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Clinical Evidence of Disease Anticipation in Families Segregating a *C9orf72* Repeat Expansion

Sara Van Mossevelde, MD; Julie van der Zee, PhD; Ilse Gijselinck, PhD; Kristel Sleegers, MD, PhD; Jan De Bleecker, MD, PhD; Anne Sieben, MD; Rik Vandenberghe, MD, PhD; Tim Van Langenhove, MD, PhD; Jonathan Baets, MD, PhD; Olivier Deryck, MD; Patrick Santens, MD, PhD; Adrian Ivanoiu, MD, PhD; Christiana Willems, MD; Veerle Bäumer, BSc; Marleen Van den Broeck, BSc; Karin Peeters, BSN; Maria Mattheijssens, BSN; Peter De Jonghe, MD, PhD; Patrick Cras, MD, PhD; Jean-Jacques Martin, MD, PhD; Marc Cruts, PhD; Peter P. De Deyn, MD, PhD; Sebastiaan Engelborghs, MD, PhD; Christine Van Broeckhoven, PhD, DSc; for the Belgian Neurology (BELNEU) Consortium

IMPORTANCE Patients carrying a *C9orf72* repeat expansion leading to frontotemporal dementia and/or amyotrophic lateral sclerosis have highly variable ages at onset of disease, suggesting the presence of modifying factors.

OBJECTIVE To provide clinical-based evidence for disease anticipation in families carrying a *C9orf72* repeat expansion by analyzing age at onset, disease duration, and age at death in successive generations.

DESIGN, SETTING, AND PARTICIPANTS This cohort study was performed from June 16, 2000, to June 1, 2016, in 36 extended Belgian families in which a *C9orf72* repeat expansion was segregating. The generational effect on age at onset, disease duration, and age at death was estimated using a mixed effects Cox proportional hazards regression model, including random-effects terms for within-family correlation and kinship. Time until disease onset or last examination, time from disease onset until death or last examination, or age at death was collected for for 244 individuals (132 proven or obligate *C9orf72* carriers), of whom 147 were clinically affected (89 proven or obligate *C9orf72* carriers).

MAIN OUTCOMES AND MEASURES Generational effect on age at onset, disease duration, and age at death.

RESULTS Among the 111 individuals with age at onset available (66 men and 45 women; mean [SD] age, 57.2 [9.1] years), the mean (SD) age at onset per generation (from earliest-born to latest-born generation) was 62.5 (8.3), 57.1 (8.2), 54.6 (10.2), and 49.3 (7.5) years. Censored regression analysis on all affected and unaffected at-risk relatives confirmed a decrease in age at onset in successive generations ($P < .001$). No generational effect was observed for disease duration or age at death.

CONCLUSIONS AND RELEVANCE The clinical data provide supportive evidence for the occurrence of disease anticipation in families carrying a *C9orf72* repeat expansion by means of a decrease in age at onset across successive generations. This finding may help clinicians decide from which age onward it may be relevant to clinically follow presymptomatic individuals who carry a *C9orf72* repeat expansion.

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Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Group Information: The Belgian Neurology (BELNEU) Consortium members are listed at the end of this article.

Corresponding Author: Christine Van Broeckhoven, PhD, DSc, VIB Center for Molecular Neurology, University of Antwerp–CDE, Universiteitsplein 1, 2610 Antwerp, Belgium (christine.vanbroeckhoven@molgen.vib-ua.be).

Frontotemporal lobar degeneration is a heterogeneous neurodegenerative disorder with predominant atrophy of the frontal and/or temporal lobes of the brain, clinically characterized by behavioral and/or language deficits. About 15% of patients with frontotemporal dementia (FTD) also develop amyotrophic lateral sclerosis (ALS), a motor neuron disease clinically characterized by progressive muscle weakness, muscular atrophy, fasciculations, and spasticity.[1] *C9orf72* (OMIM 614260) repeat expansions are the most common genetic cause of familial FTD (25% of cases) and familial ALS (37% of cases).[2] Up to 88% of patients with familial FTD or ALS with both clinical phenotypes have the *C9orf72* repeat expansion.[3,4]

Highly variable ages at onset have been reported in individuals who carry the *C9orf72* repeat expansion, which is suggestive of the influence of modifying factors.[5-7] In a Belgian cohort, evidence was provided for the existence of repeat expansions as short as 45 repeat units that are pathologic.[5] The study also showed that short repeat expansions of less than 80 repeat units are inversely correlated with later ages at onset. Repeat expansions of more than 80 repeat units are difficult to size accurately because of their large size—up to 4400 repeats corresponding to near 27 Kb[8]—and the enormous variability in repeat sizes produced by somatic mosaicism masking the true length of the *C9orf72* repeat expansion. However, an association was observed between the methylation state and the expansion size in blood and the brain. Consequently, the degree of methylation, which is measurable, is a reflection of the repeat expansion size. In informative *C9orf72* parent-child transmissions, earlier ages at onset, increasing expansion sizes, and/or increasing methylation states were identified in accordance with disease anticipation.[5]

Disease anticipation is a well-known phenomenon occurring in repeat expansion disorders that affects expression of the disease. Repeat expansions are dynamic mutations in which the copy number of simple DNA repeats is unstable. Consequently, these repeats are at risk of changes in repeat size when they are transmitted to the next generation. Clinically, in several repeat expansion disorders, a decrease in age at onset of the disorder is observed in successive generations.[9-11] In addition, in later-born generations the clinical phenotype becomes more severe in association with increasing repeat expansion sizes.[12]

The risk of increased repeat expansion in successive generations can be influenced by several factors. In Huntington disease, larger expansions are less stable than shorter ones and disease anticipation is more common in paternal transmissions,[13] while in other repeat expansion diseases anticipation can be predominant in maternal inheritance.[11] A decrease in repeat size can also occur, which might be influenced by the sex of the parent who transmits the repeat.[12]

Because of the enormous size of the *C9orf72* expansions in most carriers, it remains difficult to gather enough experimental proof for a role of genetic anticipation in most transmissions. Therefore, we aimed to provide additional clinical-based evidence for the occurrence of disease anticipation in families carrying a *C9orf72* repeat expansion by analyzing age at onset, disease duration, and age at death in successive generations.

Key Points

Question Is there clinical evidence for the occurrence of disease anticipation in families carrying a *C9orf72* repeat expansion?

Findings In this cohort study within 36 *C9orf72* pedigrees, a significant decrease in age at onset was seen across successive generations, but no generational effect was seen on disease duration or age at death.

Meaning These data provide supportive evidence for the occurrence of disease anticipation in families carrying a *C9orf72* repeat expansion and may help clinicians decide from which age onward it may be relevant to clinically follow presymptomatic individuals who carry a *C9orf72* repeat expansion.

Methods

Study Population

We investigated families with a *C9orf72* repeat expansion ascertained in Belgium. The index patients of these families belong to large cohorts of patients with a clinical diagnosis of FTD (n = 462), ALS (n = 215), both clinical phenotypes (n = 46), or Alzheimer disease dementia (n = 1221). Recruitment of patients took place from June 16, 2000, to June 1, 2016, by neurologists of different university and general hospitals, collaborating in the framework of the Belgian Neurology Consortium. Index patients were evaluated using a protocol that included a detailed personal and familial medical history, clinical neurologic examination, neuropsychological testing, biochemical analyses, and neuroimaging. The diagnosis of FTD was made according to the international consensus criteria.[14,15] Diagnosis of ALS was made according to the revised El Escorial criteria.[16,17] Diagnosis of Alzheimer disease dementia was made according to the diagnostic criteria from the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association[18] and the diagnostic criteria from the National Institute on Aging-Alzheimer's Association.[19] All available medical records were reviewed by a physician on the research team (S.V.M.).

Index patients and their relatives were contacted by trained research nurses (K.P. and M.M.). Detailed information on family history of dementia and/or ALS was gathered, and family members were asked to participate in genetic studies. Written informed consent for participation in the genetic studies was obtained from participants or their legal guardians. The informed consent forms for patient ascertainment were approved by the local ethics committees of each of the collaborating neurological centers (Antwerp University Hospital, University Hospitals Leuven, University Hospital Ghent, University Hospital Brussels, Saint-Luc University Hospital Liège, Hospital Network Antwerp, General Hospital Sint-Jan Brugge-Oostende, General Hospital Sint-Maria Halle, and Jessa Hospital Hasselt). The genetic study protocols and informed consent forms were approved by the ethics committee of the University Hospital of Antwerp, Antwerp, Belgium, and the University of Antwerp, Antwerp.

Families of index patients carrying a *C9orf72* repeat expansion were included in the current study if information was available about the ages at onset and/or ages at death of clinically affected individuals or unaffected family members carrying a *C9orf72* repeat expansion in at least two generations, after exclusion of the youngest generation with no affected family members carrying a *C9orf72* repeat expansion yet. A total of 36 families were included in the study. The ages of individuals who died at a young age (<25 years) without development of the disease were excluded. To reduce bias to cohort effects, we numbered the earliest-born generation in each pedigree as generation 4 irrespective of the amount of generations with available age data, creating a mixture of birth cohorts across generations (eTable 1 in the Supplement). Information on which family members were clinically affected, regardless of whether blood samples were available, was gathered by a physician (S.V.M.) and trained research nurses (K.P. and M.M.). Clinical files of affected family members were collected and reviewed. The ages at onset of affected family members were determined as the age at which the first symptoms occurred, based on information in the clinical files or information received from living relatives if no clinical files were available (eTable 1 in the Supplement).

Statistical Analysis

We used mixed effects Cox proportional hazard regression models (Coxme package in R [The R Foundation for Statistical Computing]) to study the generational effect on age at onset, age at death, or disease duration. This family-based analysis gives information on more than 1 consecutive generation and enabled us to account for the degree of kinship (determined using kinship2 in R) in the model, and allowed us to include both affected and unaffected individuals. In the initial mixed effects Cox proportional hazard regression models, kinship, family, sex, phenotype, source of information about age at onset (personal clinical file, clinical file of relative, or hearsay from living relative) (in models for age at onset and disease duration), mean life expectancy per birth cohort of 25 years (based on data from the Federal Public Service Statistics Belgium) (in models for disease duration and age at death), age at onset (in model for disease duration), and disease duration (in model for age at death) were included as covariates. In the final mixed effects Cox proportional hazard regression models, the covariates only included those with a significant effect ($P \leq .05$) in the initial model.

Unaffected individuals were included in the model for age at onset and censored at the age of last evaluation. Affected individuals still alive were included in the model for disease duration and censored at the age of last evaluation. In the model for age at death only affected individuals were included.

For the comparative analysis of the difference in age at onset between a mother and her offspring and a father and his offspring, an independent *t* test was used (normal distribution of difference in age at onset). When ages at onset were available for several affected children of 1 parent, the median age at onset of the children was used. All statistical testing was 2-tailed and the level of significance was set at $P < .05$.

Results

Descriptive

From the 36 families, age at onset was available for 111 affected individuals, disease duration was available for 87 deceased affected individuals, and age at death was available for 124 affected individuals. Frontotemporal dementia (for patients with age at onset available, 33 [29.7%]; for those with disease duration available, 24 [27.6%]; for those with age at death available, 26 [21.0%]) and ALS (for patients with age at onset available, 36 [32.4%]; for those with disease duration available, 29 [33.3%]; for those with age at death available, 44 [35.5%]) were the most frequent diagnoses, but other diagnoses such as Alzheimer disease dementia and Parkinson disease (PD) were present (**Table 1**). For this study, the diagnoses were grouped into 3 phenotypes: pure dementia, ALS, and combined FTD-ALS. The first group included patients with an FTD diagnosis as well as those with a diagnosis of unspecified dementia, Alzheimer disease dementia, and PD with dementia. Of the 36 families, 21 had available age data in 2 generations, 13 in 3 generations and 2 in 4 generations (eTable 1 in the Supplement).

Age at Onset

Individuals in later-born generations were more likely to develop disease at an earlier age, with a risk estimate (SE) of 1.98 (0.20) ($P < .001$ for trend) (**Table 2**), resulting in a decreasing mean (SD) age at onset in successive generations (generation 4, 62.5 [8.3] years; generation 3, 57.1 [8.2] years; generation 2, 54.6 [10.2] years; and generation 1, 49.3 [7.5] years) (Table 1 and **Figure**). When the analysis was restricted only to individuals identified as carrying the expansion, age at onset still decreased significantly across successive generations, with a risk estimate (SE) of 1.66 (0.21) ($P = .01$) (eTable 2 in the Supplement). In the analysis restricted only to individuals identified as carrying the expansion, sex was significantly associated with age at onset, with a risk estimate (SE) of 0.44 (0.28) ($P = .003$), with an earlier age at onset in males.

To investigate the influence of the sex of the transmitting parent on the occurrence of disease anticipation, we compared the mean (SD) difference in age at onset between an affected mother and her affected offspring (n = 14; 4.8 [9.4] years) with the mean (SD) difference in age at onset between an affected father and his affected offspring (n = 17; 9.9 [7.5] years); no significant difference was found ($P = .11$). When only transmissions were taken into account with an identified *C9orf72* expansion in both the parent and the child, there was also no significant difference between the mean (SD) difference in age at onset between an affected mother and her affected offspring (n = 6; 5.4 [10.2] years) and the mean (SD) difference in age at onset between an affected father and his affected offspring (n = 11; 10.0 [7.7] years) ($P = .32$).

Disease Duration

Disease duration was not significantly different between generations (risk estimate [SE], 1.03 [0.19]; $P = .87$). Shorter disease durations were correlated with later ages at onset (risk

Table 1. Demographic, Clinical, and Genetic Characteristics of Affected Family Members Carrying the *C9orf72* Repeat Expansion

Available Information	Age at Onset (n = 111)	Disease Duration[a] (n = 87)	Age at Death (n = 124)
Generation			
All			
Age, mean (SD), y	57.2 (9.1)	6.4 (5.9)	64.7 (9.1)
Age, median (range), y	57 (29-80)	5 (1-29)	65 (40-87)
4			
Age, mean (SD), y	62.5 (8.3)	7.5 (7.4)	68.3 (8.8)
No.	21	20	38
3			
Age, mean (SD), y	57.1 (8.2)	5.9 (6.0)	63.2 (8.3)
No.	58	45	60
2			
Age, mean (SD), y	54.6 (10.2)	6.6 (4.2)	64.3 (9.0)
No.	28	21	24
1			
Age, mean (SD), y	49.3 (7.5)	5	58
No.	4	1	1
Male, No. (%)	66 (59.5)	53 (60.9)	73 (58.9)
Carrier status			
Carrier, No. (%)[b]	80 (72.1)	59 (67.8)	68 (54.8)
Short, No.	3	2	2
Long, No.	57	37	38
Diagnosis, No. (%)			
Pure dementia	63 (56.8)	48 (55.2)	60 (48.4)
FTD	33 (29.7)	24 (27.6)	26 (21.0)
Unspecified dementia	16 (14.4)	15 (17.2)	22 (17.7)
Alzheimer disease dementia	12 (10.8)	7 (8.0)	9 (7.3)
PD + unspecified dementia	2 (1.8)	2 (2.3)	3 (2.4)
FTD-ALS	11 (9.9)	10 (11.5)	11 (8.9)
ALS	36 (32.4)	29 (33.3)	44 (35.5)
PD	1 (0.9)	0	2 (1.6)
Unknown	0	0	7 (5.6)
Transmission, No. (%)[c]			
Maternal	48 (43.2)	37 (42.5)	49 (39.5)
Paternal	45 (40.5)	33 (37.9)	39 (31.5)
Unknown	18 (16.2)	17 (19.5)	36 (29.0)

Abbreviations: ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; PD, Parkinson disease.

[a] Only deceased individuals included.

[b] Identified on DNA as well as obligate carriers of the mutation.

[c] Parent of transmission was determined based on carrier status or clinical status (if no DNA of the parents was available) of the parents.

estimate [SE], 1.07 [0.02]; $P < .001$). A significant association was present between phenotype and disease duration (risk estimate [SE], 3.37 [0.19]; $P < .001$): patients with pure dementia had a significantly longer disease duration than did patients with ALS, with or without FTD. Similar results were obtained when including only identified expansion carriers (for generation: risk estimate [SE], 1.05 [0.23]; $P = .82$; for age at onset: risk estimate [SE], 1.06 [0.02]; $P = .002$; and for phenotype: risk estimate [SE], 2.48 [0.20]; $P < .001$).

Age at Death

Age at death was not significantly different between generations (risk estimate [SE], 1.01 [0.15]; $P = .92$). There was a significant association with phenotype (risk estimate [SE], 1.89 [0.15]; $P < .001$), sex (risk estimate [SE], 0.60 [0.23]; $P = .02$), and life expectancy (risk estimate [SE], 1.06 [0.02]; $P = .02$). Age at death was earlier in males and in patients with ALS with or without FTD. In the analysis restricted only to individuals identified as carrying the expansion, the significant association with life expectancy was lost, but phenotype and sex were still significantly associated with age at death (risk estimate [SE], 1.91 [0.18]; $P < .001$; and 0.50 [0.29]; $P = .02$, respectively) while generation was not (risk estimate [SE], 1.18 [0.18]; $P = .36$).

Discussion

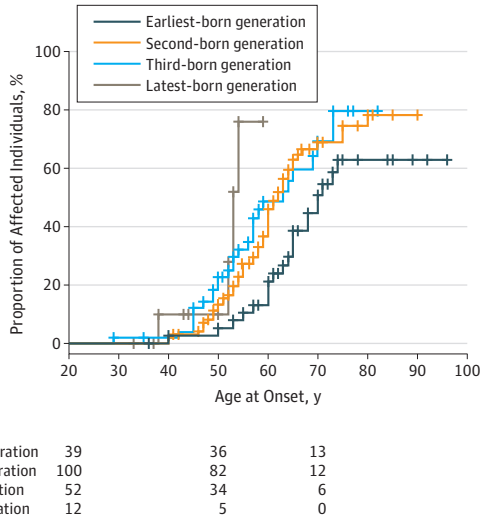
In accordance with other diseases associated with repeat expansions, one might expect that the onset of *C9orf72*-associated disease is linked with repeat length and that genetic anticipation occurs when the repeat expansion is transmitted to the next generation. Although other reports could not reveal

Table 2. Increasing Risk to Develop Disease at a Younger Age in Successive Generations

Risk	Exponent, Coefficient (SE)	*P* Value
Generational effect	1.98 (0.20)	<.001
Risk estimates per generation[a]		
Generation 3	2.26 (0.35)	.02
Generation 2	3.56 (0.44)	.004
Generation 1	12.11 (0.78)	.001

[a] Reference generation is generation 4 (earliest-born generation).

Figure. Onset Age in Successive Generations



No. at risk

Earliest-born generation	39	36	13
Second-born generation	100	82	12
Third-born generation	52	34	6
Latest-born generation	12	5	0

Kaplan-Meier curve showing the cumulative proportion of individuals carrying the *C9orf72* repeat expansion grouped by generation, affected at a certain age; unaffected individuals were censored at the age of last examination.

an association between repeat length and age at onset,[8,20-28] a recent study has demonstrated an inverse correlation between short repeat expansions and later ages at onset, owing to an assay that allowed the precise measurement of repeat sizes shorter than 80 repeat units (ie, short expansions).[5] However, few individuals who carried short expansions were identified in our cohort; therefore, a thorough study of disease anticipation in parent-offspring pairs in which the parent carried a short expansion and the offspring carried a long expansion (>80 units) was not possible and did not allow firm conclusions to be drawn regarding the occurrence of genetic anticipation in families carrying the *C9orf72* repeat expansion (eTable 3 in the Supplement). Therefore, we aimed to provide clinical evidence to support the hypothesis of disease anticipation. In accordance with other diseases associated with repeat expansions, we clinically expect ages at onset to become earlier and disease to become more severe in successive generations as a result of genetic anticipation (ie, repeat amplification).

We estimated the generational effect on age at onset, disease duration, and age at death in 36 extended pedigrees using a mixed effects Cox proportional hazards regression model. This is a more powerful approach compared with affected parent-child pairs since unaffected individuals are also included in this analysis, which avoids right-truncation bias[29,30] resulting from excluding individuals who might develop disease after the study is concluded. In accordance with the expectations of genetic anticipation, we observed a significant decrease in age at onset over successive generations. Similar to results of a published study,[31] we could not determine a significant effect of parental sex since the difference in age at onset between father and offspring and between mother and offspring was not significantly different. However, the number of affected parent-offspring pairs with available ages at onset was too small to draw any firm conclusions.

To study the hypothesis of more severe disease in successive generations, we analyzed the generational effect on disease duration. A correlation between disease duration and repeat length was reported in the cerebellum but not in the frontal lobes.[23] Our results of a lack of association between disease duration and generation could imply that cerebellar repeat length does not increase in successive generations, while frontal repeat length might or might not repeat.

Another marker of disease severity can be the incidence of ALS, which might be considered a more severe phenotype than FTD. Some authors have suggested that patients with ALS carry a larger number of repeat units than do patients with FTD.[32,33] If genetic anticipation occurs, it could result in an increasing proportion of patients with ALS in successive generations. However, for several reasons, we were not able to reliably test this hypothesis. It is likely that the presence of ALS was not always recognized in the oldest generations since clinical consultations by a neurologist were less frequent and, in contrast to symptoms of dementia, signs of motor neuron disease were not likely recognized by laymen. The first description of ALS by Charcot dates from 1869,[34] but only gained attention in 1939 when Lou Gehrig was diagnosed with ALS. Another concern is the heterogeneity in clinical diagnoses[2,35-41]: apart from diagnoses of FTD and/or ALS, patients in our cohort received diagnoses of Alzheimer disease dementia, PD, PD plus dementia, and unspecified dementia. For our analyses, we categorized all patients with a dementia diagnosis without ALS together in 1 group. The motivation to do so was not only statistical power, but also because many of these patients might actually have FTD. Fifty-one affected individuals had a diagnosis of Alzheimer disease dementia, PD plus dementia, or unspecified dementia; however, the diagnosis was based on the clinical record in only 13 patients. Consequently, many patients lacked diagnostic workup by a neurologist, which is particularly relevant since previous reports have shown that Alzheimer disease dementia or PD are likely clinical misdiagnoses since *C9orf72* repeat expansions are very rarely found in pathologically confirmed Alzheimer disease or PD.[39,41]

Whether an individual carrying the *C9orf72* repeat expansion develops FTD, ALS, or both might be influenced by several factors other than repeat length. Chiò et al[31] argued for an association between the phenotype of the parent and the phenotype of the child. We confirmed this finding in our data (eTable 4 in the Supplement): a parent with cognitive decline

is more likely to have an affected child who manifests symptoms of cognitive decline and, similarly, an affected child is more likely to develop ALS if the parent has ALS.

As for disease duration, we could not detect a significant difference of age at death between generations. The rationale to study age at death was that age at death could be a more objective marker than age at onset, since it cannot be influenced by recall bias. However, age at onset is directly determined by *C9orf72*-associated disease, while age at death, especially in patients with dementia, is generally instigated by other comorbidities. We included life expectancy as a covariate in the analysis of age at death to correct for surveillance that better treatment of comorbidities results in later age at death in successive generations. If genetic anticipation does occur in *C9orf72*-associated disease, our results indicate that the *C9orf72* repeat expansion length would not determine age at death, as previously reported.[33] This is in contrast to Huntington disease, in which a significant association of the *HTT* (OMIM 613004) CAG expansion with age at death was recently found.[42]

A complication in the study of the clinical consequences of disease anticipation is that not only repeat expansion size but also several other factors might be influencing clinical characteristics of *C9orf72*-associated disease. Chiò et al[31] suggested that the clinical phenotype influences age at onset since they observed that the co-occurrence of FTD increases age at onset in patients with ALS. In our study, we could not demonstrate a significant association between age at onset and clinical phenotype, but age at death was significantly earlier in patients with ALS than in those with pure dementia. Consequently, disease duration was significantly shorter in patients with ALS with or without FTD. Another factor that could act as a modifier is sex. We could not entirely confirm the observation of Williams et al[43] that males were significantly more likely to develop *C9orf72*-associated ALS at a younger age: we only observed an earlier onset of *C9orf72*-associated disease in males in the analysis restricted only to individuals identified as carrying the expansion. Additional studies of a potential effect of sex on age at onset are necessary.

Limitations

Our study has some limitations. First, we had to rely on information about age at onset obtained from relatives in 49 of 111 patients (44.1%), mostly from older generations, increasing the risk for recall bias. However, source of information about age at onset was not significantly associated with age at onset. Moreover, Minikel et al[44] stated that the inclusion of data on individuals ascertained retrospectively through family history can reduce or even eliminate a false signal of disease anticipation. A second limitation is that we were unable to prove experimentally the carrier status in 31 of 111 patients (27.9%) with age at onset data, and thus we could not exclude the potential inclusion of phenocopies in the families or sporadic patients with late-onset Alzheimer disease dementia. A third factor that could distort our findings is that relatives in the later-born generations might recognize the disease earlier because they are more familiar with the symptoms. Unfortunately, statistical methods to fully resolve this issue are lacking. In an attempt to decrease this factor of bias, we performed an additional analysis including only ages at onset that were determined by a physician, which still resulted in a significant generational effect on age at onset (risk estimate [SE], 3.66 [0.30]; $P < .001$) (eTable 5 in the Supplement). Finally, recall bias and societal generational aspects can lead to an overestimation of the anticipation in age at onset. However, an effort was made to account for as many as possible (known) factors of bias in this study and we believe we have provided a valuable study adding arguments for the occurrence of disease anticipation in *C9orf72*-associated disease. Nevertheless, additional clinical and molecular studies are necessary to confirm our findings and provide additional evidence.

Conclusions

We provided clinical data supporting the occurrence of disease anticipation in families carrying the *C9orf72* repeat expansion by means of an earlier age at onset in successive generations. Our results suggest that, in contrast with other diseases associated with repeat expansion, disease anticipation results in earlier ages at onset but not in more severe phenotypes. Our finding may help clinicians decide from which age onward it may be relevant to clinically follow up presymptomatic individuals who carry the *C9orf72* repeat expansion.

ARTICLE INFORMATION

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Author Affiliations: Center for Molecular Neurology, VIB, Antwerp, Belgium (Van Mossevelde, van der Zee, Gijselinck, Sleegers, Sieben, Van Langenhove, Baets, Bäumer, Van den Broeck, Peeters, Mattheijssens, De Jonghe, Martin, Cruts, Van Broeckhoven); Institute Born-Bunge, University of Antwerp, Antwerp, Belgium (Van Mossevelde, van der Zee, Gijselinck, Sleegers, Sieben, Van Langenhove, Baets, Bäumer, Van den Broeck, Peeters, Mattheijssens, De Jonghe, Cras, Martin, Cruts, De Deyn, Engelborghs, Van Broeckhoven); Department of Neurology and Memory Clinic, Hospital Network Antwerp Middelheim and Hoge Beuken, Antwerp, Belgium (Van Mossevelde, De Deyn, Engelborghs); Department of Neurology, Antwerp University Hospital, Edegem, Belgium (Van Mossevelde, Van Langenhove, Baets, De Jonghe, Cras); Department of Neurology, University Hospital Ghent and University of Ghent, Ghent, Belgium (De Bleecker, Sieben, Santens); Department of Neurosciences, Faculty of Medicine, KU Leuven, Leuven, Belgium (Vandenberghe); Department of Neurology, University Hospitals Leuven, Leuven, Belgium (Vandenberghe); Department of Neurology, General Hospital Sint-Jan Brugge-Oostende, Brugge, Belgium (Deryck); Department of Neurology, Saint-Luc University Hospital and Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium (Ivanoiu); Department of Neurology, Jessa Hospital, Hasselt, Belgium (Willems).

Author Contributions: Dr Van Broeckhoven had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Van Mossevelde, van der Zee, Sleegers, Van Broeckhoven.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Van Mossevelde, Van Broeckhoven.
Critical revision of the manuscript for important intellectual content: All authors.
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