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

Gardiner, S.L.

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

Geerte Stuitje,[1,2] Martine J. van Belzen,[2] Sarah L. Gardiner,[1,3], Willeke M. C. van Roon-Mom,[3] Merel W. Boogaard,[2] REGISTRY Investigators of the European Huntington Disease Network,[*] Sarah J. Tabrizi,[4] Raymund A. C. Roos[1] and N. A. Aziz[1,4]

[*]See Supplementary material for a list of Registry members.
Departments of [1]Neurology, [2]Clinical Genetics and [3]Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands
[4]Department of Neurodegenerative Disease, UCL Huntington's Disease Centre, University College London Institute of Neurology, London, United Kingdom

Brain: a journal of neurology. 2017; 140(7): e42

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*

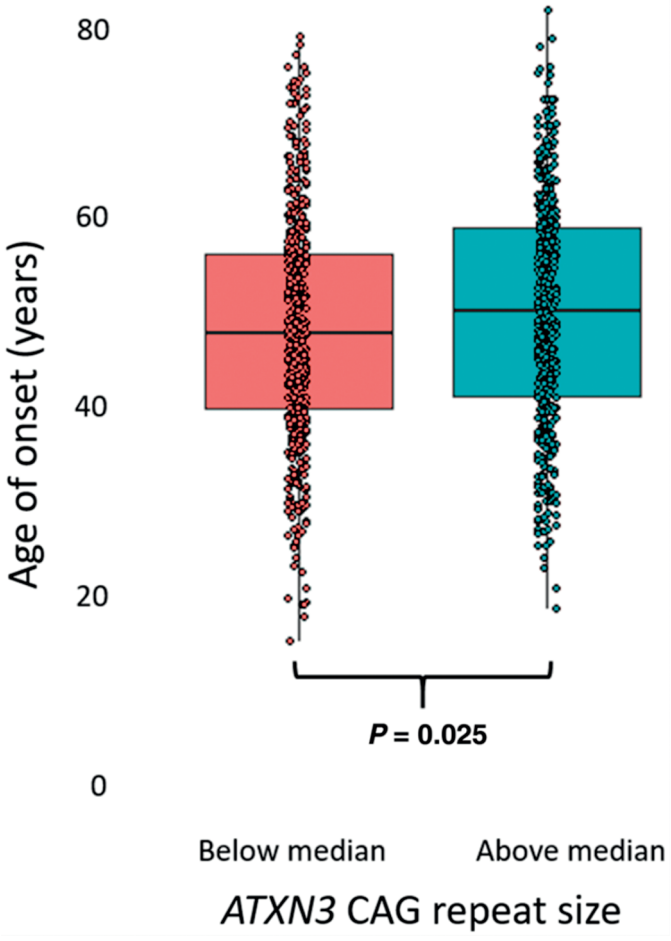


Figure 1. Association between *ATXN3* CAG repeat size and age of onset. Boxplots comparing the age of onset between participants with a below or above median number of CAG repeats in their larger *ATXN3* allele (Mann-Whiney U-test P = 0.025). Black horizontal lines represent medians, boxes display interquartile 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

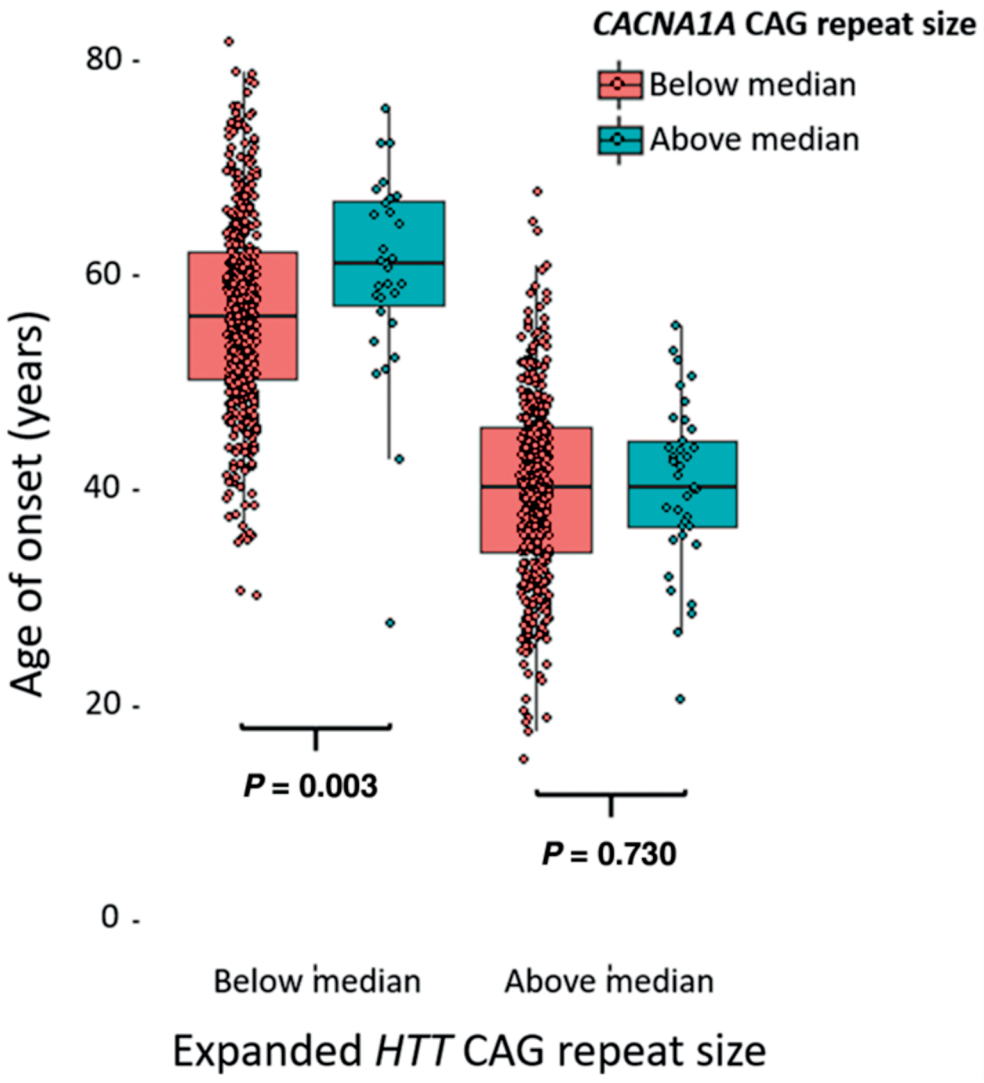


Figure 2. Interaction between the larger *CACNA1A* allele and mutant *HTT CAG* repeat size. Only in patients with mutant *HTT* CAG repeat size below median higher *CACNA1A* CAG repeat size was associated with a higher age-of-onset (Mann-Whiney U-test P = 0.003). Black horizontal lines represent medians, 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.

ACKNOWLEDGEMENTS

We would like to express our gratitude to all the patients who participated in the REGISTRY study. We also thank all the REGISTRY Investigators of the European Huntington Disease Network investigators for collecting the data (**Supplementary material**).

FUNDING

This study was supported by a seed fund (#570) from the European Huntington Disease Network. N.A.A. is supported by a VENI-grant (#91615080) from the Netherlands Organization of Scientific Research and a Marie Sklodowska-Curie Individual Fellowship grant from the European Union (Horizon 2020, #701130).

REFERENCES

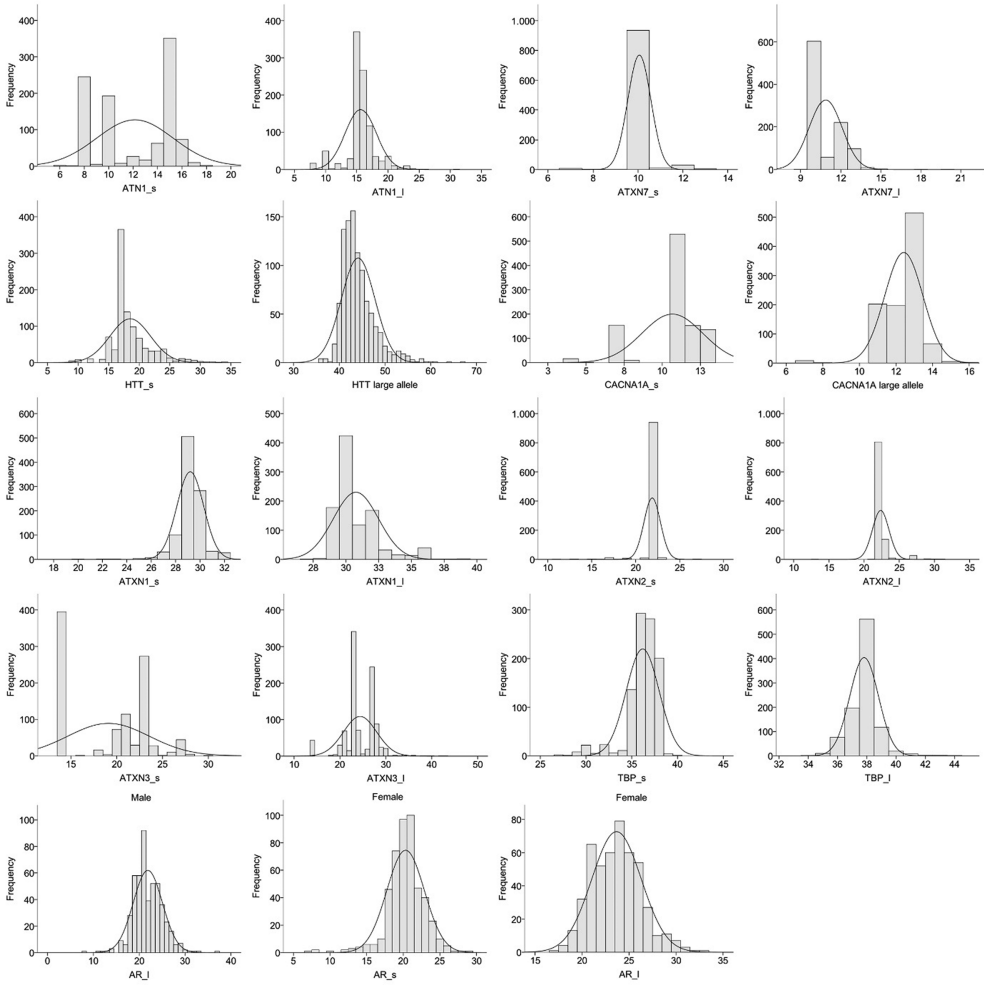
1. Tezenas du Montcel S, Durr A, Bauer P, et al. Modulation of the age at onset in spinocerebellar ataxia by CAG tracts in various genes. *Brain : a journal of neurology* 2014; **137**(Pt 9): 2444-55.
2. Chen Z, Zheng C, Long Z, et al. (CAG)n loci as genetic modifiers of age-at-onset in patients with Machado-Joseph disease from mainland China. *Brain : a journal of neurology* 2016; **139**(Pt 8): e41.
3. Cazeneuve C, Durr A. Genetic and molecular studies. In: Bates GP, Tabrizi SJ, Jones L, eds. Huntington's disease. New York, NY: Oxford University Press; 2014: 109-30.
4. Arning L, Epplen JT. Genetic modifiers of Huntington's disease: beyond CAG. *Future Neurology* 2012; **7**(1): 93-109.
5. GeM-HD C. Identification of Genetic Factors that Modify Clinical Onset of Huntington's Disease. *Cell* 2015; **162**(3): 516-26.
6. Langbehn DR, Brinkman RR, Falush D, Paulsen JS, Hayden MR. A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. *Clinical genetics* 2004; **65**(4): 267-77.
7. Aziz NA, Jurgens CK, Landwehrmeyer GB, et al. Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease. *Neurology* 2009; **73**(16): 1280-5.
8. Raposo M, Ramos A, Bettencourt C, Lima M. Replicating studies of genetic modifiers in spinocerebellar ataxia type 3: can homogeneous cohorts aid? *Brain : a journal of neurology* 2015; **138**(Pt 12): e398.
9. Tezenas du Montcel S. Reply: Replicating studies of genetic modifiers in spinocerebellar ataxia type 3: can homogeneous cohorts aid? *Brain : a journal of neurology* 2015; **138**(Pt 12): e399.

SUPPLEMENTARY MATERIAL

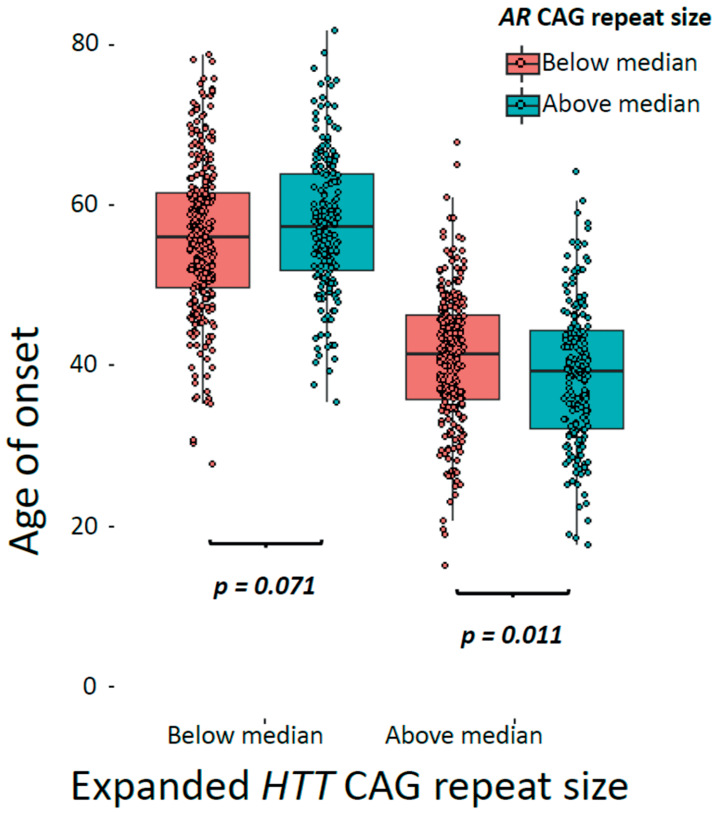
Supplementary Table 1. Distributions of the CAG repeat tracts in polyglutamine disease-associated genes.

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

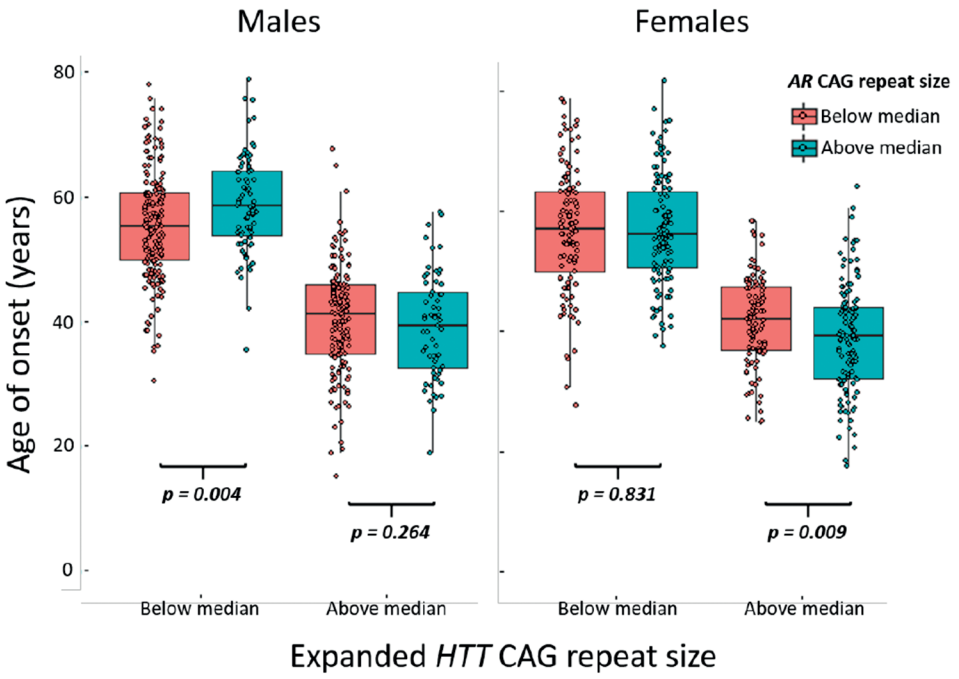
PDAG=polyglutamine disease-associated gene.



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

REGISTRY 2004- 2015

Registry Steering committee: Anne-Catherine Bachoud-Lévi, Anna-Rita Bentivoglio, Ida Biunno, Raphael M. Bonelli, Juliana Bronzova, Jean-Marc Burgunder, Stephen B. Dunnett, Joaquim J. Ferreira, Jan Frich, Joe Giuliano, Olivia J. Handley, Arvid Heiberg, Sergey Illarioshkin, Torsten Illmann, Jiri Klempir, G. Bernhard Landwehrmeyer, Jamie Levey, Tim McLean, Jørgen E. Nielsen, Susana Pro Koivisto, Markku Päivärinta, Sven Pålhagen, Oliver Quarrell, Maria Ramos-Arroyo, Raymund A.C. Roos, Carsten Saft, Ana Rojo Sebastián, Sarah J. Tabrizi, Wim Vandenberghe, Christine Verellen-Dumoulin, Tereza Uhrova, Jan Wahlström+, Jacek Zaremba

Language coordinators: Verena Baake (formerly Rödig), Katrin Barth, Monica Bascuñana Garde, Tomáš Bernard, Sabrina Betz, Reineke Bos, Adrien Come, Leonor Correia Guedes, Jenny Callaghan, Selene Capodarca, Sébastien Charpentier Wildson, Vieira da Silva, Martina Di Renzo, Daniel Ecker, Ana Maria Finisterra,, Ruth Fullam, Camille Genoves, Mette Gilling, Olivia J Handley, Carina Hvalstedt, Christine Held, Hasina Hussain, Kerstin Koppers, Claudia Lamanna, Matilde Laurà, Asunción Martínez Descals, Saul Martinez-Horta, Tiago Mestre, Sara Minster, Daniela Monza, Kristina Münkel, Lisanne Mütze, Martin Oehmen, Helene Padieu, Laurent Paterski, Nadia Peppa, Susana Pro Koivisto, Beate Rindal, Dawn Rogers, Niini Røren (formerly Heinonen), Ana Salgueiro, Pavla Šašinková, Catherine Taylor, Erika Timewell, Jenny Townhill, Patricia Trigo Cubillo, Marleen R van Walsem, Marie-Noelle Witjes-Ané, Grzegorz Witkowski, Abigail Wright, Daniel Zielonka, Eugeniusz Zielonka, Paola Zinzi

AUSTRIA

Graz (Medizinische Universitäts Graz, Psychiatrie): Raphael M. Bonelli, Karen Hecht, Brigitte Herranhof, Anna Holl (formerly Hödl), Hans-Peter Kapfhammer, Michael Koppitz, Sabine Lilek, Markus Magnet, Nicole Müller, Daniela Otti, Annamaria Painold, Karin Reisinger, Monika Scheibl, Helmut Schöggl, Jasmin Ullah

Innsbruck (Universitätsklinik Innsbruck, Neurologie): Eva-Maria Braunwarth, Florian Brugger, Lisa Buratti, Eva-Maria Hametner, Caroline Hepperger, Christiane Holas, Anna Hotter, Anna Hussl, Barbara Larcher, Philipp Mahlknecht, Christoph Müller, Bernadette Pinter, Werner Poewe, Eva-Magdalena Reiter, Klaus Seppi, Fabienne Sprenger, Gregor Wenning

BELGIUM

Charleroi (Institut de Pathologie et de Génétique (IPG)): Michel Dupuis, Cécile Minet, Pascale Ribaï, Dominique Van Paemel, Christine Verellen-Dumoulin

Leuven: (Universitair Ziekenhuis Gasthuisberg,): Andrea Boogaerts, Wim Vandenberghe, Dimphna van Reijen, Petra Weckx

CZECH REPUBLIC

Prague (Extrapyramidové centrum, Neurologická klinika, 1. LF UK a VFN): Jiří Klempíř, Veronika Majerová, Jan Roth

DENMARK

Copenhagen University Hospital (Rigshospitalet, Memory clinic): Lena Hjermind, Oda Jacobsen, Suzanne Lindquist, Jørgen Nielsen, Lisbeth Regeur, Jette Stockholm, Ida Unmack Larsen, Peter Roos, Christina Vangsted-Hansen, Tua Vinther-Jensen

FINLAND

Kuopio: Paivi Hartikainen, Mari Ollokainen

Turku-Suvituuli (Rehabilitation Centre Suvituuli): Pia Eklund, Heli Hiivola, Hannele Hyppönen, Kirsti Martikainen, Katri Tuuha

FRANCE

Angers (Centre de référence des maladies neurogénétique- CHU d'Angers): Philippe Allain, Dominique Bonneau, Marie Bost, Bénédicte Gohier, Marie-Anne Guérid, Audrey Olivier, Julie Prouzet, Adriana Prundean, Clarisse Scherer-Gagou, Christophe Verny

Bordeaux (Hôpital Pellegrin): Blandine Babiloni, Déborah Bled, Sabrina Debruxelles, Charlotte Duché, Cyril Goizet, Laetitia Jameau, Danielle Lafoucrière, Umberto Spampinato

Clermont-Ferrand (Hôpital Gabriel Montpied): Julien Couttier, Bérengère Debilly, Christine Delaigue, Franck Durif, Véronique Germain, Perrine Legendre, Sylvie Loiseau, Miguel Ulla, Tiphaine Vidal

Creteil (Hôpital Henri Mondor): Anne-Catherine Bachoud-Lévi, Farideh Badei, Marie-Françoise Boissé, Lotfi Boudali, Laurent Cleret de Langavant, Laurie Lemoine, Graca Morgado, Katia Youssov

Lille-Amiens:
Lille (CHRU Roger Salengro): Agnès Annic, Recka Barthélémy, Christelle De Bruycker, Maryline Cabaret, Anne-Sophie Carette, Nicolas Carrière, Eric Decorte, Luc Defebvre, Marie Delliaux, Arnaud Delval, Alizé Depelchin, Alain Destee, Nelly Dewulf-Pasz , Thibaut Dondaine, Florence Dugauquier, Kathy Dujardin, Lucie Hopes, Pierre Krystkowiak, Marie-Hélène Lemaire, Sylvie Manouvrier, Eugénie Mutez, Mireille Peter, Lucie Plomhause,

Bernard Sablonnière, Clémence Simonin, Céline Tard, Stéphanie Thibault-Tanchou, Isabelle Vuillaume

Amiens (CHU Sud): Marcellin Bellonet, Alexandra Benoit, Hassan Berrisoul, Stéphanie Blin, Françoise Courtin, Cécile Duru, Véronique Fasquel, Mélanie Flament, Olivier Godefroy, Pierre Krystkowiak, Béatrice Mantaux, Alicia Playe, Martine Roussel, Mélissa Tir, Béatrice Schüler, Sandrine Wannepain

Marseille (Hôpital La Timone): Jean-Philippe Azulay, Christelle Chabot, Marie Delfini, Alexandre Eusebio, Frédérique Fluchere, Christine Garreau, Hélène Grosjean, Aicha Guenam, Laura Mundler, Marielle Nowak Rolland Raseta

Paris (Hôpital de la Pitié Salpêtrière): Sandra Benaich, Alexis Brice, Sarah Boster, Perrine Charles, Alexandra Durr, Claire Ewenczyk, Hélène Francisque, Céline Jauffret, Damian Justo, Abdulrahman Kassar, Stephan Klebe, Fabien Lesne, Paolo Milani, Marie-Lorraine Monin, Tiffany Monnier, Emmanuel Roze, Alina Tataru, Maya Tchikviladzé

Strasbourg (Hôpital Civil): Mathieu Anheim, Nadia Barun, Ouhaid Lagha-Boukbiza, Nadine Longato, Christophe Marcel, Clélie Phillipps, Gabrielle Rudolf, Gisèle Steinmetz , Christine Tranchant , Caroline Wagner , Marie-Agathe Zimmermann

Toulouse (Hôpital Purpan): Leily Blondeau, Fabienne Calvas, Samia Cheriet, Helène Delabaere, Jean-François Demonet, Jérémie Pariente, Michèle Pierre, Sandrine Rolland

GERMANY

Aachen (Universitätsklinikum Aachen, Neurologische Klinik): Christoph Michael Kosinski, Eva Milkereit, Daniela Probst, Kathrin Reetz, Christian Sass, Johannes Schiefer, Christiane Schlangen, Cornelius J. Werner

Bochum (Huntington-Zentrum (NRW) Bochum im St. Josef-Hospital): Jürgen Andrich, Gisa Ellrichmann, Lennard Herrmann, Rainer Hoffmann, Barbara Kaminski, Peter Kraus, Carsten Saft, Christiane Stamm

Dinslaken (Reha Zentrum in Dinslaken im Gesundheitszentrums Lang): Herwig Lange, Robert Maiwald

Dresden (Universitätsklinikum Carl Gustav Carus an der Technischen Universität Dresden, Klinik und Poliklinik für Neurologie): Cecile Bosredon, Ulrike Hunger, Matthias Löhle, Antonia Maass, Christiana Ossig, Simone Schmidt, Alexander Storch, Annett Wolz, Martin Wolz

Freiburg (Universitätsklinik Freiburg, Neurologie): Ulrike Bergmann, Regina Böringer, Philipp Capetian, Gerit Kammel, Johann Lambeck, Miriam Mächtel, Simone Meier, Michel Rijntjes, Birgit Zucker

Hamburg (Universitätsklinikum Hamburg-Eppendorf, Klinik und Poliklinik für Neurologie): Kai Boelmans, Christos Ganos, Ines Goerendt, Walburgis Heinicke, Ute Hidding, Jan Lewerenz, Alexander Münchau, Michael Orth, Jenny Schmalfeld, Lars Stubbe, Simone Zittel

Hannover (Neurologische Klinik mit Klinischer Neurophysiologie, Medizinische Hochschule Hannover): Gabriele Diercks, Dirk Dressler, Flverly Francis, Sabine Gayde-Stephan, Heike Gorzolla, Bianca Kramer, Rebecca Minschke, Christoph Schrader, Pawel Tacik

Itzehoe (Schwerpunktpraxis Huntington, Neurologie und Psychiatrie): Michael Ribbat+

Marburg KPP (Klinik für Psychiatrie und Psychotherapie Marburg-Süd): Bernhard Longinus

München (Huntington-Ambulanz im Neuro-Kopfzentrum - Klinikum rechts der Isar der Neurologischen Klinik und Poliklinik der Technischen Universität München): Antje Lüsebrink, Mark Mühlau, Alexander Peinemann, Michael Städtler, Adolf Weindl, Juliane Winkelmann, Cornelia Ziegler

Münster (Universitätsklinikum Münster, Klinik und Poliklinik für Neurologie): Natalie Bechtel, Heike Beckmann, Stefan Bohlen, Nicole Göpfert, Eva Hölzner, Herwig Lange, Ralf Reilmann, Stefanie Rohm, Silke Rumpf, Christian Sass, Sigrun Schepers, Nathalia Weber

Taufkirchen (Isar-Amper-Klinikum - Klinik Taufkirchen (Vils)): Michael Bachmeier, Matthias Dose, Nina Hofstetter, Ralf Marquard, Alzbeta Mühlbäck

Ulm (Universitätsklinikum Ulm, Neurologie): Katrin Barth, Andrea Buck, Julia Connemann, Daniel Ecker, Carolin Geitner, Christine Held, Andrea Kesse, Bernhard Landwehrmeyer, Franziska Lezius, Jan Lewerenz, Solveig Nepper, Anke Niess, Michael Orth, Ariane Schneider, Daniela Schwenk, Sigurd Süssmuth, Sonja Trautmann, Melanie Vogel, Patrick Weydt

ITALY

Bari (Neurophysiopathology of Pain Unit, Basic Medical, Neuroscience and Sensory System Department, University of Bari): Claudia Cormio, Olimpia Difruscolo, Giovanni Franco, Vittorio Sciruicchio, Claudia Serpino, Marina de Tommaso

Bologna (DIBINEM - Alma Mater Studiorum - Università di Bologna, IRCCS Istituto delle Scienze Neurologiche di Bologna): Giovanna Calandra-Buonaura, Sabina Capellari, Pietro Cortelli, Roberto Gallassi, Roberto Poda, Cesa Scaglione

Florence (Department of Neuroscience, University of Florence & Careggi University Hospital): Elisabetta Bertini, Caterina Bartoli, Fernanda Fortunato, Elena Ghelli, Andrea Ginestroni, Claudia Mechi, Marco Paganini, Silvia Piacentini, Silvia Pradella, Anna Maria Romoli, Sandro Sorbi

Genoa (Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genova): Giovanni Abbruzzese, Monica Bandettini di Poggio, Giovanna Ferrandes, Paola Mandich, Roberta Marchese, Emilio Di Maria, Tiziano Tamburini

Milan (SODS Genetica delle Malattie Neurodegenerative e Metaboliche & U.O. Neurologia, Fondazione IRCCS Istituto Neurologico Carlo Besta): Alberto Albanese, Simona Castagliuolo, Anna Castaldo, Stefano Di Donato, Daniela Di Bella, Cinzia Gellera, Silvia Genitrini, Caterina Mariotti, Daniela Monza, Lorenzo Nanetti, Marta Panzeri, Dominga Paridi, Paola Soliveri, Francesca Spagnolo, Franco Taroni, Chiara Tomasello

Naples (Department of Neurosciences and Reproductive and Odontostomatological Sciences, Federico II University of Naples): Giuseppe De Michele, Luigi Di Maio, Carlo Rinaldi, Marco Massarelli, Silvio Peluso, Alessandro Roca, Cinzia Valeria Russo, Elena Salvatore, Pierpaolo Sorrentino, Tecla Tucci

Pozzilli (IS) (IRCCS Neuromed): Milena Cannella, Valentina Codella, Francesca De Gregorio, Annunziata De Nicola, Francesca Elifani, Tiziana Martino, Francesca Lovo, Irene Mazzante, Martina Petrollini, Maria Simonelli, Ferdinando Squitieri, Maurizio Vezza

Rome (LIRH Foundation): Barbara D'Alessio, Chiara Esposito, Irene Mazzante, Ferdinando Squitieri

Rome (Department of Neurology, Università Cattolica del Sacro Cuore; Institute of Translational Pharmacology & Institute of Cognitive Sciences and Technologies, National Research Council of Italy): Anna Rita Bentivoglio, Francesco Bove, Claudio Catalli, Raffaella Di Giacopo, Alfonso Fasano, Marina Frontali, Arianna Guidubaldi, Tamara Ialongo, Gioia Jacopini, Giovanna Loria, Anna Modoni, Martina Petracca, Carla Piano, Piccininni Chiara, Davide Quaranta, Silvia Romano, Francesco Soleti, Marcella Solito, Maria Spadaro, Flavia Torlizzi, Paola Zinzi

Rome (Azienda Ospedaliera Sant'Andrea; Department of Neuroscience, Mental Health and Sensory Organs (NESMOS), Faculty of Medicine and Psychology,

Sapienza University of Rome; Institute of Translational Pharmacology & Institute of Cognitive Sciences and Technologies, National Research Council of Italy): Giulia Coarelli, Michela Ferraldeschi, Marina Frontali, Gioia Jacopini, Giovanni Ristori, Silvia Romano, Paola Zinzi

NETHERLANDS

Enschede (Medisch Spectrum Twente): Monique S.E. van Hout, Jeroen P.P. van Vugt, A. Marit de Weert, Marloes Verhoeven

Groningen (Polikliniek Neurologie): Meike Dekker, Jesper Klooster, Nico Leenders, Joost van Oostrom, Jesper Klooster, Berry Kremer

Leiden (Leiden University Medical Centre (LUMC)): Verena Baake, Simon J. A. van den Bogaard, Reineke Bos, Eve M. Dumas, Ellen P. 't Hart, Milou Jacobs, Anne Kampstra, Raymund A.C. Roos, Anne Schoonderbeek

NORWAY

Bergen (Haukeland University Hospital, Dept of Medical Genetics and Olaviken Psychiatric Hospital): Ellen Økland Blinkenberg. (NKS Olaviken`s HD clinic): Erik Hauge, Hilde Tyvoll

Oslo University Hospital (Dept. of Medical Genetics, Dept. of Neurology, Dept. of Neurorehabilitation): Olaf Aaserud, Nils Olaf Aanonsen, Kathrine Bjørgo, Nancy Borgeød, Elisabeth Dramstad, Madeleine Fannemel, Jan C. Frich, Per F. Gørvell, Kathrine Haggag, Cecilie Haggag Johannessen, Arvid Heiberg, Lars Retterstøl, Oddveig Røsby, Jutta Rummel, Alma Sikiric, Bodil Stokke, Marleen van Walsem, Ragnhild Wehus

Trondheim (St. Olavs Hospital): Inga Bjørnevoll, Sigrid Botne Sando, Marte Gjøl Haug, Hanna Haugan Størseth, Vibeke Arntsen

POLAND

Gdansk (St. Adalbert Hospital, Gdansk, Medical University of Gdansk, Neurological and Psychiatric Nursing Dpt.): Artur Dziadkiewicz, Agnieszka Konkel, Ewa Narożańska, Malgorzata Nowak, Piotr Robowski, Emilia Sitek, Jaroslaw Slawek, Witold Soltan, Michal Szinwelski

Katowice (Medical University of Silesia, Katowice): Michał Arkuszewski, Magdalena Błaszczyk, Magdalena Boczarska-Jedynak, Ewelina Ciach-Wysocka, Agnieszka Gorzkowska, Barbara Jasińska-Myga, Aleksandra Kaczmarczyk, Gabriela Kłodowska – Duda, Grzegorz Opala, Monika Rudzińska, Daniel Stompel

Krakow (Krakowska Akademia Neurologii): Krzysztof Banaszkiewicz, Dorota Boćwińska, Kamila Bojakowska-Jaremek, Małgorzata Dec, Natalia Grabska, Malgorzata Krawczyk, Ewelina Kubowicz, Michalina Malec-Litwinowicz, Monika Rudzińska, Agata Stenwak, Andrzej Szczudlik, Elżbieta Szczygieł, Magdalena Wójcik, Anna Wasielewska

Poznan (Poznan University of Medical Sciences, Poland): Jacek Anioła Anna Bryl, Anna Ciesielska, Aneta Klimberg, Jerzy Marcinkowski, Husam Samara, Justyna Sempołowicz, Bartłomiej Wiśniewski, Daniel Zielonka

Warsaw-MU (Medical University of Warsaw, Neurology): Anna Gogol (formerly Kalbarczyk), Piotr Janik, Zygmunt Jamrozik, Anna Kaminska, Hubert Kwiecinski+, Natalia Szejko

Warsaw-IPiN (Institute of Psychiatry and Neurology Dep. of Genetics, First Dep. of Neurology): Jakub Antczak, Katarzyna Jachinska, Wioletta Krysa, Maryla Rakowicz, Przemyslaw Richter, Rafal Rola, Danuta Ryglewicz, Halina Sienkiewicz-Jarosz, Iwona Stępniak, Anna Sułek, Grzegorz Witkowski, Jacek Zaremba, Elzbieta Zdzienicka, Karolina Ziora-Jakutowicz

PORTUGAL

Coimbra – (Hospital Universitário de Coimbra): Cristina Januário, Filipa Júlio

Lisbon-HSM (Hospital de Santa Maria, Clinical Pharmacology Unit, Instituto de Medicina Molecular): Leonor Correia Guedes, Miguel Coelho, Joaquim J Ferreira, Andreia Magalhães, Tiago Mestre, Tiago Mendes, Dulce Neutel, Filipe Rodrigues, Anabela Valadas

Lisbon-HFF (Hospital Fernando da Fonseca): Cristina Costa, Helena Cardoso, Tiago Mendes, Mariana Santos

Porto- HSJ (Hospital de São João): Carlos Andrade, Andreia Costa, Carolina Garrett, Miguel Gago, Joana Guimarães, João Massano, Joana Meireles, Ana Monteiro

SPAIN

Badajoz (Hospital Infanta Cristina): Carmen Durán Herrera, Patrocinio Garcia Moreno

Barcelona-Bellvitge (Hospital Universitari de Bellvitge): Jordi Bas, Núria Busquets, Matilde Calopa, Serge Jaumà Classen, Nadia Rodríguez Dedichá

Barcelona- Clínic i Provincial (Hospital Clínic i Provincial): María Teresa Buongiorno, Andrés de la Cerda Santa María, Esteban Muñoz, Pilar Santacruz

Barcelona-Hospital Mútua de Terrassa: Miquel Aguilar Barbera, Ana Rojo Sebastián, Sonia Arribas Pardo, Dolors Badenes Guia, Noemi Calzado, Laura Casas Hernanz, Juan Pablo Tartari Díaz-Zorita, Judit López Catena, Pilar Quiléz Ferrer, Gemma Tome Carruesco

Barcelona-Merced (Hospital Mare de Deu de La Merced): Misericordia Floriach Robert, Cèlia Mareca Viladrich, Elvira Roca, Jesús Miguel Ruiz Idiago, Antonio Villa Riballo

Barcelona-Santa Cruz y San Pablo (Hospital de la Santa Creu i Sant Pau): Antonia Campolongo, Ramon Fernandez de Bobadilla, Jaime Kulisevsky Bojarsky, Saul Martinez-Horta, Javier Pagonabarraga, Jesus Perez Perez, Roser Ribosa, Carolina Villa

Burgos (Servicio de Neurología Hospital General Yagüe): Esther Cubo, Cecilia Gil Polo, Natividad Mariscal

Fuenlabrada (Hospital Universitario): Fernando Alonso-Frech, María del Valle Loarte

Madrid-Clinico (Hospital Clínico Universitario San Carlos): Fernando Alonso Frech, María del Mar Fenollar, Rocío García-Ramos García, Clara Villanueva

Madrid RYC (Hospital Ramón y Cajal, Neurología): Mónica Bascuñana, Marta Fatás Ventura, Juan García Caldentey, Guillermo García Ribas, Justo García de Yébenes, José Luis López-Sendón Moreno, Verónica Mañanes Barral, Patricia Trigo Cubillo

Madrid FJD (Madrid-Fundación Jiménez Díaz): Cici Feliz Feliz, Pedro José García Ruíz, Ana García, Juan García Caldentey, Rosa Guerrero López, Antonio Herranz Bárcenas, Asunción Martínez-Descals, Angel Martínez Pueyo, Veronica Puertas Martin, Noelia Rodríguez Martínez, María José Sainz Artiga, Vicenta Sánchez, Javier del Val Fernandez

Murcia (Hospital Universitario Virgen de la Arrixaca): Moreau María Dolores Alarcón, Carmen Antúnez Almagro, Esther Diéguez, Lorenza Fortuna, Salvadora Manzanares, Juan Marín Muñoz, María Martirio Antequera Torres, Fuensanta Noguera Perea, Laura Vivancos

Oviedo (Hospital Central de Asturias): Sonia González, Luis Menéndez Guisasola, Marta Para Prieto, René Ribacoba, Carlos Salvador, Pablo Sánchez Lozano

Palma de Mallorca (Hospital Universitario Son Espases): Juan García Caldentey, Inés Legarda Ramirez, Penelope Navas Arques, Monica Rodriguez Lopera, Barbara Vives Pastor

Pamplona (Complejo Hospitalario de Navarra): Itziar Gaston, Fermin Garcia-Amigot, Maria Dolores Martinez-Jaurrieta, Maria Antonia Ramos-Arroyo

Sevilla (Hospital Universitario Virgen del Rocío): Fátima Carrillo, María Teresa Cáceres Redondo, Pablo Mir, Laura Vargas González

Sevilla (Hospital Virgen Macarena): Fátima Damas Hermoso, José Manuel García Moreno, Carolina Mendez Lucena, Eva María Pacheco Cortegana, José Chacón Peña, Luis Redondo, Violeta Sánchez Sánchez

Valencia (Hospital la Fe): Maria Bosca, Juan Andres Burguera, Francisco Castera Brugada Carmen Peiró Vilaplana, Pilar Solís, Begoña Jeweinat Figuerola, Paloma Millan Palanca

SWEDEN

Göteborg (Sahlgrenska University Hospital): Jan Wahlström+, Ulrika Høsterey-Ugander, Gunnel Fredlund, Radu Constantinescu, Kajsa Lewin, Liselotte Neleborn-Lingefjärd, Maria Berglund, Peter Berglund, Petra Linnsand

Stockholm Karolinska University Hospital: Stanislav Benaminov, Elisabeth Björnsson, Daniel Merrick, Martin Paucar, Sven Pålhagen, Per Svenningsson, Tina Wallden

Umeå (Umeå University Hospital): Måns Berglund, Ghada Loutfi, Carina Olofsson, Eva-Lena Stattin, Laila Westman, Birgitta Wikström

Uppsala University Hospital: Camilla Ekwall, Marie-Lousie Göller, Valter Niemelä, Jimmy Sundblom

SWITZERLAND

Bern: Jean-Marc Burgunder, Jessica Koehli, Yanik Stebler (Swiss HD Zentrum), Alain Kaelin, Irene Romero, Michael Schüpbach, Sabine Weber Zaugg (Zentrum für Bewegungsstörungen, Neurologische Klinik und Poliklinik, Universität Bern)

U.K.

Aberdeen (NHS Grampian Clinical Genetics Centre & University of Aberdeen): Lorna Downie, Roisin Jack, Kirsty Matheson, Zosia Miedzybrodzka, Daniela Rae, Sheila A Simpson, Fiona Summers, Alexandra Ure, Vivien Vaughan

Birmingham (The Barberry Centre, Dept of Psychiatry): Shahbana Akhtar, Jenny Crooks, Adrienne Curtis, Jenny de Souza (Keylock), John Piedad, Hugh Rickards, Jan Wright

Bristol (North Bristol NHs Trust, Southmead hospital): Elizabeth Coulthard, Louise Gethin, Beverley Hayward, Kasia Sieradzan, Abigail Wright

Cambridge (Cambridge Centre for Brain Repair, Forvie Site): Roger A. Barker, Deidre O'Keefe, Anna Gerrtiz (nee Di Pietro), Kate Fisher, Anna Goodman, Susan Hill, Sarah Mason, Rachel Swain, Natalie Valle Guzman

Cardiff (Schools of Medicine and Biosciences, Cardiff University): Monica Busse, Cynthia Butcher, Stephen Dunnett, Catherine Clenaghan, Ruth Fullam, Sarah Hunt, Lesley Jones, Una Jones, Hanan Khalil, Sara Minster, Michael Owen, Kathleen Price, Jenny Townhill, Anne Rosser

Edinburgh (SE Scotland Genetic Service, Western General Hospital): Maureen Edwards, Carrie Ho (Scottish Huntington´s Association), Marie McGill, Mary Porteous, Pauline Pearson

Glasgow (Glasgow HD Management Clinic, Southern General Hospital): Catherine Deith, Jane Ireland, Stuart Ritchie

Gloucester (Department of Neurology Gloucestershire Royal Hospital): Pauline Brown, Liz Burrows, Amy Fletcher, Alison Harding, Fiona Laver, Mark Silva, Aileen Thomson

Hull (Castle Hill Hospital): Carol Chu, Carole Evans, Deena Gallentree, Stephanie Hamer, Alison Kraus, Ivana Markova, Ashok Raman

Leeds (Chapel Allerton Hospital, Department of Clinical Genetics): Jeremy Cosgrove, Deena Gallantree, Stephanie Hamer, Emma Hobson, Stuart Jamieson , Alison Kraus, Mandy Longthorpe, Ivana Markova, Hannah Musgrave, Caroline Peacy, Ashok Raman, Liz Rowett, Jean Toscano, Sue Wild, Pam Yardumian

Leicester (Leicestershire Partnership Trust, Mill Lodge): Carole Clayton, Heather Dipple, Dawn Freire-Patino, Caroline Hallam, Julia Middleton

London (Guy's Hospital): Thomasin Andrews, Andrew Dougherty, Charlotte Golding, Fred Kavalier, Hana Laing, Alison Lashwood, Dene Robertson, Deborah Ruddy, Alastair Santhouse, Anna Whaite

London (The National Hospital for Neurology and Neurosurgery): Thomasin Andrews, Stefanie Brown, Stefania Bruno, Elvina Chu, Karen Doherty, Charlotte Golding, Salman Haider, Davina Hensman, Nayana Lahiri, Monica Lewis, Marianne Novak, Aakta

Patel, Nicola Robertson, Elisabeth Rosser, Sarah Tabrizi, Rachel Taylor, Thomas Warner, Edward Wild

Manchester (Genetic Medicine, University of Manchester, Manchester Academic Health Sciences Centre and Central Manchester University Hospitals NHS Foundation Trust): Natalie Arran, Judith Bek, Jenny Callaghan, David Craufurd, Ruth Fullam, Marianne Hare, Liz Howard, Susan Huson, Liz Johnson, Mary Jones, Ashok Krishnamoorthy, Helen Murphy, Emma Oughton, Lucy Partington-Jones, Dawn Rogers, Andrea Sollom, Julie Snowden, Cheryl Stopford, Jennifer Thompson, Iris Trender-Gerhard, Nichola Verstraelen (formerly Ritchie), Leann Westmoreland

Newcastle-upon-Tyne (Centre for Life, Institute of Medical Genetics): Ginette Cass, Lynn Davidson, Jill Davison, Neil Fullerton, Katrina Holmes, Suresh Komati, Sharon McDonnell, Zeid Mohammed, Karen Morgan, Lois Savage, Baldev Singh, Josh Wood

Oxford (Oxford University Hospitals NHS Trust, Dept. of Neurosciences, University of Oxford): Andrea H Nemeth, Gill Siuda, Ruth Valentine, Kathryn Dixon, Richard Armstrong

Plymouth (Plymouth Huntington Disease Service, Mount Gould Hospital): David Harrison, Max Hughes, Sandra Large, John O Donovan, Amy Palmer, Andrew Parkinson, Beverley Soltysiak, Leanne Timings, Josh Williams

Poole (Brain Injury Service, Poole Hospital): John Burn, Rebecca Weekes, Janet Craven, Wendy Bailey, Caroline Coleman, Diane Haig-Brown, Steve Simpson

Preston (Neurology Department, Preston Royal Hospital): Marianne Hare, Tahir Majeed, Nicola Verstraelen (Ritchie)

Sheffield (The Royal Hallamshire Hospital– Sheffield Children's Hospital): Oliver Bandmann, Alyson Bradbury, Helen Fairtlough, Kay Fillingham, Isabella Foustanos, Paul Gill, Mbombe Kazoka, Kirsty O'Donovan, Louise Nevitt, Nadia Peppa, Oliver Quarrell, Cat Taylor, Katherine Tidswell, Kirsty O'Donovan

Southampton (Southampton General Hospital): Christopher Kipps, Lesley MacKinnon, Veena Agarwal, Elaine Hayward, Kerry Gunner, Kayla Harris, Mary Anderson, Melanie Heywood, Liane Keys, Sarah Smalley