APOE and TOMM40 in Alzheimer’s Disease: A Case of Mistaken Identity, Using Precision Tools but with Loss of Accuracy

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"The association of the apolipoprotein E (APOE) ε4 allele with AD is strong (odds ratios ranging from 3 to 10) and undisputed" [1]. In fact, in 2015 this remains generally true, yet this same paper falls to mention the association with the poly-T variant (rs10524523) in the TOMM40 gene (aka TOMM40'523) which encodes the channel component of the complex that transports proteins across the outer mitochondrial membrane. The same research group published in Archives of Neurology in 2012 a widely quoted editorial entitled "TOMM40 Association with Alzheimer's disease: Tales of APOE and Linkage Disequilibrium" [2]. In this article, Guerreiro and Hardy made the case that although APOE and TOMM40 were adjacent to each other on chromosome 19, and in linkage disequilibrium [LD]; the association of TOMM40 was entirely due to its position in LD with APOE. To further support this argument, the authors stressed that, "since the first genome-wide association studies in AD it was clear that no other locus in the genome would present such a strong association with the disease as that on chromosome 19 around the APOE locus" [2]. What the authors failed to comprehend or consider was that the GWAS SNPs representing the APOE region were actually four TOMM40 SNPs and a 3 non-coding SNP near APOE, not either coding SNPs of APOE. The two coding SNPs that determine the differences between APOE4, APOE3, and APOE2 alleles are not present in the genotyping platforms typically used in GWAS studies. Simply stated, it was TOMM40 SNPs being measured, not APOE SNPs and therefore a case of mistaken identity. Identity is equivalent to accuracy.

Lack of precision in interpreting the GWAS data has delayed the progress of targeting medicines for AD [3,4]. In fact, in 2015, there exist significant and growing support for the genetics of TOMM40 including TOMM40'523, and a plausible hypothesis for the intraneuronal interaction of apoE3 and apoE4 fragments and the Tom40 protein complex leading to potential mode of action therapeutic targets [5-6]. In addition, TOMM40'523-APOE haplotypes are more informative for disease risk than APOE4.

The TOMM40'523 locus is not a single SNP, but a structural variation with more than 25 different poly-thymidine length variants, referred to as a simple sequence repeat [SSR]. The multiple, inherited poly-thymidine sequence lengths at the rs10524523 locus can be divided into three observable size ranges: Small, less than 20 thymidine [T] length repeats; Large, 20-30 Ts; and Very Large, more than 30 Ts [7]. All of the poly-T variant lengths are at the rs10524523 position in LD with APOE, and, when measured accurately, it is clear that the different size ranges from distinct haplotypes with different APOE alleles. Humans inherit two of the three possible allele range, or alleles [S, L, VL] and APOE4-VL are localized to two highly distinct phylogenetic clades [7]. Using this phylogenetic approach it was possible to map all the TOMM40 SNPs and TOMM40'523 variants in LD [8]. This identified two distinctly separated clades that could be identified S and VL, that are in LD with the APOE4 or APOE2 alleles. Thus it became possible in 2010 to differentiate two different APOE4 types that could not be distinguished by APOE typing alone. This results in a higher proportion of informative individuals with TOMM40'523 sequencing that APOE4 alone. Caucasian individuals with the APOE4 genotype had two Long TOMM40'523 alleles. It is now possible to demonstrate that for APOE3 and APOE2 chromosomes there were two distinct haplotypes represented, allowing prognostic data for age of onset distributions from all APOE3/3 and APOE2/3 carrying individuals. Thus APOE4 distribution curves are a sub-set of the TOMM40'523 age of onset distributions, increasing the individual haplotype informative genotyping to 100% subjects, compared to 29% of subjects who carry an APOE4 allele [8].

For more than 20 years, APOE4-containing genotypes [APOE4/4, APOE3/4 and APOE2/4] were clearly associated with earlier age of onset distributions. Corder et al. identified the later effect age of onset for carriers of APOE3/3 genotypes in 1993 [9]. Saunders et al. reported the relationship of APOE4 carriage with the onset of late-onset [so-called sporadic] AD in 1993 [10]. For clarity, APOE3/3 was never AD-neutral, simply associated with later age of onset than the APOE4-containing genotypes. Over the years, clinical studies and drug trials compared the APOE4 carriers against the APOE4 non-carriers, assuming the neutrality of APOE3 and not defining the age of onset differences in the comparative groups. It has been shown in multiple clinical studies, including many therapeutic trials with APOE3/3 cases representing more than 50% of the AD prevalent population, confirming that the APOE3 variant can hardly be described as having a neutral effect. The APOE2/2 is exceedingly rare in any AD cohort [especially in neuropathologically-confirmed criteria for amyloid deposition] and APOE3/3 has the latest age of onset distribution compared to APOE3/3, APOE4/3 and APOE4/4 genotypes [8].

The relevant clinical question is to ask which measurement is better as an informative prognostic for AD: APOE4 carriers [29% of Caucasians] or APOE-TOMM40'523 haplotypes for 100% of

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Received September 22, 2015; Accepted September 22, 2015; Published September 28, 2015


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Caucasians. It was clear in 1995 when a panel of experts stated that presence of APOE4 was not sufficient to use as a diagnostic [11]. That was the reason the two SNPs defining the three isoforms were excluded from the GWAS platforms so that they could not be used in clinical diagnosis without test validation as a diagnostic. In this age of “precision medicine”, the biological effects have to be analyzed at least as accurately as the “Big Data” statistical analyses [12]. This has not been the case with regard to the genetics of AD. APOE measurements can be accurately ascertained and with suitable careful technique length variation in the homopolymer of Ts can also be accurately assessed [ +/- 1-T differences] [13,14]. Had TOMM40’523 been discovered before APOE association, APOE4 alleles would have been considered as a subgroup of APOE-TOMM40’523 haplotypes. Thus, the biological interactions with the Tom40 protein should not be excluded from consideration as an important contributor to disease progression, as has been the case in most reviews since 2010. The genetic informative relationship between APOE and TOMM40’523 has been largely excluded from discussion and therefore wider biological considerations for potential therapies [1,4,15-17].

The penalty paid in this era of precision medicine is inaccurately interpreting the roles of TOMM40’523 and APOE in disease pathogenesis, resulting in delays testing new hypotheses while other older hypotheses fail. This is not good science. Biological data relative to pathogenic hypotheses should be enlisted early, not after an additional decade with millions of new patients with AD remains inadequately treated. Amyloid plaques are viewed by a minority of investigators as a consequence [not an etiology] of the disease process in the CNS, and this concept is greatly supported by the total lack of clinical association of the APP locus with late-onset AD. Surely the Big Data of more than 2 dozen AD GWAS studies should be analyzed appropriately for genes related to the molecular etiology of disease, for both positive and negative association with specific clinical phenotypes.

Ages of onset distributions are clinically-relevant measurements [although usually poorly documented in retrospective studies]. Age of onset is especially accurate when the criteria for onset are well-defined and subjects are studied prospectively from normal cognition to the first signs of impairment [8]. The amyloid load shows a greater density in APOE4 carrying patients, yet the genetic association of the APP gene or nearby loci with late-onset AD has been consistently negative [16]. Rather, much of the AD field insists that the amyloid cascade is the etiology for AD, and therefore its expression in brain tissue is a causal phenomenon. Similar criteria for age of onset using APOE and TOMM40’523 illustrates that both contribute to a biological effect – and the fact that they are located adjacent to each other supports the need for further interaction studies, especially with other components of the translocase of the outer mitochondrial membrane complex that regulate protein and peptide entry into the mitochondrion. This is also inferred from Figure 1, demonstrating that inheriting different TOMM40’523-S or TOMM40’523-V1 haplotypes are also related to a more defined range of age of onset distributions.

The result of not considering the phylogenetic data and the age of onset distributions has led to a widespread looking over the biological role of TOMM40’523 in many/most comments and reviews of the genetics of AD. The confirmation of prospectively followed AD cohorts with respect to age of onset distributions takes years, and as more data has supported the role of TOMM40’523 the perils of ignoring the science has been further exposed.

This comes at a time when pursuit of the amyloid cascade hypotheses had not led to effective treatments even though tens of billions have been invested. Target identification based on mitochondrial and other metabolic modes of pathogenesis could lead to a myriad of potential new drug development programs at a time when the pharmaceutical industry has generally decided that most central nervous disease treatments do not represent worthwhile portfolio opportunities. The nature of CNS clinical trials for new drugs entails approximately a 12-15 year development period that is usually measured against a 20 year chemical patent. The largest single cause of failure of completed trials has been for no efficacy. Another crippling cause of failure rests on the business development philosophies of pharmaceutical companies. Actually, pharmaceutical companies do not have biases, but CEOs and Clinical Development Directors do – and they generally change with management every 4-7 years on the average - usually disrupting the continuity of long, relatively expensive clinical trials. In-house developed Futility Analyses are now the second largest cause for early termination of expensive trials [17-19].

With more than three decades with the amyloid hypothesis squeezing out most new initiatives, trying to test new hypotheses in AD results is nearly impossible. The travesty is that testing a single hypothesis over 30 years has resulted in significantly diminished funding to explore equally plausible hypotheses. How does that happen? Imagine that most everyone successful in AD academics, most doing some perturbation of amyloid hypotheses, review the grant requests for innovative new hypotheses. Most are killed “with faint praise” and miss the funding line – others are simply killed before review. The intellectual density of amyloid supporters in funding positions is not supportive of peer review, but a process of conflict of interest voting. This has been commented about over the years – but still continues [20]. The only way that new ideas will be tested is when the system changes and, especially in the field of AD, when funding is designated for new hypotheses and reviews are based on sound review of data, not editorial or peer review trump cards. “Faced with the choice between changing one’s mind and proving that there is no need to do so, almost everyone gets busy on the proof” [John Kenneth Galbraith].

References


