Supplementary Material

Mutations in TYROBP are not a common cause of dementia in a Turkish cohort

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Introduction

TYRO protein tyrosine kinase-binding protein (*TYROBP)* (also known as *DAP12*) is a gene located on the long arm of chromosome 19. It encodes a 113 amino acid long transmembrane protein that is expressed on macrophages, monocytes, lymphocytes, osteoclasts, and, in brain, on microglia (Tomasello and Vivier, 2005). TYROBP plays different potential roles including signal transduction, bone modelling, brain myelination, and inflammation (Kuroda et al., 2007). TYROBP is a key regulator of the microglia network activated in late-onset Alzheimer's disease (LOAD) and has been shown to be significantly upregulated in the brains of Alzheimer's disease (AD) patients (Frank et al., 2008; Zhang et al., 2013). Mutations in *TYROBP* and *TREM2*, following an autosomal recessive pattern of inheritance, are known to cause Nasu-Hakola disease (Paloneva et al. 2000; Paloneva et al. 2002). Recently, we and others have identified homozygous and compound heterozygous variants in *TREM2* as the cause of frontotemporal dementia (FTD) syndromes without associated bone phenotypes (Guerreiro et al., 2013a; Guerreiro et al., 2013c) and heterozygous rare variants in the same gene as associated with a significant increase in the risk of AD (Guerreiro et al., 2013b; Jonsson et al., 2013).
The close association of *TREM2* and *TYROBP* to the different dementias and the functional interaction between them provide support for looking at *TYROBP* variation in a cohort of dementia patients.

Materials and Methods

Patient cohort

All participants were recruited consecutively over 24 months (2010 - 2012) in the Behavioral Neurology and Movement Disorders Unit outpatient clinic in Istanbul Faculty of Medicine, Istanbul University. They underwent detailed clinical and neuropsychological examination and, in most cases, cerebral magnetic resonance imaging (cMRI) or positron emission tomography (PET) imaging. The diagnosis of dementia was based on the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's disease (McKhann et al., 1984). FTD was defined following the criteria of International Behavioural Variant FTD Criteria Consortium (Rascovsky et al., 2011) and the criteria developed by an international group of PPA investigators (Gorno-Tempini et al., 2011). The diagnosis of dementia with Lewy bodies (DLB) was made based on the consensus guidelines from the DLB consortium (McKeith, 2006). Diagnostic procedures for Parkinson's disease dementia (PDD) followed the recommendations of the movement disorder society task force (Dubois et al., 2007). Twenty cases were diagnosed with mild cognitive impairment (MCI). MCI is known to not be a clinically stable entity, with some cases not having an underlying neurodegenerative process. Consequently, and given that no clinical follow up was performed, it is possible that some of these patients may have improved, some may have stabilized and some may have progressed to dementia.

All patients were negative for mutations in known dementia genes: *APP, PSEN1, PSEN2, NOTCH3, TREM2, MAPT, GRN, C9orf72, CHMP2B, FUS, TARDBP, SQSTM1* and *VCP* (Lohmann et al., 2012; Guerreiro et al., 2012; Guerreiro et al., 2013a; Guven et al., 2016).

The study was approved by the Ethics Committee of Istanbul Faculty of Medicine, Istanbul University and informed consent was obtained from the patients.

DNA extraction

Peripheral blood samples were collected and genomic DNA was extracted by standard procedures using the Qiagen DNA isolation maxi kit (Qiagen, Hilden, Germany).

Whole-Genome Genotyping (WGG)

One hundred and three samples underwent WGG using Illumina Infinium Technology to identify the presence of any large structural variants (>50 Kb) and large regions of homozygosity (>1 Mb). Samples were run on HumanOmniExpress BeadChips as per the manufacturer's instructions and data were visualized using the GenomeStudio Data Analysis Software (Illumina Inc.). The *TYROBP* locus (chr19:35234335-37547219, based on hg19) was analysed, which included two SNPs, rs1802029 and rs3817624, within the *TYROBP* gene (Figure S1).

Whole-Exome Sequencing (WES)

Sixty-four samples underwent whole-exome sequencing. SeqCap EZ Exome Library version 1.0 (Roche NimbleGen) was used, as per manufacturer's protocol, to enrich sequences corresponding to all annotated human exons by hybridization. One flow cell lane was used to sequence each DNA sample, on paired-end 50-base pair HiSeq 2000 runs (Illumina Inc), yielding an average of 6 billion high quality bases per sample. Illumina pipeline (version 1.7.1) was used to perform base calling and image analysis with default parameters. The Burrows-Wheeler aligner (Li and Durbin, 2009) was used to map sequence reads to the reference genome (GRCh37/hg19) and SAMtools was used to generate BAM files (li et al., 2009). SNPs and indels were called using the Genome Analysis Toolkit (DePristo et al., 2011) and annotated with SNPEff (Cingolani et al., 2012).

Sanger Sequencing

The 5 exons of *TYROBP* were Sanger sequenced in 39 samples. Exons were amplified by polymerase chain reaction (PCR) with Roche FastStart PCR Master Mix (Roche Diagnostics Corp) and sequenced with Applied Biosystems BigDye terminator version3.1 sequencing chemistry in an ABI3730XL genetic analyzer as per the manufacturer's instructions (Applied Biosystems). Primers are available upon request. The sequences were analysed using

Sequencher software version 4.2 (Gene Codes). The same procedures were used to obtain data for exons with low coverage in WES (different exons in 32 samples with average exonic coverage below 8x).

In silico analysis

Minor allele frequency (MAF) for each variant was obtained from the ExAC database for the global, European and South Asian populations. The functional predicted impact was evaluated using SIFT (Kumar et al., 2009), PolyPhen-2 (Adzhubei et al., 2010), MutationTaster (Schwarz et al., 2014) and CADD (Kircher et al., 2014) software. ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) was also used in the variants' classification process.

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Supplementary Figures and Tables

Figure S1: Plot of the B allele frequencies (BAF) and log R ratios (LRR) values for a representative sample from the Turkish cohort visualized with Genome Studio V2011.1.
The blue dots represent variants incorporated by the HumanOmniExpress BeadChips and the red line is the smoothing series for these data. The depicted area (chr19:35234335-37547219, based on hg19) encompasses *TYROBP* (identified by the pink vertical line) and represents the analysed locus. No large CNVs or large tracts of homozygosity were identified. LRR indicates the relative abundance of the genomic DNA around the SNP while the BAF of a SNP reflects the relative abundance of B allele intensity. It is an adjusted value generated by GenomeStudio, assuming three canonical clusters (A/A: 0.0, A/B: 0.5, B/B: 1).

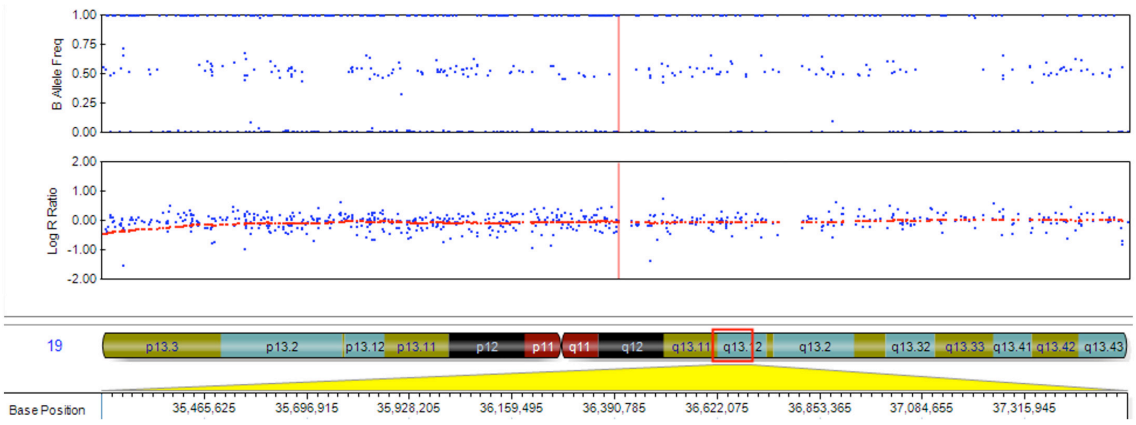

Table S1. Characteristics of the cohort studied. The majority of the cases were diagnosed with AD.

Diagnoses	# Cases	Male	Female	Average AAO (SD)
Mild Cognitive Impairment	20	12	8	66.7 (± 11.6)
Alzheimer's Disease	50	15	35	65.9 (± 9.1)
Frontotemporal Dementia	24	14	10	59.3 (± 14.2)
*Other	9	7	2	51.1 (±15.9)
Total	103	48	55	63.2 (±12.3)

*Other includes patients with CADASIL-type dementia, with Lewy Body Disease and with Corticobasal Degeneration. # Cases: number of cases, AAO: age-at-onset, SD: standard deviation.

Table S2. Coding variants identified in the studied cohort of 103 Turkish dementia cases (based on hg19). Minor allele frequencies for the studied cohort are presented in column "# Cases (MAF)". Global MAF represents minor allele frequencies for the global population and were obtained from the ExAC database. Functional impact classification resulted from the prediction obtained with SIFT, PolyPhen-2, MutationTaster and CADD software. All predictors classified the p.Val55Leu as benign. MutationTaster and CADD software also classified the synonymous variant as a polymorphism.

Variant	rsID	Position	Ref	Alt	# Cases (MAF)	Global MAF	ClinVar	Functional Impact
p.Val55Leu	rs77782321	19:36398414	C	A	3 (0.00971)	0.01518	Benign/ Likely benign	Benign
p.Gly41Gly	rs111477177	19:36398454	G	C	4 (0.01942)	0.02782	Likely benign	Benign

Ref: reference allele, Alt: alternative allele, # Cases: number of cases, MAF: minor allele frequency.