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# Relations between *C9orf72* expansion size in blood, age at onset, age at collection and transmission across generations in patients and presymptomatic carriers

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#### ABSTRACT

A (GGGGCC)<sub>n</sub> repeat expansion in *C9orf72* gene is the major cause of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). The relations between the repeats size and the age at disease onset (AO) or the clinical phenotype (FTD vs. ALS) were investigated in 125 FTD, ALS, and presymptomatic carriers. Positive correlations were found between repeats number and the AO ( $p < 10^{e-4}$ ) but our results suggested that the association was mainly driven by age at collection ( $p < 10^{e-4}$ ). A weaker association was observed with clinical presentation (p = 0.02), which became nonsignificant after adjustment for the age at collection in each group. Importantly, repeats number variably expanded or contracted over time in carriers with multiple blood samples, as well as through generations in parent-offspring pairs, conversely to what occurs in several expansion diseases with anticipation at the molecular level. Finally, this study establishes that measure of repeats number in lymphocytes is not a reliable biomarker predictive of the AO or disease outcome in *C9orf72* long expansion carriers.

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#### 1. Introduction

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Frontotemporal dementia (FTD) is the second cause of degenerative dementia in the presenium, after Alzheimer's disease. A familial history of the disease is reported in half of the patients. Most of the familial cases reflect a monogenic origin with an autosomal

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dominant mode of inheritance. Symptoms of amyotrophic lateral sclerosis (ALS) are associated in 15% of patients with FTD. A GGGGCC ( $G_4C_2$ ) repeat expansion in the first intron of chromosome 9 open reading frame 72 (*C9orf72*) is one of the major genetic cause of FTD and of ALS (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Most healthy individuals carry fewer than 24 repeats, whereas patients have up to thousands of hexanucleotide copies (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Three mechanisms might explain the pathogenicity of large repeat expansions: a C9orf72 loss-of-function, the formation of nuclear sense and anti-sense RNA foci, and dipeptide repeat proteins generated by repeat-associated non-ATG translation (Lagier-Tourenne et al., 2013).

C9orf72 disease is characterized by an important heterogeneity of clinical presentation (FTD, ALS) and of age of disease onset. Behavioral or motor symptoms can occur from the third decade of life, to a nearly incomplete penetrance in elderly mutation carriers (Le Ber et al., 2013). The size of the expansion also greatly varies among C9orf72 carriers and might explain a part of this clinical variability, particularly of the age at disease onset (AO) as reported in other repeat expansion diseases. Few studies have analyzed C9orf72 expansion sizes in blood, with controversial results concluding either to a positive (Beck et al., 2013), the absence (Dols-Icardo et al., 2014; Nordin et al., 2015; van Blitterswijk et al., 2013), or an inverse correlation with AO (Gijselinck et al., 2016; Van Mossevelde et al., 2017). Most of these studies were based on a limited number of C9orf72 carriers, thus precluding definite conclusions. Consequently, no clear information about the correlation of C9orf72 repeats number and clinical phenotypes is available to date, and further investigations are needed in large cohorts of patients.

Establishing whether the size of the expansion in blood can be used as a reliable peripheral marker predicting the age at onset and disease outcome in mutation carriers is critical when therapeutics based on antisense oligonucleotides or other molecules are being developed (Donnelly et al., 2013; Jiang et al., 2016). To address these questions, we quantified the expansion size of *C9orf72* in peripheral lymphocytes from 125 carriers, which represents the largest cohort studied so far. The correlations between the expansion size and clinical phenotype, AO or age at blood collection, and the dynamic of the expansion in lymphocytes through time and over generations were analyzed.

#### 2. Material and methods

#### 2.1. Subjects and ethics

The estimate of  $G_4C_2$  repeats number on the expanded allele was done in 125 *C9orf72* carriers, including 73 symptomatic patients and 52 presymptomatic (PS) carriers. Genetic status has been established for all of them by repeat-primed PCR before, as previously described (Le Ber et al., 2013). Clinical data and blood samples were obtained after written informed consent for clinic-genetic studies was signed. Local ethic committee approvals were obtained for studies of symptomatic (IRB: CPP IIe de France II, project #RBM 02-59) and presymptomatic carriers (RCB: 2015-A00856-43 CPP 68-15 IIe-de-France VI, for PrevDemAls and FTLD-exome studies, respectively).

A brief description of patients and PS cohorts is provided in Table 1. Among the patients (62 were French, 11 Italian), 42 presented isolated FTD, 12 had isolated ALS, and 19 had FTD and secondarily developed ALS.

Eleven carriers (8 patients, 1 PS, 2 converters) from this cohort had multiple time points (2–4 times). Time intervals between first and last blood samples ranged from 7 months to 11 years (mean interval = 57.9 months; SD = 39.2 months).

<b>Table 1</b> ndividual characteristics and de	tails of C9orf72 pathoge	enic repeats number ii	n patients and presymp	tomatic (PS) carriers					
Demographic and molecular	Overall $(n = 125)$	Overall $(n = 73)$	Patients (n = 73)						PS carriers
characteristics			Early onset <sup>a</sup> $(n = 23) AO \leq 48$	Late onset <sup>a</sup> $(n = 25) AO \ge 68$	Medium onset (n = 25) AO [49-67]	FTD (n = 42)	FTD-ALS $(n = 19)$	ALS (n = 12)	(n = 52)
Individual characteristics									
Gender (male)	57 (46%)	40 (55%)	10(44%)	14(56%)	16 (40%)	24 (57%)	11 (58%)	5 (42%)	17 (33%)
Age at onset		$57.96 \pm 11.95$	$43.22 \pm 3.57$	$\textbf{70.84} \pm \textbf{2.61}$	$58.64\pm5.30$	$59.70 \pm 11.42$	$57.00\pm10.97$	$54.42\pm14.30$	
(years) ±		(37-77)	(37-48)	(68-77)	(50-67)	(37-75)	(41-73)	(38-77)	
Age at collection	54.65 + 15.70	60.99 + 11.38	49.41 + 8.26	73.04 + 3.22	61.60 + 6.81	$63.50 \pm 10.99$	59.89 + 10.09	57.00 + 14.97	45.75 + 16.69
(years) ±	(20-88)	(39-80)	(39-70)	(69-80)	(46-73)	(39-79)	(46-75)	(39-80)	(20-88)
SD (min-max)									
G <sub>4</sub> C <sub>2</sub> pathogenic repeats numl	ber								
Mean ± SD	$2388 \pm 484.7$	$2426 \pm 480.7$	$2186 \pm 411.6$	$2741 \pm 406.9$	$2331 \pm 451.8$	$2540 \pm 476.9$	$2281 \pm 478.2$	$2228 \pm 429.1$	$2334 \pm 489.9$
Median	2394	2451	2297	2818	2546	2622	2214	2118	2332
Minimum	1294	1294	1294	2033	1363	1294	1363	1623	1459
Maximum	3889	3320	2963	3320	3235	3320	3235	3141	3889
25th percentile	2030	2116	1888	2404	1954	2297	1974	1920	1983
75th percentile	2689	2771	2451	3122	2650	2835	2475	2570	2626
(ev: FTD frontotemnoral demer	ntia: ALS amvotronhic l	lateral sclerosis							

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## 2.2. Molecular analyses and sizing of C9orf72 repeat number by Southern blot

Genomic DNA was extracted from peripheral lymphocytes. Southern blots were then performed to size the repeat expansion (Beck et al., 2013). Briefly, 15 µg of genomic DNA was digested overnight with AluI (20 u) and DdeI (20 u) before electrophoresis. DNA was transferred to positively charged nylon membrane (Roche Applied Science) by capillary blotting and was baked at 80°C for 2 hours. The hybridization probe was an oligonucleotide from Eurofins MWG Operon (Germany) and comprised 5 hexanucleotide repeats (GGGGCC)<sub>5</sub> labeled 3' and 5' with digoxigenin (DIG). Filter hybridization was undertaken as recommended in the DIG Application Manual (Roche Applied Science) except for the supplementation of DIG Easy Hyb buffer with 100 µg/mL denatured fragmented salmon sperm DNA. After prehybridization at 48°C for 4 hours, hybridization was allowed to proceed at 48°C overnight. A total of 1 ng of labeled oligonucleotide probe were used per milliliter of hybridization solution. Membranes were washed initially in  $2 \times$  standard sodium citrate (SSC) and 0.1% sodium dodecyl sulfate (SDS), while the oven was being ramped from 48°C to 65°C and then washed in fresh solution at 65°C for 15 minutes; further 15 minutes washes in  $0.5 \times$  SSC, 0.1% SDS and  $0.2 \times$  SSC, and 0.1% SDS at  $65^{\circ}$ C followed. Detection of the hybridized probe DNA was carried out with Digoxigenin antibody-HRP (Antibodies-online, 1/10,000 dilution) and reveled with ECL ("SuperSignal West Dura Extended DurationSubstrate" Fisher Scientific). Signals were visualized on Fluorescent Detection Film (Roche Applied Science) after 1-5 hours. All samples were electrophoresed against DIGlabeled DNA molecular-weight markers VII (Roche Applied Science). Hexanucleotide repeats number was estimated by visual interpolation with a plot (created in Microsoft Excel) of log10 base-pair number against migration distance and subtraction of the wild-type allele fragment size (199 bp). Mode and mean sizes were recorded for each individual with expanded repeats as described previously (Suh et al., 2015). The mode, defined as the point on the Southern blot with the highest intensity, was used for all the statistical analysis. For patients with multiple collections, modal number of repeats measured at the first sampling was used in correlation tests. Six technical duplicates were used to estimate the range of imprecision when sizing C9orf72 large expansions with this method. DNA extracted from the same blood collection of 6 patients was used in 2 different Southern blots. The absolute value of difference between 2 measures ranged from 16 to 172 repeats.

#### 2.3. Comparisons and statistical analyses

Relations between repeats number and AO, age at blood collection, and clinical phenotype (FTD vs. ALS) were evaluated. We first investigated the association between repeats number and AO in patients with extreme AO: early ( $\leq$ 48 years; n = 23 patients) or late onset ( $\geq$ 68 years; n = 25). AO, as well as the age at collection, was also considered as continuous variables in correlation analyses. Relations between the age at collection and repeats number were investigated in both symptomatic and PS carriers.

We also compared repeats number in a subgroup of youngest patients with an age at onset  $\leq$ 45 years (n = 16) and in presymptomatic carriers with an age at the time of blood collection  $\leq$ 45 years (n = 32).

To accurately evaluate the impact of the repeats number on the phenotype, we compared 2 groups of patients: those with isolated ALS (n = 12) and those with isolated FTD (n = 42); cases presenting both phenotypes (FTD and ALS) were not included in any group in this analysis.

Finally, we assessed the dynamic of the expansion in lymphocytes in a given patient over time and over successive generations in familial cases. We evaluated whether the size varied over time in mutation carriers with multiple sample collections (n = 11) by comparing the expansion at each time point, regardless of the time interval during successive time points. The repeat size transmission across generations was evaluated by studying repeat size in 16 parent-offspring pairs belonging to 12 families from the whole cohort.

The F-test was used to compare variances of *C9orf72* repeat number between PS and symptomatic patients, as the repeat number followed a normal distribution in both groups (Kolmogorov-Smirnov normality test p > 0.05). Conversely, the AO and age at collection did not pass normality tests in our cohort (Kolmogorov-Smirnov normality test p < 0.05). Consequently, Spearman's *rho* (r) was used to estimate correlation levels with associated 2tailed p value. A 2-tailed Mann-Whitney U test was performed for all statistical comparisons of means.

Since a previous study showed that gender can influence AO in patients with *C9orf72* mutations (Le Ber et al., 2013), correlations between *C9orf72* repeats number and AO were calculated using residual values of AO after adjusting for sex. In some analyses, repeats number was adjusted for the age at collection or AO. Residual values of repeats number were then used in correlation or in group comparisons analyses. GraphPad Prism Software v5.00 was used for data visualization.

#### 3. Results

## 3.1. Clinical and molecular characteristics of patients and PS carriers

The mean AO in *C9orf72* patients was 58 years (range: 37-77 years; median = 59; SD = 12.0). Although the mean AO was lower in ALS and FTD-ALS patients than in patients with isolated FTD, none of these differences was significant between the 3 groups (p > 0.05 for all comparisons).

The mean age at first blood collection was 45.8 years in PS (range: 20–88 years; median = 43; SD = 16.7) and 61 years in symptomatic subjects (range: 39–80 years; median = 62; SD = 11.4). The mean age at collection was lower in ALS and FTD-ALS compared to FTD but, here again, these differences were not significant (p > 0.05 for all mean comparisons).

The number of repeats ranged from 1459 to 3889 (mean = 2334; SD = 489.9) in PS carriers and from 1294 to 3320 (mean = 2426, SD = 480.7) in symptomatic patients. Means and variances of repeat number did not differ significantly between PS and symptomatic patients (p = 0.19 for Mann-Whitney U test and p = 0.87 for F-test). Details of repeat sizes distribution parameters in all carriers' groups are shown in Table 1 and Supplementary Fig. 1.

## 3.2. Relations between repeat expansion size, age at onset, and age at blood collection

Mean repeats number was significantly lower in patients with early onset (AO  $\leq$  48, mean = 2186) than in patients with late onset (AO  $\geq$  68, mean = 2741) (p = 0.0001; Fig. 1A). When AO was considered as a continuous variable, repeats number was positively correlated with AO (r = 0.54; p < 0.0001) (Fig. 1B).

Relations between age at collection and repeats number were investigated as well (Fig. 2). We observed similar trends of correlations between repeats number and age at collection in patients (r = 0.51; p < 0.0001). Considering that the age at collection might influence the *C9orf72* pathogenic expansion size in blood and could be a confounding factor in our analyses, repeats number was adjusted for age at collection and used in statistical analyses. The residual correlation of repeats number adjusted for age at collection with AO was no longer significant (r = -0.008; p = 0.95). In the same way, comparisons of adjusted repeats number for age at collection



**Fig. 1.** Relations between C9orf72  $G_4C_2$  repeats number and clinical features in patients. (A): Comparison of repeat number in early-onset ([EO]; AO  $\leq$  48y; n = 23) and late-onset ([LO]; AO  $\geq$  68y; n = 25). (B): Correlation between repeats number and AO. A regression line is displayed. (C): Comparison of repeats number between FTD (n = 42) and ALS patients (n = 12). For statistical comparison between 2 groups, mean and standard deviation are displayed in each group. For correlation, Spearman's *r* is indicated. For all statistical tests, asterisks represent *p* value significance (\*\*\* $p \leq$  0.0001; \*p < 0.05).

between patients with extreme AO (EO vs. LO) became nonsignificant (p = 0.84). When repeats number was adjusted for AO, the residual correlation was slightly higher (r = 0.12) but nonsignificant. As attempted, AO and age at collection were highly correlated in our cohort (r = 0.95, p < 0.0001). Importantly, the same trend of correlation was found in PS individuals. The number of  $G_4C_2$  repeats in the expanded allele was positively correlated with age at collection (r = 0.65; p < 0.0001). To go further, we compared the mean size of expansions in the subgroup of PS carriers with the youngest age at collection ( $\leq$ 45 years) and in patients who developed the disease before 45 years. Means of repeats number were lower in each subgroup compared to their respective whole cohort of patients and PS carriers. Difference of means between these 2 subgroups was not significant (2037 vs. 2147, respectively; p = 0.59).

## 3.3. Relations between repeat number and clinical phenotypes (FTD vs. ALS) in patients

Mean number of repeats was slightly lower in patients with ALS versus FTD (2228 vs. 2540, respectively; p = 0.024, Fig. 1C). Comparisons of repeats number adjusted for the age at collection between these 2 groups became nonsignificant (p = 0.33).

#### 3.4. Dynamic of the pathogenic expansion in lymphocytes over time

For a subset of the aforementioned samples (n = 11), multiple time points were available. These patients and PS individuals gave us the opportunity to measure expansion size changes over time individually and to evaluate relations with disease onset. We did not detect important repeats number differences or a clear trend toward expansion or contraction of the pathogenic expansion in the time intervals considered (Fig. 3). The absolute values of repeat number differences between collections ranged from 15 to 231. Among the carriers, 2 patients (patients 8 and 11, Fig. 3) developed symptoms between 2 blood collection times. These 2 converters did not present massive expansion or contraction of repeats number before and after disease onset. Little and opposite changes were observed for these 2 individuals (-173 repeats and +56 repeats for a time interval of 6.5 and 11 years, respectively).

## 3.5. Dynamic of the repeat expansion in parent-offspring transmissions

A total of 16 parent-offspring transmissions have been studied from the whole cohort, including 8 simple parent-offspring transmissions and 4 parents with 2 children each (Fig. 4). A reduction of repeats number from parents to children was observed in 11/16 pairs (mean = -503 repeats). Conversely, the repeats number increased over generations in 4/16 pairs (mean = +399 repeats). Nearly the same expansion size was detected in one parentoffspring pair. Expansion or contraction of repeats number over generations did not depend on the gender, the age at collection, nor the age at onset of the transmitting parent (Fig. 4).

#### 4. Discussion

The intronic  $G_4C_2$  pathogenic expansion in *C9orf72* is the major genetic cause of FTD, FTD-ALS, and ALS (Cruts et al., 2013; Le Ber



**Fig. 2.** Correlations between C9orf72  $G_4C_2$  repeats number and age at collection in patients (A) and in presymptomatic carriers (B). A regression line and Spearman's *r* are indicated. Asterisks represent *p* value significance (\*\*\**p* < 0.0001).



**Fig. 3.** *C9orf72*  $G_4C_2$  repeats number in carriers with multiple blood collections. Samples are ordered in function of time (1 > 4 when available). Carriers are ordered from the individual with the shortest to the longest time interval between first and last blood collection. Duration between first and last collection is indicated in month (mo) or years (y) above repeats number estimates. Patients 8 and 11 were converters.

et al., 2013; Majounie et al., 2012). We recently showed that genetic factors significantly influence the AO in *C9orf72* families (Barbier et al., 2017). However, modifiers of AO in *C9orf72* FTD remain not yet fully understood, and the search for genetic factors or markers predicting the disease outcome has become a priority to improve patients' genetic counseling and set up clinical trials.

Because of the particular nature of the mutation, C9orf72 FTD and/or ALS belong to the family of repeat expansion diseases. As observed in other repeat expansion disorders, such as Huntington' disease or Fragile-X syndrome, it has been proposed that the size of expansion could influence clinical features in C9orf72 disease. Estimate of repeats number from blood DNA has appeared as a potential noninvasive biomarker, despite the fact that blood estimates of repeats number may not exactly reflect what is occurring in the brain (van Blitterswijk et al., 2013). Consequently, some studies focusing on C9orf72 expansion size in peripheral lymphocytes were performed, leading to controversial conclusions (Table 2) (Beck et al., 2013; Cruts et al., 2013; Dols-Icardo et al., 2014; Gijselinck et al., 2016; Nordin et al., 2015; Russ et al., 2015; Suh et al., 2015; van Blitterswijk et al., 2013). Here, we found a positive correlation between repeats number and AO in a large cohort of 73 C9orf72 patients carrying long expansions, which is the largest cohort of carriers in whom blood expansion repeats number was measured to date.

A significant correlation between repeats number in blood and AO was found in 3 previous studies, based on smaller cohorts (van

Blitterswijk et al., 2013; Nordin et al., 2015; Dols-Icardo et al., 2014). By contrast, an inverse correlation was evidenced in a Belgian cohort study that compared a group of 6 short expansion (<100 repeats) to 66 long expansion (>100 repeats) carriers (Gijselinck et al., 2016; Van Mossevelde et al., 2017). However, the methodology used in the Belgian cohort is distinct from other studies and ours, all studying AO as a quantitative trait in long expansion carriers. Small samples sizes, or potential distinct mechanisms in short and large expansion carriers, could explain divergent results. Also, these results suggest that *C90rf72* pathogenesis could depend on crossing a repeat length, though it is unclear whether this interpretation holds in brain and other disease-relevant tissues.

Despite diverging conclusions on relations between repeats number and AO in the literature, converging evidences came out from association analyses of *C9orf72* repeats number and DNA methylation state. Repeats expansion number has been showed to influence DNA methylation at the *C9orf72* locus, longer expansions being associated with a higher methylation state in the 5' regulatory region of the gene (Gijselinck et al., 2016; Russ et al., 2015; Xi et al., 2013). In this context, at least for longer expansions, downregulation of expression of the expanded *C9orf72* allele might counterbalance deleterious effects of the pathogenic expansion and slightly delay the disease onset.

However, and besides correlations with AO, we also detected a significant correlation between repeats number and age at collection in patients. Intriguingly, a very similar correlation was found in



**Fig. 4.** *C9orf72* G<sub>4</sub>C<sub>2</sub> repeats number in parent-offspring pairs. Gender of transmitting parent, age at collection and age at onset are indicated for patients or presymptomatic (PS) carriers in the right panel. Multiple offspring are ordered according to their date of birth (#1 being the oldest). M: male, F: female.

our series of 52 PS carriers with an age at collection ranging from 20 years to 88 years, which is presently the cohort covering the most expanded range of age to look at relationship between expansion size and age at collection. This suggests that age at collection could be a major confounding variable, and that the observed correlations and associations are driven mostly by the age at collection. Indeed, when repeats number were adjusted for the age at collection, no significant result was observed from the analyses of correlation with AO, nor association with clinical phenotypes. Notably, these results are in line with those of Suh et al. who found a positive correlation between repeats number in blood and age at collection in C9orf72 patients, but not with age at onset after corrections for the age at collection and disease phenotypes (Suh et al., 2015). Finally, the collinearity between AO and age at collection prevents to determine statistically which variable, AO or age at collection, is driving the correlation with repeats number. This constitutes a limitation of this work as of other studies.

To go further, we focused on PS carriers with the youngest age at collection (<45 years) and patients who developed disease before the same age limit. Means of repeats number were not statistically significant between these young PS carriers and patients with a very early onset. Nevertheless, and assuming the hypothesis that low repeats number may be associated with earlier AO in our cohort of patients, all these young PS carriers (62% of the PS cohort) should be at high risk to develop disease in a very short time as they carry a number of repeats similar from the subset of patients who converted within the same range of age. However, the age-related penetrance before 45 years of age was previously estimated to be lower than 10% in 2 different cohorts of C9orf72 carriers (Le Ber et al., 2013; Majounie et al., 2012). All together, these results suggest that the lower number of repeats are more likely related to a younger age at collection rather than to the risk to develop disease, arguing in favor of a predominant influence of the age at collection on blood DNA repeats number estimates.

Given the correlation of the expansion size in blood with age at collection, and due to the dynamic nature of this mutation, it is reasonable to speculate that C9orf72 pathogenic expansions could evolve, expand or contract, with time in a given carrier. Data from C9orf72 individuals with multiple time points (with a mean time interval of 57.9 months) allowed to measure blood repeats number changes with time per patient, with a maximum time interval of 11 years. Importantly, we did not detect massive expansion or contraction of the expansion size with time, even in carriers with the longest follow-up interval (>10 years), or in the 2 carriers who converted between the first and last blood samples. The repeats number slightly varied over time in all cases. In addition, there was no clear and concordant trend toward expansion or contraction between first and last collections, as some carriers presented expansions, contractions, or even similar number of repeats over time. Smaller and more subtle changes might have happened in the interval between several samplings that cannot be precisely detected because of technical limitations of the Southern blot. Indeed, long expansions usually appear as a smear, preventing an accurate estimation of modal number of repeats but, up to now, this is the only method to size C9orf72 long expansions. The maximum difference due to the technical imprecision between 2 measures from the same individual's DNA was up to 172 repeats. According to the slope of the regression lines between repeats number and age at collection, number of G<sub>4</sub>C<sub>2</sub> repeats measured in blood DNA should increase by around 18 repeats per year. Theoretically, a longer period of follow-up (at least 20 years) should be needed therefore to reliably detect evolution of repeats number over time. So, in this study, the time intervals between multiple sample collections might have been too short to clearly observe the influence of the age on blood repeats number with this method. The weak

Long expansion associated with 2016 Gijselinck lower AO<sup>a</sup> et al., 57 Higher in ALS vs. FTD for age at collection No after correcting Positive correlation Suh et al., 2015 05 84 50 34 21 No correlation Nordin et al., 2015 å Russ et al., 2015 correlation Positive 26 Dols-Icardo et al., 2014 Higher in ALS vs. FTD °Z 22 60 88 van Blitterswijk et al., 2013 No variation ۶ ۶ ۶ 15 9 23 1 Positive correlation Beck et al., 2013 ۶ 57 28 28 29  $Multiple time points (n =) \qquad 11 \\ \textbf{Relations between pathologic C9orf72 repeats number and:} \\$ No after correcting for No after correcting for Positive correlation age at collection No variation age at collection In this study 42 119 52 11 73 Individual's information Presymptomatics (n =) Time points of blood Individuals (n =) Age at collection Patients (n =) collections FTD-ALS Phenotype ΕD ALS 90

Summary of studies that investigated the relations between blood C90rf72 repeats number, age at onset, and clinical data from patients and carriers

Table 2

 $^{\rm a}$  In this study the authors compared short (<100 repeats) versus long (>100 repeats) pathogenic C90rf72 expansion Key: FTD, frontotemporal dementia; ALS, amyotrophic lateral sclerosis

variations of observed repeats numbers more likely reflect the inaccuracy of the measurement method rather than true variations. Young PS *C9orf72* carriers, who will be followed for a longer period of time, should bring more valuable data to analyze the dynamics of the pathogenic expansion during lifespan and confirm, or not, whether blood repeats number increases over time.

Finally, the dynamic of long expansions through generations was investigated in 16 parent-offspring pairs. Important and measurable changes in repeats number have been observed, regardless of the gender or the age of transmitting parent. Unexpectedly, a contraction between successive generations was observed in the majority of transmissions (11/16). It is likely that a younger age at collection in offspring versus parents (mean difference of age at collection = 28 years) may partly explain this trend even though this hypothesis did not fit with all paired comparisons. Indeed, expansion or stable repeat number was observed as well, although less frequently. In line with these results, expansions as well as contractions of C9orf72 repeats have been previously reported in lymphocytes from parent-offspring pairs carrying short expansions (Gijselinck et al., 2016; Van Mossevelde et al., 2017). Importantly, these results demonstrate that blood C9orf72 long expansions do not systematically expand through generations and would argue against an anticipation phenomenon at the molecular level.

#### 5. Conclusions

This study establishes that long repeat expansions are highly instable in lymphocytes and variable across time and generations. We observed that the C9orf72 expansion size in blood correlated with age of disease onset and moderately with clinical outcome. However, the age at sampling appeared as a major confounding factor, which could contribute to the observed trends. The correlation between repeats number and age at collection in PS individuals including young carriers who are likely to be more distant from the disease onset argues in favor of the last hypothesis. This study thus clearly supports that blood measure of long repeats cannot serve as a reliable noninvasive biomarker to predict disease onset or evolution toward FTD or ALS in C9orf72 disease, conversely to several other repeat expansion diseases. The identification of alternative biomarkers, easily measurable in accessible fluids, must therefore be a major research challenge as therapeutic era is coming.

#### **Disclosure statement**

The authors have no actual or potential conflict of interest.

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#### Appendix A. Supplementary data

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#### References

- Barbier, M., Camuzat, A., Houot, M., Clot, F., Caroppo, P., Fournier, C., Rinaldi, D., Pasquier, F., Hannequin, D., Pariente, J., Larcher, K., French Clinical and Genetic Research Network on FTD/FTD-ALS, Predict-PGRN & PrevDemAls Study Groups, Brice, A., Génin, E., Sabbagh, A., Le Ber, I., 2017. Factors influencing the age at onset in familial frontotemporal lobar dementia: important weight of genetics. Neurol. Genet. 3, e203.
- Beck, J., Poulter, M., Hensman, D., Rohrer, J.D., Mahoney, C.J., Adamson, G., Campbell, T., Uphill, J., Borg, A., Fratta, P., Orrell, R.W., Malaspina, A., Rowe, J., Brown, J., Hodges, J., Sidle, K., Polke, J.M., Houlden, H., Schott, J.M., Fox, N.C., Rossor, M.N., Tabrizi, S.J., Isaacs, A.M., Hardy, J., Warren, J.D., Collinge, J., Mead, S., 2013. Large C9orf72 hexanucleotide repeat expansions are seen in multiple neurodegenerative syndromes and are more frequent than expected in the UK population. Am. J. Hum. Genet. 92, 345–353.
- Cruts, M., Gijselinck, I., Van Langenhove, T., van der Zee, J., Van Broeckhoven, C., 2013. Current insights into the C9orf72 repeat expansion diseases of the FTLD/ ALS spectrum. Trends Neurosci. 36, 450–459.
- DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.-Y.R., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knopman, D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R., Rademakers, R., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding

region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256.

- Dols-Icardo, O., García-Redondo, A., Rojas-García, R., Sánchez-Valle, R., Noguera, A., Gómez-Tortosa, E., Pastor, P., Hernández, I., Esteban-Pérez, J., Suárez-Calvet, M., Antón-Aguirre, S., Amer, G., Ortega-Cubero, S., Blesa, R., Fortea, J., Alcolea, D., Capdevila, A., Antonell, A., Lladó, A., Muñoz-Blanco, J.L., Mora, J.S., Galán-Dávila, L., Rodríguez De Rivera, F.J., Lleó, A., Clarimón, J., 2014. Characterization of the repeat expansion size in C90rf72 in amyotrophic lateral sclerosis and frontotemporal dementia. Hum. Mol. Genet. 23, 749–754.
- Donnelly, C.J., Zhang, P.-W., Pham, J.T., Haeusler, A.R., Heusler, A.R., Mistry, N.A., Vidensky, S., Daley, E.L., Poth, E.M., Hoover, B., Fines, D.M., Maragakis, N., Tienari, P.J., Petrucelli, L., Traynor, B.J., Wang, J., Rigo, F., Bennett, C.F., Blackshaw, S., Sattler, R., Rothstein, J.D., 2013. RNA toxicity from the ALS/FTD C90RF72 expansion is mitigated by antisense intervention. Neuron 80, 415–428.
- Gijselinck, I., Van Mossevelde, S., van der Zee, J., Sieben, A., Engelborghs, S., De Bleecker, J., Ivanoiu, A., Deryck, O., Edbauer, D., Zhang, M., Heeman, B., Bäumer, V., Van den Broeck, M., Mattheijssens, M., Peeters, K., Rogaeva, E., De Jonghe, P., Cras, P., Martin, J.-J., de Deyn, P.P., Cruts, M., Van Broeckhoven, C., 2016. The C9orf72 repeat size correlates with onset age of disease, DNA methylation and transcriptional downregulation of the promoter. Mol. Psychiatry 21, 1112–1124.
- Jiang, J., Zhu, Q., Gendron, T.F., Saberi, S., McAlonis-Downes, M., Seelman, A., Stauffer, J.E., Jafar-Nejad, P., Drenner, K., Schulte, D., Chun, S., Sun, S., Ling, S.-C., Myers, B., Engelhardt, J., Katz, M., Baughn, M., Platoshyn, O., Marsala, M., Watt, A., Heyser, C.J., Ard, M.C., De Muynck, L., Daughrity, L.M., Swing, D.A., Tessarollo, L., Jung, C.J., Delpoux, A., Utzschneider, D.T., Hedrick, S.M., de Jong, P.J., Edbauer, D., Van Damme, P., Petrucelli, L., Shaw, C.E., Bennett, C.F., Da Cruz, S., Ravits, J., Rigo, F., Cleveland, D.W., Lagier-Tourenne, C., 2016. Gain of toxicity from ALS/FTD-Linked repeat expansions in C90RF72 is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs. Neuron 90, 535–550.
- Lagier-Tourenne, C., Baughn, M., Rigo, F., Sun, S., Liu, P., Li, H.-R., Jiang, J., Watt, A.T., Chun, S., Katz, M., Qiu, J., Sun, Y., Ling, S.-C., Zhu, Q., Polymenidou, M., Drenner, K., Artates, J.W., McAlonis-Downes, M., Markmiller, S., Hutt, K.R., Pizzo, D.P., Cady, J., Harms, M.B., Baloh, R.H., Vandenberg, S.R., Yeo, G.W., Fu, X.-D., Bennett, C.F., Cleveland, D.W., Ravits, J., 2013. Targeted degradation of sense and antisense C90rf72 RNA foci as therapy for ALS and frontotemporal degeneration. Proc. Natl. Acad. Sci. U. S. A. 110, E4530–E4539.
- Le Ber, I., Camuzat, A., Guillot-Noel, L., Hannequin, D., Lacomblez, L., Golfier, V., Puel, M., Martinaud, O., Deramecourt, V., Rivaud-Pechoux, S., Millecamps, S., Vercelletto, M., Couratier, P., Sellal, F., Pasquier, F., Salachas, F., Thomas-Antérion, C., Didic, M., Pariente, J., Seilhean, D., Ruberg, M., Wargon, I., Blanc, F., Camu, W., Michel, B.-F., Berger, E., Sauvée, M., Thauvin-Robinet, C., Mondon, K., Tournier-Lasserve, E., Goizet, C., Fleury, M., Viennet, G., Verpillat, P., Meininger, V., Duyckaerts, C., Dubois, B., Brice, A., 2013. C90RF72 repeat expansions in the frontotemporal dementias spectrum of diseases: a flow-chart for genetic testing. J. Alzheimers Dis. 34, 485–499.
- Majounie, E., Renton, A.E., Mok, K., Dopper, E.G.P., Waite, A., Rollinson, S., Chiò, A., Restagno, G., Nicolaou, N., Simon-Sanchez, J., van Swieten, J.C., Abramzon, Y., Johnson, J.O., Sendtner, M., Pamphlett, R., Orrell, R.W., Mead, S., Sidle, K.C., Houlden, H., Rohrer, J.D., Morrison, K.E., Pall, H., Talbot, K., Ansorge, O., , Chromosome 9-ALS/FTD Consortium, French research network on FTLD/FTLD/ALS, ITALSGEN Consortium, Hernandez, D.G., Arepalli, S., Sabatelli, M., Mora, G., Corbo, M., Giannini, F., Calvo, A., Englund, E., Borghero, G., Floris, G.L., Remes, A.M., Laaksovirta, H., McCluskey, L., Trojanowski, J.Q., Van Deerlin, V.M.,

Schellenberg, G.D., Nalls, M.A., Drory, V.E., Lu, C.-S., Yeh, T.-H., Ishiura, H., Takahashi, Y., Tsuji, S., Le Ber, I., Brice, A., Drepper, C., Williams, N., Kirby, J., Shaw, P., Hardy, J., Tienari, P.J., Heutink, P., Morris, H.R., Pickering-Brown, S., Traynor, B.J., 2012. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. Lancet Neurol. 11, 323–330.

- Nordin, A., Akimoto, C., Wuolikainen, A., Alstermark, H., Jonsson, P., Birve, A., Marklund, S.L., Graffmo, K.S., Forsberg, K., Brännström, T., Andersen, P.M., 2015. Extensive size variability of the GGGGCC expansion in C9orf72 in both neuronal and non-neuronal tissues in 18 patients with ALS or FTD. Hum. Mol. Genet. 24, 3133–3142.
- Renton, A.E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A.M., Kaganovich, A., Scholz, S.W., Duckworth, J., Ding, J., Harmer, D.W., Hernandez, D.G., Johnson, J.O., Mok, K., Ryten, M., Trabzuni, D., Guerreiro, R.J., Orrell, R.W., Neal, J., Murray, A., Pearson, J., Jansen, I.E., Sondervan, D., Seelaar, H., Blake, D., Young, K., Halliwell, N., Callister, J.B., Toulson, G., Richardson, A., Gerhard, A., Snowden, J., Mann, D., Neary, D., Nalls, M.A., Peuralinna, T., Jansson, L., Isoviita, V.-M., Kaivorinne, A.-L., Hölttä-Vuori, M., Ikonen, E., Sulkava, R., Benatar, M., Wuu, J., Chiò, A., Restagno, G., Borghero, G., Sabatelli, M., , ITALSGEN Consortium, Heckerman, D., Rogaeva, E., Zinman, L., Rothstein, J.D., Sendtner, M., Drepper, C., Eichler, E.E., Alkan, C., Abdullaev, Z., Pack, S.D., Dutra, A., Pak, E., Hardy, J., Singleton, A., Williams, N.M., Heutink, P., Pickering-Brown, S., Morris, H.R., Tienari, P.J., Traynor, B.J., 2011. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72, 257-268.
- Russ, J., Liu, E.Y., Wu, K., Neal, D., Suh, E., Irwin, D.J., McMillan, C.T., Harms, M.B., Cairns, N.J., Wood, E.M., Xie, S.X., Elman, L., McCluskey, L., Grossman, M., Van Deerlin, V.M., Lee, E.B., 2015. Hypermethylation of repeat expanded Coorf72 is a clinical and molecular disease modifier. Acta Neuropathol. (Berl.) 129, 39–52.
- Suh, E., Lee, E.B., Neal, D., Wood, E.M., Toledo, J.B., Rennert, L., Irwin, D.J., McMillan, C.T., Krock, B., Elman, L.B., McCluskey, L.F., Grossman, M., Xie, S.X., Trojanowski, J.Q., Van Deerlin, V.M., 2015. Semi-automated quantification of C9orf72 expansion size reveals inverse correlation between hexanucleotide repeat number and disease duration in frontotemporal degeneration. Acta Neuropathol. (Berl.) 130, 363–372.
- van Blitterswijk, M., DeJesus-Hernandez, M., Niemantsverdriet, E., Murray, M.E., Heckman, M.G., Diehl, N.N., Brown, P.H., Baker, M.C., Finch, N.A., Bauer, P.O., Serrano, G., Beach, T.G., Josephs, K.A., Knopman, D.S., Petersen, R.C., Boeve, B.F., Graff-Radford, N.R., Boylan, K.B., Petrucelli, L., Dickson, D.W., Rademakers, R., 2013. Association between repeat sizes and clinical and pathological characteristics in carriers of C90RF72 repeat expansions (Xpansize-72): a crosssectional cohort study. Lancet Neurol. 12, 978–988.
- Van Mossevelde, S., van der Zee, J., Gijselinck, I., Sleegers, K., De Bleecker, J., Sieben, A., Vandenberghe, R., Van Langenhove, T., Baets, J., Deryck, O., Santens, P., Ivanoiu, A., Willems, C., Bäumer, V., Van den Broeck, M., Peeters, K., Mattheijssens, M., De Jonghe, P., Cras, P., Martin, J.-J., Cruts, M., De Deyn, P.P., Engelborghs, S., Van Broeckhoven, C., Belgian Neurology (BELNEU) Consortium, 2017. Clinical evidence of disease anticipation in families segregating a C9orf72 repeat expansion. JAMA Neurol. 74, 445–452.
- Xi, Z., Zinman, L., Moreno, D., Schymick, J., Liang, Y., Sato, C., Zheng, Y., Ghani, M., Dib, S., Keith, J., Robertson, J., Rogaeva, E., 2013. Hypermethylation of the CpG island near the G4C2 repeat in ALS with a C9orf72 expansion. Am. J. Hum. Genet. 92, 981–989.