 doi:10.20944/preprints202109.0109.v1

Review

Convergent Canonical Pathways in Autism Spectrum Disorder from Proteomic, Transcriptomic and DNA Methylation Data

Caitlyn Mahony[1] and Colleen O' Ryan[1]*

[1] Molecular & Cell Biology, University of Cape Town, Private Bag, Rondebosch 7700, Cape Town, South Africa

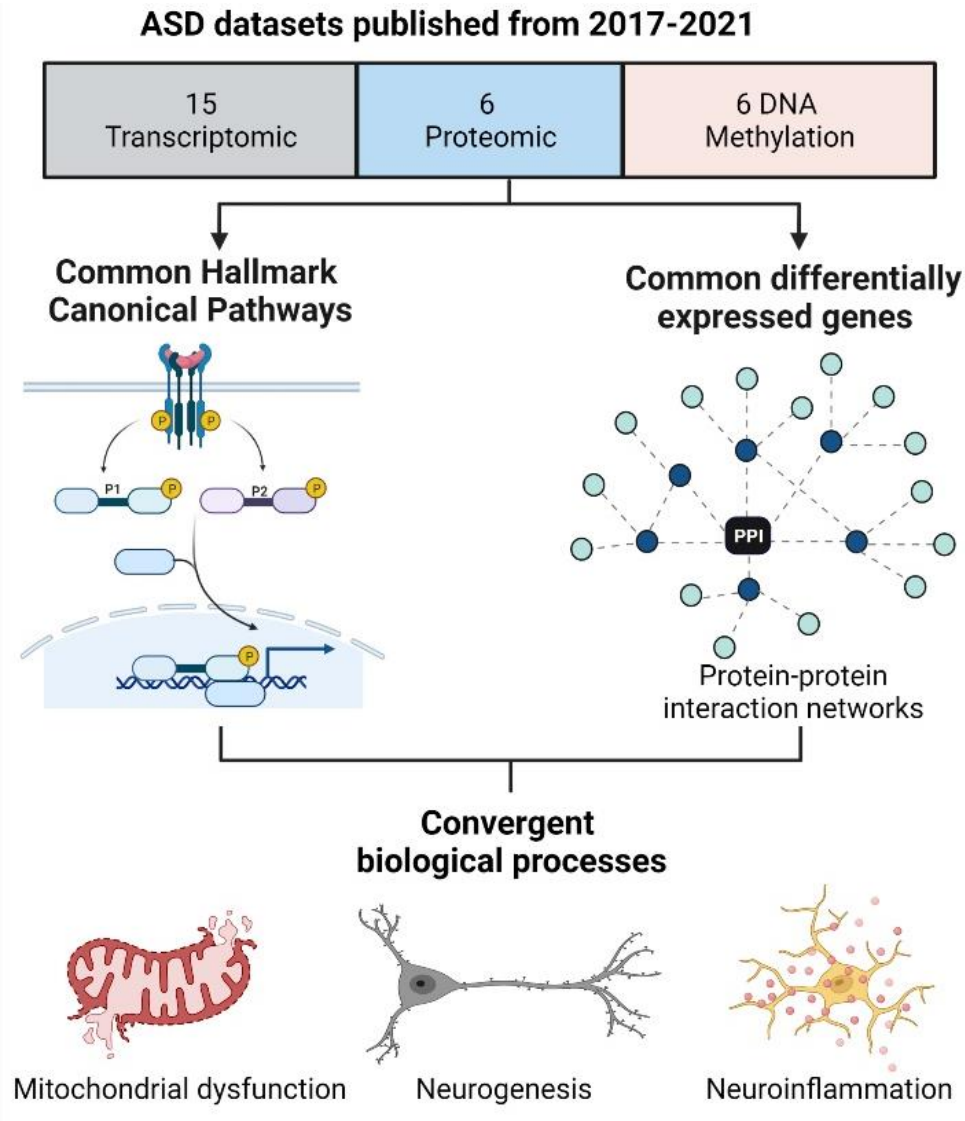
* Corresponding author: colleen.oryan@uct.ac.za

Abstract: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder with extensive genetic and aetiological heterogeneity. While the underlying molecular mechanisms involved remain unclear, significant progress has been facilitated by recent advances in high-throughput transcriptomic, epigenomic and proteomic technologies. Here, we review recently published ASD proteomic data and compare proteomic functional enrichment signatures to those of transcriptomic and epigenomic data. We identify canonical pathways that are consistently implicated in ASD molecular data and find an enrichment of pathways involved in mitochondrial metabolism and neurogenesis. We identify a subset of differentially expressed proteins that are supported by ASD transcriptomic and DNA methylation data. Furthermore, these differentially expressed proteins are enriched for disease phenotype pathways associated with ASD aetiology. These proteins converge on protein-protein interaction networks that regulate cell proliferation and differentiation, metabolism and inflammation which demonstrates a link between canonical pathways, biological processes and the ASD phenotype. This review highlights how proteomics can uncover potential molecular mechanisms to explain a link between mitochondrial dysfunction and neurodevelopmental pathology.

Keywords: proteomics; transcriptomics; DNA methylation; mitochondria; metabolism; OXPHOS; ASD; neurogenesis; gliosis; neurodevelopment

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

Graphical Abstract



1. Introduction

Autism Spectrum Disorder (ASD) is a lifelong neurodevelopmental condition that is characterized by heterogeneous genetic origins and a poorly understood aetiology [1]. Although the heritability of ASD is undisputed, the genetic contribution of ASD is only 64.6% [2] to 80% [3]. The genetic architecture of ASD is comprised of highly penetrant, rare variants, as well as more common variants which have smaller effect sizes [4]. However, no single genetic variant accounts for more than 1% of ASD cases [5], with more than 90% of ASD cases being idiopathic [6]. Recent technological advances in genomics, coupled with systems biology approaches that use functional genomic datasets, have facilitated remarkable progress in understanding the functional convergence of the genetic loci associated with ASD [7]. However, a challenge of ASD molecular research is being able to "translate gene discovery into an actionable understanding of ASD pathology" [8]. Transcriptomic and epigenomic approaches are widely used to study gene regulation and expression in ASD, and these have provided some insight into the functional changes at the molecular level. However, there have been fewer proteomic studies in ASD, due in part to the numerous challenges of this approach. Proteomic methodological approaches vary widely with respect to sample

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

preparation, instrumentation and protein quantification methods, all of which culminates in studies with limited reproducibility [9]. Additionally, the lack of unified data repositories and standardised protein identifiers make it difficult to integrate results between different studies [10]. However, as the "undertakers of biological activities" [11], proteins directly reflect the physiological processes underpinning disease aetiology. proteins directly reflect the physiological processes underpinning disease aetiology. Therefore, proteomics is an essential tool to characterise the cellular mechanisms involved in ASD aetiology and to validate the changes observed in the ASD transcriptome and epigenome.

We propose that the integration ASD proteomic data with large scale ASD transcriptomic and DNA methylation (DNAm) data may yield insight into the molecular mechanisms of ASD. Despite the aetiological and genetic complexity of ASD, this underlying heterogeneity is thought to converge on a limited number of biochemical pathways [12]. Integrative analyses of disparate molecular datasets are well-suited for the identification of common functional networks implicated in complex disorders and these networks can provide potential links between disease mechanisms and phenotypes [13]. In this review, we collate recently published data from independent transcriptomic, DNAm and proteomics studies to identify canonical pathways that are consistently implicated in ASD. Importantly, the purpose of this review is not to combine these disparate datasets in a bioinformatic analysis. Instead, these datasets will be functionally annotated in a uniform manner for comparison. First, we will examine this data to identify common shared canonical pathways across different molecular datasets. Subsequently, we will explore ASD proteomic datasets to identify differentially expressed proteins that are also implicated in the transcriptomic and DNAm data. Finally, these proteins will be characterised with respect to enriched protein-protein interaction networks to investigate the link between differentially expressed proteins, enriched canonical pathways and biological processes involved in ASD aetiology.

2. Methods

This review integrated data from large-scale quantitative meta-analyses from both transcriptomic and DNAm data, as well as from ASD proteomic datasets published over the past five years. Overall, data were collated from 19 studies and meta-analyses from ASD cohorts published between 2017 and 2021 (Table 1). The publications included six proteomic datasets [11,14–18], eight transcriptomic studies [19-26], and five DNAm screens [26–30]. The data collated from each study was the published list of genes or proteins found to be significantly associated with ASD after data quality control, normalisation, processing, statistical analyses and peer review (Supplementary Table S1). The datasets used in this review were treated as inherently and intentionally heterogeneous given that the data were: i) were derived from different cohorts and tissue types, ii) generated using different molecular approaches, iii) subject to different data processing and quality control workflows, and iv) filtered using different methods to determine statistical significance. Rather than combine the raw datasets in a bioinformatic analysis, the final gene lists from each dataset were functionally annotated and analysed to identify common canonical pathways.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

Table 1. A description of the 19 datasets included in this review, summarizing the type of study, the cohort size, the tissue type, and the definition of the final dataset used from each study. DE = differentially expressed; DM= differentially methylated.

Reference	Type of study	Tissue		Cohort	Dataset used in analysis
(Tylee et al. 2017)	Transcriptomic Meta-analysis	Blood	*Ex vivo* blood or lymphocytes	626 ASD and 447 controls across seven independent studies	90 DE genes in ASD ($p<0.05$).
(Mordaunt et al. 2019)			Umbilical cord blood samples	59 ASD, 92 non- typically developing, 120 typically developing controls	172 DE genes in ASD where $p<0.01$.
(Gao et al. 2020)			Peripheral blood samples	96 ASD and 42 controls	3624 DE genes in ASD.
(He et al. 2019)			Five data sets from blood lymphoblastoid cell lines (LCLs);	485 ASD and 398 controls	DE genes ($p<0.05$) in ASD.
(He et al. 2019)			Three data sets from postmortem brain tissue	109 ASD and 129 controls	DE genes ($p<0.05$) in ASD.
(Forés-Martos et al. 2019)			Frontal cortex tissue	34 ASD cases and 130 controls across three studies	1055 DE genes in ASD (FDR $P<0.05$)
(Rahman et al. 2020)			Post-mortem brain tissue	15 ASD and 15 controls across two studies	1567 DE genes in ASD.
(Wright et al. 2017)		Brain tissue	Postmortem brain tissue (dorsolateral prefrontal cortex)	13 ASD (3F, 10M), average age 22 (4 to 67) and 39 controls (9F, 30M), average age 22 (2 to 69)	1463 DE genes in ASD across all moderately expressed Ensembl genes (13 011) at marginal statistical ($P<0.05$) significance.
(Yao et al. 2021)			Brain tissure: cerebellum, frontal cortex, and temporal cortex	79 brain tissue samples from 19 ASD and 17 controls	364 DE genes in ASD.
(Ramaswami et al. 2020)				82 ASD samples and 74 control samples from 47 ASD and 44 control brains (Parikshak et al.)	5200 DE genes (FDR<0.05)
(Hewitson et al. 2021)	Proteomics	Blood	Blood serum	76 ASD (boys) and 78 controls (boys), 18 months–8 years of age	86 downregulated, 52 upregulated proteins in ASD (FDR < 0.05).
(Shen et al. 2019)			Blood PBMCs	24 male and 6 female ASD (2–6 years old), and age and gender-matched controls.	41 DE proteins in ASD.
(Shen et al. 2018)			Blood plasma	24 male and 6 female autistic patients (2–6 years old), and age and gender-matched controls.	24 DE proteins in ASD.
(Yang et al. 2018)			Blood serum	Han Chinese children: 68 ASD (average age = 12.42yrs) and age-matched 80 controls (average age 14.32yrs).	Eight biomarker peaks with higher expression in ASD.
(Yao et al. 2021)			Blood samples	79 brain tissue samples from 19 ASD and 17 controls; ELISA analysis of 20 ASD,	59 genes predicted to encode ASD-related blood-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

				and 20 age- and gender-matched controls.	secretory proteins; six proteins were validated using an ELISA.
(Abraham et al. 2019)		Brain tissue	Brodmann area 19 (BA19) Posterior inferior cerebellum (CB)	9 ASD cases (2-60yrs) and 9 age- and gender matched controls (1-60yrs)	146 DE proteins from BA19 between ASD and controls ($p < 0.05$); 191 DE proteins in cerebellum ($p < 0.05$).
(Mordaunt et al. 2020)		Blood	Umbilical cord blood samples	Discovery set = 74 males (35 ASD and 39 controls) and 32 females (15 ASD and 17 controls) in the MARBLES and EARLI studies. Replication set = 38 males (21 ASD and 17 controls) and 8 females (5 ASD and 3 controls).	537 DM genes in both discovery and replication sets in males
(Hu et al. 2020)	DNA Methylation analysis		Lymphoblastoid cell lines	21 ASD and 21 controls	181 DM genes that overlap between the discovery and validation groups males
(Wong et al. 2019)		Brain tissue	Post-mortem brain tissue from Prefrontal cortex, temporal cortex and cerebellum	43 ASD and 38 controls	i)Top ranked iASD-associated DM probes identified in the cross-cortex model incorporating both prefrontal cortex and temporal cortex data.
(Ramaswami et al. 2020)			Brain tissue	56 ASD samples and 41 control samples from 33 ASD and 26 control brains.	DM genes (Either Promoter or Gene body, FDR<0.05).
(Stathopoulous et al 2020)		Buccal cells	Buccal DNA	48 boys (32 ASD and 16 controls, 6–12 years old)	898 DM genes in ASD.

The gene list from each study was converted into NCBI Entrez IDs using a combination of gene ID conversion tools, namely i) Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 Gene Accession Conversion Tool (https://david.ncifcrf.gov/conversion.jsp) [31,32], ii) the g:Profiler tool g:convert for Gene ID conversion (https://biit.cs.ut.ee/gprofiler/convert) [33], iii) the GIDcon batchwise gene ID conversion tool (http://resource.ibab.ac.in/GIDCON/geneid/home.html) and iv) the SYNGO geneset analysis tool (https://syngoportal.org/convert.html). For the datasets written as Ensemble Gene IDs, the Ensemble ID History Converter (https://www.ensembl.org/Homo_sapiens/Tools/IDMapper) was used to map all identifiers to matched identifiers in the most recent release of the database. Subsequently, all Ensemble Identifiers, Gene Symbols or GenBank accession numbers not identified through any of these ID conversion tools were manually submitted to NCBI (https://www.ncbi.nlm.nih.gov/gene) to find the corresponding Gene ID. Deprecated Ensemble IDs that were not matched to an ID in the most recent release were excluded.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

Subsequently, each gene list was subjected to gene set enrichment analysis [GSEA] against the Molecular Signatures Database (MSigDB) [34], using the open-source GSEA tool developed by UC San Diego and the Broad Institute (http://www.gsea-msigdb.org/gsea/msigdb/annotate.jsp) [35,36]. Each dataset was annotated with respect to the top 10 significantly enriched Hallmark Canonical pathways where each pathway represents a well-defined biological process in one curated gene set [37]. These enrichment signatures were compared between independent datasets to identify canonical pathways that were consistently dysregulated in ASD at all three molecular levels; proteomic, transcriptomic and epigenomic.

The proteomic datasets were examined to identify a subset of differentially expressed proteins that were also implicated in transcriptomic and DNAm data in ASD. After conversion to NCBI Entrez Gene IDs, this subset of proteins was annotated with respect to significantly enriched Hallmark canonical pathways, ClinVar disease pathways and Transcription Factor Protein-Protein interactions (TF-PPIs) using the Enrichr gene list analysis tool suite (https://maayanlab.cloud/Enrichr/) [38,39]. TF-PPIs are defined by a library of datasets corresponding to a list of transcription factors and the proteins that interact with them. The top 10 significantly enriched TF-PPIs were used to generate a signalling network based on the SIGnaling Network Open Resource (SIGNOR) v2.0 database [40], using the Network Analyst web interface (https://www.networkanalyst.ca/NetworkAnalyst/home.xhtml) [41]. This network was used to explore the relationship between the canonical pathways implicated in ASD, biological processes and phenotypic aspects of ASD aetiology.

3. Results

After collating the data from 19 different studies (Table 1), we determined the top 10 significantly enriched Hallmark canonical pathways for each of the datasets (Figure S1). These enrichment signatures were compared across all 19 datasets to identify canonical pathways that were consistently supported by all three molecular approaches (Table 2). Eight Hallmark canonical pathways were enriched in seven or more different datasets (Figure 1). Of these, six canonical pathways were supported using all three molecular approaches. There were two pathways that were most frequently associated with ASD: the oxidative phosphorylation and mTORC1 signalling pathways. These two pathways were implicated in 10 different datasets and these datasets included proteomic, transcriptomic and DNAm datasets derived from *post mortem* brain tissue from individuals with ASD. There were four additional canonical pathways consistently implicated across molecular datasets: the coagulation, xenobiotic metabolism, p53 and adipogenesis pathways. Notably, three canonical pathways, oxidative phosphorylation, mTORC1 signalling and xenobiotic metabolism, were each implicated in at least three proteomic, three transcriptomic and two DNA methylation studies. The canonical pathway most consistently associated with the ASD proteomic data was the coagulation pathway. This pathway was implicated in five out of six proteomic datasets, as well as three transcriptomic datasets and one DNAm screen.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

Table 2. A summary of the Hallmark Enrichment Signatures of 19 ASD molecular datasets, highlighting canonical pathways that were most consistently implicated in ASD proteomic, transcriptomic, and DNAm datasets. Each independent dataset was annotated with respect to the top ten significantly enriched Hallmark canonical pathways (FDR<0.5); data indicate the number of enrichment signatures implicating each canonical pathway. Pathways in bold = strongly supported using all three molecular approaches; Pathways in italicised = supported by two (of three) molecular datasets.

Hallmark Canonical Pathways In 4 Or More Transcriptomic Enrichment Signatures	**No. Of Transcriptomic Datasets**	**Hallmark Canonical Pathways In 2 Or More DNAm Enrichment Signatures**	**No. Of DNAm Datasets**	**Hallmark Canonical Pathways In 2 Or More Proteomic Enrichment Signatures**	**No. Of Proteomic Datasets**
INTERFERON GAMMA RESPONSE	8	**OXIDATIVE PHOSPHORYLATION**	3	COAGULATION	5
COMPLEMENT	6	*P53 PATHWAY*	3	*COMPLEMENT*	3
MTORC1 SIGNALING	5	MITOTIC SPINDLE	3	**OXIDATIVE PHOSPHORYLATION**	3
P53 PATHWAY	5	**MTORC1 SIGNALING**	2	**MTORC1 SIGNALING**	3
ALLOGRAFT REJECTION	5	*XENOBIOTIC METABOLISM*	2	*XENOBIOTIC METABOLISM*	3
INTERFERON ALPHA RESPONSE	5	UV RESPONSE UP	2	ADIPOGENESIS	3
TNFA SIGNALING VIA NFKB	5	*UNFOLDED PROTEIN RESPONSE*	2	*UNFOLDED PROTEIN RESPONSE*	2
HYPOXIA	5	ESTROGEN RESPONSE EARLY	2	MYOGENESIS	2
INFLAMMATORY RESPONSE	5	E2F TARGETS	2	FATTY ACID METABOLISM	2
APOPTOSIS	5	DNA REPAIR	2	MYC TARGETS V1	2
OXIDATIVE PHOSPHORYLATION	4	PEROXISOME	2	ANGIOGENESIS	2
EPITHELIAL MESENCHYMAL TRANSITION	4				
KRAS SIGNALING UP	4				

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

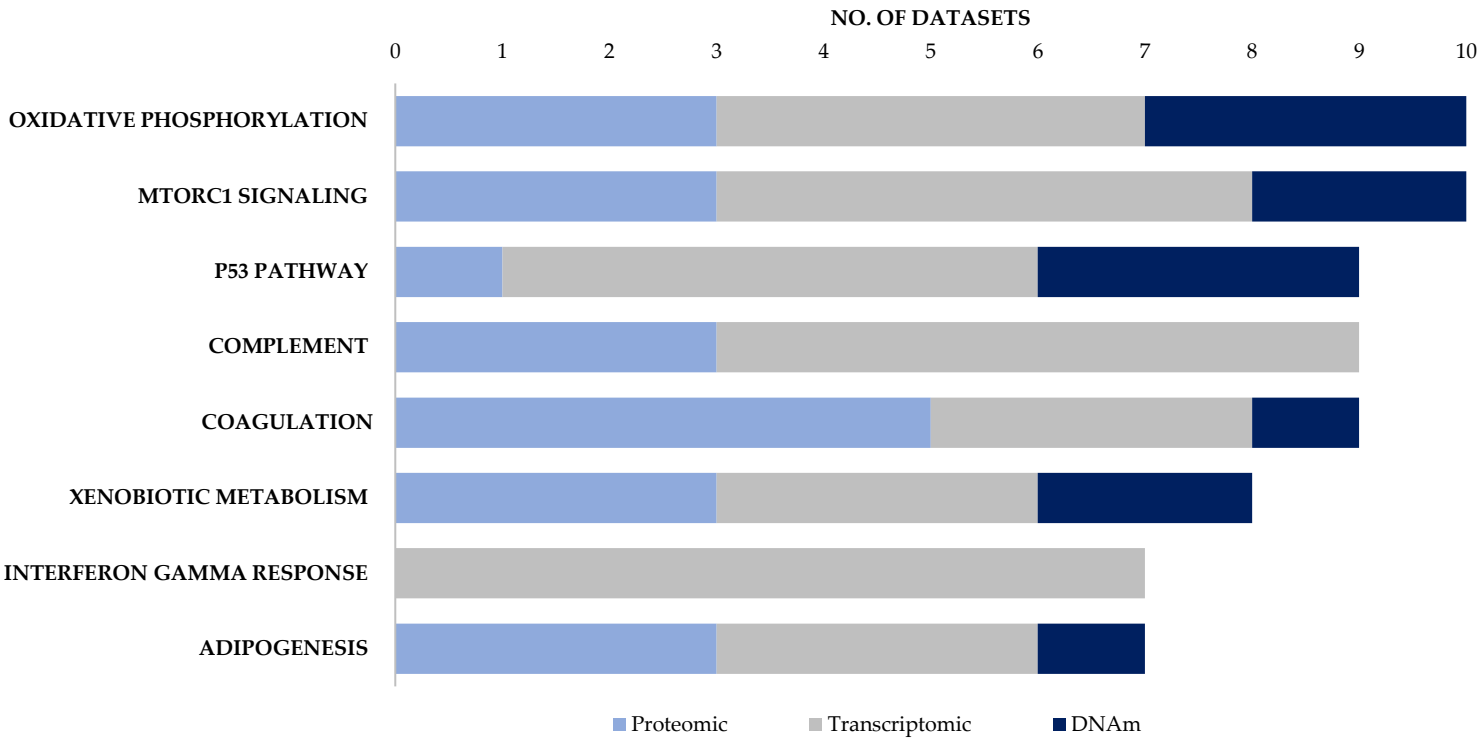


Figure 1. Hallmark Canonical Pathways most consistently implicated in ASD Molecular Data. Datasets were collated from 19 studies and meta-analyses on ASD papers published between 2017 and 2021: six proteomic, eight transcriptomic and six DNA methylation (DNAm) studies. All gene lists were converted into NCBI Entrez Gene IDs. Each dataset was annotated with respect to the top 10 significantly enriched Hallmark Canonical pathways. The Hallmark Canonical Pathways that were significantly enriched in seven or more independent ASD proteomic, transcriptomic and DNAm datasets are shown; those shown in bold are those consistently implicated using all three molecular approaches.

We next explored how the proteomic data articulated with the transcriptomic and DNAm data associated with ASD. We identified a subset of 121 proteins that were implicated in ASD using all three molecular approaches (Figure S3; Supplementary Table S2). We characterised this dataset with respect to enriched Hallmark canonical pathways, disease phenotype pathways, and downstream biological processes in order to explore the functional implications associated with these proteins. This subset of proteins was significantly enriched for Hallmark canonical pathways involved in mTOR signalling (this includes mTORC1 signalling and PI3K-AKT-mTOR signaling), metabolism (this includes oxidative phosphorylation, glycolysis, fatty acid metabolism and adipogenesis), and immune responses (this includes complement, allograft rejection and IL6-JAK-STAT3 signalling). The 10 most significantly enriched Hallmark canonical pathways in this subset of proteins included four canonical pathways that were also consistently enriched across all 19 datasets; namely, mTORC1 signalling, oxidative phosphorylation, adipogenesis and the complement response (Figure 2A). Together, the four canonical pathways from both analyses converge on signalling networks that regulate neural stem cell proliferation, differentiation, metabolism, redox homeostasis and reactive gliosis during neurodevelopment (Figure 2B).

The subset of 121 proteins that were implicated using all three molecular approaches was also enriched for five ClinVar disease pathways associated with neurological, immunological and metabolic diseases (Table 3). Notably, the most significantly enriched disease pathway was for Leigh syndrome, which is a pediatric mitochondrial disease

 doi:10.20944/preprints202109.0109.v1

that manifests with severe neuropathology [42]. Therefore, the above-mentioned proteins converge on three central components of ASD pathophysiology which may yield insight into the link between canonical pathways and the dysregulation of biological processes in ASD aetiology. Consequently, we examined the protein-protein interaction networks and downstream signalling pathways associated with this dataset by testing for significant enrichment of TF-PPIs. The top 10 significantly enriched TF-PPIs are key transcriptional regulators of mitochondrial metabolism (CREBP1A, PPARGC1A and HNF1A), lipid metabolism (PPARCG1A and STAT1), adipogenesis (NR3C1), the p53 pathway (TP53; TP63) and inflammation (NR3C1, STAT1) (Supplementary Table S4). Notably, several of these transcription factors mediate signalling via the PI3K-AKT-mTOR (HTT, TP53, CREBP1A, PPARGC1A) or ERK-mTOR (ESR1,STAT1,HNF1A) pathways. This highlights some of the transcription factors that regulate the canonical pathways implicated in ASD molecular data. The signalling network between the top 10 significantly enriched transcription factors converged on two SIGNOR stimuli (ROS and DNA damage), as well as six SIGNOR signalling pathways; namely, polarization, proliferation, apoptosis, cell death, mitochondrial biogenesis and inflammation (Figure 3). Therefore, this subset of differentially expressed proteins highlights a link between signalling pathways, biological processes and ClinVar disease phenotypes associated with ASD aetiology. Altogether, recent proteomic profiling studies, in conjunction with previously published transcriptomic and epigenomic meta-analyses, consistently implicate canonical pathways involved in neuronal metabolism, differentiation and inflammation.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

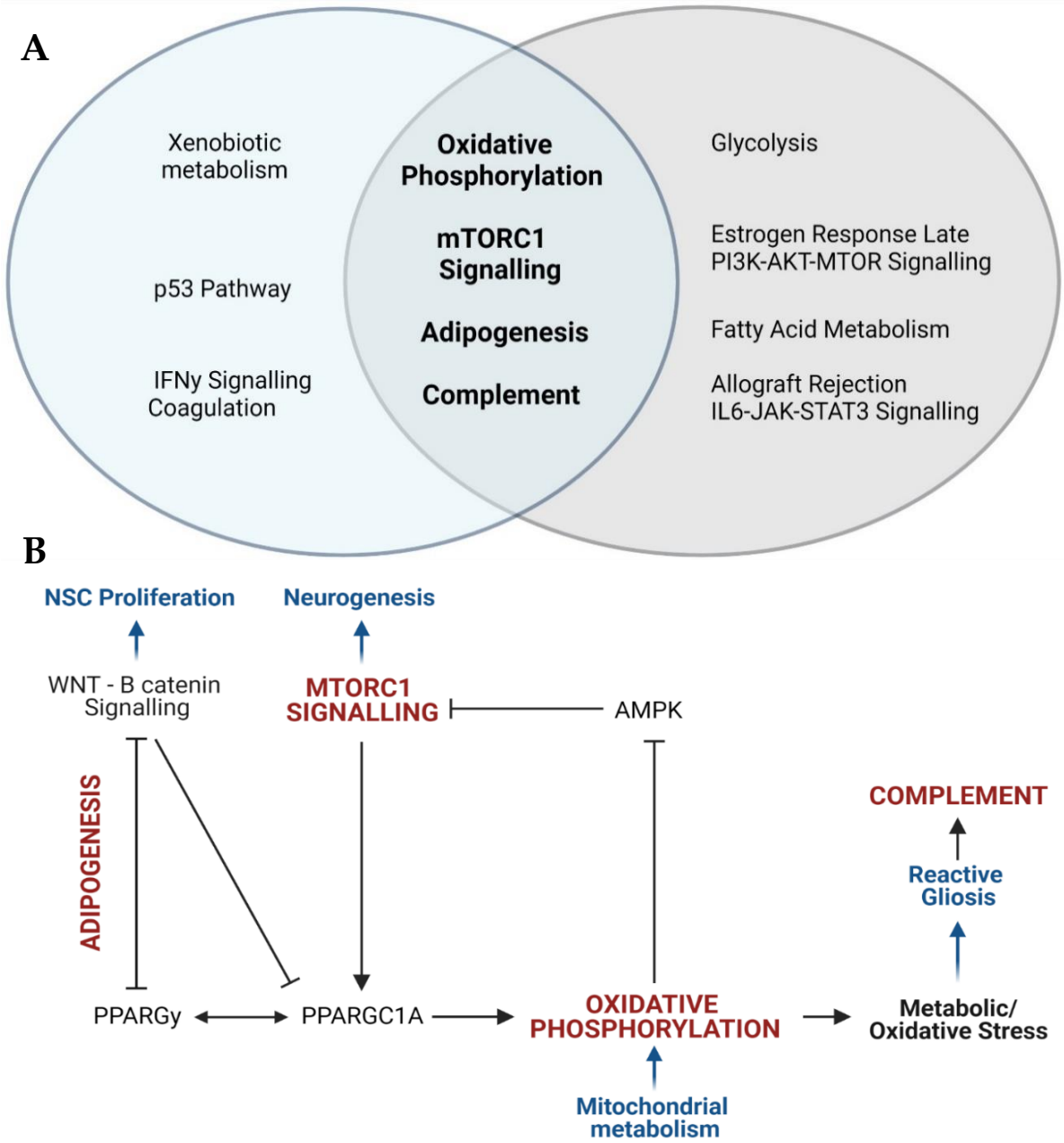


Figure 2. ASD proteomic profiles converge on four canonical pathways involved in mitochondrial metabolism, neurogenesis and neuroinflammation. A) The Venn diagram shows the Hallmark canonical pathways implicated in seven or more independent datasets (in blue), the enriched canonical pathways in the 121 differentially expressed proteins implicated in transcriptomic and DNAm datasets (light grey) and the pathways that overlap (in bold font). B) The four pathways implicated in both analyses (Figure 2A) converge on the regulation of the following biological processes (in blue): neural stem cell proliferation, neurogenesis, mitochondrial metabolism and inflammation. The mTORC1 signalling pathway induces oxidative phosphorylation via the activation of camp-responsive element binding protein 1 (CREB1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A), which regulates mitochondrial metabolism and the antioxidant response. PPARGC1A is regulated by peroxisome proliferator-activated receptor gamma (PPARGy), which acts as a master regulator of lipid metabolism and is essential to induce adipogenesis. Adipogenesis is negatively regulated by WNT signalling, which inhibits PPARGy, PPARGC1A and mTORC1 signalling to regulate stem cell proliferation and differentiation. Mitochondrial metabolism regulates metabolic and redox homeostasis which governs the inflammatory profile of microglia. Oxidative stress leads to the over proliferation of reactive microglia, which triggers the pro-inflammatory complement response.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

Table 3. ClinVar Disease Pathways that are significantly enriched in the 121 differentially expressed proteins that are also implicated in both transcriptomic and DNAm datasets.

ClinVar Disease Pathway	Disease Phenotype	P value
Leigh Syndrome	Neurological Disease	0.0027
Familial Partial Lipodystrophy		0.0299
Pyruvate Dehydrogenase Complex Deficiency	Metabolic Disease	0.0299
Mitochondrial DNA Deletion Syndromes		0.0474
Autoimmune Lymphoproliferative Syndrome	Autoimmune Disease	0.0358

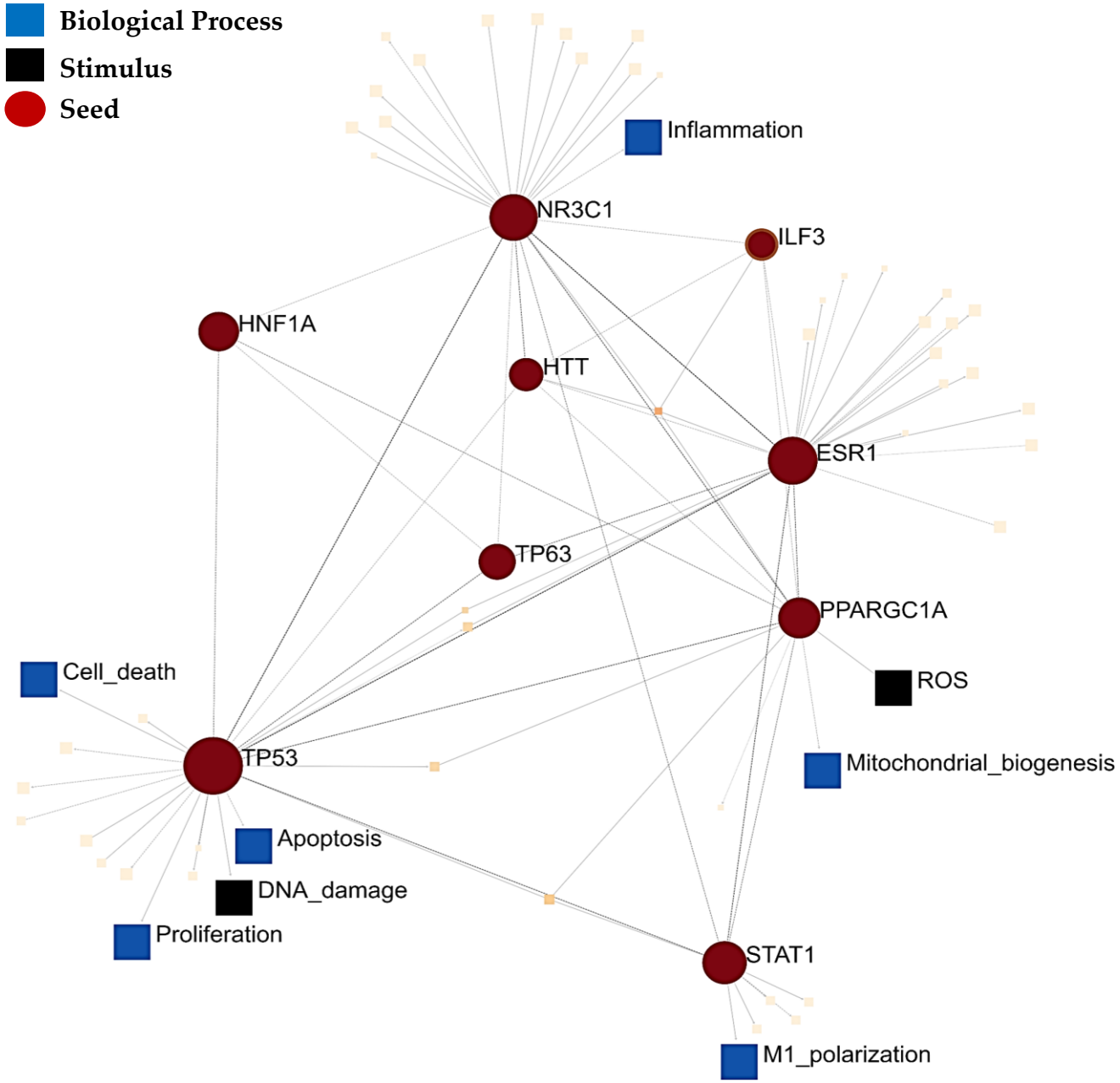


Figure 3. Signalling network between the top 10 Transcription Factor Protein-Protein Interactions (PPI) enriched in the set of 121 proteins that are supported by ASD proteomic, transcriptomic and epigenomic datasets. This PPI network was annotated with respect to signalling pathways in the SIGnaling Network Open Resource (SIGNOR) v2.0 database of manually-annotated causal relationships between proteins that participate in signal transduction.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

4. Discussion

Molecular research of ASD requires a "multi-omics" approach to comprehensively characterise the convergent biological processes associated with its complex genetic architecture. Proteomic, transcriptomic and epigenomic approaches are well-established as ways to investigate changes in gene regulation, expression and function in ASD. DNA methylation is widely recognized as an epigenetic regulator of gene expression that contributes to ASD aetiology [43]. Transcriptomic studies facilitate an understanding of the link between the genetic mutations associated with ASD and changes in gene expression and function [11]. Recent advances in high-throughput proteomic technologies have facilitated the progress of ASD proteomic profiling studies using both peripheral and brain tissue to provide insight into the cellular mechanisms involved in ASD aetiology. Proteomic approaches are uniquely able to detect different protein isoforms or post-translational modifications and have the potential to identify disease biomarkers and drug targets for the diagnosis and treatment of ASD [9,11]. Furthermore, proteomics yields insight into the dysregulation of biologically relevant protein interaction networks and signalling path- ways [18]. This review integrated the functional annotations of ASD proteomic, transcriptomic and DNAm datasets to identify common canonical pathways associated with ASD.

We found that the canonical pathways involved in metabolism, redox homeostasis, inflammation and proliferation are consistently supported by proteomic, transcriptomic and DNAm data in ASD (Figure 1, Figure 2). This review explored the link between canonical pathways, biological processes and disease phenotype pathways in ASD by functionally annotating a subset of differentially expressed proteins that were also supported by transcriptomic and DNAm data. This subset of proteins was enriched for four canonical pathways that were also implicated in seven or more independent datasets. These were the pathways for oxidative phosphorylation, mTORC1 signalling, adipogenesis and the complement response (Figure 2A). The ten most significantly enriched TF-PPIs in this subset of proteins highlighted key transcriptional regulators of these canonical pathways. These transcriptional regulators form a signalling network that highlights how these canonical pathways regulate biological processes involved in neuropathology (Figure 3). This network converges on the SIGNOR pathways for mitochondrial biogenesis and inflammation, and this is consistent with prior evidence linking mitochondrial dysfunction [44-49] and neuroinflammation [50-52] to ASD. The polarization and proliferation pathways are essential regulators of neural stem cell self renewal and differentiation [53–55], while the apoptosis and cell death signalling pathways are implicated as mechanisms whereby microglia drive synaptic pruning, and the maturation and migration of neuronal progenitors [56]. In addition, this subset of proteins was significantly enriched for five ClinVar disease pathways associated with neuropathology, mitochondrial dysfunction or auto-immune disease (Table 3). Therefore, the differentially expressed proteins highlighted in this review are linked to both molecular and phenotypic facets of ASD aetiology.

The common canonical pathways highlighted above are each established as molecular mechanisms contributing to ASD aetiology. Additionally, when these pathways are considered together in the context of neurodevelopment, they converge on a link between mitochondrial dysfunction, neurogenesis and inflammation in ASD. Firstly, a role for mTORC1 signalling is well described in ASD [57,58]. Mutations in tuberous sclerosis complex (TSC) and Phosphatase and tensin homolog (PTEN), which are both central regulators of mTORC1 signalling, are associated with autistic behaviours [59-62]. Aberrant mTOR signalling is associated with altered synaptogenesis and ASD-like neurophysiology in animal and organoid model systems [63-68]. Moreover, the mTOR signalling pathway is a promising therapeutic target in ASD [69].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

The canonical pathways for mTORC1 signalling and oxidative phosphorylation were the two most commonly implicated pathways in our analyses which is consistent with evidence showing that mitochondrial dysfunction is involved in ASD aetiology [44-49]. Mitochondrial function has recently been proposed as a central driver of neuronal differentiation [70-73] and the tight coregulation of mTORC1 signalling and mitochondrial metabolism is essential to control neurogenesis. The transition from undifferentiated neuronal stem cells (NSCs) to mature neurons relies on a metabolic shift from aerobic glycolysis to mitochondrial respiration. The latter fuels neuronal migration, dendrite formation and synaptogenesis [74]. The mTOR signalling pathway plays a central role in driving this metabolic switch, which is essential for neuronal survival and differentiation [72]. Neurogenic factors induce AKT-MTORC1 signalling, which upregulates peroxisome proliferator-activated receptor-gamma coactivator (PPARGC1A) signalling to induce mitochondrial respiration and biogenesis [71,75]. Conversely, cellular metabolism regulates mTOR signalling via the AMP-dependent protein kinase (AMPK), which is inhibited by a decrease in the AMP:ATP ratio [76]. The dysregulation of signalling between mTORC1 and mitochondrial metabolism during development has profound consequences for NSC commitment and differentiation, and the development and maintenance of mature neuronal networks.

We also observed a consistent enrichment of the adipogenesis pathway in ASD molecular data, which implicates the same networks that regulate stem cell metabolism and differentiation. Adipogenesis is regulated by the "opposite interplay" between WNT/B-catenin signalling and peroxisome proliferator-activated receptor gamma (PPARGy), each of which negatively regulates the other [77]. WNT signalling is directly involved in regulating NSC maintenance, proliferation and differentiation [78,79] and WNT responsive genes maintain aerobic glycolysis in NSCs [72]. This highlights that the WNT pathway is at the intersection between stem cell metabolism and development. Cross-talk between WNT signalling and mTORC1 signalling is linked to the "Warburg effect" that induces aerobic glycolysis and over-proliferation in cancer cells [77]. A dysregulation of these same pathways during neurodevelopment could disrupt NSC commitment and differentiation. In fact, chronic activation of WNT signalling altered mTOR signalling in human organoids, leading to increased NSC proliferation, impaired neuronal differentiation and disrupted radial glial organization [80]. Moreover, *in vivo* downregulation of WNT signalling leads to premature neurogenesis and atypical behaviours [81]. The WNT/B catenin pathway has also been implicated in ASD genetic and transcriptomic data [82-86].

On the other hand, PPARGy acts as a master regulator of lipid metabolism and regulates target genes necessary for differentiation, fatty acid transport, carbohydrate metabolism, and energy homeostasis [87]. Mitochondrial fatty acid oxidation (FAO) is essential for the self-renewal of NSCs, but fatty acid metabolism shifts towards lipogenesis during neurogenesis [88-91]. *In vitro* studies show that this shift in fatty acid metabolism is regulated by the AMPK-PPARGC1A axis [91]. The coregulation between PPARGy and PPARGC1A means that glucose and fatty acid metabolism are intrinsically linked during neurodevelopment. Disrupting FAO inhibits stem cell self-renewal [89,91] and impairs NSC differentiation in mouse models [92]. Recent reviews of ASD proteomic data also find that proteins involved in lipid metabolism are differentially expressed in ASD [93,94]. A role for PPARGy is supported by increasing evidence of mitochondrial FAO deficiencies in ASD, and PPARGy agonists have been proposed as therapeutic agents in ASD [95,96]. This highlights how PPARGy is implicated in neurodevelopment and ASD, and how the canonical pathways for mTORC1 signalling, oxidative phosphorylation and adipogenesis converge on the signalling between neuronal metabolism and differentiation.

A dysregulation of the signalling between mitochondrial metabolism and neurogenesis has significant implications for neuronal development and function. Firstly, mitochondrial oxidative phosphorylation is one of the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0109.v1

primary producers of intracellular reactive oxygen species (ROS) and disruptions to mitochondrial metabolism can lead to oxidative stress [97]. Increased oxidative stress is a well-documented aspect of ASD pathophysiology; the evidence for markers of oxidative stress associated with glutathione metabolism, lipid peroxidation, protein oxidation, DNA oxidation and antioxidant enzyme activity has been comprehensively reviewed in recent years [45,98-103]. In the context of neurodevelopment, this has significant implications for the redox regulation of neurogenesis. Undifferentiated NSCs are characterized by high levels of endogenous ROS, which plays a functional role in stem cell maintenance [105] and oxidative stress is known to promote NSC self-renewal and inhibit downstream neurogenesis [97,105]. Studies on i*n vitro* and *in vivo* model systems consistently demonstrate that increasing oxidative stress [106 - 108] or disrupting mitochondrial homeostasis and function [109-112] leads to an inhibition of neurogenesis and a shift towards gliosis; the latter is associated with neuroinflammation and is established as a hallmark of ASD aetiology. Clinical studies also report that gliosis is one of the neuropathological manifestations of mitochondrial disease [43,113-116].

Many of the canonical pathways highlighted in this review are implicated as mechanisms involved in the response to oxidative stress and gliosis during neurogenesis. Clinical data has reported mTOR signalling as a mechanism that connects mitochondrial disease to gliosis [117], with both *in vitro* - [118] and *in vitro*- studies [119] showing that gliosis can be induced by targeted disruptions to mTOR signalling. Cross-talk between the PPARGy and WNT canonical pathways is also associated with increased oxidative stress and chronic inflammation in cancer [119], and WNT/B-catenin-PPARGy signalling can be targeted to reduce reactive gliosis in models of neurodegenerative disease [120-122]. In addition, the p53 pathway and xenobiotic metabolism were each implicated in eight or more independent datasets in our analysis and these pathways were supported by all three types of molecular data (Figure 1). The p53 signalling pathway is a central regulator of inflammation, oxidative stress and apoptosis [124], while the canonical pathway for xenobiotic metabolism is comprised of genes that respond to inflammation, metabolic stress and ROS [125]. Notably, both mTOR and WNT signalling regulate p53 degradation [74,126,127]. The p53 signalling pathway also plays an important role in neural precursor cell self-renewal, neuronal commitment and reactive gliosis in response to mitochondrial dysfunction [111,112,128].

Gliosis is characterized by highly reactive microglia and astrocytes, leading to an overproduction of glial-specific fibrillary acidic protein (GFAP) and inflammatory cytokines [129]. This review highlights the consistent enrichment of inflammatory canonical pathways in ASD transcriptomic and proteomic data. The canonical pathways involved in inflammatory immune response pathways were those most frequently associated with the transcriptomic enrichment signatures: six of ten hallmark canonical pathways that were implicated in at least five independent transcriptomic datasets were related to immune responses. This is consistent with a substantial body of work implicating neuroinflammation and gliosis as mechanisms of pathology of ASD, which has been thoroughly reviewed elsewhere [50-52,130-133]. A role for inflammation, astrocyte function and microglial activation is well-supported by transcriptomic and proteomic studies in ASD brain tissue [134-136]. This is also consistent with immunohistochemistry, positron emission tomography and morphological data in ASD [130,137,138]. Notably, microglial phagocytosis is responsible for the degradation of proliferating NSCs during neurodevelopment [57]. This is essential for synaptogenesis and post-natal synaptic pruning, both of which are thought to be dysregulated in ASD [130,139,140]. Importantly, microglial metabolism is closely linked to neuroinflammatory state [141,142], and both glucose and lipid metabolism regulate microglial activation [143-146]. The dysregulation of glial and microglial metabolism, proliferation and function can alter neuronal differentiation, impair

 doi:10.20944/preprints202109.0109.v1

synaptogenesis and pruning, and change neuroinflammatory state, with profound implications for neural architecture and connectivity [147].

Our analysis of recent ASD proteomic profiles further supports a role for neuroinflammation in ASD. The complement and coagulation cascades were implicated in three and five out of six ASD proteomic studies respectively, which is supported in independent reviews of ASD proteomic data [13,93,94]. Both cascades form part of the innate immune system and interactions between them via mannose-binding lectin associated serine proteases are well documented [148-153]. Both cascades are involved in the inflammatory response by reactive microglia [154,155]. Coagulation proteins affect the morphology, proliferation and function of astrocytes [154], while the complement system is implicated as a mechanism by which microglia regulate neurogenesis, neuronal migration and synaptic pruning [155-158]. Complement proteins play a role in microglial activation, which can influence neuroinflammatory signalling and neurodevelopment [159]. Animal models show that genetic knockdown of complement proteins impairs neuronal migration, while activation of the complement system rescues this deficit [156]. There is mounting evidence for complement system dysfunction in neurodevelopmental disorders [157], which has been proposed as one of the mechanisms behind the synaptic pruning deficits, increased dendritic spine density, cortical hyperconnectivity and resultant behavioural phenotypes in ASD [158]. Therefore, the immune response cascades enriched in ASD proteomic profiles function as a link between gliosis, neurogenesis and synaptogenesis, highlighting a point of convergence between these different mechanisms in ASD pathology.

Collectively, the canonical pathways highlighted in this review are consistent with known pathways that to contribute to ASD aetiology, as well as pathways that have been implicated in independent reviews of ASD molecular data. Importantly, our review also considers the interactions between these canonical pathways, particularly in the context of neurodevelopment. The latter is often overlooked when the relevance of each canonical pathway is evaluated in isolation. Previous reviews have comprehensively described the role of mTORC1 signalling in protein synthesis, neuronal differentiation, migration and patterning [159], but these reviews have focused less on the role of mTORC1 as a regulator of mitochondrial metabolism. The same is true for reviews on the role played by WNT signalling in neurogenesis and ASD [160,161], which describe the central role played by WNT signalling in NSC proliferation and differentiation. However, our review emphasises how WNT-PPARGy signalling intersects with PPARGC1A, anti-inflammatory and antioxidant responses, and the regulation of mitochondrial biogenesis and metabolism. Given that mitochondrial dysfunction is an established component of ASD aetiology, and that metabolic state plays a central role in neuronal differentiation, our review highlights a potentially important connection between mTORC1, oxidative phosphorylation and neuropathology in ASD. Finally, our review integrates all of the above with the thoroughly reviewed evidence for neuroinflammation and oxidative stress in ASD [49-52,98-103] by considering the role of mitochondrial metabolism in gliosis and microglial activation, and the downstream consequences for synaptogenesis, neuronal connectivity and behavioural phenotypes. Therefore, our review is not only consistent with current molecular mechanisms implicated in ASD, but also highlights an under-explored link between mitochondrial dysfunction, neuroinflammation and neurodevelopmental pathology.

 doi:10.20944/preprints202109.0109.v1

5. Conclusion

The genetic and phenotypic complexity of ASD makes it challenging to identify the molecular mechanisms that contribute to its aetiology. Proteomic approaches can yield novel insight into the dysregulation of biological processes and signalling networks in ASD. Despite the power of proteomics, there are currently limitations to this approach in terms of scaling up in sample size and efficiency. In light of these caveats, we integrated recently published ASD proteomic profiles with large-scale meta-analyses of whole-genome transcriptomic and DNAm data to compare the functional enrichment signatures of disparate molecular datasets. Our analysis demonstrates that ASD proteomic, transcriptomic and DNAm data consistently support the dysregulation of mTORC1 signalling, oxidative phosphorylation, adipogenesis and the response to inflammation and oxidative stress. The mTORC1-, WNT- and PPARGy- signalling pathways are each well-established regulators of neurodevelopment that are also implicated in ASD and neurodevelopmental pathology. The mTORC1 signalling pathway regulates oxidative phosphorylation via PPARGC1A, which operates in concert with PPARGy to regulate mitochondrial respiration and fatty acid oxidation. These pathways play an essential role in the tight coupling between stem cell proliferation, differentiation and mitochondrial metabolism during neurogenesis. If this coupling is disrupted, this can induce metabolic and oxidative stress in NSCs. Both metabolic and oxidative stress are known to disrupt neurogenesis, promote gliosis and induce microglial activation. This proposes a link between mitochondrial dysfunction, oxidative stress and gliosis, each of which are well-established features of ASD aetiology. Given the central role played by glial networks in neuronal migration and synaptogenesis, gliosis is implicated as a mechanism involved in neuroinflammation and the neuropathophysiology associated with ASD. In summary, this review demonstrates that ASD molecular data converges on canonical pathways involved in mitochondrial function, neurogenesis and neuroinflammation, highlighting how these three key aspects of ASD aetiology interact, and the relevance of these interactions in the context of neurodevelopment.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, **Supplementary Figure 1: The top 10 significantly enriched Hallmark canonical pathways in 19 datasets of ASD molecular data**; the heatmap represents the FDR value of enrichment; **Supplementary Figure 2: Summary of Hallmark Enrichment Signatures across 19 datasets of ASD proteomic, transcriptomic and DNAm datasets**. The vertical axis represents the number of datasets for which each Hallmark canonical Pathway is among the top 10 significantly enriched Hallmark Pathways in that dataset; **Supplementary Figure 3. The 121 differentially expressed proteins previously implicated in both transcriptomic and DNA methylation ASD datasets.** The Venn Diagram represents 15 075 unique gene IDs implicated across the 19 datasets included in this review. This includes 12 037 unique genes reported across 11 transcriptomic studies, 5 573 genes across six genome-wide DNA methylation screens and 750 proteins across five proteomic profiling studies. We identified a subset of 121 proteins that had been implicated in ASD using Transcriptomic, Epigenomic and Proteomic methods; **Supplementary Table 1:** Full datasets collated from the ASD proteomic, transcriptomic and DNA methylation studies described in Table 1. Each dataset represents the list of genes or proteins found to be significantly associated in ASD after data processing and statistical analyses, converted into NCBI Entrez Gene IDs; **Supplementary Table 2:** The 121 proteins identified in this review that have been implicated in Transcriptomic, Epigenomic and Proteomic data in ASD; **Supplementary Table 3:** Top 10 Hallmark canonical pathways enriched in the set of 121 proteins identified in this review that have been shown to be altered on the Transcriptomic, Epigenomic and Proteomic level in ASD; **Supplementary Table 4:** The top 10 significantly enriched Transcription Factor Protein-Protein Interactions in the dataset of 121 differentially expressed proteins that are also implicated in both Transcriptomic and DNAm data.

Author Contributions: Conceptualization, methodology, analysis, writing—original draft, preparation, writing—review and editing and visualization: C. Mahony and C. O'Ryan; Project supervision administration and funding acquisition: C. O'Ryan. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Research Foundation, South Africa (Grant number 118524).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available within the article and supplementary material here.

Conflicts of Interest: The authors declare no conflict of interest.

6. References

1. Wiśniowiecka-Kowalnik, B. & Nowakowska, B. A. Genetics and epigenetics of autism spectrum disorder—current evidence in the field. *Journal of Applied Genetics* **60**, 37–47 (2019).
2. Xie, S. *et al.* The Familial Risk of Autism Spectrum Disorder with and without Intellectual Disability. *Autism Res.* **13**, 2242–2250 (2020).
3. Bai, D. *et al.* Association of Genetic and Environmental Factors With Autism in a 5-Country Cohort. *JAMA Psychiatry* **76**, 1035–1043 (2019).
4. Pintacuda, G., Martín, J. M. & Eggan, K. C. Mind the translational gap: using iPS cell models to bridge from genetic discoveries to perturbed pathways and therapeutic targets. *Molecular Autism* **12**, 10 (2021).
5. Torre-Ubieta, L. de la, Won, H., Stein, J. L. & Geschwind, D. H. Advancing the understanding of autism disease mechanisms through genetics. *Nat. Med. 2016 224* **22**, 345–361 (2016).
6. Duffney, L. J. *et al.* Epigenetics and autism spectrum disorder: A report of an autism case with mutation in H1 linker histone HIST1H1E and literature review. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet.* **177**, 426–433 (2018).
7. Choi, L. & An, J.-Y. Genetic architecture of autism spectrum disorder: Lessons from large-scale genomic studies. *Neurosci. Biobehav. Rev.* **128**, 244–257 (2021).
8. Manoli, D. S. & State, M. W. Autism spectrum disorder genetics and the search for pathological mechanisms. *American Journal of Psychiatry* **178**, 30–38 (2021).
9. Comes, A. L. *et al.* Proteomics for blood biomarker exploration of severe mental illness: pitfalls of the past and potential for the future. *Transl. Psychiatry 2018 81* **8**, 1–15 (2018).
10. Huang, H., Shukla, H., Wu, C. & Saxena, S. Challenges and Solutions in Proteomics. *Curr. Genomics* **8**, 21–28 (2007).
11. Shen, L. *et al.* Proteomics Study of Peripheral Blood Mononuclear Cells (PBMCs) in Autistic Children. *Front. Cell. Neurosci.* **13**, 105 (2019).
12. Ruggeri, B., Sarkans, U., Schumann, G. & Persico, A. M. Biomarkers in autism spectrum disorder: the old and the new. *Psychopharmacol. 2013 2316* **231**, 1201–1216 (2013).
13. Higdon, R. *et al.* The promise of multi-omics and clinical data integration to identify and target personalized healthcare approaches in autism spectrum disorders. *OMICS A Journal of Integrative Biology* **19**, 197–208 (2015).

14. Hewitson, L. *et al.* Blood biomarker discovery for autism spectrum disorder: A proteomic analysis. *PLoS One* **16**, e0246581 (2021).
15. Shen, L. *et al.* iTRAQ-Based Proteomic Analysis Reveals Protein Profile in Plasma from Children with Autism. *PROTEOMICS - Clin. Appl.* **12**, 1700085 (2018).
16. Yang, J. *et al.* Peptidome Analysis Reveals Novel Serum Biomarkers for Children with Autism Spectrum Disorder in China. *PROTEOMICS - Clin. Appl.* **12**, 1700164 (2018).
17. Yao, F. *et al.* Protein Biomarkers of Autism Spectrum Disorder Identified by Computational and Experimental Methods. **12**, 1–13 (2021).
18. Abraham, J. R. *et al.* Proteomic Investigations of Autism Brain Identify Known and Novel Pathogenetic Processes. *Sci. Rep.* **9**, 1–11 (2019).
19. Tylee, D. S. *et al.* Blood transcriptomic comparison of individuals with and without autism spectrum disorder: A combined-samples mega-analysis. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet.* **174**, 181–201 (2017).
20. Mordaunt, C. E. *et al.* A meta-analysis of two high-risk prospective cohort studies reveals autism-specific transcriptional changes to chromatin, autoimmune, and environmental response genes in umbilical cord blood. *Mol. Autism* **10**, 1–21 (2019).
21. Gao, H. *et al.* Integrated Systems Analysis Explores Dysfunctional Molecular Modules and Regulatory Factors in Children with Autism Spectrum Disorder. *J. Mol. Neurosci.* 1–11 (2020). doi:10.1007/s12031-020-01658-w
22. He, Y., Zhou, Y., Ma, W. & Wang, J. An integrated transcriptomic analysis of autism spectrum disorder. *Sci. Rep.* **9**, 1–9 (2019).
23. Forés-Martos, J. *et al.* Transcriptomic metaanalyses of autistic brains reveals shared gene expression and biological pathway abnormalities with cancer. *Mol. Autism* **10**, 17 (2019).
24. Rahman, M. R. *et al.* Comprehensive Analysis of RNA-Seq Gene Expression Profiling of Brain Transcriptomes Reveals Novel Genes, Regulators, and Pathways in Autism Spectrum Disorder. *Brain Sci.* **10**, 747 (2020).
25. Wright, C. *et al.* Altered expression of histamine signaling genes in autism spectrum disorder. *Transl. Psychiatry* **7**, e1126 (2017).
26. Ramaswami, G. *et al.* Integrative genomics identifies a convergent molecular subtype that links epigenomic with transcriptomic differences in autism. *Nat. Commun.* **11**, 1–14 (2020).
27. Mordaunt, C. E. *et al.* Cord blood DNA methylome in newborns later diagnosed with autism spectrum disorder reflects early dysregulation of neurodevelopmental and X-linked genes. *Genome Med.* **12**, 1–25 (2020).
28. Hu, V. W., Hong, Y., Xu, M. & Shu, H. T. Altered DNA methylation in a severe subtype of idiopathic autism: Evidence for sex differences in affected metabolic pathways. *Autism* 136236132097108 (2020). doi:10.1177/1362361320971085
29. Wong, C. C. Y. *et al.* Genome-wide DNA methylation profiling identifies convergent molecular signatures associated with idiopathic and syndromic autism in post-mortem human brain tissue. *Hum. Mol. Genet.* **28**, 2201–2211 (2019).
30. Stathopoulos, S. *et al.* DNA Methylation Associated with Mitochondrial Dysfunction in a South African Autism Spectrum Disorder Cohort. *Autism Res.* **13**, 1079–1093 (2020).
31. Huang, D. W., Sherman, B. T. & Lempicki, R. A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **4**, 44–57 (2009).

32. Huang, D. W., Sherman, B. T. & Lempicki, R. A. Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* **37**, 1–13 (2009).
33. Raudvere, U. *et al.* g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res.* (2019). doi:10.1093/nar/gkz369
34. Liberzon, A. *et al.* Molecular signatures database (MSigDB) 3.0. *Bioinformatics* **27**, 1739–1740 (2011).
35. Mootha, V. K. *et al.* PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* **34**, 267–273 (2003).
36. Subramanian, A. *et al.* Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 15545–15550 (2005).
37. Liberzon, A. *et al.* The Molecular Signatures Database Hallmark Gene Set Collection. *Cell Syst.* **1**, 417–425 (2015).
38. Chen, E. Y. *et al.* Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* **14**, (2013).
39. Kuleshov, M. V. *et al.* Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res.* **44**, W90–W97 (2016).
40. Licata, L. *et al.* SIGNOR 2.0, the SIGnaling Network Open Resource 2.0: 2019 update. *Nucleic Acids Res.* **48**, D504–D510 (2020).
41. Xia, J., Gill, E. E. & Hancock, R. E. W. NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. *Nat. Protoc.* **10**, 823–844 (2015).
42. Lake, N. J., Bird, M. J., Isohanni, P. & Paetau, A. Leigh Syndrome Neuropathology and Pathogenesis. *J. Neuropathol. Exp. Neurol.* **74**, 482–492 (2015).
43. Tremblay, M. W. & Jiang, Y. DNA Methylation and Susceptibility to Autism Spectrum Disorder. *https://doi.org/10.1146/annurev-med-120417-091431* **70**, 151–166 (2019).
44. Siddiqui, M. F., Elwell, C. & Johnson, M. H. Mitochondrial Dysfunction in Autism Spectrum Disorders. *Autism. Open. Access* (2016). doi:10.4172/2165-7890.1000190
45. Griffiths, K. K. & Levy, R. J. Evidence of Mitochondrial Dysfunction in Autism: Biochemical Links, Genetic-Based Associations, and Non-Energy-Related Mechanisms. *Oxid. Med. Cell. Longev.* **2017**, 4314025 (2017).
46. Rose, S. *et al.* Clinical and Molecular Characteristics of Mitochondrial Dysfunction in Autism Spectrum Disorder. *Molecular Diagnosis and Therapy* (2018). doi:10.1007/s40291-018-0352-x
47. Castora, F. J. Mitochondrial function and abnormalities implicated in the pathogenesis of ASD. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **92**, 83–108 (2019).
48. Citrigno, L. *et al.* The Mitochondrial Dysfunction Hypothesis in Autism Spectrum Disorders: Current Status and Future Perspectives. *Int. J. Mol. Sci.* **21**, 5785 (2020).
49. Thorsen, M. Oxidative stress, metabolic and mitochondrial abnormalities associated with autism spectrum disorder. in *Progress in Molecular Biology and Translational Science* **173**, 331–354 (Elsevier B.V., 2020).
50. Gładysz, D., Krzywdzińska, A. & Hozyasz, K. K. Immune Abnormalities in Autism Spectrum Disorder—Could They Hold Promise for Causative Treatment? *Molecular Neurobiology* **55**, 6387–6435 (2018).
51. Liao, X., Liu, Y., Fu, X. & Li, Y. Postmortem Studies of Neuroinflammation in Autism Spectrum Disorder: a Systematic Review. *Molecular Neurobiology* **57**, 3424–3438 (2020).
52. Pangrazzi, L., Balasco, L. & Bozzi, Y. Oxidative Stress and Immune System Dysfunction in Autism Spectrum Disorders. *Int. J. Mol. Sci. 2020, Vol. 21, Page 3293* **21**, 3293 (2020).

53. Götz, M. & Huttner, W. B. The cell biology of neurogenesis. *Nat. Rev. Mol. Cell Biol. 2005 610* **6**, 777–788 (2005).
54. Vieira, M. S. *et al.* Neural stem cell differentiation into mature neurons: Mechanisms of regulation and biotechnological applications. *Biotechnol. Adv.* **36**, 1946–1970 (2018).
55. Castro-Torres, R. D. *et al.* Involvement of JNK1 in Neuronal Polarization During Brain Development. *Cells 2020, Vol. 9, Page 1897* **9**, 1897 (2020).
56. Sierra, A. *et al.* Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* **7**, 483–495 (2010).
57. Ganesan, H. *et al.* mTOR signalling pathway - A root cause for idiopathic autism? *BMB Rep.* **52**, 424 (2019).
58. Boksha, I. S., Prokhorova, T. A., Tereshkina, E. B., Savushkina, O. K. & Burbaeva, G. S. Protein Phosphorylation Signaling Cascades in Autism: The Role of mTOR Pathway. *Biochem. 2021 865* **86**, 577–596 (2021).
59. Geschwind, D. Genetics of autism spectrum disorders. *Trends Cogn. Sci.* **15**, 409–416 (2011).
60. Zhou, J. & Parada, L. F. PTEN signaling in autism spectrum disorders. *Curr. Opin. Neurobiol.* **22**, 873–879 (2012).
61. Wiśniowiecka-Kowalnik, B. & Nowakowska, B. A. Genetics and epigenetics of autism spectrum disorder—current evidence in the field. *Journal of Applied Genetics* **60**, 37–47 (2019).
62. Masini, E. *et al.* An Overview of the Main Genetic, Epigenetic and Environmental Factors Involved in Autism Spectrum Disorder Focusing on Synaptic Activity. *Int. J. Mol. Sci.* **21**, 8290 (2020).
63. Kwon, C. H. *et al.* Pten Regulates Neuronal Arborization and Social Interaction in Mice. *Neuron* **50**, 377–388 (2006).
64. Poduri, A. *et al.* Somatic Activation of AKT3 Causes Hemispheric Developmental Brain Malformations. *Neuron* **74**, 41–48 (2012).
65. Tsai, P. T. *et al.* Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature* **488**, 647–651 (2012).
66. Tang, G. *et al.* Loss of mTOR-Dependent Macroautophagy Causes Autistic-like Synaptic Pruning Deficits. *Neuron* **83**, 1131–1143 (2014).
67. Lieberman, O. J. *et al.* mTOR Suppresses Macroautophagy During Striatal Postnatal Development and Is Hyperactive in Mouse Models of Autism Spectrum Disorders. *Front. Cell. Neurosci.* **0**, 70 (2020).
68. Zhang, W. *et al.* Cerebral organoid and mouse models reveal a RAB39b–PI3K–mTOR pathway-dependent dysregulation of cortical development leading to macrocephaly/autism phenotypes. *Genes Dev.* **34**, 580–597 (2020).
69. Basilico, B., Morandell, J. & Novarino, G. Molecular mechanisms for targeted ASD treatments. *Current Opinion in Genetics and Development* **65**, 126–137 (2020).
70. Khacho, M., Harris, R. & Slack, R. S. Mitochondria as central regulators of neural stem cell fate and cognitive function. *Nat. Rev. Neurosci.* **20**, 34–48 (2019).
71. Beckervordersandforth, R. Mitochondrial Metabolism-Mediated Regulation of Adult Neurogenesis. *Brain Plast.* **3**, 73–87 (2017).
72. Zheng, X. *et al.* Metabolic reprogramming during neuronal differentiation from aerobic glycolysis to neuronal oxidative phosphorylation. *Elife* **5**, (2016).
73. Maffezzini, C., Calvo-Garrido, J., Wredenberg, A. & Freyer, C. Metabolic regulation of neurodifferentiation in the adult brain. *Cell. Mol. Life Sci.* (2020). doi:10.1007/s00018-019-03430-9
74. Licausi, F. & Hartman, N. W. Role of mTOR complexes in neurogenesis. *International Journal of Molecular Sciences* **19**, (2018).

75. Cunningham, J. T. *et al.* mTOR controls mitochondrial oxidative function through a YY1-PGC-1α transcriptional complex. *Nature* **450**, 736–740 (2007).
76. Vadlakonda, L., Pasupuleti, M. & Pallu, R. Role of PI3K-AKT-mTOR and Wnt signaling pathways in transition of G1-S phase of cell cycle in cancer cells. *Front. Oncol.* **3 APR**, 85 (2013).
77. Vallée, A., Lecarpentier, Y., Guillevin, R. & Vallée, J. Opposite Interplay Between the Canonical WNT/β-Catenin Pathway and PPAR Gamma: A Potential Therapeutic Target in Gliomas. *Neurosci. Bull.* **34**, 573–588 (2018).
78. Faigle, R. & Song, H. Signaling mechanisms regulating adult neural stem cells and neurogenesis. *Biochimica et Biophysica Acta - General Subjects* **1830**, 2435–2448 (2013).
79. Rao, T. P. & Kuhl, M. An Updated Overview on Wnt Signaling Pathways. *Circ. Res.* **106**, 1798–1806 (2010).
80. López-Tobón, A. *et al.* Human Cortical Organoids Expose a Differential Function of GSK3 on Cortical Neurogenesis. *Stem Cell Reports* **13**, 847–861 (2019).
81. Hussaini, S. M. Q. *et al.* Wnt signaling in neuropsychiatric disorders: Ties with adult hippocampal neurogenesis and behavior. *Neurosci. Biobehav. Rev.* **47**, 369–383 (2014).
82. Caglayan, A. O. Genetic causes of syndromic and non-syndromic autism. *Dev. Med. Child Neurol.* **52**, 130–138 (2010).
83. Mulligan, K. A. & Cheyette, B. N. R. Neurodevelopmental Perspectives on Wnt Signaling in Psychiatry. *Mol. Neuropsychiatry* **2**, 219–246 (2016).
84. Iakoucheva, L. M., Muotri, A. R. & Sebat, J. Getting to the Cores of Autism. *Cell* **178**, 1287–1298 (2019).
85. Caracci, M. O., Avila, M. E. & De Ferrari, G. V. Synaptic Wnt/GSK3 β Signaling Hub in Autism. *Neural Plast.* **2016**, (2016).
86. Kwan, V., Unda, B. K. & Singh, K. K. Wnt signaling networks in autism spectrum disorder and intellectual disability. *J. Neurodev. Disord.* **8**, (2016).
87. Perera, R. J. *et al.* Identification of novel PPARγ target genes in primary human adipocytes. *Gene* **369**, 90–99 (2006).
88. Panov, A., Orynbayeva, Z., Vavilin, V. & Lyakhovich, V. Fatty acids in energy metabolism of the central nervous system. *BioMed Research International* **2014**, (2014).
89. Ito, K. *et al.* Self-renewal of a purified Tie2+ hematopoietic stem cell population relies on mitochondrial clearance. *Science (80-.).* **354**, 1156–1160 (2016).
90. Longo, N., Frigeni, M. & Pasquali, M. Carnitine transport and fatty acid oxidation. *Biochim. Biophys. Acta - Mol. Cell Res.* **1863**, 2422–2435 (2016).
91. Knobloch, M. *et al.* A Fatty Acid Oxidation-Dependent Metabolic Shift Regulates Adult Neural Stem Cell Activity. *Cell Rep.* **20**, 2144–2155 (2017).
92. Xie, Z., Jones, A., Deeney, J. T., Hur, S. K. & Bankaitis, V. A. Inborn Errors of Long-Chain Fatty Acid β-Oxidation Link Neural Stem Cell Self-Renewal to Autism. *Cell Rep.* **14**, 991–999 (2016).
93. Ristori, M. V. *et al.* Proteomics and Metabolomics Approaches towards a Functional Insight onto AUTISM Spectrum Disorders: Phenotype Stratification and Biomarker Discovery. *Int. J. Mol. Sci. 2020, Vol. 21, Page 6274* **21**, 6274 (2020).
94. Shen, L. *et al.* Biomarkers in autism spectrum disorders: Current progress. *Clin. Chim. Acta* **502**, 41–54 (2020).
95. Barone, R. *et al.* Nuclear Peroxisome Proliferator-Activated Receptors (PPARs) as therapeutic targets of resveratrol for autism spectrum disorder. *Int. J. Mol. Sci.* **20**, (2019).

96. Vallée, A., Vallée, J. N. & Lecarpentier, Y. PPARγ agonists: potential treatment for autism spectrum disorder by inhibiting the canonical WNT/β-catenin pathway. *Molecular Psychiatry* **24**, 643–652 (2019).
97. Beckervordersandforth, R. *et al.* Role of Mitochondrial Metabolism in the Control of Early Lineage Progression and Aging Phenotypes in Adult Hippocampal Neurogenesis. *Neuron* **93**, 560-573.e6 (2017).
98. Abruzzo, P. M. *et al.* Plasma peroxiredoxin changes and inflammatory cytokines support the involvement of neuro-inflammation and oxidative stress in Autism Spectrum Disorder. *J. Transl. Med. 2019 171* **17**, 1–12 (2019).
99. Bjørklund, G. *et al.* Oxidative Stress in Autism Spectrum Disorder. *Molecular Neurobiology* **57**, 2314–2332 (2020).
100. Hu, T., Dong, Y., He, C., Zhao, M. & He, Q. The Gut Microbiota and Oxidative Stress in Autism Spectrum Disorders (ASD). *Oxid. Med. Cell. Longev.* **2020**, (2020).
101. Manivasagam, T. *et al.* Role of Oxidative Stress and Antioxidants in Autism. *Adv. Neurobiol.* **24**, 193–206 (2020).
102. Morimoto, M. *et al.* Assessment of oxidative stress in autism spectrum disorder using reactive oxygen metabolites and biological antioxidant potential. *PLoS One* **15**, e0233550 (2020).
103. Chen, L. *et al.* Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies (N = 9109). *Transl. Psychiatry* **11**, (2021).
104. Le Belle, J. E. *et al.* Proliferative Neural Stem Cells Have High Endogenous ROS Levels that Regulate Self-Renewal and Neurogenesis in a PI3K/Akt-Dependant Manner. *Cell Stem Cell* **8**, 59–71 (2011).
105. Prozorovski, T., Schneider, R., Berndt, C., Hartung, H. P. & Aktas, O. Redox-regulated fate of neural stem progenitor cells. *Biochimica et Biophysica Acta - General Subjects* **1850**, 1543–1554 (2015).
106. Prozorovski, T. *et al.* Sirt1 contributes critically to the redox-dependent fate of neural progenitors. *Nat. Cell Biol.* **10**, 385–394 (2008).
107. Hou, Y. *et al.* Mitochondrial superoxide production negatively regulates neural progenitor proliferation and cerebral cortical development. *Stem Cells* **30**, 2535–2547 (2012).
108. Huang, T. T., Zou, Y. & Corniola, R. Oxidative stress and adult neurogenesis-Effects of radiation and superoxide dismutase deficiency. *Seminars in Cell and Developmental Biology* **23**, 738–744 (2012).
109. Liu, J. *et al.* Bmi1 regulates mitochondrial function and the DNA damage response pathway. *Nature* **459**, 387–392 (2009).
110. Fasano, C. A. *et al.* Bmi-1 cooperates with Foxgl to maintain neural stem cell self-renewal in the forebrain. *Genes Dev.* **23**, 561–574 (2009).
111. Wang, C., Liang, C. C., Bian, Z. C., Zhu, Y. & Guan, J. L. FIP200 is required for maintenance and differentiation of postnatal neural stem cells. *Nat. Neurosci.* **16**, 532–542 (2013).
112. Chatoo, W., Abdouh, M., Duparc, R. H. & Bernier, G. Bmi1 distinguishes immature retinal progenitor/stem cells from the main progenitor cell population and is required for normal retinal development. *Stem Cells* **28**, 1412–1423 (2010).
113. Brown, G. K. & Squier, M. V. Neuropathology and pathogenesis of mitochondrial diseases. *J. Inherit. Metab. Dis.* **19**, 553–572 (1996).
114. Chow, C. W. & Thorburn, D. R. Morphological correlates of mitochondrial dysfunction in children. *Hum. Reprod.* **15**, 68–78 (2000).
115. Saneto, R. P., Friedman, S. D. & Shaw, D. W. W. Neuroimaging of mitochondrial disease. *Mitochondrion* **8**, 396–413 (2008).

116. Jiménez-Caballero, P. E., Mollejo-Villanueva, M. & Álvarez-Tejerina, A. Mitochondrial encephalopathy due to complex i deficiency. brain tissue biopsy findings and clinical course following pharmacological. *Rev. Neurol.* **47**, 27–30 (2008).
117. Johnson, S. C. *et al.* mTOR Inhibition Alleviates Mitochondrial Disease in a Mouse Model of Leigh Syndrome. *Science (80-.).* **342**, 1524–1528 (2013).
118. Wen, Y. *et al.* Astroglial PTEN loss disrupts neuronal lamination by dysregulating radial glia-guided neuronal migration. *Aging Dis.* **4**, 113–126 (2013).
119. Sarn, N. *et al.* Cytoplasmic-predominant Pten increases microglial activation and synaptic pruning in a murine model with autism-like phenotype. *Mol. Psychiatry* 1–14 (2020). doi:10.1038/s41380-020-0681-0
120. Vallée, A. & Lecarpentier, Y. Crosstalk Between Peroxisome Proliferator-Activated Receptor Gamma and the Canonical WNT/β-Catenin Pathway in Chronic Inflammation and Oxidative Stress During Carcinogenesis. *Front. Immunol.* **9**, 1 (2018).
121. Scuderi, C. *et al.* Palmitoylethanolamide controls reactive gliosis and exerts neuroprotective functions in a rat model of Alzheimer's disease. *Cell Death Dis. 2014 59* **5**, e1419–e1419 (2014).
122. Xu, H., Xu, B., Wang, Z. X., Tan, G. W. & Shen, S. H. Inhibition of Wnt/β-catenin signal is alleviated reactive gliosis in rats with hydrocephalus. *Child's Nerv. Syst.* **31**, 227–234 (2015).
123. Lee, Y., Lee, S., Chang, S.-C. & Lee, J. Significant roles of neuroinflammation in Parkinson's disease: therapeutic targets for PD prevention. *Arch. Pharmacal Res. 2019 425* **42**, 416–425 (2019).
124. Mendrysa, S. M., Ghassemifar, S. & Malek, R. p53 in the CNS: Perspectives on Development, Stem Cells, and Cancer. *Genes Cancer* **2**, 431 (2011).
125. Silva-Adaya, D., Garza-Lombó, C. & Gonsebatt, M. E. Xenobiotic transport and metabolism in the human brain. *Neurotoxicology* **86**, 125–138 (2021).
126. Sánchez-Alegría, K., Flores-León, M., Avila-Muñoz, E., Rodríguez-Corona, N. & Arias, C. PI3K signaling in neurons: A central node for the control of multiple functions. *International Journal of Molecular Sciences* **19**, (2018).
127. Qin, J.-J. *et al.* Inhibiting β-Catenin by β-Carboline-Type MDM2 Inhibitor for Pancreatic Cancer Therapy. *Front. Pharmacol.* **9**, 5 (2018).
128. Forsberg, K. *et al.* The tumor suppressor p53 fine-tunes reactive oxygen species levels and neurogenesis via PI3 kinase signaling. *J. Neurosci.* **33**, 14318–14330 (2013).
129. Sofroniew, M. V. Astrocyte barriers to neurotoxic inflammation. *Nat. Rev. Neurosci.* **16**, 249–263 (2015).
130. Petrelli, F., Pucci, L. & Bezzi, P. Astrocytes and microglia and their potential link with autism spectrum disorders. *Front. Cell. Neurosci.* **10**, (2016).
131. Mekori-Domachevsky, E., Segal-Gavish, H. & Gross, R. Neuroinflammation and neuroprotection in schizophrenia and autism spectrum disorder. in *Neuroprotection in Autism, Schizophrenia and Alzheimer's disease* 101–122 (Elsevier, 2019). doi:10.1016/B978-0-12-814037-6.00011-2
132. Liao, X., Yang, J., Wang, H. & Li, Y. Microglia mediated neuroinflammation in autism spectrum disorder. *Journal of Psychiatric Research* **130**, 167–176 (2020).
133. Liao, X. & Li, Y. Nuclear Factor Kappa B in Autism Spectrum Disorder: A Systematic Review. *Pharmacological Research* **159**, 104918 (2020).
134. Voineagu, I. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* **474**, 380–384 (2011).
135. Laurence, J. A. & Fatemi, S. H. Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. *Cerebellum* **4**, 206–210 (2005).

136. Edmonson, C., Ziats, M. N. & Rennert, O. M. Altered glial marker expression in autistic post-mortem prefrontal cortex and cerebellum. *Mol. Autism* **5**, 3 (2014).
137. Fatemi, S. H., Folsom, T. D., Reutiman, T. J. & Lee, S. Expression of astrocytic markers aquaporin 4 and connexin 43 is altered in brains of subjects with autism. *Synapse* **62**, 501 (2008).
138. Wegiel, J. *et al.* The neuropathology of autism: Defects of neurogenesis and neuronal migration, and dysplastic changes. *Acta Neuropathol.* **119**, 755–770 (2010).
139. Clarke, L. E. & Barres, B. A. Emerging roles of astrocytes in neural circuit development. *Nature Reviews Neuroscience* **14**, 311–321 (2013).
140. Sahlender, D. A., Savtchouk, I. & Volterra, A. What do we know about gliotransmitter release from astrocytes? *Philosophical Transactions of the Royal Society B: Biological Sciences* **369**, (2014).
141. Lauro, C. & Limatola, C. Metabolic Reprograming of Microglia in the Regulation of the Innate Inflammatory Response. *Frontiers in Immunology* **11**, 493 (2020).
142. Lively, S. & Schlichter, L. C. Microglia responses to pro-inflammatory stimuli (LPS, IFNγ+TNFα) and reprogramming by resolving cytokines (IL-4, IL-10). *Front. Cell. Neurosci.* **12**, (2018).
143. Loving, B. A. & Bruce, K. D. Lipid and Lipoprotein Metabolism in Microglia. *Front. Physiol.* **0**, 393 (2020).
144. Chausse, B., Kakimoto, P. A. & Kann, O. Microglia and lipids: how metabolism controls brain innate immunity. *Semin. Cell Dev. Biol.* **112**, 137–144 (2021).
145. Baik, S. H. *et al.* A Breakdown in Metabolic Reprogramming Causes Microglia Dysfunction in Alzheimer's Disease. *Cell Metab.* **30**, 493-507.e6 (2019).
146. Bernier, L. P., York, E. M. & MacVicar, B. A. Immunometabolism in the Brain: How Metabolism Shapes Microglial Function. *Trends Neurosci.* **43**, 854–869 (2020).
147. Revuelta, M., Scheuer, T., Chew, L. J. & Schmitz, T. Glial Factors Regulating White Matter Development and Pathologies of the Cerebellum. *Neurochem. Res.* **45**, 643–655 (2020).
148. Markiewski, M. M., Nilsson, B., Ekdahl, K. N., Mollnes, T. E. & Lambris, J. D. Complement and coagulation: strangers or partners in crime? *Trends Immunol.* **28**, 184–192 (2007).
149. Amara, U. *et al.* Interaction Between the Coagulation and Complement System. *Adv. Exp. Med. Biol.* **632**, 71 (2008).
150. Amara, U. *et al.* Molecular Intercommunication between the Complement and Coagulation Systems. *J. Immunol.* **185**, 5628–5636 (2010).
151. Orsini, F., De Blasio, D., Zangari, R., Zanier, E. R. & De Simoni, M.-G. Versatility of the complement system in neuroinflammation, neurodegeneration and brain homeostasis. *Front. Cell. Neurosci.* **0**, 380 (2014).
152. Hammad, A., Westacott, L. & Zaben, M. The role of the complement system in traumatic brain injury: a review. *J. Neuroinflammation* **15**, (2018).
153. Fletcher-Sandersjöö, A., Maegele, M. & Bellander, B.-M. Does Complement-Mediated Hemostatic Disturbance Occur in Traumatic Brain Injury? A Literature Review and Observational Study Protocol. *Int. J. Mol. Sci. 2020, Vol. 21, Page 1596* **21**, 1596 (2020).
154. Luca, C. De, Virtuoso, A., Maggio, N. & Papa, M. Neuro-Coagulopathy: Blood Coagulation Factors in Central Nervous System Diseases. Int. J. Mol. Sci. 18, (2017).
155. Ratajczak, M. Z. et al. The emerging link between the complement cascade and purinergic signaling in stress hematopoiesis. Frontiers in Immunology 9, 1295 (2018).
156. Gorelik, A. *et al.* Developmental activities of the complement pathway in migrating neurons. *Nat. Commun. 2017 81* **8**, 1–12 (2017).

157. Magdalon, J. *et al.* Complement System in Brain Architecture and Neurodevelopmental Disorders. *Front. Neurosci.* **0**, 23 (2020).
158. Ziabska, K., Ziemka-Nalecz, M., Pawelec, P., Sypecka, J. & Zalewska, T. Aberrant complement system activation in neurological disorders. *Int. J. Mol. Sci.* **22**, (2021).
159. Schafer, D. P. *et al.* Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner. *Neuron* **74**, 691–705 (2012).
160. Ganesan, H. *et al.* mTOR signalling pathway - A root cause for idiopathic autism? *BMB Rep.* **52**, 424–433 (2019).
161. Kumar, S. *et al.* Impaired neurodevelopmental pathways in autism spectrum disorder: a review of signaling mechanisms and crosstalk. *J. Neurodev. Disord. 2019 111* **11**, 1–14 (2019).
162. Arredondo, S. B., Valenzuela-Bezanilla, D., Mardones, M. D. & Varela-Nallar, L. Role of Wnt Signaling in Adult Hippocampal Neurogenesis in Health and Disease. *Front. Cell Dev. Biol.* **8**, 860 (2020).