The neuropathology of autism: A systematic review of post-mortem studies of autism and related disorders

Title: The Neuropathology of Autism: A Systematic Review of Post-Mortem Studies of Autism and Related Disorders.

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Abstract:

Post-mortem studies allow for the direct investigation of brain tissue in those with autism and related disorders. Several review articles have focused on aspects of post-mortem abnormalities but none has brought together the entire post-mortem literature. Here, we systematically review the evidence from post-mortem studies of autism, and of related disorders that present with autistic features. The literature consists of a small number of small studies, but several remarkably consistent findings are evident. Cortical layering is largely undisturbed, but there are consistent reductions in minicolumn numbers and aberrant myelination. Transcriptomics repeatedly implicate aberrant synaptic, metabolic, proliferation, apoptosis and immune pathways. Sufficient replicated evidence is available to implicate non-coding RNA, aberrant epigenetic profiles, GABAergic, glutamatergic and glial dysfunction in autism pathogenesis. Overall, the cerebellum and frontal cortex are most consistently implicated, sometimes revealing distinct region-specific alterations. The literature on related disorders such as Rett syndrome, Fragile X, Williams syndrome and copy number variations (CNVs) predisposing to autism is particularly small and inconclusive. Larger studies, matched for gender, developmental stage, co-morbidities and drug treatment are required.

Keywords:

Autism, Autism Spectrum Disorder (ASD), Post-mortem studies, Systematic Review

1. Introduction

Autism is a complex neurodevelopmental disorder affecting around 1 in 100 children (Association and Association, 2013). It is characterised by difficulties in social cognition and communication, as well as restrictive and repetitive behaviours (Varghese et al., 2017). Beyond these core symptoms, individuals display a wide range of comorbidities including seizures, intellectual disability, and other cognitive impairments (Canitano, 2007).

The term Autistic Spectrum Disorders (ASD) is used in Diagnostic and Statistical Manual 5 (DSM-5: citation?) to encompass several potentially distinguishable clinical conditions that share core symptoms: autism (as above), Asperger syndrome (as above but milder and/or without communication difficulties) and Pervasive Developmental Disorders-not otherwise specified (Casanova, 2015), as well as Rett syndrome and including conditions such as Fragile X presenting with autism. The International Classification of Diseases (ICD)_10 also includes Rett syndrome and other known causes of ASDs in the F84 section (WHO, 1992). Here, we use the term autism and related disorders to encompass all those syndromes.

The pathogenesis of autism is far from fully understood. It is highly heritable, but the majority of cases are idiopathic. Various environmental factors, such as maternal immune activation and prenatal exposure to toxins, likely contribute (Amaral et al., 2018, Varghese et al., 2017, De Felice et al., 2015). There is also a known genetic component to some ASDs, which may be grouped into three classes: first, single gene mutations, contributing to around 5% of cases, including Fragile X and Rett Syndrome (Hagerman et al., 2011, Loat et al., 2008). Secondly, large genomic copy number variants (CNVs), such as deletions and duplications of a given gene segment, account for approximately 10% of the cases (Ramaswami and Geschwind, 2018, Tuzun et al., 2005). These include deletions and duplications of the human chromosomal region 16p11.2, 22q11 deletion, 15q11-q13 deletion, 15q13.3 microdeletion and 15q11-13 duplication (Fernandez et al., 2010, Wu et al., 2014, Hogart et al., 2009, Varghese et al., 2017). Third, polygenic risk factors, due to accumulation of common de novo single-nucleotide variants, probably account for at least 20% of ASD liability (Weiner et al., 2017, Sanders et al., 2012, McKenna et al., 2018).

Recent advances in genetic techniques, especially whole-exome sequencing and induced pluripotent stem cell (iPSC)-derived models have helped clarify some of the neurobiology of the disorders (Bauman and Kemper, 2005, Courchesne et al., 2019, Varghese et al., 2017). Similarly, despite the limited availability of human autopsy material and the technical limitations of studying the human brain, post-mortem studies have contributed to our understanding of ASD. Through the direct study of brain tissue, disease-related alterations at the cellular, synaptic and molecular levels can be detected, enabling characterization at the level of neuronal populations and their neural circuits (Lewis, 2002). Therefore, post-mortem studies are a complementary approach to other methodologies, providing an essential interface between the clinical presentation and the underlying molecular and cellular pathology.

Several narrative review articles have been published outlining different aspects of ASD neuropathology. These have focussed on GABAergic deficits, mitochondrial dysfunction, microglial impairments and neuroanatomical alterations, as well as the implications of epigenetic and transcriptomic studies (Varghese et al., 2017, Fatemi and Folsom, 2015, Frick et al., 2013, Bauman and Kemper, 2005, Ansel et al., 2017, Smith et al., 2019a, Wei et al., 2014). However, to date, a comprehensive systematic review that details and synthesises the nature of the various findings in post-mortem studies, including the genetic, epigenetic and transcriptomic patterns in ASD brains revealed, has not been published.

In this paper, we systematically review the evidence from post-mortem ASD studies. Unusually, but importantly, we also include post-mortem findings in disorders that commonly present with autism, namely: Fragile X syndrome (FXS), Rett syndrome (RTT), Williams syndrome (WS) as well as 15q11-13 duplication and 22q11 deletion syndromes.

2. Methods

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews were followed. The initial protocol for performing this review was published on the International prospective register of systematic reviews, PROSPERO (CRD42019130273).

2.1. Information sources and Search Strategy

The following electronic databases were searched: Web of Science, MEDLINE/PubMed, EMBASE and PsycINFO. A systematic search was conducted on 01 November 2019. The search strategy included terms relating to or describing ASD AND post-mortem studies ("ASD" OR "Autism" OR "Autism Spectrum Disorder" OR "Autistic" OR "Asperger") AND ("post mortem" OR "postmortem" OR "post-mortem" OR "Autopsy"). The search terms were adapted for use with different databases. Literature was limited to human studies only and was not restricted to the English language. Figure 1 shows a PRISMA flowchart displaying a summary of our search and review process, including the total number of articles identified, included and excluded.

2.2. Inclusion criteria and study selection

Titles and abstracts of the articles identified were screened against the inclusion criteria shown in Table 1.

2.3. Data Extraction

The titles and abstracts of studies retrieved using the search strategy were screened independently by two review authors (RF and RH) to identify studies that potentially meet the inclusion criteria. For articles passing this screening stage, the full journal articles were read and assessed for eligibility by the first author (RF) to determine whether they met study inclusion criteria. A randomly selected sample of 25% of the studies was then be checked for accuracy (RH). Disagreements over the eligibility of particular studies was resolved through discussion with a third and fourth reviewer (DP and SL). Extracted information included: (a) number of post-mortem subjects in the study; (b) descriptive variables (e.g. age, gender) of study population including controls; (c) diagnosis of subjects, including how diagnosed; (d) data pertain to the post-mortem itself (cause of death, post-mortem interval); (e) region of the brain tissue investigated (e.g. cerebellum, pre-fontal cortex); (f) aspect of post-mortem tissue investigated (e.g. neuronal populations, neocortical architecture); (g) study methodologies; (h) outcomes and findings (e.g. GABAergic deficits or other).

3. Results

Results are grouped according to the type of impairment identified, starting with large-scale neuropathological and cytoarchitectural abnormalities, then impairments at the molecular, genetic

and epigenetic level, and finally dysfunctions in systems, pathways and mechanisms. Supplementary tables 3-7 highlight the main findings for each study and the extracted information stated in section 2.3 for autism, RTT, FXS, WS, 15qdup and 22qdel respectively. There was insufficient comparable data to conduct meta-analyses. Given that, and to avoid misinterpreting statistically non-significant results as indicating no difference (Amrhein et al., 2019), we report important post-mortem findings, including statistically non-significant results. Unless indicated as such, the findings reported are statistically significant. Figure 2 summarises the major findings in ASD grouped by brain region. Supplementary table 1 lists all included literature together with the diagnosis and clinical criteria for diagnosis of the patients. Supplementary table 2 lists the literature excluded after full-text was reviewed and the reason for omission.

3.1. Post-mortem findings in ASD

Most studies simply stated that their participants had a diagnosis of autism or ASD. The diagnosis was made upon the Autism Diagnostic Interview (ADI) or Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994, Lord et al., 1997) criteria in the majority of the studies and sometimes according to Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV) (Bell, 1994) or ICD-10 criteria. A number of studies have also used Autism Diagnostic Observation Schedules (ADOS) (Lord et al., 1999)and Childhood Autism Rating Scale (CARS) (Schopler et al., 1980) as well as medical records and family interviews to assess the patients. Seven studies include cases that are diagnosed with both ASD and 15qdup and only one study had an ASD case also diagnosed with RTT (Supplementary table 7).

3.1.1. Brain Weight

An early neuropathological study proposed that megalencephaly (increased brain weight >2.5 SD above the mean) is a frequent feature of autism/ASD. Out of the six brains investigated, four were unusually large and heavy (Bailey et al., 1998). However, Courchesne et al. (1999) examined the brain weights of 21 autistic subjects and only one such case. Instead of a general tendency toward megalencephaly, Courchesne et al. (1999) proposed that brain weights in ASD fall into three groups: a large majority with normal brain weight, a minority with megalencephaly and another with micrencephaly (Courchesne et al., 1999). Indeed, several structural imaging studies

have also observed increased brain volumes in ASD subjects as a group compared to healthy controls (Hiess et al., 2015, Dougherty et al., 2016, Sacco et al., 2015).

3.1.2. Cortical layering and cellular organisation

Hutsler et al. (2007) reported disturbances in cortical cell patterning in the superior temporal gyrus, dorsolateral frontal lobes and dorsal parietal lobes including dysplasias, associated disturbances in lamination and a slightly less distinct gray/white matter boundary in 8 ASD subjects. Stoner et al. (2014) identified focal regions of reduced expression or unusual patterns of markers, which they referred to as “patches” in the dorsolateral prefrontal cortex (DL-PFC) and posterior superior temporal cortex in 10 autistic children aged 2-15 years. Deficits in the expression of excitatory cortical neuron markers was the most robust indicator of a patch region. These patches of abnormal laminar cytoarchitecture and cortical disorganization were obseved in neurons, but not glia (Stoner et al., 2014). However, there was notable variation with respect to which cell types and layers were most affected by the pathological features.

The subventricular zone of the lateral ventricles (LV-SVZ) is one of the two neurogenic niches in the brain essential for neural proliferation, migration, and differentiation, both during prenatal and postnatal development (Alvarez-Buylla and Lim, 2004, Riquelme et al., 2008, Pearson et al., 2013). Pearson et al. (2013) found no differences in cortical layering and organisation in the LV-SVZ of individuals with autism between 5-60 years of age, consistent with Hutsler et al. (2007) (Pearson et al., 2013). Kotagiri et al. (2014) found no gross morphological differences but notable differences in the SVZ niche: a lower density of total cells in the septal hypocellular gap in 4 autistic individuals (without epilepsy) compared with controls. Individuals with both autism and epilepsy also had (non-significantly) lower density of cells in the septal SVZ hypocellular gap than controls. Looking for differences in proliferation markers, Pearson et al. (2013) found that the mean number of Ki67+ proliferating cells in the LV-SVZ was higher in only the youngest one (5 years old) of several autism and age-matched control pairs. Kotagiri et al. (2014) also found a higher density of Proliferating cell nuclear antigen (PCNA), which is expressed at detectable levels throughout the cell cycle but peaks at S phase (Kurki et al., 1986), in the septal hypocellular gap and ependymal layer but not striatal side of autism samples. This increase was similar in autistic subjects with and without epilepsy (Kotagiri et al., 2014).

In summary, there is little evidence to support the hypothesis of dysregulated layer formation in ASD.

3.1.3. Minicolumnopathy

The minicolumn is a basic repeating functional unit of cortical organization, in which interactions between cells in layers VI and II occur predominantly in a radial direction, involving neurons stacked above each other (Favorov and Kelly, 1994a, Favorov and Kelly, 1994b, Casanova et al., 2002a). Casanova et al. (2002) investigated the configuration of minicolumns in 9 autism cases and 9 controls in the frontal and temporal lobes and found that the autism cases had more minicolumns of reduced width that were less compact in their cellular configuration and no global difference in neuronal density (Casanova et al., 2002a, Casanova et al., 2002b). They went on to find significant differences in minicolumnar morphometry between autism subjects and controls in the frontopolar and anterior cingulate gyrus, with no significant changes in the dorsolateral, orbitofrontal and ventrolateral prefrontal cortex (Casanova et al., 2006a). In an independent sample of 9 autism subjects, however, the DL-PFC showed the greatest reduction in minicolumnar width of four sampled areas (including primary motor cortex, primary visual cortex and primary sensory cortex) and neuronal density in autism exceeded that of controls by 23%. This increased cell density was apparently a result of a greater number of minicolumns, rather than the number of cells per minicolumn, which appeared normal (Casanova et al., 2006b).

3.1.4. Neuropathological Alterations

Neuronal size, numbers and density: One of the earliest post-mortem studies, by Coleman et al. (1985), counted the numbers of neurons and glia in several regions of the cerebral cortex in one well-documented case of autism compared to two age- and sex-matched controls. The autistic brain consistently showed a smaller glia/neuron ratio in all examined regions, but no consistent differences in cell density were found (Coleman et al., 1985). Van Kooten et al. (2008) reported reductions in neuron densities in layer III, total neuron numbers in layers III, V and VI, and mean perikaryal volumes of neurons in layers V and VI in the fusiform gyrus but not in the primary visual cortex or in the whole cerebral cortex. The results were not related to any history

of seizures (Van Kooten et al., 2008). Courchesne et al. (2011) found 79% more neurons in the DL-PFC and 29% more in mesial prefrontal cortex (M-PFC) in 7 male autistic children than in 6 controls. The autistic group also had larger than average brain weight, suggesting a pathological increase in neuron numbers (Courchesne et al., 2011). Similarly, supernumerary layer I cells appeared slightly more frequently in the superior temporal gyrus, dorsolateral frontal lobes and dorsal parietal lobes of the 8 subjects studied by Hutsler et al. (2007). On the other hand, Camacho et al. (2014) quantified the density of neurons in layer I of the superior temporal cortex and found no change in the density of neurons in layer I of the cortex of 6 subjects diagnosed with typical autism compared to 6 age-matched controls. Stoner et al. (2014) also observed no reduction in neuron density in the DL-PFC in autism. Similarly, Kim et al. (2015) found no significant differences in the number, soma volume, nor ratio of number or volume of supragranular to infragranular pyramidal neurons within the anterior superior temporal area (TA2; superior temporal gyrus) of autistic subjects.

Wegiel et al. (2014) performed a stereological study to estimate neuronal volumes in subcortical structures, the hippocampus, the archicortex, the cerebellum and brainstem in 14 autistic subjects (4-60 years-old) compared to 14 age-matched controls. They found reduced volumes of the neuronal soma within the examined regions over a very broad range from 5% to 34%. In the four 4-8 year-old autistic subjects, significant volume deficits were present in 14 subregions, whereas in the six 11-23 year-olds and four subjects ≥36 years, volume deficits were found in only 3 of 16 examined regions and 4 regions respectively. Purkinje cells and neurons in the claustrum persistently demonstrated lower neuronal soma volumes across the lifespan. Intriguingly, however, the developmental trajectory of neuronal volume changes revealed an increase in neuronal volumes in both autistic teenagers and adults, and a decrease of neuronal sizes in most regions in both older control groups, suggesting an abnormal trajectory of neuronal growth in teenagers and adults with autism (Wegiel et al., 2014).

In summary, the findings regarding neuronal numbers, densities and volumes are not consistent and it remains unclear whether such differences as there may be point to general or regional

disruptions of brain development in ASD, or whether this reflects the varying presentations of the disorder.

Axonal impairments: Zikopoulos et al. (2010) examined single axons and their ultrastructure in the white matter below the anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and lateral prefrontal cortex (LPFC). Overall axonal density between autism and control groups was similar but there were significantly fewer long-range ACC axons in the deep white matter and increased short- and medium-range ACC axons in the superficial white matter in 5 autistic subjects, with no significant differences in the proportion of thalamo-cortical axons, suggesting a reduction in long-distance cortico-cortical and cortico-subcortical pathways, such as connections to the striatum, the basal forebrain and brainstem. Moreover, higher percentages of thinner axons with more branches were observed, where most points of bifurcation were unmyelinated or arose after thinning of the myelin. In fact, the overall myelin thickness was lower in autism, with no differences in oligodendrocyte numbers (Zikopoulos and Barbas, 2010). Zikopoulos et al. (2018) then quantitatively studied the fine structure of myelinated axons in the white matter below ACC in a total of 17 cases and 15 controls between the ages of 3–67 years. Lower density of myelinated axons connecting the ACC with neighboring cortices was revealed in children with ASD (3-10 years), suggesting an early pathology in this region. The proportion of thin axons, which form short-range pathways, were increased significantly in individuals with autism, and the relative proportion of thick axons, which form long-range pathways, increased from childhood to adulthood in the control group, but decreased in ASD (Zikopoulos et al., 2018).

Similarly, Trutzer et al. (2019) revealed disorganization of cortical networks and local circuits within layer 1 within the LPFC. Children with autism had increased variability in the trajectories and thickness of myelinated axons in layer 1, while adults had a reduction in the relative proportion of thin axons, although there was no significant difference between groups in overall neuron density in layer 1 in adults, nor any age-associated change in the percent surface area occupied by cells (Trutzer et al., 2019).

In summary, thin axons with abberant myelination in ASD are consistent across three studies, albeit with a total number of only 38 cases.

Alterations in dendritic spines: Three studies have reported increased spine densities in ASD. Hutsler et al. (2010) found higher average dendritic spine densities in 10 ASD subjects than in 15 age-matched control cases in the frontal, temporal and parietal lobes. These were higher on the apical and basilar dendrites of superficial cortical layers, predominantly within layer II of all three cortical locations while oblique dendrite densities were similar across layers. Further, higher spine densities within the ASD group were not associated with a history of epileptic seizure activity, but were associated with decreased brain weights and most commonly found in ASD subjects with lower levels of cognitive functioning (Hutsler and Zhang, 2010). Tang et al. (2014) also reported increased dendritic spine density with reduced developmental spine pruning in layer V pyramidal neurons in postmortem ASD temporal lobe. From childhood through adolescence, dendritic spines decreased by ~45% in controls but only by ~16% in ASD patients suggesting a developmental defect in spine pruning in ASD (Tang et al., 2014).

In the lateral nucleus of the amygdala, Weir et al. (2018) found that both ASD and control subjects showed a similar pattern of increasing dendritic length with age well into adulthood with no differences on measures of soma volume, total dendritic length, or number of primary dendrites at any age. However, spine density was greater in young ASD cases compared to age-matched controls (<18 years old) and decreased in the amygdala as people with ASD aged. By adulthood, there was no observable difference in spine density in the amygdala between ASD and controls (>18 years old), highlighting the need for wider age-groups and age stratification to reveal putative age-specific differences in ASD (Weir et al., 2018).

Von Economo Neurons, (VENs): Kennedy et al. (2007) conducted the first stereological investigation of the number of spindle neurons, also known as Von Economo Neurons (VENs), in autism. They found no evidence of a reduction in spindle neuron number in fronto-insular cortex (Kennedy et al., 2007). Santos et al. (2011) however found increased ratios of VENs relative to pyramidal neurons in layer V of frontoinsular cortex in four young patients with autism compared

to three aged-matched controls. A non-significant trend to higher absolute numbers of VENs was also found. Moreover, VENs in autistic subjects exhibited several atypical characteristics, such as swollen somata and clumps of oligodendrocytes in close apposition, unlike the slender somata and gradually tapering apical and basal dendrites found in controls (Santos et al., 2011). Uppal et al. (2014) examined the anterior midcingulate cortex (aMCC) and found no overall differences in mean VEN population size, mean pyramidal neuron number or the mean ratio of VENs to pyramidal neurons between 7 autistic patients and 7 controls. When they subdivided the autism and control groups into young children (4-8 years) and adolescents (13-21 years), young children with autism had smaller pyramidal neurons than their matched controls. Further, in a rare brain-behaviour link, the number of von Economo neurons was positively correlated with ADI-R social interaction scores and the number of pyramidal neurons was positively correlated with restricted and repetitive behavior scores (Uppal et al., 2014). Nonetheless, work to date is not sufficient to conclude that Von Economo neuron involvement is a characteristic neuropathological feature of ASD.

Purkinje neurons: Lower Purkinje cell counts in the cerebella of autistic subjects were reported by Bauman and Kemper, (1985) and Ritvo et al. (1986). Fatemi et al. (2002b) found that the average cross-sectional areas of Purkinje cells of the patients with autism were smaller by 24% compared to controls but no differences in Purkinje cell densities were observed (Fatemi et al., 2002b). Skefos et al. (2014) examined Purkinje cell densities in four cerebellar regions, and overall Purkinje cell density was found to be lower in autistic cases, unlike Fatemi et al. (2002b)'s initial findings. This effect was most prominent in crus I and II regions, with approximately 20% lower Purkinje cell density (Skefos et al., 2014). Thus, lower cell counts, but not densities, are consistently reported in the 4 studies investigating Purkinje cell abnormalities (in a total of 18 cases).

Neuronal impairments in the Amygdala: Bauman and Kemper (1985) first reported increased cell-packing density and reduced cell sizes in the central, medial, and cortical nuclei in a 29-year-old male with autism and comorbid seizure disorder (Bauman and Kemper, 1985). This was replicated in five additional autistic subjects (Kemper and Bauman, 1993). Schumann et al. (2006)

found no differences in cell size or the overall volume of the amygdala and its subdivisions. However, there were fewer neurons in the amygdala overall and in its lateral nucleus in the autism group (Schumann and Amaral, 2006).

Although these studies consistently report abberations in the neurons of the amygdala, each reports a different aspect (cell size, density and neuronal numbers), and one can only speculate how such potentially varied aberrant development and function of the amygdala may contribute to ASD clinically. (However, data from structural and functional magnetic resonance imaging studies, are relevant – see Discussion).

Neuronal impairments in the Brainstem: Only one study has examined the brainstem in post-mortem autism tissue, specifically neurons of the medial superior olive (MSO), a part of the superior olivary complex (SOC) that is essential for the proper processing of auditory information (Kulesza Jr, 2007, Kulesza and Mangunay, 2008). Differences in cell body shape and orientation were found: MSO neurons in the 5 autistic samples were smaller in terms of cell body area, perimeter and major axis and were more round than in controls (Kulesza and Mangunay, 2008).

3.1.5. Receptor Abnormalities

Different types of receptors have been examined in the post-mortem ASD brain, but only the findings in Nicotinic receptors, which have been replicated twice in two separate regions, have been replicated.

Nicotinic receptors: Two studies demonstrated reductions in nicotinic receptors in cortex & cerebellum, respectively. Perry et al. (2001) revealed up to 65%–73% reduction in nicotinic receptors in both the parietal and frontal cortices. No difference, however, was found in the basal forebrain. Similarly, lower levels of both the alpha-4 and beta-2 nicotinic receptor subunits were found in the parietal cortex of 7 autistic subjects (Perry et al., 2001). Lee et al. (2002) further demonstrated a reduction in high affinity nicotinic receptor subunits alpha-3, alpha-4 and beta-2 in the granule cell, Purkinje and molecular layers of the cerebellum of 8 autism cases together with a 3-fold increase in the nicotinic receptor subunit alpha-7, particularly significant in the

granule cell layer. Alterations to alpha-4 and alpha-7 receptor subunits were mirrored by western blotting and immunohistochemistry in the Purkinje and granule cell layer respectively (Lee et al., 2002). How such reductions in nicotinic receptors may relate to cortical or cerebellar ASD neuropathology is unclear.

Other receptors: A decrease in mRNA and protein levels of estrogen receptor-beta (ER-β) and aromatase (CYP19A1) in the middle frontal gyrus was observed with reductions in mRNA levels of ER co-activators (Crider et al., 2014b). Higher oxytocin receptor (OXTR) binding was found in the nucleus basalis of Meynert, while lower OXTR binding was observed in the ventral pallidum (Freeman et al., 2018). Finally, reductions in glucocorticoid receptor (GR) isoforms: GRα (64 %), GRγ (48 %), GRP (20 %) and mineralocorticoid receptor (MR) (46 %) mRNA levels were observed and were recapitulated on the protein level in the middle frontal gyrus (Patel et al., 2016).

3.1.6. Gene Expression Patterns in ASD

Transcriptomics: Garbett et al. (2008) assessed the transcriptome of the temporal cortex in 6 autistic subjects and 6 controls, revealing a dramatic increase in the expression of immune system-related genes as well as alterations in transcripts of genes involved in cell communication, differentiation, cell cycle regulation and chaperone systems and cell death in autism. Moreover, greater transcript variability was observed among cases, reflecting the heterogeneity of the disorder (Garbett et al., 2008). Chow et al. (2012) investigated DNA and mRNA patterns in prefrontal cortex of both 15 young and adult postmortem autistic subjects. In 9 young autism prefrontal cortex (<14 years) samples, dysregulation in pathways governing cell number, cortical patterning and differentiation were found (Chow et al., 2012). In contrast, the 6 adult autism prefrontal cortex cases (15–56 years) exhibited dysregulation of signaling and repair pathways, development and oxidative stress pathways, as well as genes related to tissue remodeling and wound repair (Chow et al., 2012). Furthermore, several genes critical for development were dysregulated across both young and adult autism cases, such as p53 signaling and PTEN pathways. Others included: cell cycle, proliferation, apoptosis, cytoskeleton and extracellular matrix remodeling as well as growth and development functions (Chow et al., 2012).

Focusing on group differences between cases and controls risks overlooking the heterogeneity within groups. Certainly, phenotypic variability is characteristic of ASD. Therefore, Guan et al. (2016) developed a variability-centric gene expression analysis methods called “aberrant gene expression analysis” which detects the discrepancies in gene expression dispersion between groups and identifies genes with significantly different expression variability. By re-visiting a previously published RNA-seq dataset by Gupta et al. (2014), they revealed 54 functional gene sets whose expression dispersion in ASD samples was more pronounced than that in controls, the functions of which were grouped into four major categories: metabolism and biosynthesis, immune or inflammatory response, signaling pathway and vitamins and supplements; recapitulating the findings of several studies highlighting many genes implicated in ASD (Lee et al., 2005, Guan et al., 2016, Gupta et al., 2014). Guan et al. (2019) also identified eight gene sets with dysregulated expression in the ASD cortex, that were also shared by a post-mortem cohort of individuals with schizophrenia and bipolar disorder. The genes identified were enriched with functions associated with amino acid transport, synapse and neurotransmitter release, oxidative stress and nitric oxide synthase biosynthesis, immune response, protein folding, lysophosphatidic acid-mediated signaling and glycolysis (Guan et al., 2019). Schwede et al. (2018) also showed that genes associated with mitochondrial function were downregulated in the cerebral cortex in autism and were correlated with genes related to synaptic function; a finding that was validated across three different previously published microarray datasets (Schwede et al., 2018). To investigate RNA editing, a critical pathway involved in synaptic development, on a genome-wide scale, Tran et al. (2019) profiled global patterns of adenosine-to-inosine (A-to-I) editing in idiopathic ASD subjects. A global bias for hypoediting was found in the ASD frontal cortex (n=29), temporal cortex (n=30) and cerebellum (n=31) which involved many synaptic genes (Tran et al., 2019).

Proteomics and Metabolomics: Analyses of total brain gray matter proteins by Junaid et al., (2004) identified an increase in polarity (acidicity) of glyoxalase I (Glo1), an enzyme responsible for scavenging toxic products of cellular metabolic reactions, in 4/8 autistic subjects. A 38% decrease in Glo1 enzyme activity was also revealed in autism samples, together with the accumulation of advanced glycation end products (Junaid et al., 2004). Moreover, Broek et al.

(2014) conducted proteomic profiling of the prefrontal cortex and cerebellum in 16 autistic subjects and controls using selected reaction monitoring mass spectrometry (SRM-MS) and found altered levels of proteins related to myelination, synaptic vesicle regulation and energy metabolism. Decreased levels of proteins associated with myelination and increased synaptic and energy-related proteins were evident only in the prefrontal cortex. In the cerebellum, however, opposite directional changes were found for myelination and synaptic proteins (Broek et al., 2014). Finally, Graham et al. (2016) utilised high resolution metabolomics to biochemically profile the cerebellum in individuals with ASD. However, they were unable to identify or characterise any potential brain biomarkers for ASD (Graham et al., 2016).

Allele-specific expression (ASE): ASE is a form of genetic regulation where mRNA expression at a specific locus is biased toward a specific allele. Chang et al. (2018) investigated the role of ASE in ASD pathogenesis via analysing genomic DNA from parent and offspring as well as RNA from offspring's post-mortem prefrontal cortex in parent-child quartet samples, where one offspring had a diagnosis of ASD. Although both offspring had distinct ASE patterns of genes and miRNAs in the prefrontal cortex, only the offspring diagnosed with ASD exhibited a mono-to-biallelic switch for LRP2BP (LRP2 binding protein) and ZNF407 (Zinc finger protein 407) (Chang et al., 2018). Lee et al. (2019) also found shifts from major allele expression to the minor allele with lower population frequency in ASD when ASE is observed, opposite to the canonical pattern. These ASD-specific ASE genes were enriched in ASD-risk genes as well as genes downregulated in post-mortem ASD brain tissue (Lee et al., 2019a). These two studies highlight the presence of ASE abnormalities in ASD, but further investigation is required to elucidate how ASE could contribute to ASD.

Sex-bias: Male sex is a robust risk factor for ASD (Fombonne, 2009). It has been hypothesised that this is either due to ASD risk genes being sex-differentially regulated or a result of their interaction with sexually dimorphic developmental pathways. Werling et al. (2016) characterized sexually dimorphic gene expression to evaluate ASD risk genes for evidence of sex-biased expression in neocortical tissue of typically developing adults and found no evidence for systematic sex-differential expression of ASD risk genes. Overall, they found that genes

overexpressed in males were enriched for the genes upregulated in post-mortem ASD cases (Voineagu et al., 2011). These included astrocyte and microglia markers as well as other immune system function and inflammatory response genes (Werling et al., 2016). Similarly, Lee et al. (2019) found no transcripts with significant sex-dependent dysregulation, but many genes with sex-independent dysregulation were identified in the dorsolateral prefrontal cortex. Pathway analysis for the sex-independent genes identified an enrichment of ribosome-related pathways. Due to the small sample size and small number of female subjects, this analysis is likely underpowered to detect all sex-autism interactions (Lee et al., 2019b). Schumann et al. (2017) examined sexual dimorphism of microRNA and small noncoding RNA (sncRNA) in the superior temporal sulcus and the primary auditory cortex of 5 male and 5 female ASD cases and 8 control subjects. They revealed more dysregulated sncRNA, miRNA target genes, and pathways in ASD females compared to ASD males, suggesting a sexually dimorphic expression of miRNA and other sncRNA in ASD (Schumann et al., 2017). The three studies to date consistently show no evidence for sex-dependent expression, implying that an interaction with sexually dimorphic pathways is more probable.

In summary, most studies consistently implicate abberant synaptic, metabolic, proliferation, apoptosis and immune pathways in the ASD brain. Table 2 summarises the representative transcripts/proteins dysregulated. Very few studies have investigated specific aspects of gene expression and regulation. One study reported a bias for RNA-hypoediting in pathways involved in synaptic development, two showed no evidence for sex-dependent expression and another two highlighted the presence of ASE abnormalities in ASD. There is insufficient evidence to confidently conclude whether these mechanisms contribute to ASD pathology. However, their convergence on particular pathways, such as synaptic development or metabolism, provides hypotheses for further investigation.

3.1.7. Alterations in ASD-candidate genes

A number of ASD-candidate genes have been examined in the post-mortem ASD tissue. However, apart from MECP2, MET, EN-2, FMRP, RORA and BDNF most of the perurbations in

other genes are reported by single studies and have not been replicated. These are summarised in Table 3.

Methyl CpG binding protein 2 (MECP2): Mutations in MECP2, a key epigenetic regulator, have been reported in individuals with ASD, albeit uncommonly (Lobo-Menendez et al., 2003). Nagarajan et al. (2006) first demonstrated a significant reduction in MeCP2 expression in autistic subjects compared to age-matched controls in the frontal cortex and fusiform gyrus. They demonstrated a significant increase in MECP2 promoter methylation in frontal cortex of autistic male subjects which also significantly correlated with reduced MeCP2 protein expression observed in 11/14 autism cases (Nagarajan et al., 2006). Aberrant MECP2 methylation profiles were also shown in autistic female subjects (Nagarajan et al., 2008). Swanberg et al. (2009) further investigated the role of MeCP2 in the regulation of immediate early genes (IEGs), namely the activity-dependent early growth response gene 2 (EGR2), revealing a significant reduction in both EGR2 and MeCP2 in cortical samples of ASD subjects (Swanberg et al., 2009). On the other hand, Plummer et al., (2013) found no difference in MECP2 expression in temporal cortex between 15 cases and 18 controls, nor between males and females (Plummer et al., 2013).

MET: MET is another ASD-candidate gene involved in the transcriptional regulation of MECP2. Campbell et al. (2007) examined the cerebral cortex of 8 ASD subjects to find a reduction in MET mRNA and protein levels. Microarray analysis revealed a trend towards increased expression of several up-stream molecules involved in the regulation of MET signaling activity (Campbell et al., 2007). Plummer et al. (2013) also showed a significant reduction in MET expression in the temporal cortex of all 15 ASD individuals examined. Stratification by sex revealed a significant reduction in MET expression in males but not females (Plummer et al., 2013).

These two studies suggest a reduction in MET expressionin ASD, but the exact nature of disturbances in MET signaling, or its interaction with other pathways in post-mortem ASD tissue, such as glutamate neurotransmission and PTEN/AKT signalling, needs further investigation.

Engrailed-2 (EN-2): Two studies have reported an upregulation in EN-2 expression. James et al. (2013) found a significant increase in both EN-2 gene expression and protein levels, accompanied

by significant hypermethylation of the EN-2 promoter region in the cerebellar cortex of 13 autism cases. The histone H3K27 trimethylation mark (gene repression) was also significantly decreased in the EN-2 promoter, while the mean level of histone H3K4 trimethylation (gene activation) was elevated, consistent with the observed EN-2 overexpression (James et al., 2013). Similarly, Choi et al. (2014) demonstrated an increase in EN-2 mRNA expression in the cerebella of 29 cases, together with a significant increase in sonic hedgehog (SHH) and a decrease in insulin induced gene 1 (INSIG1) levels; 2 of the 5 genes that flank EN-2 and are co-expressed with EN-2 during development to coordinate similar developmental processes (Choi et al., 2014).

Fragile-X mental retardation protein (FMRP): Fatemi and colleagues found a significant reduction in FMRP protein levels in the superior frontal cortex and cerebellar vermis of 5 adult autistic cases. Since FMRP regulates the translation of several genes, Fatemi et al. (2011) also investigated the levels of its downstream molecules, to find a significant increase in mGluR5 in autistic children and a significant increase in GFAP levels in both superior frontal cortex and cerebellar vermis samples of 20 autistic children and adults (Fatemi and Folsom, 2011, Fatemi et al., 2011). Fatemi et al. (2013) then investigated the expression of four targets of FMRP signaling, namely: homer 1, amyloid beta A4 precursor protein (APP), ras-related C3 botulinum toxin substrate 1 (RAC1) and striatal-enriched protein tyrosine phosphatase (STEP) in the same brain regions. Increases in RAC1, APP (120 kDa and 80 kDa) protein levels were found in the superior frontal cortex of children with autism, with no change in the cerebellar vermis. In 12 adult cases, significant increases in RAC1 and STEP (46 kDa), together with a significant decrease in homer 1 was found in the superior frontal cortex. Additionally, RAC1 was significantly increased while APP (120), STEP (66 kDa), STEP (27 kDa), and homer 1 were significantly decreased in the vermis of 8 adult cases, with no change found in children. These differences were more prominent in the superior frontal cortex than in the cerebellar vermis (Fatemi et al., 2013). Moreover, Rustan et al. (2013) examined serine 499 phosphorylated FMRP protein levels, which acts as a translational repressor, in the superior frontal cortex and cerebellar vermis to find a significant reduction in the vermis of both autistic adults (N=5) and children (N=4) as well as in the superior frontal cortex of adults (N=10) only (Nalavadi et al., 2012, Rustan et al., 2013).

Retinoid-Related Orphan Receptor Alpha (RORA): A decrease in the expression of RORA protein was found in the prefrontal cortex and the cerebellum of 5 autism subjects (Nguyen et al., 2010). Sarachana et al. (2013) demonstrated that RORA transcriptionally regulates several ASD-associated genes, the expression of which was non-significantly reduced in the prefrontal cortex of 3 autism subjects (Sarachana and Hu, 2013). Given its regulation by sex-hormones, Hu et al. (2015) further investigated sex differences in RORA and CYP19A1 (also reffered to as aromatase; a RORA target gene) protein levels in 12 ASD frontal cortex samples. Although higher levels of RORA and aromatase proteins were found in females compared to males in the control group (non-significant) there was no apparent sex difference in RORA protein in the cortex of male and female ASD subjects. In addition, a strong positive correlation between RORA and aromatase protein levels was found in male and female subjects as well as ASD males, but not in ASD females (Hu et al., 2015).

Brain Derived Neurotrophic Factor (BDNF): Garcia et al. (2012) examined the fusiform gyrus of 11 autistic patients. BDNF mRNA levels were not altered, but total BDNF protein levels were greater in autism, suggesting that these alterations are not transcriptionally driven. Further analysis revealed higher pro-BDNF and lower truncated BDNF protein levels in autism tissue, suggesting a defective processing of pro-BDNF to its truncated form (Garcia et al., 2012). Similarly, Maussion et al. (2019) found a significant increase of BDNF expression level in the frontal cortex of 9 autistic subjects, with a trend towards higher expression levels in lobule VI of the cerebellum (non-significant) (Maussion et al., 2019).

3.1.8. Non-protein coding RNAs (ncRNAs)

Long ncRNAs (lncRNAs): Velmeshev et al. (2013) examined the prefrontal cortex (n=9), superior temporal gyrus (n=9) and cerebellum (n=7) of ASD subjects and identified natural anti-sense transcripts (NATs) at approximately 40% of loci previously implicated in ASD. These included antisense RNAs to ASD-related genes such as: FOXP1, ZNF81, SYNGAP1, CACNA1C, NIPBL, VPS13B, NHS, DHCR7, LAMP2, FOXG1 and PQBP1. All these ASD-NATs were detectable in all brain regions studied and most revealed region-dependent expression patterns,

apart from that of FOXG1, which was not detected in the cerebellar samples. Compared to controls, SYNGAP1-AS was significantly upregulated in the prefrontal cortex and superior temporal gyrus of ASD subjects, but not in the cerebellum (Velmeshev et al., 2013). Similarly, Kerin et al. (2012) showed that MSNP1-AS (moesin pseudogene 1, antisense) expression was increased in the temporal cortex of 10 ASD cases compared to 10 controls (Kerin et al., 2012).

Ziats et al. (2013) identified over 200 lncRNAs that were differentially expressed between autism and control samples (82 unique to the prefrontal cortex and 143 unique to the cerebellum), which were enriched for genomic regions containing genes related to neurodevelopment and psychiatric disorders, such as UBE3A (Ziats and Rennert, 2013). Similarly, using genome-wide transcriptome analysis, Parikshak et al., (2016) investigated the noncoding transcriptome in the frontal and temporal cortex and cerebellum of a large cohort of 48 idiopathic ASD cases and 49 controls to find that differential gene expression was overall consistently stronger in the cortex than in the cerebellum. Dysregulation of primate-specific lncRNAs was evident in ASD, particularly LINC00693 and LINC00689, both of which interact with miRNA processing complexes. They are typically downregulated during development, were upregulated in the ASD cortex (Parikshak et al., 2016).

Small ncRNAs (sncRNAs): Abu-Elneel et al. (2008) compared the expression of 466 miRNAs in samples of the cerebellar cortex to find 28 miRNAs differentially expressed in at least one of the 13 autism samples, compared to 13 controls; with no particular miRNA consistently dysregulated across all autism samples (Abu-Elneel et al., 2008). Moreover, Almehmadi et al. (2019) revealed a significant increase in miR-155p5 gene expression, a pro-inflammatory microRNA implicated in several inflammatory diseases (Tili et al., 2009), in the amygdala but not in the DL-PFC of ASD children (1-12 years of age). Interestingly, a third of the ASD samples of the 8 subjects had miR-155p5 levels similar to those of controls (Almehmadi et al., 2019).

Ander et al. (2015) examined superior temporal sulcus and primary auditory cortex - demonstrating altered miRNA expression in both regions in ASD (n=10), with no single miRNA commonly affected in both regions. Functional analysis of their gene targets revealed an

association with several nervous system, cell cycle and canonical signaling pathways, including PI3K-Akt signaling, as previously implicated in ASD (Chen et al., 2014) with miRNAs regulating immune functions only disrupted in the superior temporal sulcus. Small nucleolar RNA (snoRNA) and pre-miRNA were also dysregulated in ASD (Ander et al., 2015). Stamova et al. (2015) also assessed sncRNAs in the superior temporal sulcus and the primary auditory cortex, to find dysregulated expression of miRNAs (miR-132, miR-103 and miR-320) in 8 ASD samples, the targets of which overlapped With ASD-implicated genes (Stamova et al., 2015). In another study, genome-wide miRNA expression profiling in the frontal cortex, temporal cortex and cerebellar vermis of 55 ASD subjects revealed perturbations in several miRNAs (Wu et al., 2016). Although the miRNA expression profiles were very similar between the frontal and temporal cortex, distinct miRNA expression profiles were observed in the cerebellum, consistent with previous mRNA findings (Voineagu et al., 2011, Oldham et al., 2008). Wu et al., 2016 also demonstrated a regulatory relationship between miRNAs and their target mRNA expression in that upregulated miRNAs potentially contributed to the downregulation of neuronal and synaptic genes while the downregulated miRNAs contributed to the upregulation of immune-inflammatory genes (Wu et al., 2016).

To summarise, most of the studies investigating aberrant expression of ncRNAs have sampled from the frontal cortex, temporal cortex and cerebellum. Interestingly, distinct miRNA expression profiles were frequently observed in the cortex compared to other brain regions, implying that some regions are more vulnerable to transcriptomic alterations or that different regions respond differently according to their biological functions. Three studies demonstrated dysregulated expression patterns of lncRNAs and 5 revealed altered expression of sncRNAs, including miRNAs, snoRNAs and pre-miRNA. Most of the dysregulated ncRNAs were constantly reported to target ASD-risk genes or genomic regions containing genes related to neurodevelopment and psychiatric disorders. One can, therefore, confidently implicate ncRNAs in the molecular pathology of ASD where their dysregulation could lead to the previously mentioned ASD-associated transcriptomic changes.

3.1.9. Epigenetic Alterations

Epigenetic signatures: Shulha et al. (2012) compared the trimethylated H3K4 (H3K4me3) epigenomes in prefrontal cortex neurons in autistic subjects with ages ranging from infancy up to 70 years. Although no global difference in H3K4me3 occupancy at annotated promoters was observed, an abnormal H3K4me3 profile around the transcription start sites was observed in 4/16 cases, where H3K4me3 signatures spread from the transcription start sites into downstream gene bodies and upstream promoters. Additionally, altered H3K4me3 peaks were observed at several genes that regulate neuronal connectivity, and higher-order behaviors and loci implicated in neurodevelopmental disorders, together with altered expression of their corresponding transcripts (Shulha et al., 2012). Ladd-Acosta et al. (2014) further examined DNA methylation in the DL-PFC, temporal cortex and cerebellum of 20 ASD subjects to identify four genome-wide significant differentially methylated regions (DMRs). These DMRs implicated the genes PRRT1 (7.8% less methylation), TSPAN32 and C11orf21 (6.6% less methylation), and ZFP57 (13.9% more methylation) in the ASD temporal cortex and SDHAP3 (15.8% more methylation) in the ASD cerebellum (Ladd-Acosta et al., 2014). Wong et al. (2019) also quantified genome-wide DNA methylation patterns in the prefrontal cortex, temporal cortex and cerebellum to find widespread ASD-specific DNA methylation differences at several CpG sites, that were more consistent and pronounced in both cortical regions. These regions were enriched for genes implicated in the immune system, synaptic signalling and neuronal regulation significantly associated with ASD (Wong et al., 2019b). Similarly, Shpyleva et al. (2014) revealed an increase in the levels of 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) in genomic DNA of cerebellar tissue from 15 autistic subjects (Shpyleva et al., 2014). Cheng et al. (2018) also profiled the genome-wide distribution of 5hmC in 17 ASD cerebellar tissue and identified a set of differentially hydroxymethylated regions (DhMRs) exclusive to young ASD subjects (<18 years of age), with no significant DhMRs in older age groups. These intragenic DhMRs spanned genes involved in cell–cell communication and neurological disorders (Cheng et al., 2018). Sun et al., (2016) conducted a histone acetylome-wide association study (HAWAS) using cerebellar, prefrontal and temporal cortex tissues, identifying widespread aberrations in histone acetylation in the ASD cerebral cortex, with over 5,000 enhancer or promoter loci systematically shifted up or down. Similar histone acetylation changes were observed between the prefrontal and temporal cortex,

but the acetylation profile of the cerebellum was different, with only 247 perturbated loci, similar to the methylation patterns observed in previous studies. Functional analysis revealed an enrichment for genes associated with ion channels, synaptic function and neuronal excitability among the loci with increased H3K27ac in the ASD cereberal cortex (Sun et al., 2016).

It is therefore clear that the epigenetic signature of ASD tissue generally differs from that of controls, as shown by the aberrant methylation patterns consistently reported in cerebellar and cortical samples which implicate neurodevelopmental and ASD-related genes. The different studies summarised here each report a particular aspect of abnormal methylation profiles: H3K4me3, 5mC and 5hmC, and only one study investigated histone acetylation, which requires further validation to confirm the exact mechanism/s through which impaired DNA methylation could contribute to ASD pathogenesis.

Histone methyltransferases and demethylases: Only one study investigated histone lysine methyltransferases in ASD. No difference in gene expression levels of EHMT1 and EHMT2 was observed between 14 ASD cases and controls, and no differential expression of EHMT1 and EHMT2 isoforms was found in the prefrontal cortex of the Japanese subjects examined (Balan et al., 2014).

3.1.10. GABAergic Impairments

Interneurons (INs): Specific subpopulations of INs have been implicated in ASD, with a focus on Parvalbumin (PV)+ INs. Lawrence et al. (2010) investigated the density of subsets of anterior hippocampal INs, identified by immunohistochemical staining with PV, Calbindin (CB) and Calretinin (CR), to reveal increases in the density of CB-immunoreactive INs in the dentate gyrus, in CR-immunoreactive INs in area CA1 and in PV-immunoreactive INs in areas CA1 and CA3 in the hippocampus in 5 autistic individuals compared to 5 controls (Lawrence et al., 2010). Oblak et al. (2011) did not however replicate these findings in the posterior cingulate cortex or fusiform gyrus of 8 and 9 autistic subjects respectively, although there was a trend towards a reduced density of CB neurons in the posterior cingulate cortex (Oblak et al., 2011). Hashemi et al. (2017) did find a reduction in the number of PV+ INs, equally in the supragranular and infragranular

layers of the prefrontal cortex of 11 autism subjects, with no difference in the number of CB+ and CR+ INs (Hashemi et al., 2017). This was complementary to other findings in the prefrontal cortex also revealing lower expression of PV in autism (Edmonson et al., 2014). Analysis of differentially expressed genes in a previously published RNA-seq dataset also revealed that PV was the most strongly downregulated gene in the cerebral cortex of autism cases (Schwede et al., 2018).

Given that Purkinje cell (PC) function in the cerebellum is critically dependent on their innervation by the GABAergic basket cells (BCs) and stellate cells (SCs), Whitney et al. (2009) quantified PV+ INs in the posterior cerebellar lobe of 6 autism subjects but found no statistically significant difference in the density or number of BCs or SCs per PC (Whitney et al., 2009). However, when Edmonson et al. (2014) assessed the relative expression of PV+ INs in the cerebellum, lower expression of PV was found in 4 autism cases (Edmonson et al., 2014). Soghomonian et al. (2017) also examined PV gene expression by measuring PV mRNA levels at the single cell level in PCs of Crus II of the lateral cerebellar hemispheres, finding a reduction in PV mRNA levels in PCs in autism that was not influenced by post-mortem interval or age at death (Soghomonian et al., 2017).

Despite some discrepancies, most of these studies point towards a reduction in PV levels in the neocortex and possibly also the cerebellum. A decrease in the number of PV-expressing cells could result from decreased neuronal number or decreased PV protein expression. Since PV is critical for maintaining synaptic plasticity of GABAergic synapses, its altered expression in ASD suggests impaired synaptic plasticity, especially of the cerebellar output to other brain regions.

Reelin (RELN): Several studies have implicated RELN dysfunction in ASD. Reductions of 43-44% in mean levels of the protein (non-significant) and its predominant cleavage products (only significant for 180kDa) were revealed in the cerebellar cortex of 5 male autistic subjects compared to age, sex, and PMI matched-controls (Fatemi et al., 2001). This was partially replicated in the cerebellar and frontal cortices, and parietal areas (non-significant), of another cohort of autism subjects. The reductions in RELN mRNA levels were accompanied by increases in levels of RELN receptor, VLDLR mRNA in frontal and cerebellar cortices. The mRNA levels of Disabled-1 (Dab-

1), a cytosolic adapter protein that facilitates signalling between RELN-secreting cells and cortical pyramidal cells (Howell et al., 1997, Cooper and Howell, 1999), were reduced in the same areas (Fatemi et al., 2005). In layer I of the adult human cortex, Camacho et al., (2014) found however that 70% of cells express RELN in both control and autistic subjects, with no differences on quantification of the density of neurons in layer I of the superior temporal cortex. Thus, reduced RELN expression in the cerebral cortex of those with autism is unlikely to be a consequence of decreased numbers of RELN-expressing neurons in layer I but may result from abnormal RELN processing, or reductions in other RELN-expressing neuronal cell types (Camacho et al., 2014).

It is also possible that a decrease in RELN gene expression stems from epigenetic dysregulation. Lintas et al. (2016) examined post-mortem temporo-cortical tissue samples in 6 pairs of ASD and sex, age and PMI matched controls, showing a higher number of methylated CpG islands and heavier methylation in the 5′ region of the RELN gene promoter in ASD. Zhubi et al. (2014) investigated the binding of MeCP2 to RELN promoter and gene body in cerebella of 10 ASD patients. An increase in MeCP2 binding to the promoter region, but not to the corresponding gene body, was associated with an enrichment of local methylation patterns in the cerebellar cortex of ASD patients (Zhubi et al., 2014). This is in keeping with the significant reductions in RELN mRNA in the frontal cortex.

In summary, two studies point towards reduced RELN levels and two implicate epigenetic blunting of RELN gene expression in the ASD cerebral cortex and cerebellum.

Glutamic acid decarboxylase (GAD) alterations: GAD is the major rate-limiting enzyme that regulates GABA synthesis from L-glutamate. In the adult brain, GAD exists in two major isoforms: GAD65 and GAD67. Three studies (total 18 ASD, 21 control) consistently identified reductions in GAD levels. Fatemi et al. (2002) found both GAD65 and GAD67 were reduced by 48% (non-significant) and 61% in the parietal cortex respectively and by 50% and 51% (non-significant) in the cerebellum of autism subjects (Fatemi et al., 2002a). Moreover, Yip et al. (2007) quantified GAD67 mRNA in cerebellar Purkinje cells, and found a 40% reduction in mRNA levels in ASD subjects (Llinás et al., 2004, Yip et al., 2007). Yip et al. (2009) further measured GAD65 mRNA

levels in the dentate somata, which integrate inputs from the brainstem and spinal cord with Purkinje cell efferents (Chan-Palay et al., 1979, Yip et al., 2009). GAD65 mRNA labelling revealed two distinct subpopulations of neurons in both control and autism brains. The larger cells (about 18–20 mm in diameter) which likely feedback to inferior olivary neurons, had a 51% reduction in GAD65 mRNA levels in autism subjects, but there was no such reduction in the small-sized cells (about 10–12mm in diameter) presumed to be INs. Reduced Purkinje cell GABA input to the cerebellar nuclei could potentially interfere with the synchronous firing of inferior olivary neurons and disrupt cerebellar output to higher association cortices (Yip et al., 2009).

GABA receptor abnormalities: Fatemi et al. (2009) measured the expression of GABAA subunits in the parietal cortex, superior frontal cortex and cerebellum of 6-8 autism cases and controls. Reductions in GABRA1, GABRA2, GABRA3, and GABRB3 were observed in the parietal cortex in autism, while GABRA1 and GABRB3 were also decreased in cerebella, and GABRA1 in the superior frontal cortex as well. The presence of seizure disorder did not have a significant impact on GABAA receptor subunit expression (Fatemi et al., 2009b). In a further study (Fatemi et al., 2009a), the levels of GABBR1 were decreased in all three brain regions, while the levels of GABBR2 were reduced only in the cerebellum (Fatemi et al., 2009a). In another cohort of autism subjects, Fatemi et al. (2011) also found a decrease in the level of GABRB3 protein in the cerebellar vermis. Oblak et al. (2009) examined the number, density and distribution of GABAA receptors and benzodiazepine binding sites in the ACC. They found decreases in the mean density of GABAA receptors in the supragranular (layers I-III, 46.8%) and infragranular (layers V-VI, 20.2%) layers, as well as reductions in the density of benzodiazepine binding sites in the supragranular (28.9%) and infragranular (16.4%) lamina (Oblak et al., 2009). There was no significant difference in muscimol or flunitrazepam binding (ligands of GABAA receptors and Benzodiazepine binding sites respectively) between autism cases with and without seizures. The results from Fatemi et al. (2009) and Oblak et al. (2009) both support the notion of a GABAergic dysfunction in ASD brains. The downregulation of GABAA receptors may reflect increased GABA innervation and/or release resulting in an excitation/inhibition imbalance in output to key limbic cortical targets. Further, Crider et al. (2014) found a decrease in GABAAα1 protein, but not mRNA levels, in the middle frontal gyrus of ASD subjects - suggesting altered post-translational

regulation of $GABA_A$ receptors. They also found that the expression of SYVN1, a known ER-associated degradation (ERAD) E3 ubiquitin ligase, was higher in ASD samples at both mRNA and protein levels, indicating altered Ubiquitination pathway (Crider et al., 2014a).

In summary, consistent reductions in PV and altered levels of specific IN subpopulations as well as reductions in RELN, GABA receptors and GAD levels have been reported in 20 studies, in a total of 154 cases, providing strong evidence for GABAergic impairment in ASD.

3.1.11. Glutamatergic impairments

Glutamatergic synapses: Nicolini et al. (2015) found a decrease in the protein levels of PSD-95 in the fusiform gyrus of 11 subjects with idiopathic autism (Nicolini et al., 2015). Further, Chandley et al. (2015) investigated gene expression levels of 16 synaptic genes relevant to glutamatergic neurotransmission in pyramidal neurons from layer III of the ACC. Analysis revealed a robust decrease in NTRK2 expression, which encodes the tropomyosin receptor kinase B (TrkB), in 12 ASD samples compared to typically developing donors. There were also trends towards low expression levels of genes encoding a metabotropic glutamate receptor (GRM8), an ionotropic glutamate receptor subunit (GRIN1), a glutamate transporter (SLC1A1), and a glutamate receptor anchoring protein (GRIP1). However, the expression levels of NTRK2 and other synaptic genes in the prefrontal cortex were normal (Chandley et al., 2015).

Enzymatic dysfunction: Only one study has investigated the protein expression levels of enzymes in the glutamate-glutamine cycle and that was in the ACC of 7 autism subjects (Shimmura et al., 2013). The protein levels of kidney-type glutaminase (KGA), but not those of several other enzymes measured, were lower in subjects with autism. There were no significant differences in free glutamate or glutamine levels, but the ratio of glutamate to glutamine was lower in the autism samples than in controls, suggesting an altered turnover of the pool of each amino acid (Shimmura et al., 2013). Whether this contibutes to altered glutamatergic neurotransmission in the ACC in autism needs further investigation.

Glutamate receptors and transporters: Four studies have reported increased glutamate receptors and transporters in ASD. Purcell et al. (2001) identified higher levels of GluR1 and EAAT1 mRNA in postmortem autism cerebellum using microarray analysis confirmed with RT-PCR. They also found that the mRNA levels of two AMPA- type glutamate receptors, GluR2 and GluR3 were increased in cerebellum samples from 10 autistic subjects, as well as the mRNA of a glutamate transporter, EAAT2. Other glutamate receptor types such as kainate 1, metabotropic 3 and NMDA 1 displayed no significant difference in their expression levels. They further investigated if mRNA differences translated to the protein level. The glial glutamate transporter EAAT1 and glutamate receptor AMPA1 protein levels were increased three and 2.5-fold respectively in autism. An increase in the protein levels of EAAT2 and the NMDA 1 were also observed. They further examined the density of NMDA- and AMPA-type glutamate receptors by receptor autoradiography to find that, unlike their protein expression, AMPA glutamate receptor density was decreased in both the granule cell layer and molecular cell layer of the cerebellum, with no difference in NMDA receptor density (Purcell et al., 2001a). Fatemi et al. (2011) found substantially increased protein levels of mGluR5, a GroupI metabotropic glutamate receptor, in the superior frontal cortex of 20 children with autism. In adults, slightly elevated levels of dimerized mGluR5 and total mGluR5 did not reach statistical significance (Fatemi and Folsom, 2011). This increase in mGlur5 was also observed in the cerebellar vermis of 16 children with autism (Fatemi et al., 2011). Moreover, Chana et al. (2015) conducted a stereological investigation of the DL-PFC and found a trend to a decrease in intensity of mGluR5-positive neurons and glia, consistent with the significant reduction in mGluR5 gene expression over all age ranges in autism observed in published microarray data (Chana et al., 2015).

Thus, although excitatory-inhibitory imbalance is a long proposed theory for ASD pathology, only 7 studies with a total of 64 cases and 76 controls have investigated glutamatergic impairments in post-mortem ASD brains: 4 showing higher glutamate receptor and transporter levels in the cerebellum and frontal cortex, one revealing lower KGA in the ACC, and two which found decreases in PSD-95 protein levels and genes associated with synaptic transmission in the fusiform gyrus and ACC respectively. These findings are therefore suggestive without being conclusive.

3.1.12. Serotonergic (5-HT) dysfunction

It is hypothesised that factors which regulate 5-HT transporter (5-HTT) expression could be associated in ASD pathology. Nakamura et al. (2011) investigated syntaxin 1A (STX1A) mRNA expression, a candidate 5-HTT presynaptic regulatory protein, and found significantly lower STX1A in the 8 autism anterior cingulate gyrus samples. Lower STX1A expression was also observed in the motor cortex of 7 cases (non- significant) (Nakamura et al., 2011). Similarly, the expression of LIM homeodomain transcription factor 1b (LMX1B), that plays a role in both the development and maintenance of serotoninergic neurons in the adult brain (Dolmazon et al., 2011, Song et al., 2011), was found to be significantly reduced in the anterior cingulate gyrus of autism patients (Thanseem et al., 2011). Moreover, Iwata et al. (2014) identified N-ethylmaleimide-sensitive factor (NSF) as a novel serotonin transporter-binding protein, and on examining mRNA expression of the serotonin transporter (SLC6A4) and NSF found no significant differences in SLC6A4 expression, although NSF expression showed a trend towards a reduction (Iwata et al., 2014).

Oblak et al. (2013) further quantified the density of the presynaptic 5-HTT, as well the two major receptor subtypes: 5-HT1A, located pre- and postsynaptically, and 5-HT2A receptors, predominantly postsynaptically, in the posterior cingulate cortex and fusiform gyrus of 7 and 9 autism subjects respectively. They found a significant reduction in 5-HT_{1A} and in 5-HT_{2A} receptor-binding density in both regions. A significant reduction in 5-HTT density was also observed in the deep layers of the fusiform gyrus only. A history of seizures only had a significant effect on 5-HTT binding-density in the superficial layers of the posterior cingulate cortex, where autism subjects with seizure history had a higher density of transporters (Oblak et al., 2013). Brandenburg et al. (2019) then expanded on the previous single concentration ligand study by comparing multiple concentrations of three 5-HT ligands in a larger group of 27 autism subjects in the anterior cingulate cortex, posterior cingulate cortex and fusiform gyrus. Across all autism subjects, a significant decrease in 5-HTT and 5-HT2 in the ACC was observed, a pattern that was most prominent in the 13 adult cases (>16 years). Unlike controls, autism subjects demonstrated an age-dependent decrease in 5-HT2 density. However, normal density and ligand affinity for 5-

HT1A, 5-HT2, and 5-HTT was found in the autism posterior cingulate cortex and fusiform gyrus (Brandenburg and Blatt, 2019).

Azmitia et al. (2011a) found substantially more 5-HT axons in 13 autistic brains from donors aged 2.8–29 years, relative to 9 controls, in the principle ascending fiber bundles of the medial and lateral forebrain bundles, as well as in the innervation density of the amygdala and the piriform, superior temporal, and parahippocampal cortices. Unlike the fine, varicose, and highly branched 5-HTT axons of controls, several types of dystrophic 5-HT axons were seen in the termination fields in autism donors of 8 years or more in the amygdala, hippocampus and in temporal lobe cortices (Azmitia et al., 2011a). These findings were confirmed and extended, through captured images and morphometric analysis, in three of the major serotonin pathways: MFB, Ansa Lent, and Stria Terminalis; indicating that the increase is in all ascending pathways. A similarly consistent and dramatic increase in both the number of axons per unit section and in the area fraction of axons was seen in the superior temporal cortex and globus pallidus (Azmitia et al., 2011b). Further, Pagan et al. (2017) investigated the complete serotonin-melatonin pathway in pineal glands of ASD subjects to find a reduction in melatonin and in the two enzymes that convert serotonin into melatonin: arylalkylamine N-acetyltransferase (AANAT) and acetylserotonin O-methyltransferase (ASMT). However, no increase in N-acetylserotonin (NAS) or serotonin levels were found (Pagan et al., 2017).

In summary, factors which could regulate the expression of serotonin or its receptors include a reduction in some regulatory proteins, transcription factors and serotonin transporters. However, none of these findings have been replicated in the same brain regions, which could be attributed to the slightly different techniques used. Two studies report an increase in numbers and dystrophic features; although the potential effects of serotonin enhancing drugs as sometimes used to treat older ASD adults with anxiety or depression should also be considered.

3.1.13. Glial impairments and Neuroinflammation

Microglial organization: Morgan et al. (2012) studied microglial and neuronal organization to find increased short-distance microglia–neuron interaction in the DL-PFC of 13 autism subjects,

where microglial processes encircled neurons. Despite the small number of subjects, preliminary analysis of the 3 young subjects (<6 years old) revealed very large effect, suggesting that this alteration could be present from an early age. No difference in neuron–neuron clustering was found and microglia–microglia organization appeared normal in autism subjects of all ages (Morgan et al., 2012). Clearly, however, more studies of potentially abberent neuron-specific interactions are required.

Microglial morphology, somal volume and density: Morgan et al. (2010) revealed morphological alterations which included somal enlargement, process retraction and thickening and extension of filopodia from processes in the DL-PFC of 13 autism subjects. The average microglial somal volume was increased in white matter and showed a non-significant trend to increased gray matter microglial cell density – neirther of which was influnced by seizure history. Microglial activation was observed in a sizeable proportion of cases with autism in this study (marked activation in 5/13 cases, including 2/3 cases under the age of 6; with marginal activation in 4/13 cases) (Morgan et al., 2010). Tetreault et al. (2012) also showed significantly higher microglial density (1-21%) compared to controls in the frontoinsular and visual cortex of 11 autism patients (Tetreault et al., 2012).

Morgan et al. (2014) further assessed the cell numbers and average volumes of microglia, astrocytes and oligodendrocytes in the amygdala of 8 ASD subjects to find no overall differences in cell numbers or volumes in comparison to 10 controls. However, 2/8 ASD subjects did have strong microglial activation. Additionally, age-related analysis suggested fewer oligodendrocytes in adult ASD cases older than 20 years compared to controls in the basal and lateral (non-significant) nuclei (Morgan et al., 2014). Consistent with other studies implicating increased glial cells in ASD, Menassa et al. (2017) also found increased layer II glial cell densities in the primary olfactory cortex (piriform cortex) of 17 ASD subjects with and without epilepsy, and associated with greater symptom severity in individuals with ASD (Menassa et al., 2017).

Lee et al. (2017) quantified six structurally and functionally distinct microglia phenotypes in the temporal cortex to find no difference in the total density of all microglia phenotypes between 10

ASD cases and controls. However, a significant decrease in ramified microglia in both gray matter and white matter was seen together with a significant increase in primed microglia in gray matter of ASD cases, with no difference in densities of other microglial phenotypes. Age-related analysis revealed that at around 2–3 years of age, both ASD cases and controls had rod and reactive microglia, but controls showed less of these two morphologies. Furthermore, from 8 to 32 years, controls had more ramified microglia than primed microglia or other non-ramified microglia phenotype, while ASD subjects showed more primed microglia than ramified microglia unlike controls. Additionally, a significantly positive correlation between age and density of primed microglia was found in both gray and white matter of ASD subjects, which was not the case in controls (Lee et al., 2017).

In summary, three studies (total 41 ASD cases) consistently report an increase in microglial densities, but none of these findings has been replicated in the same brain region. Another study, however, only reports differences in specific phenotypes and not in the total microglial density and two observe differences upon age-stratification. Two studies report no effect of epilepsy on the observed increase. Whether one can generalise that microglial disturbances are not necessarily restricted to higher cognitive centers, or the nature of changes across the lifespan of ASD subjects still needs further investigation.

Glial and astrocytic marker expression: Laurence et al. (2005) quantified levels of glial fibrillary acidic protein (GFAP) in the superior frontal cortex, parietal cortex and cerebellum of 6 autism subjects and 10 controls. They found elevated GFAP levels in all three regions in autism. Additionally, analysis of the effects of seizure, mode of death and medications (psychotropic and antiepileptic) had no impact on GFAP levels in ASD subjects (Laurence and Fatemi, 2005). This elevation in GFAP was also replicated in later studies by Fatemi et al. (2011) in the superior frontal corex and cerebellar vermis of 20 adults and children with autism (Fatemi and Folsom, 2011, Fatemi et al., 2011). Compared to 14 controls, Crawford et al. (2015) found significantly higher GFAP protein levels in the ACC white matter of 14 ASD donors, with no difference observed in the gray matter. Immunoreactivity of myelin oligodendrocyte glycoprotein (MOG), another glial marker, was similar in both white and gray matter of the ACC of ASD cases and controls. Similarly,

no significant differences in GFAP- or MOG- immunoreactivity were seen in either white or gray matter of the anterior prefrontal cortex between cases and controls. Gene expression analysis also showed no differences between groups in white and gray matter of either brain regions (Crawford et al., 2015). The discrepancy between GFAP protein and gene expression levels could be due to the different response pattern in translational and transcriptional regulation of GFAP or a biphasic expression of GFAP in activated cells reported previously (Tawfik et al., 2005, Pekny et al., 2014). Unlike the previous findings where GFAP was elevated in several regions of the ASD brain, Nicolini et al. (2015) found no increase in GFAP protein levels in the fusiform gyrus of 11 autism subjects (Nicolini et al., 2015).

Fatemi et al. (2008) investigated the expression of two astrocytic markers: aquaporin 4, a transmembrane water channel protein, and connexin 43, a major component protein in astrocytic gap juctions, in the parietal cortex, superior frontal cortex and cerebella of 22 autism subjects. While Connexin 43 expression was increased significantly in the superior frontal cortex, Aquaporin 4 expression was significantly decreased in the cerebellum (Fatemi et al., 2008). Similarly, Edmonson et al. (2014) revealed significantly increased expression of microglial markers GFAP, TREM2, DAP12, and CX3CR1 in the prefrontal cortex. In the cerebellum, however, only the astrocytic marker GFAP was elevated in the 9 people with autism (Edmonson et al., 2014).

In summary, elevation of glial markers, specifically GFAP, has been consistently reported in post-mortem ASD tissue, especially in the prefrontal cortex and cerebellum. One study reported no increase in the fusiform gyrus, but the expression of glial markers in this region has not been further investigated. Collectively, 7 studies of 75 ASD cases support a broad hypothesis of glial dysfunction in ASD - which could signify gliosis, reactive injury and/or perturbed neuronal migration processes.

Potential mechanisms of microglial activation: Vargas et al. (2005) further demonstrated an activation of microglia and astroglia in the cerebral cortex, white matter and notably in cerebellum of autistic patients. This was accompanied a marked increase in levels of proinflammatory and

anti-inflammatory cytokines, macrophage chemoattractant protein-1 (MCP–1) and the tumor growth factor ß-1 (TGFß-1) respectively (Vargas et al., 2005). Young et al. (2011) measured Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) levels in post-mortem samples of OFC tissue. Increased extranuclear and nuclear translocated NF-κB p65 expression was demonstrated in neurons, astrocytes and microglia of 9 Autism Spectrum Condition (ASC) subjects compared to 9 controls, and was particularly prominent in highly activated microglia (Young et al., 2011). DiStasio et al. (2019) also reported the presence of adaptive immune cells and immune cell–mediated cytotoxic damage in the CSF–brain barrier in ~65% of a large cohort of 25 ASD subjects between 5–68 years of age. This was observed in both male and female subjects, across all ages, in most brain regions and in white and gray matter as well as leptomeninges (DiStasio et al., 2019). However, insufficient evidence has been provided to implicate a particular signalling cascade or potential mechanism for neuroinflammation in ASD.

3.1.14. Stress in ASD

Mitochondrial impairments and oxidative stress: Palmieri et al. (2010) studied the mitochondrial aspartate/glutamate carrier (AGC) in temporocortical gray matter and found higher AGC transport rates in all 6 autism cases, accompanied by an increase in neocortical Ca^{2+} levels. Similarly, increased expression of the predominant AGC isoform in brain, ACG1, and cytochrome c oxidase activity was observed. A marked increase in oxidized mitochondrial proteins was also found in 4/6 autism subjects (Palmieri et al., 2010). Anitha et al. (2012) demonstrated the aberrant expression of several genes associated with mitochondrial dysfunction in autism in the anterior cingulate gyrus, motor cortex and thalamus of 8 autistic subjects. The majority of genes showed a reduced expression and had functions related to membrane polarization and potential, mitochondrial transport, small molecule transport, targeting proteins to mitochondria, mitochondrial protein import, inner and outer membrane translocation, mitochondrial fission and fusion, mitochondrial localization or apoptosis (Anitha et al., 2012). Schwede et al. (2018) also revealed that genes associated with mitochondrial function were downregulated in autism cerebral cortex samples and these genes correlated with genes related to synaptic function (Schwede et al., 2018). Anitha et al. (2013) further compared the expression of 84 electron

transport chain (ETC) genes in the same brain regions to find reduced region-specific expression of several ETC genes in 8 autism samples (Anitha et al., 2013). Tang et al. (2013) showed that the temporal cortex of 20 ASD patients exhibited altered protein levels of mitochondrial respiratory chain protein complexes. Decreased mitochondrial antioxidant enzyme SOD2 was accompanied with greater oxidative DNA damage and higher protein levels of mitochondrial membrane proteins and mitochondrial fission proteins were found, together with decreased levels of the fusion proteins. Interestingly, most of these alterations were prominent in the cortical pyramidal neurons of ASD children but to a lesser extent, and sometimes completely absent, in adults (Tang et al., 2013). Finally, Lepagnol-Bestel et al. (2008) assessed SCL25A12 expression, a member of the SLC25A mitochondrial transporter family, in the frontal cortex and cerebellum of 9 autistic individuals to find an upregulation in the autism prefrontal cortex with no difference in its expression levels in the granule cells of cerebellum (Lepagnol-Bestel et al., 2008).

Increases in 3-nitrotyrosine (3-NT), a specific oxidative stress marker, has been reported in the cerebella of autism cases, which was associated with an elevation in neurotrophin-3 (NT-3) levels (Sajdel-Sulkowska et al., 2009). Sajdel-Sulkowska et al. 2011 further compared the different regions particularly affected by oxidative stress between two ASD and matched controls - cerebellar hemispheres and putamen showed elevated 3-NT levels in both cases, while orbitofrontal cortex, Wernicke's area, cerebellar vermis, cerebellar hemisphere and pons showed highest 3-NT levels in the older case (Sajdel-Sulkowska et al., 2011). An increase in 3-NT levels in ASD was replicated by Khan et al. (2014) in the orbitofrontal cortex, with trends towards increases in the cingulate gyrus, putamen and Wernicke's area in 7 male ASD cases, and in the brainstem of 3 female ASD subjects (Khan et al., 2014). Muratore et al. (2013) investigated if altered oxidative stress in autism affects Methionine synthase (MS) status, an enzyme whose lower activity results in an increased production of the antioxidant glutathione (GSH). Although MS mRNA levels were significantly reduced in the cerebral cortex of 20 autism subjects, particularly at younger ages, MS protein levels were similar to controls. An decrease in the sulfur-containing metabolites homocysteine (HCY) and cystathionine levels was also observed in autism tissues, implying an activation of the transsulfuration pathway that supplies cysteine for GSH synthesis, particularly during oxidative stress. However, no differences in the levels of 8-

hydroxyguanosine (8-OHG), a biomarker of oxidative stress, were found (Muratore et al., 2013). On the other hand, Shpyleva et al. (2014) evaluated the levels of 8-oxo-7-hydrodeoxyguanosine (8-oxodG), the prevalent form of oxidative DNA damage due to reactive oxygen species, in the genomic DNA of cerebellar tissues of autism cases and reported a significant elevation compared to controls (Shpyleva et al., 2014). Furthermore, Zhang et al. (2016) measured the levels of Vitamin B12, also known as cobalamin (Cbl), which serves as a cofactor for MS and methylmalonylCoA mutase, in its methyl- and adenosyl-forms (MeCbl and AdoCbl) respectively, in the frontal cortex of autism cases to find that MeCbl and AdoCbl levels were more than 3-fold lower in autism where lower MeCbl was associated with reduced MS activity and higher levels of its substrate HCY. Although lower MS activity recapitulates the previous findings of reduced MS mRNA, higher HCY levels contradicts the findings of Muratore et al., (2013) (Zhang et al., 2016).

Endoplasmic reticulum (ER) stress: One study showed a significant increase in the mRNA levels of ER stress-related genes: ATF4, ATF6, PERK, XBP1, CHOP and IRE1 in the middle frontal gyrus of 13 ASD cases. Additionally, a positive association between mRNA levels of ER stress genes and stereotyped behavior was identified in ASD subjects (Crider et al., 2017).

To summarise, an array of mitochondrial alterations have been reported in several regions of the ASD brain, most of them highlighting altered expression of mitochondrial genes. These findings suggest the presence of a compromised mitochondrial function in ASD. One study expanded its findings to reveal prominent effects in children, but this has not been investigated in other studies. Several lines of evidence also point towards the presence of oxidative stress in ASD. Four studies have demonstrated elevated oxidative stress markers 3-NT and (8-oxodG) in the ASD cerebella (3 studies), and other brain areas (1 study), and another two implicate dysfunctional MS activity in the cortex.

3.1.15. Apoptotic dysfunction

Reduced Bcl-2 levels in 5 samples of autism cerebellum have been reported (Fatemi et al., 2001). Araghi-Niknam and Fatemi (2003) also demonstrated altered levels of levels of Bcl-2 and P53, a tumor suppressor protein that controls neuronal apoptosis, in the frontal, parietal and cerebellar

cortices of 5 autistic individuals. Whereas Bcl-2 was decreased in the frontal (non-significant) and cerebellar cortices, P53 levels were increased in the same brain regions (both non-significant) (Araghi-Niknam and Fatemi, 2003, Fatemi and Halt, 2001). This was recapitulated by Sheikh et al. (2010) with reductions in Bcl-2 expression in the frontal cerebral cortex and cerebellum, accompanied by increased p53 expression in the Purkinje cells and granule cells of the cerebella (Sheikh et al., 2010b). Moreover, the levels of cathepsin D protein, a protease of ample expression in the brain that initiates apoptosis, were higher in the the frontal cortices, pyramidal and granule cells of the hippocampus, as well as in the neurons of the cerebellum in 7 autistic cases (Sheikh et al., 2010a). The same group also found an increase in caspase-3 expression, a protease essential for the regulation and execution of apoptosis, in the cerebellum of autistic individuals (Sheikh et al., 2010a). Thus, four studies, in a total of 17 subjects, consistently report either a reduction of anti-apoptotic proteins or an increase in pro-apoptotic proteins in the ASD frontal cortex and cerebellum.

3.1.16. Miscellaneous

This section summarises single studies of miscellaneous aspects of ASD neuropathology which have, as far as we are aware, not been otherwise examined.

Blood Brain Barrier: The elevation of components of tight junctions in brain endothelial cells associated with blood brain barrier integrity and function were found in the frontal cortex and cerebellum tissues of ASD subjects (Fiorentino et al., 2016).

Angiogenesis: An increase in both nestin and CD34 immunoreactivity was found in the pericytes and endothelial cells respectively, throughout the superior temporal cortex fusiform cortex, pons/midbrain and cerebellum in young ASD subjects- suggestive of splitting (intussusceptive) angiogenesis. However, the history of seizures in this group of ASD subjects could have promoted angiogenesis (Azmitia et al., 2016) – as can serotoninergic drugs, such as SSRIs.

Single nucleotide variant (SNV) patterns: More autism cases harbored protein-altering variants in known ASD-candidate genes, particularly more deleterious variants such as loss-of-function

and missense mutations as well as loss-of-function variants, such as nonsense, splice site and frameshift mutations (D'Gama et al., 2015, Sanders et al., 2012, De Rubeis et al., 2014).

Akt/mTOR pathway: Reductions in protein levels of phosphorylated and total mTOR, full-length TrkB, PI3K, Akt, as well as p70S6 kinase, eIF4B and PSD-95 were found in the fusiform gyrus of subjects with idiopathic autism. An imbalance in TrkB protein isoforms was also observed, (Nicolini et al., 2015). (Chen et al., (2014) previously reported disruptions in ncRNAs that target genes associated with PI3K-Akt signaling, but it is unclear whether this involved Akt/mTOR dysregulation.)

Thyroid deficiency: The biologically active form of thyroid hormone (TH), 3',3,5-triiodothyronine (T3) levels were reduced (non-significantly) in the cortical regions of ASD brains (OFC ~80%, putamen ~60% and Wernicke's area ~75%). The expression of several TH-dependent genes was also altered, especially the TTR family of genes, with aberrant expression of negatively regulated genes, such as DIO2, and SWAP-1, and positively regulated genes such as DIO3, BDNF, and RELN, in an apparently brain region- and sex-dependent manner (Khan et al., 2014).

3.2. Post-mortem alterations in RTT

Most Post-mortem studies in RTT do not state whether RTT patients also had a diagnosis of ASD. Studies which investigated both ASD and RTT stratified the cases into separate groups. Only four studies state that RTT diagnosis was made based on the classic criteria set by Hagberg (Hagberg et al., 1983, Hagberg et al., 1985) and/or The Rett Syndrome Diagnostic Criteria Work Group (syndrome, 1988) (Supplementary table 7).

3.2.1. Brain size and Neuropathology

Bauman et al. (1995) reported that the brains from 3 girls with RTT were smaller than matched controls. Increased neuronal cell-packing density was observed with smaller neurons - many of which appeared ghost-like with pale Nissl substance in the cerebral cortex, basal ganglia, thalamus, hippocampus, and amygdala. In the hippocampus, a substantial reduction in neuronal size was observed, most prominently in areas CA4 and CA3. In the cerebellum, a thinning of the molecular and granule cell layers was observed together with a reduction in Purkinje cells

numbers throughout the hemispheres with no evidence of obvious gliosis (Bauman et al., 1995). Another two studies by Jellinger et al., also reported cortical atrophy, reduced brain weight, occasional mild gliosis and under-pigmentation of the substantia nigra in 8 and 9 female RTT cases respectively, compared to age-matched controls (Jellinger et al., 1988, Jellinger and Seitelberger, 1986). All 12 brains were smaller, with reduced pigmentation in the substantia nigra.

Dendritic abnormalities: Belichenko et al. (1994) reported loss of dendritic spines in the frontal, temporal and motor cortical areas of 3 RTT cases (Belichenko et al., 1994). In addition to reduced brain weight, Armstrong et al. (1995) also reported shorter basal and apical dendrites of pyramidal neurons in the motor cortex as well as basal dendrites in the frontal cortex and subiculum in 22 cases (Armstrong et al., 1995). Moreover, Chapleau et al. (2009) reported lower dendritic spine density in secondary and tertiary apical dendrites of CA1 pyramidal neurons in the hippocampus, similar to the findings of Belichenko et al. (1994) in the pyramidal neurons of the motor cortex of 10 RTT subjects (Chapleau et al., 2009).

Another approach to characterizing dendritic abnormalities is through examining cytoskeletal proteins and molecules important for proper dendritic formation and function. Kaufmann et al. (2000) examined MAP-2, a member the family of microtubule associated proteins (MAPs), and neurofilaments (NF) immunoreactivity in 11 RTT cases. While both somatic and dendritic MAP-2 immunoreactivity were decreased in layers V–VI, NF immunoreactivity demonstrated an increase in dendritic immunostaining in layers II–III and a decrease in the dendrites of lower layers (Kaufmann et al., 2000). Similarly, Lipani et al. (2000) quantified Nerve growth factor (NGF), responsible for dendritic outgrowth during later developmental stages and proper synaptogenesis, and its receptor trkA expression in the frontal cortex of 9 RTT subjects. They found a reduction in both NGF and trkA expression. Remarkably, all RTT samples demonstrated NGF levels at or below the minimum level observed in controls and no NGF was detected in 3 RTT patients (Lipani et al., 2000).

These 3 studies on a total of 35 RTT patients could be summarised as showing a reduction in spine densities and shorter dendritic morphology, and layers III and V pyramidal cells of the cerebral cortex appear to be most affected in RTT. Two other studies reported aberrant expression on proteins and molecules essential for proper dendritic development. Together with evidence from gene expression analysis implicating synaptic genes (see 3.2.3.), it appears that impaired dendritic trees and differential involvement of upper and lower cortical layers involved in cortico-cortical circuits and subcortical projecting neurons respectively may alter cortical physiology in RTT.

3.2.2. Alterations in Neuropeptides and Receptors

Wenk et al. (1993) found a non-significant reduction in choline acetyltransferase (CHAT) activity in many cortical and subcortical regions of four RTT subjects (with no difference in the number of NMDA, AMPA, Y opioid and neurotensin binding sites (Wenk et al., 1993). Moreover, in a cohort of 9 female RTT subjects, Blue et al. (1999) identified regional, receptor and age-specific changes in the glutamate and GABA receptors in the basal ganglia. Overall, the densities of ionotropic receptors (NMDA, AMPA, and kainate (KA) glutamate receptors (GluRs)) were reduced by 15% and this was more prominent in the putamen of older subjects (>8 years). However, no significant group or age differences were found for mGluR density in the basal ganglia. GABA receptor density was however increased in the caudate of young RTT subjects (Blue et al., 1999). Gogliotti et al, (2016) also revealed a reduction in metabotropic glutamate receptor 5 (mGlu5) protein levels in the primary motor cortex of seven RS patients compared to eight controls (Gogliotti et al., 2016). On the other hand, Wong et al. (2018) quantified Dopamine D1 and D2 receptor (D1R and D2R) density in the caudate and putamen of a cohort of 6 girls with RTT between the ages of 2–25 years to find no group differences in either receptor density compared to controls (Wong et al., 2018). Therefore, insufficient evidence is available to associate the decline in motor and cognitive functions observed in RTT to specific neurotransmitter systems.

3.2.3. Alterations in Biogenic Amines

No compelling evidence has yet been presented on the disruption of central monoaminergic systems in RTT. Lekman et al. (1989) measured the levels of DA, 5-HT, and NA, and their respective metabolites: HVA, 5-HIAA, and hydroxymethoxyphenylglycol (HMPG) in 4 RTT cases

(12-30 years) compared to 3 controls (22-38 years). They found an apparent age-related trend, in that a 12-year-old case showed normal or close to normal levels, whereas marked reductions were found in the 20- and 30-year-old subjects. In the substantia nigra of the 2 older patients, 50% or greater reduction in all compounds, apart from HMPG, was found; consistent with the reduced melanin content in neurons of the substantia nigra previously reported in RTT patients. Older RTT subjects showed reduced 5-HT, NA and HMPG levels in the thalamus together with reduced 5-HT levels in the frontal cortex, whereas in the hippocampus, the 2 youngest RTT cases showed reduced DA levels with normal levels in the older subjects (Lekman et al., 1989). Although a controlled single case study (Sofic et al 1987) also reports a reduction in DA, 5-HT, and NA and some of their metabolites, these findings should be interpreted with caution given the very small number of cases, and confounding by e.g. long-term undernutrition (Sofic et al., 1987).

3.2.4. Gene Expression Patterns in RTT

Colantuoni et al. (2001) showed increased expression of several glial transcripts and global down-regulation of neuronal transcripts, some of which were downregulated by 50% or more in 6 RTT subjects. Remarkably consistent reductions in transcripts encoding presynaptic markers were identified (Colantuoni et al., 2001). Gibson et al. (2010) also assessed the mRNA expression profiles of the frontal and occipital cortex in 6 RTT cases. They found that a subset of genes associated with neurological disorders and synaptic vesicle cycling was differentially expressed in the frontal cortex of RTT patients, compared to RTT occipital cortex and control frontal cortex, and that MeCP2 is associated with the promoter regions of some of these genes, suggesting that loss of MeCP2 function in RTT may be responsible for their aberrant expression (Gibson et al., 2010). Furthermore, Gogliotti et al. (2018) identified 1887 altered genes in the motor cortex and 2110 genes in the cerebellum, showing a global trend towards an increased expression in 9 RTT cases. These were enriched in pathways associated with mitogen-activated protein kinase (MAPK) signalling, long-term potentiation and axon guidance. Moreover, 4/5 muscarinic acetylcholine receptors (mAChRs): CHRM1, CHRM2, CHRM3 and CHRM4, demonstrated a dysregulation in expression in at least one brain region (Gogliotti et al., 2018). To summarise, three studies have highlighted the dysregulation of genes associated with glial, neuronal and synaptic functions in several regions of the RTT brain, with one study (Gibson et al., 2010)

suggesting region-specific dysregulation. Table 2 summarises some of the representative transcripts dysregulated.

3.2.5. Alterations in MeCP2 and target genes

Armstrong et al. (2003) showed that compared to age-matched controls, fewer neurons (50%) express MeCP2 in the cortical regions of 14 RTT subjects between 6–41 years (Armstrong et al., 2003). Nagarajan et al. (2006) demonstrated a reduction in MeCP2 expression in all of 9 RTT samples, including those without MeCP2 coding mutations (Nagarajan et al., 2006). In another group of 7 RTT subjects, reductions in MECP2 protein levels were also shown (Gogliotti et al., 2016). Because MeCP2 regulates several other genes, Swanberg et al., (2009) investigated the role of MeCP2 in the regulation of EGR2, which was found to co-localise with MeCP2 and mutually regulate expression, showing a significant reduction in EGR2 in 10 RTT cortical samples (Swanberg et al., 2009). Miyake et al. (2011) also identified PCDHB1, a protocadherin essential for brain development which encodes neuronal adhesion molecules, as a MeCP2 target gene; the expression of which was up-regulated in RTT brain tissue (in 3/4 subjects) (Miyake et al., 2011). Similarly, the expression of the gene encoding MET receptor tyrosine kinase was found to be dramatically decreased in the temporal cortex of 5 female RTT cases (Plummer et al., 2013). Thus, overall, one study highlights the reduction in MeCP2 levels in all RTT cases examined, while the other three showed a dysregulation in MeCP2 target genes, where EGR2 and MET were down-regulated and PCDHB1 was upregulated. One could conclude that aberrant MeCP2 expression alters the expression of several target genes, but specififc findings have not yet been replicated, nor expanded to include more MeCP2 targets.

3.2.6. Somatic LINE-1 retrotransposition

One study investigated Human-specific LINE-1 (L1Hs) retrotransposition in RTT by profiling a large number of somatic insertions from 20 samples of 5 RTT subjects and matched controls. An increase of somatic L1Hs insertions was found in the brain compared to non-brain tissues from the same subjects. In neurons of RTT prefrontal cortex, clonal somatic insertions were enriched in introns and depleted in exons (Zhao et al., 2019).

3.3. Alterations in Fragile X Syndrome (FXS)

There is no mention on how FXS was diagnosed in the post-mortem studies below or whether or not these cases also had an ASD diagnosis except for one study (Greco et al., 2011) that includes the clinical history of all three patients, stating that one patient was also diagnosed with ASD and molecular/chromosomal analysis confirmed presence of a full mutation allele.

3.3.1. Neuropathology

Hinton et al. (1991) reported long, thin, immature spines in two 15- and 41-year old FXS subjects. Analysis of neocortical layers II–VI of the cingulate and temporal association areas, however, revealed no significant differences in neuronal counts compared to controls (Hinton et al., 1991). Irwin et al. (2001) examined the dendritic spines on layer V pyramidal cells of the temporal and visual cortices in three (48-, 48-, and 73-year-old) male FXS cases to reveal longer dendritic spines in both cortical regions with immature morphology and higher spine density on distal segments of apical and basilar dendrites (Irwin et al., 2001). A later study by Greco et al. (2011) examined the hippocampus and the cerebellar vermis of three older men, aged 57, 64 and 78 years, with FXS. Abnormalities in the hippocampus included focal thickening of hippocampal CA1 and irregularities in the appearance of the dentate gyrus. In the cerebellar vermis, all lobules as well as the lateral cortex of the posterior lobe of the cerebellum demonstrated a reduction in Purkinje cell numbers. Additionally, patchy foliar white matter axonal and astrocytic abnormalities accompanied with mild, yet excessive, undulations of the internal granular cell layer were observed. Compared to 5 age-matched controls, both anterior and posterior lobes of the vermis also demonstrated panfoliar atrophy, with preferential atrophy of the posterior lobule (VI to VII) (Greco et al., 2011).

Collectively, abnormal dendritic spine lengths and morphology have been consistently reported in FXS, which could reflect a failure of normal dendritic spine maturation and/or pruning during development which continues throughout adulthood.

3.3.2. Miscellaneous impairments in FXS

mGluR5 and relevant pathways: The mGluR theory of FXS proposes that loss of FMRP expression activates mGluRs and consequently prompts exaggerated translation of synaptic

plasticity-related mRNAs (Bear et al., 2004, Huber et al., 2002). Lohith et al. (2013) assessed mGluR5 receptor binding and protein expression in the prefrontal cortex of 14 FXS patients or carriers to find increased mGluR5 density (non-significant), with an increase in mGluR5 protein expression. However, whether the administration of psychotropic medications could affect mGluR5 expression remains a caveat to be explored (Lohith et al., 2013). Gkogkas et al. (2014) revealed a 37% increase in p-eIF4E levels, as well as a 42 % increase in MMP-9 protein levels, two downstream molecules in the Ras/ERK/Mnk pathway (Gkogkas et al., 2014). However, these findings have not been replicated and no compelling evidence for the involvement of translational control of Mmp-9 as a potential mechanism to regulate synaptic function in FXS is presented.

FMRP targets - Bone morphogenetic protein type II receptor (BMPR2): Kashima et al. (2016) demonstrated a ~2-fold increase in the full-length BMPR2 isoform in the upper to middle layers of the prefrontal cortex of 3 FXS subjects. The loss of FMRP in FXS subjects was correlated with a ~3-fold increase of full-length BMPR2 protein isoform as well as 16-fold increase of phosphorylated cofilin, a marker of LIMK1 activity(Kashima et al., 2016).

Iron Metabolism: One study investigated iron dysregulation in FXS revealing an accumulation of iron in the stroma of the choroid plexus. Additionally, a reduction in transferrin, ferroportin and ceruloplasmin levels was observed in the epithelial cells, and the distribution of transferrin receptor 1 was shifted from the basolateral membrane, as observed in the controls, to a predominantly intracellular location in 8 FXS samples (Ariza et al., 2015).

3.4. William's Syndrome (WS)

Most studies reported using somatic characteristics and/or genetic testing to confirm the. Diagnosis of WS. There is no mention on whether the cases also had a clinical diagnosis of ASD (supplementary table7).

3.4.1. Neuropathology

Galaburda and Bellugi (2000) performed neuroanatomical analysis on 4 WS brains, showing a trend towards increased cell-packing density in the cortex, although not uniform, accompanied with larger neurons and a variable neuron density in layer VI, unlike the supragranular layers

(Galaburda and Bellugi, 2000). Furthermore, quantitative analysis of brains from 3 WS subjects revealed a higher density of smaller, closely packed neurons in both granular and infragranular layers of the primary visual cortex. However, this was only true in the left hemisphere (Galaburda et al., 2002). Holinger et al. (2005) later examined the primary auditory cortex in the same 3 WS subjects to reveal large neurons bilaterally in layer II and in layer VI of the left hemisphere, with no significant differences in overall cell density (Holinger et al., 2005). Lew et al. (2017) further measured neuron density in the supragranular and infragranular layers of several cortical areas, namely: prefrontal cortex, motor cortex, primary somatosensory area and visual cortex in a cohort of 6 matched WS and controls. In the prefrontal cortex, lower neuronal density was observed, with layers V/VI showing the largest decrease in density. On the other hand, the primary somatosensory and visual areas revealed higher density compared to controls (non-significant), with no differences observed in the primary motor cortex (Lew et al., 2017).

Collectively, altered neuronal density has been consistently reported in 4 studies, with larger neurons observed in two studies. These findings also support the dorsal–ventral hypothesis of WS, which suggests that the dorsal forebrain is more affected than the ventral, in keeping with the severe visual–spatial deficits, excellent facial recognition, heightened sound sensitivity, and specific patterns of auditory evoked potentials characteristic of WS subjects.

Given that the OFC provides significant connections to subcortical structures, including the amygdala and striatum, Lew et al. (2018) quantitatively examined the amygdala of 7 WS subjects to find more neurons in the lateral nucleus. However, no significant differences in neuron number were observed in the basal, accessory basal, or central nuclei, nor were any differences in nuclei volume, neuron density or neuron soma area (Lew et al., 2018). Hanson et al. (2018) further examined the density of neurons and glia in the striatum (caudate, putamen and nucleus accumbens) of 5 WS cases. Although no significant increase in the overall neuron density was found, an increase in the density of glia in the dorsal caudate nucleus, as well as an increase in the ratio of glia to neurons in the dorsal and medial caudate nucleus was observed. Similarly, an increase in the density of oligodendrocytes was reported in the medial caudate nucleus, a region

that also receives projections from the prefrontal cortex, further implicating aberrant connectivity in WS pathogenesis (Hanson et al., 2018). Wilder et al. (2018) examined the ventromedial prefrontal cortex, which is richly connected to the amygdala and striatum, to reveal a reduction in decreased neuron density (statistically significant in the supragranular layers) accompanied by an increase in glia density and glia to neuron ratio in both supra- and infragranular layers of 7 WS cases (Wilder et al., 2018). These results suggest that cytoarchitectural abnormalities in the prefrontal cortex, together with impairments in frontostriatal and frontoamygdala circuitry could be associated with the hyper-social behaviour and anxiety frequently reported in WS.

3.5. Alterations in ASD-related CNVs

3.5.1. 15q11-13 duplication syndrome (15qdup)

Almost all 15qdup cases were reported to have a clinical diagnosis of ASD. ADI-R and clinical history were used to assess the patients and cytogenetic/molecular diagnostics was used to confirm the duplication.

Gene Expression Patterns, transcriptomics and epigenetics: Using brain tissue from a male subject with 15q11–13 hexasomy and a female with 15q11–13 tetrasomy, Hogart et al. (2009) demonstrated that both gene expression and DNA methylation correlated with parental gene dosage in the male 15qdup, who was reported to have severe cognitive impairment and seizures. Interestingly, deficiencies in the paternally expressed transcripts were observed in the female subject, together with unchanged levels of maternally expressed UBE3A compared to controls. Furthermore, this aberrant expression of paternal transcripts was consistent with enriched DNA methylation at the 15q11–13 imprinting control region (ICR), also reffered to as imprinting center of the Prader-Willi locus (PWS-IC); a differentially methylated CpG island that is normally unmethylated on the active paternal allele and heavily methylated on the silent maternal allele and controls several imprinted genes essential for neurodevelopment within the 15q11-13 region (Sutcliffe et al., 1994, Scoles et al., 2011). Moreover, a reduction in the non-imprinted, biallellically expressed, 15q11–13 GABA receptor subunit gene expression was observed, particularly in the female subject without any significant GABRB3 methylation differences (Hogart et al., 2009). Scoles et al. (2011) also showed higher methylation levels at the PWS-IC in 8 15qdup samples compared to controls and ASD subjects, suggesting a maternal origin to 15qdup. Similarly, no

increase in GABRB3 transcript or protein levels due to the increase in copy number was found in the 15qdup samples. Compared to 21 controls, paternally expressed SNRPN levels were reduced in 15qdup cases. On the other hand, analysis of the maternally expressed UBE3A transcript and protein levels revealed an increase compared to both control and ASD cases, unlike the previous findings of Hogart et al. (2009) (Scoles et al., 2011).

Collectively, these findings highlight variability in transcriptional and epigenetic changes, suggesting that gene expression within the 15q11-q13 is not solely dependent on copy number as evident by the changes in GABRB3 and SNRPN expression.

Similarities to ASD: Parikshak et al. (2016) demonstrated that 15qdup shares the core transcriptomic signature observed in idiopathic ASD in a cohort of nine 15qdup subjects. Genome wide differential gene expression as well as differential splicing profiles were very consistent between 15qdup and ASD subjects (See 3.1.6. gene expression patterns in ASD). Interestingly, more homogeneous perturbations of higher magnitude were observed in the 15qdup cortex, compared to that of idiopathic ASD subjects (Parikshak et al., 2016). Similarly, Lee et al. (2019) assessed allele-specific expression (ASE) to find an enrichment of minor allele in dup15q compared with controls, similar to the findings in ASD subjects. In both idiopathic ASD and 15qdup, this allele shifting is shown to enhance snoRNA-targeted splicing changes in ASD-relevant target genes (Lee et al., 2019a). Moreover, Tran et al. (2019) demonstrated a global bias for RNA hypoediting involving many synaptic genes in ASD, the severity of which was exacerbated in 15qdup subjects. Hypoediting sites also included differential editing at specific glutamate receptors, providing further evidence for a hypoediting landscape that is shared across ASD and 15qdup (Tran et al., 2019). Wong et al. (2019) also identified widespread differences in DNA methylation in the cerebral cortex of idiopathic ASD subjects which was as highly correlated between ASD and 15qdup patients. Co-methylation network analyses further highlighted genes involved in immune system, synaptic signalling and neuronal regulation in cortical DNA methylation changes associated with both ASD and 15qdup cases, reflecting a convergent

molecular signature in the cortex of different forms of idiopathic and syndromic ASD (Wong et al., 2019b).

Candidate genes and relevant pathways: Oguro-Ando et al. (2015) have shown an upregulation in Cytoplasmic FMR1-interacting protein 1 (CYFIP1) expression in the superior temporal gyrus of 15qdup subjects. Similarly, CYFIP1-associated mTOR dysregulation was observed in 3 15qdup cases with ASD, where an upregulation of mTOR (approximately six-fold) was seen in 15qdup samples (Oguro-Ando et al., 2015). This has however yet to be replicated.

Environmental factors: Mitchell et al. (2012) analysed the levels of Persistent organic pollutants (POPs) in post-mortem tissue of subjects with 15qdup as well as other neurodevelopmental disorders including ASD, Down, Rett, Prader-Willi and Angelman syndromes. Compared to controls, polychlorinated biphenyl 95 (PCB 95) levels were significantly higher almost exclusively in cases with maternal 15qdup or deletion in Prader-Willi syndrome, proposing that exposure to POPs could predispose to genetic copy number variation (Mitchell et al., 2012).

3.5.2. 22q11 Deletion Syndrome (22qdel)

Only one controlled study has reported neuropathological findings in subjects with 22qdel. In a cohort of 3 adult cases, Kiehl et al. (2009) reported the presence of frontal lobe bilateral periventricular neuronal heterotopia in a 44-year-old male, accompanied with excess frontal white matter neurons, cerebellar ectopia and reduced myelination in the white matter. The other two subjects, a 22-year-old male and a 52-year-old female, showed no signs of neuronal disorganization. However, several white matter abnormalities, such as severe gliosis, focal foamy macrophage infiltrates and hypertensive-like vascular changes were reported (Kiehl et al., 2009).

4. Discussion

4.1. Summary of Key findings in ASD

The majority of post-mortem findings reported here came from a small number of studies examining particular aspects of ASD brains, and only very few covered the other disorders. Nonetheless some tentative conclusions can be drawn, not least because to collect post-mortem material for further studies that may disprove them will take years. Cortical layering was largely

undisturbed in most studies, providing little evidence to support the hypothesis of dysregulated layer formation in ASD; however, reductions in minicolumn numbers and aberrant myelination were consistently reported. The findings regarding neuronal numbers, densities and volumes reported here are not sufficiently consistent in the different brain regions examined to draw a general conclusion.

Several transcriptomic studies have demonstrated atypical expression of genes associated with critical developmental processes in ASD brains, potentially serving as ASD-susceptibility genes and providing preliminary hypotheses on the possible molecular processes that could be implicated in ASD pathophysiology. Transcriptomic analysis further implicates abberant synaptic, metabolic, proliferation, apoptosis and immune pathways in the ASD brain. Dysregulated expression patterns of ncRNAs, both long and small, that target ASD-risk genes/genomic regions has also been consistently reported. Further, epigenetic signatures of ASD samples largely differed from that of controls. GABAergic impairments consistently reported in ASD include reduction in GABA receptors (5 studies), GAD levels in the cerebellum (3 studies), reductions in RELN (4 studies), PV (6 studies) and altered levels of specific IN subpopulations (2 studies). Although excitatory/inhibitory (E/I) imbalance is a well-proposed theory for ASD pathophysiology, only a small number of papers have investigated the glutamatergic impairments, with the most consistent finding being of increased glutamate receptors and transporters (4 studies). Moreover, an increase in 5-HT neuron numbers and dystrophic features were fairly consistently reported (2 studies), pointing to a potential serotonergic dysfunction. Glial findings included elevation of glial markers (7 studies), specifically GFAP, and an increase in microglial densities (3 studies). Additionally, reduced anti-apoptotic proteins and increased pro-apoptotic proteins in ASD tissue (4 studies) point towards a disruption in apoptotic mechanisms in ASD.

4.2. Putative pathophysiological mechanisms in ASD

E/I imbalance: The GABAergic and glutamatergic impairments reported are consistent with theories of an E/I imbalance of principal neurons as well as their output to key limbic cortical targets (Rubenstein and Merzenich, 2003, Zikopoulos and Barbas, 2013). These could underlie

some socio-emotional behaviours in autism and in particular those where the resulting impaired network formation could cause inhibitory deficits, such as the sensory overload and lack of stimulus discrimination often seen in ASD. Additionally, E/I imbalance could explain the liability to seizure disorders that are commonly comorbid with ASD. The post-mortem findings supporting E/I imbalance are further backed up by several neuroimaging studies (Hegarty et al., 2018, Ajram et al., 2017, Foss-Feig et al., 2017, Port et al., 2019) as well as data from mouse model studies, electrophysiology (Bozzi et al., 2018) and induced pluripotent stem cell (iPSC)-based models (Culotta and Penzes, 2020).

Disrupted neurogenesis: Aberrant apoptotic signaling could contribute to the excess neuronal numbers occasionally reported and more consistent accounts of larger brain volumes in some people with ASD. Further, there is compelling evidence from head circumference and structural neuroimaging studies of the brain that the head, brain, cortex and cerebellum are all generally enlarged in autism (Sacco et al., 2015, Stanfield et al., 2008) and in a sub-group of some 10-15% in particular. Given the presence of minicolumnopathy, possibly altered neuronal numbers and white and gray matter fibers, abnormal neurogenesis could arise due to one or more factors in particular cases, including: the total number of founder cells, cell cycle duration or number of successive cell cycles, as well as the modes of cell division and selective cell death or neuronal migration. Such generalised processes from an early age could underlie the learning disability seen in approximately a half of people meeting diagnostic criteria for ASD.

Synaptic dysfunction: PV is critical for maintaining synaptic plasticity of GABAergic synapses, and the studies showing its altered expression highlight possible impaired synaptic plasticity, especially of the cerebellar output to other brain regions. Indeed this is consistent with a large functonal MRI literature in autism (Philip et al., 2012). Altered levels of proteins related to synaptic vesicle regulation, aberrant gene expression levels of synaptic genes relevant to glutamatergic neurotransmission, and synaptic pathways all point to impaired synaptic transmission in ASD. Findings from iPSC-based studies (Taoufik et al., 2018) and mouse models (Caldeira et al., 2019) also support the synaptic dysfunction hypothesis.

Neuroinflammation: Transcriptomic analysis repeatedly implicates astrocyte and microglia markers and other immune system function and inflammatory response genes. Increased miR-155p5 gene expression, a pro-inflammatory miRNA implicated in several inflammatory diseases has also been consistently reported in ASD samples. Similarly, the increased microglial density throughout the ASD cortex suggests that microglial activation may represent an neuroimmune system response to synaptic or neuronal disturbances, which could contribute to ASD pathophysiology. Utilising animal and cellular models will further help investigating potential neuroinflammation mechanisms in ASD (Freitas et al., 2018).

Sex-bias: No evidence for sex-depended expression was evident in our review, suggesting that an ongoing interaction with sexually dimorphic pathways is a more probable explanation of the sex bias in autism. Reductions in ER-β and aromatase and a decrease in RORA protein levels, which is regulated by sex-hormones, could serve to explain some of this, although the evidence remains insufficient. In addition, some of these 'sex effects' in the literature may be spurious false positives owing to multiple hypothesis testing (Bennett et al., 2009), given the general lack of sufficiently large samples to be divided into subgroups and that some sex interactions have not been replicated. However, unlike the post-mortem findings, several human neuroimaging studies highlight brain differences between the two sexes (Lai et al., 2017). Similarly, a recent study utilising in-silico modeling and in-vivo chemogenetic manipulations in mice, together with fMRI has shown that E/I imbalance affects the medial prefrontal cortex differently in male ASD subjects compared to females (Trakoshis et al., 2020).

4.3. Region-dependent patterns in ASD

Although a wide range of cellular, molecular and genetic alterations have been identified over different brain regions and across different age-groups in post-mortem ASD samples, the cerebellum and frontal cortex are the two regions most consistently implicated in the disorder. Abnormalities demonstrated include but are not limited to altered cytoarchitecture, glial and mitochondrial function, gene expression, epigenetic patterns and non-coding RNA expression. The involvement of these two regions in ASD pathology is also supported by findings from structural imaging studies (Dougherty et al., 2016).

While some alterations are shared between the two regions, others have shown different or even opposite patterns. For example, both regions showed similar alterations in specific genes, such as RELN (Fatemi et al., 2005) and RORA (Nguyen et al., 2010) – and the levels of GABAB receptor subunit, GABBR1 were decreased in both regions, but the levels of GABBR2 were reduced only in the cerebellum (Fatemi et al., 2009a). Similarly, increased expression of several microglial and astrocytic markers was evident in prefrontal cortex, but only GFAP was elevated in the cerebellum (Edmonson et al., 2014). Higher levels of cathepsin D protein were also found in both areas, but the cerebellum showed an additional increase in caspase-3 expression (Sheikh et al., 2010a). On the other hand, some genes were only altered in one region and not the other. For example, SLC25A12 (Lepagnol-Bestel et al., 2008) and BDNF (Maussion et al., 2019) were upregulated in the frontal cortex, with no apparent difference in their expression levels in the cerebellum. Furthermore, similar patterns in epigenetic mechanisms such as a global bias for RNA-hypoediting (Tran et al., 2019) and higher overall DNA methylation (Zhu et al., 2014) were shared across both regions. In addition, although the number of unique lncRNAs differentially expressed were different in both regions, Ziats et al. (2013) have demonstrated more transcriptional homogeneity upon comparing intra-individual differences in expression of both lncRNAs and genes between these two brain regions, supporting the proposition that in ASD, anatomically distinct regions are genomically less differentiated, and therefore less specialised, from each other compared to neurotypical subjects (Minshew and Keller, 2010).

We also repeatedly found studies showing different patterns in the cortex to that of the cerebellum, particularly studies on NATs, DNA methylation and acetylation profiles, as well as gene expression patterns. Investigating the noncoding transcriptome, alternative splicing as well as upstream molecular regulators revealed a consistently stronger differential gene expression in the cortex compared to the cerebellum, implying that the cortex is particularly more vulnerable to transcriptomic alterations (Parikshak et al., 2016). Similarly, genome-wide miRNA expression profiling has shown very similar miRNA expression profiles between the frontal and temporal cortex, with distinct profiles observed in the cerebellum (Wu et al., 2016) (Voineagu et al., 2011,

Oldham et al., 2008). Certain ASD-NATs, such as FOXG1-AS, have also shown region-dependent patterns of expression according to their biological functions, reflecting possible different roles in the development and function of the distinct regions (Velmeshev et al., 2013). More pronounced and distinct genome-wide DNA methylation patterns have been identified in the prefrontal and temporal corticies compared to the cerebellum (Wong et al., 2019). The same was true for histone acetylation changes (Sun et al., 2016). Moreover, Proteomic profiling of the prefrontal cortex and cerebellum has revealed decreased levels of proteins associated with myelination and increased synaptic and energy-related proteins only in the prefrontal cortex with opposite directional changes for myelination and synaptic proteins in the cerebellum, pointing towards the presence of differentially dysregulated molecular processes in different brain regions in ASD (Broek et al., 2014).

4.4. Overlap between ASD and related disorders

ASD and related disorders display overlap in clinical presentation, as well as in the extent of molecular, cellular and genetic perturbations identified, although fewer post-mortem studies have been performed on other disorders compared to autism and ASD. Such similarities are sometimes evident in the same brain region across the different disorders and at other times the same alteration spans different regions in different disorders. Table 4 summarizes the common findings. A common loss of cerebellar Purkinje cells is apparent in ASD, RTT and FXS. Similarly, dendritic spine abnormalities have been reported in the prefrontal cortex of ASD, RTT and WS subjects and in the cingulate and temporal association areas in FXS. Alterations in neuronal size and/or density were also common in ASD and WS prefrontal cortex, and extended to the primary auditory and visual corticies in WS. Indeed, it appears that the prefrontal cortex is preferentially targeted or vulnerable in the neurodevelopmental disorders manifesting with ASD studied here. This obseravtion is supported by sociobehavioral, cognitive, structural and functional neuroimaging studies (Smith et al., 2019b, Brumback et al., 2018, Clements et al., 2018). Alterations to the serotonergic system were also identified in ASD and RTT, albeit in different brain regions. Interestingly, opposing patterns were sometimes observed in ASD and other relevant disorders. For example, a decrease in lateral nucleus neuron number in ASD contrasts

with an increase in WS. Differential gene expression of certain genes was also consistent in both ASD and other disorders. MeCP2 expression, for example, was reduced in both ASD and RTT prefrontal cortex. The differences in expression patterns between the different regions in both disorders where some regions display differential expression of MeCP2, but no differences in other regions, suggests a region-specific dysfunction of genes & transcriptional factors in ASD and related disorders.

4.5. Limitations to post-mortem studies and result variability

In general, the difficulty obtaining human autopsy material, let alone in samples with rare and specific genetic mutations, calls for caution when interpreting and analysing data from the limited number of small studies available. There are also suggestions of neuropathological heterogeneity within ASD and ASD-related syndromes – for example, where measurements of several ASD subjects fell within the normal range of controls and only a fraction of ASD subjects have distinctly abberant profiles. This has been reported in gene expression and methylation studies, as well as other studies on miRNAs, microglia and 5-HTT. Further, most studies independently investigate different brain regions in convenience samples making it harder to generalise the findings or to examine their reproducibility or consistency. It is therefore usually unclear whether any of these reported differences point to regional disruptions and desynchronization of brain development in ASD, or reflect the varying symptoms, cognitive deficits and co-morbidities in the disorder.

The acquisition and utilisation of human brain tissue poses several limitations. Apart from the experimental challenges which include lack of control over PMI and the agonal state (events such as hypoxia or trauma) that could alter the quality of RNA isolated from post-mortem tissues, many of the samples are donated from subjects with co-morbidities, for which different medications are sometimes administered; making it hard to conclude whether ASD, seizures, drug therapy or a combination of these factors is responsible for some of the observations. It is also difficult to match subjects for gender, age and developmental stage. The paucity of samples from younger patients or across a broad age-range makes it harder to investigate the early developmental processes likely to be critical in understanding a neurodevelopmental disorder like ASD. Additionally,

examining tissues long after the onset of the disorder makes it more likely that the alterations observed could be secondary consequences of the disorder. Anxiety and depression, for example, are over-represented in adults with ASD and often treated with a range of psychotropic medication. One should not overlook the effects of drugs such as SSRIs and antipsychotics which can certainly alter gene expression. Some studies utilise brain tissue from multiple brain banks with different brain-banking methodologies, where the clinical records are often only available for a minority of cases, further adding to experimental variability and the difficulty in correlating neuropathological findings with behaviour and symptom severity.

In gene expression studies, the utilisation of array platforms as well as different experimental designs, statistical approaches and control strategies makes it almost impossible to precisely compare different studies, even if the results seem similar. Limited tissue availability also sometimes dictates sampling different regions of the same brain area in different subjects, for example BA8 or BA10 of the prefrontal cortex which could confound the differential expression analysis by the tissue specificity of gene expression in the different regions. Similarly, epigenetic studies utilising arrays, such as the Illumina 450K array, albeit being a highly reliable and robust platform, come with limitations,. Across the whole genome, DNA methylation is examined in only a relatively small proportion of sites, although its content spans regulatory regions associated with the majority of known annotated genes (Wong et al., 2019a). Elucidating disease-relevant DNA methylation changes that are specific to individual brain cell types also remains a challenge, given that bulk tissue from each brain region is usually used, which is a potential confounder in DNA methylation studies (Mill and Heijmans, 2013).

In conclusion, nevertheless, post-mortem studies have allowed numerous researchers to directly investigate ASD brain tissue, thus allowing the characterization of autism and related neurodevelopmental disorders at the level of neuronal populations and the specific neural circuits that they form. Despite the limitations of a small literature, there are several consistent findings of ASD-related alterations at the global, regional, cellular, synaptic and molecular levels. Post-mortem studies remain a critical part of the neuroscientific armamentarium but would benefit from an international collaborative approach to gathering and processing samples. Complementary in-

vitro studies utilising animal, cellular and organoid models and deep phenotyping of clinical samples diseases will also be required to fill the remaining knowledge gaps.

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6. References:

ABU-ELNEEL, K., LIU, T., GAZZANIGA, F. S., NISHIMURA, Y., WALL, D. P., GESCHWIND, D. H., LAO, K. Q. & KOSIK, K. S. 2008. Heterogeneous dysregulation of microRNAs across the autism spectrum. *Neurogenetics,* 9**,** 153-161.

AJRAM, L., HORDER, J., MENDEZ, M., GALANOPOULOS, A., BRENNAN, L., WICHERS, R., ROBERTSON, D., MURPHY, C., ZINKSTOK, J. & IVIN, G. 2017. Shifting brain inhibitory balance and connectivity of the prefrontal cortex of adults with autism spectrum disorder. *Translational Psychiatry,* 7**,** e1137-e1137.

ALMEHMADI, K. A., TSILIONI, I. & THEOHARIDES, T. C. 2019. Increased Expression of miR-155p5 in Amygdala of Children With Autism Spectrum Disorder. *Autism Research.*

ALVAREZ-BUYLLA, A. & LIM, D. A. 2004. For the long run: maintaining germinal niches in the adult brain. *Neuron,* 41**,** 683-686.

AMARAL, D. G., ANDERSON, M. P., ANSORGE, O., CHANCE, S., HARE, C., HOF, P. R., MILLER, M., NAGAKURA, I., PICKETT, J., SCHUMANN, C. & TAMMINGA, C. 2018. Autism BrainNet A network of postmortem brain banks established to facilitate autism research. *In:* HUITINGA, I. & WEBSTER, M. J. (eds.) *Brain Banking.* Amsterdam: Elsevier Science Bv.

AMRHEIN, V., GREENLAND, S. & MCSHANE, B. 2019. Scientists rise up against statistical significance. Nature Publishing Group.

ANDER, B. P., BARGER, N., STAMOVA, B., SHARP, F. R. & SCHUMANN, C. M. 2015. Atypical miRNA expression in temporal cortex associated with dysregulation of immune, cell cycle, and other pathways in autism spectrum disorders. *Molecular Autism,* 6 (1) (no pagination).

ANITHA, A., NAKAMURA, K., THANSEEM, I., MATSUZAKI, H., MIYACHI, T., TSUJII, M., IWATA, Y., SUZUKI, K., SUGIYAMA, T. & MORI, N. 2013. Downregulation of the expression of mitochondrial electron transport complex genes in autism brains. *Brain Pathology,* 23**,** 294-302.

ANITHA, A., NAKAMURA, K., THANSEEM, I., YAMADA, K., IWAYAMA, Y., TOYOTA, T., MATSUZAKI, H., MIYACHI, T., YAMADA, S., TSUJII, M., TSUCHIYA, K. J., MATSUMOTO, K., IWATA, Y., SUZUKI, K., ICHIKAWA, H., SUGIYAMA, T., YOSHIKAWA, T. & MORI, N. 2012. Brain region-specific altered expression and association of mitochondria-related genes in autism. *Molecular Autism,* 3 (1) (no pagination).

ANITHA, A., THANSEEM, I., NAKAMURA, K., VASU, M. M., YAMADA, K., UEKI, T., IWAYAMA, Y., TOYOTA, T., TSUCHIYA, K. J., IWATA, Y., SUZUKI, K., SUGIYAMA, T., TSUJII, M., YOSHIKAWA, T. & MORI, N. 2014. Zinc finger protein 804A (ZNF804A) and verbal deficits in individuals with autism. *Journal of Psychiatry & Neuroscience,* 39**,** 294-303.

ANSEL, A., ROSENZWEIG, J. P., ZISMAN, P. D., MELAMED, M. & GESUNDHEIT, B. 2017. Variation in gene expression in autism spectrum disorders: an extensive review of transcriptomic studies. *Frontiers in neuroscience,* 10**,** 601.

ARAGHI-NIKNAM, M. & FATEMI, S. H. 2003. Levels of Bcl-2 and P53 Are Altered in Superior Frontal and Cerebellar Cortices of Autistic Subjects. *Cellular and Molecular Neurobiology,* 23**,** 945-952.

ARIZA, J., STEWARD, C., RUECKERT, F., WIDDISON, M., COFFMAN, R., AFJEI, A., NOCTOR, S. C., HAGERMAN, R., HAGERMAN, P. & MARTINEZ-CERDENO, V. 2015. Dysregulated iron metabolism in the choroid plexus in fragile X-associated tremor/ataxia syndrome. *Brain Research,* 1598**,** 88-96.

ARMSTRONG, D., DUNN, J. K., ANTALFFY, B. & TRIVEDI, R. 1995. Selective dendritic alterations in the cortex of Rett syndrome. *Journal of Neuropathology and Experimental Neurology,* 54**,** 195-201.

ARMSTRONG, D. D., DEGUCHI, K. & ANTALLFY, B. 2003. Survey of MeCP2 in the Rett Syndrome and the Non-Rett Syndrome Brain. *Journal of Child Neurology,* 18**,** 683-687.

ASSOCIATION, A. P. & ASSOCIATION, A. P. 2013. Diagnostic and statistical manual of mental disorders: DSM-5. Washington, DC: American psychiatric association.

AZMITIA, E., SACCOMANO, Z., ALZOOBAEE, M., BOLDRINI, M. & WHITAKER-AZMITIA, P. 2016. Persistent angiogenesis in the autism brain: An immunocytochemical study of postmortem cortex, brainstem and cerebellum. *Journal of Autism and Developmental Disorders,* 46**,** 1307-1318.

AZMITIA, E. C., SINGH, J. S., HOU, X. P. & WEGIEL, J. 2011a. Dystrophic serotonin axons in postmortem brains from young autism patients. *Anatomical Record,* 294**,** 1653-1662.

AZMITIA, E. C., SINGH, J. S. & WHITAKER-AZMITIA, P. M. 2011b. Increased serotonin axons (immunoreactive to 5-HT transporter) in postmortem brains from young autism donors. *Neuropharmacology,* 60**,** 1347-1354.

BAILEY, A., LUTHERT, P., DEAN, A., HARDING, B., JANOTA, I., MONTGOMERY, M., RUTTER, M. & LANTOS, P. 1998. A clinicopathological study of autism. *Brain: a journal of neurology,* 121**,** 889-905.

BALAN, S., IWAYAMA, Y., MAEKAWA, M., TOYOTA, T., OHNISHI, T., TOYOSHIMA, M., SHIMAMOTO, C., ESAKI, K., YAMADA, K., IWATA, Y., SUZUKI, K., IDE, M., OTA, M., FUKUCHI, S., TSUJII, M., MORI, N., SHINKAI, Y. & YOSHIKAWA, T. 2014. Exon resequencing of H3K9 methyltransferase complex genes, EHMT1, EHTM2 and WIZ, in Japanese autism subjects. *Molecular Autism,* 5 (1) (no pagination).

BAUMAN, M. & KEMPER, T. L. 1985. Histoanatomic observations of the brain in early infantile autism. *Neurology,* 35**,** 866-874.

BAUMAN, M. L. & KEMPER, T. L. 2005. Neuroanatomic observations of the brain in autism: A review and future directions. *International Journal of Developmental Neuroscience,* 23**,** 183-187.

BAUMAN, M. L., KEMPER, T. L. & ARIN, D. M. 1995. Pervasive neuroanatomic abnormalities of the brain in three cases of Rett's syndrome. *Neurology,* 45**,** 1581-1586.

BEAR, M. F., HUBER, K. M. & WARREN, S. T. 2004. The mGluR theory of fragile X mental retardation. *Trends in neurosciences,* 27**,** 370-377.

BELICHENKO, P. V., OLDFORS, A., HAGBERG, B. & DAHLSTROM, A. 1994. Rett syndrome: 3-D confocal microscopy of cortical pyramidal dendrites and afferents.

Neuroreport: An International Journal for the Rapid Communication of Research in Neuroscience, 5**,** 1509-1513.

BELL, C. C. 1994. DSM-IV: diagnostic and statistical manual of mental disorders. *Jama,* 272**,** 828-829.

BENNETT, C. M., WOLFORD, G. L. & MILLER, M. B. 2009. The principled control of false positives in neuroimaging. *Social cognitive and affective neuroscience,* 4**,** 417-422.

BLUE, M. E., NAIDU, S. & JOHNSTON, M. V. 1999. Altered development of glutamate and GABA receptors in the basal ganglia of girls with Rett syndrome. *Experimental Neurology,* 156**,** 345-352.

BOZZI, Y., PROVENZANO, G. & CASAROSA, S. 2018. Neurobiological bases of autism–epilepsy comorbidity: a focus on excitation/inhibition imbalance. *European Journal of Neuroscience,* 47**,** 534-548.

BRANDENBURG, C. & BLATT, G. J. 2019. Differential serotonin transporter (5-HTT) and 5-HT<inf>2</inf> receptor density in limbic and neocortical areas of adults and children with ASD: implications for SSRI efficacy. *Journal of neurochemistry.,* 20.

BROEK, J. A. C., GUEST, P. C., RAHMOUNE, H. & BAHN, S. 2014. Proteomic analysis of post mortem brain tissue from autism patients: Evidence for opposite changes in prefrontal cortex and cerebellum in synaptic connectivity-related proteins. *Molecular Autism,* 5 (1) (no pagination).

BRUMBACK, A. C., ELLWOOD, I., KJAERBY, C., IAFRATI, J., ROBINSON, S., LEE, A., PATEL, T., NAGARAJ, S., DAVATOLHAGH, F. & SOHAL, V. S. 2018. Identifying specific prefrontal neurons that contribute to autism-associated abnormalities in physiology and social behavior. *Molecular psychiatry,* 23**,** 2078-2089.

CALDEIRA, G. L., PECA, J. & CARVALHO, A. L. 2019. New insights on synaptic dysfunction in neuropsychiatric disorders. *Current Opinion in Neurobiology,* 57**,** 62-70.

CAMACHO, J., EJAZ, E., ARIZA, J., NOCTOR, S. C. & MARTINEZ-CERDENO, V. 2014. RELN-expressing neuron density in layer I of the superior temporal lobe is similar in human brains with autism and in age-matched controls. *Neuroscience Letters,* 579**,** 163-167.

CAMPBELL, D. B., D'ORONZIO, R., GARBETT, K., EBERT, P. J., MIRNICS, K., LEVITT, P. & PERSICO, A. M. 2007. Disruption of cerebral cortex MET signaling in autism spectrum disorder. *Annals of Neurology,* 62**,** 243-250.

CANITANO, R. 2007. Epilepsy in autism spectrum disorders. *European child & adolescent psychiatry,* 16**,** 61-66.

CASANOVA, M. F. 2015. The neuropathology of autism. *The molecular basis of autism.* New York, NY: Springer Science + Business Media; US.

CASANOVA, M. F., BUXHOEVEDEN, D. P., SWITALA, A. E. & ROY, E. 2002a. Minicolumnar pathology in autism. *Neurology,* 58**,** 428-32.

CASANOVA, M. F., BUXHOEVEDEN, D. P., SWITALA, A. E. & ROY, E. 2002b. Neuronal density and architecture (Gray Level Index) in the brains of autistic patients. *Journal of Child Neurology,* 17**,** 515-521.

CASANOVA, M. F., VAN KOOTEN, I., SWITALA, A. E., VAN ENGELAND, H., HEINSEN, H., STEINBUSCH, H. W. M., HOF, P. R. & SCHMITZ, C. 2006a. Abnormalities of cortical

minicolumnar organization in the prefrontal lobes of autistic patients. *Clinical Neuroscience Research,* 6**,** 127-133.

CASANOVA, M. F., VAN KOOTEN, I. A. J., SWITALA, A. E., VAN ENGELAND, H., HEINSEN, H., STEINBUSCH, H. W. M., HOF, P. R., TRIPPE, J., STONE, J. & SCHMITZ, C. 2006b. Minicolumnar abnormalities in autism. *Acta Neuropathologica,* 112**,** 287-303.

CHAN-PALAY, V., PALAY, S. L. & WU, J.-Y. 1979. Gamma-aminobutyric acid pathways in the cerebellum studied by retrograde and anterograde transport of glutamic acid decarboxylase antibody after in vivo injections. *Anatomy and embryology,* 157**,** 1-14.

CHANA, G., LASKARIS, L., PANTELIS, C., GILLETT, P., TESTA, R., ZANTOMIO, D., BURROWS, E. L., HANNAN, A. J., EVERALL, I. P. & SKAFIDAS, E. 2015. Decreased expression of mGluR5 within the dorsolateral prefrontal cortex in autism and increased microglial number in mGluR5 knockout mice: Pathophysiological and neurobehavioral implications. *Brain, Behavior, and Immunity,* 49**,** 197-205.

CHANDLEY, M. J., CRAWFORD, J. D., SZEBENI, A., SZEBENI, K. & ORDWAY, G. A. 2015. NTRK2 expression levels are reduced in laser captured pyramidal neurons from the anterior cingulate cortex in males with autism spectrum disorder. *Molecular Autism,* 6 (1) (no pagination).

CHANG, K. W., LIN, C. Y., WU, J. Y., COON, H., HUANG, P. H., HO, H. N., AKBARIAN, S., GAU, S. S. F. & HUANG, H. S. 2018. Allele-specific expression in a family quartet with autism reveals mono-to-biallelic switch and novel transcriptional processes of autism susceptibility genes. *Scientific reports,* 8**,** 4277.

CHAPLEAU, C. A., CALFA, G. D., LANE, M. C., ALBERTSON, A. J., LARIMORE, J. L., KUDO, S., ARMSTRONG, D. L., PERCY, A. K. & POZZO-MILLER, L. 2009. Dendritic spine pathologies in hippocampal pyramidal neurons from Rett syndrome brain and after expression of Rett-associated MECP2 mutations. *Neurobiology of Disease,* 35**,** 219-233.

CHEN, J., ALBERTS, I. & LI, X. 2014. Dysregulation of the IGF-I/PI3K/AKT/mTOR signaling pathway in autism spectrum disorders. *International Journal of Developmental Neuroscience,* 35**,** 35-41.

CHENG, Y., LI, Z. Y., MANUPIPATPONG, S., LIN, L., LI, X. K., XU, T. L., JIANG, Y. H., SHU, Q., WU, H. & JIN, P. 2018. 5-Hydroxymethylcytosine alterations in the human postmortem brains of autism spectrum disorder. *Human Molecular Genetics,* 27**,** 2955-2964.

CHOI, J., ABABON, M. R., SOLIMAN, M., LIN, Y., BRZUSTOWICZ, L. M., MATTESON, P. G. & MILLONIG, J. H. 2014. Autism Associated Gene, ENGRAILED2, and Flanking Gene Levels Are Altered in Post-Mortem Cerebellum. *Plos One,* 9**,** 9.

CHOW, M. L., PRAMPARO, T., WINN, M. E., BARNES, C. C., LI, H. R., WEISS, L., FAN, J. B., MURRAY, S., APRIL, C., BELINSON, H., FU, X. D., WYNSHAW-BORIS, A., SCHORK, N. J. & COURCHESNE, E. 2012. Age-dependent brain gene expression and copy number anomalies in autism suggest distinct pathological processes at young versus mature ages. *PLoS Genetics,* 8**,** e1002592.

CLEMENTS, C. C., ZOLTOWSKI, A. R., YANKOWITZ, L. D., YERYS, B. E., SCHULTZ, R. T. & HERRINGTON, J. D. 2018. Evaluation of the social motivation hypothesis of autism: a systematic review and meta-analysis. *JAMA psychiatry,* 75**,** 797-808.

COLANTUONI, C., JEON, O. H., HYDER, K., CHENCHIK, A., KHIMANI, A. H., NARAYANAN, V., HOFFMAN, E. P., KAUFMANN, W. E., NAIDU, S. & PEVSNER, J. 2001. Gene expression profiling in postmortem Rett Syndrome brain: Differential gene expression and patient classification. *Neurobiology of Disease,* 8**,** 847-865.

COLEMAN, P. D., ROMANO, J., LAPHAM, L. & SIMON, W. 1985. Cell counts in cerebral cortex of an autistic patient. *Journal of Autism and Developmental Disorders,* 15**,** 245-255.

COOPER, J. A. & HOWELL, B. W. 1999. Lipoprotein receptors: signaling functions in the brain? *Cell,* 97**,** 671-674.

COURCHESNE, E., MOUTON, P. R., CALHOUN, M. E., SEMENDEFERI, K., AHRENS-BARBEAU, C., HALLET, M. J., BARNES, C. C. & PIERCE, K. 2011. Neuron number and size in prefrontal cortex of children with autism. *JAMA: Journal of the American Medical Association,* 306**,** 2001-2010.

COURCHESNE, E., MULLER, R. A. & SAITOH, O. 1999. Brain weight in autism: Normal in the majority of cases, megalencephalic in rare cases. *Neurology,* 52**,** 1057-1059.

COURCHESNE, E., PRAMPARO, T., GAZESTANI, V. H., LOMBARDO, M. V., PIERCE, K. & LEWIS, N. E. 2019. The ASD Living Biology: From cell proliferation to clinical phenotype. *Molecular Psychiatry,* 24**,** 88-107.

CRAWFORD, J. D., CHANDLEY, M. J., SZEBENI, K., SZEBENI, A., WATERS, B. & ORDWAY, G. A. 2015. Elevated GFAP protein in anterior cingulate cortical white matter in males with autism spectrum disorder. *Autism Research,* 8**,** 649-657.

CRIDER, A., AHMED, A. O. & PILLAI, A. 2017. Altered Expression of Endoplasmic Reticulum Stress-Related Genes in the Middle Frontal Cortex of Subjects with Autism Spectrum Disorder. *Molecular Neuropsychiatry,* 3**,** 85-91.

CRIDER, A., PANDYA, C. D., PETER, D., AHMED, A. O. & PILLAI, A. 2014a. Ubiquitin-proteasome dependent degradation of GABA<inf>A</inf>alpha1 in autism spectrum disorder. *Molecular Autism,* 5 (1) (no pagination).

CRIDER, A., THAKKAR, R., AHMED, A. O. & PILLAI, A. 2014b. Dysregulation of estrogen receptor beta (ERbeta), aromatase (CYP19A1), and ER co-activators in the middle frontal gyrus of autism spectrum disorder subjects. *Molecular Autism,* 5 (1) (no pagination).

CULOTTA, L. & PENZES, P. 2020. Exploring the mechanisms underlying excitation/inhibition imbalance in human iPSC-derived models of ASD. *Molecular Autism,* 11**,** 1-11.

D'GAMA, A. M., POCHAREDDY, S., LI, M., JAMUAR, S. S., REIFF, R. E., LAM, A. T. N., SESTAN, N. & WALSH, C. A. 2015. Targeted DNA Sequencing from Autism Spectrum Disorder Brains Implicates Multiple Genetic Mechanisms. *Neuron,* 88**,** 910-917.

DE FELICE, A., SCATTONI, M. L., RICCERI, L. & CALAMANDREI, G. 2015. Prenatal exposure to a common organophosphate insecticide delays motor development in a mouse model of idiopathic autism. *PLoS one,* 10.

DE RUBEIS, S., HE, X., GOLDBERG, A. P., POULTNEY, C. S., SAMOCHA, K., CICEK, A. E., KOU, Y., LIU, L., FROMER, M. & WALKER, S. 2014. Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature,* 515**,** 209-215.

DISTASIO, M. M., NAGAKURA, I., NADLER, M. J. & ANDERSON, M. P. 2019. T-lymphocytes and Cytotoxic Astrocyte Blebs Correlate Across Autism Brains. *Annals of neurology.,* 08.

DOLMAZON, V., ALENINA, N., MARKOSSIAN, S., MANCIP, J., VAN DE VREDE, Y., FONTAINE, E., DEHAY, C., KENNEDY, H., BADER, M. & SAVATIER, P. 2011. Forced expression of LIM homeodomain transcription factor 1b enhances differentiation of mouse embryonic stem cells into serotonergic neurons. *Stem cells and development,* 20**,** 301-311.

DOUGHERTY, C. C., EVANS, D. W., MYERS, S. M., MOORE, G. J. & MICHAEL, A. M. 2016. A comparison of structural brain imaging findings in autism spectrum disorder and attention-deficit hyperactivity disorder. *Neuropsychology review,* 26**,** 25-43.

EDMONSON, C., ZIATS, M. N. & RENNERT, O. M. 2014. Altered glial marker expression in autistic post-mortem prefrontal cortex and cerebellum. *Molecular Autism,* 5 (1) (no pagination).

FATEMI, S., FOLSOM, T. D., REUTIMAN, T. J. & LEE, S. 2008. Expression of astrocytic markers aquaporin 4 and connexin 43 is altered in brains of subjects with autism. *Synapse,* 62**,** 501-507.

FATEMI, S., HALT, A. R., STARY, J. M., KANODIA, R., SCHULZ, S. & REALMUTO, G. R. 2002a. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. *Biological Psychiatry,* 52**,** 805-810.

FATEMI, S., SNOW, A. V., STARY, J. M., ARAGHI-NIKNAM, M., REUTIMAN, T. J., LEE, S., BROOKS, A. I. & PEARCE, D. A. 2005. Reelin Signaling is Impaired in Autism. *Biological Psychiatry,* 57**,** 777-787.

FATEMI, S., STARY, J. M., HALT, A. R. & REALMUTO, G. R. 2001. Dysregulation of Reelin and Bcl-2 proteins in autistic cerebellum. *Journal of Autism and Developmental Disorders,* 31**,** 529-535.

FATEMI, S. H. & FOLSOM, T. D. 2011. Dysregulation of fragile X mental retardation protein and metabotropic glutamate receptor 5 in superior frontal cortex of individuals with autism: A postmortem brain study. *Molecular Autism,* 2 (1) (no pagination).

FATEMI, S. H. & FOLSOM, T. D. 2015. GABA receptor subunit distribution and FMRP-mGluR5 signaling abnormalities in the cerebellum of subjects with schizophrenia, mood disorders, and autism. *Schizophrenia Research,* 167**,** 42-56.

FATEMI, S. H., FOLSOM, T. D., KNEELAND, R. E. & LIESCH, S. B. 2011. Metabotropic glutamate Receptor 5 upregulation in children with autism is associated with underexpression of both fragile X mental retardation protein and GABA<inf>A</inf> receptor beta 3 in adults with autism. *Anatomical Record,* 294**,** 1635-1645.

FATEMI, S. H., FOLSOM, T. D., KNEELAND, R. E., YOUSEFI, M. K., LIESCH, S. B. & THURAS, P. D. 2013. Impairment of fragile X mental retardation protein-metabotropic glutamate receptor 5 signaling and its downstream cognates ras-related C3 botulinum toxin substrate 1, amyloid beta A4 precursor protein, striatal-enriched protein tyrosine phosphatase, and homer 1, in autism: A postmortem study in cerebellar vermis and superior frontal cortex. *Molecular Autism,* 4 (1) (no pagination).

FATEMI, S. H., FOLSOM, T. D., REUTIMAN, T. J. & THURAS, P. D. 2009a. Expression of GABA<inf>B</inf> Receptors Is Altered in Brains of Subjects with Autism. *Cerebellum,* 8**,** 64-69.

FATEMI, S. H. & HALT, A. R. 2001. Altered levels of Bcl2 and p53 proteins in parietal cortex reflect deranged apoptotic regulation in autism. *Synapse,* 42**,** 281-284.

FATEMI, S. H., HALT, A. R., REALMUTO, G., EARLE, J., KIST, D. A., THURAS, P. & METZ, A. 2002b. Purkinje cell size is reduced in cerebellum of patients with autism. *Cellular and Molecular Neurobiology,* 22**,** 171-175.

FATEMI, S. H., REUTIMAN, T. J., FOLSOM, T. D. & THURAS, P. D. 2009b. GABA(A) receptor downregulation in brains of subjects with autism. *Journal of Autism & Developmental Disorders,* 39**,** 223-30.

FAVOROV, O. V. & KELLY, D. G. 1994a. Minicolumnar organization within somatosensory cortical segregates: I. Development of afferent connections. *Cerebral Cortex,* 4**,** 408-427.

FAVOROV, O. V. & KELLY, D. G. 1994b. Minicolumnar organization within somatosensory cortical segregates: II. Emergent functional properties. *Cerebral Cortex,* 4**,** 428-442.

FERNANDEZ, B. A., ROBERTS, W., CHUNG, B., WEKSBERG, R., MEYN, S., SZATMARI, P., JOSEPH-GEORGE, A. M., MACKAY, S., WHITTEN, K. & NOBLE, B. 2010. Phenotypic spectrum associated with de novo and inherited deletions and duplications at 16p11. 2 in individuals ascertained for diagnosis of autism spectrum disorder. *Journal of medical genetics,* 47**,** 195-203.

FIORENTINO, M., SAPONE, A., SENGER, S., CAMHI, S. S., KADZIELSKI, S. M., BUIE, T. M., KELLY, D. L., CASCELLA, N. & FASANO, A. 2016. Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. *Molecular Autism,* 7 (1) (no pagination).

FOMBONNE, E. 2009. Epidemiology of pervasive developmental disorders. *Pediatric research,* 65**,** 591-598.

FOSS-FEIG, J. H., ADKINSON, B. D., JI, J. L., YANG, G., SRIHARI, V. H., MCPARTLAND, J. C., KRYSTAL, J. H., MURRAY, J. D. & ANTICEVIC, A. 2017. Searching for cross-diagnostic convergence: neural mechanisms governing excitation and inhibition balance in schizophrenia and autism spectrum disorders. *Biological psychiatry,* 81**,** 848-861.

FREEMAN, S. M., PALUMBO, M. C., LAWRENCE, R. H., SMITH, A. L., GOODMAN, M. M. & BALES, K. L. 2018. Effect of age and autism spectrum disorder on oxytocin receptor density in the human basal forebrain and midbrain. *Translational Psychiatry,* 8 (1) (no pagination).

FREITAS, B. C., MEI, A., MENDES, A. P. D., BELTRÃO-BRAGA, P. C. & MARCHETTO, M. C. 2018. Modeling Inflammation in autism spectrum disorders using stem cells. *Frontiers in Pediatrics,* 6**,** 394.

FRICK, L. R., WILLIAMS, K. & PITTENGER, C. 2013. Microglial dysregulation in psychiatric disease. *Clinical and Developmental Immunology,* 2013 (no pagination).

GALABURDA, A. M. & BELLUGI, U. 2000. V. Multi-level analysis of cortical neuroanatomy in Williams syndrome. *Journal of Cognitive Neuroscience,* 12**,** 74-88.

GALABURDA, A. M., HOLINGER, D. P., BELLUGI, U. & SHERMAN, G. F. 2002. Williams syndrome: neuronal size and neuronal-packing density in primary visual cortex. *Archives of Neurology,* 59**,** 1461-1467.

GARBETT, K., EBERT, P. J., MITCHELL, A., LINTAS, C., MANZI, B., MIRNICS, K. & PERSICO, A. M. 2008. Immune transcriptome alterations in the temporal cortex of subjects with autism. *Neurobiology of Disease,* 30**,** 303-311.

GARCIA, K. L. P., YU, G., NICOLINI, C., MICHALSKI, B., GARZON, D. J., CHIU, V. S., TONGIORGI, E., SZATMARI, P. & FAHNESTOCK, M. 2012. Altered Balance of Proteolytic Isoforms of Pro-Brain-Derived Neurotrophic Factor in Autism. *Journal of Neuropathology and Experimental Neurology,* 71**,** 289-297.

GIBSON, J. H., SLOBEDMAN, B., KN, H., WILLIAMSON, S. L., MINCHENKO, D., EL-OSTA, A., STERN, J. L. & CHRISTODOULOU, J. 2010. Downstream targets of methyl CpG binding protein 2 and their abnormal expression in the frontal cortex of the human Rett syndrome brain. *BMC Neuroscience Vol 11 2010, ArtID 53,* 11.

GKOGKAS, C. G., KHOUTORSKY, A., CAO, R., JAFARNEJAD, S. M., PRAGER-KHOUTORSKY, M., GIANNAKAS, N., KAMINARI, A., FRAGKOULI, A., NADER, K., PRICE, T. J., KONICEK, B. W., GRAFF, J. R., TZINIA, A. K., LACAILLE, J. C. & SONENBERG, N. 2014. Pharmacogenetic Inhibition of eIF4E-Dependent Mmp9 mRNA Translation Reverses Fragile X Syndrome-like Phenotypes. *Cell Reports,* 9**,** 1742-1756.

GOGLIOTTI, R. G., FISHER, N. M., STANSLEY, B. J., JONES, C. K., LINDSLEY, C. W., CONN, P. J. & NISWENDER, C. M. 2018. Total RNA sequencing of rett syndrome autopsy samples identifies the M<inf>4</inf> muscarinic receptor as a novel therapeutic target. *Journal of Pharmacology and Experimental Therapeutics,* 365**,** 291-300.

GOGLIOTTI, R. G., SENTER, R. K., ROOK, J. M., GHOSHAL, A., ZAMORANO, R., MALOSH, C., STAUFFER, S. R., BRIDGES, T. M., BARTOLOME, J. M., DANIELS, J. S., JONES, C. K., LINDSLEY, C. W., CONN, P. J. & NISWENDER, C. M. 2016. mGlu5 positive allosteric modulation normalizes synaptic plasticity defects and motor phenotypes in a mouse model of Rett syndrome. *Human Molecular Genetics,* 25**,** 1990-2004.

GRAHAM, S. F., CHEVALLIER, O. P., KUMAR, P., TURKOGLU, O. & BAHADO-SINGH, R. O. 2016. High resolution metabolomic analysis of ASD human brain uncovers novel biomarkers of disease. *Metabolomics,* 12**,** 10.

GRECO, C. M., NAVARRO, C. S., HUNSAKER, M. R., MAEZAWA, I., SHULER, J. F., TASSONE, F., DELANY, M., AU, J. W., BERMAN, R. F., JIN, L. W., SCHUMANN, C., HAGERMAN, P. J. & HAGERMAN, R. J. 2011. Neuropathologic features in the hippocampus and cerebellum of three older men with fragile X syndrome. *Molecular Autism,* 2 (1) (no pagination).

GUAN, J., CAI, J. J., JI, G. & SHAM, P. C. 2019. Commonality in dysregulated expression of gene sets in cortical brains of individuals with autism, schizophrenia, and bipolar disorder. *Translational Psychiatry,* 9 (1) (no pagination).

GUAN, J., YANG, E., YANG, J., ZENG, Y., JI, G. & CAI, J. J. 2016. Exploiting aberrant mRNA expression in autism for gene discovery and diagnosis. *Human Genetics,* 135**,** 797-811.

GUPTA, S., ELLIS, S. E., ASHAR, F. N., MOES, A., BADER, J. S., ZHAN, J., WEST, A. B. & ARKING, D. E. 2014. Transcriptome analysis reveals dysregulation of innate

immune response genes and neuronal activity-dependent genes in autism. *Nature communications,* 5**,** 1-8.

HAGBERG, B., AICARDI, J., DIAS, K. & RAMOS, O. 1983. A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society,* 14**,** 471-479.

HAGBERG, B., GOUTIÈRES, F., HANEFELD, F., RETT, A. & WILSON, J. 1985. Rett syndrome: criteria for inclusion and exclusion. *Brain & development,* 7**,** 372-373.

HAGERMAN, R., AU, J. & HAGERMAN, P. 2011. FMR1 premutation and full mutation molecular mechanisms related to autism. *Journal of neurodevelopmental disorders,* 3**,** 211.

HANSON, K. L., LEW, C. H., HRVOJ-MIHIC, B., GROENIGER, K. M., HALGREN, E., BELLUGI, U. & SEMENDEFERI, K. 2018. Increased glia density in the caudate nucleus in williams syndrome: Implications for frontostriatal dysfunction in autism. *Developmental neurobiology,* 78**,** 531-545.

HASHEMI, E., ARIZA, J., ROGERS, H., NOCTOR, S. C. & MARTINEZ-CERDENO, V. 2017. The number of parvalbumin-expressing interneurons is decreased in the medial prefrontal cortex in autism. *Cerebral Cortex,* 27**,** 1931-1943.

HEGARTY, J. P., WEBER, D. J., CIRSTEA, C. M. & BEVERSDORF, D. Q. 2018. Cerebro-cerebellar functional connectivity is associated with cerebellar excitation–inhibition balance in autism spectrum disorder. *Journal of autism and developmental disorders,* 48**,** 3460-3473.

HIESS, R. K., ALTER, R., SOJOUDI, S., ARDEKANI, B., KUZNIECKY, R. & PARDOE, H. 2015. Corpus callosum area and brain volume in autism spectrum disorder: quantitative analysis of structural MRI from the ABIDE database. *Journal of autism and developmental disorders,* 45**,** 3107-3114.

HINTON, V., BROWN, W., WISNIEWSKI, K. & RUDELLI, R. 1991. Analysis of neocortex in three males with the fragile X syndrome. *American journal of medical genetics,* 41**,** 289-294.

HOGART, A., LEUNG, K. N., WANG, N. J., WU, D. J., DRISCOLL, J., VALLERO, R. O., SCHANEN, N. C. & LASALLE, J. M. 2009. Chromosome 15q11-13 duplication syndrome brain reveals epigenetic alterations in gene expression not predicted from copy number. *Journal of Medical Genetics,* 46**,** 86-93.

HOLINGER, D. P., BELLUGI, U., MILLS, D. L., KORENBERG, J. R., REISS, A. L., SHERMAN, G. F. & GALABURDA, A. M. 2005. Relative sparing of primary auditory cortex in Williams syndrome. *Brain research,* 1037**,** 35-42.

HOWELL, B. W., HAWKES, R., SORIANO, P. & COOPER, J. A. 1997. Neuronal position in the developing brain is regulated by mouse disabled-1. *Nature,* 389**,** 733-737.

HU, V. W., SARACHANA, T., SHERRARD, R. M. & KOCHER, K. M. 2015. Investigation of sex differences in the expression of RORA and its transcriptional targets in the brain as a potential contributor to the sex bias in autism. *Molecular Autism,* 6 (1) (no pagination).

HUBER, K. M., GALLAGHER, S. M., WARREN, S. T. & BEAR, M. F. 2002. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proceedings of the National Academy of Sciences,* 99**,** 7746-7750.

HUTSLER, J. J. & ZHANG, H. 2010. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Research,* 1309**,** 83-94.

IRWIN, S. A., PATEL, B., IDUPULAPATI, M., HARRIS, J. B., CRISOSTOMO, R. A., LARSEN, B. P., KOOY, F., WILLEMS, P. J., CRAS, P. & KOZLOWSKI, P. B. 2001. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. *American journal of medical genetics,* 98**,** 161-167.

IWATA, K., MATSUZAKI, H., TACHIBANA, T., OHNO, K., YOSHIMURA, S., TAKAMURA, H., YAMADA, K., MATSUZAKI, S., NAKAMURA, K., TSUCHIYA, K. J., MATSUMOTO, K., TSUJII, M., SUGIYAMA, T., KATAYAMA, T. & MORI, N. 2014. N-ethylmaleimide-sensitive factor interacts with the serotonin transporter and modulates its trafficking: Implications for pathophysiology in autism. *Molecular Autism,* 5 (1) (no pagination).

JAMES, S. J., SHPYLEVA, S., MELNYK, S., PAVLIV, O. & POGRIBNY, I. P. 2013. Complex epigenetic regulation of Engrailed-2 (EN-2) homeobox gene in the autism cerebellum. *Translational Psychiatry,* 3 (no pagination).

JELLINGER, K., ARMSTRONG, D., ZOGHBI, H. & PERCY, A. 1988. Neuropathology of Rett syndrome. *Acta neuropathologica,* 76**,** 142-158.

JELLINGER, K. & SEITELBERGER, F. 1986. Neuropathology of Rett syndrome. *American Journal of Medical Genetics,* 24**,** 259-288.

JUNAID, M. A., KOWAL, D., BARUA, M., PULLARKAT, P. S., SKLOWER BROOKS, S. & PULLARKAT, R. K. 2004. Proteomic studies identified a single nucleotide polymorphism in glyoxalase I as autism susceptibility factor. *American Journal of Medical Genetics. Part A,* 131**,** 11-7.

KASHIMA, R., ROY, S., ASCANO, M., MARTINEZ-CERDENO, V., ARIZA-TORRES, J., KIM, S., LOUIE, J., LU, Y., LEYTON, P., BLOCH, K. D., KORNBERG, T. B., HAGERMAN, P. J., HAGERMAN, R., LAGNA, G. & HATA, A. 2016. Augmented noncanonical BMP type II receptor signaling mediates the synaptic abnormality of fragile X syndrome. *Science Signaling,* 9 (431) (no pagination).

KAUFMANN, W. E., MACDONALD, S. M. & ALTAMURA, C. R. 2000. Dendritic cytoskeletal protein expression in mental retardation: An immunohistochemical study of the neocortex in Rett syndrome. *Cerebral Cortex,* 10**,** 992-1004.

KEMPER, T. L. & BAUMAN, M. L. 1993. The contribution of neuropathologic studies to the understanding of autism. *Neurologic clinics,* 11**,** 175-187.

KENNEDY, D. P., SEMENDEFERI, K. & COURCHESNE, E. 2007. No reduction of spindle neuron number in frontoinsular cortex in autism. *Brain and Cognition,* 64**,** 124-129.

KERIN, T., RAMANATHAN, A., RIVAS, K., GREPO, N., COETZEE, G. A. & CAMPBELL, D. B. 2012. A noncoding RNA antisense to moesin at 5p14.1 in autism. *Science Translational Medicine,* 4 (128) (no pagination).

KHAN, A., HARNEY, J. W., ZAVACKI, A. M. & SAJDEL-SULKOWSKA, E. M. 2014. Disrupted brain thyroid hormone homeostasis and altered thyroid hormone-dependent brain gene expression in autism spectrum disorders. *Journal of Physiology and Pharmacology,* 65**,** 257-272.

KIEHL, T., CHOW, E., MIKULIS, D., GEORGE, S. & BASSETT, A. 2009. Neuropathologic features in adults with 22q11. 2 deletion syndrome. *Cerebral Cortex,* 19**,** 153-164.

KOTAGIRI, P., CHANCE, S. A., SZELE, F. G. & ESIRI, M. M. 2014. Subventricular zone cytoarchitecture changes in Autism. *Developmental Neurobiology,* 74**,** 25-41.

KULESZA JR, R. J. 2007. Cytoarchitecture of the human superior olivary complex: medial and lateral superior olive. *Hearing research,* 225**,** 80-90.

KULESZA, R. J. & MANGUNAY, K. 2008. Morphological features of the medial superior olive in autism. *Brain Research,* 1200**,** 132-137.

KURKI, P., VANDERLAAN, M., DOLBEARE, F., GRAY, J. & TAN, E. 1986. Expression of proliferating cell nuclear antigen (PCNA)/cyclin during the cell cycle. *Experimental cell research,* 166**,** 209-219.

LADD-ACOSTA, C., HANSEN, K. D., BRIEM, E., FALLIN, M. D., KAUFMANN, W. E. & FEINBERG, A. P. 2014. Common DNA methylation alterations in multiple brain regions in autism. *Molecular psychiatry,* 19**,** 862-871.

LAI, M. C., LERCH, J. P., FLORIS, D. L., RUIGROK, A. N., POHL, A., LOMBARDO, M. V. & BARON-COHEN, S. 2017. Imaging sex/gender and autism in the brain: Etiological implications. *Journal of neuroscience research,* 95**,** 380-397.

LAURENCE, J. A. & FATEMI, S. H. 2005. Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. *Cerebellum,* 4**,** 206-210.

LAWRENCE, Y. A., KEMPER, T. L., BAUMAN, M. L. & BLATT, G. J. 2010. Parvalbumin-, calbindin-, and calretinin-immunoreactive hippocampal interneuron density in autism. *Acta Neurologica Scandinavica,* 121**,** 99-108.

LEE, A. S., AZMITIA, E. C. & WHITAKER-AZMITIA, P. M. 2017. Developmental microglial priming in postmortem autism spectrum disorder temporal cortex. *Brain, Behavior, and Immunity,* 62**,** 193-202.

LEE, C., KANG, E. Y., GANDAL, M. J., ESKIN, E. & GESCHWIND, D. H. 2019a. Profiling allele-specific gene expression in brains from individuals with autism spectrum disorder reveals preferential minor allele usage. *Nature Neuroscience,* 22**,** 1521-1532.

LEE, H. J., SONG, J. Y., KIM, J. W., JIN, S.-Y., HONG, M. S., PARK, J. K., CHUNG, J.-H., SHIBATA, H. & FUKUMAKI, Y. 2005. Association study of polymorphisms in synaptic vesicle-associated genes, SYN2 and CPLX2, with schizophrenia. *Behavioral and Brain Functions,* 1**,** 15.

LEE, M., MARTIN-RUIZ, C., GRAHAM, A., COURT, J., JAROS, E., PERRY, R., IVERSEN, P., BAUMAN, M. & PERRY, E. 2002. Nicotinic receptor abnormalities in the cerebellar cortex in autism. *Brain: A Journal of Neurology,* 125**,** 1483-1495.

LEE, S. C., QUINN, T. P., LAI, J., KONG, S. W., HERTZ-PICCIOTTO, I., GLATT, S. J., CROWLEY, T. M., VENKATESH, S. & NGUYEN, T. 2019b. Solving for X: Evidence for sex-specific autism biomarkers across multiple transcriptomic studies. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics,* 180**,** 377-389.

LEKMAN, A., WITT, E., GOTTFRIES, J., HAGBERG, B. A., PERCY, A. K. & SVENNERHOLM, L. 1989. Rett syndrome: Biogenic amines and metabolites in postmortem brain. *Pediatric Neurology,* 5**,** 357-362.

LEPAGNOL-BESTEL, A. M., MAUSSION, G., BODA, B., CARDONA, A., IWAYAMA, Y., DELEZOIDE, A. L., MOALIC, J. M., MULLER, D., DEAN, B., YOSHIKAWA, T., GORWOOD, P., BUXBAUM, J. D., RAMOZ, N. & SIMONNEAU, M. 2008. SLC25A12 expression is associated with neurite outgrowth and is upregulated in the prefrontal cortex of autistic subjects. *Molecular Psychiatry,* 13**,** 385-397.

LEW, C. H., BROWN, C., BELLUGI, U. & SEMENDEFERI, K. 2017. Neuron density is decreased in the prefrontal cortex in Williams syndrome. *Autism Research,* 10**,** 99-112.

LEW, C. H., GROENIGER, K. M., BELLUGI, U., STEFANACCI, L., SCHUMANN, C. M. & SEMENDEFERI, K. 2018. A postmortem stereological study of the amygdala in Williams syndrome. *Brain Structure and Function,* 223**,** 1897-1907.

LEWIS, D. A. 2002. The human brain revisited: opportunities and challenges in postmortem studies of psychiatric disorders. *Neuropsychopharmacology,* 26**,** 143-154.

LINTAS, C., SACCO, R., GARBETT, K., MIRNICS, K., MILITERNI, R., BRAVACCIO, C., CURATOLO, P., MANZI, B., SCHNEIDER, C., MELMED, R., ELIA, M., PASCUCCI, T., PUGLISI-ALLEGRA, S., REICHELT, K. L. & PERSICO, A. M. 2009. Involvement of the PRKCB1 gene in autistic disorder: Significant genetic association and reduced neocortical gene expression. *Molecular Psychiatry,* 14**,** 705-718.

LIPANI, J. D., BHATTACHARJEE, M. B., COREY, D. M. & LEE, D. A. 2000. Reduced nerve growth factor in Rett syndrome postmortem brain tissue. *Journal of Neuropathology and Experimental Neurology,* 59**,** 889-895.

LLINÁS, R., WALTON, K. & LANG, E. 2004. The synaptic organization of the brain. Oxford Univ Press, New York.

LOAT, C., CURRAN, S., LEWIS, C., DUVALL, J., GESCHWIND, D., BOLTON, P. & CRAIG, I. 2008. Methyl-CpG-binding protein 2 polymorphisms and vulnerability to autism. *Genes, Brain and Behavior,* 7**,** 754-760.

LOBO-MENENDEZ, F., SOSSEY-ALAOUI, K., BELL, J. M., COPELAND-YATES, S. A., PLANK, S. M., SANFORD, S. O., SKINNER, C., SIMENSEN, R. J., SCHROER, R. J. & MICHAELIS, R. C. 2003. Absence of MeCP2 mutations in patients from the South Carolina autism project. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics,* 117**,** 97-101.

LOHITH, T. G., OSTERWEIL, E. K., FUJITA, M., JENKO, K. J., BEAR, M. F. & INNIS, R. B. 2013. Is metabotropic glutamate receptor 5 upregulated in prefrontal cortex in fragile X syndrome? *Molecular Autism,* 4**,** 8.

LORD, C., PICKLES, A., MCLENNAN, J., RUTTER, M., BREGMAN, J., FOLSTEIN, S., FOMBONNE, E., LEBOYER, M. & MINSHEW, N. 1997. Diagnosing autism: analyses of data from the Autism Diagnostic Interview. *Journal of autism and developmental disorders,* 27**,** 501-517.

LORD, C., RUTTER, M. & DILAVORE, P. C. 1999. Autism Diagnostic Observation Schedule--Generic. *Dissertation Abstracts International Section A: Humanities and Social Sciences*.

LORD, C., RUTTER, M. & LE COUTEUR, A. 1994. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with

possible pervasive developmental disorders. *Journal of autism and developmental disorders,* 24**,** 659-685.

MAUSSION, G., MOALIC, J. M., SIMONNEAU, M., GORWOOD, P. & RAMOZ, N. 2019. Increased expression of BDNF mRNA in the frontal cortex of autistic patients. *Behavioural Brain Research,* 359**,** 903-909.

MCKENNA, B., KOOMAR, T., VERVIER, K., KREMSREITER, J. & MICHAELSON, J. J. 2018. Whole-genome sequencing in a family with twin boys with autism and intellectual disability suggests multimodal polygenic risk. *Molecular Case Studies,* 4**,** a003285.

MENASSA, D. A., SLOAN, C. & CHANCE, S. A. 2017. Primary olfactory cortex in autism and epilepsy: increased glial cells in autism. *Brain Pathology,* 27**,** 437-448.

MILL, J. & HEIJMANS, B. T. 2013. From promises to practical strategies in epigenetic epidemiology. *Nature Reviews Genetics,* 14**,** 585-594.

MINSHEW, N. J. & KELLER, T. A. 2010. The nature of brain dysfunction in autism: functional brain imaging studies. *Current opinion in neurology,* 23**,** 124.

MITCHELL, M. M., WOODS, R., CHI, L. H., SCHMIDT, R. J., PESSAH, I. N., KOSTYNIAK, P. J. & LASALLE, J. M. 2012. Levels of select PCB and PBDE congeners in human postmortem brain reveal possible environmental involvement in 15q11-q13 duplication autism spectrum disorder. *Environmental and Molecular Mutagenesis,* 53**,** 589-598.

MIYAKE, K., HIRASAWA, T., SOUTOME, M., ITOH, M., GOTO, Y. I., ENDOH, K., TAKAHASHI, K., KUDO, S., NAKAGAWA, T., YOKOI, S., TAIRA, T., INAZAWA, J. & KUBOTA, T. 2011. The protocadherins, PCDHB1 and PCDH7, are regulated by MeCP2 in neuronal cells and brain tissues: Implication for pathogenesis of Rett syndrome. *BMC Neuroscience,* 12 (no pagination).

MORGAN, J. T., BARGER, N., AMARAL, D. G. & SCHUMANN, C. M. 2014. Stereological study of amygdala glial populations in adolescents and adults with autism spectrum disorder. *PLoS ONE,* 9 (10) (no pagination).

MORGAN, J. T., CHANA, G., ABRAMSON, I., SEMENDEFERI, K., COURCHESNE, E. & EVERALL, I. P. 2012. Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism. *Brain Research,* 1456**,** 72-81.

MORGAN, J. T., CHANA, G., PARDO, C. A., ACHIM, C., SEMENDEFERI, K., BUCKWALTER, J., COURCHESNE, E. & EVERALL, I. P. 2010. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biological Psychiatry,* 68**,** 368-376.

MURATORE, C. R., HODGSON, N. W., TRIVEDI, M. S., ABDOLMALEKY, H. M., PERSICO, A. M., LINTAS, C., DE LA MONTE, S. & DETH, R. C. 2013. Age-Dependent Decrease and Alternative Splicing of Methionine Synthase mRNA in Human Cerebral Cortex and an Accelerated Decrease in Autism. *PLoS ONE,* 8 (2) (no pagination).

NAGARAJAN, R. P., HOGART, A. R., GWYE, Y., MARTIN, M. R. & LASALLE, J. M. 2006. Reduced MeCP2 expression is frequent in autism frontal cortex and correlates with aberrant MECP2 promoter methylation. *Epigenetics : official journal of the DNA Methylation Society,* 1**,** e1-11.

NAGARAJAN, R. P., PATZEL, K. A., MARTIN, M., YASUI, D. H., SWANBERG, S. E., HERTZ-PICCIOTTO, I., HANSEN, R. L., VAN DE WATER, J., PESSAH, I. N., JIANG, R.,

ROBINSON, W. P. & LASALLE, J. M. 2008. MECP2 promoter methylation and X chromosome inactivation in autism. *Autism research : Official Journal of the International Society for Autism Research,* 1**,** 169-78.

NAKAMURA, K., IWATA, Y., ANITHA, A., MIYACHI, T., TOYOTA, T., YAMADA, S., TSUJII, M., TSUCHIYA, K. J., IWAYAMA, Y., YAMADA, K., HATTORI, E., MATSUZAKI, H., MATSUMOTO, K., SUZUKI, K., SUDA, S., TAKEBAYASHI, K., TAKEI, N., ICHIKAWA, H., SUGIYAMA, T., YOSHIKAWA, T. & MORI, N. 2011. Replication study of Japanese cohorts supports the role of STX1A in autism susceptibility. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 35**,** 454-8.

NALAVADI, V. C., MUDDASHETTY, R. S., GROSS, C. & BASSELL, G. J. 2012. Dephosphorylation-induced ubiquitination and degradation of FMRP in dendrites: a role in immediate early mGluR-stimulated translation. *Journal of Neuroscience,* 32**,** 2582-2587.

NGUYEN, A., RAUCH, T. A., PFEIFER, G. P. & HU, V. W. 2010. Global methylation profiling of lymphoblastoid cell lines reveals epigenetic contributions to autism spectrum disorders and a novel autism candidate gene, RORA, whose protein product is reduced in autistic brain. *The FASEB Journal,* 24**,** 3036-3051.

NICOLINI, C., AHN, Y., MICHALSKI, B., RHO, J. M. & FAHNESTOCK, M. 2015. Decreased mTOR signaling pathway in human idiopathic autism and in rats exposed to valproic acid. *Acta neuropathologica communications,* 3**,** 3.

OBLAK, A., GIBBS, T. T. & BLATT, G. J. 2009. Decreased GABA<inf>a</inf> receptors and benzodiazepine binding sites in the anterior cingulate cortex in autism. *Autism Research,* 2**,** 205-219.

OBLAK, A., GIBBS, T. T. & BLATT, G. J. 2013. Reduced serotonin receptor subtypes in a limbic and a neocortical region in autism. *Autism Research,* 6**,** 571-583.

OBLAK, A. L., ROSENE, D. L., KEMPER, T. L., BAUMAN, M. L. & BLATT, G. J. 2011. Altered posterior cingulate cortical cyctoarchitecture, but normal density of neurons and interneurons in the posterior cingulate cortex and fusiform gyrus in autism. *Autism Research,* 4**,** 200-211.

OGURO-ANDO, A., ROSENSWEIG, C., HERMAN, E., NISHIMURA, Y., WERLING, D., BILL, B. R., BERG, J. M., GAO, F., COPPOLA, G., ABRAHAMS, B. S. & GESCHWIND, D. H. 2015. Increased CYFIP1 dosage alters cellular and dendritic morphology and dysregulates mTOR. *Molecular Psychiatry,* 20**,** 1069-1078.

OLDHAM, M. C., KONOPKA, G., IWAMOTO, K., LANGFELDER, P., KATO, T., HORVATH, S. & GESCHWIND, D. H. 2008. Functional organization of the transcriptome in human brain. *Nature neuroscience,* 11**,** 1271.

PAGAN, C., GOUBRAN-BOTROS, H., DELORME, R., BENABOU, M., LEMIERE, N., MURRAY, K., AMSELLEM, F., CALLEBERT, J., CHASTE, P., JAMAIN, S., FAUCHEREAU, F., HUGUET, G., MARONDE, E., LEBOYER, M., LAUNAY, J. M. & BOURGERON, T. 2017. Disruption of melatonin synthesis is associated with impaired 14-3-3 and miR-451 levels in patients with autism spectrum disorders. *Scientific reports,* 7**,** 2096.

PALMIERI, L., PAPALEO, V., PORCELLI, V., SCARCIA, P., GAITA, L., SACCO, R., HAGER, J., ROUSSEAU, F., CURATOLO, P., MANZI, B., MILITERNI, R., BRAVACCIO, C., TRILLO, S., SCHNEIDER, C., MELMED, R., ELIA, M., LENTI, C., SACCANI, M., PASCUCCI, T., PUGLISI-ALLEGRA, S., REICHELT, K. L. & PERSICO, A. M. 2010. Altered calcium

homeostasis in autism-spectrum disorders: evidence from biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier AGC1. *Molecular Psychiatry,* 15**,** 38-52.

PARIKSHAK, N. N., SWARUP, V., BELGARD, T. G., IRIMIA, M., RAMASWAMI, G., GANDAL, M. J., HARTL, C., LEPPA, V., UBIETA, L. T., HUANG, J., LOWE, J. K., BLENCOWE, B. J., HORVATH, S. & GESCHWIND, D. H. 2016. Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism. *Nature,* 540**,** 423-427.

PATEL, N., CRIDER, A., PANDYA, C. D., AHMED, A. O. & PILLAI, A. 2016. Altered mRNA levels of glucocorticoid receptor, mineralocorticoid receptor, and co-chaperones (FKBP5 and PTGES3) in the middle frontal gyrus of autism spectrum disorder subjects. *Molecular Neurobiology,* 53**,** 2090-2099.

PEARSON, B. L., CORLEY, M. J., VASCONCELLOS, A., BLANCHARD, D. C. & BLANCHARD, R. J. 2013. Heparan sulfate deficiency in autistic postmortem brain tissue from the subventricular zone of the lateral ventricles. *Behavioural Brain Research,* 243**,** 138-145.

PEKNY, M., WILHELMSSON, U. & PEKNA, M. 2014. The dual role of astrocyte activation and reactive gliosis. *Neuroscience letters,* 565**,** 30-38.

PERRY, E. K., LEE, M. L., MARTIN-RUIZ, C. M., COURT, J. A., VOLSEN, S. G., MERRIT, J., FOLLY, E., IVERSEN, P. E., BAUMAN, M. L. & PERRY, R. H. 2001. Cholinergic activity in autism: abnormalities in the cerebral cortex and basal forebrain. *American journal of psychiatry,* 158**,** 1058-1066.

PHILIP, R. C., DAUVERMANN, M. R., WHALLEY, H. C., BAYNHAM, K., LAWRIE, S. M. & STANFIELD, A. C. 2012. A systematic review and meta-analysis of the fMRI investigation of autism spectrum disorders. *Neuroscience & Biobehavioral Reviews,* 36**,** 901-942.

PLUMMER, J. T., EVGRAFOV, O. V., BERGMAN, M. Y., FRIEZ, M., HAIMAN, C. A., LEVITT, P. & ALDINGER, K. A. 2013. Transcriptional regulation of the MET receptor tyrosine kinase gene by MeCP2 and sex-specific expression in autism and Rett syndrome. *Translational Psychiatry,* 3 (no pagination).

PORT, R. G., OBERMAN, L. M. & ROBERTS, T. P. 2019. Revisiting the excitation/inhibition imbalance hypothesis of ASD through a clinical lens. *The British journal of radiology,* 92**,** 20180944.

PURCELL, A., JEON, O., ZIMMERMAN, A., BLUE, M. & PEVSNER, J. 2001a. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. *Neurology,* 57**,** 1618-1628.

PURCELL, A. E., ROCCO, M. M., LENHART, J. A., HYDER, K., ZIMMERMAN, A. W. & PEVSNER, J. 2001b. Assessment of neural cell adhesion molecule (NCAM) in autistic serum and postmortem brain. *Journal of Autism and Developmental Disorders,* 31**,** 183-194.

RAMASWAMI, G. & GESCHWIND, D. H. 2018. Genetics of autism spectrum disorder. *Handbook of clinical neurology.* Elsevier.

RIQUELME, P. A., DRAPEAU, E. & DOETSCH, F. 2008. Brain micro-ecologies: neural stem cell niches in the adult mammalian brain. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 363**,** 123-137.

RUBENSTEIN, J. & MERZENICH, M. M. 2003. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes, Brain and Behavior,* 2**,** 255-267.

RUSTAN, O. G., FOLSOM, T. D., YOUSEFI, M. K. & FATEMI, S. H. 2013. Phosphorylated fragile X mental retardation protein at serine 499, is reduced in cerebellar vermis and superior frontal cortex of subjects with autism: Implications for fragile X mental retardation protein-metabotropic glutamate receptor 5 signaling. *Molecular Autism,* 4 (1) (no pagination).

SACCO, R., GABRIELE, S. & PERSICO, A. M. 2015. Head circumference and brain size in autism spectrum disorder: a systematic review and meta-analysis. *Psychiatry Research: Neuroimaging,* 234**,** 239-251.

SAJDEL-SULKOWSKA, E. M., XU, M. & KOIBUCHI, N. 2009. Increase in cerebellar neurotrophin-3 and oxidative stress markers in autism. *The Cerebellum,* 8**,** 366-372.

SAJDEL-SULKOWSKA, E. M., XU, M., MCGINNIS, W. & KOIBUCHI, N. 2011. Brain region-specific changes in oxidative stress and neurotrophin levels in autism spectrum disorders (ASD). *The Cerebellum,* 10**,** 43-48.

SANDERS, S. J., MURTHA, M. T., GUPTA, A. R., MURDOCH, J. D., RAUBESON, M. J., WILLSEY, A. J., ERCAN-SENCICEK, A. G., DILULLO, N. M., PARIKSHAK, N. N. & STEIN, J. L. 2012. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature,* 485**,** 237-241.

SANTOS, M., UPPAL, N., BUTTI, C., WICINSKI, B., SCHMEIDLER, J., GIANNAKOPOULOS, P., HEINSEN, H., SCHMITZ, C. & HOF, P. R. 2011. Von Economo neurons in autism: A stereologic study of the frontoinsular cortex in children. *Brain Research,* 1380**,** 206-217.

SARACHANA, T. & HU, V. W. 2013. Genome-wide identification of transcriptional targets of RORA reveals direct regulation of multiple genes associated with autism spectrum disorder. *Molecular Autism,* 4 (1) (no pagination).

SCHOPLER, E., REICHLER, R. J., DEVELLIS, R. F. & DALY, K. 1980. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). *Journal of autism and developmental disorders,* 10**,** 91-103.

SCHUMANN, C. M. & AMARAL, D. G. 2006. Stereological Analysis of Amygdala Neuron Number in Autism. *The Journal of Neuroscience,* 26**,** 7674-7679.

SCHUMANN, C. M., SHARP, F. R., ANDER, B. P. & STAMOVA, B. 2017. Possible sexually dimorphic role of miRNA and other sncRNA in ASD brain. *Molecular Autism,* 8 (1) (no pagination).

SCHWEDE, M., GARBETT, K., MIRNICS, K., GESCHWIND, D. H. & MORROW, E. M. 2014. Genes for endosomal NHE6 and NHE9 are misregulated in autism brains. *Molecular Psychiatry,* 19**,** 277-279.

SCHWEDE, M., NAGPAL, S., GANDAL, M. J., PARIKSHAK, N. N., MIRNICS, K., GESCHWIND, D. H. & MORROW, E. M. 2018. Strong correlation of downregulated genes related to synaptic transmission and mitochondria in post-mortem autism cerebral cortex. *Journal of Neurodevelopmental Disorders Vol 10 2018, ArtID 18,* 10.

SCOLES, H. A., URRACA, N., CHADWICK, S. W., REITER, L. T. & LASALLE, J. M. 2011. Increased copy number for methylated maternal 15q duplications leads to changes in gene and protein expression in human cortical samples. *Molecular Autism,* 2 (1) (no pagination).

SHEIKH, A., LI, X., WEN, G., TAUQEER, Z., BROWN, W. & MALIK, M. 2010a. Cathepsin D and apoptosis related proteins are elevated in the brain of autistic subjects. *Neuroscience,* 165**,** 363-370.

SHEIKH, A. M., MALIK, M., WEN, G., CHAUHAN, A., CHAUHAN, V., GONG, C. X., LIU, F., BROWN, W. T. & LI, X. 2010b. BDNF-Akt-Bcl2 antiapoptotic signaling pathway is compromised in the brain of autistic subjects. *Journal of neuroscience research,* 88**,** 2641-2647.

SHIMMURA, C., SUZUKI, K., IWATA, Y., TSUCHIYA, K. J., OHNO, K., MATSUZAKI, H., IWATA, K., KAMENO, Y., TAKAHASHI, T., WAKUDA, T., NAKAMURA, K., HASHIMOTO, K. & MORI, N. 2013. Enzymes in the glutamate-glutamine cycle in the anterior cingulate cortex in postmortem brain of subjects with autism. *Molecular Autism,* 4 (1) (no pagination).

SHPYLEVA, S., IVANOVSKY, S., DE CONTI, A., MELNYK, S., TRYNDYAK, V., BELAND, F. A., JAMES, S. J. & POGRIBNY, I. P. 2014. Cerebellar oxidative DNA damage and altered DNA methylation in the BTBR T+tf/J mouse model of autism and similarities with human post mortem cerebellum. *PLoS ONE,* 9 (11) (no pagination).

SHULHA, H. P., CHEUNG, I., WHITTLE, C., WANG, J., VIRGIL, D., LIN, C. L., GUO, Y., LESSARD, A., AKBARIAN, S. & WENG, Z. P. 2012. Epigenetic Signatures of Autism Trimethylated H3K4 Landscapes in Prefrontal Neurons. *Archives of General Psychiatry,* 69**,** 314-324.

SKEFOS, J., CUMMINGS, C., ENZER, K., HOLIDAY, J., WEED, K., LEVY, E., YUCE, T., KEMPER, T. & BAUMAN, M. 2014. Regional alterations in Purkinje cell density in patients with autism. *PLoS ONE,* 9 (2) (no pagination).

SMITH, A., STORTI, S., LUKOSE, R. & KULESZA, R. J. 2019a. Structural and Functional Aberrations of the Auditory Brainstem in Autism Spectrum Disorder. *The Journal of the American Osteopathic Association,* 119**,** 41-50.

SMITH, E. H., HORGA, G., YATES, M. J., MIKELL, C. B., BANKS, G. P., PATHAK, Y. J., SCHEVON, C. A., MCKHANN, G. M., HAYDEN, B. Y. & BOTVINICK, M. M. 2019b. Widespread temporal coding of cognitive control in the human prefrontal cortex. *Nature neuroscience***,** 1-9.

SOFIC, E., RIEDERER, P., KILLIAN, W. & RETT, A. 1987. Reduced concentrations of ascorbic acid and glutathione in a single case of Rett syndrome: A postmortem brain study. *Brain and Development,* 9**,** 529-531.

SOGHOMONIAN, J. J., ZHANG, K. Z., REPRAKASH, S. & BLATT, G. J. 2017. Decreased parvalbumin mRNA levels in cerebellar Purkinje cells in autism. *Autism Research,* 10**,** 1787-1796.

SONG, N.-N., XIU, J.-B., HUANG, Y., CHEN, J.-Y., ZHANG, L., GUTKNECHT, L., LESCH, K. P., LI, H. & DING, Y.-Q. 2011. Adult raphe-specific deletion of Lmx1b leads to central serotonin deficiency. *PLoS One,* 6**,** e15998.

STAMOVA, B., ANDER, B. P., BARGER, N., SHARP, F. R. & SCHUMANN, C. M. 2015. Specific Regional and Age-Related Small Noncoding RNA Expression Patterns Within Superior Temporal Gyrus of Typical Human Brains Are Less Distinct in Autism Brains. *Journal of Child Neurology,* 30**,** 1930-1946.

STANFIELD, A. C., MCINTOSH, A. M., SPENCER, M. D., PHILIP, R., GAUR, S. & LAWRIE, S. M. 2008. Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. *European psychiatry,* 23**,** 289-299.

STONER, R., CHOW, M. L., BOYLE, M. P., SUNKIN, S. M., MOUTON, P. R., ROY, S., WYNSHAW-BORIS, A., COLAMARINO, S. A., LEIN, E. S. & COURCHESNE, E. 2014. Patches of disorganization in the neocortex of children with autism. *The New England Journal of Medicine,* 370**,** 1209-1219.

SUDA, S., IWATA, K., SHIMMURA, C., KAMENO, Y., ANITHA, A., THANSEEM, I., NAKAMURA, K., MATSUZAKI, H., TSUCHIYA, K. J., SUGIHARA, G., IWATA, Y., SUZUKI, K., KOIZUMI, K., HIGASHIDA, H., TAKEI, N. & MORI, N. 2011. Decreased expression of axon-guidance receptors in the anterior cingulate cortex in autism. *Molecular Autism,* 2 (1) (no pagination).

SUN, W. J., POSCHMANN, J., DEL ROSARIO, R. C. H., PARIKSHAK, N. N., HAJAN, H. S., KUMAR, V., RAMASAMY, R., BELGARD, T. G., ELANGGOVAN, B., WONG, C. C. Y., MILL, J., GESCHWIND, D. H. & PRABHAKAR, S. 2016. Histone Acetylome-wide Association Study of Autism Spectrum Disorder. *Cell,* 167**,** 1385-+.

SUTCLIFFE, J. S., NAKAO, M., CHRISTIAN, S., ÖRSTAVIK, K. H., TOMMERUP, N., LEDBETTER, D. H. & BEAUDET, A. L. 1994. Deletions of a differentially methylated CpG island at the SNRPN gene define a putative imprinting control region. *Nature genetics,* 8**,** 52-58.

SWANBERG, S. E., NAGARAJAN, R. P., PEDDADA, S., YASUI, D. H. & LASALLE, J. M. 2009. Reciprocal co-regulation of EGR2 and MECP2 is disrupted in Rett syndrome and autism. *Human Molecular Genetics,* 18**,** 525-534.

TANG, G., GUDSNUK, K., KUO, S. H., COTRINA, M. L., ROSOKLIJA, G., SOSUNOV, A., SONDERS, M. S., KANTER, E., CASTAGNA, C., YAMAMOTO, A., YUE, Z., ARANCIO, O., PETERSON, B. S., CHAMPAGNE, F., DWORK, A. J., GOLDMAN, J. & SULZER, D. 2014. Loss of mTOR-Dependent Macroautophagy Causes Autistic-like Synaptic Pruning Deficits. *Neuron,* 83**,** 1131-1143.

TANG, G., GUTIERREZ RIOS, P., KUO, S. H., AKMAN, H. O., ROSOKLIJA, G., TANJI, K., DWORK, A., SCHON, E. A., DIMAURO, S., GOLDMAN, J. & SULZER, D. 2013. Mitochondrial abnormalities in temporal lobe of autistic brain. *Neurobiology of Disease,* 54**,** 349-361.

TAOUFIK, E., KOUROUPI, G., ZYGOGIANNI, O. & MATSAS, R. 2018. Synaptic dysfunction in neurodegenerative and neurodevelopmental diseases: an overview of induced pluripotent stem-cell-based disease models. *Open biology,* 8**,** 180138.

TAWFIK, V. L., LACROIX-FRALISH, M. L., NUTILE-MCMENEMY, N. & DELEO, J. A. 2005. Transcriptional and translational regulation of glial activation by morphine in a rodent model of neuropathic pain. *Journal of pharmacology and experimental therapeutics,* 313**,** 1239-1247.

TETREAULT, N. A., HAKEEM, A. Y., JIANG, S., WILLIAMS, B. A., ALLMAN, E., WOLD, B. J. & ALLMAN, J. M. 2012. Microglia in the cerebral cortex in autism. *Journal of Autism and Developmental Disorders,* 42**,** 2569-2584.

THANSEEM, I., ANITHA, A., NAKAMURA, K., SUDA, S., IWATA, K., MATSUZAKI, H., OHTSUBO, M., UEKI, T., KATAYAMA, T., IWATA, Y., SUZUKI, K., MINOSHIMA, S. & MORI, N. 2012. Elevated transcription factor specificity protein 1 in autistic brains alters the expression of autism candidate genes. *Biological Psychiatry,* 71**,** 410-418.

THANSEEM, I., NAKAMURA, K., ANITHA, A., SUDA, S., YAMADA, K., IWAYAMA, Y., TOYOTA, T., TSUJII, M., IWATA, Y., SUZUKI, K., MATSUZAKI, H., IWATA, K., SUGIYAMA, T., YOSHIKAWA, T. & MORI, N. 2011. Association of transcription factor gene LMX1B with autism. *PLoS ONE,* 6 (8) (no pagination).

TILI, E., CROCE, C. M. & MICHAILLE, J.-J. 2009. miR-155: on the crosstalk between inflammation and cancer. *International reviews of immunology,* 28**,** 264-284.

TRAKOSHIS, S., ROCCHI, F., CANELLA, C., YOU, W., CHAKRABARTI, B., RUIGROK, A. N., BULLMORE, E. T., SUCKLING, J., MARKICEVIC, M. & ZERBI, V. 2020. Intrinsic excitation-inhibition imbalance affects medial prefrontal cortex differently in autistic men versus women. *Elife,* 9**,** e55684.

TRAN, S. S., JUN, H. I., BAHN, J. H., AZGHADI, A., RAMASWAMI, G., VAN NOSTRAND, E. L., NGUYEN, T. B., HSIAO, Y. H. E., LEE, C., PRATT, G. A., MARTINEZ-CERDENO, V., HAGERMAN, R. J., YEO, G. W., GESCHWIND, D. H. & XIAO, X. 2019. Widespread RNA editing dysregulation in brains from autistic individuals. *Nature Neuroscience,* 22**,** 25-36.

TRUTZER, I. M., GARCIA-CABEZAS, M. A. & ZIKOPOULOS, B. 2019. Postnatal development and maturation of layer 1 in the lateral prefrontal cortex and its disruption in autism. *Acta neuropathologica communications,* 7**,** 40.

TUZUN, E., SHARP, A. J., BAILEY, J. A., KAUL, R., MORRISON, V. A., PERTZ, L. M., HAUGEN, E., HAYDEN, H., ALBERTSON, D. & PINKEL, D. 2005. Fine-scale structural variation of the human genome. *Nature genetics,* 37**,** 727-732.

UPPAL, N., WICINSKI, B., BUXBAUM, J. D., HEINSEN, H., SCHMITZ, C. & HOF, P. R. 2014. Neuropathology of the anterior midcingulate cortex in young children with autism. *Journal of Neuropathology and Experimental Neurology,* 73**,** 891-902.

VAN KOOTEN, I. A. J., PALMEN, S. J. M. C., VON CAPPELN, P., STEINBUSCH, H. W. M., KORR, H., HEINSEN, H., HOF, P. R., VAN ENGELAND, H. & SCHMITZ, C. 2008. Neurons in the fusiform gyrus are fewer and smaller in autism. *Brain,* 131**,** 987-999.

VARGAS, D. L., NASCIMBENE, C., KRISHNAN, C., ZIMMERMAN, A. W. & PARDO, C. A. 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of Neurology,* 57**,** 67-81.

VARGHESE, M., KESHAV, N., JACOT-DESCOMBES, S., WARDA, T., WICINSKI, B., DICKSTEIN, D. L., HARONY-NICOLAS, H., DE RUBEIS, S., DRAPEAU, E. & BUXBAUM, J. D. 2017. Autism spectrum disorder: neuropathology and animal models. *Acta neuropathologica,* 134**,** 537-566.

VELMESHEV, D., MAGISTRI, M. & FAGHIHI, M. A. 2013. Expression of non-protein-coding antisense RNAs in genomic regions related to autism spectrum disorders. *Molecular Autism,* 4 (1) (no pagination).

VOINEAGU, I., WANG, X., JOHNSTON, P., LOWE, J. K., TIAN, Y., HORVATH, S., MILL, J., CANTOR, R. M., BLENCOWE, B. J. & GESCHWIND, D. H. 2011. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature,* 474**,** 380-384.

WEGIEL, J., FLORY, M., KUCHNA, I., NOWICKI, K., MA, S. Y., IMAKI, H., COHEN, I. L., LONDON, E., BROWN, W. T. & WISNIEWSKI, T. 2014. Brain-region-specific alterations of the trajectories of neuronal volume growth throughout the lifespan in autism. *Acta neuropathologica communications,* 2**,** 28.

WEI, H., ALBERTS, I. & LI, X. 2014. The apoptotic perspective of autism. *International Journal of Developmental Neuroscience,* 36**,** 13-18.

WEINER, D. J., WIGDOR, E. M., RIPKE, S., WALTERS, R. K., KOSMICKI, J. A., GROVE, J., SAMOCHA, K. E., GOLDSTEIN, J. I., OKBAY, A. & BYBJERG-GRAUHOLM, J. 2017. Polygenic transmission disequilibrium confirms that common and rare variation act additively to create risk for autism spectrum disorders. *Nature genetics,* 49**,** 978-985.

WEIR, R. K., BAUMAN, M. D., JACOBS, B. & SCHUMANN, C. M. 2018. Protracted dendritic growth in the typically developing human amygdala and increased spine density in young ASD brains. *Journal of Comparative Neurology,* 526**,** 262-274.

WENK, G. L., O'LEARY, M., NEMEROFF, C. B., BISSETTE, G., MOSER, H. & NAIDU, S. 1993. Neurochemical alterations in Rett syndrome. *Developmental Brain Research,* 74**,** 67-72.

WERLING, D. M., PARIKSHAK, N. N. & GESCHWIND, D. H. 2016. Gene expression in human brain implicates sexually dimorphic pathways in autism spectrum disorders. *Nature Communications,* 7 (no pagination).

WHITNEY, E. R., KEMPER, T. L., ROSENE, D. L., BAUMAN, M. L. & BLATT, G. J. 2009. Density of cerebellar basket and stellate cells in autism: evidence for a late developmental loss of Purkinje cells. *Journal of Neuroscience Research,* 87**,** 2245-54.

WILDER, L., HANSON, K. L., LEW, C. H., BELLUGI, U. & SEMENDEFERI, K. 2018. Decreased Neuron Density and Increased Glia Density in the Ventromedial Prefrontal Cortex (Brodmann Area 25) in Williams Syndrome. *Brain Sciences,* 8**,** 13.

WONG, C. C. Y., SMITH, R. G., HANNON, E., ASSARY, E., PARIKSHAK, N., PRABHAKAR, S., GESCHWIND, D. & MILL, J. 2019a. Convergent Methylomic Signatures between Autism Associated with Duplications of Chromosome 15q and Idiopathic Autism. *European Neuropsychopharmacology,* 29 (Supplement 3)**,** S983.

WONG, C. C. Y., SMITH, R. G., HANNON, E., RAMASWAMI, G., PARIKSHAK, N. N., ASSARY, E., TROAKES, C., POSCHMANN, J., SCHALKWYK, L. C., SUN, W. J., PRABHAKAR, S., GESCHWIND, D. H. & MI, J. 2019b. Genome-wide DNA methylation profiling identifies convergent molecular signatures associated with idiopathic and syndromic autism in post-mortem human brain tissue. *Human Molecular Genetics,* 28**,** 2201-2211.

WONG, D. F., BLUE, M. E., BRASIC, J. R., NANDI, A., VALENTINE, H., STANSFIELD, K. H., ROUSSET, O., BIBAT, G., YABLONSKI, M. E., JOHNSTON, M. V., GJEDDE, A. & NAIDU, S. 2018. Are dopamine receptor and transporter changes in Rett syndrome reflected in Mecp2-deficient mice? *Experimental Neurology,* 307**,** 74-81.

WRIGHT, C., SHIN, J. H., RAJPUROHIT, A., DEEP-SOBOSLAY, A., COLLADO-TORRES, L., BRANDON, N. J., HYDE, T. M., KLEINMAN, J. E., JAFFE, A. E., CROSS, A. J. & WEINBERGER, D. R. 2017. Altered expression of histamine signaling genes in autism spectrum disorder. *Translational Psychiatry,* 7 (5) (no pagination).

WU, P., TEOT, L., MURDOCH, G., MONAGHAN-NICHOLS, A. P. & MCFADDEN, K. 2014. Neuropathology of 22q11 deletion syndrome in an infant. *Pediatric & Developmental Pathology,* 17**,** 386-92.

WU, Y. E., PARIKSHAK, N. N., BELGARD, T. G. & GESCHWIND, D. H. 2016. Genome-wide, integrative analysis implicates microRNA dysregulation in autism spectrum disorder. *Nature Neuroscience,* 19**,** 1463-1476.

XIA, K., GUO, H., HU, Z., XUN, G., ZUO, L., PENG, Y., WANG, K., HE, Y., XIONG, Z., SUN, L., PAN, Q., LONG, Z., ZOU, X., LI, X., LI, W., XU, X., LU, L., LIU, Y., HU, Y., TIAN, D., LONG, L., OU, J., LIU, Y., LI, X., ZHANG, L., PAN, Y., CHEN, J., PENG, H., LIU, Q., LUO, X., SU, W., WU, L., LIANG, D., DAI, H., YAN, X., FENG, Y., TANG, B., LI, J., MIEDZYBRODZKA, Z., XIA, J., ZHANG, Z., LUO, X., ZHANG, X., ST CLAIR, D., ZHAO, J. & ZHANG, F. 2014. Common genetic variants in 1p13.2 associate with risk of autism. *Molecular Psychiatry,* 19**,** 1212-1219.

YIP, J., SOGHOMONIAN, J. J. & BLATT, G. J. 2007. Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. *Acta Neuropathologica,* 113**,** 559-68.

YIP, J., SOGHOMONIAN, J. J. & BLATT, G. J. 2009. Decreased GAD65 mRNA levels in select subpopulations of neurons in the cerebellar dentate nuclei in autism: An in situ hybridization study. *Autism Research,* 2**,** 50-59.

YOUNG, A. M. H., CAMPBELL, E., LYNCH, S., SUCKLING, J. & POWIS, S. J. 2011. Aberrant NF-kappaB expression in autism spectrum condition: A mechanism for neuroinflammation. *Frontiers in Psychiatry,* 2 (MAY) (no pagination).

ZHANG, Y. T., HODGSON, N. W., TRIVEDI, M. S., ABDOLMALEKY, H. M., FOURNIER, M., CUENOD, M., DO, K. Q. & DETH, R. C. 2016. Decreased Brain Levels of Vitamin B12 in Aging, Autism and Schizophrenia. *Plos One,* 11**,** 19.

ZHAO, B., WU, Q., YE, A. Y., GUO, J., ZHENG, X., YANG, X., YAN, L., LIU, Q. R., HYDE, T. M., WEI, L. & HUANG, A. Y. 2019. Somatic LINE-1 retrotransposition in cortical neurons and non-brain tissues of Rett patients and healthy individuals. *PLoS genetics,* 15**,** e1008043.

ZHU, L., WANG, X., LI, X. L., TOWERS, A., CAO, X., WANG, P., BOWMAN, R., YANG, H., GOLDSTEIN, J., LI, Y. J. & JIANG, Y. H. 2014. Epigenetic dysregulation of SHANK3 in brain tissues from individuals with autism spectrum disorders. *Human Molecular Genetics,* 23**,** 1563-78.

ZHUBI, A., CHEN, Y., DONG, E., COOK, E. H., GUIDOTTI, A. & GRAYSON, D. R. 2014. Increased binding of MeCP2 to the GAD1 and RELN promoters may be mediated

by an enrichment of 5-hmC in autism spectrum disorder (ASD) cerebellum. *Translational Psychiatry,* 4 (no pagination).
ZIATS, M. N. & RENNERT, O. M. 2013. Aberrant Expression of Long Noncoding RNAs in Autistic Brain. *Journal of Molecular Neuroscience,* 49**,** 589-593.
ZIKOPOULOS, B. & BARBAS, H. 2010. Changes in prefrontal axons may disrupt the network in autism. *The Journal of Neuroscience,* 30**,** 14595-14609.
ZIKOPOULOS, B. & BARBAS, H. 2013. Altered neural connectivity in excitatory and inhibitory cortical circuits in autism. *Frontiers in Human Neuroscience Vol 7 2013, ArtID 609,* 7.
ZIKOPOULOS, B., LIU, X. F., TEPE, J., TRUTZER, I., JOHN, Y. J. & BARBAS, H. 2018. Opposite development of short- and long-range anterior cingulate pathways in autism. *Acta Neuropathologica,* 136**,** 759-778.

7. Figure captions:

Figure 1: Preferred reporting items for systematic reviews and meta-analysis (PRISMA) flowchart. Figure outlines search and review process with the total number of articles included and excluded in this review.

Figure 2: Regional impairments in ASD. Figure summarises the major findings in ASD grouped by brain region.

8. Tables:

Table 1: Inclusion and exclusion criteria to be eligible for inclusion in the current systematic review.

Inclusion Criteria	Exclusion Criteria
Post-mortem studies examining ASD subjects.	Studies not involving post-mortem brain tissue.
Any form of ASD (idiopathic or genetic, such as FragileX, Rett Syndrome, 15q11-13, etc…).	Studies lacking healthy controls for comparison
Subjects of any ages and either or both sexes.	Studies which have not undergone peer-review
Studies with controls.	

Table 2: Summary of pathways and representative transcripts or proteins identified by gene expression studies.

First Author (year)	Diagnosis	Region	Pathways implicated	Representative transcripts/proteins
Garbett et al. (2008)	ASD	Temporal cortex	Cell communication	SERPINH1, SPP1, CHI3L1, DTNA, SPP1, RFX4
			Differentiation	FGF12, MYT1L, GAS7
			Cell cycle regulation and chaperone systems	GADD45B, IFI16, HSPA6, BCL6, TAP1, RIT1

			Cell death	NFKB, TNFR2, P38MAPK, TID, 41BB, CASPASE, FAS pathway
Chow et al. (2012)	ASD	prefrontal cortex	(young: <14 years)	
			Cell cycle and apoptosis	BRCA1 and CHK2,
			cell proliferation, cell fate	WNT3
			neural patterning and differentiation	FGF1, HOXD1, NDE1, NODAL, PCSK6 and GREM1
			(Adult: 15–56 years)	RELN, BTRC, BMP4, MAPK10 and NTRK3 as well as MAPK12, CDKN1A, NTRK3, PRKAR1A, PIK3CA,
			Signaling, development and oxidative stress, tissue remodeling and wound repair	CASP9, MAPK10, ADCY6 and MAGED1
			(young and adult)	14-3-3 (YWHAZ), CDC25A, CDCD25C, ATRX
			cell cycle	CTNNB1 (beta- catenin), FSHB, PRKACB, PRKCZ],
			proliferation	BAD, CASP8, CASP10, MDM2

			apoptosis	ErbB4, MMP2, NID1, TIMP1, COL4A3
			cytoskeleton and extracellular matrix remodeling	RELN, ROBO1, ADORA2A, p21 (CDKN1A), 14-3-3, HGF, FGFRL1, TSC1
			growth and development	
Guan et al. (2016)	ASD	Cerebral cortex, anterior prefrontal Cortex, part of the frontal cortex (previously published dataset by Gupta et al., (2014)	Metabolism and biosynthesis	H6PD, PRPS2, PFKP, SC5DL, NSDHL, DHCR7, SQLE, HSD17B7, HMGCR, DLD, HIBADH, MCCC2
			Immune/Inflammatory response	SELPLG, C3, ITGB1, MAPK8, ATF2, MAPK14, CHUK, RBX1, BTRC, STAT1, PTPN1, JAK1
			Signalling pathway	JUN, CSNK2A1, ELK1, MAPK8, MAPK14, JUN, NDUFV3, PISD, FOS, PRKAA1, CAB39, TSC1
			Vitamins and supplements	SLC2A3, COL4A2, SLC2A1, GCHFR, PTS, AKT1

Guan et al. (2019)	ASD	Cerebral cortex	amino acid transport	CLN8, KCNJ10, SLC1A1, SLC1A2, SLC6A1
			synapse and neurotransmitter release	ALDH5A1, NF1, NTRK2, SLC1A2, SLC6A1, NLGN3, RIMS1, CNR1, OPHN1 and STXBP1
			oxidative stress and nitric oxide synthase biosynthesis	GPX1 and SOD1
			immune response	ADA, CX3CR1, FOXP1, IFNG, IL6, KIT, LAT, PIK3CG, RORA and TSC1
			protein folding	NLGN4, HSPH1, HSPE1, DNAJB1, CHCHD4
			lysophosphatidic acid- mediated signaling	ADCY3 and ADCY5
			glycolysis	ALDOA, ALDOB, PFKFB1, PGK1 and PPP2CA
Schwede et al. (2018)	ASD	Cerebral cortex	Mitochondria	AGK, TTC19, NDUFS1, MAT2B, MAPK9, SDHA, SLC25A3, SLC25A14, SLC25A36, ARMC1, PPP2R1A,

				OCIAD1, TIMM17A, TOMM20, PPP3CA, GABARAPL1, C12orf10, SLC8A1, KIF1BP, FIS1
			Synapse	ICA1, ARHGAP44, GABRA1, GABRB2, DNM1L, GLRB, KCNA1, ARFGEF2, NRN1, SYT11, SYT2, TOR1A, VAMP1, CHRNB3, ANK3
Broek et al. (2014)	ASD	Prefrontal cortex, cerebellum	myelination proteins	MAG and myelin proteolipid protein, PLP1
			synaptic proteins	synapsin-2, SYN2 and syntaxin-binding protein 1, STXBP1
			energy-related proteins (in prefrontal cortex only)	creatine kinase B-type, CKB
Ander et al. (2015)	ASD	superior temporal sulcus and primary auditory cortex	Nervous system, cell cycle and canonical signalling pathways	superior temporal sulcus: miRNAs: miR-4753-5p, miR-1 snoRNAs: ACA39, SNORA11C, SNORA27 pre-miRNAs: mir-1204, mir-1913, mir-605, mir-3152, mir-4730, mir-1287, mir-19b-1 and mir-4490 primary auditory cortex:

				miRNAs: miR-664-3p, miR-4709-3p, miR-4742-3p and miR-297 pre-miRNAs: mir-3158-1, mir-3194, mir-4314, mir-4436a, mir-138-2, mir-146b and mir-548g
Stamova et al. (2015)	ASD	superior temporal gyrus, namely: the superior temporal sulcus and the primary auditory cortex	Ephrin receptor, ephrin B, netrin and reelin signalling; NGF, neurotrophin, CNTF and melatonin signaling.	miRNAs: miR-132, miR-103 and miR-320
Schumann et al. (2017)	ASD	superior temporal sulcus and primary auditory cortex	oligodendrocyte differentiation neuronal differentiation anxiety	miR-219 and miR-338 miR-125 miR-488

Anitha et al. (2012)	ASD	anterior cingulate gyrus, motor cortex and thalamus	membrane polarization and potential, mitochondrial transport, small molecule transport, targeting proteins to mitochondria, mitochondrion protein import, inner and outer membrane translocation, mitochondrial fission and fusion, mitochondrial localization, and apoptosis.	MTX2, NEFL, SLC25A27, DNAJC19, DNM1L, LRPPRC, SLC25A12, SLC25A14, SLC25A24 and TOMM20
Colantuoni et al. (2001)	RTT	Cerebral cortex	glial transcripts	alpha- B-crystallin, glial fibrillary acidic protein (GFAP), glial excitatory amino acid transporter 1 (EAAT1) and S100 A13
			neuronal transcripts	GABA and glutamate receptors (GABA A B3 receptor, AMPA receptors 1,2,3, mGluR1, mGluR8), neuronal pentraxin 1, calcineurin, CAM kinase II-beta, neuronal olfactomedin-related ER localized protein (NOEL), Ca2+ ATPases, neuronatin alpha and beta, neuron specific enolase and PCP4, MAP-2, MAP-1B,

			Presynaptic markers	neurofilament proteins of 66 and 200 kDa, tau and tau kinase II
Gibson et al. (2010)	RTT	frontal and occipital cortex	Genes associated with neurological disorders	annexin VI, syntaxin 1A, DOC2A and synaptosomal associated protein of 25 kDa (SNAP-25) clusterin (CLU) and cytochrome c oxidase subunit 1 (CO1)
			synaptic vesicle cycling	dynamin 1 (DNM1)

Table 3: Differential expression of ASD-candidate genes in unreplicated studies.

Reference	**Gene**	**Differential expression**	**Region**
(Schwede et al., 2014)	Endosomal Na+/H+ Exchanger 6 (NHE6 or SLC9A6)	Downregulated	cerebral cortex
	NHE9	Upregulated	
(Lintas et al., 2009)	PRKCB1-1 and PRKCB1-2	Downregulated	temporocortical gray matter
(Suda et al., 2011)	EFNB3, PLXNA4A and ROBO2	Downregulated	anterior cingulate cortex

(Thanseem et al., 2012)	Specificity protein 1 (Sp1), CD38, ITGB3, MAOA, MECP2, OXTR and PTEN	Upregulated	anterior cingulate gyrus
	HTR2A	Downregulated	
(Purcell et al., 2001b)	Neural cell adhesion molecule-180 (NCAM-180)	Downregulated	cerebellar cortex
(Shimamoto et al., 2014).	Fatty acid binding protein 7 (FABP7)	Upregulated	frontal and parietal corticies
(Xia et al., 2014)	TRIM33, NRAS	Upregulated	Frontal and temporal cortex
(Anitha et al., 2014)	zinc finger protein 804A (ZNF804A)	Downregulated	anterior cingulate gyrus
(Zhu et al., 2014).	SHANK3	Downregulation	cerebellum and cerebral cortex
(Wright et al., 2017).	HDC, HNMT, HRH1, HRH2, HRH3 and HRH4	No differential expression	dorsolateral prefrontal cortex

Table 4: Summary of common impairments in ASD and ASD-relevant disorders.

Aspect	ASD	RTT	FXS	WS
Lower Purkinje cell counts	Yes	Yes	Yes	N/a
Altered neuronal size/density	Yes, PFC	Yes, cerebral cortex, basal ganglia, thalamus,	N/A	Yes, PFC

		hippocampus, and amygdala		
Dendrite/spine abnormalities	Yes, PFC, Temporal, parietal cortex, amygdala	Yes, PFC, Hippocampus, Motor cortex	Yes, Cingulate and temporal association areas	Yes, PFC, Primary visual and auditory cortex
Altered MecP2 expression	Yes, PFC, fusiform gyrus, cerebellum	Yes, Cerebral cortex and fusiform gyrus	N/A	N/A
5-HT impairments	Yes, ACC	Yes, PFC, thalamus	N/A	N/A
Gene Expression patterns	synaptic, metabolic, proliferation, apoptosis and immune pathways	glial, neuronal and synaptic functions	N/A	N/A