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The pathogenetic basis for a disease continuum in early- and late-onset ataxia-dystonia supports a unified genetic diagnostic approach

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ABSTRACT

Introduction: Genetically inherited ataxic disorders are classified by their age of disease presentation into early- and late-onset ataxia (EOA and LOA, presenting before or after the 25th year-of-life). In both disease groups, comorbid dystonia co-occurs frequently. Despite overlapping genes and pathogenetic features, EOA, LOA and dystonia are considered as different genetic entities with a separate diagnostic approach. This often leads to diagnostic delay. So far, the possibility of a disease continuum between EOA, LOA and mixed ataxia-dystonia has not been explored *in silico.* In the present study, we analyzed the pathogenetic mechanisms underlying EOA, LOA and mixed ataxia-dystonia.

Methods: We analyzed the association of 267 ataxia genes with comorbid dystonia and anatomical MRI lesions in literature. We compared anatomical damage, biological pathways, and temporal cerebellar gene expression between EOA, LOA and mixed ataxia-dystonia.

Results: The majority (≈65%) of ataxia genes were associated with comorbid dystonia in literature. Both EOA and LOA gene groups with comorbid dystonia were significantly associated with lesions in the cortico-basal-ganglia-pontocerebellar network. EOA, LOA and mixed ataxia-dystonia gene groups were enriched for biological pathways related to nervous system development, neural signaling and cellular processes. All genes revealed similar cerebellar gene expression levels before and after 25 years of age and during cerebellar development.

Conclusion: In EOA, LOA and mixed ataxia-dystonia gene groups, our findings show similar anatomical damage, underlying biological pathways and temporal cerebellar gene expression patterns. These findings may suggest the existence of a disease continuum, supporting the diagnostic use of a unified genetic approach.

1. Introduction

Genetically inherited cerebellar ataxia comprises a clinically and diagnostically heterogeneous group of movement disorders, mainly characterized by impaired balance and coordination [1]. Ataxic disorders are classified by the age of disease presentation into early-onset ataxia (EOA) and late-onset ataxia (LOA), beginning respectively before or after the 25th year of life [2]. Both EOA and LOA often present in combination with comorbid dystonia [3,4], as reported in 65% of patients in our clinical EOA cohort [5].

Despite the different age of disease onset in EOA and LOA patients, there are many similarities between both groups, such as the substantial overlap in genes [5,6], the involved anatomical motor networks within the nervous system (including the cerebellum) [2,5,7] and the underlying biological mechanisms [5], suggesting a shared pathogenesis [5,6]. Analogously, there are also many similarities between EOA, LOA and dystonia patients. In literature, neuroradiological damage and abnormal signaling of the cerebello-thalamo-cortical network [6,8–10] and/or the cortico-basal-ganglia-cerebellar network through the pedunculopontine tegmental nucleus (PPTg) [11,12] have been reported to underlie movement disorders in ataxia and dystonia [6,8–12]. Moreover, overlapping biological pathways have been identified between EOA and dystonia, including cellular energy depletion and network signal transduction [5], and between spinocerebellar ataxia (SCA, dominantly inherited LOA) and dystonia, including synaptic transmission and

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Abbreviations

EOA	Early-onset ataxia
LOA	Late-onset ataxia
PPTg	Pedunculopontine tegmental nucleus
SCA	Spinocerebellar ataxia
UMCG	University Medical Center Groningen
EOAD+	Early-onset ataxia with comorbid dystonia
EOAD	Early-onset ataxia without comorbid dystonia
LOAD+	Late-onset ataxia with comorbid dystonia
LOAD	Late-onset ataxia without comorbid dystonia
OMIM	Online Mendelian Inheritance in Man
MRI	Magnetic resonance imaging
GO	Gene ontology
GO:BP	Gene ontology biological pathway
CBPC	Cortico-basal-ganglia-pontocerebellar (network)

nervous system development [6]. In this perspective, EOA, LOA and mixed ataxia-dystonia phenotypes could be attributable to the same disease continuum, rather than being entirely different disorders.

Regardless of the overlapping phenotypes, genotypes, sites of anatomical damage and biological mechanisms, EOA and LOA with or without comorbid dystonia are still considered as distinct entities in clinical practice [2,13]. As a result, disease-specific gene panels for either EOA, LOA or dystonia are still being used in clinical genetic diagnostics [2,14–17]. This often leads to diagnostic delay, especially in patients presenting with mixed phenotypes [5,11,18,19].

So far, the pathogenetic foundation for the distinction between EOA and LOA as separate disease groups has not been studied. In the present *in silico* study, we therefore aimed to investigate and compare the anatomical damage, underlying biological processes and temporal gene expression patterns between EOA, LOA and mixed ataxia-dystonia. We reasoned that such data could elucidate the potential existence of one disease continuum, which would support the use of a unified genetic approach and could improve diagnostic efficacy.

2. Methods

We proceeded in a five-step approach, depicted schematically in Fig. 1.

2.1. Step 1: EOA and LOA gene inclusion

We included EOA and LOA genes listed in the respective gene panels from the Department of Genetics of the University Medical Center of Groningen (UMCG, Groningen, the Netherlands; version 18, accessed before November 2021) [20]. We supplemented these with newly reported SCA genes by Nibbeling et al. (2017) [6] and with the complete list of EOA genes reported by the European EOA expert working group (2019) [2]. EOA and LOA genes were included irrespective of the type of pathogenic variant, inheritance pattern, age of onset, severity of disease, presence of additional comorbid movement disorders other than dystonia or other neurological phenotypes.

2.2. Step 2: association of EOA and LOA genes with dystonic phenotypes

We investigated whether EOA and LOA genotypes were phenotypically associated with dystonia in literature, and subclassified the EOA and LOA genes accordingly. EOA and LOA genes reported with dystonia were characterized as EOAD+ and LOAD+; genes not reported with dystonia as EOAD- and LOAD- (Supplementary Files I and II). The following sources were consulted: 1) Online Mendelian Inheritance in Man (OMIM; https://omim.org); 2) the Genomics England PanelApp

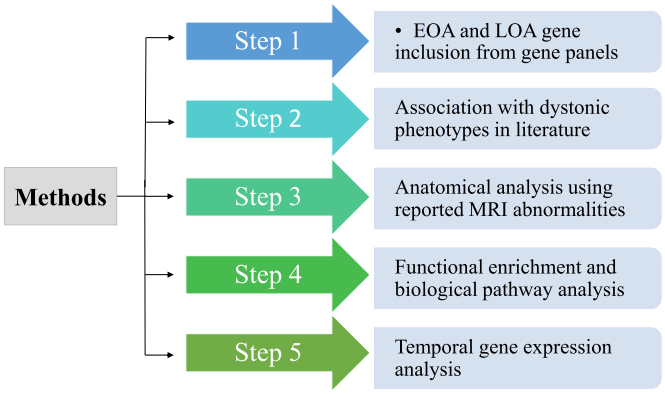

Fig. 1. Schematic representation of methods. We performed a five-step approach:
1) We included EOA and LOA genes from a) the University Medical Center Groningen gene panels, b) novel spinocerebellar ataxia genes (Nibbeling et al., 2017) and c) EOA genes from the expert review of the European EOA working group (Brandsma et al., 2019).
2) We investigated whether genes underlying EOA and LOA were associated with comorbid dystonia *in silico* (from literature).
3) We investigated MRI abnormalities reported in literature for each gene and used these data to perform anatomical analysis.
4) We performed functional enrichment- and biological pathway analysis for the EOAD+ and LOAD + genes.
5) We explored the cerebellar expression of EOAD+ and LOAD + genes in 9 developmental stages (Supplementary Fig. 1).
To determine whether comorbid dystonia had an influence on the underlying pathogenetic mechanisms and on the expression patterns in the cerebellum, or whether the results were comparable, we performed step four and five also for EOA and LOA genes and compared these with results in EOAD+ and LOAD + genes. EOA = Early-onset ataxia; LOA = Late-onset ataxia; EOAD+ = Early-onset ataxia with comorbid dystonia; LOAD+ = Late-onset ataxia with comorbid dystonia; MRI = magnetic resonance imaging.

(https://panelapp.genomicsengland.co.uk), from which we only selected genes with a high or moderate level of evidence; and 3) PubMed of the National Center for Biotechnology Information (NCBI; https://pubmed.ncbi.nlm.nih.gov). Our search query consisted of the following terms: ("Gene name") AND ("Dystonia" [Mesh] OR "Dystoni*" [tiab]).

2.3. Step 3: anatomical analysis using magnetic resonance imaging abnormalities reported in literature

We recorded the location of anatomical damage reported on MRI in patients with pathogenic variants in EOAD ± and LOAD ± genes, using OMIM and PubMed. Our search query in PubMed consisted of the following terms: ("Gene name") AND ("Neuroimaging"[Mesh] OR "Neuroimaging"[tiab] OR "Magnetic Resonance Imaging"[Mesh] OR "Magnetic Resonance Imaging" [tiab]). Then, in the EOAD ± and LOAD ± gene subgroups we investigated the frequency of reported anatomical MRI damage in a) either cerebellar or extracerebellar structures, and b) multiple brain structures concomitantly.

2.4. Step 4: functional enrichment and biological pathway analysis

To expose shared pathogenetic mechanisms between EOA, LOA and mixed ataxia-dystonia, we generated shared brain-specific gene co-expression networks of the EOAD+, LOAD+, EOA and LOA gene groups using MetaBrain (https://network.metabrain.nl) [21]. Each gene group was procedurally enriched with 200 predicted co-expressed genes (Supplementary Files III-VI). Subsequently, pathway analysis between the shared EOAD+ and LOAD + gene network was performed using the meta-analysis function of Metascape (version 3.5, http://metascape.org)

[22]. Here, terms with a p-value <.01 and a minimum count of three were collected and grouped into the top 20 clusters based on their membership similarities (i.e., similar biological functions). Terms enriched in both gene lists and with the strongest *p*-values (expressed in log10) were automatically used as cluster representatives (i.e., umbrella terms for the whole cluster; reported as "summary row" in the Supplementary File XII). Clusters were therefore named after their cluster representative term. From all the available sources in Metascape (i.e., Gene Ontology, KEGG, Reactome, WikiPathways, among others), we only selected Gene Ontology (GO) biological pathways (GO:BPs). Clustered data was visualized through Cytoscape (https://cytoscape.org). We considered an adjusted *p*-value of $\leq 10^{-5}$, corrected for multiple testing (Benjamini-Hochberg), statistically significant.

To verify whether the shared GO:BPs were also enriched in the single EOAD+ and LOAD + gene co-expression networks, we performed GO term enrichment analysis using gProfiler (https://biit.cs.ut.ee/gprofiler/gost; database version: Ensembl 104, Ensembl Genomes 51, Wormbase ParaSite 15). We annotated only the top significant GO:BPs, considered as an adjusted p-value of $\leq 10^{-5}$, corrected for multiple testing.

We then performed the same procedure for both the shared and single gene networks in the EOA and LOA gene groups.

2.5. Step 5: Temporal gene expression analysis in the cerebellum over nine developmental stages

To investigate whether the clinical distinction into early- and late-onset ataxia, with the cut-off point at 25 years of age [2,15], is reflected by distinct temporal expression levels of EOAD + versus LOAD + genes, and EOA versus LOA genes, we compared average cerebellar expression levels before and after 25 years of age between EOAD+ and LOAD+, and between EOA and LOA. RNAseq data of 16 human brain structures over 26 time points (indicating age, given in postconceptional weeks, months, or years) were publicly available as reads per kilobase per million (RPKM) from BrainSpan (Gencode v10, Atlas of the Developing Human Brain, https://www.brainspan.org/static/home). Additional information on the background of the RNA samples can be found on the abovementioned website. For our analysis, we extracted RNAseq data of the cerebellum.

To analyze possible differences in gene expression levels over different time-segments, we classified the 26 time points into nine developmental stages (S1–S9, from the pre-to postnatal period; see Supplementary Fig. 1), according to Eidhof et al. (2019) [23]. We then removed duplicate genes between the groups and visualized the cerebellar expression of merged EOA/LOA and EOAD+/LOAD + genes over the nine developmental stages. For this purpose, a hierarchical clustered heatmap was generated using Pearson correlation through the Morpheus analysis software (https://software.broadinstitute.org/morpheus). Finally, we compared cerebellar expression levels of the EOAD+ and LOAD+, and of the EOA and LOA genes in the stages encompassing cerebellar development (S1–S8), taking the age of 18 years as cut-off point for the end of cerebellar development, as described in literature [11,24–27].

2.6. Statistical analyses

Statistical analyses were performed using SPSS, version 28.0 (IBM SPSS Statistics for Windows, 2021). To analyze associations between the MRI lesions and dystonic comorbidity, we used the Chi-square Test of Independence or the Fisher's Exact Test. Afterwards, to test the strength and the direction of these relationships, we used the Bivariate Pearson Correlation.

We used log10-transformed (i.e., normalized) RNA-seq data to perform unpaired t-tests and compare the gene expression levels between EOAD+ and LOAD+, and between EOA and LOA before and after the age of 25 years, as described in section 2.1.5. The same methodology was used for the analysis of gene expression levels during cerebellar development (S1–S8). We then plotted these data in box and whisker plots using GraphPad Prism (version 9.4.0, for Windows; GraphPad Software, San Diego, California, USA, 2022. Available at: www.graphpad.com).

To investigate differences in gene expression levels over time, we first performed descriptive statistics of the nine developmental stages per each gene group, followed by ordinary one-way repeated measures ANOVA to compare mean expression levels of the nine developmental stages with each other. We set the significance level at $\alpha = .05$.

3. Results

3.1. Frequency of comorbid dystonia in EOA and LOA

We generated comprehensive lists of EOA and LOA genes, comprising 241 and 93 genes respectively (Supplementary Files I and II). Comorbid dystonia was reported in cases with pathogenic variants in 117 of the 241 EOA genes (48.5%, EOAD+) and in 75 of the 93 LOA genes (80.6%, LOAD+) (Supplementary Files I and II). 67 genes were identified as overlapping between EOA and LOA groups, and 43 genes were overlapping between EOA, LOA and mixed ataxia-dystonia (Supplementary Files VII and VIII, Supplementary Figs. 3 and 4).

3.2. Anatomical lesions in EOA and LOA with and without comorbid dystonia

For all reported cases with pathogenic variants in EOAD ± and LOAD ± genes, anatomical MRI damage was described in four cerebellar and eight extracerebellar regions (Supplementary File IX). Reported prevalence of cerebellar lesions was similar for all gene groups (Table 1, Supplementary Tables I and II). Extracerebellar lesions at the a) pons and/or basal ganglia and/or thalamus, and b) cerebral cortex and/or basal ganglia and/or thalamus were significantly associated with both EOAD+ and LOAD+ (Table 1), but not with EOAD- or LOAD-gene subgroups (Supplementary Tables I and II).

3.3. Gene network-, pathway-, and clustering analysis in EOA, LOA and mixed ataxia-dystonia

To investigate whether EOAD+ and LOAD + share underlying biological pathways, we generated a shared brain-specific gene co-expression network for both gene groups, followed by GO term

Table 1
Anatomical MRI findings reported in both EOAD+ and LOAD + gene groups.

Brain structure	EOAD+	LOAD+
Cerebellum (*in toto*)	82.1% $p = .212$	89.3% $p = .238$
Pons AND/OR basal ganglia AND/OR thalamus	58.1% $p = <.001$ $r = .22$, $^{\#}p = <.001$	44.0% $p = .010$ $r = .27$, $^{\#}p = .009$
Basal ganglia AND/OR thalamus AND/OR cerebral cortex	74.4% $p = <.001$ $r = .22$, $^{\#}p = <.001$	25.3% $p = .019$ $r = .25$, $^{\#}p = .046$

Footnote. Frequency (expressed in percentage, %) of anatomical MRI lesions and the statistical significance (given by the *p*-value, Chi-square/Fisher's exact test) of their association with comorbid dystonia in the EOAD+ and LOAD + groups. The correlation coefficient (*r*) is given for significant associations, along with the significance level ($^{\#}p$) of the correlation. The complete overview of anatomical damage in EOAD ± and LOAD ± can be found in Supplementary Tables I and II, respectively. AND/OR = lesions reported in both or either of these structures; EOAD+/− = early-onset ataxia with or without comorbid dystonia, LOAD+/− = late-onset ataxia with or without comorbid dystonia.

biological pathways (GO:BP) enrichment and clustering analysis. In this shared gene co-expression network, we identified 158 enriched clusters, comprising a total of 855 GO:BPs (Supplementary Files X, XI and XII). The top significant clusters shared between EOAD+ and LOAD + included pathways related to signaling, homeostatic-, metabolic- and cellular processes, such as localization, transport, and organization (Table 2, Fig. 2, Supplementary File XII).

We then investigated whether the GO:BP clusters found in the shared EOAD+ and LOAD + gene co-expression network were also enriched in the shared EOA and LOA gene co-expression network. Similar significant clusters were observed for EOA and LOA as for EOAD+ and LOAD+ (Supplementary File XIII). These clusters were enriched for GO:BPs involved in neural signaling, nervous system development, metabolic- and cellular processes, including transport and organization (Table 3, Fig. 3, Supplementary File XIV). The GO:BP brain development (including cerebellar and hindbrain development) was enriched in both the EOA and LOA gene groups (Table 3), as well as in the EOAD+ and LOAD + gene groups (Supplementary File X).

Moreover, when we subsequently investigated the single gene co-expression networks for EOAD+, LOAD+, EOA and LOA gene groups, we noted that these were all significantly enriched for the GO:BP nervous system development (Supplementary Tables III and IV).

3.4. Temporal cerebellar expression analysis of EOA, LOA and mixed ataxia-dystonia genes

To investigate whether the clinical distinction of ataxia into EOA and LOA corresponds with different temporal expression levels of EOAD+, LOAD+, EOA and LOA genes in the cerebellum, we analyzed cerebellar gene expression levels before and after the clinical cut-off at 25 years of age, and compared these between EOAD + versus LOAD+, and between EOA versus LOA genes. We observed no significant difference in average cerebellar expression levels before versus after 25 years of age in all gene groups (Fig. 4).

When investigating possible differences in gene expression levels over time, we observed that hierarchical clustering of the temporal cerebellar expression data of the merged EOAD+/LOAD+ and EOA/LOA gene groups did not reveal marked clusters over the nine developmental stages (Supplementary Figs. 6 and 7). We also did not observe a significant difference in mean expression levels between the developmental stages of each gene group (Supplementary Tables V-VIII). Finally, the comparison of average gene expression levels during cerebellar development between EOAD+ and LOAD+, and between EOA and LOA showed no significant difference (Fig. 5).

Table 2
Top significant GO:BP clusters enriched in the shared EOAD+ and LOAD + gene co-expression network.

Parent terms	GO:BP	*p*-value (Log10)
Signaling	Synaptic signaling	−18.97
Cellular process - Cellular localization	Intracellular protein transport	−15.76
Cellular process - Cellular component organization	Membrane organization	−14.62
Cellular process - Cellular localization	Vacuolar transport	−12.24
Cellular process – Transport	Vesicle-mediated transport in synapse	−11.75
Cellular process – Cellular metabolic process	Energy derivation by oxidation of organic compounds	−11.61
Cellular process – Transport	Cation transmembrane transport	−11.18
Cellular process - Cellular localization	Organelle localization	−10.61
Cellular process – Transport	Golgi vesicle transport	−9.61
Cellular process - Cellular component organization	Mitochondrion organization	−9.47
Biological regulation – Homeostatic process	Regulation of membrane potential	−9.08
Metabolic process	Autophagy	−8.95
Cellular process – Regulation of cellular process	Regulation of vesicle-mediated transport	−8.75

Footnote. Top GO:BP clusters enriched in the shared EOAD+ and LOAD + gene co-expression network. The gene co-expression network consisted of EOAD + genes, LOAD + genes and predicted genes from Metabrain. The *p*-value is expressed in log-base 10, corrected for multiple testing. Only GO:BP cluster representative terms (i.e., umbrella terms for the whole cluster) were automatically selected. Parent terms are given for each GO:BP, indicating the broader terms of which the specific GO:BP is part of; i.e., "intracellular protein transport" is a form of "cellular localization", which in turn is a "cellular process". For the complete list of GO:BP enriched clusters, see Supplementary File X. GO:BP = gene ontology biological pathway; EOAD+ = early-onset ataxia with comorbid dystonia; LOAD+ = late-onset ataxia with comorbid dystonia.

4. Discussion

To the best of our knowledge, this is the first *in silico* study exploring the possibility of one pathogenetic disease continuum between EOA, LOA and mixed ataxia-dystonia. To do so, we investigated the association of a large set of 267 ataxia genes with phenotypes of comorbid dystonia in literature and linked these phenotypes with reported anatomical MRI damage and underlying pathogenetic mechanisms. Our data revealed strong similarities between the EOA, LOA and mixed ataxia-dystonia gene groups. In both EOA and LOA, our findings revealed an association between comorbid dystonia and extracerebellar MRI abnormalities at the anatomical cortico-basal-ganglia-pontocerebellar (CBPC) network. Furthermore, the functions of EOA, LOA and mixed ataxia-dystonia genes converged into similar biological pathways, including brain development, neural signaling, metabolic- and cellular processes, such as transport and organization. Finally, between the EOA, LOA and mixed ataxia-dystonia groups there was neither a difference in cerebellar gene expression before or after the clinical cut-off age of 25 years, nor in temporal gene expression during cerebellar development. Overall, our findings in EOA, LOA and mixed ataxia-dystonia suggest the presence of one disease continuum. These data question the clinical relevance of classifying and diagnosing ataxia as separate EOA and LOA disease groups.

The first indication for a pathogenetic continuum arises from our anatomical results. The substantial percentage (≈65%) of mixed ataxia-dystonia cases reported in literature, along with the 65% of comorbid dystonia cases in a clinical EOA cohort [5], may reduce the likelihood that these disorders incidentally co-occur as single entities. Also, as expected, our *in silico* work confirms that EOA, LOA and mixed ataxia-dystonia can be regarded as network disorders [5,8,28,29], related to damage of the anatomical nodes within the CBPC network, 30 including the basal ganglia, pons, thalamus, cerebral cortex or all these extracerebellar structures concomitantly. Furthermore, the absent association of extracerebellar damage in EOA and LOA without comorbid dystonia may indicate that damage to one or multiple extracerebellar nodes within the CBPC network may specifically lead to mixed ataxia-dystonia phenotypes. One may accordingly speculate that the heterogeneity of these mixed movement disorder phenotypes could result from a combination of coexisting lesion types in the cerebellum and in extracerebellar structures [31]. As a consequence of genetic defects, especially during the highly vulnerable period of brain development, impaired neuroplasticity may affect the cerebellum and its connections, causing altered synaptic homeostasis and neural transmission [11,32,33]. This might in turn result in dysfunctional interactions between the interconnected motor centers within the CBPC network and to compensatory reactions in non-damaged structures [29,30,32], hindering the motor output of the CBPC network and possibly leading to mixed ataxia-dystonia [11].

On a biological level, the many shared GO:BPs between EOA, LOA and mixed ataxia-dystonia reinforce the suggestion of a disease continuum. These shared biological pathways comprised synaptic signaling, energy metabolism-related processes, and brain development, including

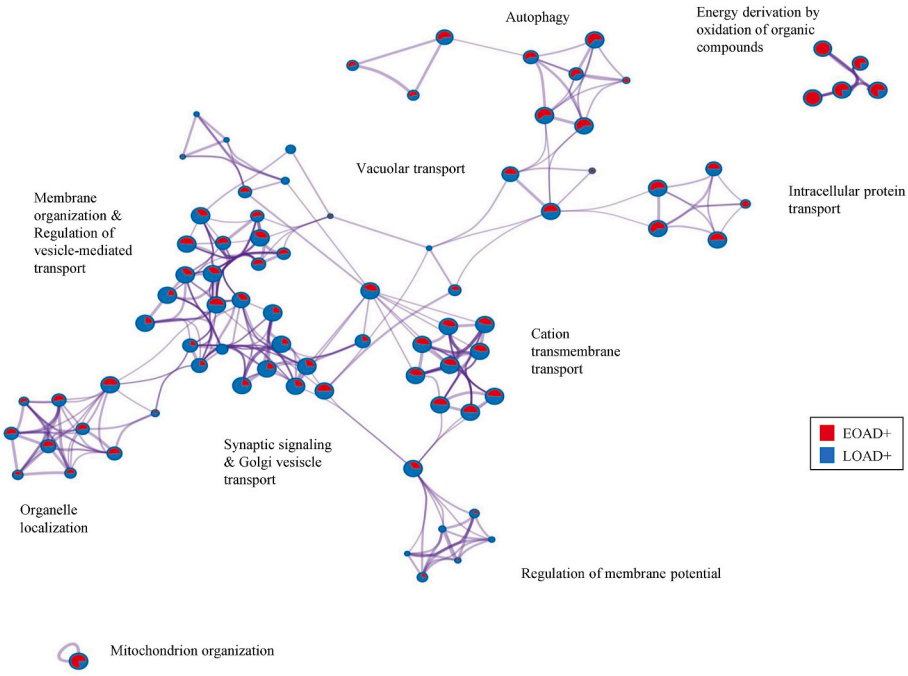

Fig. 2. Top significant GO:BP clusters enriched in the shared EOAD+ and LOAD + gene co-expression network. Network plot of the top significant enriched GO:BPs in the shared EOAD+ and LOAD + gene co-expression network. GO:BP terms are represented as color-coded pie charts based on their enrichment in the EOAD+ and/or LOAD + genes, where red = enriched in the EOAD + genes, and blue = enriched in the LOAD + genes. All the major GO:BP terms are shared between EOAD+ and LOAD + genes. Biological pathways related to energy metabolism included energy derivation by oxidation of organic compounds and autophagy. Homeostatic processes included the GO:BP regulation of membrane potential. Biological pathways related to cellular transport included intracellular protein-, vacuolar, cation transmembrane- and Golgi vesicle transport, vesicle-mediated transport in synapse, and regulation of vesicle-mediated transport. Finally, GO:BPs related to cellular organization were membrane and mitochondrion organization. See Supplementary File X for a detailed description of the clusters and their corresponding pathways. GO:BP = gene ontology biological pathway; EOAD+ = early-onset ataxia with comorbid dystonia; LOAD+ = late-onset ataxia with comorbid dystonia. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Top significant GO:BP clusters enriched in the shared EOA and LOA gene co-expression networks.

Parent terms	GO:BP	*p*-value (Log10)
Cellular process - Cellular localization	Intracellular protein transport	−16.11
Nervous system development	Brain development	−14.59
Signaling	*Trans*-synaptic signaling	−14.58
Cellular process - Cellular component organization	Membrane organization	−13.15
Nervous system development	Cerebellum development	−10.43
Cellular process - Cellular component organization	Vesicle organization	−11.84
Cellular process – Transport	Golgi vesicle transport	−11.05
Cellular process - Cellular component organization	Organelle organization	−11.04
Metabolic process	Protein catabolic process	−10.94
Cellular process	Microtubule-based process	−10.62
Cellular process - Cellular localization	Vacuolar transport	−10.50
Metabolic process	Cellular amide metabolic process	−10.37
Cellular process - Cellular component organization	Mitochondrion organization	−10.00

Footnote. Top GO:BP clusters enriched in the shared EOA and LOA gene co-expression networks. The gene co-expression network consisted of EOA genes, LOA genes and predicted genes from Metabrain. The *p*-value is expressed in log-base 10, corrected for multiple testing. Only GO:BP cluster representative terms (i.e., umbrella terms for the whole cluster) were automatically selected. Parent terms are given for each GO:BP, indicating the broader terms of which the specific GO:BP is part of; i.e., "intracellular protein transport" is a form of "cellular localization", which in turn is a "cellular process".
For the complete list of GO:BP enriched clusters, see Supplementary File XIII. GO:BP = gene ontology biological pathway; EOA = early-onset ataxia; LOA = late-onset ataxia.

hindbrain and cerebellum, among others. Aberrations of these biological mechanisms were previously described in both recessive and dominant ataxia, and in dystonia [5,6,11,23,34]. As such, these biological mechanisms could be involved in the pathogenesis of these disorders regardless of the phenotype. Disturbances of these processes may have different consequences depending on the underlying genetic defect and its effect at protein level [23]. For example, impaired synaptic signaling due to Purkinje cell degeneration may be related to pathogenetic variants in: 1) mitochondrial tRNA aminoacylation genes, such as *RARS2*, leading to EOA(D+) [35–37], or 2) transcriptional regulator genes, like *ATNX1*, leading to LOA(D+) [23,38,39]. Interestingly, biological processes related to cellular organization and nervous system development were enriched in all gene groups, possibly suggesting a common developmental origin for ataxia-dystonia. However, although our work may give some insight in the shared underlying biology of EOA, LOA and mixed ataxia-dystonia, it remains difficult to pinpoint the exact mechanisms underlying these disorders. This is mainly because the above-mentioned biological processes can converge into similar molecular mechanisms. As such, aberrations of one biological process may often lead to dysregulation of another.

Regarding the timing of gene expression, our findings indicate that the clinical classification of ataxia into EOA and LOA is not reflected by distinct temporal gene expression patterns. This is shown by the fact that for all genes no difference was observed in cerebellar gene expression levels before versus after 25 years of age, nor during cerebellar development. As such, our data may question the utility of the clinical classification of ataxia into EOA and LOA, and of the cut-off at 25 years of age. An example against this distinction may be illustrated by carriers of some SCA gene mutations. In these subjects, moderately high gene expression in early developmental stages (Supplementary Figs. 4 and 5), along with early anatomical damage to cerebellar pathways [40], may result in prodromal symptoms up to 18 years before clinical presentation, such as deterioration of motor performance, quantified by increased SARA scores [41,42], sensory abnormalities and/or cognitive impairments [43]. Therefore, on a pathogenetic level, the distinction between EOA and LOA with or without comorbid dystonia is not fully justifiable. Until now, the underlying cause for the variability of age of disease onset is still unclear. Based on our findings, we suggest that the timing of disease presentation, either early or later in life, may result from an interplay of factors, encompassing anatomical damage, the underlying genetic variant and the resulting effects at protein level, as outlined above. Moreover, the presence of congenital malformations of the posterior fossa [2], triggering environmental factors, such as trauma, metabolic derangement or fever [2,11], and biological phenomena, such

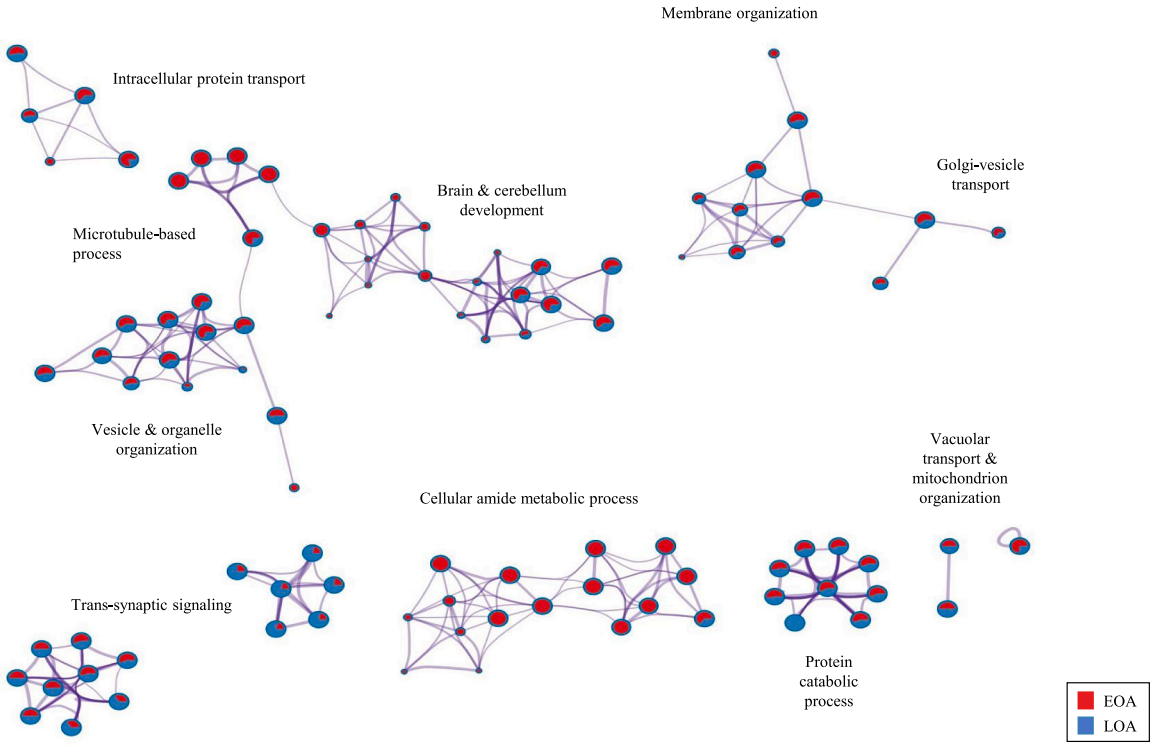

Fig. 3. Top significant GO:BP clusters enriched in the shared EOA and LOA gene co-expression network. Network plot of top significant enriched GO:BPs in shared gene-networks. GO:BP terms are represented as color-coded pie charts based on the gene affiliation (i.e., enriched in the EOA and/or LOA genes), where red = enriched in the EOA genes, and blue = enriched in the LOA genes. All the major GO:BP terms are shared between EOA and LOA genes, besides the GO:BP cellular amide metabolic processes, being mainly enriched for EOA genes. Biological pathways related to nervous system development included brain and cerebellum development, those related to energy metabolism included protein catabolic- and cellular amide metabolic processes. GO:BPs related to cellular transport included intracellular protein-, Golgi vesicle and vacuolar transport. Finally, cellular organization processes included the GO:BPs membrane, vesicle, organelle, mitochondrion organization and microtubule-based processes. A detailed description of the clusters and their corresponding pathways can be found in Supplementary File XIII. GO:BP = gene ontology biological pathway; EOA = early-onset ataxia; LOA = late-onset ataxia. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

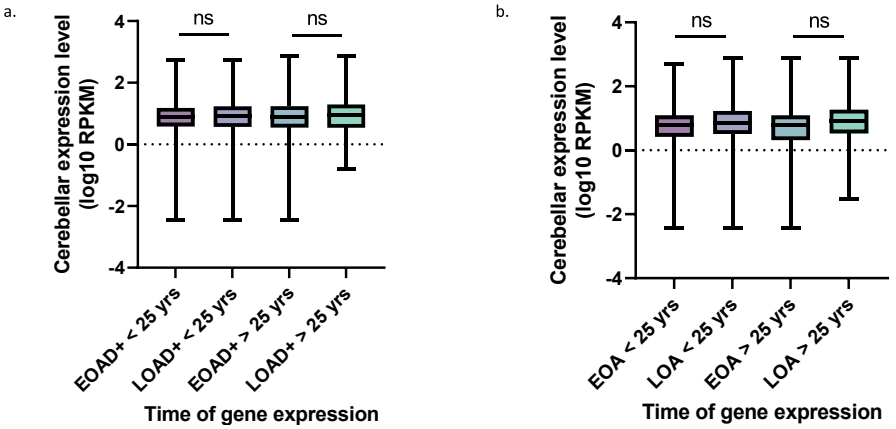

Fig. 4. Comparison of average expression levels before and after 25 years of age. Box and whisker plots showing average cerebellar expression levels (given in Reads per Kilobase Million, RPKM, in log10) before and after 25 years of age in a. EOAD+ and LOAD + genes, and b. EOA and LOA genes Data was extracted from the BrainSpan database. The age of 25 years was chosen as cut-off point according to the clinical distinction into early- and late-onset ataxia. An unpaired *t*-test showed no significant difference in the cerebellar expression levels before and after the age of 25 years, between a. EOAD+ and LOAD+, and b. EOA and LOA genes. ns = not significant; EOAD+ = early-onset ataxia with comorbid dystonia; LOAD+ = late-onset ataxia with comorbid dystonia; EOA = early-onset ataxia; LOA = late-onset ataxia; <25 yrs = before 25 years of age; >25 yrs = after 25 years of age.

as genetic heterogeneity [44] and phenotypic pleiotropy [45], may also influence the age of disease onset.

We are aware that the diagnostic algorithm for EOA differs from that of LOA with regards to the early-stage screening for congenital malformations with MRI [2]. However, there are strong similarities in the genetic work-up of both gene groups, as genes frequently overlap between EOA and LOA. For this reason, and under the premise that EOA, LOA and mixed ataxia-dystonia can be regarded as one pathogenetic disease continuum, our study supports the use of broader diagnostic genetic investigations in clinical practice. For example, using a combined WES-based movement disorder gene panel, where (new) genes of interest can be included in the filter strategy, may reduce the diagnostic delay [46] and the probability of uncovering unsolicited findings [47]. However, in clinical practice the application of WES data may still be challenging, especially with regards to variant interpretation [48] and detection of structural variations and repeat sequences [49]. In the near

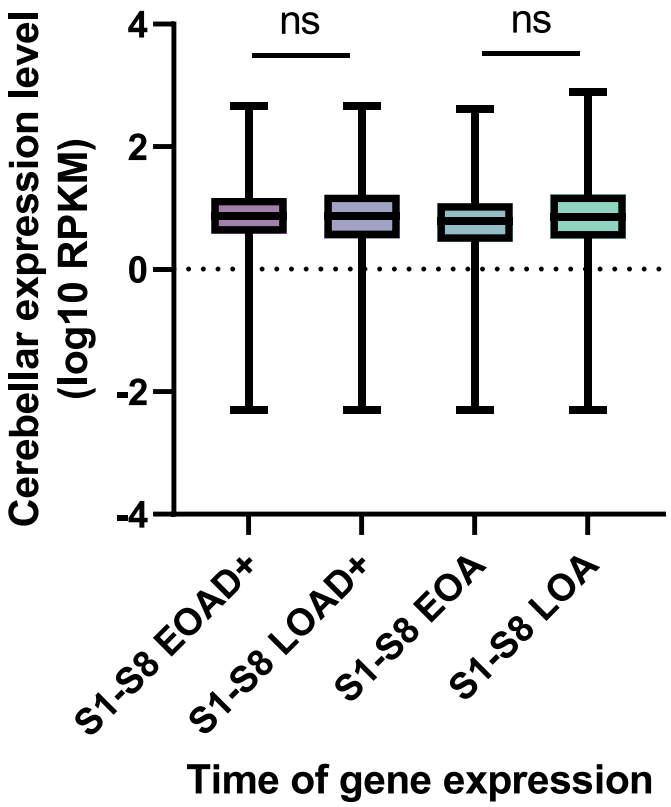

Fig. 5. Comparison of average expression levels during cerebellar development. Box and whisker plots showing average cerebellar expression levels (given in Reads per Kilobase Million, RPKM, in log10) during cerebellar development stages per each gene group. Data was extracted from the BrainSpan database. We compared the developmental stages during cerebellar development (S1–S8, embryonic stages until 18 years of age) in EOAD + vs LOAD+, and in EOA vs LOA. The description of each developmental stage is found in Supplementary Fig. 1. An unpaired *t*-test showed no significant difference between the expression levels in each gene group during cerebellar development. ns = not significant; EOA = early-onset ataxia; EOAD+ = early-onset ataxia with comorbid dystonia; LOA = late-onset ataxia; LOAD+ = late-onset ataxia with comorbid dystonia.

future, the use of long-read sequencing may hopefully overcome the latter technical issue.

We recognize some limitations to this study. First, we performed this study using the ataxia UMCG gene panels and supplemented these with genes recommended by the European EOA expert working group2 and with new candidate SCA genes.6 However, we are aware that gene panels may differ per genetic center and per version update. This may illustrate the importance of using common sets of genes, shared between genetic centers, over local gene panels. Furthermore, we are aware that the numerical imbalance between the lists of EOA and LOA genes could introduce a statistical bias in our study. Nonetheless, as our data is retrieved from literature, these numbers are fixed for the time being. Finally, *in silico* algorithms detect patterns within the data and may consequently overlook specific gene characteristics.

5. Conclusion

Altogether, our findings suggest the existence of a disease continuum for EOA, LOA and mixed ataxia-dystonia phenotypes. These data question the clinical relevance of classifying and diagnosing ataxia according to the age of onset into separate EOA and LOA gene groups, with or without comorbid dystonia. Our findings may thus support a unified genetic diagnostic approach for ataxic and dystonic movement disorder phenotypes.

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Author contribution

Conceptualization: MG, DSV, DAS; methodology: MG, DSV, DAS; analysis: MG; writing – original draft: MG DSV, DAS; writing – review and editing: MG, FV, DSV, DAS. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

D.A. Sival and D.S. Verbeek are members of the European Reference Network for Rare Neurological Diseases. The authors declare no conflict of interest.

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2023.02.005.

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