Negative results

Heterozygous TYROBP deletion (PLOSLFIN) is not a strong risk factor for cognitive impairment

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A B S T R A C T
Biallelic loss-of-function mutations in TYROBP and TREM2 cause a rare disease that resembles early-onset frontotemporal dementia with bone lesions called polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL). Some PLOSL-causing variants in TREM2 have also been associated with Alzheimer’s disease when heterozygous. Here, we studied the PLOSLFIN TYROBP deletion that covers 4 of the gene’s 5 exons. We genotyped 3220 older Finns (mean age 79, range 58–104) and found 11 deletion carriers (mean age 78, range 60–94). The carrier prevalence was 0.0034 (1 in 293) that matches previous findings in younger cohorts suggesting no significant early mortality. By comparing Mini-Mental State Examination (MMSE) scores and diagnoses of dementia, we did not find any significant differences between TYROBP deletion carriers and noncarriers (all p-values >0.5). Neuropathological analysis of 2 deletion carriers (aged 89 and 94 years) demonstrated only minimal beta amyloid pathology (Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) score 0). Collectively these results suggest that heterozygous carriership of the TYROBP deletion is not a major risk factor of cognitive impairment.

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1. Introduction

Triggering receptor expressed on myeloid cells 2 (TREM2) and TYRO protein tyrosine kinase binding protein (TYROBP) are part of the same receptor-signaling complex (Paloneva et al., 2002). Biallelic loss-of-function mutations in TYROBP or TREM2 genes cause polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL, Nasu-Hakola disease) (Paloneva et al., 2000, 2002), a rare disease characterized by pathologic fractures, personality changes, and presenile dementia (Hakola, 1972). In TYROBP, also known as DAP12, compound heterozygous mutations have been reported to cause PLOSL (Kuroda et al., 2007), whereas compound heterozygous mutations in TREM2 also lead to a frontotemporal dementia–like phenotype (Guerreiro et al., 2013). TREM2 and TYROBP have also been genetically or functionally associated with Alzheimer’s disease (AD), both late-onset (Jonsson et al., 2013; Sims et al., 2017; Zhang et al., 2013) and early-onset forms (Pottier et al., 2016; Slattery et al., 2014). While the association of TREM2 and AD seems strong, less data on TYROBP is available, and it is inconclusive. However, a recent gene-regulatory network analysis of postmortem brains suggested TYROBP as an important regulator of gene expression in AD (Zhang et al., 2013). In a cohort of 103 Turkish patients with different forms of dementia (average age of onset 66.7 years), a TYROBP p.Val155Leu variant was found in 3 individuals, but this variant was not considered pathogenic.
In this article, we studied the effect of PLOSLFIN deletion of TYROBP on cognitive impairment.

In this study, we genotyped 3220 older Finns for the 5.3 kb deletion in TYROBP that causes PLOSL in individuals of Finnish ancestry (Paloneva et al., 2000) and found no association with cognitive impairment.

2. Material and methods

2.1. Cohorts

We studied 4 population-derived cohorts from the Finnish capital region (Helsinki area) from whom the 5.3 kb TYROBP PLOSLFIN deletion was genotyped and information on cognition was available. We also analyzed APOE alleles and APP p.Ala673Thr variant to compare the background genetic risk burden for cognitive impairment between TYROBP deletion carriers and noncarriers. APOE ε4 is the most prevalent genetic risk factor for cognitive impairment and APP p.Ala673Thr is a strong protective mutation against AD and age-associated cognitive decline (Jonsson et al., 2012; Kero et al., 2013).

The Helsinki Birth cohort study consists of 8760 individuals born in Helsinki in 1934–1944 (Barker et al., 2005; Eriksson et al., 2006). In 2001–2004, a random sample of 2003 individuals participated in a clinical study including DNA extraction (Kajantie et al., 2012; Yliharsila et al., 2007) and a random subsample’s cognition was assessed with MMSE and CERAD. In addition, registry information up till December 31, 2013 was checked for dementia hospitalizations and dementia deaths. This included data on diagnoses of any organic dementias, given by physicians in inpatient (1969–2013) and outpatient (1998–2013) care (codes 290.00–290.10 from International Classification of Diseases (ICD)-9, 290, 2912A, 2928C, 2941A, 3310A, and 3311A from ICD-9, and F00, F01, F03, F051, and G30 from ICD-10) and of AD (codes 310.0 and 319.1 from ICD-9 and G30 and F00 from ICD-10) until December 31, 2013 was derived from the Finnish Hospital Discharge and Causes of Death Registers (Lahti et al., 2014).

HelsinkiBusinessmen Study originally consists of 3490 healthy Finnish men, born in 1919–1934. All participants were businessmen or executives with similar and high socioeconomic status. In 2002–2003, 672 individuals who lived at home were randomly selected for analyses, and 650 of them gave a venous blood sample. Participants’ cognition was tested using the MMSE (Strandberg et al., 2016). In 2014, cognition was evaluated with a questionnaire.

The DEBATE cohort consists of a random sample of 4800 individuals living in Helsinki, Finland. In 2000, 400 home-living individuals with stable cardiovascular disease (coronary artery disease, stroke or transient ischemic attack, peripheral artery disease) were clinically examined, and cognitive functions were assessed with MMSE (Uusvaara et al., 2013). In 2014, dementia diagnoses were screened from death certificates. Vantaa 85+ Study (VV85+) includes 601 individuals, aged 85 years and older, who were living in the city of Vantaa, Finland. Five hundred fifty-three individuals underwent neurological examination including MMSE by 2 neurologists in 1991–1992, with follow-up studies in 1994, 1996, 1999, and 2001. Neuropathological autopsy was performed on 281 individuals (Polvikoski et al., 1995).

The study was approved by the Coordinating Ethics Committee of the Helsinki University Central Hospital. The VV85+ was also approved by the Ethics Committee of the Health Centre of the City of Vantaa.

2.2. Genotyping

Genomic DNA was available of 3260 samples from the 4 different cohorts. Samples from VV85+ were whole-genome amplified DNA derived from peripheral blood leukocytes. We used polymerase chain reaction (PCR) assay with 1 forward and 2 reverse primers to genotype the samples. Samples without the deletion produce 1 amplicon (~500 bp), samples heterozygous for the deletion produce 2 amplicons (~500 bp and ~700 bp), and samples homozygous for the deletion produce only the larger amplicon (~700 bp). Primer sequences were 5' GCCGACATCCGTATGACTG 3' for forward primer, 5' TAGTATGCAGTCGATTTCTCA 3' for reverse primer 1, and 5' CIAGTCTGGCGGTGCATTTC 3' for reverse primer 2. We used a positive control in all PCR assays.

We performed PCR in 20-μL reactions containing 100 ng of DNA, 0.50 μL of DyNAzyme II DNA polymerase (Thermo Scientific, Waltham, USA), 1.0-M Betaine (Affymetrix, Santa Clara, USA), 0.3-nM of dNTP (Thermo Scientific), 0.5 mM of each primer (Sigma-Aldrich, St. Louis, USA), and 1.0x optimized DyNAzyme PCR buffer (Thermo Scientific, Waltham, USA). PCR was started with an initial denaturation at 94°C for 12 minutes; followed by 40 cycles of 30 seconds at 94°C, 15 seconds at 60°C (~1°C for every cycle until at 55°C), and 30 seconds at 72°C; and a final extension at 72°C for 10 minutes. Amplicons were visualized in 2% agarose gel electrophoresis with ethidium bromide to determine the genotype of every sample.

APOE was genotyped as previously described (Mylllykangas et al., 1999). APP p.Ala673Thr was genotyped as previously described (Kero et al., 2013).

2.3. Assessment of cognitive impairment

In the first analysis, we analyzed MMSE scores as a dichotomized variable where people with an MMSE score < 24 were considered to have cognitive impairment. In the second analysis, MMSE scores were treated as a continuous variable. In the third analysis, we used cohort-specific information as complementary information. This information was, depending on cohort, a clinical diagnosis of dementia, any dementia diagnosis on death certificate, a questionnaire study with self-reported diagnoses, and medications or hospital discharge and cause of death register information on any dementia diagnosis. In this third analysis, individuals with MMSE scores ≤ 24 or who had any diagnosis of dementia were considered to have cognitive impairment.

2.4. Statistical analyses

Analyses were conducted with SPSS v.24.0 (IBM Corp. Released, 2016. IBM SPSS Statistics for Windows, version 24.0. Armonk, NY: IBM Corp.). We used Fisher’s exact test and Mann-Whitney U test to test for the association between cognitive impairment and the deletion. We used the same tests to study the differences in age, sex, APOE ε4, and APP p.Ala673Thr between deletion carriers and noncarriers.

Statistical power was calculated with the Genetic Power Calculator (http://zzz.bwh.harvard.edu/gpc/), accessed August 10, 2017 (Purcell et al., 2003). Assuming deletion prevalence of 0.0034 based on our sequencing results, 20% prevalence of cognitive impairment in ≥75-year-old Finnish population (Rahkonen et al., 2003), relative risk of 2.5, case-control ratio of 4, and α = 0.05, 423 cases were required for 80% power. This number of cases was achieved in all analyses.
3. Results

All cohorts together, we genotyped successfully 3220 (98.7%) individuals, and 11 were heterozygous for the deletion, giving a total frequency of 0.0034. DEBATE was the only cohort with no deletion carriers (Supplementary Table 1). No sample was homozygous for the deletion as could be expected. A summary of the characteristics of the 11 deletion carriers is shown in Supplementary Table 2.

MMSE scores were available for 2390 individuals, including 7 deletion carriers. In the first analysis, 497 noncarriers (21%) and 1 deletion carrier (14%) had cognitive impairment (MMSE ≤ 24) (Fisher’s exact test p = 1). When MMSE scores were analyzed as a continuous variable, there was no significant difference between deletion carriers and noncarriers (Mann-Whitney U test p = 0.68). In the third analysis, any information on cognition (MMSE, questionnaire, diagnosis of dementia) was retrieved from 3192 individuals including all 11 deletion carriers. Of the deletion carriers, 3 (27%) and of the noncarriers, 692 (22%) had cognitive impairment (Fisher’s exact test p = 0.71). In all 3 analyses, there were no statistically significant differences between carriers and noncarriers in age, sex, APP p.Ala673Thr, or APOE ε4 status. In the first and second analyses, deletion carriers versus noncarriers had the following characteristics: mean age 77.9 versus 76.1 years (mean ranks 1350 vs. 1195, p = 0.71), and 28.8% versus 32.4% had the APOE ε4 allele (p = 1.0). In the third analysis, deletion carriers versus noncarriers had the following characteristics: mean age 77.6 versus 78.9 years (mean ranks 1463 vs. 1596, p = 0.63), 54.5% versus 46.7% females (p = 0.76), and 45.5% versus 32.9% (p = 0.52). There were 23 (0.7%) carriers of the APP p.Ala673Thr variant, none among the TYROBP deletion carriers (p = 1.0 in all analyses).

Two subjects of the VV85+ were autopsied at the ages of 89 and 94 years. Their mean percentages of the cortex covered by metachromine silver-positive senile plaques in sections cut from 4 neocortical samples (neocortical beta amyloid percentages) were 0% and 0.18%, both had a CERAD score of 0 and Braak stages 3 and 4. These neuropathological features do not show significant signs of AD pathology. The mean beta amyloid percentages in the VV85+ neocortical samples (neocortical beta amyloid percentages) were 0.68% (mean ranks 1463 vs. 1596, p = 0.08). In the third analysis, any information on cognition (MMSE, questionnaire, diagnosis of dementia) was retrieved from 3192 individuals including all 11 deletion carriers. Of the deletion carriers, 3 (27%) and of the noncarriers, 692 (22%) had cognitive impairment (Fisher’s exact test p = 0.71). In all 3 analyses, there were no statistically significant differences between carriers and noncarriers in age, sex, APP p.Ala673Thr, or APOE ε4 status. In the first and second analyses, deletion carriers versus noncarriers had the following characteristics: mean age 77.9 versus 76.1 years (mean ranks 1350 vs. 1195, p = 0.55), 57.1% versus 44.9% females (p = 0.71), and 28.6% versus 32.4% had the APOE ε4 allele (p = 1.0).

In the third analysis, deletion carriers versus noncarriers had the following characteristics: mean age 77.6 versus 78.9 years (mean ranks 1463 vs. 1596, p = 0.63), 54.5% versus 46.7% females (p = 0.76), and 45.5% versus 32.9% (p = 0.52). There were 23 (0.7%) carriers of the APP p.Ala673Thr variant, none among the TYROBP deletion carriers (p = 1.0 in all analyses).

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We performed logistic regression analyses to study the effect of APOE ε2 and ε4 carriageships on cognitive impairment and used age and sex as covariates. APOE ε4 carriageship associated with cognitive impairment in both MMSE score–based analysis (p = 2.3 × 10−4, odds ratios [OR] = 1.7 [1.3–2.3]) and when all information available on cognition was used (p = 2.4 × 10−5, OR = 1.9 [1.5–2.4]), APOE ε2 associated with better cognition when using cognitive impairment status derived from MMSE scores (p = 0.019, OR = 0.6 [0.39–0.92]) and showed a trend for better cognition when all information available on cognition was used (p = 0.07, OR = 0.74 [0.54–1.03]).

4. Discussion

We used MMSE scores and dementia diagnoses to study cognitive impairment on PLOSfAD deletion carriers and noncarriers and found no statistically significant differences. Neuropathological data on 2 deletion carriers brings additional support that the deletion is not a major risk factor for AD. On the contrary, both had very low neocortical beta amyloid percentages and CERAD scores, which is noteworthy because it is known that TREM2 and TYROBP expressing microglia accumulate around senile plaques but whether the effect is beneficiary or harmful is still under debate.

To the best of our knowledge, we report the first data on cognition and neuropathological examination of older individuals with a heterozygous PLOS-causing TYROBP variant. Our results suggest that the deletion is not a major risk factor for cognitive impairment. The main limitations of our study arise from the rarity of the PLOSfAD deletion and larger studies are needed to exclude minor effects on cognition. Moreover, it is not known how much TYROBP protein levels decrease in the deletion carriers because we were not able to study the expression level of TYROBP.

High socioeconomic status is also known to be a protective factor against cognitive impairment, and MMSE test may lose some of its sensitivity with highly educated people. Unfortunately, we did not know the socioeconomic status of most of the participants and could not include the effect of socioeconomic status into our analyses.

The VV85+ samples were whole-genome amplified DNA, whereas other samples were genomic DNA extracted from the blood. Amplification is a potential source of error when studying larger deletions. In our study, however, the frequency of the studied deletion in the VV85+ samples was in line with the other cohorts suggesting no error occurred during whole-genome amplification.

Disclosure statement

The authors have no actual no potential conflicts of interest.

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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.2017.12.008.

References


