet 2003, 73:682-687.

13. Li JL, Hayden MR, Warby SC, Durr A, Morrison PJ, Nance M, Ross CA, Margolis RL, Rosenblatt A, Squitieri F, et al. Genome-wide significance for a modifier of age at neurological onset in Huntington’s disease at 6q23-24: the HD MAPS study. BMC Med Genet 2006, 7:71.

14. Gayán J, Brocklebank D, Andresen JM, Alkorta-Aranburu G, US-Venezuela Collaborative Research Group, Zameel Cader M, Roberts SA, Cherry SS, Wexler NS, Cardon LR, Housman DE. Genomewide linkage scan reveals novel loci modifying age of onset of Huntington’s disease in the Venezuelan HD kindreds. Genet Epidemiol 2008, 32:445-453.

15. Gil JM, Rego AC. Mechanisms of neurodegeneration in Huntington’s disease. Eur J Neurosci 2008, 27:2803-2820.

16. Pugg Kushner P, Spiegelman BM. Peroxisome proliferator-activated receptor-γ coactivator 1a (PGC-1a): transcriptional coactivator and metabolic regulator. Endocr Rev 2003, 24:78-90.

17. Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell 2006, 127:59-69.

18. St-Pierre J, Drori S, Ulery M, Silvaggi JM, Rhee J, Jäger S, Handschin C, Zheng K, Lin J, Yang W, Simon DK, Bachoo R, Spiegelman BM. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. Cell 2006, 127:397-408.

19. Weydt P, Pineda VV, Torrence AE, Libby RT, Satterfield TF, Lazarowski ER, Gilbert ML, Morton GJ, Bamminger TK, Strand AD, Cui L, Beyer RP, Esley CN, Smith AC, Krainc D, Luquet S, Sweet IR, Schweitz MW, La Spada AR. Thermoregulatory and metabolic defects in Huntington’s disease transgenic mice implicate PGC-1alpha in Huntington’s disease neurodegeneration. Cell Metab 2006, 4:349-62.

20. Iglseder B, Oberkofler H, Felder TK, Klein K, Paulweber B, Krempler F, Tregouet DA, Patsch W. Associations of PPARGCA1 haplotypes with plaque score but not with intima-media thickness of carotid arteries in middle-aged subjects. Stroke 2006, 37:2260-2265.

21. Werner T. Computer-assisted analysis of transcription control regions. Matinspector and other programs. Methods Mol Biol 2000, 132:337-349.

22. Weydt P, Soyal S, Gellera C, DiDonato S, Wellingering C, Oberkofler H, Landwehrmeyer B, Patsch W. The gene coding for PGC-1alpha modifies age at onset in Huntington’s Disease. Molecular Neurodegeneration 2009, 4:3.