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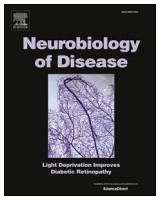

Review

Integrative human and murine multi-omics: Highlighting shared biomarkers in the neuronal ceroid lipofuscinoses

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ABSTRACT

Neuronal ceroid lipofuscinosis (NCL) is a group of neurodegenerative disorders whose molecular mechanisms remain largely unknown. Omics approaches are among the methods that generate new information on modifying factors and molecular signatures. Moreover, omics data integration can address the need to progressively expand knowledge around the disease and pinpoint specific proteins to promote as candidate biomarkers.

In this work, we integrated a total of 62 proteomic and transcriptomic datasets originating from humans and mice, employing a new approach able to define dysregulated processes across species, stages and NCL forms. Moreover, we selected a pool of differentially expressed proteins and genes as species- and form-related biomarkers of disease status/progression and evaluated local and spatial differences in most affected brain regions.

Our results offer promising targets for potential new therapeutic strategies and reinforce the hypothesis of a connection between NCLs and other forms of dementia, particularly Alzheimer's disease.

1. Introduction

The collective term neuronal ceroid lipofuscinosis (NCL) refers to a group of inherited neurodegenerative disorders that affect children and young adults, and are characterized by retinopathy leading to blindness, ataxia and gait abnormalities, drug-resistant epilepsy, mental deterioration, and early death. The genetic landscape of NCL, also known as Batten disease, is highly heterogeneous with thirteen known disease forms to date (Table 1), associated with over 400 mutations in several genes entered in the NCL database (*NCL resource mutation database,* 2021). The NCLs are usually inherited according to an autosomal recessive pattern, although a rare, autosomal dominant adult-onset form has been identified (Mole et al., 2012).

The disease has a worldwide distribution with an incidence range calculated to be 1.28/100.000 live births, and there are about 6–700 new diagnoses each year. In around 9.7% of cases, however, mutations cannot be demonstrated in any of the known NCL genes in spite of a typical NCL clinical presentation (Santorelli et al., 2013; Simpson et al., 2014; Sleat et al., 2016). These cases remain molecularly undefined.

Cases are classified by: *i*) the age at disease onset (congenital, infantile, late infantile, juvenile, adult), *ii*) the designation of the mutated gene (*CLN*), *iii*) the characteristics of autofluorescent storage material accumulated in lysosomes and *iv*) the ultrastructural features of the cytosomes (Simonati and Williams, 2022).

No treatment other than palliative care is currently available for NCL (Iwan et al., 2021; Kohlschütter et al., 2019), with the exception of a cohort of patients with neuronal ceroid lipofuscinosis type 2 (CLN2 disease) undergoing enzyme replacement therapy (Brineura™, Cerliponase alfa) (Specchio et al., 2020). Poor information on disease pathophysiology, and therefore on possible disease biomarkers, is a major obstacle to clinical trials in this field. Exploration of disease pathways likely shared by NCLs, such as oxidative phosphorylation, mitochondrial bioenergetics, autophagy (either macro- or mitophagy), and lysosomal clearance, may facilitate the process of biomarker discovery, making it possible to identify novel targets useful for monitoring disease status and progression, and accelerating trial readiness in patients.

The research field has recently benefited from the development of omics approaches allowing the generation of huge amounts of

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information even from small numbers of biological samples or cases.

Transcriptomics, in particular, has advanced considerably in recent years. Microarray platforms and high-throughput RNA sequencing have allowed high coverage of the human transcriptome, as well as that of the main animal models of the disease.

Proteomics, on the other hand, involving the use of mass spectrometry-based technologies, provides the most accurate insight into cellular physiology and disease biomarkers. Furthermore, new emerging disciplines are allowing the assessment of metabolomic and lipidomic analyses, increasing available information on the interplay between genes and the environment.

In this setting, one of the major challenges when seeking to determine the mechanisms underlying biological processes is sample complexity, given the possible presence of splice variants and/or post-translational modification (Al-Amrani et al., 2021); another is the large volume of generated data, which require a validation process using different, *non*-omics approaches.

Although human samples, such as biological fluids, patient fibroblasts, and post-mortem tissues are, traditionally, the main samples used to investigate genetic, clinical, and biochemical factors, the increasing availability of *in vitro* and *in vivo* disease models has facilitated our understanding of disease pathophysiology and the screening of possible therapeutic targets.

Both spontaneous and engineered animal models (listed in Mole and Gardner), as well as KO and overexpressing cell lines (Doccini et al., 2020; Pezzini et al., 2017; Scifo et al., 2013, 2015) and multicellular model organisms (Huber et al., 2020), have been developed for several NCL forms.

The use of murine models has uncovered novel aspects of the disease, identifying certain brain areas, including the thalamus, cortex, and cerebellum, and more recently the spinal cord, as the most vulnerable affected regions (Cooper et al., 2006; Nelvagal et al., 2020b; Radke et al., 2015), clinically relevant for defining status and progression of the disease (Doccini et al., 2020; Eaton et al., 2019; Hirz et al., 2017; Katz et al., 2017; Nelvagal et al., 2020b; Oswald et al., 2005, 2008; Perentos et al., 2016; Russell et al., 2018; Tikka et al., 2016).

Despite the increasing information obtained through research on NCL, the molecular networks that regulate the onset and progression of the disease are still not completely known.

Even though the various NCLs differ clinically, their genes seem to be part of a common network where abnormal lysosomal function leads to different pathological conditions, including defective autophagy and bioenergetic dysfunction, that undermine cell survival. Phenotypic, pathophysiological similarities between CLN3, CLN6, and CLN8, and their protein interactomes suggested that the three proteins participate in shared pathways essential for neuronal function (Rechtzigel et al., 2022). Moreover, together with the lysosomal hydrolases PPT1 and TPP1, they complement each other in the modulation of cell growth and apoptosis (Yap et al., 2021). Co-immunoprecipitation and *in vitro* binding assays revealed several physical interactions between NCL proteins including CLN2, CLN3, CLN5, CLN6, CLN8, CLN12 (Bajaj et al., 2020; Lyly et al., 2009; Vesa et al., 2002; Wang et al., 2023) suggesting a key role in lysosome biogenesis and autophagic defects. Shared interacting partners between CLN3 and CLN5 proteins were also identified by Tandem Affinity Purification coupled to Mass Spectrometry (TAP-MS), with bridging proteins involved in autophagy, mitochondrial dysfunction and calcium binding (Scifo et al., 2013). Targeting autophagy in NCLs, may represent a powerful strategy for the development of effective therapies. Particular attention has been paid to the coordinated lysosomal expression and regulation (CLEAR) transcription factor binding site, which controls the expression of many *endo*-lysosomal proteins, and this, in turn, has led to the definition of a pathway, which includes the transcription factor EB (TFEB) and mTORC1, with a considerable influence on cellular functions (Fraldi et al., 2016; Settembre et al., 2013; Sharma et al., 2018). To date, TFEB, a master regulator of autophagy which promotes the clearance of cellular storage material, is one of the most promising targets for the development of new treatments in NCLs and other lysosomal storage diseases (Kim et al., 2022; Palmieri et al., 2017; Soldati et al., 2021).

The mitochondrial compartment, too, is implicated in several NCLs,

Table 1
Human neuronal ceroid lipofuscinoses variants.

Disease	Eponym	Affected Gene	Clinical Phenotype	Gene Product	Biochemical phenotype
CLN1	Haltia–Santavuori	*CLN1/PPT1*	Classic infantile, late infantile, juvenile, adult*	PPT1 (soluble protein)	lysosomal enzyme (palmitoyl-thioesterase)
CLN2	Janský–Bielschowsky	*CLN2/TPP1*	Classic late infantile, juvenile*	TPP-1 (soluble protein)	lysosomal enzyme (serine protease)
CLN3	Spielmeyer–Sjögren	*CLN3*	Juvenile*	CLN3/battenin	membrane protein (6 TMD)
CLN4	Parry	*CLN4/ DNAJC5*	Adult autosomal dominant*	DNAJC5 (soluble protein)	co-chaperone involved in *exo*-endocytosis cytosol
CLN5	Finnish variant late infantile, variant juvenile (previously CLN9)	*CLN5*	Late infantile variant, juvenile, adult*	CLN5 (soluble protein)	lysosomal enzyme bis(monoacylglycero) phosphate synthase
CLN6	Early juvenile (Lake Cavanaugh), late infantile Costa Rican-Indian variant, adult Kuf type A	*CLN6*	Late infantile variant*, adult (Kuf, type A)*	CLN6	membrane protein (7 TMD)
CLN7	Turkish variant late infantile	*CLN7/ MFSD8*	Late infantile variant*, juvenile*, adult*	MFSD8	membrane protein (12 TMD); transporter?
CLN8	Northern epilepsy, progressive EPMR	*CLN8*	Late infantile variant EPMR*	CLN8	membrane protein (5 TMD)
CLN10	Congenital	*CLN10/ CTSD*	Congenital classic*, late infantile*, adult*	Cathepsin D	lysosomal enzyme aspartyl-endopeptidase
CLN11	Adult variant	*CLN11/ GRN*	Adult*	Progranulin	granular protein
CLN12	Juvenile variant	*CLN12/ ATP13A2*	Juvenile, Kufor–Rakeb syndrome*	P-type ATPase	membrane protein (10 TMD)
CLN13	Adult Kuf type B	*CLN13/ CTSF*	Adult Kuf type*	Cathepsin F	lysosomal enzyme cysteine protease
CLN14	Infantile	*CLN14/ KCTD7*	Infantile, progressive myoclonus epilepsy 3*	Potassium channel tetramerization domain- containing protein 7 (soluble protein)	unknown

Abbreviation: EPMR, epilepsy with mental retardation; TMD trans-membrane domains.
* These diseases have neurological involvement.

playing a key role in the initiation of the apoptotic cascade that triggers the process of neuronal death. Several mitochondrial genes/proteins have been found to be highly compromised in different NCLs, and mitochondrial dysfunction has emerged as a significant pathological pathway (Das et al., 1999; Doccini et al., 2020; Jolly et al., 2002; Kline et al., 2020; Pezzini et al., 2017; Scifo et al., 2013; Tikka et al., 2016).

Another important issue is the role of Ca^{2+} signaling in NCL pathophysiology, given that it acts as an upstream regulator of neuronal excitability (Demontis et al., 2020) and is also directly connected with the accumulation of the disease hallmark (intracellular subunit C of mitochondrial ATP synthase) (McGeoch and Guidotti, 2001). Mounting evidence points to a role for CLN3 protein in Ca^{2+} homeostasis and autophagic flux as a leading cause of the pathology (An Haack et al., 2011; Bosch and Kielian, 2019; Chandrachud et al., 2015; Chang et al., 2007); moreover, in CLN8 neurons a reduced mitochondrial Ca^{2+} buffering capacity has been demonstrated, leading to the neurodegenerative signs of the disease (Kolikova et al., 2011). A putative calmodulin-binding domain has also been identified within the sequence of almost all NCL proteins (Mathavarajah et al., 2018), making this, too, a possible therapeutic target. However, the precise links between Ca^{2+} signaling and the NCL proteins remain to be elucidated, and further efforts are needed to clarify the involvement of mitochondrial and autophagic processes (Moloudizargari et al., 2017).

The development of omics approaches has facilitated the identification of disease signatures and the cellular mechanisms involved in complex pathological conditions, including neurodegeneration (Mirza et al., 2019; Song et al., 2020).

Despite an emerging role for metabolomics and lipidomics in efforts to understand NCL pathogenesis (Evers et al., 2017; Sindelar et al., 2018), to date, it is proteomics and transcriptomics that have provided the majority of available datasets. Data are currently available for most of the NCLs, particularly CLN1, CLN2, CLN3 and CLN5 (Fig. 1).

Several omics studies have been carried out on different tissues and fluids derived from patients and mice, as well as on engineered cell models. However, other vertebrate and invertebrate organisms have been used, too, including sheep and *Dictyostelium* (Huber, 2017; Huber and Mathavarajah, 2018, 2019; Iwan et al., 2021).

By means of proteomic and transcriptomic approaches, several palmitoyl protein thioesterase 1 (PPT1) interactor partners have been identified, expanding the role of *CLN1* in the de-palmytoilation process and the ensuing effects on synaptic function and NCL phenotypes (Gorenberg et al., 2022; Pezzini et al., 2017; Scifo et al., 2015). In $Cln1^{-/-}$ mice, progressive glial activation, early vulnerability of spinal interneurons, and affected functional modules emerged from proteomic profiling (Nelvagal et al., 2020a) and a region-specific proteomic analysis (Tikka et al., 2016).

The transcriptomic profile in the $Cln2^{-/-}$ murine model (Domowicz et al., 2019) linked the loss of TPP1 enzyme activity to neuronal injury highlighting pathways linked to microgliosis and astrogliosis, particularly at cerebellar level.

Recurrent pathological features associated with altered bioenergetics and autophagy induction have also been proposed for CLN3 disease where the use of proteomics on a $Cln3^{-/-}$ mouse model made it possible to identify molecular modulators related to the increased synaptic vulnerability (Llavero Hurtado et al., 2017; Zhong et al., 2020). Lysosomal proteomics in the *in vitro* CLN3 disease model also showed altered lysosomal homeostasis and cellular trafficking processes, functionally connecting CLN3 disease with proteins and signaling pathways specific to neurons (Schmidtke et al., 2019).

We demonstrated oxidative stress, together with autophagy processes and impaired bioenergetics, by means of an organelle-specific quantitative proteomic approach in CLN5 disease models. Altered mitochondrial and lysosomal compartments point to a possible crosstalk between organelles through mitophagy activation and the involvement of lipid metabolism (Doccini et al., 2020, 2022). Using the same approach, affected $Cln5^{-/-}$ mice were analyzed at different disease stages, making it possible to identify molecular processes related to progression, including redox imbalance which already occurs in the early stage of the disease.

A double KO mouse model ($Cln1^{-/-}/Cln5^{-/-}$) (Blom et al., 2013) supported the hypothesis of convergent pathological pathways between these two forms of the disease and highlighted a worst pathological phenotype characterized by early demyelination, cortical astrocytosis, and microglial activation.

Analyzing results provided by a single omics approach may often lead to misinterpretations, as the lack of standardized protocols and of a harmonized toolkit for data analysis makes findings difficult to replicate between laboratories. On the other hand, the increasing number of omics studies has allowed the identification of recurrent pathological mechanisms related to different NCL forms, while the possibility to interpret datasets using multi-omics workflows has clarified the cross-correlation of the disease processes, as well as the trans-species connections (Sun and Hu, 2016). A systematic integrative analysis using the available omics datasets can potentially shed light on dynamic interactions and altered processes with further implications for the identification of disease markers that may be used for early diagnosis or as therapeutic targets.

In two recent studies, Sleat and colleagues (Sleat et al., 2017, 2019) identified several candidate biomarkers related to CLN1, CLN2, and CLN3 disease, highlighting a significant change in expression of different lysosomal proteins and markers of neuroinflammation. Furthermore, they identified altered proteins shared between the analyzed forms, matrixes, and/or species.

Recently, Iwan and colleagues (Iwan et al., 2021) investigated the relevance of candidate biomarkers obtained by proteomic analysis of urine samples from CLN2 patients as well as ovine models of CLN5 and CLN6 disease. The protein expression levels were further analyzed by a targeted mass spectrometry assay on an extended cohort of patients affected by CLN1, CLN3, CLN5, CLN6, and CLN7. The results suggested the presence of at least four candidate proteins that might be developed as possible surrogate biomarkers of disease status.

Although overlap analyses, as well as single omics datasets, are

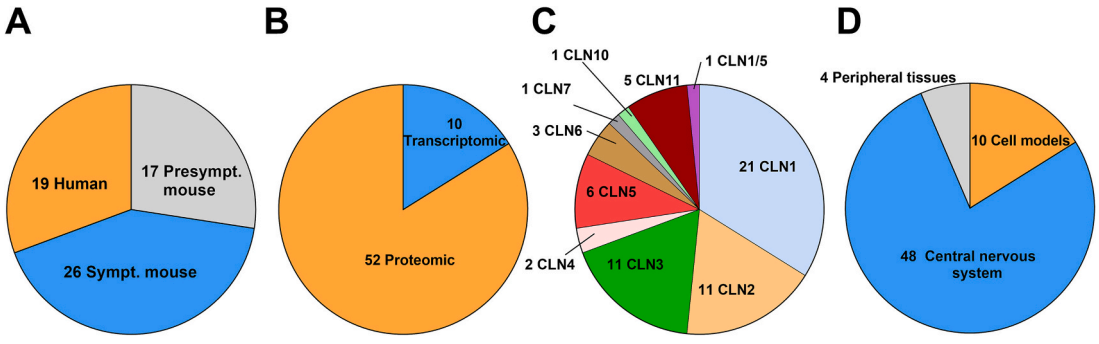

Fig. 1. Classification of datasets by: (A) species; (B) type of omics data; (C) NCL forms; (D) tissues.

offering growing evidence concerning the molecular networks underlying the disease, an increasing number of cross-omics approaches are driving the development of integrative bioinformatics analyses able both to enhance the specificity of the bioinformatic data and increase the amount of information produced (Rotroff and Motsinger-Reif, 2016).

The first attempt to integrate available omics data on NCL was made in 2019 by Kline and colleagues who superimposed proteomic datasets referring to humans and mouse models (Kline et al., 2020). Overlap analysis revealed conserved alterations in oxidative phosphorylation and mitochondrial dysfunction as the main dysregulated canonical pathways across all studies. The data obtained from the CLN1–3 datasets further consolidated the finding of mitochondrial dysfunction, showing a conserved alteration in the electron transport chain components. Furthermore, the analysis revealed activation of the rapamycin-insensitive companion of mammalian target of rapamycin (RICTOR) gene as a master gene regulating downstream protein changes in NCLs. Despite the high transversality of the study, the increasing availability of data suggests that there is now a need for further studies, to better explain the differences between analyzed species, and for the use of omics techniques, to further expand and harmonize the information.

For the present study, we systematically collected and reviewed all the proteomic and transcriptomic studies available in the NCL literature and set out to provide an integrative perspective by developing a proper bioinformatic filtering system to dissect common pathological features of the disease.

We applied an efficient integrative approach, seeking to identify shared dysregulated pathways across the NCLs by combining information from untargeted proteomic and transcriptomic studies. Moreover, we evaluated possible trans-species connections by analyzing human results and murine datasets at different disease stages.

Our analyses raised the possibility of relationships between NCL and other more common forms of dementia.

2. Material and methods

A total of 62 omics datasets from both proteomic and transcriptomic studies were collected through a literature search and by consulting the lists generated for our recent studies. They refer to central nervous system ($n = 48$ datasets), cell models ($n = 10$), and peripheral tissues (n = 4) (Fig. 1). Supplementary Table S1 reports the references of all the datasets included in our database. Fig. 2 outlines the filtering strategy and subsequent bioinformatic investigation. Filter parameters are detailed in Supplementary Table S2.

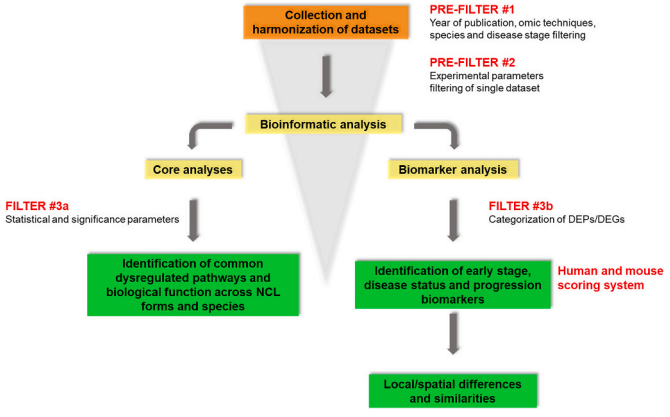

Fig. 2. Description of filtering strategy and subsequent bioinformatic investigation.

2.1. Setup of the filtering system and generation of the harmonized database

For each dataset, proteins and genes were selected by considering a significance value <0.05 for all comparisons between the control and disease groups. For proteomic datasets we considered accession identifiers (IDs) with ≥2 counted unique peptides, whereas for transcriptomic datasets, we excluded transcripts that did not have any Ensembl protein ID (ENSP ID, www.ensembl.org) counterpart. In this way, we selected only transcripts referable to a protein product. To harmonize all the omics datasets, all IDs were converted into Ensembl gene IDs (ENSG IDs), used as unique reference accession numbers.

Datasets referring to the same disease form were classified based on expression data and common IDs were averaged following outlier removal performed according to Tukey (k = 1.5).

A single list of proteins/genes per NCL was provided, keeping the information obtained from proteomic and transcriptomic studies separate. For each omics technique, we also prepared a single dataset including averaged data (average proteomic, average transcriptomic) for all available NCL forms in order to facilitate the bioinformatic analysis and more easily pinpoint common features in Batten disease. Supplementary Tables S3-S5 are the final data sheets used for the overlap analyses. Bioinformatic analysis was conducted using Ingenuity Pathway Analysis (IPA™) (Qiagen, Hilden, Germany; IPA Summer Release - July 2021; version 73,620,684).

An expression filtering system was applied to all datasets to select differentially expressed proteins (DEPs) and genes (DEGs) with expression levels diverging by >20%, both in up- and in down-regulation, in disease groups *versus* the control group.

A core analysis workflow was used for dataset interpretation, and to identify dysregulated pathways and biological functions cross-correlated in NCLs. The significance values associated with the core analysis were calculated using Fisher's Exact Test and statistically estimate the overlap between the datasets used and various sets of molecules in the reported annotations. Only annotations with -Log(p-value) > 1.3 were considered significant. Moreover, a z-score was calculated as a parameter able to predict the activation or inhibition of altered pathways by measuring how the expression pattern of molecules in the datasets of interest compared to the pattern expected for a specific annotation. The z-score calculation is described in (Krämer et al., 2014). Values > |1.5| were considered for network analyses. The 10 most dysregulated pathways emerging across the various NCL forms, from both human and mouse analyses, were selected for their transversal meaning.

2.2. Candidate early stage, disease status and progression biomarkers

The identification of candidate biomarkers for neurodegenerative diseases, especially with the ultimate aim of preventing the progression (or even onset) of symptoms, is a major challenge. To date, no specific biomarkers have been identified for NCLs. In this setting, research has largely been oriented toward the identification of predictive and progression biomarkers. In this regard, an integrative bioinformatic analysis was proposed to pinpoint specific molecules to promote as candidate biomarkers, able to define disease status or progression. Once we had selected DEPs/DEGs present in at least two different NCL omics datasets, a biomarker filter tool in IPA was used to filter molecules based on species, node type, and disease form, and select biomarkers related to hereditary, neurological, metabolic, inflammatory or skeletal muscle disorders; they had to be detectable at least in plasma/serum.

Moreover, a further scoring system was set up in order to select DEPs/DEGs with high confidence scores as promising biomarkers. This system evaluated: *i*) the entity of the differential expression; *ii*) the number of NCL forms that show the DEPs/DEGs; *iii*) the origin (central or peripheral tissue) of the omics-analyzed samples. Supplementary Table S2 reports the parameters used to set up the scoring system. We

selected only DEPs/DEGs that reached a threshold score of 8, as assigned by the scoring system, and were identified as candidate analytes by the IPA "biomarker analysis tool". The human biomarker list is shown in Supplementary Table S6a.

Proteomic datasets of mouse pre-symptomatic and symptomatic stages were analyzed using the same bioinformatic tool. Biomarker results were cross compared to identify dysregulated molecules that might be promoted as possible early stage, disease status or progression biomarkers. The number of available transcriptomic datasets precluded any analysis, and so we simply selected DEPs common to the two stages by applying the same scoring system used for the human datasets, although the origin parameter was not considered since almost all the datasets referred to central nervous system. Only DEPs over a threshold value of 9 were selected. The murine scoring system is detailed in Supplementary Table S2. The mouse biomarker list is shown in Supplementary Table S6b.

To better distinguish between early stage, disease status and progression biomarkers, we applied an additional filter based on the calculation, for each selected biomarker, of the ratio between $\log_2$(FC) in the symptomatic stage and $\log_2$(FC) in the pre-symptomatic stage. Values higher than 1.5 were considered possible progression biomarkers. Values between 0.5 and 1.5 suggested early biomarkers. In order to better explain events related to disease progression, the following exclusion criteria were applied: ratios <0, a $\log_2$(FC) that was lower in the symptomatic than in the pre-symptomatic phase, or a $\log_2(FC) < |0.26|$. By means of this approach, we excluded DEPs with dissimilar $\log_2$(FC) signs between stages, and DEPs not consistent with a progressive trend or too low to match with a biomarker.

3. Results

3.1. Convergent pathways across different NCL forms

Bioinformatic analysis of human datasets showed several common biological annotations involved in NCL pathophysiology, highlighting the presence of altered bioenergetics (*Mitochondrial Dysfunction*, *Oxidative Phosphorylation*, *TCA Cycle II*), neuroinflammation processes (*Sirtuin Signaling Pathway*, *Clathrin Mediated Endocytosis Signaling*, *Phagosome Maturation*, *SNARE Signaling Pathway*), and senescence of cells (*Mitochondrial Dysfunction*, *Clathrin Mediated Endocytosis Signaling*). The top 10 canonical pathways were sorted based on their significance (Fig. 3A), reporting the activation z-score values (Fig. 3B). The most dysregulated pathways also showed the same inhibition or activation state across NCL forms.

Network analysis revealed a set of focus molecules implicated in the *Mitochondrial Dysfunction,* the *Oxidative Phosphorylation*, the *Sirtuin Signaling Pathway*, and the *Synaptogenesis Signaling Pathway*, predicting a biological context able to generate a more accurate hypothesis about the altered nodes and their impact on the affected functional annotations in NCLs (Fig. 3C).

In order to evaluate transspecies overlap, we evaluated the degree of dysregulation of the pathways found to be most altered in humans, also in mouse models in both disease stages. With the exception of the *TCA cycle II (Eukaryotic),* the altered pathways in humans were maintained in mice with significant levels of dysregulation (Fig. 3D). However, the bioinformatics analysis did not allow calculation of the activation z-scores beyond the threshold value ($\pm$ 1.5), other than for a small number of annotated pathways (Fig. 3E). This bioinformatic inability to predict up- or downregulations might be due to a high heterogeneity of collected datasets that included several different analyzed matrices or focused on various specific brain structures.

Dysregulation of *Oxidative Phosphorylation* and *Synaptogenesis Signaling Pathway* already emerged in the pre-symptomatic stage, suggesting early involvement, transversal to several NCL forms. Interestingly, the *Synaptogenesis Signaling Pathway* in the early stage was upregulated with consistent activation z-score values across NCL forms. However, following onset of the major disease symptoms, this pathway drastically modified the extent of the z-score, which turned to an inhibitory state, particularly in CLN1 and CLN10 (activation z-score = −2.287; activation z-score = −2.0, respectively), better reflecting the human results.

Comparing stages, the early involvement of the *Sirtuin Signaling Pathway* was highlighted in the CLN5 form. Furthermore, an activation z-score of 1.789 was shown in the symptomatic stage of the CLN1 form, suggesting an attempt to modulate the response to neuronal degeneration through activation of autophagy/mitophagy.

3.2. Bioinformatic analysis of mouse datasets highlighted lysosomal and synaptic dysregulation throughout the disease course

Bioinformatic analysis of murine datasets showed alteration of both lysosomal and synaptic processes. As in humans, the *Synaptogenesis Signaling Pathway* and *Phagosome Maturation* emerged as the most dysregulated pathways in both the disease stages, suggesting a crucial role throughout the disease course. The top 10 canonical pathways were sorted and compared based on their significance.

The early-stage results highlighted pre-synaptic alterations (*Oxytocin Signaling Pathway*, *Calcium Signaling*, *SNARE Signaling Pathway*) and active neuroinflammatory processes (*Clathrin Mediated Endocytosis Signaling*, *Phagosome Maturation*, *Granzyme A Signaling*) (Fig. 4 A-B), whereas the symptomatic stage dataset highlighted alteration of neuroinflammatory processes (*Phagosome Maturation, Semaphorin Neuronal Repulsive Signaling Pathway, 14–3-3 Mediated Signaling*), and of neural circuit formation (*Synaptogenesis Signaling Pathway*, *Axonal Guidance Signaling*) (Fig. 4— C-D).

However, in the pre-symptomatic stage, only the Cln1 and Cln3 datasets provided an activation z-score for the proposed pathways; in the symptomatic one, on the other hand, the bioinformatic analyses in several forms, including Cln6, Cln7, Cln10 and Cln11, showed only few annotations with consistent z-score values or rejected pathways.

In order to hypothesize meaningful pathways related to disease progression, we compared the results observed between the pre-symptomatic and symptomatic stage to identify possible biomarkers of phenoconversion (Fig. 4 E-F).

As already seen in the human proteomic results, in the mouse model, too, dysregulation of the *Synaptogenesis Signaling Pathway* seemed to play a key role in NCL, with possible implications for disease progression, particularly in the Cln1 and Cln10 forms, as already described in the comparison with human datasets. Data referring to the Cln1 and Cln2 forms highlighted upregulation of the *CLEAR Signaling Pathway* and *SNARE Signaling Pathway*, with implications for lysosomal function (Palmieri et al., 2011; Settembre et al., 2013; Settembre and Medina, 2015) and synaptic transmission (Margiotta, 2021; Urbina and Gupton, 2020), respectively. The Cln1 form showed significant impairment of the *CLEAR Signaling Pathway* even before symptom onset (activation z-score = 2.2, in the pre-symptomatic stage), whereas the Cln2 form showed a positive activation z-score of 3.1 in the symptomatic stage. Furthermore, the Cln2 mouse model showed a progressive impairment of the *SNARE Signaling Pathway*, changing from an activation z-score = −1.2 in the pre-symptomatic stage to an activation z-score = −1.8 in the symptomatic one.

Common *Disease and Function* annotations across stages were compared using the comparison analysis bioinformatic tool in IPA, in order to identify trends and similarities across analyses. Increased engulfment of cells was transversally recognized with a progressive upregulation (activation z-score = 1.610 in the pre-symptomatic stage; activation z-score = 4.475 in the symptomatic stage). Commonly dysregulated genes were identified as part of this disease function, including ones related to neuroinflammation (*Anxa5, C1qa, Icam1, Mapk8, Mapk14*) and NCL genes (*Ppt1, Grn*). Endocytosis was reported as slightly upregulated in the pre-symptomatic stage and drastically increased in the disease status stage (activation z-score = 1.314 in the

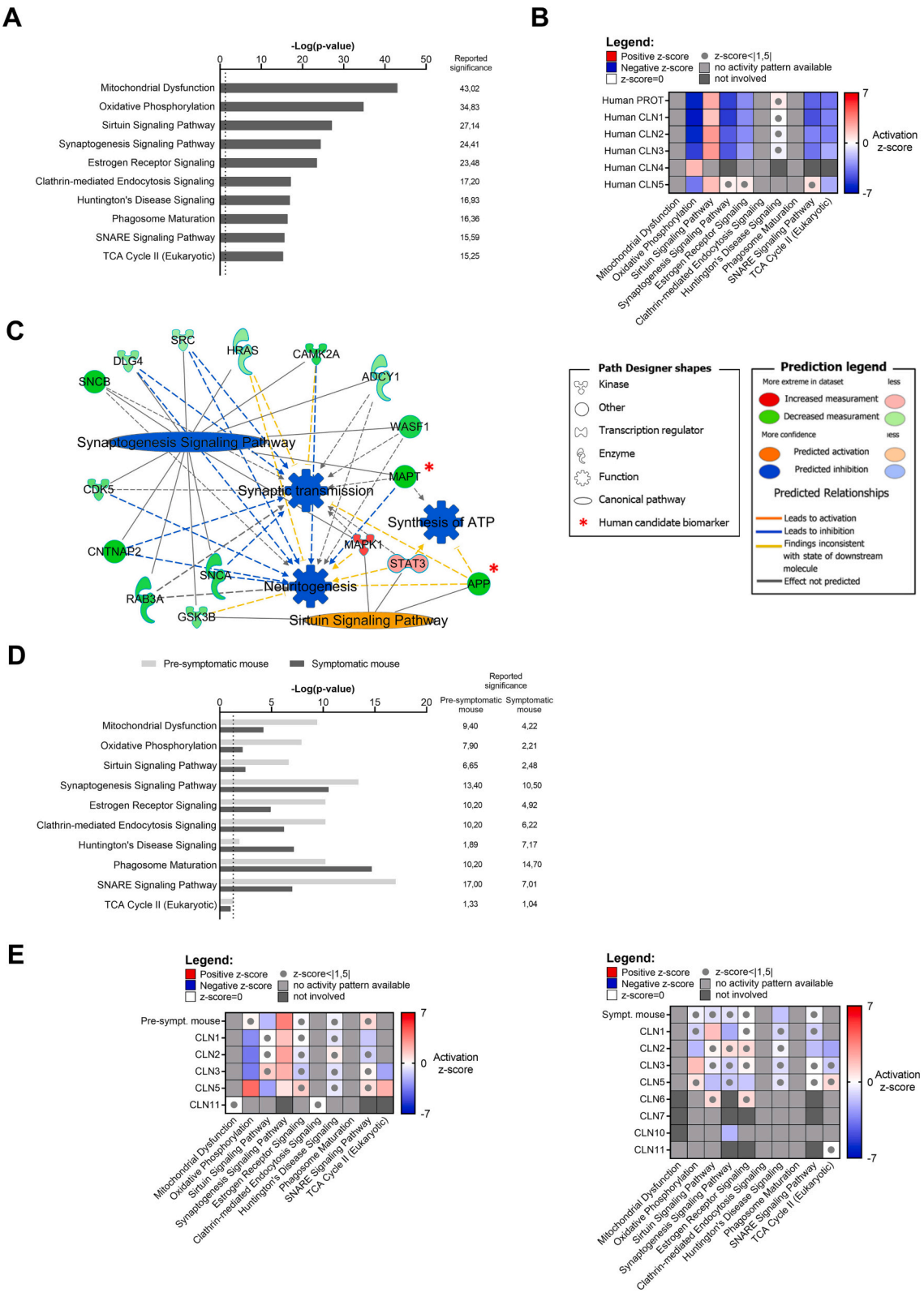

Fig. 3. (A) Bar chart representation of the 10 most dysregulated pathways identified in human datasets, sorted by their significance. Significant values are reported alongside the fig. (B) Heat map representation of the most dysregulated pathways through the different forms. (C) Molecular network encompassing DEPs involved in Mitochondrial Dysfunction, with molecular nodes reporting association with Oxidative Phosphorylation, Sirtuin Signaling Pathway and Synaptogenesis Signaling Pathway. (D) Bar chart representation showing the significant values, in pre-symptomatic and symptomatic mice, of the 10 most dysregulated pathways in humans. Significant values are reported alongside the fig. (E) Heat map representation showing the activation state, in mouse datasets (both stages), of the 10 most dysregulated pathways in humans.

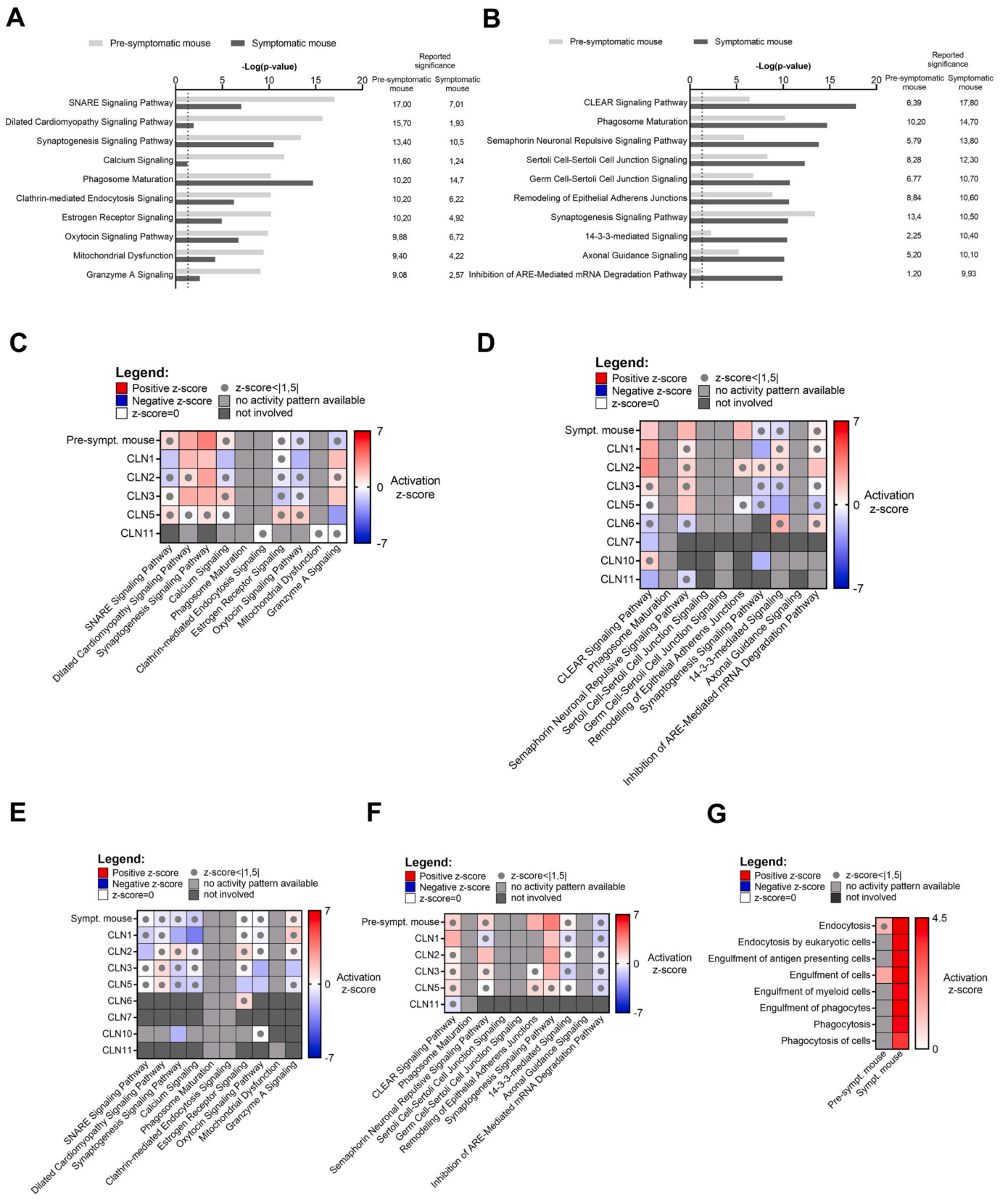

Fig. 4. (A-B) Bar chart representation of the 10 most dysregulated pathways identified in pre-symptomatic and symptomatic mouse datasets, sorted by their significance; significant values are reported alongside the figure; heat map representing the activation state of the 10 most dysregulated pathways in (C) pre-symptomatic and (D) symptomatic mice. Disease progression was evaluated on the basis of inhibition or up-regulation of meaningful pathways in mice. Heat-map representations show the top 10 dysregulated pathways of the mouse dataset in the (E) pre-symptomatic and (F) symptomatic stages. In (G) we depicted the heat map representing increased processes throughout the course of the disease.

pre-symptomatic stage; activation z-score = 4.417 in the symptomatic stage). Specific involvement of *Synaptic Vesicle Endocytosis* was shown by the common dysregulation of a gene subset (*Bin1, Grn, Itsn1, Syt1*), together with up−/downregulation of NCL-related genes (*Ppt1, Grn*) (Fig. 4 G). The complete set of common altered genes associated with the disease and function annotations related to its progression are listed in Supplementary Table S7.

3.3. Biomarker analysis of human and mouse datasets highlighted valuable biomarkers for evaluating disease status and progression

Biomarker analysis performed on human datasets revealed 50 common DEPs/DEGs, including 22 with a high confidence score (value ≥8) (Table 2).

Several DEPs/DEGs were found to be related to neurodegenerative processes (CLU, MAP1B, APP, ANK3), sphingolipid synthesis and metabolism (CTSD, HEXA, HEXB, NEU1, PGK1, PSAP), as well as vesicular (HEXB, NEU1, NSF, PFN1) and synaptic processes (ANK3, MAP1B). Gene Ontology cellular localization highlighted mitochondria (HSPD1, LETM1, NNT) and lysosomes (CTSD, HEXA, HEXB, NEU1, PSAP) as the most involved organelles, reinforcing the previously reported hypothesis of altered bioenergetics and autophagic processes.

Table 2
Candidate biomarkers with high confidence score common to both human proteomic and transcriptomic datasets.

Gene symbol	Gene Name	Confidence score	Averaged transcriptomic data log_2(FC)	Averaged proteomic data log_2(FC)
CLU	clusterin	9.86	1.82	−0.61
RBMX	RNA binding motif protein X-linked	9.71	−0.76	−0.61
PSAP	prosaposin	9.39	0.56	1.60
CST3	cystatin C	9.33	0.51	−0.88
HEXB	hexosaminidase subunit beta	9.28	0.63	0.54
FTH1	ferritin heavy chain 1	8.90	0.76	−2.16
MAP1B	microtubule associated protein 1B	8.88	−1.18	−0.76
TUBB4B	tubulin beta 4B class IVb	8.88	−0.96	−2.71
APP	amyloid beta precursor protein	8.80	0.59	−0.89
QDPR	quinoid dihydropteridine reductase	8.80	−1.46	−0.71
NEU1	neuraminidase 1	8.75	0.61	−0.92
HEXA	hexosaminidase subunit alpha	8.71	0.61	1.51
HSPD1	heat shock protein family D (Hsp60) member 1	8.67	−0.82	0.48
PGK1	phosphoglycerate kinase 1	8.67	−0.71	−0.45
ANK3	ankyrin 3	8.50	1.57	−0.64
ENO2	enolase 2	8.36	−0.58	−0.73
CTSD	cathepsin D	8.30	0.77	0.38
NSF	N-ethylmaleimide sensitive factor. Vesicle fusing ATPase	8.21	−0.73	−0.35
LETM1	leucine zipper and EF-hand containing transmembrane protein 1	8.13	−0.65	−0.37
ANXA5	annexin A5	8.07	0.52	0.87
NNT	nicotinamide nucleotide transhydrogenase	8.07	−0.70	−0.35
PFN1	profilin 1	8.00	−0.53	0.79

A network analysis highlighted direct relationships between proposed biomarkers and several NCL proteins and recognized a key role for amyloid precursor protein (APP), which upstream was affected by the expression levels of the proposed protein set, and downstream regulated the molecular processes related to the synthesis and metabolism of sphingolipids (Fig. 5).

Among the biomarkers in the list, clusterin (CLU) emerged with the highest confidence score (9.86) and appeared to be dysregulated in four NCL forms in the human dataset, being upregulated in CLN1 and CLN4 (log_2(FC) of 0.8 and 1.1, respectively), and downregulated in CLN2 and CLN3 (log_2(FC) of −2.06 and − 2.27, respectively). Furthermore, upregulated levels of prosaposin (PSAP) were reported in CLN1, CLN2, CLN3 and CLN4, suggesting a transversal pathophysiological role as already reported by Sleat and colleagues for human NCL brains (Sleat et al., 2017). Taken together, these results suggest a transversal role for both CLU and PSAP in NCL, which might therefore be valid status biomarkers.

The biomarker analysis of mouse datasets revealed 14 DEPs with high confidence scores and consistency across the disease (Table 3). Ctsd, HexB and Psap showed the highest confidence scores also in the human counterpart (together with HexA). Their dysregulation dramatically increased from the pre-symptomatic to the symptomatic stage in the Cln1, Cln2 and Cln11 disease forms, and in specific neuronal areas related to Cln1 and known to be mainly involved in disease progression, such as the frontal cortex and spinal cord (Nelvagal et al., 2020a; Shyng et al., 2017). The dysregulation of C1qa and C1qb proteins indicated involvement of the immune system and neuroinflammatory processes possibly related to neurodevelopment, the aging process and synaptic pruning (Cho, 2019; Hammond et al., 2019).

Other candidate biomarkers with medium confidence scores (7 < score < 9) were identified to be transversal across species, including Anxa5, Ank3 and Clu. Together with Apoe, they showed low consistency across NCL forms, even though their differential expression at symptom onset was increased compared with the pre-symptomatic data, enough to hypothesize a role as progression biomarkers specifically related to some disease forms.

4. Discussion

Recent methodological advances in multi-omics approaches have revolutionized research in rare diseases by balancing the low availability of samples and the poor information about pathophysiology with the generation of “big data”, which has greatly enhanced our understanding of the molecular complexity underlying these diseases. Omics data can be integrated correlating them from different sources and technologies in order to identify affected disease pathways, biomarkers, as well as new pharmaceutical targets.

In the brain, common dysregulated pathways have been identified not only in NCLs, but also in almost every late-onset neurodegenerative and aging-related disorder, suggesting that targeting these common pathways may open up promising new therapeutic opportunities (Kline et al., 2020; Kodam et al., 2023; Schilder et al., 2022).

In this review, we explored the pathogenesis of NCL disease, evaluating human data in order to define common molecular networks underlying the top dysregulated processes. Moreover, by distinguishing between pre-symptomatic and symptomatic stages, we were able to evaluate disease progression in murine models from both a cell-specific and an anatomical point of view. We evaluated the cellular microenvironment, highlighting the main altered pathways and pinpointing processes implicated across species and in single forms. The results generated pave the way for identifying the different brain regions primarily affected by the disease.

Using available human (Doccini et al., 2020, 2022; Henderson et al., 2016; Iwan et al., 2021; Nosková et al., 2011; Pezzini et al., 2017; Santi et al., 2020; Sleat et al., 2017, 2019; Zhong et al., 2020) and mouse (Best et al., 2021; Doccini et al., 2020, 2022; Domowicz et al., 2019; Evers

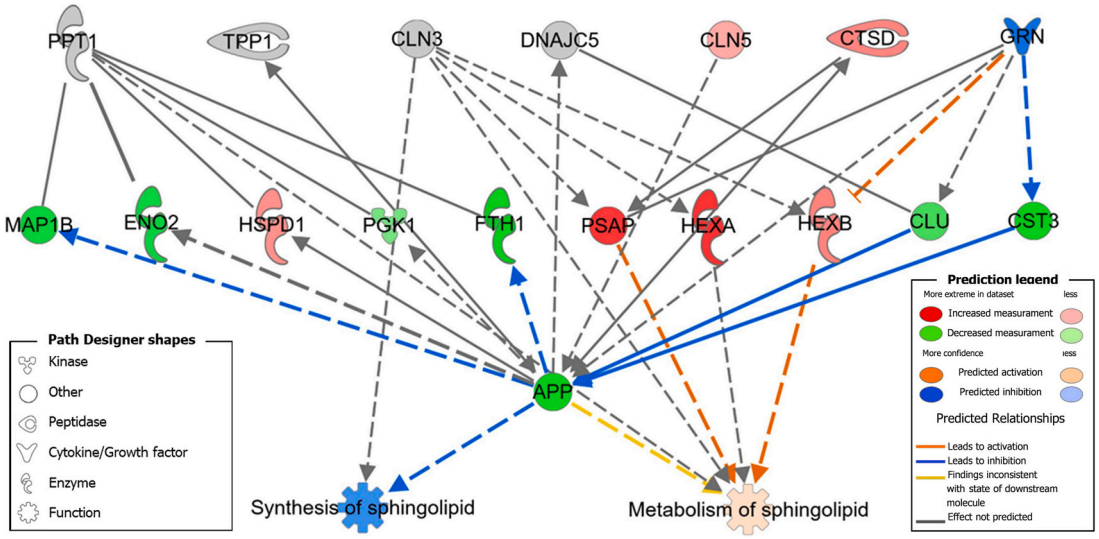

Fig. 5. Network analysis highlight direct connection between DEPs that emerged from the biomarker analyses and NCL mutated proteins.

Table 3
Candidate biomarkers with high confidence score common to pre-symptomatic and symptomatic proteomic datasets.

Gene symbol	Gene name	Confidence score	Averaged proteomic data log_2(FC)	
			Pre-symptomatic mouse	Symptomatic mouse
PSAP	Prosaposin	13.5	0.35	0.69
CTSD	Cathepsin D	12	0.48	0.45
HEXB	Hexosaminidase subunit beta	12	0.28	0.41
MBP	Myelin basic protein	12	0.43	−0.29
CTSZ	Cathepsin Z	11	0.41	0.44
GFAP	Glial fibrillary acidic protein	11	0.73	0.91
LAMP2	Lysosomal associated membrane protein 2	11	0.43	0.59
C1QA	Complement c1q a chain	10	0.46	1.01
CTSS	Cathepsin S	10	0.28	0.59
C1QB	Complement c1q b chain	9	0.43	0.81
COL1A1	Collagen type i alpha 1 chain	9	0.82	0.63
COL1A2	Collagen type i alpha 2 chain	9	0.89	0.68
HEXA	Hexosaminidase subunit alpha	9	0.26	0.48
KRT5	Keratin 5	9	0.88	0.80

et al., 2017; Geier et al., 2019; Huang et al., 2020; Klein et al., 2017; Koch et al., 2013; Llavero Hurtado et al., 2017; Nelvagal et al., 2020b; Schmidtke et al., 2019; Segal-Salto et al., 2017; Sleat et al., 2019; Tikka et al., 2016; Tuermer et al., 2021) proteomic and transcriptomic datasets, and a *Cln1/Cln5* double KO dataset (Blom et al., 2013), we analyzed 62 omics datasets and defined analytes shared between NCL forms that could be used to identify: *i*) common pathways across species; *ii*) candidate biomarkers of disease status; *iii*) disease progression; and *iv*) local/spatial differences and similarities.

The integrative analysis highlighted common molecular signatures across species, with involvement of mitochondrial dysfunction, synaptogenesis, lysosomal impairment, and neuroinflammatory processes.

Impaired mitochondrial function emerged in both human and mouse datasets as a result of altered levels of electron transport chain protein and reduced efficiency in energy production. Previous studies in various disease models have demonstrated morphological and functional alterations of the mitochondrial compartment, suggesting a potential role for proteins in mitochondrial dynamics. Mitochondrial shape is closely linked to many physiological processes such as cell cycle, immunity, apoptosis, and mitochondrial quality control (Tilokani et al., 2018), and damaged mitochondrial dynamics has been reported in NCL patient fibroblasts and animal models (Das et al., 1999; Jolly et al., 2002; Pezzini et al., 2011). Furthermore, disorganization of mitochondrial cellular distribution and cristae pattern has been reported in iPSCs obtained from reprogrammed CLN3 patients' fibroblasts, cerebellar precursor cells in a *Cln3*$^{\Delta ex7/8}$ model, and *Cln3*$^{-/-}$ neurons (Fossale et al., 2004; Lojewski et al., 2014; Luiro et al., 2006), whereas differentially expressed mitochondrial proteins have linked the lack of CLN5 with oxidative stress, bioenergetic impairment, and autophagy induction resulting in an activation of mitophagy (Doccini et al., 2020). Dysfunctional energy metabolism has been implicated in infantile, late infantile and juvenile NCL, also highlighting early dysregulation of the oxidative phosphorylation and the TCA cycle (Doccini et al., 2020; Kline et al., 2020; Luiro et al., 2006; Nelvagal et al., 2020a; Tikka et al., 2016). Compromised energy production in NCL correlates with selective loss of inhibitory GABAergic interneurons and neuronal loss primarily in brain areas most metabolically active and rich in mitochondria, providing indirect evidence of mitochondrial involvement in the disease pathogenesis.

In mouse profiles, pre-synaptic compartment alterations with dysregulation of the *Oxytocin Signaling pathway*, *Calcium Signaling*, as well as the *Exocytosis Process* have been recognized, suggesting that the influx of Ca^{2+} at pre-synaptic level represents a crucial step in synaptic vesicle exocytosis and neurotransmitter release (Catterall, 2011; He et al., 2018). Oxytocin directly increases neuronal excitability by regulating the activity of ion channels in the membrane and thus modulating synaptic transmission (Bakos et al., 2018), in line with the role of PPT1 in mediating synaptic functions (Gorenberg et al., 2022). Synaptic vesicle recycling is modulated by clathrin-mediated endocytosis (Gan and Watanabe, 2018; Royle and Lagnado, 2010), found to be one of the most dysregulated pathways in human analyses.

A progressive neuroinflammatory response emerged both in human and mouse data. Microglial activation has been reported in *Cln1*, *Cln3*, *Cln6* and *Cln11* KO mouse models in the pre-symptomatic stage, as well as in NCL patient brains (Blom et al., 2013; Francelle and Mazzulli,

2022; Mirza et al., 2013; Nelvagal et al., 2020a; Pontikis et al., 2005; Shyng et al., 2017; Tyynelä et al., 2004) in response to protein aggregates and undegraded storage material associated with chronic neuroinflammation and neuronal cell loss (Francelle and Mazzulli, 2022). The comparison between the pre- and symptomatic stages highlighted a set of DEPs associated with cell engulfment. These included Ppt1 and Grn (the protein products of *Cln1* and *Cln11*, respectively), as well as C1qa and Icam1, which also have a key role in the inflammatory response (Bui et al., 2020; Lopez et al., 2012).

New information about the *Sirtuin Signaling Pathway* in NCL has been obtained through reconstruction of a molecular network including the *Synaptogenesis Signaling Pathway*, *Neuroinflammation* and *Autophagy*, and sirtuin-related proteins implicated in mitophagy, in the regulation of mitochondrial quantity and quality, and in ROS scavenging (Fig. 3C). Emerging evidence has also connected this molecular network with anti-aging processes (Lee, 2019; Wan et al., 2022), cellular senescence (Lee et al., 2019), and late stages autophagosome maturation (Weidberg et al., 2010; Zhu et al., 2013).

A set of promising biomarkers was obtained by processing the harmonized dataset with the IPA "biomarker analysis tool". Human biomarker analysis highlighted APP, CLU, CTSD, and PSAP as potentially valuable status biomarkers, further reinforcing the hypothesis of synaptic dysregulation, as well as alteration of the metabolism of endogenous toxic agents (*i.e.*, α-syn; β-amyloid), of vesicle exocytosis, and of sphingolipid metabolism. The identification of CLU and APP as potential biomarkers also suggests a pathophysiology overlap between the cellular engulfment in NCL and the processes of accumulation of beta amyloid deposits underlying AD. The precursor protein of saposins (PSAP) is directly related to lysosomal disorders and NCLs since its cleavage is mediated by CTSD (Cárcel-Trullols et al., 2017) involved in the enzymatic hydrolysis of sphingolipids (Hiraiwa et al., 1997). Overexpressed PSAP levels were transversally recorded in both proteomic and transcriptomic datasets, in line with what has previously been described in CLN10 astrocytes (Di Spiezio et al., 2021), and the hypothesis of a functional role for GRN/CLN11 in lysosomes through a physical PSAP-PGRN interaction (Holler et al., 2017).

Biomarker analysis of mouse datasets identified Ctsd, Hexa, Hexb and Psap as trans-species biomarkers. Although Ctsd was found to be a status biomarker, Hexa, Hexb and Psap showed increased expression from the pre-symptomatic to the symptomatic stage, suggesting a role as biomarkers of disease progression. Their upregulated levels were consistent with the increased lysosomal dysfunction that emerged in our bioinformatic analysis and are transversally implicated in NCL pathogenesis. Biomarker analysis also identified C1qa and Gfap as valuable progression biomarkers. Different studies in NCL mouse models (Blom et al., 2013; Lui et al., 2016; Mirza et al., 2013; Sleat et al., 2019) have also suggested that aberrant activation of C1q is involved in NCL pathogenesis, playing a prominent role in neuroinflammation processes, as previously reported. Increased Gfap mRNA levels were observed in astrocytes of Tpp1-deficient mice (Domowicz et al., 2019). Also, Ppt1 can depalmitoylate Gfap at cysteine-291, a finding suggesting that the only palmitoylated residue in Gfap might be a valuable pharmaceutical target for treating infantile NCL (Yuan et al., 2021). Evidence for both molecules indicated a hallmark role in microglia (Färber et al., 2009) and astrocytes (Hol and Pekny, 2015), whose crosstalk is relevant also in many neurological disorders (Jha et al., 2019; Matejuk and Ransohoff, 2020). A better understanding of microglia-astrocyte interplay could be crucial in defining new therapeutic strategies to avoid the neuroinflammatory processes in NCLs.

A convergent omic profile with nine upregulated mouse biomarkers (Ctsd, C1qa, C1qb, Ctsz, Gfap, Hexa, Hexb, Lamp2 and Psap) emerged in the symptomatic stage in whole-brain datasets of Cln1, Cln2 and Cln11 forms, highlighting a distinctive molecular fingerprint across NCLs. All nine molecules, together with the medium confidence mouse biomarkers Apoe and Clu, were upregulated in the frontal cortex and spinal cord Cln1 KO datasets, with increased levels in the symptomatic stage, which possibly implicates them in CLN1 disease progression. The latter two biomarkers were also highly dysregulated in human datasets, in line with the hypothesized role of Apoe in the pathogenesis of infantile NCL. However, normal Apoe levels were detected in Ppt1$^{\Delta ex4}$ serum samples (Lyly et al., 2008), discouraging its possible use as a surrogate biomarker. Shared pathological alterations at spinal cord and frontal cortex level occur at different disease stages (Nelvagal et al., 2020a). Moreover, microglioses and astrocytosis were reported in Ppt1 and Cln5 mutant mice, with involvement of the spinal cord and thalamo-cortical system (Blom et al., 2013; Nelvagal et al., 2020a; Tikka et al., 2016). These features fitted with the impairment of glia cells in Cln1, even though no evidence was found in omic datasets referring to the prefrontal cortex from the Cln5 KO mouse model.

Dysregulated pathways, DEPs/DEGs, and biomarkers have also been identified in other neurodegenerative disorders, including Alzheimer's dementia (AD), Parkinson's disease (PD), frontotemporal dementia (FTD), and Huntington's disease, and there is mounting evidence that NCL genes are allelic to AD (CLN5) (Qureshi et al., 2018), PD (CLN1/PPT1, CLN12/ATP13A2) (Bras et al., 2012; Dearborn et al., 2015), and FTD (CLN7/MFSD8, CLN11/GRN) (Canafoglia et al., 2014; Geier et al., 2019; Smith et al., 2012). Autophagy, mitochondrial impairment and synaptic loss were identified in the pathogenesis of several neurodegenerative diseases (Croce and Yamamoto, 2019; Festa et al., 2021; Hou et al., 2020; Kim et al., 2022; Misrani et al., 2021; Park et al., 2018; Wang et al., 2020; Wu et al., 2019; Zhang et al., 2023). Emerging evidence on the association between NCL genes and endosomal trafficking has linked NCL to AD, with several points of both etiological and clinical convergence and overlap (Qureshi and Baez, 2020). Moreover, *CLU* is a major genetic risk factor for late-onset AD, together with *BIN1* and *APOE*, bioinformatically implicated in synaptic processes and proposed as medium confidence biomarkers. High CLU plasma levels were detected in AD patients compared with healthy controls (Yang et al., 2019), whereas mutations in APP resulted in the rare, familial, early-onset forms of AD (Foster et al., 2019). Increased GFAP levels have been widely associated with neuronal damage and have also been proposed as an early marker of neurodegeneration (Heller et al., 2020; Pereira et al., 2021; Zhu et al., 2021). Alterations of mRNA levels and protein expression of CTSD were observed in AD (Benes et al., 2008; Cataldo et al., 1991; Chai et al., 2019; Qureshi and Baez, 2020; Rojas et al., 2008), and a missense mutation was identified in CLN5 that segregates with AD (Qureshi et al., 2018). Taken together, this evidence suggests that a broad omics analysis in rare neurodegenerative disorders is able to provide critical teaching points also in the more common forms of neurodegeneration and could be exploited to identify new treatment opportunities for the different forms of autophagy-dependent neurodegenerative disorders, including childhood diseases like NCLs, and senile disorders including AD, PD, and FTD.

5. Conclusion

In summary, a comprehensive harmonized dataset was developed as an open system capable of being extended, updated, or subjected to new analyses, both bioinformatic and statistical. In future, the integrated use of other omics technologies (lipidomics and metabolomics) as well as an improved bioinformatic power, would allow a more focused analysis inside disease pathophysiology. This would overcome the actual limitation of our study in combining data from different disease phenotypes, analyzed samples and omic techniques. Nevertheless we thought that the filtering system used for data interpretation and analyses overcome, at least in part, this shortcoming facilitating transversal information across NCL forms both *intra* and *inter*-species, and the construction of an NCL molecular *fingerprint*. By this approach we could define a comprehensive list of candidate status and progression biomarkers ready to be validated in *in vivo* models and then in patients using both single and multiplexed analysis strategies. In future, the application of omics techniques, including the most advanced technologies such as single-cell

or spatial omics, may help to further validate the identified biomarkers and provide a deeper understanding of the cellular state and its functions, as well as better characterize possible molecular convergences and divergences between different diseases. The new information on the druggability profile of the identified DEPs/DEGs will likely form the basis for designing original therapeutic interventions to be transferred into clinical studies and promote future trial readiness in both rare and common dementias.

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CRediT authorship contribution statement

N. Gammaldi: Conceptualization, Writing – original draft, Visualization. **F. Pezzini:** Software, Resources. **E. Michelucci:** Investigation. **N. Di Giorgi:** Investigation. **A. Simonati:** Supervision, Writing – review & editing. **S. Rocchiccioli:** Investigation, Supervision. **F.M. Santorelli:** Project administration, Supervision, Writing – review & editing, Funding acquisition. **S. Doccini:** Conceptualization, Data curation, Writing – review & editing.

Data availability

Data will be made available on request.

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