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High percentage of blood-based T-cell receptor gamma V9-JP recombinations associated with amyotrophic lateral sclerosis: extensive retention of the JP KKIK amino acid motif

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Abstract

Exome and RNAseq files prepared from blood samples can be mined for adaptive immune receptor recombinations and thus for the complementarity determining region-3 (CDR3) amino acid (AA) sequences, important for antigen binding. In this report, the T-cell receptor gamma (*TRG*) recombinations were mined from amyotrophic lateral sclerosis (ALS) blood sample exome and RNAseq files, mainly inspired by: (i) a high level of gamma-delta T-cells in Parkinson's disease and (ii) TRG CDR3 AA features associated with a higher Braak stage in Alzheimer's disease. Results indicated a high percentage of V9-JP recombinations from ALS blood sample genomics files, in comparison to *TRG* recombinations obtained from a large number of blood and other tissue samples not representing ALS. This result is discussed in the context of potential phospholipid sponging by adaptive immune receptors and potential impacts on membrane rigidity and amyloid development.

Keywords

Amyotrophic lateral sclerosis, T-cell receptor gamma, phospholipids, amyloid development

Multiple sclerosis (MS) has long been recognized as a neurological condition that develops due to anomalous function of the adaptive immune system, largely due to the connection of MS to specific human leukocyte antigen (HLA) alleles [1, 2]. Other neurological diseases have also been considered to be impacted by the immune system, but in the absence of clear and significant HLA allele linkages to disease development, the linkage of other neurological diseases to the anomalous function of the adaptive immune system has been limited. However, there is apparently no role for HLA antigen presenting molecules in the function of gamma-delta T-cells, and in the case of Parkinson's disease (PD), there has been a report of higher levels of gamma-delta T-cells [3]. Also, it has been recently reported that a higher percentage of positive charges, i.e., a higher isoelectric point, among T-cell receptor gamma (TRG) complementarity

determining region-3 (CDR3) amino acids (AAs) in Alzheimer's disease is associated with a higher Braak stage [4], representing more neurofibrillary tangles and a worse clinical situation. Thus, the possibility was considered that specific *TRG* recombination features could be associated with amyotrophic lateral sclerosis (ALS).

Whole exome sequencing (WXS) blood files representing ALS database of genotypes and phenotypes (dbGaP, phs000101) dataset were mined for the *TRG* recombination reads, the variable (V)- and joining (J)-gene segments on the reads were identified, and the intervening TRG CDR3 AA sequences were obtained. It is important to note that the algorithm used for the identification of each *TRG* recombination read requires a validated V and J on one read, thereby identifying the CDR3 to a very high confidence level [5–8]. In general, the application of this algorithm to WXS or RNAseq files does lead to a lower level of recovery of adaptive immune receptor recombination reads than other algorithms, not surprising given the relatively high standard of a verifiable V- and J-gene segments on each sequencing read [8].

The ALS *TRG* recombinations were compared to *TRG* recombinations obtained in the same way, using precisely the same algorithm, from other, non-ALS samples (Table 1). Also, the supporting online material (SOM) files (Tables S1–S15) include the complete data collection for the TRG results from mining the genomics files indicated in Table 1 (with the exception of the sequencing read, which is controlled access material). For all comparisons of the ALS *TRG* recombinations with the *TRG* recombinations represented by the other datasets, it was clear that there was a very high level of recombinations of the V9-JP gene segments among the ALS cases. The only exception to these disease comparisons and the result of V9-JP specificity for ALS were the *TRG* recombination reads obtained from the PD blood sample exome files (Table 1).

To further validate the observation of the comparatively high level of *TRG* V9-JP recombinations from ALS blood exome files, the *TRG* recombination, sequencing reads were mined from ALS blood RNAseq files (dbGaP, phs002055). The same algorithm was used to mine three RNAseq file datasets, with results indicating that the *TRG* V9-JP recombination read frequencies from these non-ALS datasets were significantly lower (Table 1).

The data in Table 1, discussed above, suffer from two limitations. First, the recovery of *TRG* recombination reads from the ALS blood exomes is relatively small. As noted above, these data are sufficient for a relatively high standard of statistical significance (Table 1), but there is no doubt that a much higher level of extraction of recombination reads, inevitable with future datasets with higher fold coverage, would be important for verification of the result reported here. Second, there was no opportunity to compare the recovery of *TRG* recombination reads from the RNAseq files representing ALS blood samples to the recovery of TRG recombination reads from RNAseq files representing blood samples of other conditions or controls (the non-ALS RNAseq files in Table 1 represent tumor samples). Again, blood to blood sample comparisons will almost certainly be possible in the future due to an increased emphasis on blood biomarkers discoverable via blood-based RNAseq files.

Despite the limitations of the above set of comparisons, it is difficult to ignore a proposal for the development of ALS, and likely other amyloid-based neurological diseases, whereby inflexible cellular membranes, in currently uncertain but possibly disease-specific cell types, facilitate amyloid development [9–11]. Thus, a reduction in, or an immobilization of cell membrane phospholipids could lead to a level of increased cell membrane stiffness that could in turn facilitate amyloid development and cellular dysfunction. And for several decades, there has been published evidence of the beneficial effect of dietary phospholipids in the setting of multiple amyloid-based, neurological diseases [12–16]. This scenario raises the question of whether certain biases in *TRG* V-J usage, that, for example, favor a juxtaposition of lysines or positive charges, as in the JP gene segment, could lead to the sponging of the negatively charged phospholipids, thereby increasing cell membrane rigidity. Anti-phospholipid antibodies, where positively charged arginines are important for phospholipid binding [17], are causative for anti-phospholipid antibody syndrome [18], a condition that, however, generally does not include symptoms related to ALS or any other amyloid-based, neurological disease, raising questions about the likelihood of TRG phospholipid

Table 1. *TRG* gene segment usage as indicated by the recovery of *TRG* recombination reads from exome or RNAseq files

Source of recombination reads	Disease	Blood or other tissue processed	Fraction V9 of all *TRG* recombination reads	Fraction JP of all *TRG* recombination reads	Fraction V9-JP recombination of all *TRG* recombination reads	Two-proportion test comparing V9-JP percentages, for ALS/PD	Fraction retention of JP KKIK motif	SOM file	Project
TRG recombination reads mined from exome files	ALS, familial	Blood	15/16	16/16	15/16	The data in preceding column of this row is used for below 2-proportion tests, left-side value in succeeding rows	16/16	Table S1	phs000101
	PD	Blood	33/37	37/37	33/37	The data of the preceding column of this row is used for below 2-proportion tests, right-side value in succeeding rows	35/37	Table S2	phs001172
	ESCA	Blood	23/136	5/136	5/136	< 0.0001/< 0.0001	5/5	Table S3	TCGA-ESCA
	LUSC	Blood	74/493	23/493	20/493	< 0.0001/< 0.0001	22/23	Table S4	TCGA-LUSC
	COAD	Blood	55/239	23/239	21/239	< 0.0001/< 0.0001	20/23	Table S5	TCGA-COAD
	STAD	Blood	102/449	41/449	30/449	< 0.0001/< 0.0001	40/41	Table S6	TCGA-STAD
	Melanoma	Blood	223/953	81/953	72/953	< 0.0001/< 0.0001	81/81	Table S7	TCGA-SKCM
	Multiple myeloma	Blood	1,007/9,081	223/9,081	134/9,081	< 0.0001/< 0.0001	207/223	Table S8	COMPASS-MMRF
	WT	Blood	113/620	19/620	11/620	< 0.0001/< 0.0001	17/19	Table S9	TARGET-WT
	NBL	Blood	283/725	138/725	129/725	< 0.0001/< 0.0001	135/138	Table S10	TARGET-NBL
TRG recombination reads mined from RNAseq files	ALS	Blood	11,381/29,170	6,189/29,170	6,078/29,170	The data of the preceding column in this row is used for the 2-proportion tests in succeeding rows	6,014/6,189	Table S11	phs002055
	WT	Tumor	28/65	11/65	11/65	NS/NA	11/11	Table S12	TARGET-WT
	DLBCL	Lymph node	2,169/9,911	253/9,911	211/9,911	< 0.0001/NA	233/253	Table S13	NCICCR-DLBCL
	NBL	Tumor	456/1,799	99/1,799	98/1,799	< 0.0001/NA	98/99	Table S14	TARGET-NBL

All 2-proportion test comparisons of ALS and PD are with non-neurological diseases, and the recovery of all *TRG* recombination reads are in the SOM Tables. Note, WXS results were compared to WXS results; RNAseq results were compared to RNAseq results (Table S15). Phs numbers can be accessed at dbGaP. The acronyms in the last column are defined here and at the genomic data commons. NS: not significant; NA: not applicable; TCGA: The Cancer Genome Atlas; ESCA: esophageal cancer; LUSC: lung squamous cell carcinoma; COAD: colon adenocarcinoma; STAD: stomach adenocarcinoma; SKCM: skin cutaneous melanoma; MMRF: Multiple Myeloma Research Foundation; TARGET: Therapeutically Applicable Research to Generate Effective Treatment; WT: Wilms tumor; NCICCR: National Cancer Institute Center for Cancer Research; DLBCL: diffuse large B-cell lymphoma; NBL: neuroblastoma

sponging as having a role in amyloid-based, neurological disease. However, questions could also be raised about the differences in the mechanism or even tissue residency for anti-phospholipid antibodies *versus* gamma-delta T-cells. This issue of specificity of effect also arises in consideration of other neurological diseases that may be traceable to amyloid development in turn, potentially, indirectly traceable to gamma-delta T-cell sponging of phospholipids. There are no data at all to speak to such specificity of pathological effect as it relates to gamma-delta T-cells, but depending on how future studies may support a role for gamma-delta T-cells, it may be that questions regarding specificity of the pathological effect will be addressed by examining varying gamma-delta T-cell effects on different cell or tissue types or varying effects traceable to subtle differences in gamma-delta receptor-phospholipid affinities.

Finally, regardless of the potential mechanistic role of gamma-delta T-cells, and the JP KKIK AA motif in ALS development, the data reported here raise the question of whether that data can be used for biomarker development to identify persons at risk for ALS or to monitor disease course and severity.

Abbreviations

AA: amino acid

ALS: amyotrophic lateral sclerosis

CDR3: complementarity determining region-3

dbGaP: database of genotypes and phenotypes

HLA: human leukocyte antigen

J: joining

PD: Parkinson's disease

SOM: supporting online material

TRG: T-cell receptor gamma

V: variable

WXS: whole exome sequencing

Supplementary materials

The supplementary Tables for this article are available at: https://www.explorationpub.com/uploads/Article/file/1003124_sup_1.xlsx and https://www.explorationpub.com/uploads/Article/file/1003124_sup_2.pdf.

Declarations

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Author contributions

GB: Conceptualization, Supervision, Formal analysis, Methodology, Visualization, Writing—review & editing. TIH: Conceptualization, Formal analysis, Methodology, Software. KJC: Formal analysis, Methodology. GA: Formal analysis, Methodology, Writing—review & editing. JJS: Formal analysis, Methodology, Software.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The dataset genomics files were accessed according dbGaP approvals listed for George Blanck (according to the dataset identifying information in Table 1) at https://www.ncbi.nlm.nih.gov/gap/. Other data are included in the manuscript and the supplementary files.

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