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This is a pre print version of the following article:

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1673173 since 2018-08-11T17:00:43Z

Published version:

DOI:10.1080/21678421.2017.1413118

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17 April 2024

Letter to the editor: Reconsidering the causality of TIA1 mutations in ALS

Project Mine ALS Sequencing Consortium
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In a recent study published in Neuron, Mackenzie and colleagues prioritised T cell-restricted intracellular antigen-1 (TIA1) as a novel amyotrophic lateral sclerosis (ALS) related gene. The authors state that their findings indicate a causal role for TIA1 in ALS/FTD, which has already been adopted in a resource frequently used in the clinic (Neuromuscular Disease Center). Although the biology of TIA1 is very appealing and the functional experiments have been well-performed, current guidelines stress that these experiments do not prove causality (MacArthur et al. 2014). Regarding the evidence that is provided for causality, we feel obliged to share concerns that affect the validity of the conclusion that TIA1 mutations cause ALS. In short, we argue that the presented pedigree merely yields TIA1 as a candidate gene. The subsequent burden analysis lacks crucial methodological details, is potentially flawed, yields inconclusive results and without a replication effort, rare TIA1 mutations cannot be regarded as causal in ALS.

Mackenzie and colleagues performed whole exome sequencing in a single pair of second degree relatives. After filtering rare non-synonymous variants present in both relatives, 17 genes containing variants remained. Five of the 17 genes were expressed in brain and were predicted deleterious (CADD score >20). Out of these 5 genes, the variant P362L in *TIA1* was picked as most interesting candidate. It affects a conserved residue and the variant was predicted to be possibly damaging or deleterious by several in silico prediction algorithms. It is important to note that 4 more candidate genes were present in this pedigree derived from these filtering steps, with similar predictions for deleteriousness and CADD scores. In this context and without evidence for segregation, interesting observations, such as allele-frequency in public databases, phylogenic conservation and pathogenicity in other diseases can be used to prioritise candidate genes, but do not prove causality. To justify claims of causality, the candidate gene needs to pass the threshold for multiple testing in robust association analyses (in both a discovery and replication phase).

Mackenzie and colleagues indeed proceed with a burden analysis, where they present a p-value for *TIA1* variants in exon 11-13 ($p = 8.7 \times 10^{-6}$, SKAT-O adjusted for sex and age). It might be tempting to judge the evidence by this p-value alone (Leek and Peng 2015). However, we experienced difficulties in interpreting the p-value in the absence of methodological details.

First, in order to correctly interpret the presented p-value, we would be very interested in the following methodological details: (1) which criteria were used to include variants in the burden analysis? (2) Were cases and controls matched for ancestry? Imperfect matching can severely inflate association statistics, especially in the analysis of rare variants (Do, Kathiresan, and Abecasis 2012). (3) Were related individuals excluded from the analysis? (4) Were any quality control measures performed? (5) Of the 3,036 controls, 959 are from a difference cohort and sequenced using a different technique (Sanger sequencing vs. next generation sequencing). Did the authors ensure that both methods were equally sensitive

to detect all variants? Considering these methodological concerns, the interpretation of association analyes requires evidence of a well-behaved test-statistic (i.e. non-inflated QQ-plot) that ensures known and unknown confounders have been addressed adequately.

Second, it remains unclear how the authors derived a p-value of 8.7×10^{-6} , considering they detected 6 variant carriers among 1,039 patients and 0 variants among 3,036 controls. These numbers can be used to reconstruct their burden test using simulated data. Our simulation includes the same number of cases and controls, with identical allele-counts. The SKAT-O test yields a p-value of ~1.37×10^{-4}. Alternative aggregation tests such as SKAT, Firth logistic regression and Fisher exact, yield similar p-values (R-script available on https://bitbucket.org/ProjectMinE/tia1). This is, of course, an approximation because we do not have the exact same covariate data. Permuting the test 10,000 times with respect to the covariates does not yield a p-value < 1.13×10^{-4}. Without knowing the details of the burden test performed, the reported p-value of 8.7×10^{-6} might, therefore, be very well higher (i.e. less significant).

Third, in the context of a discovery phase experiment, the presented p-value does not justify rejection of the null-hypothesis. If we assume adequate methodology, we interpret this p-value in the context of an exome-wide hypothesis-generating search because *TIA1* can only be regarded as a candidate gene at this point. Therefore, it requires a stringent multiple testing correction of at least 1.7×10^{-6} (0.05, Bonferroni corrected for 21,000 protein-coding genes and 9,000 long noncoding RNA genes)(MacArthur et al. 2014). However, if a variety of tests and variant subsets have been considered, $p < 5 \times 10^{-7}$ is the recommended threshold (Do, Kathiresan, and Abecasis 2012). Furthermore, Mackenzie and colleagues only test variation within a specific domain of *TIA1*. Depending on the definition of a domain the number of domains in our genome far exceeds the number of genes(Sillitoe et al. 2015). Therefore, it can be argued that an even more stringent multiple testing correction is needed. The reported p-value does not meet any of these multiple testing criteria.

Fourth, replication is required in order to claim causality. To our knowledge there are 3 easily-available ALS-specific case-control cohorts (Table 1) including considerably more cases and controls than in the presented burden test. It is well-acknowledged that association analyses, such as the presented burden test, benefit from the largest number of cases and controls available to decrease the chances of both false negative and false positive results. We performed burden analysis on *TIA1* exon 11-13 in the Project MinE Consortium dataset. Data acquisition and quality control has been described previously (Project MinE Consortium et al. 2017). We assumed the same filtering was performed as in the pedigree exomes that prioritised *TIA1* as the candidate gene. We extracted heterozygous variants that alter the amino acid sequence, that were seen 2 or fewer times in ExAC and were not observed in the Exome Variant Server. This did not yield a significant

result ($p > 0.32$; SKAT-O adjusted for first 10 principal components, sex and platform; $\lambda_{GC} = 1.01$). We acknowledge that this burden test could still be underpowered and do not consider this as definite evidence that *TIA1* mutations are *not* association with ALS. To increase power and provide a more definite answer, we strongly encourage the combined analysis of all available data for the region of interest (interpreted as discovery phase).

	Method	**Cases**	**Controls**
Cirulli et al. 2015	WES	2,869	6,405
Kenna et al. 2016	WES	1,022 (FALS)	7,315
Project MinE Consortium et al. 2017	WGS	4,389	1,846

Table 1. WES = Whole Exome Sequencing, WGS = Whole Genome Sequencing, FALS = Familial ALS cases

In conclusion, even though the extensive functional experiments performed by Mackenzie and colleagues shed light on the biological function of *TIA1* and show that stress granule dynamics and phase separation may be a crucial aspect of ALS pathophysiology, there is currently insufficient genetic evidence to support a *causal* role for *TIA1* mutations in ALS. We encourage research initiatives to provide robust genetic evidence before claiming causality. Therefore, we fully support the author's remark that future studies in ALS and FTD patient cohorts and control populations will be critical to evaluate the contribution of *TIA1* in ALS/FTD. Until then, we would advise against routine screening of *TIA1* mutations in ALS/ FTD patients in the clinic.

Cirulli, Elizabeth T., Brittany N. Lasseigne, Slavé Petrovski, Peter C. Sapp, Patrick A. Dion, Claire S. Leblond, Julien Couthouis, et al. 2015. “Exome Sequencing in Amyotrophic Lateral Sclerosis Identifies Risk Genes and Pathways.” *Science* 347 (6229): 1436–41.

Do, Ron, Sekar Kathiresan, and Gonçalo R. Abecasis. 2012. “Exome Sequencing and Complex Disease: Practical Aspects of Rare Variant Association Studies.” *Human Molecular Genetics* 21 (R1): R1–9.

Kenna, Kevin P., Perry T. C. van Doormaal, Annelot M. Dekker, Nicola Ticozzi, Brendan J. Kenna, Frank P. Diekstra, Wouter van Rheenen, et al. 2016. “NEK1 Variants Confer Susceptibility to Amyotrophic Lateral Sclerosis.” *Nature Genetics* 48 (9): 1037–42.

Leek, Jeffrey T., and Roger D. Peng. 2015. “Statistics: P Values Are Just the Tip of the Iceberg.” *Nature* 520 (7549): 612.

MacArthur, D. G., T. A. Manolio, D. P. Dimmock, H. L. Rehm, J. Shendure, G. R. Abecasis, D. R. Adams, et al. 2014. “Guidelines for Investigating Causality of Sequence Variants in Human

Disease.” *Nature* 508 (7497): 469–76.
Mackenzie, Ian R., Alexandra M. Nicholson, Mohona Sarkar, James Messing, Maria D. Purice, Cyril Pottier, Kavya Annu, et al. 2017. “TIA1 Mutations in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia Promote Phase Separation and Alter Stress Granule Dynamics.” *Neuron* 95 (4): 808–16.e9.
Neuromuscular Disease Center, retrieved September 19, 2017, from http://neuromuscular.wustl.edu/musdist/distal.html#welander
Project MinE Consortium, Wouter Van Rheenen, Sara L. Pulit, Annelot M. Dekker, Ahmad Al Khleifat, William J. Brands, Alfredo Iacoangeli, et al. 2017. “Project MinE: Study Design and Pilot Analyses of a Large-Scale Whole-Genome Sequencing Study in Amyotrophic Lateral Sclerosis.” *bioRxiv*. doi:10.1101/152553.
Sillitoe, Ian, Tony E. Lewis, Alison Cuff, Sayoni Das, Paul Ashford, Natalie L. Dawson, Nicholas Furnham, et al. 2015. “CATH: Comprehensive Structural and Functional Annotations for Genome Sequences.” *Nucleic Acids Research* 43 (Database issue): D376–81.

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