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A unique common ancestor introduced P301L mutation in *MAPT* gene in frontotemporal dementia patients from Barcelona (Baix Llobregat, Spain)

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ABSTRACT

The County of Baix Llobregat (Barcelona, Catalonia, Spain) presents a high prevalence of familial frontotemporal dementia (FTD) in the presence of P301L mutation in the *MAPT* gene. To evaluate a possible unique founder effect of P301L, and its age, the analysis of 20 single-nucleotide polymorphisms covering 50 kb and 12 single-nucleotide polymorphisms located along 30 Mb around the mutation was performed by developing 2 multiplex single-base extension reactions. In addition, families with affected and healthy individuals from France and Italy were analyzed. The FTD-affected individuals from Barcelona carried the same 50-kb haplotype linked to P301L mutation, suggesting a unique common ancestor, as opposed to French patients. Italian patients are also probably descendants of a unique ancestor, which would be different from that of Barcelona. Diversity of 30-Mb haplotypes found in Barcelona and the inference of the mutation age in these populations, among other reasons, suggest that prevalence of FTD linked to P301L *MAPT* mutation is the result of a locally originated mutation.

1. Introduction

Frontotemporal dementia (FTD) is a heterogeneous neurodegenerative disorder with a strong genetic component. Mutations in the microtubule-associated protein tau (*MAPT*) gene, located on chromosome 17, cause familial FTD with 3 repeats, 4 repeats, or 3R+4R tau deposits depending on the mutation. P301L mutation (c.902C>T; rs63751273) is one of the most frequent mutations of *MAPT* gene identified in familial FTD (Rademakers et al., 2004) and is associated with 4R tauopathy (Fig. 1). The presence of this mutation in different European populations, with noteworthy frequency, has been suggested that might be explained by an ancestral mutation. In Spain, it is noticeable the high incidence of familial FTD linked to the P301L *MAPT* mutation that has been found in the county of Baix Llobregat (Barcelona, Catalonia, Spain) (Fortea et al., 2011). This notable frequency in such a small region might reflect a unique ancestral origin of the chromosome carrying this mutation, either by a newcomer mutation carrier or by a locally originated mutation.

In this study, 2 different multiplex panels have been designed to simultaneously analyze several single-nucleotide polymorphisms

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(SNPs) related to P301L mutation (Fig. 1). The 50-kb panel includes P301L mutation and 20 tag SNPs located around the region of *MAPT* gene and spread along 50 kb around the mutation. The aim of this 50-kb panel is to determine the haplotype linked to P301L mutation in FTD-affected patients to know if they bear common or different 50-kb haplotypes. The 30-Mb panel, around P301L mutation, includes 12 SNPs to study the edges of the haplotype carrying the mutation.

These panels have been analyzed in families affected by FTD linked to P301L *MAPT* mutation from Barcelona (Spain), France, and Italy. Samples from Barcelona has been analyzed to evaluate a possible founder effect of this mutation in the region, whereas samples from France and Italy were included in the study to evaluate whether similar haplotypes are shared along different European regions and to value the existence of unique founder effects in these populations.

2. Materials and methods

2.1. Analyzed samples

DNA samples of unrelated families affected by FTD linked to P301L *MAPT* mutation from Barcelona (Catalonia, Spain), France, and Italy have been analyzed. DNA samples from Barcelona used in this study belong to 20 individuals (15 carriers of the P301L *MAPT* mutation and 5 noncarriers) from 9 different and unrelated families affected by FTD (Fig. S1). All these families share a common geographical origin, the County of Baix Llobregat, in the province of Barcelona, and individuals affected by FTD present a median age of onset of 53.5 years (Borrego-Écija et al., 2017). DNA samples from France, 38 individuals (22 carriers of P301L) of 14 families (Fig. S2), and from Italy, 16 individuals (11 carriers of the mutation) from 4 different families (Fig. S3), were also studied. French and Italian individuals do not belong to specific geographic regions in their respective countries.

In addition, 60 unrelated and healthy individuals (30 men and 30 women) from the province of Barcelona (Catalonia, Spain), provided by National DNA Bank of Spain (Universidad de Salamanca, Spain), were analyzed as control population.

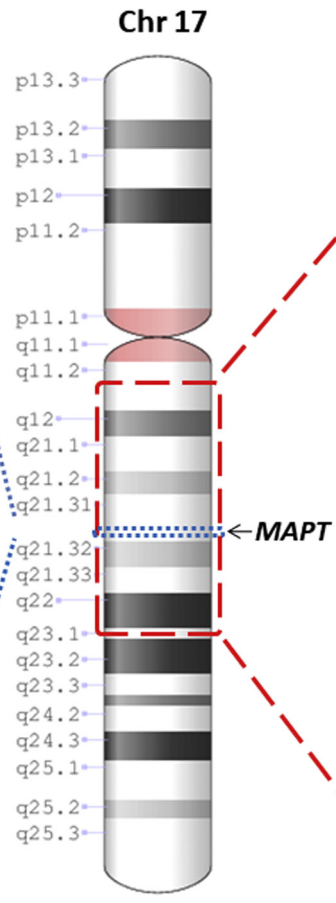
All participants gave their written informed consent, following the ethical standards of the Helsinki declaration. The study was approved by the ethics committee of the Hospital Clinic of Barcelona.

2.2. Selection of SNPs and design and development of SBE reactions

Multiplex single-base extension (SBE) reactions were designed to analyze 2 panels of SNPs. The first one, panel of 50 kb, includes

50 kb panel

SNPs	Location
rs754593	45977330
rs2435206	45980745
rs3785885	45981274
rs2435207	45981562
rs2435211	45985878
rs8079215	45987485
rs2435200	45994485
rs2471738	45998697
rs8067056	46006582
rs4792897	46008901
rs63751273 (P301L)	46010389
rs733966	46012197
rs17573858	46013430
rs916896	46021431
rs9468	46024197
rs1052594	46025323
rs5820605	46025316-17
rs17574040	46025499
rs16940799	46025567
rs7687	46025930
rs7521	46028029



30 Mb panel

SNPs	Location
rs8078405	30011231
rs72825740	32011337
rs56406346	34011717
rs1617208	36011843
rs371080205	38136692
rs11654626	42012932
rs189052	44009134
rs8067216	48010831
rs203001	52010197
rs9674910	54011050
rs1864650	56010382
rs3928386	60013350

Fig. 1. Location of the *MAPT* gene and analyzed SNPs in chromosome 17. The *MAPT* gene, located in 17q21.31 (between 45,894,382 and 46,028,334 in GRCh38.p12), is marked with dotted line. SNPs analyzed in both panels are included at both sides of the chromosome. In the left side, 20 SNPs located around P301L mutation and spread along the *MAPT* gene are showed (50-kb panel). In the right side, the analyzed 12 SNPs encompassing a region of 30 Mb around *MAPT*, marked with dashed line, are included. Image of chromosome modified from Genome Decoration Page (NCBI). Abbreviation: SNP, single-nucleotide polymorphism.

P301L mutation (rs63751273) and 20 tag SNPs located around (10 upstream and 10 downstream) encompassing a region of 50 kb (Figs. 1 and S4). SNPs were selected using the data of CEU (Utah Residents (CEPH) with Northern and Western European ancestry) population available from the International HapMap Project (International HapMap Consortium, 2003) and analyzing them with HaploView v4.2 software (Barrett et al., 2005), with the criterion of having a minor allele frequency $\geq$ 0.2.

The second one, 30-Mb panel, includes 12 SNPs covering 30 Mb around P301L, 7 SNPs upstream, and 5 SNPs downstream (Figs. 1 and S5). All the SNPs were selected using data available in Ensembl (Zerbino et al., 2018), choosing those with a minor allele frequency between 0.3 and 0.1, when possible, to ensure allelic variability and minimize random allelic coincidences (random match probability).

The amplification for the first panel (21 SNPs) was a single multiplex PCR (Table S1), whereas the amplification on the second panel (12 SNPs) needs to be performed in 2 separated multiplex PCR reactions (Table S2). These multiplex PCR reactions were achieved in C1000 Thermal cycler (Bio-Rad, Hercules, CA, USA), in a final volume of 10 μL including 5 μL of Multiplex PCR Master Mix (QIAGEN, Hilden, Germany), 1 μL of 10× primer premix, and 10 ng of target DNA. Thermocycling conditions were as follows: initial denaturalization at 95 °C–15 minutes; 38 cycles of 95 °C–30 seconds, 60 °C–45 seconds, and 72 °C–45 seconds; and a final extension at 72 °C–10 minutes. To check the amplification, the products were electrophoretically separated in 1.5% agarose gels at 100 V for 30 minutes and revealed by GelRed (Biotium Inc, Hayward, CA, USA) fluorescence and UV light (UVItec Ltd, Cambridge, UK). Two microliter of amplified products were purified by enzymatic digestion with a mix of 0.28 μL of Exo I (1 U/μL; Takara Bio Inc, Japan) and 0.72 μL of SAP (1 U/μL; Takara Bio Inc, Japan), by incubation at 37 °C for 45 minutes and 80 °C for 15 minutes.

Minisequencing or SBE of each panel of SNPs was performed in a single reaction (Table S1 and Table S2). Final volume was 7 μL, with 1 μL of the multiplex amplification product purified with Exo + SAP, 0.7 μL of SBE-primer premix (10×), and 3 μL of SNaPshot Master Mix (AB/LT/TFS; Applied Biosystems, Life Technologies, ThermoFisher Scientific, Waltham, MA, USA). Reaction was performed in C1000 Thermal cycler (Bio-Rad, Hercules, CA, USA), under the following conditions: 25 cycles of 96 °C–10 seconds, 50 °C–5 seconds, and 60 °C–30 seconds. The product of minisequencing reaction was purified by enzymatic digestion with SAP, mixing 2 μL of SBE reaction product with 0.75 μL of SAP (1 U/μL) and incubating 60 minutes at 37 °C and 15 minutes at 80 °C. Finally, 1 μL of purified product was mixed with 0.25 μL of GeneScan 120 LIZ Size Standard (AB/LT/TFS) and 10 μL of Hi-Di Formamide (AB/LT/TFS), denatured by incubating it at 96 °C for 6 minutes and 4 °C for 4 minutes, and capillary electrophoresis was performed in a ABI 3130 Genetic Analyzer (AB/LT/TFS). Electropherograms were analyzed using GenMapper v4.1 software (AB/LT/TFS).

2.3. Statistical analyses

Haplotypes of P301L mutation carriers from Barcelona, France, and Italy were inferred from their familial links for both panels of SNPs. Arlequin v3.5 software (Excoffier and Lischer, 2010) was used to check the Hardy-Weinberg equilibrium of SNPs included in the 50-kb panel in the control population from Barcelona and to infer the haplotypes of the control population by Expectation–Maximization algorithm. This software was also used to study the linkage disequilibrium of SNPs included in the 30-Mb panel analyzed in patients from Barcelona and France.

Approximate Bayesian computation analyses were performed with DIYABC v2.1.0 software (Cornuet et al., 2014) to infer the demographic history of these families suffering FTD linked to P301L *MAPT* mutation, and the age of the mutation was estimated with DMLE+ v2.3 software (Reeve and Rannala, 2002). For mutation age estimation, available data of Iberian population in Spain (IBS) and Toscani in Italy (TSI) from 1000 Genomes Project (1000 Genomes Project Consortium et al., 2015) were used to know the haplotypes present in normal population, and prevalence data of each country were obtained from previous studies (Coyle-Gilchrist et al., 2016; López-Pousa et al., 2002; Onyike and Diehl-Schmid, 2013).

3. Results and discussion

For this study, 2 different SNaPshot panels were developed. The 50-kb panel analyzes a set of SNPs, which shows high linkage disequilibrium. These SNPs are closely enough to be inherited as a block, without recombination among them. Therefore, the analysis of this set of SNPs enables us to detect whether families carrying P301L mutation descent from the same common ancestor or, on the contrary, they have different origins.

The 30-Mb panel was thought to analyze SNPs distant enough to allow recombination among them. This reduces the linkage disequilibrium between these SNPs and increases the haplotype diversity, even into the same family. The analysis of this set of SNPs allows us to define the edges of the haplotype carrying the mutation and to estimate the age of P301L mutation.

3.1. The 50-kb haplotype of P301L carriers from Barcelona

Twenty individuals from 9 apparently unrelated families settled in the County of Baix Llobregat (Barcelona, Catalonia, Spain) and affected by FTD linked to P301L *MAPT* mutation were analyzed with the 50-kb panel. The analysis of patients and relatives (Table S3 in Supplemental Material) showed that P301L *MAPT* carriers from the 9 Barcelonan families shared the same 50-kb haplotype linked to the P301L *MAPT* mutation, ACGGCTACTATCTTT-GATTA (Fig. 2A).

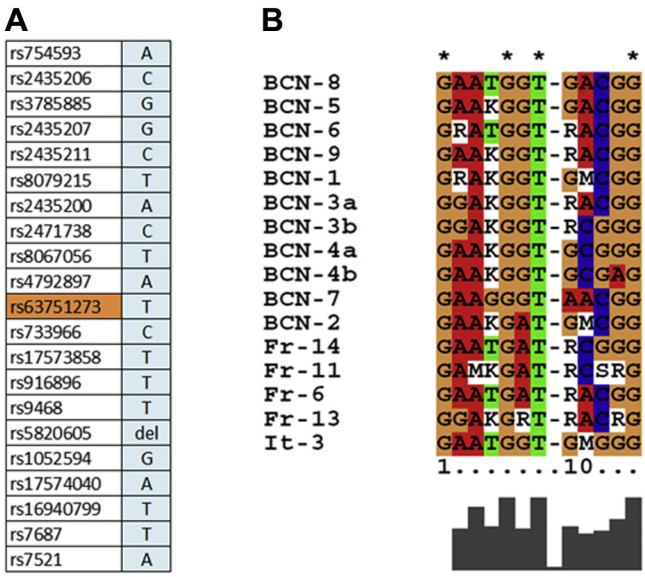


Fig. 2. Haplotypes of 50 kb and 30 Mb found in families from Barcelona. (**A**) Haplotype of the 50-kb panel shared by every carrier of P301L *MAPT* mutation (rs63751273) analyzed from Barcelona; (**B**) Alignment of haplotypes of the 30-Mb panel found in patients sharing the 50-kb haplotype of Barcelona. Those alleles which cannot be defined are indicated with the International Union of Pure and Applied Chemistry nucleotide code, and the dash (-) indicates the place where the shared 50-kb haplotype and P301L mutation are located into this 30-Mb haplotype. The asterisk marks the positions of the alignment for which every samples share the same allele.

The analysis of a sample of unaffected population from Barcelona showed numerous haplotypes for this set of SNPs (Table S4). The 50-kb haplotype linked to P301L *MAPT* mutation in affected individuals match with one of the haplotypes present in Barcelonan population. This haplotype, harboring the ancestral allele of rs63751273 in the unaffected sample, is the fifth most frequent haplotype (0.083) in the population of Barcelona.

These results suggest that in this region P301L *MAPT* mutation derives from a unique common ancestor because the mutation is linked to a specific haplotype, ACGGCTACTATCTTT-GATTA, in all the FTD-affected patients studied here. Moreover, the same haplotype, harboring the healthy allele, was found in the 8.3% of the unaffected population. This unique common ancestor could have been originated in situ or have been brought from another region, producing a founder effect in the region of Baix Llobregat. In other populations of the European continent with relatively high frequencies of P301L mutation, a possible founder effect has been also suggested (Dumanchin et al., 1998; Rademakers et al., 2004; Rizzu et al., 1999; Rosso et al., 2003).

3.2. The 50-kb haplotype of P301L carriers from France and Italy

To study if the haplotype linked to P301L mutation found in Barcelona is present in other European populations, we analyzed samples from France and Italy, which are geographically close and have had economic and historical relationships with Barcelona (Dandelet and Marino, 2007; Vicens Vives, 2015). The panel of 20 SNPs covering 50 kb around P301L mutation was analyzed in affected families from France and Italy. Thirty-eight individuals of 14 families from France and 16 individuals belonging to 4 families from Italy were studied (Table S3). As in the Barcelonan families, the haplotype of each family was attempted to be inferred through the analysis of patients carrying P301L and their healthy close relatives.

Unlike what was found in Barcelona, different haplotypes in the range of 50 kb were identified in French families (Table S5), although the haplotypes of 4 of these families could not be defined (Fr-2, Fr-3, Fr-7, and Fr-9). In remaining 10 families, at least 5 different haplotypes were identified. Two of the haplotypes were found in unique families (Fr-10 and Fr-12), and other 2 were shared each one by 2 different families (Fr-1 and Fr-5; Fr-4 and Fr-8). The fifth haplotype identified matched the one found in Barcelonan carriers (ACGGCTACTATCTTT-GATTA) and was present at least in one French family (Fr-13). The remaining 3 French families showed haplotypes that could be compatible with the Barcelonan one (Fr-6, Fr-11, and Fr-14). The reason for this compatibility is the matching of most of the alleles linked to P301L mutation, except for 1 or 2 SNPs in each haplotype, where the heterozygous state did impossible to identify the linked allele.

The Italian families showed that 3 of them shared the same 50-kb haplotype, which is different to that found in Barcelona, with nonmatching alleles in 5 of the 20 SNPs. Only one family from Italy showed a haplotype which could be compatible with the Barcelonan one, but the linked allele in 5 SNPs could not to be assigned because of their heterozygous state and the lack of more relatives to compare.

These results show that French families affected by FTD linked to P301L *MAPT* mutation analyzed in this study are descended from different ancestors. On the contrary, most of the Italian families studied here share the same haplotype linked to P301L mutation. Thus, it is possible that the appearance of this mutation in the Italian families was the result of a founder effect.

On the whole, families from Barcelona, France, and Italy affected by FTD have different haplotypes, and thus, it is feasible to think that several founder effects have happened in different regions. This also suggests that codon 301 in exon 10 of *MAPT* gene may be considered as a mutational hot spot. The French families show a noteworthy diversity of haplotypes in contrast to the presence of unique haplotypes in the Italian and Barcelonan families studied here. One of the French families shares the same 50-kb haplotype observed in families from Barcelona, whereas another Italian family and 3 additional French families could also be sharing this haplotype. This finding suggests that these familial groups could be descendant from the same common ancestor.

3.3. The 30-Mb haplotype linked to P301L mutation

Given that the Barcelonan families, one French family, and probably an Italian and 3 French families share the same 50-kb haplotype linked to P301L mutation, a new panel of 12 SNPs spreading 30 Mb around the mutation was developed to define the edges of the haplotype carrying the mutation. The results in Barcelonan, French, and Italian families sharing the same 50-kb haplotype, or a compatible one, are shown in Table S6.

Some haplotypes were incomplete due to the heterozygous state of some SNPs in analyzed families, which made impossible to determine the allele linked to the mutation in them. For comparisons, these unknown alleles were passed over, except if they were beside the SNP that differed from other haplotypes.

The 30-Mb panel revealed 11 haplotypes in the 9 families from Barcelona, showing that some affected individuals of the same family carried different 30 Mb haplotypes (BCN-3 and BCN-4). Five families had the same haplotype (BCN-1, BCN-5, BCN-6, BCN-8, and BCN-9), whereas the remaining 6 families harbored unique haplotypes (Table 1). Based on these different haplotypes, the edges of the haplotype shared by families from Barcelona go from P301L mutation (rs63751273) to rs189052, which is 2 Mb upstream (Table 1 and Fig. 2B). However, every haplotype shares at least 8 Mb with the most common 30-Mb haplotype.

In the case of the French families, there could be a haplotype shared by 2 families (Fr-11 and Fr-14), but it is incomplete in both. The other 2 French families have different haplotypes, going the edges of the possible French common haplotype from rs56406346 (12 Mb upstream) to P301L mutation (rs63751273) because the allele of rs8067216 (2 Mb downstream) was undetermined in all the haplotypes. These French haplotypes are different to those found in Barcelona, however, passing over the undetermined alleles, Fr-13 could share up to 26 Mb with the most common haplotype found in Barcelona.

On the other hand, the haplotype found in Italian family shares at least 18 Mb with the most common 30-Mb haplotype found in Barcelona. This haplotype shares all the alleles of SNPs located upstream the mutation, but downstream, the edge of this haplotype is clearly marked by the different allele found in rs9674910 (8 Mb downstream). However, it was impossible to determine the allele linked to P301L of the previous SNP (rs203001, 6 Mb downstream).

The joint analysis of 30-Mb haplotypes of every family that shares the Barcelona 50-kb haplotype revealed that all of them keep a common haplotype of up to 2 Mb linked to P301L mutation (Table 1 and Fig. 2B). This pointed to a common ancestor from which all of these families from Barcelona, France, and Italy descended.

The 30-Mb panel was also analyzed in the 3 Italian families that shared the same 50-kb haplotype among them (It-2, It-4, and It-16). One of these families (It-4) showed 2 different 30-Mb haplotypes linked to P301L (Table S6). All the alleles upstream were shared by all the haplotypes, passing over 2 undetermined SNPs of It-16. Downstream, both haplotypes found in It-4 showed a clear difference in rs9674910 (8 Mb), whereas haplotypes of It-2 and It-16 presented several undetermined SNPs. Thus, the common Italian haplotype could be from 8 Mb to up to 22 Mb long.

Table 1
Haplotypes of 12 SNPs covering up to 30 Mb around P301L of patients sharing the 50-kb haplotype found in Barcelona

Region/ Country	Family	rs8078405 (16 Mb)	rs72825740 (14 Mb)	rs56406346 (12 Mb)	rs1617208 (10 Mb)	rs371080205 (8 Mb)	rs11654626 (4 Mb)	rs189052 (2 Mb)	rs63751273 (P301L)	rs8067216 (2 Mb)	rs203001 (6 Mb)	rs9674910 (8 Mb)	rs1864650 (10 Mb)	rs3928386 (14 Mb)	Minimum length of the shared haplotype (Mb)
Barcelona	BCN-8	G	A	A	T	G	G	T	T	G	A	C	G	G	30
Barcelona	BCN-5	G	A	A	–	G	G	T	T	G	A	C	G	G	30
Barcelona	BCN-6	G	–	A	T	G	G	T	T	–	A	C	G	G	30
Barcelona	BCN-9	G	A	A	–	G	G	T	T	–	A	C	G	G	30
Barcelona	BCN-1	G	–	A	–	G	G	T	T	G	–	C	G	G	30
Barcelona	BCN-3	G	**G**	A	–	G	G	T	T	–	A	C	G	G	26
Barcelona	BCN-3	G	**G**	A	–	G	G	T	T	–	**C**	**G**	G	G	12–14
Barcelona	BCN-4	G	A	A	–	G	G	T	T	G	**C**	**G**	G	G	18
Barcelona	BCN-4	G	A	A	–	G	G	T	T	G	**C**	**G**	**A**	G	18
Barcelona	BCN-7	G	*A*	*A*	**G**	G	G	T	T	**A**	*A*	C	G	G	8
Barcelona	BCN-2	G	*A*	*A*	–	G	**A**	T	T	G	–	C	G	G	16
France	Fr-14	G	A	A	T	G	A	T	T	–	C	G	G	G	30/2–4
France	Fr-11	G	A	–	–	G	A	T	T	–	C	–	–	G	30/2–4
France	Fr-6	G	A	A	T	G	A	T	T	–	**A**	**C**	G	G	16–22/16
France	Fr-13	G	**G**	A	–	G	–	T	T	–	**A**	**C**	–	G	12–18/26
Italy	It-3	G	A	A	T	G	G	T	T	G	–	G	G	G	30/18–22

SNPs at the left of the mutation are located upstream in the chromosome, and those at the right are downstream. In brackets is showed the distance of each SNP to P301L. Alleles that differ from the common haplotype of each population are marked in bold and italicized. In the haplotypes, a dash (–) indicates those SNPs where the allele linked to the mutation could not be inferred.
Key: SNP, single-nucleotide polymorphism.

3.4. Relation between analyzed populations

Due to the presence of the same 50-kb haplotype in patients with FTD carrying P301L mutation from Barcelona (Spain), France, and, probably, Italy, we tried to study their ancestral relationship. For this aim, approximate Bayesian computation inferences were performed comparing all the individuals that share the same Barcelonan 50-kb haplotype, by using genotypic data of the 30-Mb panel (12 SNPs).

The result of this analysis showed that the most likely scenario is that in which French and Italian patients diverged at the same time from the affected population of Barcelona (Fig. S6). However, the likelihood of this scenario is low and it is only slightly higher to that obtained by the scenario in which French population is the branch from which Barcelona and Italian patients diverged (Fig. S6b). Because only one patient sharing this 50-kb haplotype was found in Italy, the same analysis was repeated removing this Italian patient (Fig. S7). Nevertheless, the same results were obtained being the most likely scenario that in which French affected patients diverged from population of Barcelona, but only with somewhat higher likelihood than the contrary scenario.

There are several factors that might point to a common ancestor of the 50-kb haplotype in Barcelona. Affected families from Barcelona present a slightly higher and more variable diversity of 30-Mb haplotypes than French and Italian families sharing the Barcelona 50-kb haplotype (Table 1). The core of 30-Mb haplotype shared by Barcelonan patients is the smaller one, with only 2-Mb in common among all of them. Besides, some haplotypes found in French and Italian families were also found in families from Barcelona. Furthermore, Barcelona is the group where more families share the ACGGCTACTATCTTT-GATTA 50-kb haplotype, being the only haplotype found in the population. This later point could reinforce the hypothesis of being a locally originated haplotype.

3.5. Age of P301L in these populations

Once the ancestry of P301L mutation has been studied for families from Barcelona, France, and Italy, the age of P301L has been calculated with DMLE+ software using the haplotypes that appeared in more than one family from each geographic region (Table 2 and Table S7).

The inference of mutation age is influenced by several issues that need to bear in mind when using DMLE+ software. The number of generations elapsed from the origin of the mutation that is estimated by this software shows great variation depending on the growth rate used (Aller et al., 2010; Porfirio et al., 2016; Sanchez-Jimeno et al., 2013). Moreover, the values of population growth have suffered great fluctuations over time. Therefore, every calculation was carried out with 2 different growth rates, 0.05 and 0.01, the range where they might have been for longer. On the other hand, age estimations also varied depending on the size of population sampled. For this reason, the age of P301L for families from Barcelona was calculated considering both the population of the County of Baix Llobregat where they originated and the total population of Spain. French and Italian samples, on the contrary, do not belong to a specific region, and thus, total populations of their countries were considered. In addition, the age of P301L was also calculated considering populations of France and Barcelona (County of Baix Llobregat) together, because these are the regions where the same 50-kb haplotype has been found.

The lowest mutation age was obtained in Barcelona (Baix Llobregat), regardless of what growth rate is used, which seems to be consequence of its population size because it is considerably smaller than Spanish or French populations, as previously mentioned. Ages obtained in Spanish and French populations are

Table 2
Estimation of the age of P301L

Population	Population size	Proportion of population sampled	Growth rate	Mutation age (no. of generations)	CI 95%
Baix Llobregat (Barcelona, Spain)	806,651	0.06641	0.05	45.965	16.2372–98.8144
			0.01	109.591	48.3171–273.588
Spain	46,549,045	0.00115	0.05	129.011	98.9806–178.292
			0.01	509.736	416.894–655.129
France	66,952,000	0.00054	0.05	114.275	64.7386–193.084
			0.01	522.852	166.315–655.846
Baix Llobregat + France	67,758,651	0.00136	0.05	141.634	105.650–205.034
			0.01	544.911	406.433–743.041

Proportion of population sampled was calculated with prevalence data of FTD for each country: 14:100,000 in Spain (López-Pousa et al., 2002) and 11:100,000 in France (Coyle-Gilchrist et al., 2016).
Key: FTD, frontotemporal dementia.

similar, being P301L slightly older in Spain when 0.05 growth rate is considered, and somewhat younger than in France for 0.01 growth rate.

Finally, when population of the County of Baix Llobregat (Barcelona) and France were taken into account together, they showed the oldest ages for both growth rates. These could be the most accurate data because they encompass the geographic area where the ACGGCTACTATCTTT-GATTA 50-kb haplotype has been found. Therefore, if it is considered that each generation spans for 20 years, P301L *MAPT* mutation would have appeared in this region between 2,832.68 and 10,898.22 years ago.

4. Conclusions

The prevalence of FTD linked to P301L *MAPT* mutation in the region of Baix Llobregat (Barcelona, Spain) seems to be due to a single founder effect. A possible founder effect has been also suggested for other European populations with noteworthy frequency of P301L (Dumanchin et al., 1998; Rademakers et al., 2004; Rizzu et al., 1999; Rosso et al., 2003). This could be the case of Italian sample analyzed in this study because most of the patients but one share the same 50-kb haplotype linked to P301L. On the contrary, French patients showed diverse 50-kb haplotypes linked to P301L, what discards a unique ancestral origin of this mutation in French population. However, the existence of unique local ancestors in different regions of France cannot be discarded, and further regional studies should be conducted to confirm it.

The 50-kb haplotype found in most of the Italian families is different to that found in Barcelona, and hence, most Italian patients affected by this mutation are unrelated to the ancestor of families from Barcelona. However, the only Italian patient whose haplotype differs from the others, even if it is partially defined, could match the one found in Barcelona. Some French families could also share the ancestor with those from Barcelona because at least one of them shows the same 50-kb haplotype linked to P301L.

The analysis of the additional set of 12 SNPs spread along 30 Mb around the mutation in patients from Barcelona, France, and Italy carrying the same 50-kb haplotype showed that diversity of this haplotype linked to P301L is slightly higher in the population of Barcelona because 2 families showed more than one 30-Mb haplotype among their affected relatives. Moreover, the 30-Mb haplotypes found in Barcelona showed the smallest shared common region into a population, which is of 8 Mb, whereas the analyzed families from France share 12 Mb. The ABC analyses also point to Barcelona as the population from which the French and Italian patients diverged. However, the likelihood obtained in these analyses is low, and it cannot be discarded the hypothesis of population of Barcelona diverging from the French. The estimation of the mutation age showed similar results in Barcelona and France, not being possible to determine in which population P301L is oldest. However, the most ancient, and probably accurate estimation, was obtained when both populations of Baix Llobregat (Barcelona) and France were analyzed together, as one. This result suggest that P301L mutation of the haplotype found in Barcelona could appear in an area encompassing France and the northeast of Spain, where the County of Baix Llobregat is placed.

All in all, results of this study suggest that prevalence of FTD linked to P301L *MAPT* mutation in Barcelona is due to a unique mutational event because every patient from this region has the same 50-kb haplotype linked to the mutation, being descended from a unique common ancestor. These results also suggest that it is a locally originated mutation, in an area that may include northeast of Spain and a part of France. In the same way, the analyzed Italian patients seem to be descendants from a common ancestor, which would be different to the one of Barcelonan population. Finally, on the contrary to Italian and Barcelonan patients, there is a wide diversity of 50-kb haplotypes in France, being clearly descendants of different ancestors, and probably result of several founder effects. Thus, it seems clear that there have been several founder effects of P301L mutation along Europe, where some regions seem to have a unique ancestor, whereas others show diverse origins. The existence of so many different founder effects of P301L mutation suggests that codon 301 in exon 10 of *MAPT* gene may be a mutational hot spot.

Disclosure

The authors declare no conflict of interest.

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All participants gave their written informed consent, and all procedures performed in this study were in accordance with the ethical standards of the Helsinki declaration. The study was approved by the Hospital Clinic ethics committee (HCB/2014/0331).

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All the authors have read and approved the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2019.08.015.

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