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REPLY

Reply to Neupane et al.: Replication study of AD-associated rare variants

In a recent study[1] we used whole genome sequencing (WGS) data from family-based and case-control cohorts to identify novel candidate genes associated with Alzheimer's disease (AD). Focusing on rare variants (MAF < = 1%) we performed single variant and region-based analyses in a family-based cohort followed by replication in unrelated subjects from a case-control cohort. We identified 13 novel AD-associated loci (4 single variant-based and 9 region-based) containing rare variants in non-coding regions, with follow up analyses implicating synaptic function. Neupane et al. attempted to confirm our findings in independent family-based and case-control datasets but limited their analysis to only exonic coding regions (+/- 100 bp), which provide coverage of <2% of variants found with WGS, therefore largely missing the single variants and the full regions which we reported as our findings.

Rare variant WGS studies are challenging, in general, and particularly in AD, given the high cost of generation of such data and the late onset of disease, which limits the number of subjects used in family-based designs. We addressed these challenges, as described in our initial article, by carefully balancing our ability to detect novel rare variants while keeping the false-positive rate under control and using an independent validation dataset. We also emphasized the importance of testing for replication of rare variant findings in additional independent datasets. Assuming that the same methodology, study design and phenotype is being used and the exact same variants are being tested, the replication rate also highly depends on sample size, effect size and minor allele frequency.[2] In addition, associations are subject to random variability due to sampling, confounding effects, and sequencing errors.[3,4]

Neupane et al. restricted their analysis to exons (+/- 100 bp) in an attempt to find exonic variants associated with AD in the 13 closest protein-coding genes implied by our WGS-based rare variant analysis. Thus, neither of the actual AD-associated genomic regions and variants we tested in Prokopenko et al. (neither the top single variants from Table 1 in,[1] nor the full top regions from Table 2 in[1]) could be directly assessed by Neupane et al. Nonetheless, in Neupane et al., exploratory analysis of exonic variants of our candidate genes identified three nominally significant loci (single variants in *C2CD3* and *NALCN*, and a region with 39 rare variants in *CTNNA2* with a p-value of 0.05, which the authors did not discuss). Analysis of those loci in our datasets revealed a very similar pattern. Rs181256373 (*C2CD3*) had a p-value of 0.05 for protection in non-Hispanic white (NHW) unrelated individuals and rs1151376 (*NALCN*) was nominally significant for protection in NIMH families (p = 0.036), but not replicated in NHW unrelated individuals (Table 1). Unfortunately, we couldn't confirm whether the effect direction is the same as in Neupane et al. since they did not provide this information. Burden of rare variants in the exonic region of CTNNA2 was not significant in our datasets, however, we note that we were not able to assess the exact same 39 rare variants as those in Neupane et al. due to the lack of this information.

In summary, while the exploratory analysis of Neupane et al. took on the challenging task of attempting replication of association signals from non-coding rare variants, using limited exonic data, we were pleased to see that of 13 candidate AD genes presented in our study, two (*NALCN*, *C2CD3*) still harbored rare exonic variants showing nominal significance for association with AD, in two independent studies, thereby providing corroboration and confirmation of a subset of our findings.

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

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TABLE 1 Single variant association results

Chromosome	Position	SNP (rsID)	Reported gene	Allele frequency, gnomAD v3	Allele frequency, non-Finnish Europeans, gnomad v3	Effect allele	Other allele	Neupane et al P-value	Discovery dataset (NIMH + NIA families)				Replication dataset NHW ADSP				Meta-analysis		
									Effect allele frequency	Z-score	P-value		Effect allele frequency	Z-score	P-value		Effect direction	Z-score	P-value
11	74118187	rs181256373	C2CD3	0.002	0.003	T	C	0.047	NA	NA	NA		0.003	−1.954	0.051		?-	−1.954	0.051
13	101095770	rs1151376	NALCN	0.061	0.004	A	G	0.024	0.022	−2.102	0.036		0.004	0.086	0.931		−+	−0.106	0.916

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