The TOMM40 poly-T rs10524523 variant is associated with cognitive performance among non-demented elderly with type 2 diabetes

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Abstract

The variable length poly-T, rs10524523 ('523') located within the TOMM40 gene, was recently associated with several phenotypes of cognitive function. The short (S) allele is associated with later AD onset age and better cognitive performance, compared to the longer alleles (long and very-long (VL)). There is strong linkage disequilibrium between variants in the TOMM40 and APOE genes. In this study, we investigated the effect of '523' on cognitive performance in a sample of cognitively normal Jewish elderly with type 2 diabetes, a group at particularly high risk for cognitive impairment. Using a MANCOVA procedure, we compared homozygous carriers of the S/S allele (N=179) to carriers of the VL/VL allele (N=152), controlling for demographic and cardiovascular covariates. The S/S group performed better than the VL/VL group (p=0.048),

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specifically in the executive function (p=0.04) and episodic memory (p=0.050) domains. These results suggest that previous findings of an association of the TOMM40 short allele with better cognitive performance, independently from the APOE variant status, are pertinent to elderly with diabetes.

1. Introduction

Type 2 diabetes (T2D) is one of the most established cardiovascular risk factors for cognitive compromise in aging (Beeri et al., 2009; McCrimmon et al., 2012). It is associated with increased risk for several cognitive outcomes, ranging from cognitive impairment in healthy individuals to dementia susceptibility (both Alzheimer's disease (AD) and vascular dementia) (Biessels et al., 2008; Luchsinger et al., 2001, 2007). For example, in a large systematic review T2D was associated with 50–100% increased AD risk, and 100–150% increased risk of vascular dementia (Biessels et al., 2006). Diabetic patients demonstrated substantially greater rates of cognitive decline compared to non-diabetic patients (Arvanitakis et al., 2004; Knopman et al., 2001), which is related to disease duration (Gregg et al., 2000) and complications (Rotkiewicz-Piorun et al., 2006). Among the potential mechanisms underlying these cognitive deficits are cerebrovascular diseases (white matter disease and small infarcts), brain's insulin resistance and hyperinsulinemia, as well as accumulation of advanced glycation end products (Beeri et al., 2009; McCrimmon et al., 2012). Therapeutic interventions (e.g. Rosigitazone) may have beneficial effects on cognitive compromise (Luchsinger, 2012).

Recently, single nucleotide polymorphisms (SNPs) within the TOMM40 (Translocase of the outer mitochondrial membrane 40) gene were associated with a number of cognitive phenotypes (Davies et al., 2014; Kim et al., 2011; Potkin et al., 2009). In addition, a variable length deoxythymidine homopolymer (poly-T), rs10524523 ('523'), located within intron 6 of the TOMM40 gene (chr19:45,403,049–45,403,083, human genome reference assembly GRCh37/hg19), was reported to be associated with cognitive decline, AD susceptibility and age at onset (Caselli et al., 2012; Hayden et al., 2012; Johnson et al., 2011; Li et al., 2013; Roses et al., 2010). A wide range of T-repeat lengths are observed for this variant (11 to more than 50), but it is currently categorized as short (S) (21T<), long (L) (21–29T) or very long (VL) (30T and more) (Hayden et al., 2012; Lutz et al., 2010). There is strong linkage disequilibrium (LD) between variants in TOMM40 and the near-by APOE gene, a major genetic risk variant for AD (Corder et al., 1993). Among Caucasians, the APOE ε3 allele is linked with either a short or very long '523' allele, while APOE ε4 is linked usually to the long allele (Linnertz et al., 2012; Lutz et al., 2010). Biologically, TOMM40 encodes the protein Tom40, an important component of a pore within the mitochondrial outer membrane, through which proteins enter this organelle (Humphries et al., 2005). This channel is essential for mitochondrial survival, a mechanism that may be involved in AD etiology (Ferencz et al., 2012). It was suggested that the Tom40 pore enables passage of beta amyloid

into the mitochondria, inducing oxidative stress and eventually leading to mitochondrial damage and neurotoxicity (Ferencz et al., 2012).

The '523' variant has been shown to contribute to AD susceptibility and is associated with the age at onset (AAO) of late onset AD (Crenshaw et al., 2013; Roses et al., 2010). Among APOE ε3/4 carriers, the shorter allele was associated with later AAO, compared to the very long allele (Roses et al., 2010). Johnson found that among healthy middle aged (mean age 55 years) APOE ε3 homozygous individuals, '523' genotype is associated with impaired cognition (when comparing the S/S homozygous group to the VL/VL group), and also to decreased brain gray matter volume, for example in the ventral posterior cingulate region (Johnson et al., 2011). These findings are consistent with those reported by Hayden et al., where the '523' S/S group was associated with better performance in cognitive domains of memory and executive control, compared to other genotypes (Hayden et al., 2012). The '523' effect of age-related memory decline was also supported by findings in a large APOE ε4 enriched cohort of normal individuals with a large range of ages (Caselli et al., 2012). However, other studies based on meta analysis of very large cohorts have failed to confirm an APOE-independent effect of '523' on cognitive related phenotypes, mainly for AD AAO (Cruchaga et al., 2011; Jun et al., 2012). This inconsistency may be explained by several technical and methodological differences between studies (reviewed in Roses et al. (2013)). The biological influence of the '523' variant is still unclear, but significant higher TOMM40 mRNA expression in human temporal and occipital cortices was shown among the VL/VL homozygous group, compared to the S/S carriers group, among healthy and AD patients (Linnertz et al., 2014). Similar results were also observed in cell based (neurobalstoma and hepatoma) luciferase reporter system (Linnertz et al., 2014).

Due to the previous findings regarding the association of the TOMM40 Poly-T variant '523' with both cognitive decline in non-demented elderly individuals and AD AAO, we investigated the effect of '523' on cognitive performance in our sample of cognitively normal diabetic elderly, a group at high risk for cognitive impairment. Following Johnson et al. (2011), Caselli et al. (2012) and Linnertz et al. (2014) findings, and in order to observe the most detectable effect, independent of APOE ε4, we decided a-priori to contrast cognitive function in those homozygous for the '523' S allele, compared to individual homozygous for the '523' VL allele. We hypothesized that the S/S group will have better cognitive performance than the VL/VL group.

2. Experimental procedures

2.1. Sample

Subjects are participants of the Israel Diabetes and Cognitive Decline (IDCD) study, a longitudinal study assessing the relationship of T2D characteristics and cognitive decline. Methods and procedures were described in detail previously (Ravona-Springer et al., 2013). In short, our sample consists of elderly (65 years old) Israeli Jewish T2D individuals, cognitively normal at study baseline (based on a multidisciplinary consensus committee). Subjects were randomly selected from the diabetes registry of the Maccabi Healthcare Services (MHS), the second largest Israeli HMO. Entry criteria to the registry are any of the following: (1) HbA1c >7.25%, (2) Glucose >200 mg/dl on two exams more than three

months apart, (3) purchase of diabetic medication twice within three months supported by a HbA1c >6.5% or Glucose >125 mg/dl within half a year, (4) clinical diagnosis of T2D by the primary or secondary practitioner, supported by a HbA1c >6.5% or Glucose >125 mg/dl within half a year.

The IDCD includes individuals from Israel's central region, who did not suffer from major medical, psychiatric, or neurological conditions that affect cognitive performance. All participants speak Hebrew fluently, and informed consent to participate was given from each. For additional details, see Ravona-Springer et al. (2013).

2.2. Cognitive assessment

All eligible patients underwent cognitive evaluation (described below), by a neuropsychologist who administered a comprehensive cognitive battery, and additional questionnaires to the subject and informant, on cognitive and functional impairment. All subjects' cognitive data are discussed by a multidisciplinary team in order to define the subjects' cognitive status (normal, mild cognitive impairment (MCI) or dementia), and only cognitively normal participants are included at baseline. The first follow up wave is ongoing and this study presents results pertinent to the first cognitive assessment only.

Factor analysis revealed four cognitive domains, which were then scored as totals of z scores: episodic memory factor (included word list immediate and delayed recall, and recognition from the CERAD neuropsychological battery) (Beeri et al., 2006), semantic categorization factor (Fernaeus and Almkvist, 1998) (included the letter (Spreen and Benton, 1977) and category fluency (Cauthen, 1978), and similarities (Wechsler, 1981)), attention/ working memory factor (included the diamond cancellation test, digit span forward and backward (Wechsler, 1987)), and an executive factor (included the trails making A and B (Reitan, 1955) and the digit symbol test (Wechsler, 1981)). In addition, an overall cognition measure was created by summarizing the four domains.

2.3. SNPs selection and genotyping

Samples were genotyped for APOE and TOMM40 '523' at Polymorphic DNA Technologies (Polymorphicdna.com, Alameda, CA). The APOE genotype (ε2/3/4) was defined by the rs429358 and rs7412 SNPs. The homopolymer poly-T rs10524523 was genotyped as described elsewhere (Caselli et al., 2012; Johnson et al., 2011; Roses et al., 2010). Alleles of the '523' variant were classified as accepted in literature (Hayden et al., 2012; Roses et al., 2010), according to the length of the poly-T repeats: short (S) (<21T), long (L) (21–29T) and very long (VL) (>29 T). Each sample was genotyped twice using different primer sets to account for the presence of rare variants in the flanking sequence. The consensus calls of the '523' genotypes based on the replicate assays were used for the genetic analysis.

2.4. Data analysis

We compared the '523' S/S group with the VL/VL group. T-tests and the chi-squared tests were used to compare the demographic and cardiovascular characteristics between groups. Separate evaluations for each of five cognitive outcomes would introduce a problem of increased probability of Type I error due to multiple tests of significance. To reduce this,

multivariate analysis of covariance (MANCOVA) was employed to evaluate the association of the '523' genotype on cognitive performance in the four domains of interest (executive function, episodic memory, attention and semantic categorization). As a comprehensive evaluation of significance for multiple domains, an advantage of MANCOVA over Bonferroni-based adjustment for multiple tests is that it takes into account the covariate structure of the cognitive measures. In a secondary analysis, we included the S/VL genotype carriers group, and repeated the analysis using MANCOVA. For post-hoc analysis, we used the least significant difference (LSD) procedure.

As we have previously done (Ravona-Springer et al., 2013), and based on evidence that these factors are associated with cognitive decline and dementia (Beeri et al., 2009), we included in the model all relevant socio-demographic and cardiovascular covariates. The socio-demographic covariates were age at the time of cognitive assessment, years of education, sex, number of follow-up years in the registry (a surrogate of duration of disease) and ancestry (Ashkenazi vs. non-Ashkenazi). The cardiovascular covariates were Hemoglobin A1c (HbA1c), body mass index (BMI), creatinine, diastolic and systolic blood pressure, total cholesterol, triglycerides, low and high density lipoprotein (LDL and HDL). For the cardiovascular covariates, we used the average of all the subject's measurements available in the MHS Diabetes Registry. Lastly, we included the APOE status (APOE ε2 vs. APOE ε3 allele carriership) to account for a possible effect on cognition of the rarer APOE ε2 allele over the more common APOE ε3. Note that APOE ε4 carriers (APOE ε3/4, APOE ε2/4 and APOE ε4/4) individuals were not included in the analysis in order to remove the effect of the APOE ε4 allele.

A p value of 0.05 (two sided) was used to determine statistical significance level. For analysis, we used SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Genotyping

In the whole sample genotyped for TOMM40 (N=841), aged between 65 and 88, the following '523' TOMM40 genotypes were observed: S/S (N=194, 23.06%), S/L (N=56, 6.65%), S/VL (N=365, 43.4%), L/L (N=7, 0.83%), L/VL (N=55, 6.54%), VL/VL (N=164, 19.5%); 113 were carriers of the APOE ε4 allele. Data regarding the '523' allele frequencies (S/L/VL) in the whole sample and in the Ashkenazi subset sample, as well as the Poly-T length, is shown in Table 1. Focusing on the S/S and the VL/VL groups of interests, 24 individuals were excluded due to missing cognitive, demographic or cardiovascular data, or lack of APOE genotype. In the VL/VL group, 3 individuals were carriers of the APOE ε4 allele. Due to the well established effect of APOE ε4 genotype on cognition, they were also excluded from the final analysis – allowing determination of the TOMM40 independent association with cognition.

The final sample included 331 cognitively normal Israeli Jewish T2D patients. 179 were '523' S/S, and 152 '523' VL/VL homozygous; 114 (34.4%) of the sample were females. The average age at cognitive assessment was 71.98 years, and 221 (63.7%) of them were from Ashkenazi origin. Forty seven (14.2%) were carriers of the APOE ε2 allele. Additional

demographic and cardiovascular details are shown in Table 2. Subjects carrying the S/S and VL/VL genotype did not differ in any of the potential covariates (see Table 2). In the secondary analysis, 345 S/VL participants were included (after exclusion of individuals with missing data).

To evaluate the effect of the '523' S/S genotype compared to VL/VL genotype, we performed a MANCOVA procedure controlling for demographic and cardiovascular covariates (see methods). The overall model Wilk's lambda was statistically significant ($F(4,310)=2.42$; $p=0.048$). To examine the contributions of each cognitive domain and overall cognition to the MANCOVA results, parallel univariate analyses of covariance were performed for those outcomes. The S/S group had statistically better performance than the VL/VL group in executive function ($F(1,313)=4.24$; $p=0.04$) and episodic memory ($F(1,313)=3.87$; $p=0.050$). A trend was observed in the overall cognitive performance ($F(1,313)=2.29$; $p=0.13$). There were no statistically significant differences between the groups in attention ($F(1,313)=0.06$; $p=0.8$)) and semantic categorization ($F(1,313)=0.12$; $p=0.72$)).

Although APOE ε2 carriership status was controlled in the analysis above, we repeated the same analysis in a smaller sample of homozygous APOE ε3/3 subjects (151 S/S and 133 VL/VL). Results were consistent with the former analysis although not reaching significance level (probably due to smaller sample size). The overall model Wilk's lambda was nearly significant ($F(4,264)=1.99$; $p=0.096$), and the S/S group had better performance than the VL/VL group in executive function ($F(1, 267)=2.83$; $p=0.094$), episodic memory ($F(1, 267)=3.75$; $p=0.054$) and overall cognitive measure ($F(1, 267)=1.79$; $p=0.182$). In a sub-analysis including only the Ashkenazi origin participants ($N=211$), the MANCOVA model was not statistically significant. In a univariate analyses of covariance, the S/S ($N=118$) group had better episodic memory performance at a near significance level compared to the VL/VL group ($N=93$) ($F(1,195)=3.62$; $p=0.059$). Results were not significant for the other domains (executive and overall), but the pattern of better performance among S/S carriers compared to VL/VL was preserved, in line with the findings for the primary analysis.

In a secondary analysis, in order to evaluate the possible additive effect for the three genotype levels (S/S, S/VL, VL/VL) on cognitive performance, we repeated the analysis, including also the '523' S/VL genotype carriers, controlling for the same demographic and cardiovascular covariates, including ancestry and carriership status of APOE ε2 allele (see description in Supp. Table 1). The overall MANCOVA was not statistically significant, but the '523' genotype effect on executive function, demonstrated near significance level ($F(2,657)=2.94$; $p=0.053$). The post-hoc analysis demonstrated that the effect was driven by differences in effect between the '523' S/S and VL/VL groups ($p=0.017$). The adjusted (estimated) mean for executive function of the S/VL group was, as expected, at intermediate level, between S/S and VL/VL (Table 3). In the MANCOVA, the '523' genotype effect on episodic memory and overall cognition was similar in trend, but not statistically significant ($F(2,657)=1.86$; $p=0.16$; $F(2,657)=1.92$; $p=0.15$ respectively). Nevertheless, the adjusted mean for the S/VL group was again at intermediate level between the two extreme genotypes (Table 3). These results support the conclusion that the '523' effect on cognitive

performance is rendered by the effect of the homozygous extreme alleles (S/S and VL/VL), mainly on executive function.

4. Discussion

The aim of this study was to investigate the association of the TOMM40 '523' poly-T variant with cognitive performance among a sample of elderly individuals with T2D, who are at particularly high risk for cognitive decline and dementia (Beeri et al., 2009; McCrimmon et al., 2012). In agreement with previous reports, we demonstrated that in this population, carriers of the '523' S/S genotype performed significantly better than individuals who have the '523' VL/VL genotype on executive function and episodic memory tasks, when controlling for multiple relevant demographic and cardiovascular variables. Importantly, the effect of '523' was independent from the APOE genotype, since no APOE ε4 carriers were included (therefore excluding the known, strong effect of APOE ε4 on cognition) and since APOE ε2 carriage was included as a covariate in the statistical model. Our results suggest that among individuals who are carriers of the APOE ε3 and ε2 alleles, including '523' genotype may be beneficial when estimating the association between genetic factors (including APOE) and cognitive performance, and potentially to enhance the accuracy of cognitive status prediction.

In this study, to enable comparisons with previous findings (Caselli et al., 2012; Hayden et al., 2012; Johnson et al., 2011), we decided a-priori to contrast the '523' S/S and VL/VL homozygous genotype groups. Since six '523' genotypes are present in the population, including all of them in the statistical analysis would create multiple testing burden. In addition, the L allele is in high LD with the APOE ε4 allele, making it difficult to disentangle the independent effect of TOMM40 '523' on cognitive performance, as discussed above. Moreover, it is biologically reasonable to expect that the most prominent differences in cognitive phenotypes are found when comparing individuals who carry the two homozygous genotypes. Similar to our study, several previous studies investigating the effect of '523' on cognition among healthy individuals found that the most noticeable cognitive differences were detected by comparing S/S to VL/VL (Caselli et al., 2012; Johnson et al., 2011). In addition, in a secondary analysis we included the S/VL genotype group, and found similar results concerning the '523' genotype effect on executive function (p=0.053), supporting the primary analysis. In the three genotypes model, post-hoc analysis demonstrated that the effect was primarily driven by differences in effect between the S/S and the VL/VL genotypes. Notably, the effect observed in the S/VL genotype was intermediate between the two homogeneous genotypes, as would have been expected.

Interestingly, the '523' S/L/VL allele frequency data (48.1%, 7.4%, 44.5% among the whole sample and 48.2%, 8.8%, 43% among Ashkenazi ancestry), as well as the Poly-T lengths range (12–39), is very similar to those previously reported in White and Hispanic population within the United States (Linnertz et al., 2012). As expected, the allele frequency distribution in our Jewish population is different from those observed among African American (where allele S frequency is 65% and VL only 25%), or population from Far Eastern ethnicity (enriched for the VL allele (52–72%) with reduced frequency of the S allele (20–38%)) (Linnertz et al., 2012). In addition, the Poly-T range in the African

American population is much longer (from 14 to 54T residues) compared to other populations, including our Jewish sample, the US white and Hispanic (14–39 residues) and Far eastern populations(Linnertz et al., 2012). Taking together, this data emphasize the important influence of ethnicity on distribution of the '523' allele frequency and the Poly-T lengths.

A limitation of this study is the relatively small sample size (n=331). However, the sample participants were cognitively normal, and despite a narrow range of cognitive functions (compared to the larger ranges found in samples including participants with cognitive impairment), '523' associations with executive function and episodic memory were statistically significant (albeit modest), after accounting for numerous potential demographic, genetic (APOE), and cardiovascular confounders. Our findings are consistent with previous reports concerning the same genetic variant ('523') in terms of the direction of association (Caselli et al., 2012; Hayden et al., 2012; Johnson et al., 2011) in healthy elderly subjects.

Another limitation to be acknowledged is that the study was based on the baseline cognitive assessment of participants. This measure provides a single time point snapshot of cognitive performance. A recent longitudinal study in Caucasians showed that fraction of individuals with dementia who possess the '523' S/S and VL/VL genotypes rapidly increases in the time frame of ages 75 to mid-90s (Crenshaw et al., 2013). Since the current study is limited to cognitive test results obtained at a single age, it is not possible to study the transition from normal cognition to dementia over an extended period of time or to place the results from the current report in context of the age of onset curves from the Crenshaw and collaborators study. Interestingly, there is a shift in the relative proportions of S/S and VL/VL individuals in the oldest members of our cohort in comparison to the youngest that occurs at ages in the mid 70s. At younger ages (65–74), the proportions of individuals carrying the two genotypes (S/S and VL/VL) are approximately equal. At older ages (75–84 and older), there is an approximately 8% enrichment for the S/S genotype compared to the VL/VL genotype. Longitudinal follow-up of the IDCD cohort is ongoing, and will enable investigating the influence of '523' on the rate of the cognitive decline and onset of AD and dementia, and characterize how age modulates it.

A clear advantage of the current study is the exquisitely well-characterized IDCD sample. All subjects were Israelis from Jewish ancestry, with validated T2D diagnosis and normal cognition (ascertained by a multidisciplinary consensus conference), and a broad cognitive assessment. In addition, we accounted for multiple potential confounders, including glycemic control and duration of T2D, that have been associated with T2D, cognitive decline and AD. Importantly, we used averages of multiple measurements of the cardiovascular risk factors representing more closely the course of T2D then single risk factor measurement.

The TOMM40 gene encodes the Tom40 protein, a translocase of the outer mitochondrial protein (Humphries et al., 2005). Mitochondria play a significant role in brain aging and AD processes (Garcia-Escudero et al., 2013; Swerdlow and Kish, 2002). Ferencz et al. (2012) suggested that TOMM40 affects the mitochondrial dysfunction cascade in AD, which leads

to structural and functional changes in the medial temporal lobes (in particular the hippocampus), and changes in connectivity patterns of this area with other brain regions. According to their model, TOMM40 regulates influx of amyloid beta into the mitochondria, both independently and in interaction with APOE dependent mechanisms (Ferencz et al., 2012). The net result is insertion of the neurotoxic amyloid into mitochondria, generation of reactive oxygen species and eventually apoptosis.

Several studies have investigated the potential functional effect of the '523' poly-T variant. Based on brain voxel-based morphometry, the '523' VL allele was associated with decreasing gray matter volume in several brain regions which are affected early in AD, such as ventral posterior cingulate and medial ventral precuneus (Johnson et al., 2011). Li et al. (2013) used human brain tissue taken from APOE ε3/3 carriers, and found that a longer poly-T allele (similar to our category of VL) was associated with high level of neuritic tangles and increased frequency of pathologically defined AD. The fact that among '523' VL homozygous carriers, the TOMM40 transcript expression was significantly higher compared to '523' S homozygous carriers (but not compared to the S/VL group) (Linnertz et al., 2014) may suggest that higher TOMM40 expression is a risk factor for AD, both pathologically (decreased brain volume and neuropathological changes) and clinically (lower cognitive functioning and earlier AD onset).

In addition to the '523' variant, several other SNPs within the TOMM40 gene were associated with AD and phenotypes related to it, such as nonpathological cognitive function in cohorts of older adults, or bilateral hippocampal and left amygdalar volumes (Davies et al., 2014; Potkin et al., 2009; Shen et al., 2010). The biological contribution of additional SNPs within the TOMM40-APOE region to cognition should be further investigated.

To conclude, we were able to confirm previous reports regarding the contribution of the TOMM40 '523' poly-T variant to cognitive function in a cognitively normal sample of elderly with T2D. We showed that carriers of the TOMM40 '523' VL/VL genotype performed significantly worse than carriers of the TOMM40 '523' S/S genotype. This effect was independent from the influence of the APOE ε4 allele. Future genetic studies (in larger samples, including longitudinal ones, from various ethnic groups) as well as a better biological understanding of the TOMM40 role in cognitive decline and AD are required.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

Arvanitakis Z, Wilson RS, Bienias JL, Evans DA, Bennett DA. Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. Arch Neurol. 2004; 61:661–666. [PubMed: 15148141]

Beeri MS, Ravona-Springer R, Silverman JM, Haroutunian V. The effects of cardiovascular risk factors on cognitive compromise. Dialogues Clin Neurosci. 2009; 11:201–212. [PubMed: 19585955]

Beeri MS, Schmeidler J, Sano M, Wang J, Lally R, Grossman H, Silverman JM. Age, gender, and education norms on the CERAD neuropsychological battery in the oldest old. Neurology. 2006; 67:1006–1010. [PubMed: 17000969]

Biessels GJ, Deary IJ, Ryan CM. Cognition and diabetes: a lifespan perspective. Lancet Neurol. 2008; 7:184–190. [PubMed: 18207116]

Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol. 2006; 5:64–74. [PubMed: 16361024]

Caselli RJ, Dueck AC, Huentelman MJ, Lutz MW, Saunders AM, Reiman EM, Roses AD. Longitudinal modeling of cognitive aging and the TOMM40 effect. Alzheimers Dement. 2012; 8:490–495. [PubMed: 23102119]

Cauthen NR. Verbal fluency: normative data. J Clin Psychol. 1978; 34:126–129. [PubMed: 641163]

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science. 1993; 261:921–923. [PubMed: 8346443]

Crenshaw DG, Gottschalk WK, Lutz MW, Grossman I, Saunders AM, Burke JR, Welsh-Bohmer KA, Brannan SK, Burns DK, Roses AD. Using genetics to enable studies on the prevention of Alzheimer's disease. Clin Pharmacol Ther. 2013; 93:177–185. [PubMed: 23249780]

Cruchaga C, Nowotny P, Kauwe JS, Ridge PG, Mayo K, Bertelsen S, Hinrichs A, Fagan AM, Holtzman DM, Morris JC, Goate AM. Association and expression analyses with single-nucleotide polymorphisms in TOMM40 in Alzheimer disease. Arch Neurol. 2011; 68:1013–1019. [PubMed: 21825236]

Davies G, Harris SE, Reynolds CA, Payton A, Knight HM, Liewald DC, Lopez LM, Luciano M, Gow AJ, Corley J, Henderson R, Murray C, Pattie A, Fox HC, Redmond P, Lutz MW, Chiba-Falek O, Linnertz C, Saith S, Haggarty P, McNeill G, Ke X, Ollier W, Horan M, Roses AD, Ponting CP, Porteous DJ, Tenesa A, Pickles A, Starr JM, Whalley LJ, Pedersen NL, Pendleton N, Visscher PM, Deary IJ. A genome-wide association study implicates the APOE locus in nonpathological cognitive ageing. Mol Psychiatry. 2014; 19:76–87. [PubMed: 23207651]

Ferencz B, Karlsson S, Kalpouzos G. Promising genetic biomarkers of preclinical Alzheimer's disease: the influence of APOE and TOMM40 on brain integrity. Int J Alzheimers Dis. 2012; 2012:421452. [PubMed: 22550605]

Fernaeus SE, Almkvist O. Word production: dissociation of two retrieval modes of semantic memory across time. J Clin Exp Neuropsychol. 1998; 20:137–143. [PubMed: 9777467]

Garcia-Escudero V, Martin-Maestro P, Perry G, Avila J. Deconstructing mitochondrial dysfunction in Alzheimer disease. Oxid Med Cell Longev. 2013; 2013:162152. [PubMed: 23840916]

Gregg EW, Yaffe K, Cauley JA, Rolka DB, Blackwell TL, Narayan KM, Cummings SR. Is diabetes associated with cognitive impairment and cognitive decline among older women? Study of Osteoporotic Fractures Research Group. Arch Intern Med. 2000; 160:174–180. [PubMed: 10647755]

Hayden KM, McEvoy JM, Linnertz C, Attix D, Kuchibhatla M, Saunders AM, Lutz MW, Welsh-Bohmer KA, Roses AD, Chiba-Falek O. A homopolymer polymorphism in the TOMM40 gene contributes to cognitive performance in aging. Alzheimers Dement. 2012; 8:381–388. [PubMed: 22863908]

Humphries AD, Streimann IC, Stojanovski D, Johnston AJ, Yano M, Hoogenraad NJ, Ryan MT. Dissection of the mitochondrial import and assembly pathway for human Tom40. J Biol Chem. 2005; 280:11535–11543. [PubMed: 15644312]

Johnson SC, La Rue A, Hermann BP, Xu G, Koscik RL, Jonaitis EM, Bendlin BB, Hogan KJ, Roses AD, Saunders AM, Lutz MW, Asthana S, Green RC, Sager MA. The effect of TOMM40 poly-T

length on gray matter volume and cognition in middle-aged persons with APOE epsilon3/epsilon3 genotype. Alzheimers Dement. 2011; 7:456–465. [PubMed: 21784354]

Jun G, Vardarajan BN, Buros J, Yu CE, Hawk MV, Dombroski BA, Crane PK, Larson EB, Mayeux R, Haines JL, Lunetta KL, Pericak-Vance MA, Schellenberg GD, Farrer LA. Comprehensive search for Alzheimer disease susceptibility loci in the APOE region. Arch Neurol. 2012; 69:1270–1279. [PubMed: 22869155]

Kim S, Swaminathan S, Shen L, Risacher SL, Nho K, Foroud T, Shaw LM, Trojanowski JQ, Potkin SG, Huentelman MJ, Craig DW, DeChairo BM, Aisen PS, Petersen RC, Weiner MW, Saykin AJ. Genome-wide association study of CSF biomarkers Abeta1–42, t-tau, and p-tau181p in the ADNI cohort. Neurology. 2011; 76:69–79. [PubMed: 21123754]

Knopman D, Boland LL, Mosley T, Howard G, Liao D, Szklo M, McGovern P, Folsom AR. Cardiovascular risk factors and cognitive decline in middle-aged adults. Neurology. 2001; 56:42–48. [PubMed: 11148234]

Li G, Bekris LM, Leong L, Steinbart EJ, Shofer JB, Crane PK, Larson EB, Peskind ER, Bird TD, Yu CE. TOMM40 intron 6 poly-T length, age at onset, and neuropathology of AD in individuals with APOE epsilon3/epsilon3. Alzheimers Dement. 2013; 9:554–561. [PubMed: 23183136]

Linnertz, C., Anderson, L., Gottschalk, W., Crenshaw, D., Lutz, MW., Allen, J., Saith, S., Mihovilovic, M., Burke, JR., Welsh-Bohmer, KA., Roses, AD., Chiba-Falek, O. Alzheimers Dement. 2014. The cis-regulatory effect of an Alzheimer's disease-associated poly-T locus on expression of TOMM40 and apolipoprotein E genes.

Linnertz C, Saunders AM, Lutz MW, Crenshaw DM, Grossman I, Burns DK, Whitfield KE, Hauser MA, McCarthy JJ, Ulmer M, Allingham R, Welsh-Bohmer KA, Roses AD, Chiba-Falek O. Characterization of the poly-T variant in the TOMM40 gene in diverse populations. PLoS One. 2012; 7:e30994. [PubMed: 22359560]

Luchsinger JA. Type 2 diabetes and cognitive impairment: linking mechanisms. J Alzheimers Dis. 2012; 30(Suppl 2):S185–S198. [PubMed: 22433668]

Luchsinger JA, Reitz C, Patel B, Tang MX, Manly JJ, Mayeux R. Relation of diabetes to mild cognitive impairment. Arch Neurol. 2007; 64:570–575. [PubMed: 17420320]

Luchsinger JA, Tang MX, Stern Y, Shea S, Mayeux R. Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort. Am J Epidemiol. 2001; 154:635–641. [PubMed: 11581097]

Lutz MW, Crenshaw DG, Saunders AM, Roses AD. Genetic variation at a single locus and age of onset for Alzheimer's disease. Alzheimers Dement. 2010; 6:125–131. [PubMed: 20298972]

McCrimmon RJ, Ryan CM, Frier BM. Diabetes and cognitive dysfunction. Lancet. 2012; 379:2291–2299. [PubMed: 22683129]

Potkin SG, Guffanti G, Lakatos A, Turner JA, Kruggel F, Fallon JH, Saykin AJ, Orro A, Lupoli S, Salvi E, Weiner M, Macciardi F. Hippocampal atrophy as a quantitative trait in a genome-wide association study identifying novel susceptibility genes for Alzheimer's disease. PLoS One. 2009; 4:e6501. [PubMed: 19668339]

Ravona-Springer R, Heymann A, Schmeidler J, Guerrero-Berroa E, Sano M, Preiss R, Koifman K, Hoffman H, Levy A, Silverman JM, Schnaider-Beeri M. Haptoglobin 1-1 genotype is associated with poorer cognitive functioning in the elderly with type 2 diabetes. Diabetes Care. 2013; 36:3139–3145. [PubMed: 23990521]

Reitan RM. The relation of the trail making test to organic brain damage. J Consult Psychol. 1955; 19:393–394. [PubMed: 13263471]

Roses AD, Lutz MW, Amrine-Madsen H, Saunders AM, Crenshaw DG, Sundseth SS, Huentelman MJ, Welsh-Bohmer KA, Reiman EM. A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease. Pharmacogenomics J. 2010; 10:375–384. [PubMed: 20029386]

Roses AD, Lutz MW, Crenshaw DG, Grossman I, Saunders AM, Gottschalk WK. TOMM40 and APOE: requirements for replication studies of association with age of disease onset and enrichment of a clinical trial. Alzheimers Dement. 2013; 9:132–136. [PubMed: 23333464]

Rotkiewicz-Piorun AM, Al Snih S, Raji MA, Kuo YF, Markides KS. Cognitive decline in older Mexican Americans with diabetes. J Natl Med Assoc. 2006; 98:1840–1847. [PubMed: 17128695]

Shen L, Kim S, Risacher SL, Nho K, Swaminathan S, West JD, Foroud T, Pankratz N, Moore JH, Sloan CD, Huentelman MJ, Craig DW, Dechairo BM, Potkin SG, Jack CR Jr, Weiner MW, Saykin AJ. Whole genome association study of brain-wide imaging phenotypes for identifying quantitative trait loci in MCI and AD: a study of the ADNI cohort. Neuroimage. 2010; 53:1051–1063. [PubMed: 20100581]

Spreen, O., Benton, AL. Neurosensory Center Comprehensive Examination for Aphasia: Manual of Instructions (NCCEA) (rev.). University of Victoria; Victoria, BC: 1977.

Swerdlow RH, Kish SJ. Mitochondria in Alzheimer's disease. Int Rev Neurobiol. 2002; 53:341–385. [PubMed: 12512346]

Wechsler, D. Wechsler Adult Intelligence Scale-Revised Manual. The Psychological Corporation; San Antonio: 1981.

Wechsler, D. Wechsler Memory Scale-Revised Manual. The Psychological Corporation; San Antonio: 1987.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.euroneuro.2014.06.002.

Table 1

'523' Allele frequencies in the whole IDCD cohort, and among the Ashkenazi subset sample. There were no statistical differnces in allele frequency distribution between the Ashkenazi origin participants and the whole sample.

Group	Subject (N)	S (%)	L (%)	VL (%)	Poly-T length (range)
Whole cohort	841	48.10	7.40	44.50	12–39
Ashkenazi ancestry	526	48.20	8.80	43	12–39

Table 2

Demographic and clinical description of the analyzed sample, comparing the '523' S/S and VL/VL groups. No statistically-significant difference in any of the variables was found between the groups (S/S vs. VL/VL).

Variable	All sample	%/SD	S/S	%/SD	VL/VL	%/SD
Number	331		179		152	
Males (N)	217	65.6%	118	65.92%	99	65.10%
Ashkenazi origin (N)	211	63.7%	118	65.92%	93	61.18%
APOE e2 allele carriers (N)	47	14.2%	28	15.64%	19	12.50%
Age at cognitive evaluation	71.98	4.87	72.44	5.03	71.43	4.63
Years of education	13.36	3.18	13.16	3.15	13.60	3.21
Hemoglobin A1c (mean)	6.79	0.82	6.76	0.78	6.83	0.86
Creatinine (mean)	1.01	0.27	1.01	0.23	1.00	0.31
BMI (mean)	28.85	4.71	28.80	4.60	28.91	4.85
Total cholesterol (mean)	181.49	23.10	181.78	23.35	181.15	22.89
HDL (mean)	47.62	10.24	47.76	9.97	47.45	10.58
LDL (mean)	103.11	18.36	103.36	18.47	102.83	18.29
Triglyceride (mean)	155.36	56.25	155.59	54.77	155.09	58.13
Duration of disease (years)	10.62	0.85	10.61	0.74	10.64	0.98
Diastolic BP (mean)	85.02	16.10	83.54	15.37	86.76	16.79
Systolic BP (mean)	166.68	9.70	165.79	9.93	167.72	9.34

Table 3

Adjusted (estimated) performance means for executive function, episodic memory and overall cognition (accounting for covariates) across the '523' S/S, S/VL and VL/VL genotypes.

Cognitive function	'523' Genotype	Mean	Std. Error	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
Executive	S/S	0.32	0.188	0.053	−0.048	0.689
	S/VL	−0.066	0.135		−0.332	0.199
	VL/VL	−0.342	0.204		−0.742	0.058
Episodic memory	S/S	0.264	0.16	0.16	−0.05	0.578
	S/VL	0.068	0.115		−0.158	0.294
	VL/VL	−0.191	0.174		−0.533	0.15
Overall	S/S	0.785	0.456	0.15	−0.11	1.679
	S/VL	−0.052	0.328		−0.696	0.593
	VL/VL	−0.481	0.495		−1.453	0.49