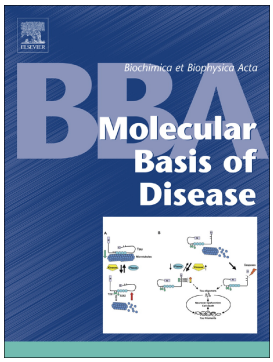

Applying modern Omic technologies to the Neuronal Ceroid Lipofuscinoses

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Applying modern Omic technologies to the Neuronal Ceroid Lipofuscinoses

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Highlights

- We discuss the benefit of application of various Omics technologies to the NCLs
- We discuss the functional Omics based studies so far applied to NCLs
- We summarise the findings of biomarker-based Omics studies for NCL
- We show a comparative systems biology analysis of data from published NCL Omics studies

Abstract

The neuronal ceroid lipofuscinoses are a group of severe and progressive neurodegenerative disorders, which generally present during childhood. With new treatments emerging on the horizon, there is a growing need to understand the specific disease mechanisms as well as identify prospective biomarkers for use to stratify patients and monitor treatment. The use of Omics technologies to NCLs have the potential to address this need. We discuss the recent use and outcomes of Omics to various forms of NCL including identification of interactomes, affected biological pathways and potential biomarker candidates. We also identify common pathways affected in NCL across the reviewed studies.

Keywords: Neuronal ceroid lipofuscinoses; Batten disease; CLN1-14; proteomics; metabolomics;

Introduction

In recent years there has been a revolution in the field of biological understanding driven by technological advances in mass screening approaches. This has resulted in an ever-expanding list of available "Omics" technologies. Omics include the genome (DNA) through to the transcriptome (RNA) and the proteome (protein), and more recently the metabolome (metabolic products). It is the integrated study of data derived from investigations of these distinct Omics that gives rise to systems biology, allowing us to use the data to answer basic fundamental questions and when applied to a disease context, to identify affected biological pathways and potential disease mechanisms or drug targets.

Genomics technologies have progressed significantly over the last few years with the rapid development of next-generation sequencing (NGS) and enabling the initiation of national programmes such as the 100, 000 genomes project in the UK [1] and the Precision Medicine initiative in the US to sequence 1 million genomes [2].

Transcriptomic technologies have also advanced in recent years. Such technologies largely consist of microarray platforms and high throughput RNA sequencing (RNA-Seq) [3]. Such technologies confer profound traction in the field given that a high degree of coverage of the transcriptome may be achieved routinely. By comparison, technologies for studying the other Omics are not nearly as automated. However, given the large volumes of data emerging from transcriptomic initiatives, there is a foreseeable need for the biochemical validation of many findings. Ultimately, investigation at the protein level is required to gain the most accurate insight into the molecular dynamics of the cell. For example, it has recently been reported that transcriptomic output is heavily influenced by epigenetic factors whilst this is not the case for the proteome [4].

Mass spectrometry-based technologies are typically used for the study of proteomics and metabolomics and their offshoot Omics such as peptidomics and glycomics. However, advances in their analysis have not been as rapid in development when compared to the other Omics already discussed. This is partially due to the complexity of the sample mixture. For example, there are approximately eight splice variants per gene, and potentially ten post-translational modifications per splice variant. There are other confounding factors for proteomics which must be accounted for in terms of the technological development [5]. For example, unlike genomics or transcriptomics methods, there is no equivalent to the DNA polymerase reaction; therefore, while the endogenous protein signal or "starting material" may be enriched, it cannot be amplified [6]. It should also be noted that approximately 97% of cellular protein (w/w) is encoded by only 3% of the genome.

Metabolomics is the most downstream and last of the "core" Omics and typically performed using either (or both) 1H NMR or mass spectrometry-based platforms. Untargeted metabolomics in IEM is a growing area as the emergence of NGS has resulted in the increase of diagnostic testing to confirm Variants of Unknown Significance (VUS). For new or uncharacterised disorders there are no clinical tests available and this is where untargeted metabolic profiling is proving valuable [7].

Why apply Omics technologies to the Neuronal Ceroid Lipofuscinoses (NCLs)?

The NCLs are part of a larger group of the IEM and occur due to mutations in over a dozen individual genes [8]. While Omics approaches have been traditionally utilised in the study of common diseases, they have only more recently been applied to rare conditions, in particular in the IEMs/NCLs.

Critically, the study of rare diseases including the NCLs is confined by comparably small patient populations and accompanying complications, such as limited tissue sampling availability and poor public awareness. Consequentially, the research support network and associated available funding for the NCLs and other rare diseases are not as well developed as those for conditions which are more prominent in the public eye, i.e. diseases associated with advancing age (e.g. Alzheimer's, Parkinson's, or diabetes). While the NCLs are clinically stratified by their causative gene mutation, outlined previously in this review and in the table below, their aetiological diversity appears to converge upon a spectrum of common clinical phenotypes. It is therefore likely that the molecular origins underpinning the individual NCLs also converge upon common pathological cascades, resulting in unifying cellular pathogeneses including defects in lysosomal function, mitochondrial dysfunction, and alterations in the endoplasmic reticulum and endo-lysosomal trafficking [9-11]. Table 1 highlights some recent studies incorporating these Omics technologies and some of the exciting findings from these studies will be discussed in more detail below. This work represents a window into what is achievable in terms of Omics application to such disorders. Critically, due to advances in the tools and technologies underpinning these -omics investigations we can likely expect a dramatic increase in our understanding of the molecular mechanisms underpinning these conditions in the coming years.

Omics to characterise the NCL interactome

Proteomic technologies can be used not only to look at the proteome at the level of whole tissues or cells but also through the application to functional studies of proteins. For example, extensive work at the University of Helsinki using interactome-centric proteomics has elucidated interacting partners of CLN3, CLN5 [12] and CLN1 [13]. In these studies, the authors used neuronal SH-SY5Y cells a twice-subcloned cell line derived from the SK-N-SH neuroblastoma cell line which serves as a model for neurodegenerative disorders. SH-SY5Y were transfected with tagged CLN proteins and subjected to a technique known as tandem affinity purified mass spectrometry (TAP-MS). Tagged CLN proteins were isolated from the SH-SY5Y cells by affinity purification. Isolated CLN complexes were analysed using proteomic techniques. Analysis of the CLN3 interactome confirmed 16 known interactors as well as identifying a further 43 novel candidates. Functional annotation of these CLN3 interactors revealed 'transmembrane transport' as a key annotated function for the interacting proteins. This same procedure was applied to CLN5 protein and identified 31 interactors. Interestingly eighteen of these were identified as being in common with the CLN3 interactome. Many of these overlapping CLN3/5 common interactors were mitochondrial carriers associated with neurological disease and calcium binding roles.

In a separate study the same group used TAP-MS methodology to characterise the binding partners of palmitoyl protein thioesterase 1 (PPT1) the affected gene in CLN1. Scifo *et al* [12] identified 23 other proteins in complex with PPT1. Three of these proteins were predicted to be palmitoylated substrates whilst others were associated largely with mitochondrial synthesis and other mitochondrial functions. Another very recent CLN1 interactome study on mouse brain lysate [14] corroborates some of the interacting proteins found in the previous studies, in particular, the protein - Transitional endoplasmic reticulum ATPase (VCP) is consistently detected in the CLN1 interactome. VCP is associated with or causative for forms of other neurodegenerative disorders such as amyotrophic lateral sclerosis types 8 or 14, with or without frontotemporal dementia [15]. VCP has also been implicated as a regulator of Wallerian degeneration [16]. Other PPT1 interactors ATP synthase subunit beta, mitochondrial (ATP5B), dihydropyrimidinase-related protein 1 (CRMP1), microtubule-associated protein 1B (MAP1B) and pyruvate dehydrogenase E1 component subunit

alpha (PDHA1), were confirmed to be differentially regulated in previous work by the same group [17]. The nature of PPT1, its role as a protein modifying enzyme means that many potential biological pathways are affected giving rise to the complex metabolic phenotype observed in CLN1. The amoeboid organism Dictyostelium has been shown to act as a useful model to study CLN5. Using this model Huber et al have determined CLN5 to be a glycoside hydrolase and used immunoprecipitation coupled with mass spectrometry to identify interacting proteins that were also associated and implicated in the pathogenesis of other of CLN diseases such as Tpp1 (Cln2), cathepsin D (Cln10) and cathepsin F (Cln13) [18].

Omics to identify the molecular consequences of NCL causing mutations

CLN1: Tikka *et al* [17] have performed proteome analysis of laser-captured thalamus regions of CLN1 knockout mice models. Looking at pre-symptomatic and symptomatic stages of disease they were able to identify 36 proteins altered pre-symptomatically of which 5 were previously identified in the CLN1 interactome study. These included CMRP1/MAP1B and PDHA1. The key downregulated pathways identified in pre-symptomatic CLN1 brain tissues were biological processes important for the proper function of neurons, including neuritogenesis, branching, and microtubule dynamics. Pathways identified at early affected stages (3-month old thalamus) ranged from those associated with nervous system development, cellular signalling, assembly and organisation. Common molecular features (as determined by gene ontology analysis) that spanned the pre and symptomatic stages included metabolic pathways involving the 2-ketoglutarate dehydrogenase complex TCA cycle and mitochondrial dysfunction [17].

Similar findings from the same group were made using transcriptomic profiling of transfected SH-SY5Y with CLN1 from overexpressing WT CLN1 and 5 selected patient mutations [19]. At the RNA level, they confirmed changes in gene expression of genes associated with neurite formation and neuronal transmission. Specifically, neuritogenesis and proliferation of neuronal processes which ties in convincingly with the proteomic data reported for CLN1 murine thalamic samples (as described above) [17].

Segal-Salto *et al* [20] used a targeted approach to functionally characterise CLN1 affected pathways. Their approach was to specifically target the membrane proteins of neuronal-like SH-SY5Y cells and enrich the acylated membrane proteins. Their study showed 88 proteins were altered in CLN1 membranes. Of these were ciliogenesis regulating proteins Rab3IP, Rab8 and Rab11. This led to the authors to look more closely at cilia in CLN1 tissues where they found reduced palmitoylation of Rab3IP. This effectively results in incorrect intracellular localisation of Rab3IP and ultimately results in defective cilia. The authors, therefore, propose that CLN1 should also be considered as a ciliopathy.

CLN3: Llavero Hurtado *et al* [21] used proteomics on isolated pre-synaptic populations from Cln3 −/− mouse brains to identify molecular modulators of synaptic stability and degeneration. Key pathways identified as correlating with regional synaptic vulnerability and validated using human post-mortem brain samples included valine catabolism and Rho signalling pathways. These pathways when assessed for potential to modulate disease processes *in vivo* using a Drosophila CLN3 model were indeed capable of altering phenotypic presentation following genetic and/or pharmacological targeting.

CLN4: Using proteomics on human adult CLN4 brain tissue and DNAJC5/CLN4 knock out mice Henderson *et al* [22] identified mislocalisation and upregulation of PPT1. Further analysis revealed that presynaptic co-chaperone CSP (encoded by DNAJC5/CLN4) is a substrate of PPT1 and appears to

have a functional effect on PPT1 as its absence affects the palmitoylation of other PPT1 substrates, particularly lysosomal and synaptic proteins. This study indicates a direct cross-talk between these two CLN disease proteins implicating a shared pathological mechanism between these two forms of NCL.

CLN10: Mutations in the Cathepsin D gene are known to cause the severe NCL CLN10. Koch *et al* [23] also from the Helsinki University research group proteomically profiled isolated synapses from a mouse knock out model of Cathepsin D and found 453 significantly altered proteins. Subsequent bioinformatics analyses indicated key affected pathways were cytoskeletal disruption and cell spreading. Wound healing assays in cathepsin D deficient cells confirmed strongly compromised spatial orientation, associated with changes in the distribution of focal adhesions and integrin assembly. It has been proposed that such changes may contribute to the early synaptic alterations and subsequent neuronal loss observed in CLN10.

CLN11: The NCLs, in particular CLN11, have attracted attention from the adult neurodegenerative field due to the shared pathology with adult frontal temporal dementia (FTD) [24]. Individuals heterozygous for the progranulin gene *GRN* also display similar findings such as skin biopsies showing enlarged lysosomes containing lamellar, pseudomembranous “fingerprint”-like inclusions of the type seen in NCL. However, the neurodegeneration in these patients is adult onset and thus occurs much later. Therefore, the pathogenesis of FTD and NCL caused by *GRN* deficiency may share gene-dosage-dependent mechanisms involving lysosomal dysfunction [25]. Evers *et al* [26] performed lipidomic and transcriptomic analysis of heterozygote (*GRN*+/-) and homozygote (*GRN*-/-) mice tissues as well as GRN FTD patient samples to identify the key pathways affected by GRN deficiency. The unbiased lipidomic analysis highlighted an increase of triacylglycerol’s (TAGS) and a reduction of phosphatidylserines (PS) and phosphatidylethanolamines (PE) in the mice. Complementary transcriptomic analysis performed alongside with the lipidomic analysis included another neurodegenerative lysosomal storage disorder, Niemann pick C (NPC) disease as a disease control. This analysis revealed a subset of altered lysosomal genes in the *GRN* mutant mice. Only a small proportion of the differently expressed genes overlapped with NPC. Those that didn’t overlap included immune and lipid metabolic related genes specific to *GRN* NCL molecular pathology.

Omics for NCL Biomarkers

In recent years there has been increasing focus on using Omics technologies directly on patient material, for biomarker studies. Biomarkers are needed for the monitoring of new treatments such as the recently FDA and EMA approved enzyme replacement therapy for CLN2 [27] and for other emerging novel therapies [28]. Whist the mutation and defective protein and presence of lipofuscin deposits are the gold standard way to diagnose an NCL patient, they are not suitable for monitoring treatment or predicting disease severity. Other inborn errors of metabolism often result in an accumulated or reduced substrate due to a block in a pathway such as accumulation of glycosaminoglycans in the mucopolysaccharidoses disorders, or glycosphingolipids in the glycosphingolipidoses disorders. For the NCLs, this is comparably more difficult due to both their complex genetic aetiologies as well as major gaps in the understanding of the molecular pathogenesis of the disease. Applying Omics technologies to the NCLs may reveal downstream affected molecules that could serve as biomarkers if they were to both correlate appropriately with disease progression and respond to treatment. Functional studies using Omics technologies may provide potential pathways to probe for candidate biomarkers. However, an ideal biomarker must be both accessible and ideally non-invasive (ie. from urine, plasma or bloodspots) as well as easily detectable by immune-based or mass spectrometry methods for clinical laboratory analysis. Additionally, it must be robust (ie. not easily affected by environmental conditions) and

reproducible. Many candidate biomarkers eventually fail due to not meeting these criteria. The ideal approach to biomarker discovery using Omics technologies is analysing an appropriate cohort of samples. This can often address the efficacy issues. With NCL being a rare disease acquiring the appropriate cohort of patient samples are very challenging. Animal models can help address this issue but even if candidate biomarkers are found from animal studies these biomarkers still need to be validated in humans if they are to eventually be of clinical use.

Recent attempts at biomarker discovery using Omics approaches include work by Hersrud *et al* [29]. The authors used a multiple proteomic approach to screen plasma for biomarker discovery in juvenile NCL. They began by using a global unbiased 2D Difference Gel electrophoresis method combined with 2 multiplex immunoassay panels MILLIPLEX® MAP magnetic immunoassay panel (26 serum protein panel) and the DiscoveryMAP ® 1.0 immunoassay from Myriad-RBM (MR) (190 protein panel) before attempting validation of differently expressed proteins by western blot. Ultimately, the immunoassay panels proved the most effective and identified candidates already associated with neurodegeneration Brain-derived neurotrophic factor, Neuronal cell adhesion molecule, Clusterin, Adiponectin, Apolipoprotein E, Vascular cell adhesion protein 1, and Myoglobin were significantly elevated in JNCL.

CSF-based biomarker discovery in NCLs has been explored by Sleat *et al* [30] by proteomic profiling of post-mortem brain and CSF from patients with CLN1, CLN2, and CLN3 disease. Their findings reported profound changes in the proteomes of all NCL patients compared to non-NCL controls. Interestingly, they observed that CLN2 and CLN3 exhibited a greater similarity in changes than with CLN1 which corresponds to the relationship between the respective observed clinical phenotypes. Importantly, the CSF profiles of all diseases showed 18 proteins commonly altered in all 3 diseases as well as some altered in specific NCLs. Promising candidates include vimentin which is a cellular cytoskeletal protein and also cellular retinoic acid-binding protein 1. The candidates presented in the Sleat study serve as potential disease biomarker candidates; however, until their efficacy can be demonstrated alongside an appropriately altered treatment response profile, their utility remains undetermined.

Sindelar *et al* [31] used untargeted metabolomic profiling on CSF from CLN2 patients and identified disease severity metabolite markers by correlating with clinical disease severity scores, identifying 29 metabolites that reflected disease severity. Using tandem mass spectrometry and target fragmentation they were able to confidently identify 8 of these compounds as being down-regulated in CLN2 CSF. Seven of these identified metabolites were acetylated amino acids which led the authors to speculate that the reduction of these modified amino acids could be a result of the lack of tripeptide cleavage by the CLN2 defective TPP1 enzyme leaving less n-terminal peptides available for acetylation. Two of the key metabolites affected in this study were N-acetylaspartyglutamic acid a common neurotransmitter and Glycero-3-phosphoinositol. Glycero-3-phosphoinositol is converted to myo-inositol and glycerol 3-phosphate by glycerophosphodiester phosphodiesterase an enzyme implicated in neurite formation which is a pathway previously mentioned in CLN1 proteomics studies [17, 19]. Interestingly, some of these CLN2 identified brain metabolites such as myo-inositol have been described altered in another neurodegenerative IEM, mucopolysaccharidoses II by quantitative in vivo brain magnetic resonance spectroscopic monitoring [32]. These findings therefore may additionally serve as general markers of early onset neurodegeneration.

Common features of NCLs – A comparison of existing NCL Omics studies

The capacity for a systems biology-based approach is not only advantageous in contextualising –omics results into mechanisms driving a specific disease, but also invites the possibility to identify molecular overlaps uniting the NCLs. Similar approaches have previously highlighted conserved molecular profiles uniting members of the motor neuron disease family [33-35] and the muscular dystrophies [36]. Although many –omics experiments generate large and often seemingly impenetrable datasets, the application of stringent filtering approaches and an unbiased analysis methodology to a diverse set of independent NCL studies suggests several conserved key features.

Here we will highlight shared molecular features uniting the NCLs, derived from our analysis linking independent proteomic studies of CLN1, CLN2, CLN3 and CLN4 post-mortem tissue, and disease models. Detailed methods used for this comparative analysis are given in supplementary information.

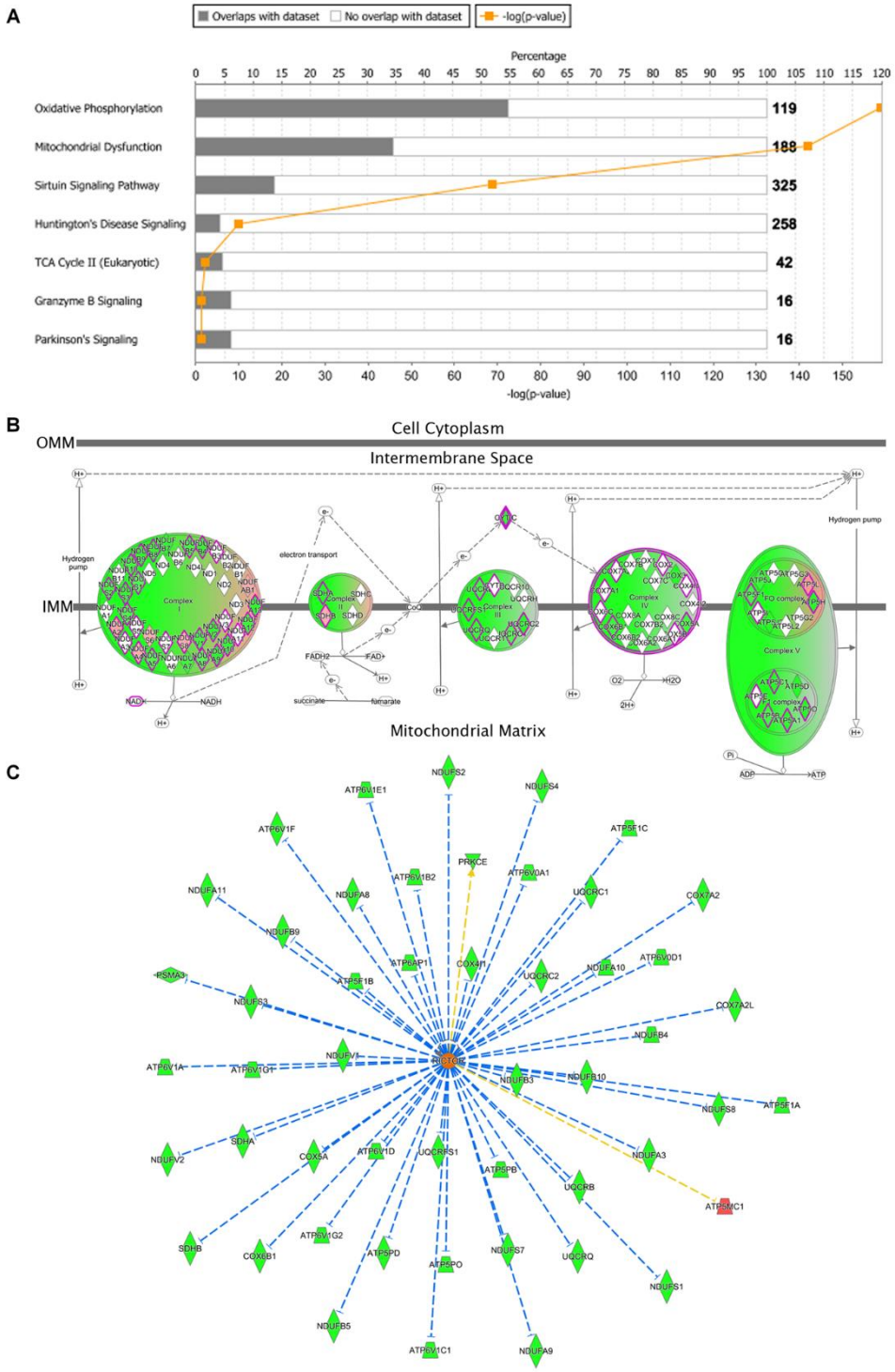

Figure 1. (A) Overlay of independent proteomic analyses of post-mortem brain in CLN1 [30] [17] [20], CLN2 [30], CLN3 and CLN4 [22]patients and a CLN3 mouse model [21] [30] as well as the neuronal PPT1 interactome [13] highlights conserved alterations in oxidative phosphorylation and mitochondrial dysfunction as top altered canonical pathways occurring across all studies. Chart was created in Ingenuity Pathway Analysis from an overlay of datasets comprised of proteomic changes in respective CLN1-4 tissue compared to wildtype control and the PPT1 interactome [13]. Canonical pathway scores and subsequent ranking for all analyses including this comparison are derived from a

Fisher's Exact Test calculating overlap between molecules in each respective input dataset and number of molecules comprising canonical pathway defined by Ingenuity Systems Database. Changes in oxidative phosphorylation comprised the top conserved canonical pathway according to Fisher's exact test calculated across all studies. Canonical pathway identification and ranking were initially performed under omission of protein expression changes between studies to account for the PPT1 interactome [13]. After top canonical pathways were determined by this method, data derived from all CLN1-3 sources described previously was consolidated in a cross-study comparative analysis inclusive of individual protein changes within and between studies as represented by (B) Conserved alterations in electron transport chain components comprise the top canonical pathway dysregulated across all studies. Schematic is derived from Ingenuity Pathway Analysis overlay of datasets following conversion into fold-change ratios representing expression alteration in the respective disease model compared to wildtype control. Subunits highlighted in purple are present across all input sources; green represents a downregulation in gene expression, while red represents an upregulation in gene expression. Highlighted white subunits indicate that no expression profile data is available for one individual input source (eg. interactome study [13]).

(C) Overlay of independent proteomic analyses highlights Rapamycin-insensitive companion of mTOR (RICTOR) as the top "master regulator" linking all proteomic changes occurring across studies. Schematic depicts top causal network as defined by z-score weighing the predicted expression change of molecules as defined by Ingenuity Knowledge Database against actual expression change of molecules reported in input dataset(s). Activation status of causal master regulator (RICTOR) in relationship to downstream protein changes is orange to represent "activation." Expression profile of target molecules or nodes within input datasets are depicted in red (upregulation) or green (downregulation); expression profiles were confirmed to exhibit the same directionality across all input sources. Relationships of upstream regulator activation to molecular changes present across all aforementioned proteomic datasets are conveyed by color of line: blue represents inhibition of expression of target molecules, orange represents activation of expression of target molecules, and yellow represents disagreement, e.g. target molecule is predicted to be inhibited by RICTOR but is reported to be upregulated in expression within one or more input datasets.

Canonical pathway analysis shows a conserved dysregulation in oxidative phosphorylation processes across independent studies of CLN1-4 disease models.

As a means of gaining initial insight into any commonly dysregulated processes attributed to the individual protein alterations reported by independent proteomic studies of CLN1, CLN2, CLN3 and CLN4 disease models and patient post-mortem tissue, we used the canonical pathways function within Ingenuity Pathway Analysis (IPA) (see Methods supplementary information and Figure 1). Interestingly we identified several canonical pathways which appeared to be consistently perturbed between CLN1, CLN2, CLN3 and CLN4 post-mortem tissue and disease models; top cascades are outlined below in Figure 1A and are strikingly dominated by conserved disruptions to mitochondrial and oxidative phosphorylation processes. An example cascade represented by Figure 1B highlights those select and specific subunits of the electron transport chain appear differentially expressed across the CLN1, CLN2, CLN3 and CLN4 studies comprising this analysis. It is of interest to note that, while it is tempting to infer that this data represents a generalised mitochondrial deficit, specific subunits of Complexes I, II and IV of the electron transport chain, highlighted in red (indicating an increase in expression), are consistently changed in opposition to the remainder of the pathway components between CLN1, CLN2 CLN3 and CLN4, suggesting that in the context of the NCLs, it may be important to regard individual components of mitochondrial biology rather than generalizing a

global alteration. It is, however, difficult to ascertain without *in vivo* validation across multiple disease models whether these subunit mis-expression data confer a causal contribution rather than a downstream consequence of the neurodegeneration unifying the NCLs. While an Omics approach provides a unique advantage in pinpointing molecular overlaps between studies that may otherwise remain unrecognised, these findings would best serve as a springboard for future studies, such as a more thorough dissection of a pre-symptomatic proteomic signature within and between the NCLs, in order to better understand how this reported molecular dysfunction promotes a common NCL pathophysiology.

Converging analysis of independent CLN1-4 disease studies identifies Rapamycin-insensitive companion of mTOR (RICTOR) as a top upstream regulator of proteomic dysregulation present across the NCLs.

Our analyses thus far have identified conserved "pre-symptomatic" protein changes that we have been able to track between studies of CLN1, CLN2, CLN3 and CLN4 post-mortem tissue and mouse models, which putatively comprise a unified dysregulation in several key cellular pathways across discreet NCLs. It was, therefore, next of interest to determine whether these conserved protein changes were altered as the result of a common upstream regulatory perturbation, which may provide further insight into disease pathogenesis as well as offer the possibility for a therapeutically targetable molecular "signature" between conditions. To do this, we performed an upstream regulatory analysis using IPA using the same input data sources, with the addition of a "symptomatic" dataset by Tikka et al [17]. to include the possibility of tracing downstream effector regulation over the disease time course. Interestingly, RICTOR was reported to be the top upstream regulator of protein changes reported in independent studies of CLN1-4 disease (Table 2). Through the causal network function in IPA, it was possible to extract which specific molecular changes within each CLN1-4 dataset have been previously reported in the literature to be downstream of RICTOR activation (Table S3). By overlaying each target molecule of RICTOR altered within each individual proteomic study, it was possible to then generate a conserved "interactome" comprising protein targets within our input datasets in relation to RICTOR, with graphical representation of the predicted effect of RICTOR upon its targets compared to the actual changes reported within the datasets (Figure 1C). Interestingly, separate targeting of the TORC1 and TORC2 (including RICTOR) pathways have been strongly implicated in the maintenance of neuronal stability and modification of phenotype in vivo from *Drosophila* to mouse models of NCLs [37-39].

Analysis	Tissue	Upstream Regulator	Predicted Activation State	Activation z-score	p-value of overlap	Target molecules in dataset
Sleat et al [25]	CLN1 Brain	RICTOR	Activated	6.582	3.75E-38	ATP5F1A, ATP5F1B, ATP5F1C, ATP5MG, ATP5PB, ATP5PD, ATP5PO, ATP6AP1, ATP6V0A1, ATP6V0D1, ATP6V1A, ATP6V1B2, ATP6V1C1, ATP6V1D, ATP6V1E1, ATP6V1F, ATP6V1G1, ATP6V1G2, ATP6V1H, COX4I1, COX5A, COX6B1, COX7A1, COX7A2, COX7A2L, CYC1, FABP5, LHPP, NCAM2, NDUFA10, NDUFA11, NDUFA2, NDUFA3, NDUFA4, NDUFA5, NDUFA8, NDUFA9, NDUFB10, NDUFB3, NDUFB4, NDUFB5, NDUFB8, NDUFB9, NDUFC2, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NDUFV3, PRKCB, PRKCE, PRKCG, PSMB2, PSMC1, PSMC2, PSMC3, PSMC4, PSMD13, PSMD3, PSME2, PTEN, RPL18, RPL28, RPL4, RPL7, RPL8, SDHA, SDHB, UQCRB, UQCRC1, UQCRC2, UQCRFS1, UQCRQ
Sleat et al [25]	CLN2 Brain	RICTOR	Activated	7.057	1.18E-36	ATP5F1A, ATP5F1B, ATP5F1C, ATP5F1D, ATP5MC1, ATP5MG, ATP5PB, ATP5PD, ATP5PO, ATP6AP1, ATP6V0A1, ATP6V0D1, ATP6V1A, ATP6V1B2, ATP6V1C1, ATP6V1D, ATP6V1E1, ATP6V1F, ATP6V1G1, ATP6V1G2, ATP6V1H, COX4I1, COX5A, COX6B1, COX7A1, COX7A2, COX7A2L, CYC1, FABP5, NDUFA10, NDUFA11, NDUFA3, NDUFA4, NDUFA8, NDUFA9, NDUFB10, NDUFB3, NDUFB4, NDUFB5, NDUFB8, NDUFB9, NDUFC2, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS7, NDUFS8, NDUFV1, NDUFV2, PPA1, PRKCE, PSMA3, PSMA4, PSMA6, PSMB1, PSMB5, PSMB6, RPS18, SDHA, SDHB, UQCRB, UQCRC1, UQCRC2, UQCRFS1, UQCRQ
Sleat et al [25]	CLN3 Brain	RICTOR	Activated	6.505	1.16E-31	ATP5F1A, ATP5F1B, ATP5F1C, ATP5MC1, ATP5PB, ATP5PD, ATP5PO, ATP6AP1, ATP6V0A1, ATP6V0D1, ATP6V1A, ATP6V1B2, ATP6V1C1, ATP6V1D, ATP6V1E1, ATP6V1F, ATP6V1G1, ATP6V1G2, COX4I1, COX5A, COX6B1, COX7A2, COX7A2L, NDUFA10, NDUFA11, NDUFA3, NDUFA8, NDUFA9, NDUFB10, NDUFB3, NDUFB4, NDUFB5, NDUFB9, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS7, NDUFS8, NDUFV1, NDUFV2, PRKCE, PSMA3, SDHA, SDHB, UQCRB, UQCRC1, UQCRC2, UQCRFS1, UQCRQ
Henderson et al [26]	CLN4 Brain	RICTOR	Inhibited	-2	1.55E-0.5	NDUFA8, NDUFA9, COX7A2, NDUFA5
Llavero Hurtado	CLN3 Thala	RICTOR	Activated	4.259	5.63E-43	ATP5F1A, ATP5F1B, ATP5F1C, ATP5F1D, ATP5MF, ATP5MG, ATP5PB, ATP5PD, ATP5PF, ATP5PO, ATP6V0A1, ATP6V0D1, ATP6V1A, ATP6V1B2, ATP6V1C1, ATP6V1E1, ATP6V1F,

et al [27]	mus					ATP6V1G2, ATP6V1H, COX5A, Cox5b, COX6B1, Cox6c, CYC1, NCAM2, NDUFA10, NDUFA2, NDUFA4, NDUFA5, NDUFA7, NDUFA8, NDUFA9, NDUFAB1, NDUFB10, NDUFB4, NDUFB5, NDUFB7, NDUFB8, NDUFB9, NDUFC2, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS8, NDUFV1, NDUFV2, PPA1, PPA2, PRKCA, PRKCG, PSMA1, PSMD12, PSMD13, PSME1, RPL10, RPL17, RPL6, RPL7, RPS10, RPS15, RPS27A, RPS8, SDHA, SDHB, UQCRB, UQCRC1, UQCRC2, UQCRFS1, UQCRHL, UQCRQ
Tikka et al [19]	CLN1 Thalamus	RICTOR	Inhibited	-2	2.24E-05	NDUFA8, NDUFS3, RPL6, RPS3
Scifo et al[18]	PPT1 Interactome	RICTOR			4.85E-33	ATP5F1A, ATP5F1B, ATP5F1C, ATP5MF, ATP5MG, ATP5PO, ATP6AP1, ATP6V1A, FAU, NDUFA4, NDUFA5, NDUFA8, NDUFAB1, NDUFS2, NDUFS3, NDUFS8, PSMA1, PSMA3, PSMA4, PSMA5, PSMA6, PSMA7, PSMA8, PSMB2, PSMB5, PSMB6, PSMC6, PSMD1, PSMD11, PSMD12, PSMD3, PSMD8, PSME1, RPL10A, RPL12, RPL13A, RPL18, RPL22, RPL23, RPL30, RPL4, RPL6, RPL7, RPL7A, RPL8, RPL9, RPLP0, RPLP2, RPS10, RPS11, RPS13, RPS18, RPS19, RPS2, RPS27A, RPS3, RPS5, RPS6, RPS8, RPS9, RPSA, UQCR10

Table 2. RICTOR is predicted to be the top upstream causal regulator resulting from an alignment of independent proteomic analyses of brain in CLN1 [30] [17, 20], CLN2 [30], CLN3 [21] [30] and CLN4 [22] *in vivo* models as well as the neuronal PPT1 interactome [13]. Predicted activation z-score is calculated by weighing the predicted expression change of target molecules as defined by Ingenuity Knowledge Database against the actual expression change of target molecules reported in input dataset(s). An activation z-score >2 or <-2 is considered statistically significant. P-value of overlap is derived from a Fisher's exact test are derived from a Fisher's Exact Test calculating overlap between molecules in each respective input dataset and number of molecules comprising a canonical pathway defined by Ingenuity Systems Database (in this case, known downstream interactors of RICTOR). Target molecules present within each proteomic dataset predicted to be activated or inhibited by RICTOR accompany their respective input source. As the Scifo et al [12] study dataset was published as an interactome, no expression profile is available and therefore no activation z-score calculation nor predicted activation state is possible

Conclusions

Overall, relatively few studies have applied Omics technologies to the NCLs with the majority being in the last few years. Yet, these studies have helped to expand our understanding of the molecular cascades underpinning these disorders. Here, we have used these existing data sets to identify pathways which are potentially conserved irrespective of the initiating mutation, model system used or omic application used in the initial discovery phase. Whilst this serves to further the proposal that there may be converging regulatory mechanisms of vulnerability and or degeneration, in the future we can expect such techniques (when used in conjunction with ever more sophisticated models and clearly defined patient populations) to lead to the identification of new regulatory cascades and clinically relevant biomarkers for disease diagnosis, progression tracking, and treatment response reporting.

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Table 1. Recent NCL Omics studies

NCL	Disease Model	Omics platform	Profiled tissue	Year	Reference
NCL	Human	Reverse Phase Protein Microarrays	Muscle biopsy	2015	[40]
jNCL	Human Plasma	Proteomics	Multiplex immunoassays, 2D DiGE	2016	[29]
CLN1	Human Cells	Proteomics	Human ppt1 expressing sh-sy5y	2015	[13]
CLN1	Mouse	Proteomics	Laser-captured thalamus	2016	[17]
CLN1	Mouse	Proteomics	Brain membrane protein cilia	2017	[20]
CLN1	Mouse	Proteomics	Mouse brain	2019	[14]
CLN2	Human	Metabolomics	Csf	2018	[31]
CLN3	Human Cells	Proteomics	Human cln3 expressing sh-sy5y	2013	[12]
CLN3	Mouse	Proteomics	Synapse	2017	[21]
CLN1/CLN2/CLN3	Human	Proteomics	Csf and brain	2017	[30]
CLN4	Human/ Mouse	Proteomics	Brain	2016	[22]
CLN1/ CLN5	Mouse	Transcriptomics	Brain cortex	2013	[41]
CLN5	Dictyostelium	Proteomics	Amoeba cells	2018	[18]
CLN10	Mouse	Proteomics	Synapse	2013	[23]
CLN11	Human And Mouse	Multi-Omics Lipidomics, Transcriptomics	Lipidomics – mouse embryonic fibroblast and brain tissue and human	2017	[26]

			brain lysosome enriched organelles, transcriptomics on mouse brain		

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Highlights

- We discuss the benefit of application of various Omics technologies to the NCLs
- We discuss the functional Omics based studies so far applied to NCLs
- We summarise the findings of biomarker-based Omics studies for NCL
- We show a comparative systems biology analysis of data from published NCL Omics studies