NMDA receptor gene variations as modifiers in Huntington disease: a replication study

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Carsten Saft, Jorgtepplen, Stefan Wieczorek, G. Bernhard Landwehrmeyer, Raymund A.C. Roos, Justo Garcia de Yebenes, Matthias Dose, Sarah J Tabrizi, David Craufurd, the REGISTRY investigators of the European Huntington's Disease Network
Larissa Arning

Abstract

Several candidate modifier genes which, in addition to the pathogenic CAG repeat expansion, influence the age at onset (AO) in Huntington disease (HD) have already been described. The aim of this study was to replicate association of variations in the N-methyl D-aspartate receptor subtype genes GRIN2A and GRIN2B in the “REGISTRY” cohort from the European Huntington Disease Network (EHDN). The analyses did replicate the association reported between the GRIN2A rs2650427 variation and AO in the entire cohort. Yet, when subjects were stratified by AO subtypes, we found nominally significant evidence for an association of the GRIN2A rs1969060 variation and the GRIN2B rs1806201 variation. These findings further implicate the N-methyl D-aspartate receptor subtype genes as loci containing variation associated with AO in HD.

Introduction

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder characterised by motor disturbances, cognitive decline, and neuropsychiatric symptoms. It is caused by a CAG repeat expansion (>36 repeats) in exon 1 of the HTT gene. [1] The lengths of the expanded CAG tract is inversely related to the age at clinical onset of HD, accounting for more than half of the overall variance in age at onset (AO). [2] Despite this strong correlation, there remains considerable variation of over 40 years in AO in individuals with identical repeat lengths. Several candidate modifier genes of HD have already been described in independent studies. [3] [4] [5] [6] [7] [8] [9] In order to confirm the associations between modifier gene variations and AO, independent replication studies are compulsory. Here, we tested the primary hypothesis of an original study[4], that variations in the NR2A and NR2B glutamate receptor subunit genes (GRIN2A, GRIN2B) explain additional variance in AO for HD.

Methods

The study cohort comprised 1,211 individuals of European ancestry with HD collected by the EHDN “REGISTRY” study prior to October 14, 2008. “REGISTRY” is a multi-centre, multi-national observational study which aims to obtain natural history data on a wide spectrum of the European HD population (http://www.euro-hd.net/html/registry).[10] In order to test previously reported HD genetic modifiers in this cohort, HD patients with available data on age, sex, age at symptom onset, mutant CAG repeat size and body mass index (BMI) were included (initial n = 1211; n = 1069; 529 men and 540 women had a complete data set).

The expanded trinucleotide repeats ranged from 40 to 89 with a mean (± SD) of 45±4.7 CAGs, and AO ranged from 6 to 74 years, with an onset (mean ± SD) of 42 ±11.8 years. AO was defined as the age at which, according to the rater, the first signs of HD appeared. Five hundred and thirty-eight patients first presented with motor disturbances (mean ± SD motor AO = 43.4±11.6 years), 241 with psychiatric problems (mean ± SD psychiatric AO = 39.9±10.8 years), and 112 with cognitive decline (mean ± SD cognitive AO = 38.6±13.1 years). For the remaining patients no specific symptoms were listed (mean ± SD AO = 42.1±11.8 years). Genotyping of three SNPs was conducted as described before.[4]

Results

None of the SNPs deviated from Hardy–Weinberg Equilibrium (HWE). Considering the earliest AO (n = 1,069), we did find evidence of association of the GRIN2A SNP rs2650427 (table 1). The R² statistic rose modestly (from 0.634 to 0.635) but significantly (p=0.028) when GRIN2A genotypes were added to the regression model. The analysis did not, however, replicate the association reported between the SNP rs1969060 in intron 2 of the GRIN2A gene and SNP C2664T (rs1806201) in exon 12 of the GRIN2B gene (table 1); but when dividing the cohort according to the nature of the symptoms presented initially, both the GRIN2B C2664T and the GRIN2A rs1969060 polymorphisms explained a small but considerable amount of additional variance.
in residual AO in the respective samples. Inclusion of the GRIN2B genotypes in the model for motor AO (n = 538) increased the \( R^2 \) statistic from 0.620 to 0.623 (p=0.046) and in the study of 241 patients with psychiatric AO, the \( R^2 \) statistic of the exponential regression rose from 0.515 to 0.523 with the GRIN2A rs1969060 genotypes included (p=0.026, table 1).

Interestingly, the association of cognitive AO (n = 112) with the GRIN2A rs2650427 polymorphism shows the highest nominal significance as compared to the other models in the study (0.770 to 0.775, p=0.014). Yet, the results remain statistically significant when excluding the patients with CAGs over >70 (n=4).

<table>
<thead>
<tr>
<th>Model</th>
<th>Genotypes</th>
<th>CAG mean ± SD</th>
<th>Most recent AO mean ± SD</th>
<th>( R^2 )</th>
<th>( P ) value</th>
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<td>HD CAG 40-89 (n=1069)</td>
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<th>Genotypes</th>
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<th>Motor AO mean ± SD</th>
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<th>( P ) value</th>
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<th>Psychiatric AO mean ± SD</th>
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<th>( P ) value</th>
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<td>HD CAG 40-67 (n=241)</td>
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<td>+ GRIN2A rs1969060</td>
<td>TT (n=172)</td>
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<td>TC (n=63)</td>
<td>44.01±3.7</td>
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In order to control the effect of sex-specific associations, we further analysed each combination of genotype with sex, but there was no trend towards significance. Moreover, on average, psychiatric and cognitive symptoms significantly predate clinical motor onset by 3.5 and 4.8 years ($p<0.001$), thus confirming that affective and cognitive symptoms could be early manifestations of neuronal dysfunction.

**Discussion**

Of the three polymorphisms tested, GRIN2A rs2650427 showed the most consistent evidence of replication in the EHDN Registry study sample. This is in accordance with another replication study in the large set of kindreds from Venezuela, where GRIN2A variation also explained a small but considerable amount of additional variance in residual AO.[5]

Yet, the interpretation of the association of cognitive AO with the GRIN2A rs2650427 polymorphism should be considered with caution since the sample size of this subgroup (n=112) is too small to provide the statistical power required.

Unfortunately, none of the SNPs associated has been validated functionally and it is most likely that the polymorphisms analysed are not the functional variations, but represent markers in linkage disequilibrium with variations that modify the AO. Although, synonymous SNPs like GRIN2B rs1806201 might be pathogenetically relevant via influencing mRNA splicing, protein stability and structure.

The failure to replicate the sex-specific effect of rs1806201 suggests that the original observation may have been false positive, emphasizing the need for stringent statistical thresholds. On the other hand, since linkage disequilibrium is not uniform across populations, the mixed ancestry in the EHDN REGISTRY study sample could account for heterogeneous results. Inconsistent results may also occur because of difficulties in exact AO definitions. The data stresses the need for precise phenotyping in order to reduce heterogeneity, and to facilitate the discovery of clinically relevant biological pathways.

**Table 1** The variability in AO attributable to the CAG repeat length was assessed by linear regression using the logarithmically transformed AO as the dependent variable and GRIN genotypes as independent variables. *R^2* illustrates the relative improvement of the regression model, when the genotypes are considered in addition to the CAG repeats.

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Although the associations replicated explain only a small fraction of the variance of AO, the observed correlations with HD phenotypes demonstrate that GRIN2A and GRIN2B remain promising candidate genes, worth to be studied further in more detail.

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Correspondence to Dr Larissa Arning, Ruhr-University, Department of Human Genetics, Universitätsstr. 150, MA5/39, 44801 Bochum, Germany, larissa.arning@rub.de

Competing interests
The authors have declared that no competing interests exist.

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Ethics approval
This study was conducted with the approval of the local ethics committee of the different clinical centres.

Expansion of Collaborator List
Investigators of the European Huntington’s Disease Network
K Barth, Language coordinator
M Bascuñana Garde, Language coordinator
R Bos, Language coordinator
D Ecker, Language coordinator
OJ Handley, Language coordinator
N Heinonen, Language coordinator
C Held, Language coordinator
M Laurà, Language coordinator
A Martínez Descals, Language coordinator
T Mestre, Language coordinator
D Monza, Language coordinator
J Naji, Language coordinator
M Orth, Language coordinator
H Padieu, Language coordinator
S Pro Koivisto, Language coordinator
A Rialland, Language coordinator
P Sasinková, Language coordinator
P Trigo Cubillo, Language coordinator
M van Walsem, Language coordinator
Sigrid Botne Sando, St. Olavs Hospital, Trondheim, Norway

Jarosław Slawek, Specialist Hospital, Gdansk Zaspa, Gdansk, Poland

Witold Soltan, Specialist Hospital, Gdansk Zaspa, Gdansk, Poland

Emilia Sitek, Specialist Hospital, Gdansk Zaspa, Gdansk, Poland

Magdalena Boczarzewska-Jedynak, Silesian Medical University Katowice, Poland

Barbara Jasinska-Myga, Silesian Medical University Katowice, Poland

Gregorz Opala, Silesian Medical University Katowice, Poland

Andrzej Szczudlik, Krakowska Akademia Neurologii, Krakow, Poland

Monika Rudzińska, Krakowska Akademia Neurologii, Krakow, Poland

Magdalena Wójcik, Krakowska Akademia Neurologii, Krakow, Poland

Krzysztof Banaszkiewicz, Krakowska Akademia Neurologii, Krakow, Poland

Małgorzata Krawczyk, Krakowska Akademia Neurologii, Krakow, Poland

Daniel Zielonka, Medical University of Pozna?, Poland

Jerzy Marcinkowski, Medical University of Pozna?, Poland

Anna Ciesielska, Medical University of Pozna?, Poland

Justyna Sempo?owicz, Medical University of Pozna?, Poland

Anna Bryl, Medical University of Pozna?, Poland

Aneta Klimberg, Medical University of Pozna?, Poland

Piotr Janik, Medical University of Warsaw, Neurology, Warsaw-MU, Poland

Anna Kalbarczyk, Medical University of Warsaw, Neurology, Warsaw-MU, Poland

Hubert Kwiecinski, Medical University of Warsaw, Neurology, Warsaw-MU, Poland

Zygmunt Jamro?ik, Medical University of Warsaw, Neurology, Warsaw-MU, Poland

Grzegorz Witkowski, Institute of Psychiatry and Neurology Dep. of Genetics, Dep. of Neurology, Warsaw-IPiN, Poland

Danuta Ryglewicz, Institute of Psychiatry and Neurology Dep. of Genetics, Dep. of Neurology, Warsaw-IPiN, Poland

Jakub Antczak, Institute of Psychiatry and Neurology Dep. of Genetics, Dep. of Neurology, Warsaw-IPiN, Poland

Maria Rakowicz, Institute of Psychiatry and Neurology Dep. of Genetics, Dep. of Neurology, Warsaw-IPiN, Poland

Katarzyna Jachinska, Institute of Psychiatry and Neurology Dep. of Genetics, Dep. of Neurology, Warsaw-IPiN, Poland

Elżbieta Zdzienicka, Institute of Psychiatry and Neurology Dep. of Genetics, Dep. of Neurology, Warsaw-IPiN, Poland

Przemysław Richter, Institute of Psychiatry and Neurology Dep. of Genetics, Dep. of Neurology, Warsaw-IPiN, Poland

Jacek Zaremba, Institute of Psychiatry and Neurology Dep. of Genetics, Dep. of Neurology, Warsaw-IPiN, Poland

Miguel Coelho, Neurological Clinical Research Unit, Institute of Molecular Medicine, Lisbon-Santa Maria, Portugal

Joaquim J Ferreira, Neurological Clinical Research Unit, Institute of Molecular Medicine, Lisbon-Santa Maria, Portugal

Tiago Mestre, Neurological Clinical Research Unit, Institute of Molecular Medicine, Lisbon-Santa Maria, Portugal

Mário M Rosa, Neurological Clinical Research Unit, Institute of Molecular Medicine, Lisbon-Santa Maria, Portugal

Anabela Valadas, Neurological Clinical Research Unit, Institute of Molecular Medicine, Lisbon-Santa Maria, Portugal

Miguel Gago, Hospital São João E.P.E., Porto-São João, Portugal

Carolina Garrett, Hospital São João E.P.E., Porto-São João, Portugal

Maria Rosalia Guerra, Hospital São João E.P.E., Porto-São João, Portugal
Colin Bourne, Leicestershire Partnership Trust, Mill Lodge, Leicester, UK
Carole Clayton, Leicestershire Partnership Trust, Mill Lodge, Leicester, UK
Heather Dipple, Leicestershire Partnership Trust, Mill Lodge, Leicester, UK
Jackie Clapton, Leicestershire Partnership Trust, Mill Lodge, Leicester, UK
Janet Grant, Leicestershire Partnership Trust, Mill Lodge, Leicester, UK
Diana Gross, Leicestershire Partnership Trust, Mill Lodge, Leicester, UK
Caroline Hallam, Leicestershire Partnership Trust, Mill Lodge, Leicester, UK
Julia Middleton, Leicestershire Partnership Trust, Mill Lodge, Leicester, UK
Ann Murch, Leicestershire Partnership Trust, Mill Lodge, Leicester, UK
Dawn Patino, Leicestershire Partnership Trust, Mill Lodge, Leicester, UK
Thomasin Andrews, The National Hospital for Neurology and Neurosurgery, London, UK
Stefania Bruno, The National Hospital for Neurology and Neurosurgery, London, UK
Elvina Chu, The National Hospital for Neurology and Neurosurgery, London, UK
Karen Doherty, The National Hospital for Neurology and Neurosurgery, London, UK
Nayana Lahiri, The National Hospital for Neurology and Neurosurgery, London, UK
Marianne Novak, The National Hospital for Neurology and Neurosurgery, London, UK
Aakta Patel, The National Hospital for Neurology and Neurosurgery, London, UK
Sarah Tabrizi, The National Hospital for Neurology and Neurosurgery, London, UK
Rachel Taylor, The National Hospital for Neurology and Neurosurgery, London, UK
Thomas Warner, The National Hospital for Neurology and Neurosurgery, London, UK
Edward Wild, The National Hospital for Neurology and Neurosurgery, London, UK
Natalie Arran, Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
David Craufurd, Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Ruth Fullam, Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Liz Howard, Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Susan Huson, Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Lucy Partington-Jones, Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Nichola Verstraelen (formerly Ritchie), Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Julie Snowden, Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Andrea Sollom, Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Cheryl Stopford, Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Jennifer Thompson, Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
References


