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Journal
Movement Disorders, 30(7)

ISSN
0885-3185

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Publication Date
2015-06-01

DOI
10.1002/mds.26238

Peer reviewed

RESEARCH ARTICLE

Novel Candidate Blood-Based Transcriptional Biomarkers of Machado-Joseph Disease

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ABSTRACT: Background: Machado-Joseph disease (or spinocerebellar ataxia type 3) is a late-onset polyglutamine neurodegenerative disorder caused by a mutation in the *ATXN3* gene, which encodes for the ubiquitously expressed protein ataxin-3. Previous studies on cell and animal models have suggested that mutated ataxin-3 is involved in transcriptional dysregulation. Starting with a whole-transcriptome profiling of peripheral blood samples from patients and controls, we aimed to confirm abnormal expression profiles in Machado-Joseph disease and to identify promising up-regulated genes as potential candidate biomarkers of disease status.

Methods: The Illumina Human V4-HT12 array was used to measure transcriptome-wide gene expression in peripheral blood samples from 12 patients and 12 controls. Technical validation and validation in an independent set of samples were performed by quantitative real-time polymerase chain reaction (PCR).

Results: Based on the results from the microarray, twenty six genes, found to be up-regulated in patients, were selected for technical validation by quantitative real-time PCR (validation rate of 81% for the up-regulation trend). Fourteen of these were further tested in an independent set of 42 patients and 35 controls; 10 genes maintained the up-regulation trend (*FCGR3B*, *CSR2RA*, *CLC*, *TNFSF14, SLA, P2RY13, FPR2, SELPLG, YIPF6,* and *GPR96*); *FCGR3B, P2RY13,* and *SELPLG* were significantly up-regulated in patients when compared with controls.

Conclusions: Our findings support the hypothesis that mutated ataxin-3 is associated with transcription dysregulation, detectable in peripheral blood cells. Furthermore, this is the first report suggesting a pool of up-regulated genes in Machado-Joseph disease that may have the potential to be used for fine phenotyping of this disease.

Key Words: spinocerebellar ataxia type 3; polyglutamine disease; gene expression; ataxin-3; microarray

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Funding agencies: This study was supported by FEDER funds through the Operational Competitiveness Programme—COMPETE and by National Funds through FCT—Fundação para a Ciência e a Tecnologia under the project FCOMP-01-0124-FEDER-028753 (PTDC/DTP/PIC/0370/2012). A PhD fellowship M3.1.2/F/006/2011 (M.R.) and postdoctoral fellowships M3.1.7/F/031/2011 (A.R.) and M3.1.3/F/004/2009 (N.K.) were supported by Fundo Regional para a Ciência (FRC), Governo dos Açores, and SFRH/BPD/33611/2009 (A.J.R.) was funded by FCT—Fundação para a Ciência e a Tecnologia. C.B. is supported by the UK Medical Research Council (MRC).

Relevant conflicts of interest/financial disclosures: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received: 1 October 2014; **Revised:** 27 January 2015; **Accepted:** 2 March 2015

Published online 25 April 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.26238

Machado-Joseph disease (MJD; MIM #109150; ORPHA98757), also named spinocerebellar ataxia type 3, is the most common autosomal dominant ataxia worldwide[1] and the second most common polyglutamine (polyQ) disorder, following Huntington's disease (HD).[2] Machado-Joseph disease is a clinically heterogeneous neurodegenerative disorder with an average onset around the fourth decade of life.[3,4] It is characterized by a wide range of manifestations, including ataxia, progressive external ophthalmoplegia, pyramidal and non-pyramidal signs, dystonia with rigidity, and distal muscular atrophy.[3,4] A widespread neuronal loss in the cerebellum, pons, medulla oblongata, basal ganglia, thalamus, midbrain, and cerebral cortex is common in this disease.[5] Effective treatment for MJD is still lacking, and only symptomatic therapeutics are available.[6] The MJD gene—*ATXN3*—mapped to 14q32.1 contains a (CAG)n tract at exon 10, which in mutant allele is expanded, typically beyond 51 repeat units.[7,8] A negative correlation between the number of CAG repeats in the expanded allele and the age at onset has been widely described, with the size of the CAG tract accounting for nearly 50% to 75% of the variation in the age at onset.[6] The *ATXN3* gene encodes for ataxin-3, a polyQ protein ubiquitously expressed in neuronal and non-neuronal tissues. Expansions of the polyQ tract above the pathological threshold initiates a cascade of pathogenic events that are being extensively studied.[9,10] Similarly to MJD, in other polyQ diseases the expanded repeat triggers conformational changes in the corresponding proteins, leading to the formation of intracellular inclusions, considered the hallmark of this group of disorders.[11] In MJD, however, the characteristic neuronal intranuclear inclusions do not seem to be the main toxic entity (revised in Evers et al.[9]). Mutated ataxin-3 seems to be involved in transcriptional regulation via two processes: (1) recruitment of transcription factors to polyQ-rich inclusions[12-14] and (2) altered interactions with transcription factors and co-activators.[15-19] So far, patterns of transcriptional dysregulation caused by mutated ataxin-3 have been studied only in cellular and animal models. In such models, transcriptional alterations of genes involved in inflammatory processes, cell signaling, and cell surface–associated proteins has been described.[17,20,21] Studies of disease-modifying compounds, in the context of clinical trials, have been recently initiated for MJD,[22,23] but gross clinical evaluation alone might not be sensitive enough to monitor disease progression and detect subtle therapeutic benefits; this is particularly true in diseases of slow progression, such as MJD. The development of disease-related biomarkers is therefore urgently needed. Gene expression profiling arrays, by identifying disease-specific transcriptional changes in blood, an easily accessible tissue, can boost the identification of potential biomarkers (revised in Coppola and Geschwind[24]).

TABLE 1. Characterization of the studied subjects according to biological group and study design

	Study Design	
	Microarray and Technical Validation	Independent Set Validation
MJD Patients		
N	12	42
Female/male	10/2	26/16
Age (y)	42 ± 6 [34-52]	47 ± 15 [22-82]
Age at onset (y)	34 ± 6 [28-46]	37 ± 13 [12-70]
CAG repeats		
Normal allele	20 ± 6 [14-29]	21 ± 5 [14-29]
Expanded allele	72 ± 2 [69-75]	71 ± 4 [62-79]
Disease duration (y)	8 ± 3 [6-17]	10 ± 8 [0-30]
Controls		
N	12	35
Female/male	6/6	23/12
Age (y)	37 ± 13 [19-61]	46 ± 12 [24-77]

All quantitative variables were presented as mean ± standard deviation [range].

These transcriptional changes have not been investigated in MJD patients, and their potential as biomarkers of disease remains to be evaluated. In this study we used microarrays to generate gene expression profiles in peripheral blood samples of MJD patients, aiming to confirm the presence of altered gene expression patterns in MJD and to identify up-regulated genes as potential candidate biomarkers of disease status.

Methods

Subjects and Sampling

Peripheral blood was collected from MJD Azorean patients and control individuals. A set of 12 patients and 12 controls was used for the gene expression microarray analysis and technical validation (Table 1). To ensure a homogeneous group of subjects, patients with an age at onset around the average (~40 y) in our series of cases, and presenting mainly cerebellar alterations, were selected for this phase of the study. An independent set of 42 patients and 35 controls was used for further validation (Table 1). All patients had a clinical and a molecular diagnosis of MJD (confirmed carriers of the *ATXN3* mutation). Patients were recruited through the Department of Neurology—Hospital Divino Espírito Santo (HDES, Ponta Delgada, Azores, Portugal). This study was approved by the Ethics Committee of the HDES, and all participants provided written informed consent.

RNA Isolation and Gene Expression Microarrays

Whole-blood samples were collected in Tempus™ Blood RNA tubes (Applied Biosystems, Waltham,

MA, USA), and total RNA was isolated using the Tempus™ Spin RNA Isolation Kit (Applied Biosystems), following the manufacturer's protocol. The integrity of total RNA samples was assessed using the RNA 6000 Nano Kit on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

RNA was amplified, biotin-labeled, and hybridized on a transcriptome-wide expression Illumina Human V4-HT12 array. Slides were scanned using Illumina BeadStation, and the signal was extracted using the Illumina BeadStudio software. All arrays were performed in the same core facility.

Technical Validation of Candidate Blood-Based Biomarkers

Up-regulation is more reliably detected than down-regulation, because it is not dependent on the lower detection limit of the quantification methods; therefore, for the next phases of the study, we ranked and selected only those genes that in the gene expression microarray were up-regulated in patients. Up-regulated genes with a Benjamini-Hochberg false discovery rate (FDR) adjusted *P*-value less than 0.05 and a log ratio greater than 1.2 were ranked. Consistency of the log ratio between pairs and maximal values of expression levels were further evaluated as criteria for the ranking procedure. Twenty-six genes that fulfilled the previously described criteria were selected for quantitative real-time polymerase chain reaction (PCR; qPCR) technical validation, using the same set of samples previously used in the microarrays.

One microgram of total RNA was used to synthesize complementary DNA, using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), according to the manufacturer's protocol. The TaqMan Gene Expression Master Mix and prevalidated TaqMan Gene Expression Assays (TaqMan IDs are described in Table 2) were used for qPCR (Applied Biosystems). TaqMan gene expression assays were designed, tested, and optimized to address all Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines.[25] More information on these assays is given in the white paper by Life Technologies (available at: https://tools.lifetechnologies.com/content/sfs/brochures/cms_088754.pdf). The *PPIB* (peptidylpropyl isomerase B; Hs00168719_m1) gene was used as the reference gene for qPCR validation. This reference gene is one of the most stably expressed genes in human blood, suitable for normalization in qPCR studies.[26] Moreover, the *PPIB* gene was identified as an appropriated gene for expression normalization in blood of HD patients and R6/2 mice.[27] Quantitative PCR was performed in a 7900HT Fast Real-Time PCR System (Applied Biosystems). Each sample was run in triplicate alongside with the reference gene.

Validation in an Independent Set of Samples

From the list of 26 genes subject to the technical validation, the 14 genes showing a consistent up-regulation trend in the technical validation phase, with the highest fold change and/or the lowest *P*-value, were further investigated in an independent set of samples (42 patients and 35 controls). The qPCR methodology was as described previously (*Technical Validation of Candidate Blood-Based Biomarkers* subsection). Gene ontology annotations were performed in Qiagen's interactive pathways analysis software (QIAGEN Redwood City, www.qiagen.com/ingenuity).

Statistical Analysis

Raw data obtained from the Illumina Human V4-HT12 arrays were analyzed with Bioconductor packages, as previously described.[28] Briefly, quality assessment included inter-array Pearson correlation and clustering based on top variant genes. Raw expression values were log2 transformed and normalized using quantiles. Batch effects were corrected using the ComBat algorithm.[29] Differential expression analysis (patients vs. controls) was performed using the limma package.[28] Up-regulated genes were considered for further analysis when FDR-adjusted *P*-value was lower than 0.05, and log ratio was higher than 1.2-fold. Because controls were hybridized in separate batches, an increased rate of false positives was expected. To minimize this issue, we applied a batch-correction algorithm (see earlier description), adopted a very conservative statistical threshold (FDR < 0.05), and verified all selected candidates by qPCR.

In the qPCR validation phases of this study (technical validation and validation in an independent set of samples), relative expression values were normalized to the reference gene (*PPIB*), and fold change values were calculated by using the $2^{-\Delta\Delta Ct}$ method.[30] A two-sample, two-tailed Student's *t* test was used to compare the ΔC_q (C_q, also named cycle threshold [Ct], is quantification cycle Cq, as suggested by MIQE guidelines[25]) values between the two biological groups (patients vs. controls), and a *P*-value was calculated. All statistical procedures were performed by using the DataAssist v3.0 software (Applied Biosystems).

Results

Microarray-based gene expression profiling was used to analyze global gene expression in peripheral blood samples of MJD patients and controls. We identified a total of 5,523 up-regulated probes, whereas 6,232 were down-regulated (FDR adjusted *P*-value < 0.05) when comparing patients with controls.

From the 26 top candidate biomarkers (Table 2) selected for technical validation by qPCR, we confirmed an up-regulation trend in 21 of 26 genes

TABLE 2. Candidate blood-based transcriptional biomarkers *selected* for the qPCR technical validation and validation in an independent set

				Microarray Data		Technical Validation		Independent Validation	
TaqMan ID	Gene ID	Symbol[a]	Full Name[a]	Log Ratio[b]	*P-Value*[c]	*FC*[d]	*P-Value*	*FC*[d]	*P-Value*
Transmembrane receptors[e]									
Hs00275547_m1	2215	***FCGR3B***	Fc fragment of IgG, low-affinity IIIb, receptor (CD16b)	1.943	1.18E-02	4.0582	6.80E-03	2.597	9.20E-03
Hs04191069_gH	3802	***KIR2DL1***	Killer cell immunoglobulin-like receptor, 2 domains, long cytoplasmic tail, 1	1.767	1.30E-03	1.8267	1.94E-01	Not selected	
Hs00427106_m1	3805	***KIR2DL4***	Killer cell immunoglobulin-like receptor, 2 domains, long cytoplasmic tail, 4	1.396	1.19E-03	1.2968	4.38E-01	Not selected	
Hs00538900_m1	1438	***CSF2RA***	Colony-stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	1.294	5.80E-05	1.5071	1.44E-01	1.331	1.37E-01
Enzymes									
Hs01055743_m1	1178	***CLC***	Charcot-Leyden crystal protein	1.763	4.91E-03	3.4077	1.70E-02	2.041	1.13E-01
Hs01587865_g1	4051	***CYP4F3***	Cytochrome P450, family 4, subfamily F, polypeptide 3	1.512	2.14E-03	4.0785	4.77E-02	0.911	8.01E-01
Hs00193422_m1	3029	***HAGH***	Hydroxyacylglutathione hydrolase	1.368	4.84E-03	0.7296	3.10E-01	Not selected	
G-protein coupled receptors									
Hs01891184_s1	3579	***CXCR2***	Chemokine (C-X-C motif) receptor 2	2.040	4.99E-03	1.9372	2.13E-01	Not selected	
Hs02759175_s1	2358	***FPR2***	Formyl peptide receptor 2	1.541	4.56E-03	1.7242	9.79E-02	1.370	2.77E-01
Hs00416889_m1	222487	***GPR97***	G protein-coupled receptor 97	1.315	8.40E-03	3.3142	7.61E-02	1.107	7.77E-01
Hs03043902_s1	53829	***P2RY13***	Purinergic receptor P2Y, G-protein coupled, 13	1.313	1.37E-03	2.1724	1.07E-02	1.665	6.20E-03
Peptidases									
Hs00153519_m1	4311	***MME***	Membrane metallo-endopeptidase	1.476	4.07E-02	2.1544	1.84E-01	0.994	9.90E-01
Hs01073631_m1	64167	***ERAP2***	Endoplasmic reticulum aminopeptidase 2	1.410	2.42E-03	0.5328	5.63E-02	Not selected	
Cytokines									
Hs00542477_m1	8740	***TNFSF14***	Tumor necrosis factor (ligand) superfamily, member 14	1.562	4.33E-04	5.6394	3.00E-04	1.687	9.02E-02
Others									
Hs00331399_m1	57535	***KIAA1324***	KIAA1324	2.101	1.77E-04	1.3916	2.65E-01	Not selected	
Hs00846590_s1	11026	***LILRA3***	Leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 3	2.037	2.98E-05	0.5844	5.40E-02	Not selected	
Hs00747812_m1	3813	***KIR3DS1***	Killer cell immunoglobulin-like receptor, three domains, short cytoplasmic tail, 1	1.993	1.49E-03	1.1759	6.18E-01	Not selected	
Hs00962914_m1	7057	***THBS1***	Thrombospondin 1	1.684	4.68E-05	0.7767	5.77E-01	Not selected	
Hs00160066_m1	5266	***PI3***	Peptidase inhibitor 3, skin-derived	1.628	3.79E-02	3.1875	4.40E-02	0.525	1.74E-01
Hs01066294_m1	23762	***OSBP2***	Oxysterol binding protein 2	1.471	2.66E-03	0.9111	8.40E-01	Not selected	
Hs00190581_m1	8875	***VNN2***	Vanin 2	1.465	4.46E-03	1.4282	2.87E-01	Not selected	
Hs00275682_s1	3310	***HSPA6***	Heat shock 70kDa protein 6 (HSP70B')	1.344	1.22E-04	1.2347	3.82E-01	Not selected	
Hs00218346_m1	55363	***HEMGN***	Hemogen	1.328	8.09E-03	3.7584	4.84E-02	0.534	8.01E-02
Hs00277129_m1	6503	***SLA***	Src-like-adaptor	1.308	6.37E-05	2.5137	1.60E-03	1.333	1.15E-01
Hs00356602_m1	6404	***SELPLG***	Selectin P ligand	1.251	4.06E-06	1.9473	1.60E-03	1.324	4.04E-02
Hs00397742_m1	286451	***YIPF6***	Yip1 domain family, member 6	1.248	1.98E-04	2.5564	1.06E-02	1.136	5.17E-01

[a]Official gene symbol and full name provided by HUGO Gene Nomenclature Committee.
[b]Log ratio = $\log_2$ (Cy3'/Cy5').
[c]*P*-value calculated by Student's *t* test and adjusted for multiple comparisons by FDR (false discovery rate) correction.
[d]Fold Change (FC) = geometric mean $2^{(-\Delta Cqpatients)}$/geometric mean $2^{(\Delta Cqcontrols)}$.
[e]Gene ontology annotations for technical validated genes were performed in Qiagen's interactive pathways analysis (IPA) software (QIAGEN, Redwood City, www.qiagen.com/ingenuity).

(validation rate of 81%), although only 10 were statistically significant. From these 21 genes, 14 (Table 2) were selected for validation by qPCR in an independent set of MJD patients and controls. Given the fact that the sex ratio in our first set of MJD samples was skewed toward females, we choose a 1:1 male to female ratio in our independent set of samples (both cases and controls) to ensure that the observed up-regulation was not caused by a sex effect. In this phase, a validation rate of 71% was obtained, with 10 of 14 genes showing an up-regulation trend. In the new set of samples, the expression levels of *FCGR3B*, *CSF2RA*, *CLC*, *FPR2*, *SLA*, *GPR97*, *P2RY13*, *TNFSF14*, *SELPLG*, and *YIPF6* were 1.11- to 2.60-fold higher in patients when compared with controls (Fig. 1; Table 2). Noteworthy, *FCGR3B*, *P2RY13*,

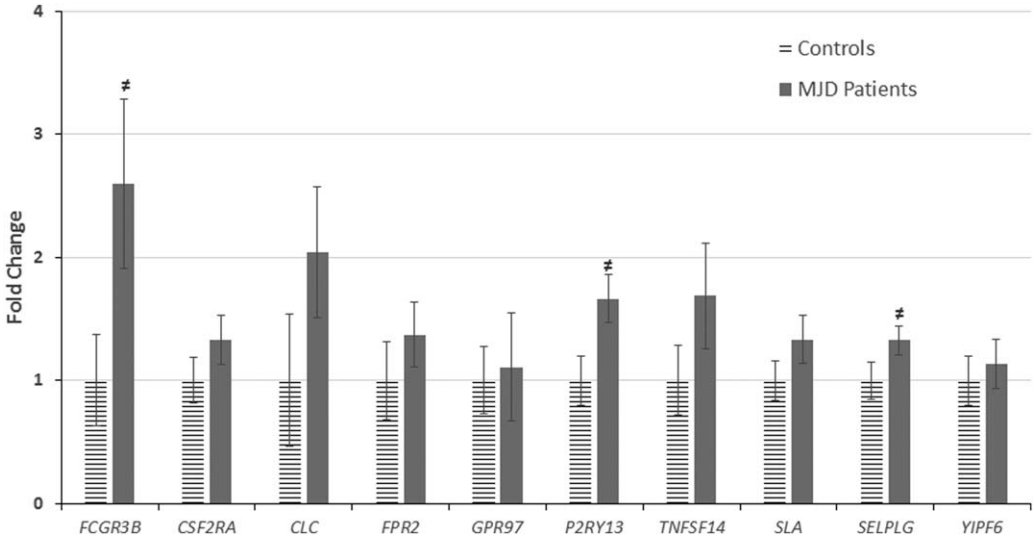

FIG. 1. Fold change (FC) values for the 10 genes maintaining an up-regulation trend in an independent set of MJD patients versus controls. Error bars were obtained by the standard error of mean (SEM) difference of the ΔCq values, and presented as FC × (2^{SEM} – 1) as described elsewhere.[37] FC = 1 implies the absence of expression change. ≠ The difference is statistically significant (two-tailed T test, *P*-value < 0.05).

and *SELPLG* genes were significantly up-regulated ($P < 0.05$). According to gene ontology functional annotations, these genes are mostly related to the immune system response (*FCGR3B, CSF2RA, FPR2, TNFSF14*, and *SELPLG*) and G-protein coupled receptor signaling (*FPR2, GPR97*, and *P2RY13*).

Within the patients group (patients from technical and independent set validation), our data show a trend for higher expression levels in patients with shorter disease duration compared with patients with longer disease duration, especially for *FCGR3B* and *CLC*, which were statistically significant (Fig. 2). The comparison between fold change values in patients grouped by CAG size in expanded allele failed to produce significant results (data not shown).

Discussion

In this study, we confirmed that transcriptional dysregulation can be detected in peripheral blood samples of MJD patients. We report a set of 10 genes—*FCGR3B, CSF2RA, CLC, FPR2, SLA, GPR97, P2RY13, TNFSF14, SELPLG*, and *YIPF6*—that consistently show an up-regulation trend in MJD patients when compared with controls. Three of these genes—*FCGR3B*, *P2RY13*, and *SELPLG*—were significantly up-regulated and therefore should be considered in future studies as biomarkers of disease status. Moreover, the significantly increased levels of *FCGR3B and CLC* messenger RNAs (mRNA) observed in early MJD stages suggests that, even in a nonaffected tissue (such

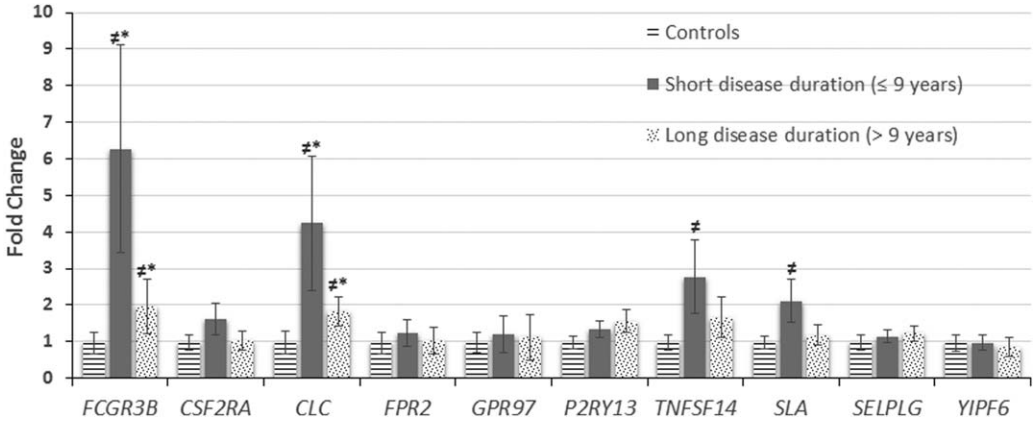

FIG. 2. Fold change (FC) values of the 10 genes, previously validated as biomarkers of MJD status, in controls, patients with 9 or fewer years elapsed since onset (short disease duration), and with more than 9 years elapsed since onset (long disease duration). Disease duration was defined as the number of years elapsed from reported age at onset to the collection of blood samples for each patient. Error bars were obtained by the standard error of mean (SEM) difference of the ΔCq values, and presented as (FC) × (2^{SEM-1}) as described elsewhere.[30] FC = 1 implies the absence of expression change. ≠The difference between each of the grouped patients and controls is statistically significant (*P*-value < 0.05); * the difference between each group of patients is statistically significant (*P*-value < 0.05).

as blood), a cellular response closer to the disease onset is activated in the presence of mutated ataxin-3; with disease progression (specifically more than 10 y after onset), expression levels of these genes fall to levels closer to those found in healthy individuals. These findings suggest a potential of the reported genes to monitor disease stages; further investigation is nevertheless required. The potential of molecular alterations in the fine phenotyping of a polyQ disease was shown by Bjorkqvist and collaborators,[31] who observed higher levels of interleukin 6 in plasma of HD gene carriers, on average 16 y before the predicted onset of clinical symptoms. This study also reported that interleukin 12 and granulocyte-macrophage colony-stimulating factor levels significantly decreased from early to moderate stages of the disease.[31]

To our knowledge, from the above-mentioned genes, only *TNFSF14, CSF2RA,* and *FPR2* have been previously associated with neurodegenerative disorders. *TNFSF14* has been associated with amyotrophic lateral sclerosis (ALS),[32] and the latter two genes with Alzheimer's disease (AD).[33,34] *TNFS14*, which is known to be expressed in immature dendrocytes, activated lymphocytes, monocytes, natural killer cells, motor neurons, and astrocytes, has been shown to be up-regulated in the spinal cord of ALS patients.[35] This cytokine is known to be important for both innate and adaptive immune processes, and it also functions with interferon-γ to induce a singular slow apoptotic death in tumor cells. Soluble TNFSF14 produced by astrocytes in ALS acts as a death-inducing ligand in motor neurons.[35] The genetic ablation of the *tnfsf14* gene (orthologue to human *TNFSF14*) in an ALS mouse model has been related to a slow disease progression as well as an extended life expectancy, suggesting a possible modifier role for this gene.[32]

CSF2RA encodes the alpha subunit of the heterodimeric receptor for colony-stimulating factor 2 (CSF2), a hematopoietic factor. In recent years, *CSF2* has been shown to be an important neurotrophic factor in the central nervous system via binding to its receptor. Both *CSF2* and *CSF2RA* are expressed in neurons throughout the central nervous system, astrocytes, ependymal cells, and choroid plexus cells. In AD, expression of the protein encoded by *CSF2RA* has been found to be dysregulated in the hippocampus of patients.[34] No further links to neurodegenerative diseases have been established for this gene.

The formyl peptide receptor 2 (encoded by *FPR2*) is a G-protein–coupled receptor (GPCR) of bacterial chemotactic peptides expressed in B cells, mononuclear phagocytes, and microglia. In AD models, the interaction between Aβ42 and *FPR2* is clearly associated with microglial cell activation. Moreover, a persistent internalization of Aβ42/*FPR2* complexes, which culminates in intracellular fibrillar formation and apoptotic death of the cells, suggests that *FPR2* can be a possible therapeutic target for AD.[33] Other members of the GPCR superfamily, which in our study includes two dysregulated genes (*P2RY13* and *GPR97*), have been previously reported in polyQ-associated neurodegeneration. In MJD models, the involvement of the adenosine A2a receptor (A2aR), a GPCR subtype, has been reported.[36] Striatal pathological conditions associated with mutant *ATXN3* overexpression in a lentiviral-based model can be abolished by inactivation of A2aR, providing the first evidence that manipulation of a neuromodulation system operated by A2aRs is effective in controlling the initial cascade of events triggered by the pathogenic ataxin-3 protein (synaptotoxicity and gliosis).[36] The *P2Y5* gene, a member of the same purinergic receptor family as *P2RY13*, has been validated as a candidate biomarker in HD blood cells.[37]

FCGR3B and *SELPLG* genes were significantly up-regulated in MJD patients. The Fc fragment of immunoglobulin G (IgG), low-affinity IIIb, receptor (FCGR3B) is an Fc gamma receptor (FcγR), which belong to a family of immunoglobulin-like receptors that bind to the Fc portion of IgG and mediate the response of effector cells to immune complexes.[38] Activation of these receptors can result in a pro-inflammatory response including the release of cytokines and other mediators. Evidence concerning the ligation of specific FcγRs in the central nervous system by IgG and alternate ligands, which promote neuroinflammation or enhance neurodegeneration, were previously reported.[38] Selectin P ligand (*SELPLG*) gene encodes a glycoprotein that functions as a high-affinity counter-receptor for the cell adhesion molecules P-, E-, and L-selectin expressed on myeloid cells and stimulated T lymphocytes.[39] The selectin family mediates the tethering and rolling of leukocytes on the vascular wall during the process of leukocyte migration into the tissues under physiological and pathological conditions.[40] The role of selectins in leukocyte–endothelial interactions in the pathogenesis of neurological diseases had been debated.[40] Rodrigues et al.[41] reported that depletion of *ATXN3,* using small-interference RNA in human and mouse cells, causes a decrease in expression of important cell adhesion molecules, indicating that the extracellular matrix–cell or cell–cell interconnection was compromised.[41]

Five of the 10 candidate biomarkers identified in the present study play a role in immune system pathways. This is not surprising given the cellular source of the mRNA used in our analysis. Noteworthy, this finding is in line with previous studies, which have shown that mutant ataxin-3 mediates up-regulation of several cytokines and cytokine-inducible transcription factors in cell models and in brain tissue of MJD patients,[17,20,42] although none of the previously reported genes were

up-regulated in our study. Immune system alterations also have been reported in peripheral blood of HD patients, inclusively, before the manifestation of motor symptoms.[31,43,44] Globally, genes involved in immune system and GPCRs may constitute good candidates for biomarkers in polyQ disorders and should be considered in future studies of MJD progression.

In summary, our results support the hypothesis that mutated ataxin-3 is associated with transcription dysregulation in peripheral blood cells. Based on the transcriptional profile of MJD patients, this study identified for the first time a pool of up-regulated genes in this disease. The understanding of how the up-regulation of these genes relates to clinical-associated progressive features during the natural history of MJD will be crucial in clinical trials measuring the effectiveness of new drugs. ■

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