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EDITORIAL

Reconsidering the causality of TIA1 mutations in ALS

RICK A. VAN DER SPEK^{1*} , WOUTER VAN RHEENEN^{1*}, SARA L. PULIT¹ , KEVIN P. KENNA², NICOLA TICOZZI^{3,4}, MAARTEN KOOYMAN⁵, RUSSELL L. MCLAUGHLIN⁶, MATTHIEU MOISSE^{7,8,9} , KRISTEL R. VAN EIJK¹, JOKE J. F. A. VAN VUGT¹, ALFREDO IACOANGELI¹⁰, PETER ANDERSEN¹¹, A. NAZLI BASAK¹², IAN BLAIR¹³, MAMEDE DE CARVALHO^{14,15}, ADRIANO CHIO^{16,17} , PHILIPPE CORCIA^{18,19}, PHILLIPE COURATIER^{18,19}, VIVIAN E. DRORY²⁰, JONATHAN D. GLASS^{21,22}, ORLA HARDIMAN^{6,23}, JESÚS S. MORA²⁴, KAREN E. MORRISON²⁵, MIGUEL MITNE-NETO²⁶, WIM ROBBERECHT^{7,8,9}, PAMELA J. SHAW²⁷ , MONICA P. PANADÉS²⁸, PHILIP VAN DAMME^{7,8,9}, VINCENZO SILANI^{3,4}, MARC GOTKINE²⁹, MARKUS WEBER³⁰, MICHAEL A. VAN ES¹, JOHN E. LANDERS², AMMAR AL-CHALABI³¹ , LEONARD H. VAN DEN BERG¹, JAN H. VELDINK¹ ; PROJECT MINE ALS SEQUENCING CONSORTIUM

¹Department of Neurology, Brain Center Rudolf Magnus University Medical Center Utrecht, Utrecht, The Netherlands; ²Department of Neurology, University of Massachusetts Medical School, Worcester, MA, USA; ³Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy; ⁴Department of Pathophysiology and Transplantation, ‘Dino Ferrari’ Center-Università degli Studi di Milano, Milan, Italy; ⁵SURFsara, Amsterdam, The Netherlands; ⁶Population Genetics Laboratory, Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Republic of Ireland; ⁷Department of Neurosciences, Experimental Neurology and Leuven Research Institute for Neuroscience and Disease (LIND), KU Leuven - University of Leuven, Leuven, Belgium; ⁸Laboratory of Neurobiology, VIB, Center for Brain & Disease Research, Leuven, Belgium; ⁹Department of Neurology, University Hospitals Leuven, Leuven, Belgium; ¹⁰Department of Biostatistics and Health Informatics, Institute of Psychiatry, Psychology and Neuroscience, King’s College London, London, UK; ¹¹Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden; ¹²Suna and Inan Kırac Foundation Neurodegeneration Research Laboratory, Bogazici University, Istanbul, Turkey; ¹³Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, New South Wales, Australia; ¹⁴Institute of Physiology, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal; ¹⁵Department of Neurosciences, Hospital de Santa Maria-CHLN, Lisbon, Portugal; ¹⁶‘Rita Levi Montalcini’ Department of Neuroscience, ALS Centre, University of Torino, Turin, Italy; ¹⁷Department of Neuroscience, Azienda Ospedaliera Città della Salute e della Scienza, Turin, Italy; ¹⁸Centre SLA, CHRU de Tours, Tours, France; ¹⁹Federation des Centres SLA Tours and Limoges, LITORALS, Tours, France; ²⁰Department of Neurology Tel-Aviv Sourasky Medical Centre, Israel; ²¹Department Neurology, Emory University School of Medicine, Atlanta, GA, USA; ²²Emory ALS Center, Emory University School of Medicine, Atlanta, GA, USA; ²³Department of Neurology, Beaumont Hospital, Dublin, Republic of Ireland; ²⁴ALS Unit, Hospital San Rafael, Madrid, Spain; ²⁵Faculty of Medicine, University of Southampton, Southampton, UK; ²⁶Human Genome Research Center, Bioscience Institute, University of São Paulo, SP, Brazil; ²⁷Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, UK; ²⁸Neurology Department, Hospital Universitari de Bellvitge, Barcelona, Spain; ²⁹Department of Neurology, The Agnes Ginges Center for Human Neurogenetics, Hadassah-Hebrew University Medical Center, Israel; ³⁰Neuromuscular Diseases Unit/ALS Clinic, Kantonsspital St. Gallen, St. Gallen, Switzerland; ³¹Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, King’s College London, London, UK

Correspondence: Jan H. Veldink, Department of Neurology and Neurosurgery, University Medical Centre Utrecht, Department of Neurology G03.228, P.O. Box 85500, 3508 GA, Utrecht, The Netherlands. E-mail: J.H.Veldink@umcutrecht.nl

*Both authors contributed equally to this work.

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T-cell-restricted intracellular antigen-1 (*TIA1*) has been recently reported as a novel amyotrophic lateral sclerosis (ALS) related gene, and has already been adopted in a resource frequently used in the clinic (Neuromuscular Disease Center) (1,2). The Project MinE Consortium has reviewed the data that support this inclusion, and suggest that the inclusion of this gene as causative is premature.

Although the biology of *TIA1* is very appealing and the published functional experiments have been well-performed, it is also the case that current guidelines stipulate that experimental data must be interpreted with clinical caution, as they do not always prove causality (3). Here, we argue that data from the published pedigree that supports *TIA1* is insufficient to prove causality. We hold that burden analysis lacks crucial methodological details, and is therefore potentially flawed. Because of this, without a replication effort, we hold that rare *TIA1* mutations cannot be regarded as causal in ALS, and that it is premature to include this gene in diagnostic panels for ALS and FTD.

This caution is not to criticize the quality of the work that was performed in characterizing this gene, which is exemplary, and worth describing in detail. The purpose is to systematically evaluate the provided genetic evidence as an exercise in demonstrating the best possible methodologies to establish genetic causality.

In the *TIA1* study, whole-exome sequencing was performed in a single pair of second-degree relatives. Variants not observed in the Exome Variant Server and observed less than three times in ExAC were extracted. After filtering non-synonymous variants present in both relatives, 17 genes containing variants remained. Five of the 17 genes were expressed in brain and contained variants that were predicted deleterious (CADD score >20). Out of these five genes, the variant P362L (rs757332023) in *TIA1* was picked as the 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. However, it is also important to note that four 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, phylogenetic conservation and pathogenicity in other diseases can be used to prioritize 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) and demonstrate phenotype-relevant downstream effects in functional follow-up.

The group correctly proceeded with a burden analysis in 1039 patients and 3036 controls, and

generated a p -value for *TIA1* variants in exon 11–13 ($p = 8.7 \times 10^{-6}$, SKAT-O adjusted for sex and age). However, this p -value remains difficult to interpret in the absence of specific methodological details (4). These should include: (1) a description of the criteria that were used to include variants in the burden analysis; (2) evidence of matching for ancestry or the use of covariates (e.g. principal components) used to correct for ancestry. This is because imperfect matching can severely inflate association statistics, especially in the analysis of rare variants (5); (3) the need to excluded related individuals from the analysis, or to account for relatedness methodologically; (4) the use of quality control measures; and (5) the use of uniform sequencing methodologies (Sanger sequencing vs. whole-exome sequencing), or to demonstrate that different methods are equally sensitive to detect all variants. Finally, the interpretation of association analyses requires evidence of a well-behaved test-statistic (i.e. through production of a QQ-plot and/or reporting LambdaGC) that ensures confounders have been addressed adequately.

For example, in this *TIA1* study describing this gene as causative in ALS, it is not entirely clear how a p -value of 8.7×10^{-6} was obtained, given a test of six variant carriers among 1039 patients and 0 variants among 3036 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 $\sim 1.37 \times 10^{-4}$. Alternative aggregation tests such as SKAT, Firth logistic regression and Fisher's 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 $< 1.13 \times 10^{-4}$. 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).

When authors focus on gene discovery, they must consider multiple testing burden. If we assume adequate methodology, we interpret the described p -value for *TIA1* in the context of an exome-wide hypothesis-generating search, because *TIA1* can only be regarded as a candidate gene at this point. Therefore, as with all potential newly discovered variants, stringent multiple testing correction of at least 1.7×10^{-6} (0.05, Bonferroni corrected for 21,000 protein-coding genes and 9000 long non-coding RNA genes) (3) is required. However, if a variety of tests and variant subsets have been considered, $p < 5 \times 10^{-7}$ is the recommended threshold (5). This becomes an issue if test variation is performed solely within a specific domain, as was the case for *TIA1*. Depending on the definition of a domain the number of domains in the human

Table 1. Datasets available for replication.

	Method	Cases	Controls
Cirulli et al 2015. (6)	WES	2,869	6,405
Kenna et al. 2016 (7)	WES	1,022 (FALS)	7,315
Project MinE Consortium et al. (8)	WGS	4,389	1,846

WES: Whole Exome Sequencing; WGS: Whole Genome Sequencing; FALS: Familial ALS cases.

genome far exceeds the number of genes (9). Therefore, it can be argued that, in a discovery phase, an even more stringent multiple testing correction is needed. Otherwise, the reported *p*-value does not meet any of these multiple testing criteria.

Finally, to avoid erroneous attribution of causation, replication is required. Association analyses, such as the SKAT-O burden test, benefit from the largest number of cases and controls available to decrease the chances of false-positive results. There are at least three available ALS-specific case-control cohorts (Table 1) including thousands of cases and controls.

To test the validity of *TIA1* as a possible causative gene in ALS, we performed burden analysis on *TIA1* exon 11–13 in the Project MinE Consortium dataset (4389 cases and 1846 controls). Data acquisition and quality control has been described previously (8). Genic SKAT-O tests in the whole dataset are well-behaved (lambdaGC 1.005). We assumed the same filtering was performed as in the pedigree exomes that prioritized *TIA1* as the candidate gene. We extracted heterozygous variants that alter the amino acid sequence, that were seen two 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). We acknowledge that this burden test could still be underpowered and/or may not explicitly test the variants tested in the initial report implicating *TIA1*. The absence of evidence does not eliminate *TIA1* mutations as potentially associating with ALS. However, as is the case in all potentially causative genes, to increase power and provide a more definitive answer, we strongly encourage the combined analysis of all available data for the region of interest. Otherwise we are not providing an adequate service for our patients.

In conclusion, the extensive functional experiments performed by Mackenzie et al. 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. However, 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.

ORCID

Rick A. Van Der Spek  <http://orcid.org/0000-0001-9983-204X>

Sara L. Pulit  <http://orcid.org/0000-0002-2502-3669>

Matthieu Moisse  <http://orcid.org/0000-0001-8880-9311>

Adriano Chio  <http://orcid.org/0000-0001-9579-5341>

Pamela J. Shaw  <http://orcid.org/0000-0002-8925-2567>

Ammar Al-Chalabi  <http://orcid.org/0000-0002-4924-7712>

Jan H. Veldink  <http://orcid.org/0000-0001-5572-9657>

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