Common neurodegeneration-associated proteins are physiologically expressed by antigen-presenting cells and are interconnected via the inflammation/autophagy-related proteins TRAF6 and SQSTM1

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ABSTRACT

There is circumstantial evidence that, under neurodegenerative conditions, peptides deriving from aggregated or misfolded brain proteins elicit adaptive immune responses. On another hand, several genes involved in familial forms of neurodegenerative diseases were found to exert key innate immune functions. However, whether or not such observations are causally linked remains unknown. To start addressing this issue, we followed a systems biology strategy based on the mining of large proteomics, genomics and immunopeptidomics databases. First, we retrieved at the mRNA and protein levels the expression patterns of common neurodegeneration-associated genes in two professional antigen-presenting cells, namely B-cells and dendritic cells. Surprisingly, we found that under physiological conditions, numerous neurodegeneration-associated proteins are abundantly expressed by human B-cells. Moreover, a survey of the human proteome allowed us to map a unique protein-protein interaction network linking common neurodegeneration-associated proteins and their first shell interactors in human B-cells. Network connectivity analysis identified two major hubs that both relate with inflammation and autophagy: the neurodegeneration-associated protein SQSTM1 (Sequestosome-1) and the signaling molecule TRAF6 (TNF Receptor Associated Factor 6). Interestingly, the mapped network also comprised two hub proteins involved in both inflammation and autoimmunity: HSPA8 (Heat Shock Protein Family A Member 8 also known as HSC70) and HSP90AA1 (Heat Shock Protein 90 Alpha Family Class A Member 1). Finally, we explored the Immune Epitope Database (IEDB-AR) and found that a large share of neurodegeneration-associated proteins provide endogenous MHC class II-binding peptides in human B-cells. For several neurodegeneration-associated proteins such as amyloid beta A4 protein, sequestosome-1 and profilin-1, the reported MHC class II restrictions of derived peptides covered multiple MHC class II alleles. In contrast, a limited set of neurodegeneration-

associated proteins appeared to provide B-cell endogenous peptides whose binding properties are restricted to specific MHC class II alleles. This is notably the case for microtubule-associated protein tau, presenilin 2 and serine/threonine-protein kinase TBK1 from which derived peptides were exclusively reported to bind HLA-DR15, a recently-identified susceptibility gene for late onset Alzheimer's disease. Overall, our work indicates that immunization against neurodegeneration-associated proteins might be a physiological process which is shaped, at least in part, by B-cells.

INTRODUCTION

Multiple studies have now established that neurodegenerative disorders are not cell-autonomous. The pathophysiological processes leading to neurodegeneration involve and target not only neurons but also glial cells, including astrocytes, microglia and oligodendrocytes (1–3). Moreover, beyond central nervous system (CNS) cells, the adaptive immune system has emerged as a potentially important player. When considering only T-cell responses, T-cell reactivity against amyloid beta peptides was already reported more than a decade ago in both patients suffering from Alzheimer's disease (AD) and aged healthy subjects (4). Recent works further demonstrated that alpha-synuclein (SNCA)-derived peptides elicit helper and cytotoxic T-cell responses in a subgroup of Parkinson's disease (PD) patients harboring specific major histocompatibility complex (MHC) alleles (5,6). Similarly, in animal models of neurodegenerative disorders, T-cell responses against neurodegeneration-associated peptides were also demonstrated. In particular, in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model of PD, pathogenic brain-infiltrating T-cells directed against SNCA were found to target the substantia nigra of diseased mice (7,8). Along this line, in a mouse model of tauopathy, CD8 cytotoxic T-cells directed against tau protein were shown to infiltrate the hippocampus and to drive cognitive alterations (9). Finally, besides AD and PD, several studies provided evidence that T-cells are activated during the course of Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and/or their animal models (10–13). Nevertheless, the putative autoantigens targeted under these conditions remain unknown.

Extending the functional results mentioned above, genome-wide association studies (GWAS) have unraveled the central position of autoimmunity-related genes in the genetic susceptibility landscape of neurodegenerative diseases (14–19). This is notably the case for HLA-DRB1 alleles which were recently demonstrated to confer increased risks of developing

PD (15) or late-onset AD (14). Further supporting the hypothesis of an autoimmune/neurodegeneration pathway, two genes involved in familial forms of PD, namely PINK1 (PTEN-induced putative kinase 1) and PRKN (Parkin RBR E3 Ubiquitin Protein Ligase also named PARK2 or Parkin) were shown to regulate the presentation of mitochondria-derived antigens in the context of MHC class I molecules (20). Finally, innate immune functions were demonstrated for several genes causatively-linked to familial forms of PD or ALS. These comprise C9ORF72 (21–23), LRRK2 (Leucine-rich repeat kinase 2) (24–27), GRN (Granulin Precursor) (28,29) as well as PRKN and PINK1 (30–34).

However, advocating for the role of autoimmunity in neurodegenerative disorders requires yet addressing several important issues. In particular, it appears crucial to determine how T-cells recognizing neurodegeneration-associated antigens may be primed in the periphery. The extent to which autoimmunity against neurodegeneration-associated antigens might be a physiological event needs also to be assessed. Last but not least, a global view on the expression of neurodegeneration-associated proteins by professional antigen-presenting cells (APCs) is still lacking. In an attempt to address these issues, we used here a systems biology approach embracing a large range of previously-published experimental data. We notably explored in normal human APCs the expression patterns and protein-protein interaction profiles of the most common neurodegeneration-associated proteins. Overall, our results indicate that in human B-cells, a large majority of neurodegeneration-associated proteins are expressed at the mRNA and protein levels and form a complex network centered by the inflammation/autophagy-related molecules SQSTM1 (Sequestosome-1) and TRAF6 (TNF Receptor Associated Factor 6). In addition, analysis of MHC class II immunopeptidome databases provides evidence that neurodegeneration-associated proteins expressed by

human B-cells are a source of endogenous peptides which are presented in the context of HLA class II molecules.

MATERIALS AND METHODS

Workflow

A scheme summarizing the workflow followed in the present work is shown in Figure 1.

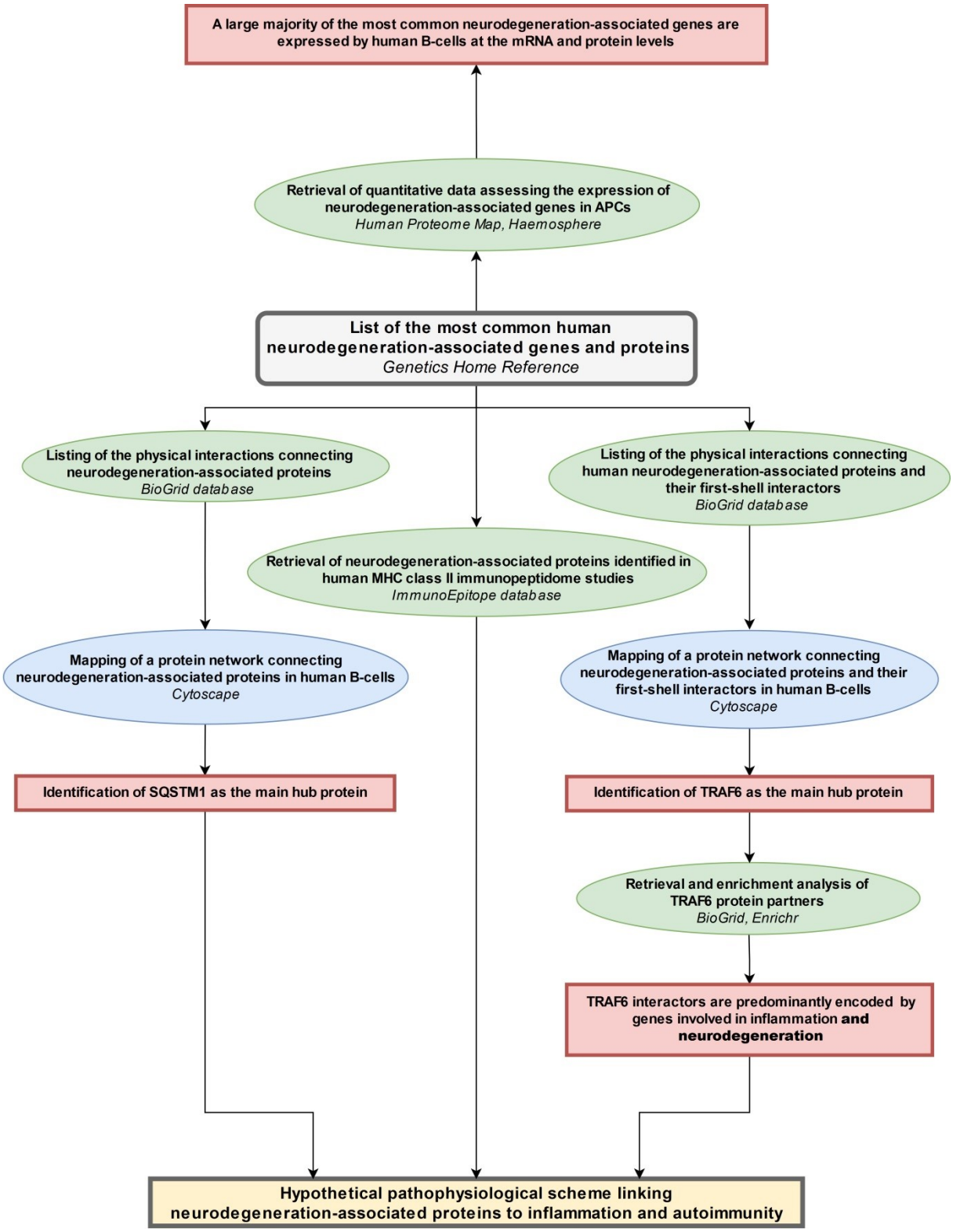


Figure 1: Workflow of the sudy. The workflow starts from the upper central grey rectangle. Other rectangles (yellow or red) frame the main results obtained following each of the analytical steps which are briefly described in ellipse shapes (green or blue). Terms in italics correspond to the name of the bioinformatics tools used for each analytical step. MHC: major histocompatibility complex; APCs: antigen-presenting cells.

Data mining and bioinformatics tools

All the bioinformatics analyses were performed at least 3 times between March 2018 and June 2019. Bioinformatic tools and corresponding tasks performed in this study are described below.

- The Genetics Home Reference website (35), is a regularly updated consumer health resource from the National Library of Medicine. It provides information to the general public about the effects of genetic variation on human health. In the present paper, we used the "Genetics Home Reference" website to select without *a priori* the most common genetic variations/alterations that are linked to the following neurodegenerative disorders: Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, fronto-temporal dementia (FTD).

- The enrichment web platform Enrichr (36) perform enrichments analyses on queried lists of genes. The Enrichr website allows surveying simultaneously 132 libraries gathering 245,575 terms and their associated lists of genes or proteins. Enrichment analysis tools provided by the Enrichr bioinformatics platform provides adjusted P-values computed from the Fisher's exact test, Z-scores assessing deviation from an expected randomly obtained rank of p-values, and combined scores which are computed from the Z-scores and adjusted P-values. We focused our analysis on the "Jensen DISEASES" ontology library (37) which is based exclusively on text-mining and allow determining whether a list of genes is significantly associated with terms depicting specific diseases.

- The BioGrid database (38) compiles 29 169 experimentally-proven protein-protein interactions connecting 23 200 human proteins. Querying the BioGrid database allows retrieving for any given human protein the current list (update in 2019) of experimentally-established and published human protein partners.

- The Haemosphere database (39) compiles RNA-seq data obtained in purified populations of peripheral blood mononuclear cells derived from healthy human subjects. Two independent studies may be surveyed via the “Heamosphere” database: i) the “Haemopedia” study (39) in which normalized expression data were obtained from 42 samples in 12 immune cell types across 7 lineages and ii) the Linsley study (40) in which normalized expression data were obtained from 24 samples in 6 immune cell types across 5 lineages. We focused our initial searches on the “Haemopedia” dataset as, contrary to the “Linsley” dataset, it includes results obtained in the dendritic cell lineage. However, the “Linsley” dataset was used to perform confirmatory investigations on the B-cell lineage. Regarding the “Haemopedia” dataset which can be queried via the “Haemosphere” server, mean values obtained for the B-cell lineage were calculated from pooled data obtained by the analysis of naïve or memory B-cells. For the DC lineage, mean values were calculated from pooled data obtained by the analysis of myeloid DCs, myeloid CD123+ DCs or plasmacytoid DCs.

- The Human Proteome Map database (41) compiles protein expression data obtained by mass spectrometry from human normal tissues and cells. A total of 85 samples were analyzed and the protein profiles of 6 blood-circulating cell types were established.

- The Immune Epitope Database and Analysis Resource (IEDB-AR) (42) compiles experimental data on antibody and T-cell epitopes in humans, non-human primates and other animal species. Our search strategy followed 3 steps described below.

Step 1: retrieval of a list of human MHC class II binding peptides and their parent proteins

- in the "Epitope" tab, the "Any Epitopes" item was marked
- in the "Assay" tab, the "MHC Ligand Assays" item was marked
- in the "Antigen" tab, "Homo sapiens" was entered in the "Organism" line

- in the "MHC restriction" tab, the "MHC class II" item was marked

- in the "Host" tab, the "Humans" item was marked

- in the "Disease" tab, the "Any Disease" item was marked.

Search was then launched and, from the results page, the "Assays" tab was selected and a list of currently known ligands (peptides and parent proteins) of human MHC molecules was retrieved.

Step 2: filtering the results

We retained only results which were both i) obtained by mass spectrometry analysis of peptides eluted from MHC class II molecules and ii) generated from cells of the B-cell lineage, in the absence of immunization or stimulation protocols.

Step 3: checking the reference IDs associated to each identified parent protein

For each selected study and/or set of results, the reference ID associated to each parent protein was checked on the UniProt website (43) in order to avoid redundant or obsolete IDs.

- The Cytoscape software (44) is an open source software allowing to visualize complex networks. We used the function "Network analysis" to identify hubs as determined by the number of degrees (connected objects) of each object in the generated networks.

Statistics

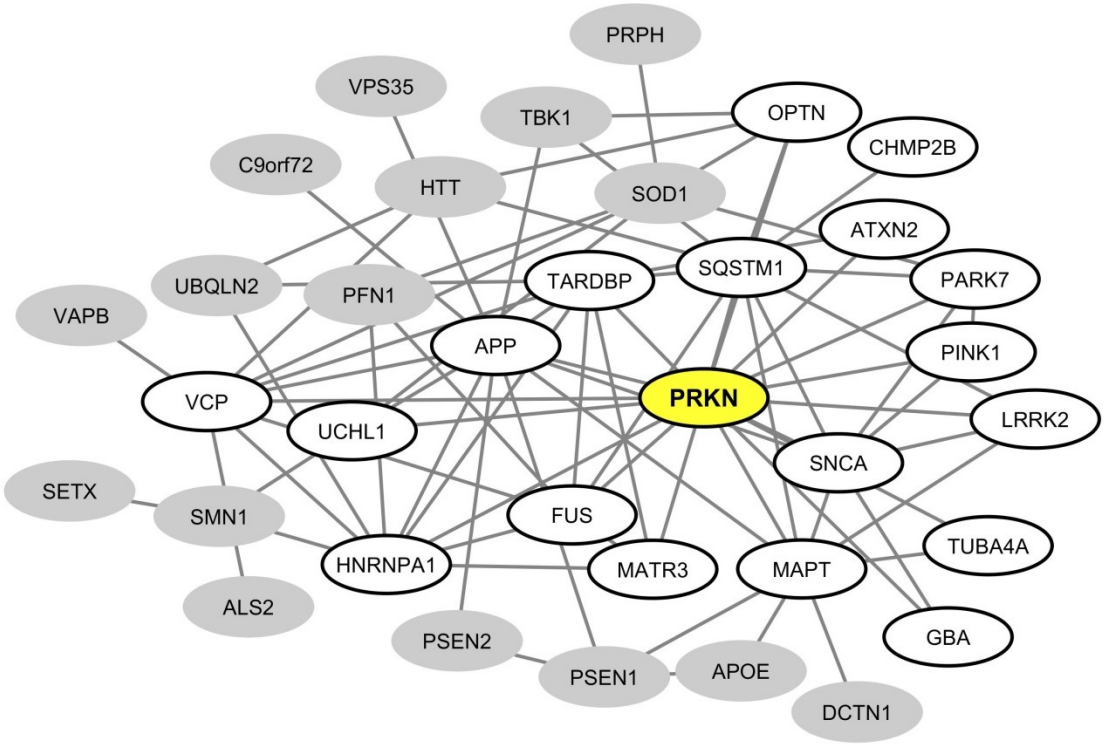
When not embedded in bioinformatics webtools, statistics for enrichment analyses were performed using the Fisher exact test. In particular, we assessed for several identified hub proteins whether their retrieved list of partners (obtained via the BioGrid database) was statistically enriched in neurodegeneration-associated proteins. To calculate enrichment factors, we set the expected reference ratio as 0.002 which corresponds to the number of

common neurodegeneration-associated proteins (i.e 48 according to the "Home Genetics Reference" website) over the number of coding genes for which interactors can be retrieved from the BioGrid database (i.e 23098). The obtained p-values were then adjusted using Bonferroni correction. A similar approach using the Fisher exact test was used to determine whether parent proteins from which derive human MHC class II-binding peptides are significantly enriched in neurodegeneration-associated proteins.

RESULTS

1- Neurodegeneration-associated proteins form a unique interaction network in which PRKN is the main hub

From the most recently updated version of the human proteome, compiled in the "BioGrid" database, we extracted the currently published and experimentally-proven protein-protein interactions connecting the most common neurodegeneration-associated proteins. Self-interactions were excluded from our analysis. From these retrieved interactions we were able to build and visualize a protein network which encompasses 35 (72%) of the 48 most common neurodegeneration-associated proteins (Figure 2). Interestingly, network analysis showed that PRKN harbored the highest number of interacting partners (n=17). Moreover, when considering the whole list of known PRKN protein partners, the calculated enrichment factor in neurodegeneration associated protein reached 18.97 and was highly significant (Fisher exact test p-value: $3.35E^{-15}$). This result points to a yet unnoticed property of PRKN as a major hub protein connecting a large array of proteins involved in not only PD but also ALS, AD, Huntington's disease and/or FTD.

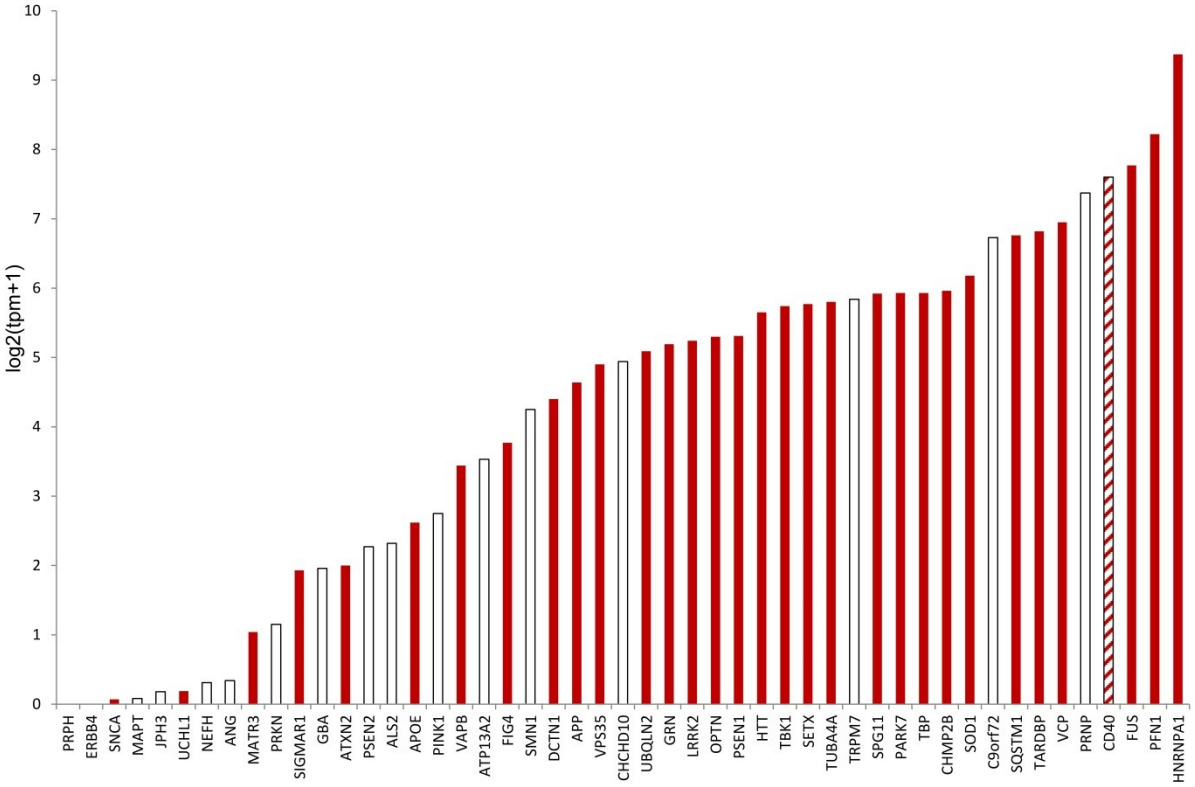


<u>Figure 2: Mapping of the protein-protein interaction network linking common neurodegeneration-associated proteins irrespective of the cell type considered.</u> A survey of the human proteome was performed by querying the protein-protein interaction database BioGRID (38). Each node represents a protein indicated by its protein symbol and each edge represents an experimentally-demonstrated protein-protein interaction. In this network, the "PRKN" (Parkin) node, highlighted in yellow, exhibits the highest degree (i.e the highest number of direct interactors). White nodes correspond to the first-shell partners of PRKN. Grey nodes are not direct interactors of PRKN.

2- A majority of common neurodegeneration-associated genes are expressed by human B-cells at the mRNA and protein levels

To determine whether genes coding for neurodegeneration-associated proteins are expressed by APCs under physiological conditions, we queried the "Haemosphere" database which compiles normalized RNA-seq data obtained from the analysis of pure subpopulations of peripheral blood mononuclear cells (39). In the "Haemosphere" database, we first surveyed the "Haemopedia" dataset (39) in which normalized RNA-seq expression data were obtained from 42 samples in 12 immune cell types across 7 lineages. Our search was restricted to 2 cell lineages which harbor demonstrated antigen-presenting functions: B-cells (45–47) and

dendritic cells (48–50). For these two cell lineages, mean values were retrieved as described in the Material and Methods section of the present paper. As a confirmatory investigation, we explored the “Linsley” dataset in which normalized expression data were obtained from 24 samples in 6 immune cell types across 5 lineages including B-cells but not dendritic cells (40). When possible, i.e for B-cells but not for dendritic cells, we checked that the retrieved values obtained from the “Haemopedia” dataset correlated with those obtained from the “Linsley” dataset (data not shown). In parallel, for B-cells, we retrieved quantitative proteomics data obtained by mass spectrometry analysis and compiled in the “Human Proteome Map” database (41). Since DCs are not covered by the “Human Proteome Map” database, we also explored results from a recent study assessing by mass spectrometry the proteomic profile of distinct human blood-circulating DC subsets (namely myeloid DCs, BDCA3+ DCs, plasmacytoid DCs and CD16+ DCs) (51). From these databases, we retrieved expression data obtained at the mRNA and protein levels for the 48 most common neurodegeneration-associated genes (Figure 3 and 4). As shown in Figure 3, It is striking that 32 (66%) of those 48 genes are detectable at the mRNA and protein levels in B-cells (Figure 3). Since mass spectrometry is a relatively poorly sensitive technique, one may consider that detected proteins are abundantly expressed.



<u>Figure 3: Expression pattern of common neurodegeneration-associated genes and proteins in human B-cells.</u> A survey of the ”Haemosphere” (39) and ‘Human Proteome Map” (41) databases was performed. The x-axis shows the protein symbols of the explored proteins and corresponding genes. CD40 (hatched red bar), a recognized B-cell marker, was assessed as a reference positive control. For each explored gene, mean expression values obtained by RNA-seq analysis are expressed in log2(tpm+1) with tpm meaning “Transcripts Per Kilobase Million”. Red bars (plain or hatched) indicate that the corresponding protein is detectable by mass spectrometry in human B-cells. White bars indicate that the corresponding protein is not detectable by mass spectrometry in human B-cells.

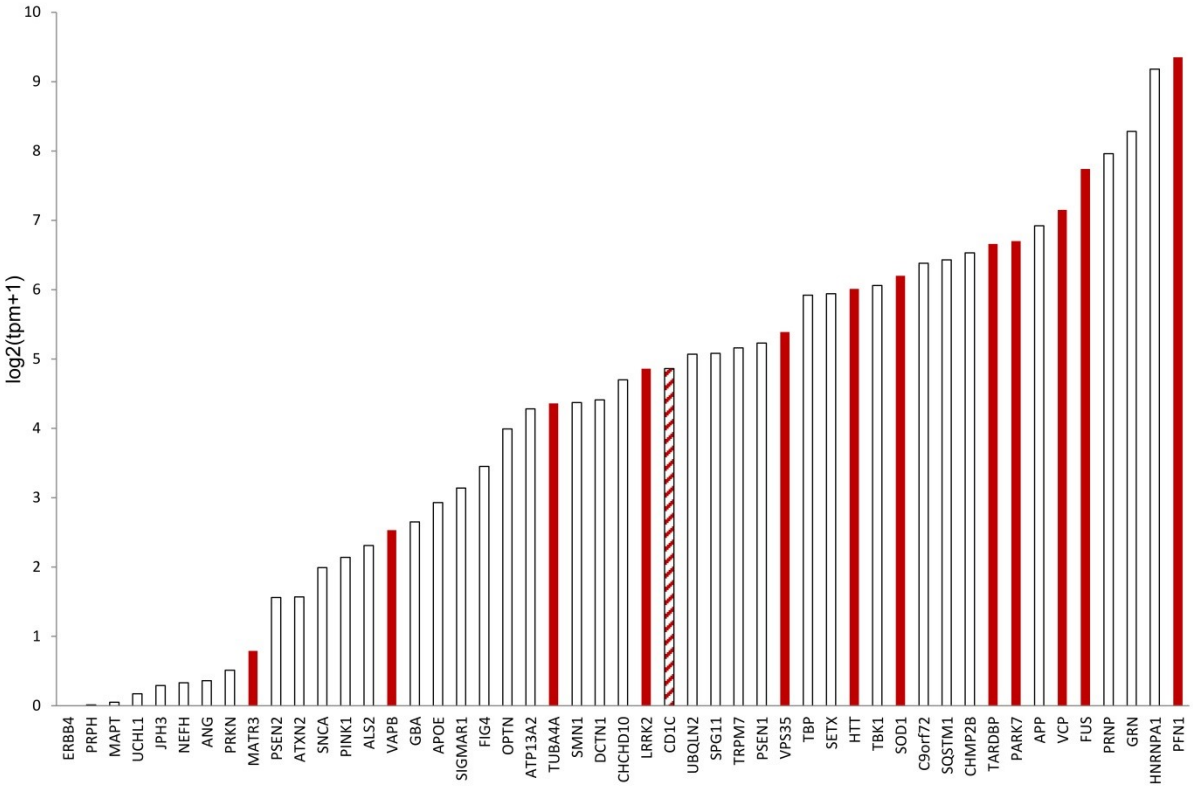
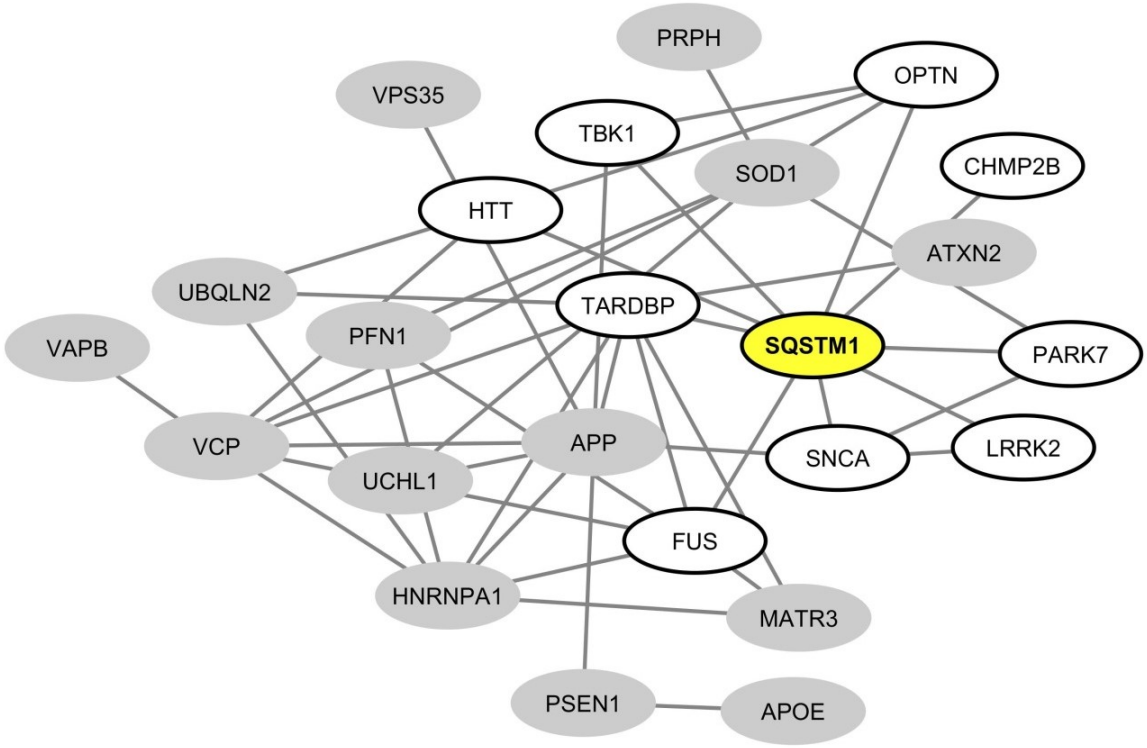


Figure 4: Expression pattern of common neurodegeneration-associated genes and proteins in human dendritic cells. The ”Haemosphere” database (39) and a proteomics dataset obtained from the analysis of human dendritic cells (51) were surveyed. The x-axis shows the protein symbols of the explored proteins and corresponding genes. CD1c (hatched red bar), a recognized dendritic cell marker, was assessed as a reference positive control. For each explored gene, mean expression values obtained by RNA-seq analysis are expressed in log2(tpm+1) with tpm meaning “Transcripts Per Kilobase Million”. Red bars (plain or hatched) indicate that the corresponding protein is detectable by mass spectrometry in human dendritic cells. White bars indicate that the corresponding protein is not detectable by mass spectrometry in human dendritic cells.

However, in both human B-cells and DCs, PRKN was not detected at the protein level by mass spectrometry. This finding urged us to identify the main hub protein(s) which, in B-cells, may interconnect abundantly-expressed neurodegeneration-associated proteins.

3- Neurodegeneration-associated proteins expressed by human B-cells form a unique interaction network in which the inflammation/autophagy-related protein SQSTM1 is the main hub

From the interaction network depicted in Figure 2 we only retained nodes corresponding to neurodegeneration-associated genes that are expressed at the mRNA level and detectable by mass spectrometry in human B-cells. We observed that in human B-cells, 24 neurodegeneration-associated proteins are predicted to form a tight interaction network which is centered by the inflammation/autophagy-related protein SQSTM1.



<u>Figure 5: Mapping of the protein-protein interaction network linking common neurodegeneration-associated proteins in human B-cells.</u> A survey of the human proteome was performed by querying the protein-protein interaction database BioGRID (38). Each node represents a protein indicated by its protein symbol and each edge represents an experimentally-demonstrated protein-protein interaction. In this network, the "SQSTM1" (Sequestosome-1) node, highlighted in yellow, exhibits the highest degree (i.e the highest number of direct interactors). White nodes correspond to the first-shell partners of SQSTM1 in human B-cells. Grey nodes are expressed by B-cells but are not direct interactors of SQSTM1.

3- The protein network formed by neurodegeneration-associated proteins connects with a set of first shell interactors among which the B-cell inflammation/autophagy-related protein TRAF6 is the main hub

To extend these data, we then retrieved from the "BioGrid" database the currently known and experimentally-demonstrated interactors of the most common neurodegeneration-associated proteins, irrespective of their levels of expression in human B-cells. From this list, we extracted candidate hub proteins interacting with more than 10 neurodegeneration-associated proteins. For each of these candidate hubs, total lists of protein interactors were then retrieved from BioGrid and a Fisher exact test was applied so to determine if neurodegeneration-associated proteins were actually significantly enriched in each established list. By this mean, we were able to assign an enrichment factor and an associated p-value to each candidate hub. This approach allowed us to identify 20 hub proteins (Table 1) with which common neurodegeneration-associated proteins are specifically connected. Among these 20 hub proteins, 11 are expressed by human B-cells at the mRNA and protein levels as assessed via the "Haemosphere" and the "Human Proteome Map" databases. Moreover, from the 10 hub proteins exhibiting the most significant and highest enrichment factors, 8 are abundantly expressed by human B-cells. These include: i) the heat shock proteins HSPA8 (also named HSC70), HSPA4 (also named HSP70) and HSP90AA1 (also named HSP90) which are all involved in both antigen presentation by MHC class II molecules (52–54) and the modulation of T-cell responses (55–57) and ii) YWHAZ and YWHAQ proteins also known as 14-3-3 protein zeta and theta respectively, which bind MHC class II molecules (58,59) and are implicated in various neurodegenerative diseases (60–62). Finally, unexpectedly, the most significant hub we identified was TRAF6 (TNF receptor associated factor 6), an inflammation/autoimmunity-related molecule (63,64) playing a major role in the control of B-

cell activation (65,66). Indeed, the whole list of TRAF6 interactors comprises 17 neurodegeneration-associated proteins which correspond to an enrichment factor of 27.68 and an adjusted p-value of $1.57E^{-16}$.

Table 1

	protein partners	Neurodegeneration-associated protein partners	enrichment factor	adjusted p-value
TRAF6	307	17	27.68	1.57E-16
PRKN	448	17	18.97	7.72E-14
HSPA4	363	13	17.90	1.82E-10
SQSTM1	294	12	20.40	2.33E-10
HSPA8	708	16	11.29	9.43E-10
YWHAZ	412	12	14.56	1.08E-08
HSP90AA1	839	15	8.93	7.65E-08
YWHAQ	476	11	11.55	5.43E-07
CYLD	620	12	9.67	9.38E-07
CTNNB1	616	11	8.92	6.90E-06
UBC	1050	14	6.66	7.52E-06
TP53	1062	14	6.59	8.58E-06
CUL7	658	11	8.35	1.30E-05
EGFR	1224	14	5.71	4.29E-05
RNF4	1251	14	5.59	5.47E-05
MCM2	940	11	5.85	3.55E-04
BRCA1	962	11	5.71	4.37E-04
NTRK1	1948	16	4.10	5.22E-04
CUL3	1227	13	5.29	8.32E-04
ESR2	2249	13	2.89	4.12E-03

Legend: A survey of the human proteome was performed by querying the protein-protein interaction database BioGRID (38). Candidate hub proteins interacting with more than 10 common neurodegeneration-associated proteins were retrieved. The lists of partners currently demonstrated for each of these candidate hubs were then retrieved. For each of these lists, an enrichment factor in neurodegeneration-associated proteins and an adjusted p-value were calculated as described in the Materials and Methods section. From left to right, the protein symbols of the identified hub proteins are shown in the first column, the corresponding total numbers of partners and total numbers of neurodegeneration-associated protein interactors are shown in the second and third column. The corresponding enrichment factors and adjusted p-values are shown in the last two columns. Proteins that are detectable by mass spectrometry in human B-cells, according to the "Human Proteome Map" database (41), are highlighted in yellow. The corresponding mRNAs are all detectable by RNAseq in B-cells according to the "Haemosphere" database (39).

However, one may argue that the list of currently-known TRAF6 partners might be enriched in not only neurodegeneration-associated proteins but also other sets of proteins which do not relate with neurodegeneration. To address this issue, we performed on the whole list of TRAF6 partners a non *a priori* enrichment analysis using the "JENSEN Disease" text-mining webtool (37) embedded in the "Enrichr" analysis platform (36). Results shown in Table 2 indicate that the 5 disease-related text-mining terms with which TRAF6 partners are the most significantly associated comprise the terms "Frontotemporal dementia" and "Neurodegenerative disease". This finding points to a specific link between TRAF6 and neurodegeneration.

Table 2

Disease Enrichment			
Term	Adjusted p-value	Z-score	Combined score
Hypohidrotic ectodermal dysplasia	7.38e-7	-2.53	46.48
Mulibrey nanism	7.38e-7	-1.64	30.18
Frontotemporal dementia	7.21e-7	-1.49	28.21
Neurodegenerative disease	7.21e-7	-1.48	28.04
Toxic encephalopathy	7.38e-7	-1.42	26.41

Legend: A survey of the human proteome was performed by querying the protein-protein interaction database BioGRID (38). The list of currently known and experimentally-demonstrated TRAF6 partners was retrieved. From this list of interactors, an enrichment analysis with the "JENSEN Disease" text-mining webtool (37) embedded in the "Enrichr" analysis platform (36) was then performed. The 5 most significant terms associated with the list of TRAF6 partners are shown. Relevant terms are highlighted in yellow.

We then built and visualized a B-cell protein-protein interaction network encompassing the most significant hub proteins and their neurodegeneration-associated interactors expressed in B-cells (Figure 5). We observed that from the 32 neurodegeneration-associated proteins expressed by B-cells, 22 (68%) are first shell interactors of HSPA4, HSPA8, TRAF6 or SQSTM1. These results unravel a yet unknown function of these molecules as major hub proteins

connecting in B-cells a large array of proteins involved in PD, ALS, AD, Huntington's disease and/or FTD.

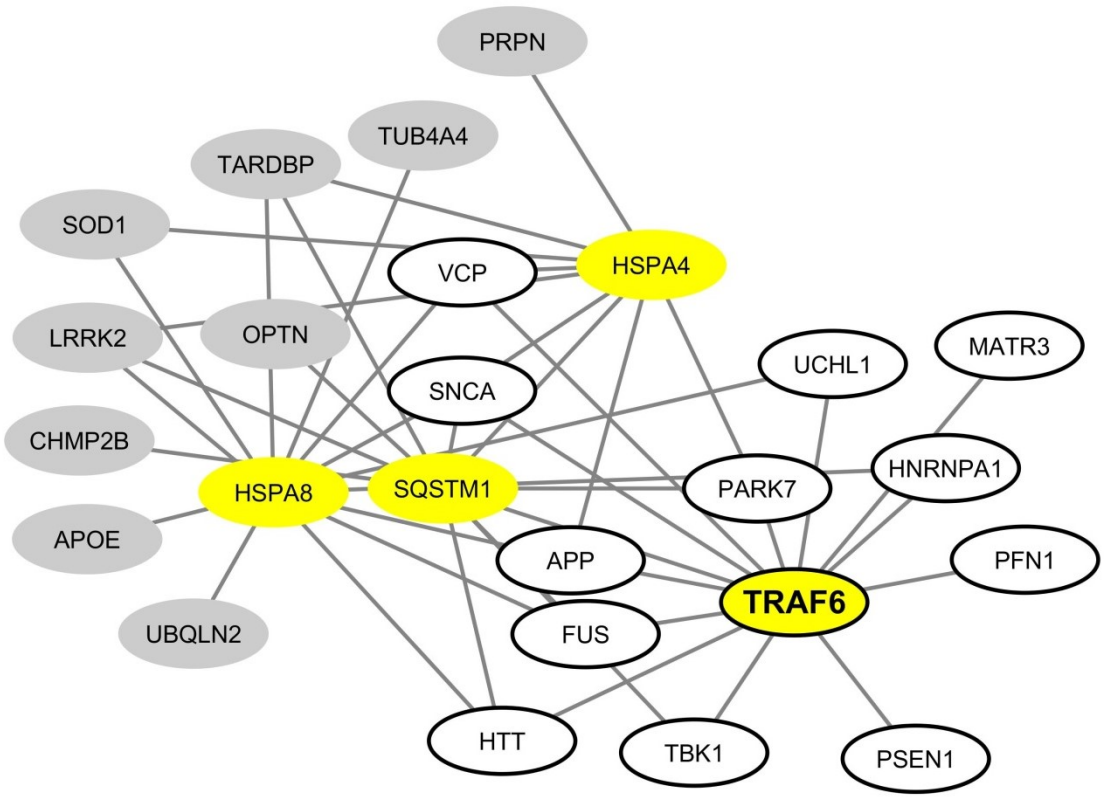


Figure 6: Mapping of the protein-protein interaction network linking hub protein partners and common neurodegeneration-associated proteins in human B-cells. A survey of the human proteome was performed by querying the protein-protein interaction database BioGRID (38). Each node represents a protein indicated by its protein symbol and each edge represents an experimentally-demonstrated protein-protein interaction. Only proteins expressed by human B-cells according to the "Human Proteome Map" database (41) were taken into account. Expression of the corresponding mRNAS in human B-cells was checked on the "Haemosphere" database (39). The 4 hub proteins expressed by B-cells and whose partners exhibited the most significant and highest enrichment factors in neurodegeneration-associated proteins are highlighted in yellow. For each of these hubs, the interacting neurodegeneration-associated proteins expressed by B-cells are shown in grey nodes or in white nodes (for TRAF6 interactors only).

4- Neurodegeneration-associated proteins provide a source of endogenous MHC class II ligands in human B-cells

While peptides presented by MHC class II molecules are classically generated by the proteolysis of phagocytized exogenous antigens, the presentation of endogenous peptides by

MHC class II molecules is an alternate pathway which has been robustly documented (67–70). We thus surveyed the Immune Epitope DataBase (IEDB-AR) (42) to assess whether peptides derived from neurodegeneration-associated proteins had been previously identified as binding MHC class II molecules in immunopeptidome studies which used human cells of the B-cell lineage as a source of endogenous peptides. Of note, we excluded studies assessing the MHC binding of exogenously-provided specific peptides and retained only works relying on the systematic mass spectrometry-based identification of peptides eluted from MHC class II molecules. In addition, we excluded experiments in which immunization or stimulation protocols were applied to B-cells. On this basis, we retained 19 studies (data supplement 1) which were performed on cells of the B-cell lineage including predominantly Epstein-Barr virus (EBV)-transformed B-cells. When screening these studies, we found that 22 out of 48 common neurodegeneration-associated proteins were reported to provide endogenous MHC class II-binding peptides in B-cells (Table 3 and data supplement 1). The most frequently identified neurodegeneration-associated parent proteins are abundantly expressed in B-cells and comprise notably: i) the AD-related proteins APP and PSEN1, ii) the ALS/FTD-related proteins PFN1, SQSTM1, GRN, SOD1 and VCP and iii) the PD-related protein PARK7 (Figure 1).

Table 3

Neurodegeneration-associated proteins	Reported MHC class II restrictions
Amyloid beta A4 protein (*APP*)	HLA class II, HLA-DQ, HLA-DR, HLA-DR3, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2, HLA-DRB1*15:01 (HLA-DR15), HLA-DRB5*01:01 (HLA-DR51), HLA-DRB1*04:05 (HLA-DR4), HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5)
Sequestosome-1 (*SQSTM1*)	HLA class II, HLA-DR, HLA-DQ,

	HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2, HLA-DR15/DR51, HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5)
Profilin-1 (*PFN1*)	HLA class II, HLA-DR, HLA-DQ, HLA-DR1, HLA-DR3, HLA-DRB1*04:05 (HLA-DR4), HLA-DR15/DR51
Superoxide dismutase [Cu-Zn] (*SOD1*)	HLA class II, HLA-DR, HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2
Transitional endoplasmic reticulum ATPase (*VCP*)	HLA class II, HLA-DR, HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2
Presenilin-1 (*PSEN1*)	HLA class II, HLA-DQ, HLA-DQ2.2, HLA-DR15/DR51, HLA-DRB1*04:05 (HLA-DR4)
Ubiquilin-2 (*UBQLN2*)	HLA class II, HLA-DR1, HLA-DQ7.5, HLA-DQ2.2, HLA-DRB1*04:05 (HLA-DR4)
Major prion protein (*PRNP*)	HLA-DQ, HLA-DR, HLA-DR1, HLA-DQ7.5, HLA-DQ2.2
Vacuolar protein sorting-associated protein 35 (*VPS35*)	HLA class II, HLA-DR , HLA-DR15/DR51, HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5)
Optineurin (*OPTN*)	HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2
Protein deglycase DJ-1 (*PARK7*)	HLA class II, HLA-DR1, HLA-DRB1*04:01 (HLA-DR4), HLA-DRB1*04:05 (HLA-DR4)
Progranulin (*GRN*)	HLA class II, HLA-DQ, HLA-DQ7.5
Spatacsin (*SPG11*)	HLA-DQ7.5, HLA-DQ2.2
Ubiquitin carboxyl-terminal hydrolase isozyme L1 (*UCHL1*)	HLA class II, HLA-DR
RNA-binding protein FUS (*FUS*)	HLA class II
Probable cation-transporting ATPase 13A2 (*ATP13A2*)	HLA-DR
Heterogeneous nuclear ribonucleoprotein A1 (*HNRNPA1*)	HLA class II
Charged multivesicular body protein 2b (*CHMP2B*)	HLA-DQ7.5
Huntingtin (*HTT*)	HLA-DR
Serine/threonine-protein kinase TBK1 (*TBK1*)	HLA-DR15/DR51
Presenilin-2 (*PSEN2*)	HLA-DR15/DR51

Microtubule-associated protein tau (*MAPT*)	HLA-DR15/DR51

Legend: A survey of the immune epitope database (IEDB-AR) (42) was performed. Only parent protein names and corresponding from which endogenous peptides eluted from HLA class II molecules were identified by mass spectrometry were taken into account. Only results obtained on human cells of the B-cell lineage in the absence of immunization or stimulation protocols were taken into account. The retrieved list of parent proteins associated to the reported MHC class II restriction of derived peptides was then crossed with the list of 48 common neurodegeneration-associated proteins we established. The protein names and corresponding gene symbols are shown in the left column; the corresponding reported MHC class II restrictions of derived peptides are shown in the right column. When needed, aliases frequently used in the nomenclature HLA class II alleles are indicated in brackets.

Finally, from the 19 studies we retained, the whole list of parent proteins identified as providing MHC class II-binding endogenous peptides in B-cells was retrieved and an enrichment analysis was performed to determine whether such a list was significantly enriched in neurodegeneration-associated proteins. We found that neurodegeneration-associated proteins encompassed 0.6% (22 out of 3588) of such parent proteins, which corresponds to an enrichment of 2.54 (p-value = 0.0006, Fisher exact test) when considering the whole number of human protein-coding genes as roughly 20 000 (71). Such an enrichment factor reached 5.34 (p-value = 0.0003, Fisher exact test) when considering only parent proteins of peptides eluted from HLA-DR15 molecules. This is of particular interest since HLA-DR15 haplotype was recently identified as a risk factor for sporadic forms of late onset AD (14). Moreover, based on the IEDB-AR survey we performed, HLA-DR15 molecules are the only HLA class II alleles reported to bind B-cell endogenous peptides deriving from Microtubule-associated protein tau, Presenilin-2 or Serine/threonine-protein kinase TBK1 (Table 3 and data supplement 1).

5- Hub molecules linking common neurodegeneration-associated proteins provide a source of endogenous MHC class II ligands in human B-cells

From the 19 relevant B-cell studies we retained on IEDB-AR, we also attempted to determine whether the hub molecules we identified as linking neurodegeneration-associated proteins in B-cells (Table 1) were, in parallel, reported to provide endogenous ligands for MHC class II molecules in B-cells. We found that from 11 candidate hubs abundantly expressed by B-cells, 8 were reported to provide endogenous ligands for MHC class II molecules in human B-cells (Table 4 and data supplement 1).

Table 4

Hub molecules linking common neurodegeneration-associated proteins	Reported MHC class II restriction
Heat shock cognate 71 kDa protein (*HSPA8*)	HLA class II, HLA-DR, HLA-DQ, HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2, HLA-DR3, HLA-DRB1*15:01 (HLA-DR15), HLA-DR15/DR51, HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5), HLA-DRB1*04:01 (HLA-DR4), HLA-DRB1*04:05 (HLA-DR4)
Sequestosome-1 (*SQSTM1*)	HLA class II, HLA-DR, HLA-DQ, HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2, HLA-DR15/DR51, HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5)
Heat shock protein HSP 90-alpha (*HSP90AA1*)	HLA class II, HLA-DR, HLA-DQ, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2, HLA-DRB1*04:05 (HLA-DR4), HLA-DRB1*15:01 (HLA-DR15) HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5)
14-3-3 protein zeta/delta (*YWHAZ*)	HLA class II, HLA-DR, HLA-DQ, HLA-DR1, HLA-DRB1*04:05 (HLA-DR4)
14-3-3 protein theta (*YWHAQ*)	HLA class II, HLA-DQ, HLA-DRB1*04:05 (HLA-DR4)
Heat shock 70 kDa protein 4 (*HSPA4*)	HLA-DR, HLA-DR3
Ubiquitin carboxyl-terminal hydrolase CYLD (*CYLD*)	HLA-DRB1*04:05 (HLA-DR4)
TNF receptor-associated factor 6 (*TRAF6*)	HLA-DR1

Legend: A survey of the immune epitope database (IEDB-AR) (42) was performed. Only parent protein names and corresponding from which endogenous peptides eluted from HLA class II molecules were identified by mass spectrometry were taken into account. Only results obtained on human cells of the B-cell lineage in the absence of immunization or stimulation protocols were taken into account. The retrieved list of parent proteins associated to the reported MHC class II restriction of derived peptides was then crossed with the list of 11 hub molecules linking common neurodegeneration-associated proteins and being abundantly expressed by human B-cells. The protein names and corresponding gene symbols are shown in the left column; the corresponding reported MHC class II restrictions of derived peptides are shown in the right column. When needed, aliases frequently used in the nomenclature HLA class II alleles are indicated in brackets.

Among these 8 hubs, HSPA8 was the parent protein being the most frequently identified as providing endogenous peptides which bind MHC class II molecules in human B-cells (Table 1).

DISCUSSION

In the present work, we mined large publically-available databases to provide experiment-based evidence of a link between neurodegeneration and autoimmunity. Using a systems biology approach we report that a large range of common neurodegeneration-associated proteins: i) are expressed by human B-cells under physiological conditions, ii) form a comprehensive and functionally-relevant protein-protein interaction network in human B-cells and iii) provide endogenous peptides which bind MHC class-II molecules in human B-cells. Patients suffering from neurodegenerative conditions exhibit T-cell or antibody-mediated responses directed against major neurodegeneration-associated proteins such as amyloid beta A4 protein, SNCA and tau protein (4–6,72). However, naturally-occurring antibodies against amyloid-beta (73), alpha-synuclein (74–76) and tau protein (72,76,77) were also demonstrated in cohorts of healthy subjects. Similarly, robust T-cell responses against peptides deriving from tau protein were recently demonstrated to occur in humans apart from

any pathological context (78).These findings suggest that the autoimmune processes described in patients with neurodegenerative conditions might be shaped by pre-existing physiological autoimmune responses directed against common neurodegeneration-associated proteins. It is worth noting that, while physiological autoimmunity was firmly demonstrated nearly 50 years ago (79–81), the intimate nature of the links bridging physiological autoimmunity to its pathological counterpart is still matter of debate. Numerous functions have been assigned to physiological autoimmunity (82,83), including, more recently, a support to cognition (84–89). In line with these findings, our data indicate that a specific set of brain antigens expressed by B-cells and involved in neurodegenerative diseases might prime a neuroprotective and, possibly, cognition-promoting T-cell response under physiological conditions.

B-cells are now recognized as professional APCs and, as such, are able to prime naïve CD4 T-cells with a similar efficiency than DCs (90,91). The autophagy of cytosolic and nuclear proteins in B-cells was shown to provide a continuous source of endogenous MHC class-II ligands (92). Moreover, such autophagosome-derived peptides induce the proliferation of autologous T-cells under *in vitro* conditions (93). However, the phenotype of autoreactive T-cells generated via this mechanism has not been explored yet. More generally, one has to keep in mind that no consensus has been currently reached regarding the phenotype of physiological autoreactive T-cells. Both autoreactive Tregs and autoreactive pro-inflammatory T-cells belong to the physiological T-cell repertoire and were both found to exert neuroprotective effects (94–97). Finally, the picture might be even more tangling since Tregs were demonstrated to prevent autoimmunity by restraining autophagy in APCs (98). Several genes involved in familial forms of neurodegenerative disorders exert key functions in the autophagy pathway. These notably comprise PRKN, PINK1 and SQSTM1 (99–102). In the

recent years, major works provided evidence that, in neurons and immune cells, functional defects in such genes hamper mitophagy (a specialized form of autophagy), stimulate the inflammasome pathway and foster the presentation of mitochondrial antigens by MHC class I molecules (33,103,104). However, these findings do not render account for the existence of HLA class II-restricted T-cell responses against neurodegeneration-associated proteins. Moreover, antigens targeted by autoimmunity during neurodegenerative conditions are far from deriving only from the mitochondrial compartment. In this regard, our work suggests that in B-cells, the inflammation/autophagy-related molecules SQSTM1 and TRAF6 are crucially involved in the presentation of neurodegeneration-associated antigens by MHC class II molecules.

The mining of previously published mass spectrometry analyses performed on MHC class II-eluted peptides showed that, in human B-cells, several neurodegeneration-associated proteins provide endogenous peptides which bind a large range of MHC class II alleles. This is notably the case for amyloid beta A4 protein, sequestosome-1 and profilin-1. Similarly, peptides deriving from HSPA8, a hub molecule which links a high number of neurodegeneration-associated proteins, bind multiple MHC class II alleles. Our observations suggest that these 4 molecules are likely to elicit immune responses in a large range of the human population. Determining whether or not such antigens are targeted by cognition-promoting autoimmunity is a potentially important issue. On another hand, in human B-cells, several neurodegeneration-associated antigens appear to provide endogenous peptides harboring an allele-specific MHC class II restriction in B-cells. For e.g. endogenous peptides deriving from microtubule-associated protein tau, PSEN2 and Serine/threonine-protein kinase TBK1 were reported to bind HLA-DR15 but none of the other MHC class-II alleles explored under the experimental conditions described above. Since HLA-DR15 is a susceptibility gene

for late onset AD (14), this may prove to be of interest in the context of AD pathophysiology. However, additional studies are clearly needed in order to establish, under strictly comparable conditions, the endogenous immunopeptidomes of distinct MHC class II alleles in B-cells. Moreover, it should be reminded that the endogenous immunopeptidome of MHC class II molecules does not match the corresponding repertoire of exogenous peptides (105). Since a widespread T-cell reactivity against Tau peptides was recently demonstrated in the general human population (78), one may anticipate that individuals bearing HLA-DR15 alleles exhibit functional specificities regarding their anti-Tau T-cell responses.

We previously proposed that the genetic polymorphism of the HLA-DRB1 locus, which, among primates, is extremely high in the human species (106), might allow the allele-specific presentation of distinct sets of "brain superautoantigens" (107,108). In turn, distinct sets of cognition-promoting T-cells would be generated, leading possibly to a high level of cognitive diversity, and thus cognitive complementarity, in Homo sapiens. The present work indicates that common neurodegeneration-associated proteins might represent an important share of brain superautoantigens. Interestingly, recent magnetic resonance imaging studies reported that, in healthy subjects, specific HLA-DR alleles correlate with the volume ranges of specific brain structures (109,110). Future studies should be designed to determine whether HLA-DR polymorphism might match both cognitive diversity (as assessed by brain imaging and/or cognitive tests) and the diversity of T-cell responses against common neurodegeneration-associated proteins.

Acknowledgments: no specific funds were used for this study. SN performed the bioinformatics analyses and wrote the paper. MG and LP performed quality controls of bioinformatics analyses and wrote the paper. We thank Céline Auxenfans and Pascale Pascal

from the "Bank of Tissues and Cells" of the "Hospices Civils de Lyon" for leaving us the opportunity to develop a bioinformatics research group.

Conflicts of interest statement: The authors declares that there is no conflict of interest regarding the publication of this article.

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