RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Investigation of Association of Serotonin Transporter and Monoamine Oxidase-A Genes With Alzheimer's Disease and Depression in the VITA Study Cohort: A 90-Month Longitudinal Study

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Manuscript Received: 6 March 2013; Manuscript Accepted: 20 December 2013

Alzheimer's disease (AD) and depression (DE) are common psychiatric disorders strongly intertwined with one another. Nevertheless, etiology and early diagnosis of the disorders are still elusive. Several genetic variations have been suggested to associate with AD and DE, particularly in genes involved in the serotonergic system such as the serotonin transporter (*SERT/SLC6A4*), responsible for the removal from the synaptic cleft, and the monoamine-oxidase-A (*MAOA*), responsible for the presynaptic degradation of serotonin. Here, we attempt to characterize this pleiotropic effect for the triallelic SERT gene-linked polymorphic region (5HTTLPR) and for the MAOA-uVNTR, in participants in the Vienna-Transdanube-Aging (VITA)-study. The VITA-study is a community-based longitudinal study following a birth cohort (75 years old at baseline examination, n = 606) from Vienna for a period of 90 months with a regular follow-up interval of 30 months. Our main finding, confirming previous reports, is that the 5HTTLPR S-allele is a risk allele for DE (OR = 1.55 CI 95% 1.03–2.32) and its carriers had a steeper increase in SGDS sum score. No association to AD was found. MAOA-uVNTR did not associate with either AD or DE. However, in AD MAOA-uVNTR S-allele carriers a steeper increase of HAMD and STAI1 sum scores ($P < 0.05$) was observed. Although the VITA-study cohort is rather small with low power to detect gene alterations, the uniqueness of this very thoroughly investigated and homogenous cohort strengthens the results through exceptional data collection. Still, reinvestigation in a larger cohort similar to

How to Cite this Article:
Scholz CJ, Jungwirth S, Danielczyk W, Weber H, Wichart I, Tragl KH, Fischer P, Riederer P, Deckert J, Grünblatt E. 2014. Investigation of association of serotonin transporter and monoamine oxidase-A genes with Alzheimer's disease and depression in the VITA study cohort: A 90-month longitudinal study.
Am J Med Genet Part B 165B:184–191.

Susanne Jungwirth, Karl-Heinz Tragl, Peter Fischer, Peter Riederer, and Edna Grünblatt designed the study and wrote the protocol. Susanne Jungwirth and Peter Fischer contributed substantially to acquisition of subjects and data. Claus-Jürgen Scholz, Susanne Jungwirth, Wichart Ildiko, Heike Weber, Jürgen Deckert, and Edna Grünblatt managed the literature searches and analyses. Claus-Jürgen Scholz undertook the statistical analysis. Claus-Jürgen Scholz, Peter Riederer, Jürgen Deckert, and Edna Grünblatt wrote the first draft of the manuscript. All the authors contributed to and have approved the final manuscript.
None of the authors has any actual or potential conflict of interest, including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence, or be perceived to influence, this work.
Grant sponsor: Ludwig Boltzmann Institute of Aging Research, Vienna, Austria; Grant sponsor: Alzheimer Forschung Initiativ (AFI), Germany; Grant sponsor: IZKF grant; Grant number: Z-6.
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Article first published online in Wiley Online Library (wileyonlinelibrary.com): 17 January 2014
DOI 10.1002/ajmg.b.32220

this, as well as a meta-analysis, is important to confirm these results.

Key words: *SLC6A4*; **serotonin transporter;** *MAOA*; **Alzheimer's disease; depression; polymorphism**

[1]INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, accounting for more than 60% of all cases [World Health Organization, 2012]. Neurodegenerative loss of cognitive function, which is characteristic of AD, has been partly attributed to allelic variation. But candidate genes vary with the age of onset. In the highly heritable, but rare, early onset (EO) AD, which typically presents at ages less than 65 years, mutations in gene coding for the amyloid precursor proteins (*APP*) presenilin 1 and 2 (*PSEN1* and *PSEN2*, respectively) are thought to be causal (reviewed in [Bertram and Tanzi, 2012]). The majority of AD cases are, however, late-onset (LO), and underlying genetic influences are more complex (reviewed in [Bertram and Tanzi, 2012]). Among all the examined variants, the ε4 allele of the apolipoprotein E gene (*APOE*) [Ward et al., 2012] displays the strongest risk factor by far, and has the most-replicated effect [Bertram et al., 2007]. Among the nongenetic risk factors, advanced age plays a major role in LOAD, reflected by the steep increase in prevalence from the age of 65 on [Prince and Jackson, 2009]. Also, a history of depression (DE), particularly late-life DE, is known to increase the risk of AD [Grünblatt et al., 2009; Barnes et al., 2012; Mossaheb et al., 2012]. Unipolar DE is the most frequent of the mood disorders, which taken together have a 12-month prevalence of almost 10% [Kessler et al., 2005]. The prevalence of DE is more than 60% higher in females, making the individual's gender an important risk factor [Wilhelm et al., 2003]. DE is often divided into early-onset (EODE) and late-onset, or geriatric, (LODE) entities. The cut-off is often unclear, but recent debates put it at 40 years of age [Korten et al., 2012]. On the other hand, geriatric DE has been recently defined as starting after the age of 65 and having its own risk factors, particularly genetic factors and comorbidities [Laks and Engelhardt, 2010]. Targets for genetic risk factors are predicted by the monoamine hypothesis, which states that DE patients suffer from impaired levels of monoamine neurotransmitters, for example, serotonin [Stockmeier, 2003]. Accordingly, polymorphisms in genes affecting the serotonin production rate (tryptophan hydroxylase-1 (*TPH1)* and *TPH2*), removal from the synaptic cleft (*SLC6A4*, formerly known as serotonin transporter (*SERT)* or *5HTT*), and presynaptic degradation (monoamine-oxidase-A (*MAOA*)) of serotonin and its various receptors, have long been a research focus [Lopez-Leon et al., 2008].

The high proportion of LOAD patients with a positive life-time diagnosis of DE raises the question of whether DE is a prodrome, or the result of LOAD (reviewed in [Tsuno and Homma, 2009]). Furthermore, the high degree of comorbidity implies common underlying mechanisms. As a consequence, polymorphisms that are predisposing to DE might display an independent association signal with LOAD. In the present study, we aimed at characterizing this pleiotropic effect for both the triallelic serotonin transporter gene-linked polymorphic region (5HTTLPR), and the *MAOA*-upstream variable number of tandem repeats (MAOA-uVNTR) in participants of the Vienna Transdanube Aging (VITA) study, since these two polymorphisms have been proposed to play a role in AD and DE in prospective studies (detailed overview see Supplementary Table S1) Previous studies have addressed similar issues in the same sample, but restricted their analyses to the biallelic 5HTTLPR and its influence on LOAD and DE diagnoses up to the first follow-up [Grünblatt et al., 2006b, 2009]. In this study, we used diagnoses up to the latest follow-up data available, corresponding to examination of the third follow-up at a mean age of 82.5 years. Due to the high clinical importance of genetic variants in serotonin-system related genes, we included the triallelic genotypes of the 5HTTLPR through the inclusion of rs25531 (an A/G polymorphism nested in the long allele of the biallelic 5HTTLPR [Hu et al., 2006; Wendland et al., 2006]), and used the cross-sectional LOAD and DE diagnoses obtained from the baseline examination and three subsequent follow-up examinations. Moreover, the association of the MAOA-uVNTR with AD and DE was newly added into this equation, with the assumption of its pleiotropic involvement. The VITA study is a community-based longitudinal study following a birth cohort of inhabitants registered in Vienna's 21st and 22nd district for a period of 90 months with a regular follow-up interval of 30 months. At baseline examination, all 606 participants were 75 years old [Fischer et al., 2002].

MATERIALS AND METHODS

Study Sample

The design and recruitment of the VITA study sample has been described in great detail elsewhere [Fischer et al., 2002; Grünblatt et al., 2009; Jungwirth et al., 2009]. It consists of 606 individuals inhabiting districts 21 and 22 of Vienna, Austria, who were all born between May 1925 and June 1926, thus forming a birth cohort that was age 75 at baseline ($t = 0$) examination. All recruited volunteers completed an extensive general physical health check, questionnaires for education and psychosocial activities, and neuropsychological examinations at baseline and in the follow-ups. Follow-up of participants was scheduled every 30 months. The first follow-up ($t = 30$) was completed by 476 attendees. Between $t = 0$ and $t = 30$, 38 of the 606 VITA participants were deceased, 70 refused to participate in the follow-up investigation, and 10 were only willing to take part in a telephone interview. The second follow-up ($t = 60$) was possible for 362 attendees; 81 subjects were deceased between $t = 0$ and $t = 60$, 92 subjects refused to take part in the follow-up investigation, and 68 subjects were only willing to take part in a telephone interview or a house visit, thus providing minimal information. Three further participants could not be contacted despite repeated attempts. At 90 months after the baseline examination ($t = 90$), 342 subjects attended the third follow-up; thereof, 264 were examined in the Danube hospital, 53 were examined at home, and 25 were interviewed on the phone. A total of 109 subjects refused to attend the follow-up and a further four could not be reached. Between $t = 0$ and $t = 90$, 151 subjects in total were deceased.

For the present study, we used life-time diagnoses for AD and DE made between the baseline examination and the third follow-up. AD cases consisted entirely of late-onset diagnoses, and fulfilled the NINCDS-ADRDA criteria—in their last follow-up examination at least—for possible or probable AD [McKhann et al., 1984]. Assessment for unipolar (i.e., minor or major) DE was performed at any of the four study examinations in accordance with DSM-IV, and was retrieved from anamnesis data for the time before study onset, and for intervals between study examinations, respectively. DE cases experienced at least one depressive episode before or during the study period [Grünblatt et al., 2006a, 2006b]. Affection by EODE was defined by the first occurrence of DE being before the age of 65 years [Laks and Engelhardt, 2010]. Accordingly, LODE refers to study attendees with their first depressive episode at the age of 65 or later. Since early- and late-onset DE are mutually exclusive, individuals affected by one type of depression were excluded in the analysis of the other type. Cross-sectional estimates for dimensional phenotypes [Mini-Mental-State-Examination (MMSE)] [Folstein et al., 1983], the Fuld Objective Memory Test (FULD) [Fuld, 1980], the Hamilton Rating Scale for Depression (HAMD) [Williams, 1988], the Short Geriatric Depression Scale (SGDS) [Yesavage, 1988], and the State-Trait Anxiety Inventory (STAI) [Spielberger and Vagg, 1984] were obtained from the worst scores available per individual. For MMSE and FULD, this was the minimum score; for HAMD, SGDS, and STAI, this was the maximum score across four study examinations. We furthermore performed linear regressions of score versus examination number within each individual and used the regression slope as summary of sum score change across the study period. Of the 606 individuals attending the VITA study, 15 participants were excluded from analysis due to unclear DE diagnoses. Notably, the dropouts included one case of untreated schizophrenia. A summary of demographic data for the remaining study sample ($n = 591$) can be found in Supplementary Table S2. All participants of the VITA study gave a written, informed consent. The study conformed to the latest version of the Declaration of Helsinki and was approved by the Ethics Committee of the City of Vienna, Austria.

Genomic DNA Isolation

DNA was prepared from 2 ml of EDTA blood using proteinase K, as described previously [Grünblatt et al., 2005]. The DNA was divided into aliquot of 50 μl each in cryo-vials (Nunc GmbH, Wiesbaden, Germany) and frozen at −70°C until processing.

Genotyping

The genotyping of MAOA-uVNTR alleles was carried out as previously described [Deckert et al., 1999], yielding four different variants: 3, 3a, 4, and 5. Triallelic genotyping of the 5HTTLPR was carried out as previously described [Lesch et al., 1996; Wendland et al., 2006]. A PCR assay was followed by a restriction fragment length polymorphism analysis using BamHI (Fermentas; Burlington, Canada) for the rs25531 located within the 5HTTLPR L-allele, resulting in L_A, L_G, and S variants. The *APOE* genotype was determined by means of a PCR assay as described elsewhere [Grünblatt et al., 2009], distinguishing the ε2, ε3, and ε4 variants of *APOE*.

Statistical Analysis

The triallelic 5HTTLPR genotypes were dichotomized according to functional evidence [Hu et al., 2006]. Hence, the long allele [Lesch et al., 1996] with the nested A/G polymorphism rs25531 [Hu et al., 2006; Wendland et al., 2006] was termed "L" only if paired with the A allele (L_A) and "S" when paired with G allele or determined already as S allele (i.e., L_G or S). The MAOA-LPR alleles 3, 3a, 4, and 5 were also collapsed to a biallelic mode according to experimental evidence [Deckert et al., 1999]. "S" designates the allele 3, whereas the other alleles were coded with "L". Genotypic risk was determined for the rarer S alleles of the respective polymorphisms. No significant departures from the Hardy–Weinberg equilibrium (HWE) were found (see Supplementary Table S3). Associations of the variable of sex and the presence of *APOE* ε4 alleles with binary disease outcomes were examined by a univariate logistic and linear regression in the case of cross-sectional sum scores. Associated parameters were included in multivariate regressions used for genetic association testing; logistic regression was employed for categorical and linear regression for dimensional phenotypes. The optimal genetic effect model for each polymorphism in each examined trait was determined using the Akaike Information Criterion (AIC, see Supplementary Table S4). The significance threshold used in this study was set to 5%. Confidence intervals (CI) for the observed effects were generally wide, therefore most *P*-values did not pass the significance threshold. This is due to restricted power imposed by sample-size limitations in the VITA study cohort. Power estimates for the detected effects, and the required sample sizes for nominally significant associations of the particular effect sizes, are given in Supplementary Table S5. All analyses were performed with R version 2.13.2 (http://www.r-project.org/, Department of Statistics and Mathematics of the WU Vienna, Austria) and PGA (power calculator for case-control genetic association analyses [Menashe et al., 2008]).

RESULTS

Of the 591 individuals examined in this study, 126 belonged to the LOAD and 308 belonged to the DE (85 EODE, 223 LODE) groups, with 144 participants being deceased during the study period (Supplementary Table S2). While fewer female participants diseased ($OR = 0.53$, $P < 10^{-2}$), AD patients more often deceased during the study period ($OR = 1.61$, $P < 0.05$, see Table I); no association was found for death and DE (data not shown).

Carriers of *APOE* ε4 alleles were significantly more frequent in the LOAD group ($OR = 2.34$, $P < 10^{-4}$), however no association was found for the total DE, EODE, and LODE groups (see Table I). Concordantly, *APOE* ε4 carriers differed significantly from ε4-free individuals in MMSE, FULD, and STAI1 scores, but not in HAMD, SGDS, and STAI2 scores (see Table II). By contrast, females were more frequently affected by DE (irrespective of age of onset), but not by LOAD (see Table I). This was also reflected by gender difference in MMSE, FULD, HAMD, SGDS, and STAI scores (see Table II). Furthermore, co-occurrence of LOAD and DE was higher than expected by chance, which largely accounts for LODE ($P < 10^{-5}$) and, to a weaker extent, for EODE ($P < 0.05$; see Table I).

TABLE I. Associations With Categorical Outcomes

		Deceased (no/yes)	DE (no/yes)	EODE (no/yes)	LODE (no/yes)	AD (no/yes)
Gender (female/male)	*P*-value	**1.15×10^{-3}**	**3.74×10^{-7}**	**6.08×10^{-5}**	**2.19×10^{-5}**	0.22
	OR (CI)	**0.53 (0.37–0.78)**	**2.39 (1.71–3.35)**	**3.01 (1.76–5.15)**	**2.2 (1.53–3.17)**	1.29 (0.86–1.94)
APOE ε4 carrier	*P*-value	0.56	0.3	0.81	0.23	**2.34×10^{-5}**
(yes/no)	OR (CI)	0.87 (0.54–1.4)	1.24 (0.83–1.85)	1.08 (0.59–1.98)	1.3 (0.85–2.01)	**2.61 (1.67–4.06)**
AD (yes/no)	*P*-value	**0.03**	**4.43×10^{-6}**	**1.1×10^{-2}**	**2.82×10^{-6}**	—
	OR (CI)	**1.61 (1.05–2.49)**	**2.7 (1.77–4.13)**	**2.18 (1.2–3.98)**	**2.92 (1.86–4.57)**	—

AD, Alzheimer's Disease; CI, 95% confidence interval; DE, depression; EODE, early-onset depression; LODE, late-onset depression; OR, odds ratio.
Associations between diseases and known risk factors were examined with logistic regression. Significant associations ($P < 0.05$) are shown in bold.

Genotypes for the triallelic 5HTTLPR and the MAOA-uVNTR were determined in all study attendees. There was no indication of genotyping errors (according to HWE tests, see Supplementary Table S3).

For each examined outcome, associated variables were used as covariates to determine the independent effects of the 5HTTLPR and the MAOA-uVNTR. In this analysis setting, we found the 5HTTLPR to be associated with DE (OR = 1.55, $P < 0.05$; Table III), but not AD. Likewise, each 5HTTLPR S allele leads to a steeper increase of SGDS sum scores between study examinations (slope = 0.16, $P < 0.05$, Table III). The MAOA-uVNTR did not show associations with categorical or dimensional phenotypes in the complete VITA sample (Table III). However, when the analysis of emotional changes was restricted to AD patients, the MAOA-uVNTR S/S or S/0 genotype displayed a steeper increase of HAMD and STAI1 sum scores (increase =2.37 and 2.77, $P < 0.05$, respectively) between study examinations than L allele carriers (Table III). No associations between survival and genotype could be observed (Table III).

DISCUSSION

In the present study, we examined two of the most frequently evaluated polymorphisms in psychiatric genetics—5HTTLPR and MAOA-uVNTR [Nishimura et al., 2005; Lopez-Leon et al., 2008; Clarke et al., 2010; Fan et al., 2012; Haenisch et al., 2012; Polito et al., 2012]—for pleiotropic effects on LOAD, DE and its EODE, and LODE forms in an age-matched VITA longitudinal study cohort. Our main finding, confirming many previous reports, is for the 5HTTLPR S-allele being the risk allele for DE [Clarke et al., 2010; Haenisch et al., 2012]. Consistent with this, we demonstrated that homozygous carriers of the 5HTTLPR S-allele scored 1.15 points higher for the HAMD. However, we did not find any significant association between 5HTTLPR and LOAD, which fits the recent meta-analysis reported by Polito et al. [2012], findings by AlzGene, and other reports, which did not show any significant association between 5HTTLPR and AD [Bertram et al., 2007; Feher et al., 2013]. In contrast, several studies reported

TABLE II. Associations With Dimensional Outcomes

	Gender (female/male)		*APOE* ε4 carrier (yes/no)	
Sum Score	**Increase**	***P*-Value**	**Increase**	***P*-Value**
MMSE[a]	**−1.027 (−1.751 to −0.302)**	**0.006**	**−1.251 (−2.103 to −0.4)**	**0.004**
MMSE[b]	−0.27 (−0.564 to 0.025)	0.073	**−0.372 (−0.725 to −0.02)**	**0.039**
FULD[a]	**−2.086 (−3.811 to −0.361)**	**0.018**	**−4.102 (−6.165 to −2.038)**	**1.092×10^{-4}**
FULD[b]	**−0.85 (−1.509 to −0.19)**	**0.012**	**−1.762 (−2.561 to −0.964)**	**1.777×10^{-5}**
HAMD[a]	**3.071 (1.987 to 4.154)**	**4.179×10^{-8}**	0.158 (−1.194 to 1.511)	0.819
HAMD[b]	0.433 (−0.14 to −1.006)	0.139	0.188 (−0.507 to 0.884)	0.596
SGDS[a]	**0.916 (0.4 to 1.433)**	**0.001**	0.043 (−0.592 to 0.678)	0.894
SGDS[b]	**0.272 (0.087 to 0.457)**	**0.004**	0.055 (−0.172 to 0.282)	0.634
STAI1[a]	**3.442 (1.709 to 5.175)**	**1.108×10^{-4}**	**2.164 (0.011 to 4.316)**	**0.049**
STAI1[b]	0.393 (−0.36 to 1.146)	0.307	−0.065 (−0.991 to 0.861)	0.89
STAI2[a]	**4.227 (2.512 to 5.942)**	**1.741×10^{-6}**	0.9 (−1.248 to 3.048)	0.412
STAI2[b]	−0.191 (−0.835 to 0.453)	0.562	−0.113 (−0.907 to 0.681)	0.78

FULD, Fuld object-memory test; HAMD, Hamilton depression scale; MMSE, mini mental state examination; SGDS, short geriatric depression scale; STAI, State-Trait Anxiety Inventory.
Associations between sum scores and known risk factors were examined with linear regression. Significant associations ($P < 0.05$) are shown in bold.
[a]Longitudinal summary given as worst sum score of all study examinations for each participant of the VITA study.
[b]Longitudinal summary given as increase of sum scores from each participant of the VITA study.

TABLE III. Results From Genetic Association Testing

	5HTTLPR s-allele		MAOA-uVNTR s-allele	
Outcome	**Effect (min. to max. 95% CI)**	***P*-value**	**Effect (min. to max. 95% CI)**	***P*-value**
Alzheimer[a]	1.39 (0.87 to 2.21)	0.169	0.82 (0.48 to 1.4)	0.474
DE[a]	1.55 (1.03 to 2.32)	0.036	0.77 (0.5 to 1.19)	0.242
Early-onset DE[a]	1.73 (0.96 to 3.13)	0.068	0.77 (0.38 to 1.57)	0.477
Late-onset DE[a]	1.54 (0.99 to 2.39)	0.055	0.75 (0.47 to 1.21)	0.244
Death[a]	1.05 (0.69 to 1.6)	0.821	1.08 (0.67 to 1.76)	0.741
MMSE[b,c]	−0.59 (−1.34 to 0.17)	0.127	−0.26 (−1.16 to 0.64)	0.575
MMSE[b,d]	−0.25 (−0.56 to 0.06)	0.12	−0.1 (−0.39 to 0.19)	0.504
MMSE[b,e]	−0.65 (−1.83 to 0.52)	0.279	−0.97 (−2.31 to 0.37)	0.16
FULD[b,c]	−1.38 (−3.2 to 0.43)	0.137	1.13 (−1.03 to 3.29)	0.307
FULD[b,d]	−0.62 (−1.32 to 0.08)	0.083	0 (−0.66 to 0.67)	0.989
FULD[b,e]	−1.23 (−3.49 to 1.04)	0.291	−0.37 (−2.42 to 1.68)	0.725
HAMD[b,c]	1.15 (−0.11 to 2.41)	0.073	0.12 (−1.26 to 1.5)	0.861
HAMD[b,d]	0.12 (−0.49 to 0.74)	0.693	0.44 (−0.14 to 1.01)	0.136
HAMD[b,e]	0.22 (−1.44 to 1.88)	0.794	2.37 (0.5 to 4.25)	0.015
SGDS[b,c]	0.47 (−0.13 to 1.08)	0.123	−0.04 (−0.7 to 0.62)	0.904
SGDS[b,d]	0.16 (0.04 to 0.28)	0.012	0.1 (−0.08 to 0.29)	0.281
SGDS[b,e]	0.3 (−0.19 to 0.78)	0.232	−0.11 (−0.55 to 0.33)	0.63
STAI1[b,c]	1.76 (−0.28 to 3.8)	0.092	−0.47 (−2.71 to 1.78)	0.683
STAI1[b,d]	0.46 (−0.35 to 1.27)	0.265	0.69 (−0.06 to 1.44)	0.07
STAI1[b,e]	−2.09 (−4.37 to 0.2)	0.076	2.77 (0.21 to 5.33)	0.036
STAI2[b,c]	1.88 (−0.12 to 3.89)	0.067	−0.34 (−2.54 to 1.86)	0.76
STAI2[b,d]	−0.37 (−1.06 to 0.32)	0.294	−0.08 (−0.73 to 0.57)	0.808
STAI2[b,e]	−0.6 (−1.9 to 0.71)	0.373	2.27 (−0.15 to 4.69)	0.068

CI, confidence interval; DE, depression; FULD, Fuld object-memory test; HAMD, Hamilton depression scale; MMSE, mini mental state examination; SGDS, short geriatric depression scale; STAI, State-Trait Anxiety Inventory.
Genetic effects on categorical outcomes were estimated with multivariate logistic regressions using associated risk factors (see Table I) as covariates. Genetic effects on dimensional outcomes were derived from multivariate linear regressions, stratified by associated risk factors (see Table II).
[a]Effect size presented as odds ratio.
[b]Effect size presented as linear increase.
[c]Longitudinal summary given as worst sum score of all study examinations for each participant of the VITA study.
[d]Longitudinal summary given as increase of sum scores from each participant of the VITA study.
[e]Longitudinal summary given as increase of sum scores from each AD patient participating the VITA study.

the 5HTTLPR S-allele to associate as risk factor with AD [Li et al., 1997; Oliveira et al., 1998; Lorenzi et al., 2010]. These conflicting results might arise from a possible protective effect of L-allele carriers in regard to survival and longevity. This hypothesis was discussed in several studies observing old-age population and allele frequencies of the 5HTTLPR, which proposed the L-allele to be associated with longevity [Gondo et al., 2005; Holsinger et al., 2012] while the S-allele appeared to associate with less successful aging [O'Hara et al., 2012]. This, however, is not supported by our study in which we could not detect any significant association between death and 5HTTLPR genotype.

The MAOA variation studied in this report is a polymorphic repeat in the promoter region of MAOA, termed MAOA-uVNTR. This polymorphism consists of a 30-bp repeat element with 3, 3.5, 4, 5, or 6 copies. The 3-repeat allele results in decreased expression of MAOA and lower levels of homovanillic acid in cerebrospinal fluid [Sabol et al., 1998; Deckert et al., 1999]. Several studies indicate that the MAOA gene may be involved in the pathogenesis of DE and major depressive disorder [Schulze et al., 2000; Gutierrez et al., 2004], with meta-analyses pointing to differences in gender, ethnicity, and polymorphisms as an explanation for inconsistent associations to DE [Fan et al., 2012; Reif et al., 2012]. In our current study, we could not find any association between MAOA-uVNTR polymorphism and DE or LOAD. Due to the lower event rate, associations with AD are in our sample generally more unlikely than with DE (see Supplementary Table S5), and the latter phenotype is strongly biased towards females (see Table I). To overcome issues related to the gender-specific MAOA-uVNTR load, we have used a recessive genetic modeling; however, the proportion of three-repeat homozygous females is considerably lower than that of S-carrying males (11.11% vs. 40%), meaning that the more likely affected gender carries less genetic risk in our cohort, which ultimately results in a low study power. Nevertheless, a recent publication by Arlt et al. [2013] reported a significant association in AD subjects with major DE, mainly in females, in which the MAOA-uVNTR low activity allele three-repeat homozygotes showed an increased risk for suffering from major DE.

Co-occurrence of LOAD and DE, as described previously in the VITA study cohort [Grünblatt et al., 2009; Mossaheb et al., 2012] and confirmed again in the follow-up, supports previous reports which suggest DE to be a risk factor for AD [Ownby et al., 2006], while other suggest DE to be prodromal to AD [Preuss et al., 2009].

Recently, Caraci et al. [2010] discuss the common pathways and processes linking DE and AD together, such as neuritic plaques and neurofibrillary tangles but also chronic inflammation and hyperactive HPA axis common to both disorders. Moreover, recent longitudinal cohort study reported that in particular late-life DE could be part of the AD prodrome, while recurrent DE is rather associated more with increased risk of vascular dementia [Barnes et al., 2012]. The fact that in our cohort LODE was stronger associated with AD than EODE is consistent with this notion.

Serotonin depletion or enhancement has been shown to influence sensory and cognitive functions in humans and nonhuman primates, including long-term memory, decision making, response inhibition, and reversal learning [Clark et al., 2004, 2005]. Recently, Enge et al. [2011] demonstrated that both 5HTTLPR and MAOA-uVNTR play an important role in the executive control of inhibitory mechanisms during response selection, essential for working-memory performance. They showed that S/S carriers of the 5-HTTLPR and MAO-H (the longer genotype related to higher enzymatic activity), subjects of the MAOA-uVNTR, had a more efficient executive control of working, memory-related performance, as evidenced by behavioral and neurophysiological measures [Enge et al., 2011]. Another interesting, recently published finding in adolescent DE is the four-way interaction of 5HTTLPR, MAOA-uVNTR, negative life events at the age of 13, and gender that can predict depressive symptoms at the age of 15 [Priess-Groben and Hyde, 2012]. However these findings were obtained in young healthy controls, and the negative results of the present study were obtained in aged individuals.

In conclusion, in this report we confirm the association of 5HTTLPR S-allele as a risk allele for DE, though it is not associated with LOAD. MAOA-uVNTR did not associate with either LOAD or DE. To evaluate these results it would be of great importance to reinvestigate them in a larger age cohort similar to the VITA study with its unique characteristics of the participants: (1) living in the same district (strengthening ethnicity), (2) recruited at the same age, (3) re-examined exactly every 30 months for up to a 90-month period, and (4) having gone through extensive health and neuropsychiatric checks, been seen by an internist and been given thorough magnetic resonance imaging. All of these factors contribute to a very homogenous cohort with extensive data for each participant, which is usually not the case in a case-control study.

ACKNOWLEDGMENTS

We would like to give special thanks to the dedicated technical assistance of Miss Miryame Hofmann. The Ludwig Boltzmann Society and AFI had no further role in study design; or in collection, analysis, and interpretation of data; or in writing the manuscript and submitting it for publication.

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