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Early onset ataxia with comorbid myoclonus and epilepsy: A disease spectrum with shared molecular pathways and cortico-thalamo-cerebellar network involvement

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ABSTRACT

Objectives: Early onset ataxia (EOA) concerns a heterogeneous disease group, often presenting with other comorbid phenotypes such as myoclonus and epilepsy. Due to genetic and phenotypic heterogeneity, it can be difficult to identify the underlying gene defect from the clinical symptoms. The pathological mechanisms underlying comorbid EOA phenotypes remain largely unknown. The aim of this study is to investigate the key pathological mechanisms in EOA with myoclonus and/or epilepsy.

Methods: For 154 EOA-genes we investigated (1) the associated phenotype (2) reported anatomical neuroimaging abnormalities, and (3) functionally enriched biological pathways through *in silico* analysis. We assessed the validity of our *in silico* results by outcome comparison to a clinical EOA-cohort (80 patients, 31 genes).

Results: EOA associated gene mutations cause a spectrum of disorders, including myoclonic and epileptic phenotypes. Cerebellar imaging abnormalities were observed in 73–86% (cohort and *in silico* respectively) of EOA-genes independently of phenotypic comorbidity. EOA phenotypes with comorbid myoclonus and myoclonus/epilepsy were specifically associated with abnormalities in the cerebello-thalamo-cortical network. EOA, myoclonus and epilepsy genes shared enriched pathways involved in neurotransmission and neurodevelopment both in the *in silico* and clinical genes. EOA gene subgroups with myoclonus and epilepsy showed specific enrichment for lysosomal and lipid processes.

Conclusions: The investigated EOA phenotypes revealed predominantly cerebellar abnormalities, with thalamo-cortical abnormalities in the mixed phenotypes, suggesting anatomical network involvement in EOA pathogenesis. The studied phenotypes exhibit a shared biomolecular pathogenesis, with some specific phenotype-dependent pathways. Mutations in EOA, epilepsy and myoclonus associated genes can all cause heterogeneous ataxia phenotypes, which supports exome sequencing with a movement disorder panel over conventional single gene panel testing in the clinical setting.

1. Introduction

Early onset ataxia (EOA) refers to a rare group of heterogeneous, genetically heritable coordination disorders with symptom onset before the 25th year of life. EOA is caused in large by autosomal recessively inherited genes. Phenotypically, EOA can present with a wide range of symptoms including ataxia with comorbid dystonia, chorea, myoclonus and epilepsy [1]. For instance, in our previous EOA study, mildly

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dystonic features were observed in over 60% of EOA cases [2]. It remains unknown whether comorbid EOA presentations are due to distinct neurological conditions or can be considered a part of a disease continuum. In the clinical setting it is challenging to predict a single genotype from the phenotype, and vice versa. The evaluation of the underlying gene defects and corresponding molecular pathways could help to understand the pathogenic processes that cause the heterogeneous EOA presentations and thereby improve testing strategies.

Previous research on EOA with comorbid dystonia, identified neurotransmission and neurodevelopment as shared pathological mechanisms [2]. The finding that EOA and dystonia converge in common molecular pathways, suggests that rather than discrete entities, these comorbid presentations might form a disease spectrum. As stated, clinical presentations of EOA also often include comorbid myoclonus and epilepsy. The pathogenesis of EOA shares a myriad of biological pathways with myoclonus and epilepsy [7–9] such as ion channel or GABA-ergic signalling dysfunction, but which pathways cause the comorbid presentations remains unclear. It is likely that the underlying pathways cause cellular dysfunction in multiple neuronal subtypes and brain areas, resulting in a combination of neurological symptoms. Recent research has suggested the involvement of different brain regions in EOA including the (sensorimotor) cortex, thalamus, basal ganglia, brainstem and cerebellum, and it has consequently been proposed that EOA could be a network disorder emerging from cerebello-thalamo-basal ganglia-cortical pathway (CTC) disturbance [3,4]. Since epilepsy is generally considered to have a cortical origin and genetic myoclonus is considered to be of subcortical or cortical origin [5,6] we therefore hypothesized that CTC network dysfunction could underlie EOA with comorbid myoclonus and/or epilepsy. The relation between the locus of regional CTC network dysfunction and the EOA phenotype is still unclear.

In the present study, for EOA phenotypes presenting with comorbid myoclonus and/or epilepsy we therefore investigated the relation between localized anatomical damage as reported in imaging studies, and underlying biological pathways from disease genes.

2. Materials and methods

2.1. Generation of clinically relevant gene list

Genetic panels from the Department of Genetics of the University Medical Centre Groningen (UMCG) were consulted in January 2021 and used to generate gene lists for EOA (154 genes), early onset myoclonus (94 genes, myoclonus onset before 18 years of age) and epilepsy (203 genes) (Supplementary Table I). Genes with exclusively adult-onset phenotypes were excluded. We standardised gene names to HGNC nomenclature using GeneCards (https://www.genecards.org) and OMIM (https://omim.org/). Genes were clustered into groups according to potential phenotype, namely EOA with comorbid myoclonus and epilepsy (EOAM + E+), EOA with comorbid myoclonus (EOAM+) and EOA with comorbid epilepsy (EOAE+). The group of ataxia genes without myoclonic or epileptic comorbidities (EOA-) was used as control.

2.2. Imaging abnormalities in EOA subgroups

For all EOA gene mutations, we recorded presence, type and location of brain abnormalities on magnetic resonance imaging (MRI) in affected patients, based on reported abnormalities in the OMIM database and additional literature search in PubMed (performed February 2021). The MRI abnormalities were categorized based on: presence of white- or gray matter abnormalities, type of abnormalities (atrophy, developmental abnormalities or other), and anatomical location.

Additionally, in a previously described clinical cohort of 80 EOA-patients from the UMCG, the Netherlands [2], we retrospectively recorded imaging (MRI) abnormalities according to presence and location of neuroanatomical damage. Patients were grouped based on the clinically observed phenotype (EOA, EOAM+, EOAE+, EOAM + E+).

For dichotomous outcome variables, Fisher exact tests were performed. For categorical variables logistic regression was performed. Significance level was set at $\alpha < 0.05$.

2.3. Biological pathway enrichment analysis

Biological pathway analysis was performed to explore the shared and unique biological mechanisms that underlie the phenotype in the described gene subgroups. To do so, we used an integrated functional enrichment analysis tool (g:GOSt feature) g:Profiler [10]. In this tool, genes are associated with available functional information, namely signalling and biochemical pathways. Subsequently the significantly overrepresented 'enriched' terms are identified. We performed (1) a comparative multi-query analysis of all early onset ataxia, myoclonus and epilepsy genes to identify overrepresented shared pathways and (2) a pathway enrichment analysis in the specific gene subsets with phenotypic overlap to identify biological pathways unique to these phenotypes. Considering the multi-query input gene list size, biological pathways were considered significantly enriched when $\alpha < .01$ whereas $\alpha < 0.05$ was used for the enrichment analysis in the gene subsets. Correction for multiple testing was performed with the g:SCS algorithm, as previously described [11].

In addition, in order to assess clinical relevance of the *in silico* analysis, we performed a third analysis using genetic data from the historical cohort. Genes were grouped according to the clinical presentation with epilepsy and/or myoclonus, in line with the approach of the *in silico* analysis. To expand the clinical dataset, we performed network enrichment using the clinical genes as seeds. In agreement with previously published methods [12], we used GeneNetwork, a co-expression tool integrating 31,499 public RNA-seq samples [13] to generate gene co-expression networks. GeneNetwork automatically performed cluster analysis. Subsequently, functional enrichment of biological pathways was performed as described above, with significance at $\alpha < 0.05$.

3. Results

3.1. Shared genes between EOA, myoclonus and epilepsy

We identified a total of 13 genes shared in EOA, myoclonus and epilepsy (EOAM + E+) gene panels. Additionally, we identified 35 shared genes in EOA and myoclonus (EOAM+) gene panels, and 26 shared genes in EOA and epilepsy gene panels (EOAE+) (Table 1). The gene functions were heterogeneous, as reflected by the former group of gene mutations linked to EOAM+E+ phenotypes which associated with transportopathies (*SLC2A1, FOLR1*), channelopathies (*CACNA1A*, *SCN8A*), synaptopathies (*STXBP1*), mitochondrial function (*POLG:*

Table 1
Shared genes in EOA, myoclonus and epilepsy based on gene panels. A total of 35 genes are shared in EOA and myoclonus (EOAM+), 26 genes in EOA and epilepsy (EOAE+), and 13 genes in EOA, myoclonus and epilepsy (EOAM + E+).

Phenotypic overlap	Genes
EOAM+	*ATM, ATP7A, ATP7B, BRAT1, CACNA1A, CACNB4, CAMTA1, CLCN2, CLN3, CLN5, COQ8A, CSTB, CYP27A1, EIF2B5, FOLR1, GFAP, GOSR2, HEXA, HEXB, KCNC3, KCND3, NKX2-1, NPC1, NPC2, PIGA, POLG, PPT1, PRKCG, PSAP, SACS, SCN8A, SEMA6B, SLC2A1, STXBP1, TPP1*
EOAE+	*ATP1A3, BRAT1, CACNA1A, CLN3, CLN5, FOLR1, GOSR2, KCNJ10, OPHN1, PIGA, PIGN, PLP1, PNKP, POLG, PPT1, PRRT2, QARS1, SCN8A, SLC1A3, SLC2A1, SLC9A6, STXBP1, SYNGAP1, TPP1, UBA5, WWOX*
EOAM + E+	*BRAT1, CACNA1A, CLN3, CLN5, FOLR1, GOSR2, PIGA, POLG, PPT1, SCN8A, SLC2A1, STXBP1, TPP1*

mDNA replication, *BRAT1:* DNA damage), intracellular trafficking (*GOSR2, PIGA*), and lysosomal function (*TPP1, CLN3, CLN5, PPT1*).

3.2. Imaging abnormalities

For all EOA genes (n = 154), the presence and type of associated imaging abnormalities were recorded (shown in Supplementary Tables II and III). The anatomical location of the abnormalities included cerebellum, cerebral cortex, corpus callosum, thalamus, hippocampus, basal ganglia, pons, brainstem and spinal cord. Cerebellar abnormalities were reported in 86% of EOA genes from all gene subgroups. Additionally, EOAM+E+ genotypes were significantly associated with abnormalities at the thalamus (OR 6.75; 95% CI 1.47 to 31.01, $p = .030$), cortex (OR 9.15, 95% CI 1.16 to 72.30, p=.016), and cortex and/or thalamus (OR 8.89; 95% CI 1.13–70.24, $p = .016$). Thalamocortical abnormalities were also significantly associated with EOAM + genotypes (thalamus: OR 8.00; 95% CI 1.89 to 33.92, $p = .005$, cortex and/or thalamus: OR 2.71; 95% CI 1.14–6.45, p=.030), whereas EOAE + genotypes were not significantly associated with lesions at a specific location. White matter abnormalities were more frequently present in the EOAE + gene subgroup (OR 2.85, 95% CI 1.10 to 6.89, p=.029) than in the other gene subgroups. The most frequently reported abnormalities at the thalamus were signalling abnormalities, including T2 hypointensity (e.g. in neuronal ceroid lipofuscinosis) and T2 hyperintensity (e.g. Wilson's disease, mitochondrial disease). For the cerebellum atrophy was most frequent (global in most, limited to the vermis in about 30%). In some, developmental abnormalities such as agenesis of the cerebellum were described. No marked correlations were observed between the gene subgroups and the type of damage.

For illustration, we included some characteristic findings in Fig. 1.

Last, we assessed whether the phenotypes from our clinical cohort were attributable to the underlying imaging abnormalities. The MRI data was available for 64 patients (49 with known gene mutation), and imaging abnormalities were found in 58% (37/64) of the cases (Supplementary Table IV). When abnormalities were observed, these were located within the cerebello-thalamo-cortical network in 86% of patients (32/37, p=<.001), to which the cerebellar abnormalities were the greatest contributor (73% of patients). Consecutive sub-analysis per phenotypic group showed no significant associations with a specific intracranial location. However, we did observe EOA M+ phenotypes were significantly associated with damage within cerebello-thalamo-cortical network locations (OR 3.67; 95% CI 1.30–10.32, p=.023).

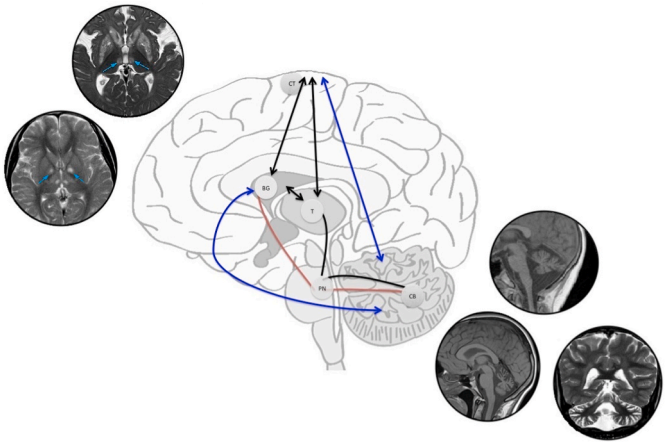

Fig. 1. Schematic representation of the cerebello-thalamus-basal ganglia-cortical circuitry
The images (clockwise, starting top left) illustrate reported thalamic abnormalities, here T2 hyperintensity in patients with an mtDNA gene mutation (Kearns Sayre Syndrome and Leigh Syndrome); frequently seen cerebellar (vermis) atrophy in patients with *AOA4* and *CACNA1A* mutations, and global and (<) cerebellar atrophy in *CLN2*. It is important to note many patients with EOA present without imaging abnormalities. The arrows show the interconnected circuitry. Black: 'traditional' pathways from cerebellum through pontine nuclei, via thalamus and potentially basal ganglia to cortex and vice versa; Red: 'Basal ganglia' pathways, via pons and subthalamic nucleus, without thalamic relay; Blue: 'Hyperdirect' pathways, namely direct connections between cerebellum and cortex and via basal ganglia (substantia nigra/globus pallidus and striatum) without relay in the pons
Abbreviations BG: basal ganglia, CB: cerebellum, CT: cortex, PN: pontine nuclei, T: thalamus. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. Biological pathway enrichment analysis

3.3.1. Shared biological pathways in EOA, childhood myoclonus and epilepsy

Through comparative enrichment analysis of the EOA, myoclonus and epilepsy gene sets, we generated an integrated enrichment map. We identified 29 biological pathways that are significantly enriched in all three gene sets (Fig. 2, Supplementary Table V). The most significantly enriched pathways were central nervous system development and synaptic signalling. Additionally, significantly enriched pathways included transmembrane transport, neuromuscular processes, locomotory behaviour and organelle organization.

3.3.2. Biological pathways in EOA gene subgroups

We further explored the biological pathways enriched in the gene subgroups for the comorbid phenotypes (Fig. 3). In the EOAM+E+ gene subgroup, we obtained 19 significantly enriched biological pathways (Supplementary Table VI), predominantly involved in lysosomal processes. The top three pathways were lysosomal lumen acidification ($p = 5.3 \times 10^{-5}$), regulation of lysosomal lumen pH ($p = 2.0 \times 10^{-4}$) and lysosome organization ($p = 2.6 \times 10^{-4}$). In the EOAM + gene subgroup, there were 13 significantly enriched biological pathways, which were similarly involved in metabolic processes (Supplementary Table VII). The top three pathways were locomotory behaviour ($p = 2.6 \times 10^{-4}$), glycosphingolipid catabolic process ($p = 9.4 \times 10^{-4}$) and glycolipid catabolic process ($p = .0014$). Sub-analysis of the genes in the 'locomotory behaviour' pathway showed involvement in glycosaminoglycan catabolic and phospholipid biosynthetic processes. Last, the EOAE + gene subgroup analysis revealed 14 significant biological pathways (Supplementary Table VIII) associated with synaptic transmission. The top three pathways were import into cell ($p = 3.77 \times 10^{-4}$), import across plasma membrane ($p = .0042$) and chemical synaptic transmission ($p = .0043$). In the EOA gene subgroup (EOA-, without epileptic or myoclonic comorbidity) there were 23 significantly enriched biological pathways involved in a variety of processes such as neurogenesis, mitochondrial function, ion transport, and DNA regulation (Supplementary Table IX).

3.3.3. Comparative analysis in a clinical cohort

Genetic information was available for 65 patients in our cohort of 80 EOA patients [2], (Supplementary Table IV). Clinically, comorbid myoclonus was present in 37% (24/65) and comorbid epilepsy in 23% (15/65) of the EOA patients. The observed clinical phenotypes strongly correlated with the expected phenotype based on genetic mutation information from gene panels (Pearson's $r = 0.671$, p = .000 for EOAE+, Pearson's $r = 0.494$, $p = .000$ for EOAM+). A total of 31 different mutated genes were present in the cohort, which were grouped according to phenotype: 15 mutated genes in patients with EOA without comorbidity, 7 mutated genes in patients with comorbid epilepsy, and 17 mutated genes in patients with comorbid myoclonus. Mutations in the *ATP1A3*, *CACNA1A* and *SPTBN2* genes revealed phenotypic pleiotropy for the investigated comorbidity (see Supplementary Table X). The gene-gene co-expression network analysis yielded gene networks for EOAE+, EOAM+ and EOA-. Consistent with our in silico findings, the EOAM+ and EOAE + gene networks were enriched for biological

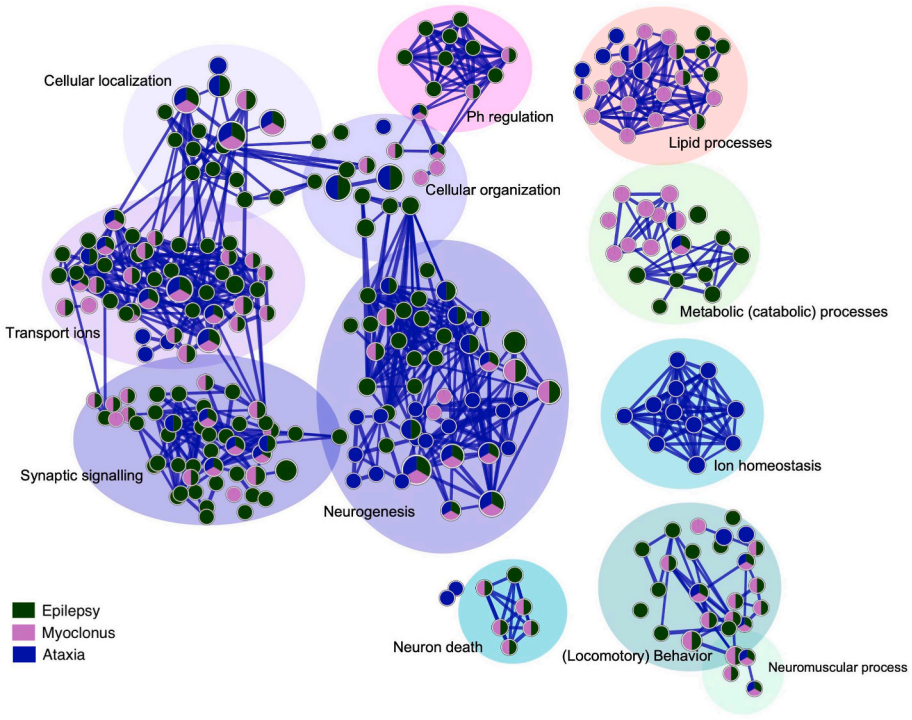

Fig. 2. Enrichment map for biological pathways in the EOA, myoclonus and epilepsy gene sets. Nodes represent significantly enriched biological pathways in the gene sets ($\alpha < .01$). Node size corresponds to the number of genes in the set. Node color represents the gene set in which the biological pathway is enriched (blue: early onset ataxia genes, green: epilepsy genes, pink: myoclonus genes). Visualization generated with Enrichment map in Cytoscape (version 3.8.2). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

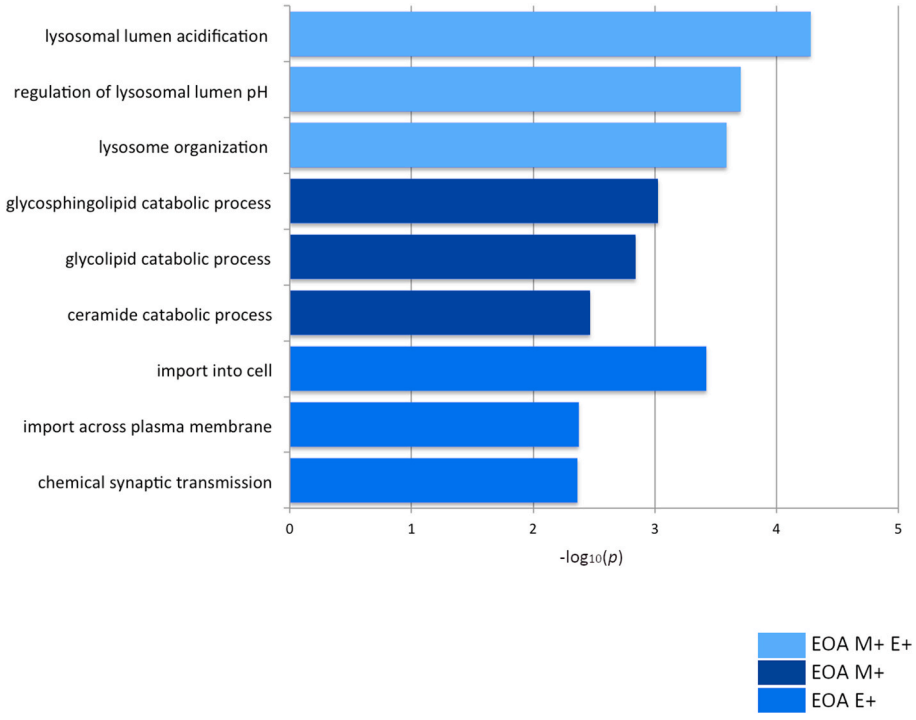

Fig. 3. Top enriched biological pathways from the *in silico* analysis in the genetic subgroups for EOAM + E+, EOAE+ and EOAM+.

pathways involved in neurogenesis and metabolic processes (see Supplementary Table XI).

4. Discussion

In the present study we investigated shared anatomical and biological pathways for EOA with comorbid myoclonus and epilepsy. Our study highlights the association between thalamic and cortical abnormalities and EOA with comorbid myoclonus and epilepsy. The movement disorder comorbidity in EOA could therefore be attributed to involvement of a larger neural network, in interplay with cerebellar damage. Neurogenesis and neurotransmission were the most significantly enriched biological pathways shared by EOA, epilepsy and myoclonus genes. The observed overlap in biological pathways and potential network involvement suggest the mixed EOA-phenotypes are part of a disease spectrum. In addition to shared pathways, we also

found specific pathways enriched in the comorbid EOA gene groups, importantly lysosomal pathways and lipid metabolic pathways in EOAM+E+ and EOAM + genes respectively, and synaptic transmission pathways in EOAE+. These unique phenotype-specific biological pathways highlight the importance of adequate clinical phenotyping in the diagnostic process as it points towards the likelihood of specific genetic involvement.

We report specific cortical and thalamic abnormalities in cases with gene mutations linked to EOAM+E+ and EOAM+, which point at a role for extra-cerebellar damage in EOA. Analogous to the association of cerebello-basal ganglia-thalamo-cortical (CTC) networks with EOA-dystonia phenotypes, the observed abnormalities in this work reflect CTC involvement in EOA with comorbid myoclonus and epilepsy as well. This association was also observed in the clinical cohort, where CTC abnormalities predominated and were significantly associated with comorbid myoclonus phenotypes. Taken together this indicates EOA should be considered a network disorder. Network involvement is increasingly being recognized in the pathogenesis of neurological disorders, including epilepsy and movement disorders such as dystonia [3,15–17]. Tractography studies have demonstrated the presence of intricate interconnected bidirectional pathways in the CTC loop [18,19]. We therefore suggest that the etiological heterogeneity in EOA phenotypes might be attributed to interference in the cortico-cerebellar pathways (Fig. 1). Considering that epilepsy is generally associated with cortical dysfunction and genetic myoclonus with (sub-)cortical dysfunction, the thalamo-cortical associations to EOA with epileptic and/or myoclonic comorbidity were not unexpected. Previous research has demonstrated a role for the thalamus in juvenile myoclonic epilepsy and generalized epilepsy, including thalamic volume reduction and/or thalamocortical network alterations [20–22]. Similarly, a growing body of evidence points at involvement of basal ganglia-cerebellar connections in EOA-dystonia phenotypes, with multiple cases of secondary dystonia emerging from cerebellar lesions [15,22]. How ataxia and myoclonus could arise from thalamic damage, or ataxia and dystonia from cerebellar damage, can best be explained though the network perspective: a pathological process in one area triggers a reorganization within the connected circuitry causing a variety of seemingly unrelated symptoms. In addition, the network perspective contributes to understanding why gene mutations with expression in multiple cell types and brain regions not limited to the cerebellum, cause phenotypes with cerebellar pathology. We are aware that the reported correlation between phenotype and anatomical damage needs to be verified in a large clinical EOA cohort. In our current EOA cohort, imaging abnormalities in the cerebellum predominated, with abnormalities in thalamic and cortical structures being less frequent (thalamic T2 signal abnormalities were present in one patient). The relatively large number of patients we reported without imaging abnormalities in the cohort might be explained by the childhood onset and evaluation of these disorders, potentially presenting with MRI abnormalities later in the disease course (e.g. due to neurodegeneration). However, when abnormalities were present in our patient cohort, these were related to the CTC network in 86% of cases. Considering EOA a network disorder opens a new perspective in understanding the pathogenesis, which might widen treatment opportunities. For instance, deep brain stimulation (DBS) of the thalamus has previously been shown to reduce seizure frequency [23,24], improve myoclonus [25,26] and essential tremor [27], and might therefore be used in this patient group. However thalamic (CTC) stimulation should be considered carefully, as ataxia can be a side effect of DBS [27]. In the future, we hope that larger multi-center trials and application of more advanced imaging techniques such as diffusion tensor imaging or functional MRI in EOA patients will elucidate the phenotypic effect of underlying network dysfunction to further extent.

The observed anatomical damage might be related to dysfunction in biological pathways. The unique phenotype-specific pathways found in the EOAM+E+ and EOAM + genes, which were largely involved in metabolic processes, illustrate this. First, we found significantly enriched lysosomal pathways in the EOAM+E+ genes. Lysosomes are dynamic organelles that participate in cellular homeostasis, especially in glycoprotein and lipid homeostasis, and regulate autophagy and mitophagy [28,29]. Additionally, lysosomes can influence signal transduction, as shown in mammalian mTORC1 pathways [30,31]. In lysosomal diseases, accumulation of undigested macromolecules causes inflammation and neurodegeneration in vulnerable regions, such as the thalamus and cerebellum [32]. Altered thalamic signal intensity and severe neurodegeneration are a hallmark both in patients and mice models of lysosomal diseases [33–35]. During cerebellar development, increased lysosomal activity is observed in synapse elimination around Purkinje cells [36,37], suggesting dysfunctional lysosome activity disrupts synaptic pruning. Additionally, Purkinje cell loss can be induced by interference with autophagosome-lysosome fusion and aberrant mitophagy [38–40], with resulting cell death independently of metabolite accumulation [41]. Not surprisingly lysosomal dysfunction has also been implicated in (late onset) ataxias such as SCA6 and SCA7 [42,43], and underlies neurodegeneration in progressive myoclonus epilepsies [31,44]. Therefore, in EOAM+E+ phenotypes, lysosomal dysfunction is likely linked to the preferential degeneration of cerebellum and thalamus. Second, in EOAM + genes, we report enrichment of lipid catabolic processes, suggesting dysfunctional lipolysis might also play a role in the comorbid phenotype. Ataxia disorders have previously been associated with dysfunctional lipid homeostasis, such as in SCA3, SCA34, SCA38, Friedreich's ataxia and Niemann Pick type C [35,45–48] and EOA with comorbid dystonia [2]. Lipid metabolism within the brain is tightly regulated to maintain neuronal structure and function [49]. Dysfunctional lipolysis can cause progressive accumulation of macromolecules, with consequent neuronal stress and degeneration [44], specifically in vulnerable regions such as the described thalamus and cerebellum. Furthermore, lipid composition of neuronal and glial cell membranes affects cell function and neurotransmission [49,50]. This is illustrated by cholesterol, which is required for normal prenatal development, regulation of neurotransmission [49,51,52], and has been specifically linked to Purkinje cell functioning in mice [53]. We hypothesize that mutations in genes operating in these metabolic molecular pathways could cause both EOAM+E+ and EOAM + phenotypes. With at least 900 genes involved in lysosomal pathways and lipid homeostasis [53], potential candidates for genetic contribution to myoclonus and/or epilepsy EOA phenotypes are expected to be identified in the future. The association of mixed phenotypes with neurometabolic pathways shows the importance of clinical phenotyping for diagnostic recognition and application of testing strategies in EOA patients.

Despite the importance of phenotyping, the large overlap in biological pathways in the studied groups underlines the need for early complete genetic testing. We found shared neurogenesis and neurotransmission pathways in EOA, epilepsy and myoclonus genes. Previously, we have also reported these pathways in shared EOA and dystonia genes, strengthening the notion that shared pathophysiology may cause these mixed EOA phenotypes (see Fig. 4). In addition, the enriched pathways from the clinical cohort genes were in line with *in silico* shared EOA, myoclonus and epilepsy biological pathways. The overlap between the *in silico* based pathways and the clinical cohort based pathways confirms these findings may have important implications for the diagnostic approach in EOA patients. Genes involved in the development of the nervous system and synaptic function can affect different neuronal subtypes both in cortical and subcortical networks, and thus thereby may lead to a complex clinical picture with ataxia, epilepsy or other motor dysfunction. The overlap in molecular pathways shows how a range of EOA, epilepsy and myoclonus gene mutations can all cause mixed ataxia presentations. Therefore, genetic testing with a single EOA gene panel might cause diagnostic delay. Whole Exome Sequencing (WES) with a complete movement disorder panel/filter and copy number variation analysis is predicted to increase diagnostic yield [14], and is therefore advised as an early step in the clinical setting.

We recognize several limitations to this study. First, we used

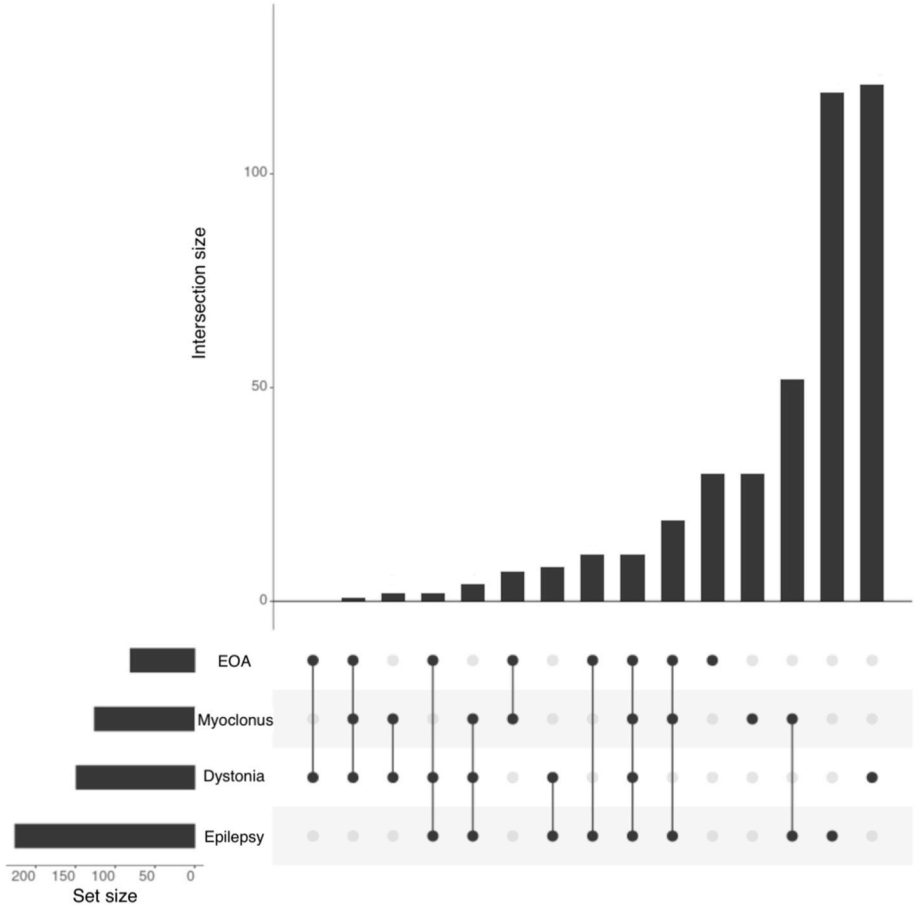

Fig. 4. Biological pathway overlap between EOA, epilepsy, myoclonus and dystonia. The connected circles represent overlapping pathway groups. The intersection size shows unique overlap patterns. Note more than half of EOA pathways are shared with comorbid disorders, most notably myoclonus and epilepsy. Diagram generated with UpSetR [56].

predefined clinical gene panels, which may not be complete as the discovery of new genes continuously expands these gene lists. The current ongoing effort to revise the nomenclature of genetically determined movement disorders [54] will hopefully standardize these in the future. However, to the best of our knowledge, most relevant and common up-to-date EOA, myoclonus and epilepsy genes are included. Second, we did not consider the mutation types in the genes. For the phenotype, this could be relevant, for instance considering the potential different consequences between gain-of-function and loss-of-function genetic mutations [9,55]. Last, we did not assess gene expression in different brain regions to correlate biological pathway and structural abnormalities. For this work we chose reported imaging abnormalities: most of the gene mutations underlying complex EOA mixed phenotypes are expressed in multiple cells and brain regions and therefore, expression was predicted to be insufficient to explain the phenotypic differences.

In summary, we explored the underlying anatomical and biological pathways in EOAM+E+ and showed shared CTC network involvement with concurrent pathogenetic pathways. Involvement of CTC network structures was previously reported in EOA-dystonia, strengthening the notion that EOA should be evaluated as a network disorder. Specific lysosomal and lipid metabolism pathways emerged in the comorbid EOAM+E+ phenotypes. Clinically, this finding emphasizes the importance of biochemical (metabolic laboratory) testing in the comorbid myoclonic phenotypes, and the need of future genetic research to further explore metabolic pathways. Neurodevelopment and synaptic transmission are shared pathophysiological pathways in all EOA phenotypes, and shared with epilepsy, myoclonus and dystonia. Considering the large number of relatively uncommon ataxia genotypes with phenotypic overlap, this data supports diagnostic genetic testing through Whole Exome Sequencing (WES) with complete movement disorder panel and copy number variation analysis, instead of testing a single EOA gene panel. Through unraveling the genetic pathways of mixed EOA phenotypes, this study hopes to increase the pathophysiological understanding of childhood ataxia, which contributes to the identification of new candidate genes and might provide therapeutic targets in the future.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2023.05.009.

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