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Variants in triggering receptor expressed on myeloid cells 2 are associated with both behavioral variant frontotemporal lobar degeneration and Alzheimer's disease

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Abstract

Recent evidence suggests that rare genetic variants within the TREM2 gene are associated with increased risk for Alzheimer's disease. TREM2 mutations are the genetic basis for a condition characterized by polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOS) and an early-onset dementia syndrome. TREM2 is important in the phagocytosis of

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apoptotic neuronal cells by microglia in the brain. Loss of function might lead to an impaired clearance and accumulation of necrotic debris and subsequent neurodegeneration. In this study, we investigated a consanguineous family segregating autosomal recessive behavioral variant FTL D from Antioquia, Colombia. Exome sequencing identified a nonsense mutation in *TREM2* (p.Trp198X) segregating with disease. Next, using a cohort of clinically characterized and neuropathologically verified sporadic AD cases and controls we report replication of the AD risk association at rs75932628 within *TREM2*. These data suggest that mutational burden in *TREM2* may serve as a risk factor for neurodegenerative disease in general and that potentially this class of *TREM2* variant carriers with dementia should be considered a molecularly distinct form of neurodegenerative disease.

1. Introduction

TREM2, or triggering receptor expressed on myeloid cells 2, is an immunoreceptor expressed on activated macrophages, osteoclast, immature dendritic cells and microglia (Colonna, 2003). It is a 26kDa transmembrane glycoprotein that consists of a single extracellular immunoglobulin-like domain, a transmembrane region with a charged lysine residue and a short cytoplasmic tail lacking any signaling motifs (Colonna, 2003). *TREM2* forms a receptor signaling complex with *TYROBP* (Paloneva et al., 2002). The charged lysine in the transmembrane domain of *TREM2* is needed for its association with *TYROBP* (Bouchon et al., 2000; Bouchon et al., 2001) and as *TREM2* lacks an intracellular signaling tail, it is completely dependent on the presence of the adaptor protein *TYROBP* (Colonna, 2003). The *TREM2*/*TYROBP* complex regulates key signaling events involved in immune responses, differentiation of dendritic cells and osteoclasts and phagocytic activity in microglia (Bouchon et al., 2001; Hsieh et al., 2009; Otero et al., 2012).

Following neuronal injury, microglia initiate repair by phagocytizing dead neurons without eliciting inflammation. *TREM2* has been shown to play a role in the phagocytosis of apoptotic neuronal cells by microglia and resolution of inflammation (Hsieh et al., 2009). *TREM2* can directly bind to neuronal cells, with increased binding to apoptotic neuronal cells. When neuronal cells undergo apoptosis, they increase the expression of *TREM2*-ligands, which mediate signal transduction by *TREM2* on microglia and promote phagocytosis (Hsieh et al., 2009). In osteoclasts, *TREM2* has been shown to regulate bone mass by regulating the rate of osteoclast generation (Otero et al., 2012).

Genetic mutations in either *TREM2* or *TYROBP* cause a similar clinical phenotype, the Nasu-Hakola syndrome (or polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy; PLOSL) (Figure 1), which is characterized with cystic-like lesions of the bone and brain demyelination that lead to fractures and presenile dementia (Paloneva et al., 2002). The disease is characterized by different stages. The first symptoms present with an osseous stage at the third decade of life with pathological bone fractures. This is followed by the early neuropsychiatric stage in the fourth decade, presenting a frontal lobe syndrome, and the late neuropsychiatric stage, with profound dementia and usually death by the age of 50 (Bianchin et al., 2004; Numasawa et al., 2011). Neuropathological findings include loss of myelin and axons in the brain, with reactive astrocytosis and microglial activation (Klunemann et al., 2005). Mutations in *TREM2* have also been described in pure early-onset dementia without bone cysts, and frontotemporal dementia (FTD)-like syndrome (Chouery et al., 2008). Recently, a variant in *TREM2* (rs75932628) has also been implicated as a risk factor for both early-onset and late-onset Alzheimer's disease (R. Guerreiro et al., 2012; Jonsson et al., 2012; Pottier et al., 2013).

In this study, we identified a nonsense mutation in *TREM2* in a consanguineous Colombian family segregating autosomal recessive FTL D. Frontotemporal lobar degeneration (*FTLD*)

is the second most common cause of early-onset dementia and fourth most common cause of late-onset dementia, and is characterized by atrophy of the prefrontal and anterior temporal lobes. FTLD is a clinically and genetically heterogeneous degenerative disorder. Patients usually show prominent behavioral and/or language deficits, which evolve gradually into cognitive impairment and dementia (McKhann et al., 2001; Neary et al., 1998). The most common clinical manifestation of FTLD consists of patients who present behavioral or personality changes (behavioral variant frontotemporal dementia or bvFTD) (Neary et al., 1998). Two other prototypic clinical phenotypes that occur in FTLD are language impairment disorders: semantic dementia (SD) and progressive nonfluent aphasia (PNFA) (Neary et al., 1998).

In this manuscript, we report that a nonsense mutation in *TREM2* is the cause of FTLD in a Colombian family from the province Antioquia. Additionally, we provide replicative evidence demonstrating a role for the rs75932628 *TREM2* variant in Alzheimer's disease thereby suggesting *TREM2* mutations as a risk factor for neurodegenerative disease in general.

2. Material and methods

2.1 Clinical diagnosis

A large consanguineous Colombian family segregating autosomal recessive FTLD was collected through the Grupo Neurociencias, University of Antioquia, Colombia (Figure 2). Three patients and five unaffected relatives from the family were included. Our patients met published criteria for behavioral variant FTD (Rascovsky et al., 2011). The index case was a female offspring of first cousins who first showed symptoms of sexual disinhibition at age 47. She was excessively familiar with strangers and had abandoned all her responsibilities in the home. A paternal uncle and a brother had similar symptoms prior to age 60.

Description of the clinical tests—All patients and controls underwent a standard medical and neurological history, physical examination, and office-based clinical cognitive assessment. Patient III:16 (index patient) and her brother (patient III:10) additionally underwent more detailed neuropsychological assessment that included:

General: mental status examinations (maximum score 30 points)

Memory: 10 item, 3 learning trials word-list learning with delayed free recall and recognition, and the Rey-Osterrieth Complex Figure Test (copy and recall, 36 points maximum for each)

Attention/Psychomotor Speed: Trail Making Test (seconds to complete), Wechsler Adult Intelligence Scale-Revised Digit Symbol Substitution Test (raw total score)

Language: FAS letter fluency (numbers of words generated in one minute for each letter), Category Fluency (Animals named in one minute), and sentence writing

Executive functions: Wisconsin Card Sorting Test (Categories and perseverative responses)

This study was conducted according to the guidelines of the ethical committee at the University of Antioquia. Informed consent was obtained from all individuals or from family members in cases of impaired cognition.

2.2 Exome sequencing

Exome sequencing was performed on two affected individuals and two unaffected individuals from the family (identify in the family tree who they are). First, 1.2ug of DNA

was fragmented to 300–400bp fragments with a Covaris E210 instrument (Covaris Inc., Woburn, US). Next, libraries were prepared with Illumina's Truseq DNA Sample Preparation Kit v2 (Illumina inc, San Diego, USA), following the manufacturer's protocol. All four samples were labeled using four different indices. Final PCR enriched fragments were validated on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, US). In addition, samples were quantified with PicoGreen (Molecular Probes, Eugene, Oregon, USA), and 500ng of each sample was pooled before entering Truseq 62Mb Exome Enrichment Kit (Illumina inc, San Diego, USA). The final library was then validated on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, US), quantified using qPCR (7900 HT, Applied Biosystems, Foster City, CA; Kapa Biosystems, Woburn, MA) and sequenced by 100bp paired-end sequencing on a HiSeq2000 instrument (Illumina inc, San Diego, USA).

2.3 Data-analysis

Reads were aligned to the Human genome (Hg19/GRC37) using the Burrows-Wheeler transform (BWA v.0.5.9) (Li and Durbin, 2009). PCR duplicates were removed using Picard v1.51 (H. Li et al., 2009) and base quality recalibration, indel realignment and SNP and INDEL discovery was performed using the Genome analysis toolkit (GATK v1.1-31-gdc8398e) (McKenna et al., 2010). Data were filtered against dbSNP135, 1000 genomes, an in-house exome database, and then annotated with snpEff v2.0.2 against ensembl v63 to identify new damaging mutations (nonsense, splice site, frameshift, missense) (Cingolani, 2011). Only homozygous variants present in the two affected individuals and either heterozygous or not present in the controls were considered as candidates.

2.4 Sanger DNA sequencing

The c.594G>A mutation in *TREM2* was confirmed with Sanger sequencing in the samples used for exome sequencing along with three additional controls and one additional case from the family. Primers surrounding the identified variants were designed using Primer3. Polymerase chain reaction (PCR) was carried out under standard conditions. Direct sequencing of the PCR product was performed on an ABI3130XL sequencer (Applied Biosystems Inc., Foster City, USA).

2.5 APOE genotyping

APOE genotyping was performed on all family members by the method of Crook et al (Crook et al., 1994).

2.6 Clinically Characterized and Neuropathologically Verified Subjects

Our US series was obtained from 21 National Alzheimer's Coordinating Center (NACC) brain banks and from the Miami Brain Bank as previously described (Corneveaux et al., 2010; Myers et al., 2007; Webster et al., 2009). Additional cohorts from the United Kingdom and Europe were obtained in the same manner as the original US series. Our criteria for inclusion were as follows: self-defined ethnicity of European descent (in an attempt to control for the known allele frequency differences between ethnic groups), neuropathologically confirmed AD or no neuropathology present, and age of death greater than 65. Neuropathological diagnosis was defined by board-certified neuropathologists according to the standard NACC protocols (Beekly et al., 2004). Samples derived from subjects with a clinical history of stroke, cerebrovascular disease, Lewy bodies, or comorbidity with any other known neurological disease were excluded. AD or control neuropathology was confirmed by plaque and tangle assessment with 45% of the entire series undergoing Braak staging (Braak and Braak, 1995). Samples were de-identified before receipt, and the study met human studies institutional review board and HIPPA regulations. This work is declared not human-subjects research and is IRB exempt under

regulation 45 CFR 46. See the Acknowledgements section for a list of individual sites that contributed samples to this effort.

2.7 Genome-wide SNP Genotyping

Genomic DNA samples were analyzed on the Genome-Wide Human SNP 6.0 Array (Affymetrix, Inc. Santa Clara, CA) according to the manufacturer's protocols (Affymetrix Genome-Wide Human SNP Nsp/Sty 6.0 User Guide; Rev. 1 2007) and as described previously (Corneveaux et al., 2010).

2.8 SNP Imputation

Genotypes were imputed using MACH v.1.0.16 (Y. Li et al., 2009) as described previously (Corneveaux et al., 2010).

3. Results

3.1 Clinical syndrome

Initial symptoms began between ages 45 and 50 involving altered social behavior in all three, as well as an oropharyngeal tic in one that subsequently developed in the other two as well. Two of the three developed the new onset of substance use including alcohol and tobacco as well as complex partial seizures characterized by versive head and eye turning and speech arrest. Eventually, all three developed a severe pan-frontal syndrome with apathy, disinhibition and impulsivity, obsessive and perseverative behavior, impaired social behavior and cognitive decline that included psychomotor slowing and impaired problem solving with increased perseverative errors, as well as constructional apraxia, and memory loss (Table 1). Physical exam revealed frontal release signs but no evidence of motor neuron disease. MRI (index patient) showed asymmetric bifrontal atrophy (Figure 3).

Patient III:16 (Index patient) began to express noticeable behavioral and personality changes at age 47, starting with sexual and social dis-inhibition, mood changes and neglect of personal hygiene. In addition, her abstract reasoning, decision-making, planning and judgment decreased severely. Her brother (patient III:10), presented behavioral changes at the age of 50. He became apathetic, showed progressive memory loss and obsessive compulsive behaviors. Later, he suffered from insomnia, presented motor and phonetic tics, and seizures characterized by versive head movement, gaze deviation, and speech arrest. The older sister (patient III:14), started showing personality changes, dis-inhibition and progressive memory loss at the age of 45. She emits guttural speech sounds and presents similar seizures as her brother characterized by a versive head, eye motions and speech arrest. All three patients' functionality has been reduced to only basic self-caring activities.

3.2 Identification of a nonsense mutation in *TREM2*

Two patients (III:10 and III:14) and two controls (III:11 and III:12) from the Colombian family were sequenced using the Illumina's 62Mb Truseq Exome Enrichment Kit. Variants identified by next generation sequencing were filtered by dbSNP135, the 1000 genomes project and an in-house list of exomes. Only variants homozygous in the two patients were further prioritized. This led to the identification of a nonsense mutation in exon 4 of the gene *TREM2*, that leads to premature truncation of the protein (c.594G>A;p.Trp198X). We confirmed the presence of the mutation in the patients by Sanger sequencing, and subsequently checked the inheritance of the mutation in the family (Figure 2).

No possible disease-causing variants were detected in the known FTLN genes (*GRN*, *MAPT*, *VCP*, *CHMP2B*, *TARDP* or *C9ORF72* and *TMEM106B*). In addition, all family members were APOE E3/E3 homozygotes.

3.3 *TREM2* as a risk factor for Alzheimer disease

Recent studies show that the p.Arg47His variant (rs75932628) is associated with a significantly increased risk for Alzheimer's disease (R. Guerreiro et al., 2012; Jonsson et al., 2012; Pottier et al., 2013). We saw an enrichment of this variant by a factor of 2.8 in our collection of clinically characterized and neuropathologically confirmed AD cases which was significant when combined with the data of Guerreiro et al, 2012 (Table 2) (R. Guerreiro et al., 2012).

4. Discussion

TREM2 is a membrane protein that forms a receptor-signaling complex with TYROBP that triggers activation of the immune response, differentiation of dendritic cells and osteoclasts and phagocytosis in microglia. We identified a mutation in exon 4 of the *TREM2* gene (p.Trp198X) in a consanguineous Colombian family segregating autosomal recessive FTLD (Figure 2). The clinical phenotype of our family seems very similar to PLOSL, except for the complete absence of a bone phenotype in our family. Chouery and colleagues also reported a mutation in *TREM2* (c.40+3delAGG) in a Lebanese family segregating pure early-onset dementia without bone cysts (Figure 1) (Chouery et al., 2008). The affected individuals presented personality and behavioral changes at age of 30–35, culminating in severe dementia. Brain MRI showed either a diffuse brain atrophy or cortical atrophy in the temporal lobes (Chouery et al., 2008). Similar to our patients, a subsequent report in Turkish patients (R.J. Guerreiro et al., 2012), also described a frontotemporal dementia (FTD)- like syndrome without bone cysts. Neither prior report described the tics we found in our patients, but two of the three Turkish patients described had generalized seizures.

Also recently, a rare missense mutation in *TREM2* (p.Arg47His; rs75932628), was found to significantly increase the risk of late-onset Alzheimer disease (R. Guerreiro et al., 2012; Jonsson et al., 2012) and early-onset Alzheimer disease (Pottier et al., 2013). The mutation may lead to a decreased affinity of *TREM2* for its natural ligand or affect its signaling. Although this variant is less frequent than APOE E4, it confers a risk for Alzheimer's disease with an effect similar to that of APOE E4 (Jonsson et al., 2012). The clinical phenotype in all *TREM2* associated dementia cases reported to date have striking panfrontal syndromes quite distinct from the typical AD phenotype. How different genetic variants result in topographically altered patterns of pathology is not yet known, but it has been previously shown that presenilin 1 mutations, unlike sporadic AD or APOE e4 carrier cases, are associated with early striatal deposition of amyloid (Klunk et al., 2007).

We saw an enrichment of this variant in *TREM2* (p.Arg47His) in our cohort of clinically characterized and neuropathologically verified Alzheimer's disease cases and control (Table 2) (Corneveaux et al., 2010), which is in line with the data of Guerreiro et al. (R. Guerreiro et al., 2012). Because *TREM2* has been shown to be involved in PLOSL, FTD-like disease, Alzheimer's disease and now also FTLD, we hypothesize that *TREM2* might be a risk factor for neurodegeneration in general, and other neurodegenerative disease cohorts should be examined for this *TREM2* variant to better understand its role in the pathophysiology of neurodegeneration.

It is also important to note that the rarity of the p.Arg47His variant results in most carriers existing in the heterozygous state in the general population. This is important as they likely express a wild type version of *TREM2* in addition to the mutant version. Under the assumption that the *TREM2* mutant doesn't function as a dominant negative molecule this may represent a therapeutic avenue for treatment or prevention of the neurodegenerative disease in the p.Arg47His heterozygotes. Chemical or biological agonists of *TREM2* could be explored for use in these individuals.

Mutations in *TREM2* leading to PLOSL are mainly loss of function mutations caused by premature truncation of the protein, lacking the complete transmembrane and cytoplasmic domains (Figure 1) (Klunemann et al., 2005). Although the effect of some of the non-synonymous mutations might be unclear, the p.Lys186Asn mutation changes the positively charged lysine in the transmembrane part of the protein to an asparagine, which disrupts the association with and expression of *TYROBP*, also resulting in a loss-of-function (Bouchon et al., 2000; Klunemann et al., 2005; Paloneva et al., 2002). The c.40+3delAGG mutation, which leads to pure early-onset dementia without bone cysts, weakens the splice site at the 5 donor site of intron 1. Quantitative RT-PCR showed an over two-fold down regulation of wild-type *TREM2* transcripts because of this mutation (Chouery et al., 2008). The presence of functional wild-type protein in these patients, although reduced, might explain why these patients only present a neurodegenerative phenotype and lack a bone phenotype.

A genotype-phenotype correlation is much less clear in our family. The p.Trp198X mutation truncates only the last 33 AA of the protein (Figure 1), leaving the extracellular and transmembrane part of the protein almost intact, but missing the short cytoplasmic domain (Bouchon et al., 2000). Since the transmembrane region mediates the formation of a complex with *TYROBP*, the protein might therefore still be expressed and be able to form a partly functional complex with *TYROBP* on the cell surface, leading to a less severe phenotype in our family. However a genotype-phenotype correlation is less clear for the p.Gln33X mutation, which has been described to cause both FTD-like syndrome and PLOSL. This suggests that environmental factors of modifier genes also influence the phenotype associated with *TREM2* mutations. In addition, heterozygote carriers of p.Gln33X were reported to show memory deficits or and might have a higher risk for developing late-onset Alzheimer disease (R. Guerreiro et al., 2012).

The Colombian family in this study originates from Antioquia, which is a young admixture that was founded in the 16–17th century by only a few hundred American natives and immigrants mainly from Spain (Carvajal-Carmona et al., 2003). The people in the region have remained relatively isolated since its origin (Carvajal-Carmona et al., 2003). Young genetic isolates confer an advantage to gene-mapping, and are usually enriched for specific Mendelian traits that often occur in the population because of a founder effect (Sheffield et al., 1998). In addition to the founder effect, small effective population size and bottlenecks increase consanguinity, and therefore increase the opportunity to map recessive diseases. Rare Mendelian disease in isolated populations is almost always attributable to a single mutation that is shared identical-by-descent among affected individuals in the population. In the Antioquia population, founder mutations have been identified previously, for example, the c.G736A mutation in the *PARK2* gene that causes early onset Parkinson's Disease (Pineda-Trujillo et al., 2006). It is unclear however when the p.Trp198X *TREM2* mutation arose in Antioquia. If the mutation originated before or close to the founding of the population, the carrier frequency could be very high in Antioquia. However, an analysis of the 1,000 Genomes Project dataset revealed no carriers of this variant in 60 individuals from Medellin, Colombia (referred to as "CLM") suggesting a frequency of less than 1 in 120 chromosomes.

In conclusion, we identified a mutation in *TREM2* that leads to FTLD in a consanguineous family from Colombia. The mechanisms of *TREM2* mutations leading to FTLD and neurodegeneration in PLOSL are unknown, but we can hypothesize that lack of *TREM2* impairs the clearance of apoptotic neurons in microglia, leading to the accumulation of necrotic debris. We also replicate the association of the *TREM2* p.Arg47His variant and Alzheimer's disease risk. The involvement of *TREM2* genetic variants in PLOSL, FTD-like syndrome, Alzheimer's disease and now also FTLD, suggests that the *TREM2* locus may be associated with neurodegenerative disease in general and raises the possibility that this

variant in the context of other genetic variants or environmental factors are the determinants of the form of neurodegenerative disease that a carrier patient presents with.

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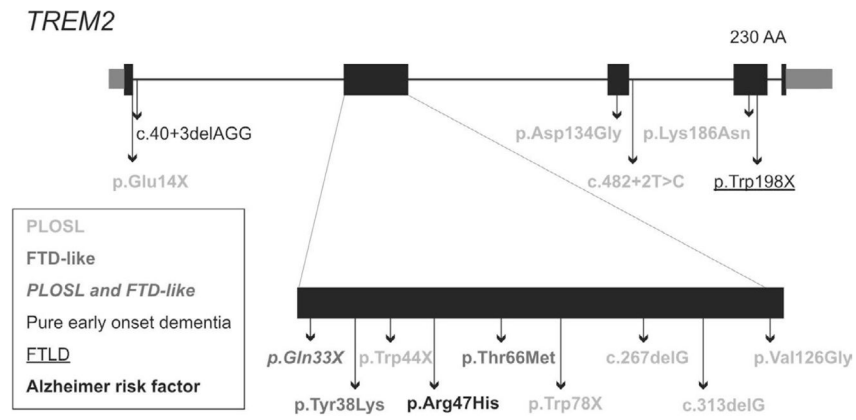


Figure 1.
Overview of the mutations found in *TREM2*. ENST00000373113.3; ENSP00000362205.3.

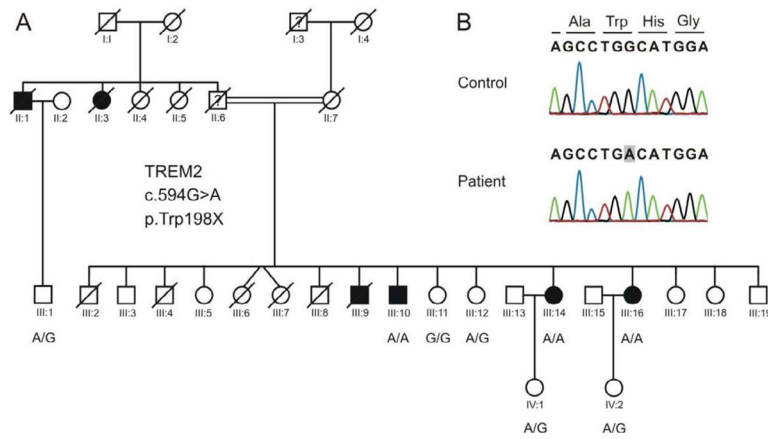


Figure 2.

A. Inheritance of the nonsense mutation in *TREM2* in the Colombian family. B. Sanger sequencing results. hg19/GRC37:chr6:41126693T>C; ENST00000373113.3:c.594G>A; ENSP00000362205.3:p.Trp198X.

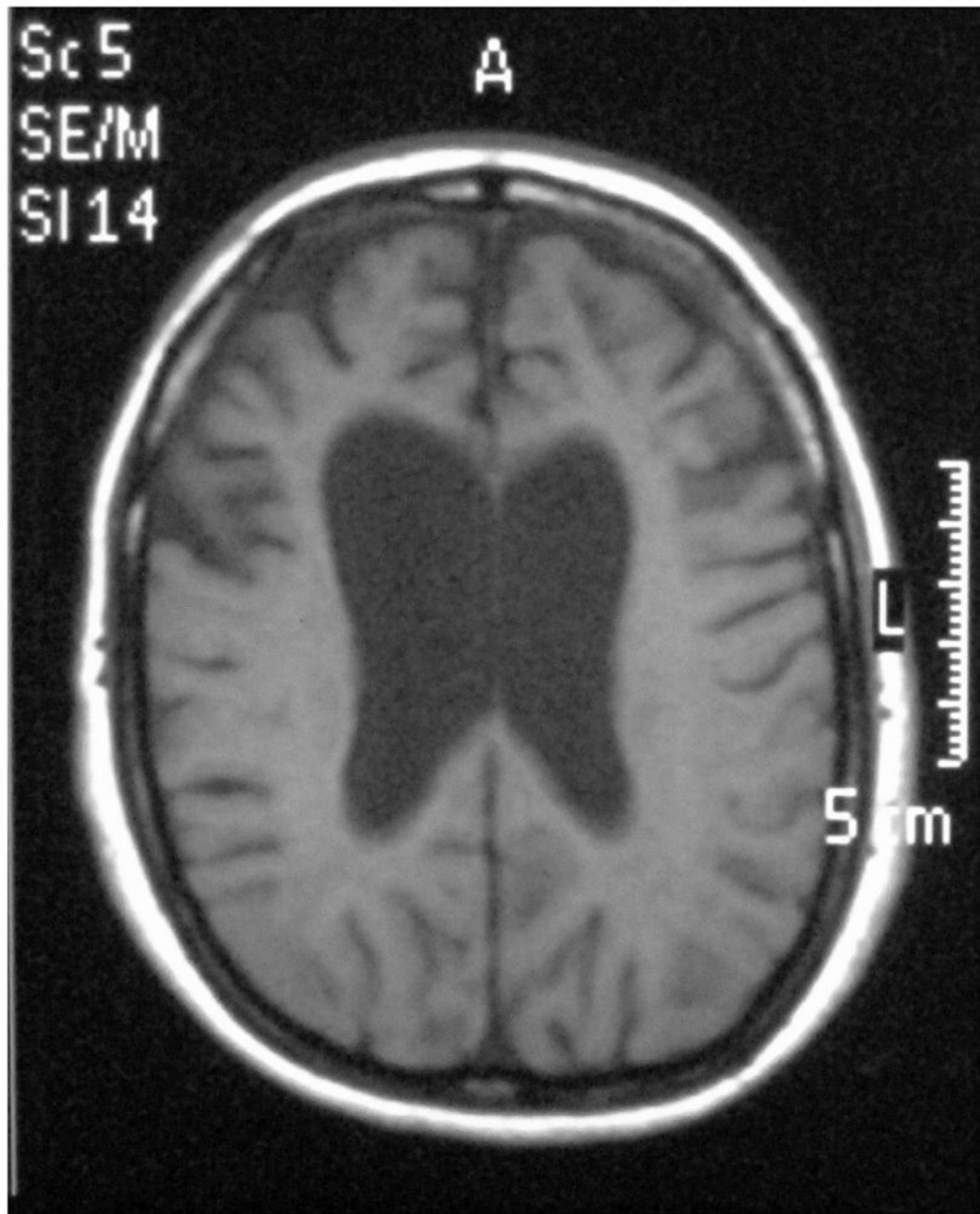


Figure 3.
MRI of the index case patient demonstrating the frontal lobe atrophy.

Table 1

Neuropsychological Test Scores in two TREM2 carrier members of the Colombian kindred.

	Patient 1	Patient 2
Age years	48	55
Gender	F	M
Education years	10	14
Handedness	Right	Right
Occupation	Homemaker	Unemployed
Minimental Status Exam	24/30	23/30
Trail Making Test-A (seconds)	99	154
Trail Making Test-B (seconds)	278	na
WAIS-R * Digit Symbol (raw total)	23	11
10 word list		
-3 learning trials	2--3--3	2--3--3
-spontaneous recall	1	0
-true positive recognition	7	1
-true negative recognition	9	10
Complex Figure Test-copy	18.5	16.5
-recall (%)	5 (27%)	0 (0%)
Animal fluency (1 minute)	8	3
Wisconsin Cart Sorting Test		
-Categories	1	0
perseverative responses (%)	28 (58.3%)	47 (97.9%)
FAS letter fluency	15	2
Frontal Behavioral Inventory	34/72	54/72

* WAIS-R = Wechsler Adult Intelligence Scale-Revised

Table 2

Association of p.Arg47His in a cohort of clinically characterized and neuropathologically verified Alzheimer's disease cases and controls ("TGenII"). AD Meta-Analysis: combination of TGenII Cohort results and Guerreiro et al., 2012.

Group	MAF case	MAF control	Allele case	Allele control	Enrichment	p-value
TGen II AD	0.0025	0.0009	5 1990	1 1158	2.8	0.4242
AD meta-analysis	0.0088	0.0027	27 3081	6 2263	3.3	0.0044