Neurogenetics (2013) 14:173–179
DOI 10.1007/s10048-013-0364-y

ORIGINAL ARTICLE

# Candidate glutamatergic and dopaminergic pathway gene variants do not influence Huntington's disease motor onset

**Eliana Marisa Ramos · Jeanne C. Latourelle · Tammy Gillis · Jayalakshmi S. Mysore · Ferdinando Squitieri · Alba Di Pardo · Stefano Di Donato · Cinzia Gellera · Michael R. Hayden · Patrick J. Morrison · Martha Nance · Christopher A. Ross · Russell L. Margolis · Estrella Gomez-Tortosa · Carmen Ayuso · Oksana Suchowersky · Ronald J. Trent · Elizabeth McCusker · Andrea Novelletto · Marina Frontali · Randi Jones · Tetsuo Ashizawa · Samuel Frank · Marie-Helene Saint-Hilaire · Steven M. Hersch · Herminia D. Rosas · Diane Lucente · Madaline B. Harrison · Andrea Zanko · Ruth K. Abramson · Karen Marder · James F. Gusella · Jong-Min Lee · Isabel Alonso · Jorge Sequeiros · Richard H. Myers · Marcy E. MacDonald**

Received: 26 March 2013 / Accepted: 18 April 2013 / Published online: 4 May 2013


**Abstract** Huntington's disease (HD) is a neurodegenerative disorder characterized by motor, cognitive, and behavioral disturbances. It is caused by the expansion of the *HTT* CAG repeat, which is the major determinant of age at onset (AO) of motor symptoms. Aberrant function of *N*-methyl-D-aspartate receptors and/or overexposure to dopamine has been suggested to cause significant neurotoxicity, contributing to HD pathogenesis. We used genetic association analysis in 1,628 HD patients to evaluate candidate polymorphisms in *N*-methyl-D-aspartate receptor subtype genes (*GRIN2A* rs4998386 and rs2650427, and *GRIN2B* rs1806201) and functional polymorphisms in genes in the dopamine

E. M. Ramos · T. Gillis · J. S. Mysore · D. Lucente · J. F. Gusella · J.-M. Lee · M. E. MacDonald (✉)
Center for Human Genetic Research, Massachusetts General Hospital, Simches Research Building, Room 5414, 185 Cambridge Street,
Boston, MA 02114 USA
e-mail: macdonam@helix.mgh.harvard.edu

E. M. Ramos · I. Alonso · J. Sequeiros
UnIGENe, IBMC—Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal

J. C. Latourelle · S. Frank · M.-H. Saint-Hilaire · R. H. Myers
Department of Neurology, Boston University School of Medicine, Boston, MA 02118 USA

F. Squitieri · A. Di Pardo
Centre for Neurogenetics and Rare Diseases, IRCCS Neuromed, 86077 Pozzilli, IS, Italy

S. Di Donato · C. Gellera
Fondazione IRCCS Istituto Neurologico Carlo Besta, Via Celoria 11,
20133 Milan, Italy

M. R. Hayden
Center for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, BC V5Z 4H4 Canada

P. J. Morrison
Regional Medical Genetics Centre, Belfast HSC Trust, Belfast BT9 7AB UK

M. Nance
Hennepin County Medical Center, 701 Park Avenue, Minneapolis, MN 55415 USA

C. A. Ross · R. L. Margolis
Department of Psychiatry and Behavioral Sciences, Johns Hopkins University, Baltimore, MD 21287 USA

E. Gomez-Tortosa
Department of Neurology, Fundación Jiménez Diaz, Madrid 28040 Spain

C. Ayuso
Department of Genetics, IIS-Fundación Jiménez Diaz, CIBERER, Madrid 28040 Spain

O. Suchowersky
Department of Medicine, University of Alberta, Edmonton, Alberta T6G 2B7 Canada

R. J. Trent · E. McCusker
Sydney Medical School, University of Sydney, Sydney, NSW 2006 Australia

pathway (*DAT1* 3′ UTR 40-bp variable number tandem repeat (VNTR), *DRD4* exon 3 48-bp VNTR, *DRD2* rs1800497, and *COMT* rs4608) as potential modifiers of the disease process. None of the seven polymorphisms tested was found to be associated with significant modification of motor AO, either in a dominant or additive model, after adjusting for ancestry. The results of this candidate-genetic study therefore do not provide strong evidence to support a modulatory role for these variations within glutamatergic and dopaminergic genes in the AO of HD motor manifestations.

**Keywords** Huntington's disease · Glutamate receptors · Dopamine pathway · Genetic modifiers

## Introduction

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder, usually of adult onset, characterized by involuntary choreiform movements, cognitive impairment, and behavioral changes. HD is caused by the expansion of an unstable polymorphic CAG repeat in *HTT* [1]. Age at onset (AO) of diagnostic clinical symptoms is inversely correlated with the size of the expanded CAG repeat. It explains about 50–70 % of the variance in motor AO [2–4], while the remainder is highly heritable, strongly implying the existence of genetic factors that modulate the rate of the pathogenic process that leads to onset of symptoms [5–7].

The neuropathological changes that comprise the pathological grading system for HD are found in the striatum, where there is a selective and progressive neuronal loss of medium spiny neurons (MSNs) [8, 9]. Glutamatergic and dopaminergic pathways are well known to regulate striatal neuronal function by interacting and modulating each other, suggesting that both glutamate and dopamine receptors may act coordinately in causing deregulation of calcium homeostasis [10–12] with consequent mitochondrial depolarization and caspase activation [13, 14]. Both pathways have been implicated in HD pathogenesis, suggesting that variation in function or expression of glutamate receptor subunits and/or dopamine pathway genes might modulate excitotoxic cell death, thereby modulating AO of symptoms. Indeed, polymorphisms within the genes that encode the NR2A and NR2B glutamate receptors (*GRIN2A* and *GRIN2B*) have been implicated in genetic studies with HD patients as potential modifiers of clinical AO [15–18].

Based upon these observations, the aim of the present study was to utilize common and multi-allele functional polymorphisms to test the possibility that genetic variation in genes of the glutamatergic (*GRIN2A* and *GRIN2B*) and dopaminergic (*COMT*, *DRD2*, *DRD4*, and *DAT1*) pathways may explain some of the variation in AO of HD motor manifestations, in a large and well-described cohort of 1,628 HD patients of European ancestry.

E. McCusker
Department of Neurology, Westmead Hospital, Westmead, Sydney, NSW 2145 Australia

A. Novelletto
Department of Biology, University Tor Vergata, 00133 Rome, Italy

M. Frontali
Institute of Translational Pharmacology, CNR, 00133 Rome, Italy

R. Jones
Department of Neurology, Emory University, Atlanta, GA 30329 USA

T. Ashizawa
Department of Neurology, University of Florida, Gainesville, FL 32610 USA

S. M. Hersch · H. D. Rosas
MIND, Massachusetts General Hospital, 114 16th Street, Charlestown, MA 02129 USA

M. B. Harrison
Department of Neurology, University of Virginia, Charlottesville, VA 22908 USA

A. Zanko
Department of Pediatrics, University of California, San Francisco, CA 94143 USA

R. K. Abramson
Department of Neuropsychiatry and Behavioral Science, University of South Carolina School of Medicine, Columbia, SC 29208 USA

K. Marder
College of Physicians and Surgeons, Columbia University, New York, NY 10032 USA

J. F. Gusella · J.-M. Lee · M. E. MacDonald
Harvard Medical School and Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA 02142 USA

I. Alonso · J. Sequeiros
CGPP, IBMC—Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal

I. Alonso · J. Sequeiros
ICBAS—Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal

P. J. Morrison
University of Ulster, Cromore Road, Coleraine BT52 15A UK

O. Suchowersky
Department Medical Genetics, University of Alberta, Edmonton, Alberta T6G 2B7 Canada

## Material and methods

*Subjects* We analyzed 1,628 DNA samples from HD patients participating in research from collaborating investigators (HD-MAPS), the HD observational study COHORT, the Harvard Tissue Resource Center Bank (McLean's Hospital, Belmont, MA) and the National Neurological Research Bank (VAMC Wadsworth Division, Los Angeles, CA). Our cohort comprises a well-described set of HD samples [19] with CAG repeat sizes ranging from 40 to 53 repeats, known motor AO, ancestry, and familial relationship.

*Genotyping* Repeat sizes of the *HTT* CAG alleles and *DAT1* and *DRD4* variable number tandem repeats (VNTRs) were determined using previously established polymerase chain reaction (PCR) amplification assays [20–22]. The size of the products was determined using the ABI PRISM 3730xl automated DNA Sequencer (Applied Biosystems, Foster City, CA) and GeneMapper version 3.7 software. Genotyping of the polymorphisms in *GRIN2A* (rs4998386 and rs2650427), *GRIN2B* (rs1806201), *COMT* (rs4680), and *DRD2* (rs1800497) was performed by real-time PCR using commercially available TaqMan Genotyping probes (Applied Biosystems, Foster City, CA) and carried out on the LightCycler® 480 (Roche Diagnostics, Mannheim), following the manufacturer's instructions.

*Statistical analysis* Multivariate analyses were conducted using generalized estimating equations (GEE) to assess the association of the different polymorphisms with residual HD motor onset, adjusting for familial component and ancestry. The weighted GEE was computed assuming an independent correlation structure and using the robust estimator of the variance to account for familial relationships. All statistical analyses were performed using PASW Statistics (version 18).

## Results

*Association with GRIN2A and GRIN2B* The genetic evidence supporting a role for *GRIN2A* or *GRIN2B* in modulating AO of HD symptoms is equivocal. A candidate gene study with 167 German HD patients reported an association between AO and rs1969060 in *GRIN2A* and two polymorphisms in *GRIN2B* (rs1806201 and rs890) [17]. However, in a follow-up study, the same authors found that two other SNPs, rs8057394 and rs2650427, in *GRIN2A* had a stronger association with AO [16]. A subsequent study in 1,211 European HD individuals found an association of *GRIN2A* rs2650427, and when stratified by AO subtypes, they found a nominally significant association with rs1969060 (*GRIN2A*) and rs1806201 (*GRIN2B*) [15]. On the other hand, in a Venezuelan sample, no evidence was found for the *GRIN2B* polymorphisms, and a weak association was found for *GRIN2A* rs1969060 [18]. We attempted to replicate the apparent genetic association of the polymorphisms with the greatest evidence of association with HD AO, namely rs2650427 in *GRIN2A* and rs1806201 in *GRIN2B*, as well as an interesting *GRIN2A* polymorphism associated with decreased Parkinson's disease (PD) risk in conjunction with heavy coffee consumption (rs4998386) [23]. However, the results of association analysis for each polymorphism failed to demonstrate significant association with HD motor AO (Table 1).

*Association with dopamine pathway genes* Dopamine pathway genes have not previously been assessed as genetic HD AO modifiers. Therefore, we tested functional polymorphisms in *DRD2* and *DAT1* believed to affect neurotransmission primarily in the striatum, in addition to polymorphisms in *COMT* and *DRD4*, known to have an impact on the frontal cortex function. The Val158Met *COMT* polymorphism has been shown to affect, in a co-dominant mode, the activity level of the COMT enzyme that metabolizes dopamine [24–26]. The TaqIA polymorphism in the vicinity of *DRD2* is reported to be a genetic marker for D2 receptor density in the brain, with the minor allele being associated with a lower density of this receptor especially in the striatum [27–30]. However, the results of our genetic analysis failed to reveal significant evidence of association of the functional polymorphism in *DRD2* or in *COMT* with HD motor AO (Table 1).

We then evaluated the *DRD4* gene, as it has been suggested that different repeat sequences of the multi-allele 48-bp VNTR in *DRD4* may differentially affect the gene's expression and consequently alter D4 receptor density in the brain. The seven-repeat allele had a lower expression compared with two- and four-repeat alleles [31]. Given this observation, our analysis specifically tested the potential association of the seven-repeat allele with motor HD AO. The results demonstrated that the presence of this allele did not explain any variance of AO in our cohort of HD patients (Table 1).

We also assessed the *DAT1* gene by evaluating the multi-allele 40-bp VNTR polymorphism. This polymorphism was chosen because it has been reported that individuals with 10/10 repeats have lower dopamine transporter density than individuals with at least one copy of the nine-repeat allele who exhibit more effective dopamine removal at the synapse [32, 33]. However, despite evidence for biological effects, the results of our analysis did not reveal a significant association of the ten-repeat allele with HD motor AO (Table 1).

## Discussion

The circuitry of the striatum, where MSNs are particularly vulnerable to the effects of the HD mutation [8, 9], has

**Table 1** Multivariate correlation of the polymorphisms in the glutamatergic (*GRIN2A* and *GRIN2B*) and dopaminergic (*COMT*, *DRD2*, *DRD4*, and *DAT1*) pathway genes with residual age at motor onset

| Gene | Polymorphism | Number of samples | Dominant model | | Additive model | |
|---|---|---|---|---|---|---|
| | | | Standardized coefficient | *p* value[a] | Standardized coefficient | *p* value[a] |
| Glutamatergic pathway | | | | | | |
| *GRIN2A* | rs4998386 | 1,585 | 0.087 | 0.108 | 0.074 | 0.144 |
| | rs2650427 | 1,619 | −0.014 | 0.739 | 0.004 | 0.878 |
| *GRIN2B* | rs1806201 | 1,602 | −0.056 | 0.164 | −0.060 | 0.053 |
| Dopaminergic pathway | | | | | | |
| *COMT* | rs4680 | 1,620 | −0.047 | 0.222 | 0.025 | 0.333 |
| *DRD2* | rs1800497 | 1,625 | 0.051 | 0.196 | 0.035 | 0.326 |
| *DRD4* | Exon 3 48-bp VNTR | 1,527 | −0.043 | 0.322 | −0.035 | 0.322 |
| *DAT1* | 3′ UTR 40-bp VNTR | 1,614 | 0.062 | 0.363 | 0.022 | 0.487 |

[a] *p* values were derived using GEE to account for familial relationships and ancestry

provided a rich source of candidate HD genetic modifiers. Aberrant function of *N*-methyl-D-aspartate receptors (NMDAR) and overexposure of MSNs to dopamine cause neurotoxicity [34–36], suggesting that variation in expression or function of glutamate receptor subunits and/or dopamine pathway genes could modulate excitotoxic death and thereby affect HD AO.

Association of AO with specific polymorphisms in *NR2A* and *NR2B*, encoding NMDAR subunits, has been previously reported [15–18]. However, in our sample of European ancestry, we found no definitive evidence of association for either of the two *GRIN2A* or for the *GRIN2B* SNPs that were tested with the residual AO after accounting for the effect of *HTT* CAG repeat length. One SNP, rs1806201 in *GRIN2B*, was close to nominal significance ($p$=0.053 in the additive model). Though this value would not survive correction for the multiple hypotheses tested in our study, it may be of interest for future modifier studies given the effects previously reported [15–17]. The lack of replication in our sample of the reported associations might be explained by different study designs, including the patient populations and definition of the phenotypic trait. We have previously shown that stringent sample selection and analysis criteria are critical factors in HD association studies. Indeed, genetic background related to ancestry [19] and non-normal distribution of CAG allele size [37] can have a profound confounding effect when testing for the effects of potential genetic modifiers.

Our test of the *GRIN2A* rs4998386 polymorphism that has been recently associated with decreased risk of developing PD in individuals who are heavy coffee drinkers [23] was an attempt to assess a neurodegenerative disease-associated risk allele that may interact with a common environmental factor. We did not find evidence of association of this particular SNP with AO of HD motor symptoms. Though coffee consumption data are not available on our study subjects, this negative result is consistent with previous genetic findings showing that the *HTT* CAG repeat polymorphism is not a modifier of PD onset [38], strongly suggesting that the pathogenic process that culminates in HD manifestations may be distinct from the neurodegenerative disease process that leads to PD symptoms.

Genes in the dopamine pathway have not previously been evaluated as potential modifiers of the AO of overt motor symptoms in HD. We selected polymorphisms in four dopaminergic pathway genes that have been investigated in other neurological disorders because they are believed to affect neurotransmission by affecting the level of the enzyme that metabolizes dopamine, the density of dopamine receptors, and the activity of dopamine transporter. Our results did not reveal a significant modifying effect of any of the four polymorphisms, in *COMT*, *DRD2*, *DRD4*, or *DAT1*, on the onset of HD motor symptoms and therefore fail to support a role for these functional variants in the disease process that leads to the onset of neurological symptoms in HD.

In summary, the results of our study did not provide evidence for an association of DNA variants that affect the biology of particular genes in the glutaminergic pathway (*GRIN2A*, *GRIN2B*) or the dopaminergic pathway (*DRD2*, *DRD4*, *DAT1*, *COMT*) with the *HTT* CAG repeat length-dependent disease process that leads to the onset of clinical motor manifestations of HD. However, our study does not preclude the possibility that other DNA variants in these genes, or in other genes involved in these pathways, may act as genetic AO modifiers. Moreover, it is also possible that genes in the glutaminergic and dopaminergic pathways may serve to modify the rate of progression of the distinct processes that determine the rate of decline in the ~18-year period between the age at clinical diagnosis of HD and death, which is independent of the size of the *HTT* CAG repeat [39].

**Acknowledgments** The authors thank Drs. Ignacio Muñoz-Sanjuan, Seung Kwak, and Jamshid Arjomand from CHDI Foundation, Inc., as well as the HD families, whose participation in genetic studies made this work possible; the COHORT co-investigators and contributors (see "Appendix"); contributors to the HD-MAPS study, Drs. Alexandra Durr, Adam Rosenblatt, Luigi Frati, Susan Perlman, P. Michael Conneally, Mary Lou Klimek, Melissa Diggin, Tiffany Hadzi, and Ayana Duckett; the Harvard Brain Tissue Resource Center at McLean Hospital; and the National Neurological Research Specimen Bank at the VA West Los Angeles Healthcare Center. EMR is the recipient of a scholarship from the Fundação para a Ciência e a Tecnologia of Portugal (SFRH/BD/44335/2008). Study funding was supported by NIH grants from the NINDS NS16367 (The Massachusetts HD Center Without Walls) and NS32765, and the CHDI Foundation, Inc.

**Ethical standards** This study used only deidentified, previously collected DNA samples and phenotypic data in a manner approved by the Institutional Review Board of Partners HealthCare, Inc.

**Conflict of interest** The authors declare that they have no conflict of interest.



## Appendix

Huntington Study Group COHORT investigators

*Steering committee*

University of Rochester, Rochester, NY: Ira Shoulson, MD (principal investigator), James Gusella, PhD (co-principal investigator), Tatiana Foroud (co-principal investigator), Irina Antonijevic, Dan van Kammen (CHDI Foundation Inc.)

*Publications and data use committee*

Tatiana Foroud (Chair), Ray Dorsey (Co-Chair), John Warner (CHDI), Joe Giuliano, Louise Vetter, Oksana Suchowersky, Christopher Beck, David Oakes

*Participating investigators and coordinators*

F. Marshall, University of Rochester;
K. Marder, S. Frucht, C. Moskowitz, R. Clouse, P. Wasserman, Columbia University Medical Center;
K. Shannon, J. Jaglin, Rush University Medical Center;
J. Jankovic, A. Palao, Baylor College of Medicine;
M. Harrison University of Virginia;
C. Singer, M. Quesada, University of Miami;
S. Hersch, D. Rosas, K. Tanev, K. Malarick, Massachusetts General Hospital;
A. Colcher University of Pennsylvania;
J. Sanchez-Ramos, University of South Florida;
S. Kostyk, Ohio State University;
J. Paulsen, University of Iowa;
J. Perlmutter, S. Tabbal, Washington University;
C. Ross, F. Nucifora, Johns Hopkins University;
R. Dubinsky, H. Dubinsky University of Kansas Medical Center;
O. Suchowersky, M.L. Klimek, University of Calgary;
R. Jones, C. Testa, S. Factor, Emory University School of Medicine;
D. Jennings, Institute for Neurological Disorders;
J. Morgan Medical College of Georgia;
D. Higgins, E. Mohlo, Albany Medical College;
J. Adams, The Centre for Addiction and Mental Health, Toronto, Canada;
S. Frank, M. Saint-Hilaire, M. Diggin, Boston University;
S. Furtado, University of Alberta;
F. Walker, C. O'Neill, V. Hunt, Wake Forest University School of Medicine;
K. Quaid, Indiana University School of Medicine;
M. LeDoux, University of Tennessee Health Science Center;
L. Raymond, B. Leavitt, J. Decolongon, University of / British Columbia, Canada;
S. Perlman, University of California, Los Angeles;
J. Corey-Bloom, G. Peavy, J. Goldstein, University of California San Diego;
R. Kumar, Colorado Neurological Institute;
E. McCusker, J. Griffith, C. Loy, Westmead Hospital, NSW, Australia;
V. Wheelock, T. Tempkin, A. Martin, University of California Davis;
M. Nance, Hennepin County Medical Center;
U. Kang, University of Chicago;
W. Mallonee, G. Suter, Hereditary Neurological Disease Center, Kansas;
F. Revilla, M. Gartner University of Cincinnati/Cincinnati Children's Hospital;
C. Drazinic, M.J. Fitzpatrick, University of Connecticut;
M. Panisset, Hôtel-Dieu Hôpital-CHUM, Canada;
K. Duff, University of Utah;
B. Scott, Duke University Medical Center;
W. Weiner, B. Robottom, University of Maryland School of Medicine;
E. Chiu, O. Yastrubetskaya, A. Churchyard, St Vincent's Aged Mental Health Service (SVAMHS) Melbourne, Australia;
T. J. Greenamyre; University of Pittsburgh;
P. Agarwal, Booth Gardner Parkinson's Care Center, WA;

Biostatistics/Coordination Center: University of Rochester—D. Oakes, C. Beck, S. Robertson, K. Eaton, P. Lindsay, L. Deuel;

DNA Genotyping Center: M. MacDonald, Center for Human Genetic Research, Massachusetts General Hospital

*Contributors*

C. Hickey, University of Rochester; L. Muratori, E. Louis, Columbia University Medical Center; A. Leserman, N. Doucette, Ohio State University; E. Uc, R. Rodnitzky, S. Vik, University of Iowa, R. Davis, S. Dietrich, University of Virginia; V. Segro, D. Erickson, Colorado Neurological Institute; V. Hunt, Wake Forest University School of Medicine; N. Lucarelli, University of Pittsburgh; J. Broyles, University of Kansas Medical Center; J. DeLaRosa, University of Texas (Galveston); E. Louis, Columbia University Medical Center; P. Panegyres, Neurodegenerative Disorders Research, WA, AUS; A. Schmidt, S. Barton, Washington University; E. Sperin, C. Testa, Emory University; F. Thiede, University of California, Los Angeles; S. E. Zauber, M. Wesson, Indiana University; R. McInnis, Massachusetts General Hospital; C. Welsh, Johns Hopkins University; M. Wesson, Indiana University School of Medicine; A. Coleman, University of British Columbia, Canada

## References


1. The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72(6):971–983
2. Snell RG, MacMillan JC, Cheadle JP, Fenton I, Lazarou LP, Davies P, MacDonald ME, Gusella JF, Harper PS, Shaw DJ (1993) Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. Nat Genet 4(4):393–397. doi:10.1038/ng0893-393
3. Duyao M, Ambrose C, Myers R, Novelletto A, Persichetti F, Frontali M, Folstein S, Ross C, Franz M, Abbott M et al (1993) Trinucleotide repeat length instability and age of onset in Huntington's disease. Nat Genet 4(4):387–392. doi:10.1038/ng0893-387
4. Andrew SE, Goldberg YP, Kremer B, Telenius H, Theilmann J, Adam S, Starr E, Squitieri F, Lin B, Kalchman MA et al (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet 4(4):398–403. doi:10.1038/ng0893-398
5. Wexler NS, Lorimer J, Porter J, Gomez F, Moskowitz C, Shackell E, Marder K, Penchaszadeh G, Roberts SA, Gayan J, Brocklebank D, Cherny SS, Cardon LR, Gray J, Dlouhy SR, Wiktorski S, Hodes ME, Conneally PM, Penney JB, Gusella J, Cha JH, Irizarry M, Rosas D, Hersch S, Hollingsworth Z, MacDonald M, Young AB, Andresen JM, Housman DE, De Young MM, Bonilla E, Stillings T, Negrette A, Snodgrass SR, Martinez-Jaurrieta MD, Ramos-Arroyo MA, Bickham J, Ramos JS, Marshall F, Shoulson I, Rey GJ, Feigin A, Arnheim N, Acevedo-Cruz A, Acosta L, Alvir J, Fischbeck K, Thompson LM, Young A, Dure L, O'Brien CJ, Paulsen J, Brickman A, Krch D, Peery S, Hogarth P, Higgins DS Jr, Landwehrmeyer B (2004) Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. Proc Natl Acad Sci U S A 101(10):3498–3503. doi:10.1073/pnas.0308679101
6. Gusella JF, MacDonald ME (2009) Huntington's disease: the case for genetic modifiers. Genome Med 1(8):80. doi:10.1186/gm80
7. Djousse L, Knowlton B, Hayden MR, Almqvist EW, Brinkman RR, Ross CA, Margolis RL, Rosenblatt A, Durr A, Dode C, Morrison PJ, Novelletto A, Frontali M, Trent RJ, McCusker E, Gomez-Tortosa E, Mayo Cabrero D, Jones R, Zanko A, Nance M, Abramson RK, Suchowersky O, Paulsen JS, Harrison MB, Yang Q, Cupples LA, Mysore J, Gusella JF, MacDonald ME, Myers RH (2004) Evidence for a modifier of onset age in Huntington disease linked to the HD gene in 4p16. Neurogenetics 5(2):109–114. doi:10.1007/s10048-004-0175-2
8. Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP Jr (1985) Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol 44(6):559–577
9. Vonsattel JP, DiFiglia M (1998) Huntington disease. J Neuropathol Exp Neurol 57(5):369–384
10. Tang TS, Chen X, Liu J, Bezprozvanny I (2007) Dopaminergic signaling and striatal neurodegeneration in Huntington's disease. J Neurosc Off J Soc Neurosci 27(30):7899–7910. doi:10.1523/JNEUROSCI.1396-07.2007
11. Starling AJ, Andre VM, Cepeda C, de Lima M, Chandler SH, Levine MS (2005) Alterations in *N*-methyl-D-aspartate receptor sensitivity and magnesium blockade occur early in development in the R6/2 mouse model of Huntington's disease. J Neurosci Res 82(3):377–386. doi:10.1002/jnr.20651
12. Cepeda C, Ariano MA, Calvert CR, Flores-Hernandez J, Chandler SH, Leavitt BR, Hayden MR, Levine MS (2001) NMDA receptor function in mouse models of Huntington disease. J Neurosci Res 66(4):525–539
13. Zeron MM, Hansson O, Chen N, Wellington CL, Leavitt BR, Brundin P, Hayden MR, Raymond LA (2002) Increased sensitivity to *N*-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. Neuron 33(6):849–860
14. Zeron MM, Fernandes HB, Krebs C, Shehadeh J, Wellington CL, Leavitt BR, Baimbridge KG, Hayden MR, Raymond LA (2004) Potentiation of NMDA receptor-mediated excitotoxicity linked with intrinsic apoptotic pathway in YAC transgenic mouse model of Huntington's disease. Mol Cell Neurosci 25(3):469–479. doi:10.1016/j.mcn.2003.11.014
15. Saft C, Epplen JT, Wieczorek S, Landwehrmeyer GB, Roos RA, de Yebenes JG, Dose M, Tabrizi SJ, Craufurd D, Arning L (2011) NMDA receptor gene variations as modifiers in Huntington disease: a replication study. PLoS Curr 3:RRN1247. doi:10.1371/currents.RRN1247
16. Arning L, Saft C, Wieczorek S, Andrich J, Kraus PH, Epplen JT (2007) NR2A and NR2B receptor gene variations modify age at onset in Huntington disease in a sex-specific manner. Hum Genet 122(2):175–182. doi:10.1007/s00439-007-0393-4
17. Arning L, Kraus PH, Valentin S, Saft C, Andrich J, Epplen JT (2005) NR2A and NR2B receptor gene variations modify age at onset in Huntington disease. Neurogenetics 6(1):25–28. doi:10.1007/s10048-004-0198-8
18. Andresen JM, Gayan J, Cherny SS, Brocklebank D, Alkorta-Aranburu G, Addis EA, Cardon LR, Housman DE, Wexler NS (2007) Replication of twelve association studies for Huntington's disease residual age of onset in large Venezuelan kindreds. J Med Genet 44(1):44–50. doi:10.1136/jmg.2006.045153
19. Ramos EM, Latourelle JC, Lee JH, Gillis T, Mysore JS, Squitieri F, Di Pardo A, Di Donato S, Hayden MR, Morrison PJ, Nance M, Ross CA, Margolis RL, Gomez-Tortosa E, Ayuso C, Suchowersky O, Trent RJ, McCusker E, Novelletto A, Frontali M, Jones R, Ashizawa T, Frank S, Saint-Hilaire MH, Hersch SM, Rosas HD, Lucente D, Harrison MB, Zanko A, Marder K, Gusella JF, Lee JM, Alonso I, Sequeiros J, Myers RH, Macdonald ME (2012) Population stratification may bias analysis of PGC-1$\alpha$ as a modifier of age at Huntington disease motor onset. Hum Genet 131(12):1833–1840. doi:10.1007/s00439-012-1205-z

20. Warner JP, Barron LH, Brock DJ (1993) A new polymerase chain reaction (PCR) assay for the trinucleotide repeat that is unstable and expanded on Huntington's disease chromosomes. Mol Cell Probes 7(3):235–239. doi:10.1006/mcpr.1993.1034
21. Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, Uhl GR (1992) Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics 14(4):1104–1106
22. Biederman J, Petty CR, Ten Haagen KS, Small J, Doyle AE, Spencer T, Mick E, Monuteaux MC, Smoller JW, Faraone SV (2009) Effect of candidate gene polymorphisms on the course of attention deficit hyperactivity disorder. Psychiatr Res 170(2–3):199–203. doi:10.1016/j.psychres.2008.12.016
23. Hamza TH, Chen H, Hill-Burns EM, Rhodes SL, Montimurro J, Kay DM, Tenesa A, Kusel VI, Sheehan P, Eaaswarkhanth M, Yearout D, Samii A, Roberts JW, Agarwal P, Bordelon Y, Park Y, Wang L, Gao J, Vance JM, Kendler KS, Bacanu SA, Scott WK, Ritz B, Nutt J, Factor SA, Zabetian CP, Payami H (2011) Genome-wide gene-environment study identifies glutamate receptor gene GRIN2A as a Parkinson's disease modifier gene via interaction with coffee. PLoS Genet 7(8):e1002237. doi:10.1371/journal.pgen.1002237
24. Tunbridge EM, Harrison PJ, Weinberger DR (2006) Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiatry 60(2):141–151. doi:10.1016/j.biopsych.2005.10.024
25. Mannisto PT, Kaakkola S (1999) Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev 51(4):593–628
26. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR (2001) Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci U S A 98(12):6917–6922. doi:10.1073/pnas.111134598
27. Thompson J, Thomas N, Singleton A, Piggott M, Lloyd S, Perry EK, Morris CM, Perry RH, Ferrier IN, Court JA (1997) D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. Pharmacogenetics 7(6):479–484
28. Ritchie T, Noble EP (2003) Association of seven polymorphisms of the D2 dopamine receptor gene with brain receptor-binding characteristics. Neurochem Res 28(1):73–82
29. Pohjalainen T, Rinne JO, Nagren K, Lehikoinen P, Anttila K, Syvalahti EK, Hietala J (1998) The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. Mol Psychiatr 3(3):256–260
30. Jonsson EG, Nothen MM, Grunhage F, Farde L, Nakashima Y, Propping P, Sedvall GC (1999) Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. Mol Psychiatr 4(3):290–296
31. Schoots O, Van Tol HH (2003) The human dopamine D4 receptor repeat sequences modulate expression. Pharmacogenomics J 3(6):343–348. doi:10.1038/sj.tpj.6500208
32. van Dyck CH, Malison RT, Jacobsen LK, Seibyl JP, Staley JK, Laruelle M, Baldwin RM, Innis RB, Gelernter J (2005) Increased dopamine transporter availability associated with the 9-repeat allele of the SLC6A3 gene. J Nucl Med Off Publ Soc Nucl Med 46(5):745–751
33. van de Giessen E, de Win MM, Tanck MW, van den Brink W, Baas F, Booij J (2009) Striatal dopamine transporter availability associated with polymorphisms in the dopamine transporter gene SLC6A3. J Nucl Med Off Publ Soc Nucl Med 50(1):45–52. doi:10.2967/jnumed.108.053652
34. Wersinger C, Chen J, Sidhu A (2004) Bimodal induction of dopamine-mediated striatal neurotoxicity is mediated through both activation of D1 dopamine receptors and autoxidation. Mol Cell Neurosci 25(1):124–137. doi:10.1016/j.mcn.2003.10.002
35. Perez-Navarro E, Canals JM, Gines S, Alberch J (2006) Cellular and molecular mechanisms involved in the selective vulnerability of striatal projection neurons in Huntington's disease. Histol Histopathol 21(11):1217–1232
36. Fan MM, Raymond LA (2007) *N*-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. Prog Neurobiol 81(5–6):272–293. doi:10.1016/j.pneurobio.2006.11.003
37. Lee JM, Ramos EM, Lee JH, Gillis T, Mysore JS, Hayden MR, Warby SC, Morrison P, Nance M, Ross CA, Margolis RL, Squitieri F, Orobello S, Di Donato S, Gomez-Tortosa E, Ayuso C, Suchowersky O, Trent RJ, McCusker E, Novelletto A, Frontali M, Jones R, Ashizawa T, Frank S, Saint-Hilaire MH, Hersch SM, Rosas HD, Lucente D, Harrison MB, Zanko A, Abramson RK, Marder K, Sequeiros J, Paulsen JS, Landwehrmeyer GB, Myers RH, Macdonald ME, Gusella JF (2012) CAG repeat expansion in Huntington disease determines age at onset in a fully dominant fashion. Neurology 78(10):690–695. doi:10.1212/WNL.0b013e318249f683
38. McNicoll CF, Latourelle JC, MacDonald ME, Lew MF, Suchowersky O, Klein C, Golbe LI, Mark MH, Growdon JH, Wooten GF, Watts RL, Guttman M, Racette BA, Perlmutter JS, Ahmed A, Shill HA, Singer C, Saint-Hilaire MH, Massood T, Huskey KW, DeStefano AL, Gillis T, Mysore J, Goldwurm S, Pezzoli G, Baker KB, Itin I, Litvan I, Nicholson G, Corbett A, Nance M, Drasby E, Isaacson S, Burn DJ, Chinnery PF, Pramstaller PP, Al-Hinti J, Moller AT, Ostergaard K, Sherman SJ, Roxburgh R, Snow B, Slevin JT, Cambi F, Gusella JF, Myers RH (2008) Huntington CAG repeat size does not modify onset age in familial Parkinson's disease: the GenePD study. Mov Disord 23(11):1596–1601. doi:10.1002/mds.22186
39. Gusella JF, Persichetti F, MacDonald ME (1997) The genetic defect causing Huntington's disease: repeated in other contexts? Mol Med 3(4):238–246