The neurobiology of autism spectrum disorders

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ABSTRACT

Data is progressively and robustly accumulating regarding the biological basis of autism. Autism spectrum disorders (ASD) are currently considered a group of neurodevelopmental disorders with onset very early in life and a complex, heterogeneous, multifactorial aetiology. A comprehensive search of the last five years of the Medline database was conducted in order to summarize recent evidence on the neurobiological bases of autism. The main findings on genetic influence, neuropathology, neurostructure and brain networks are summarized. In addition, findings from peripheral samples of subjects with autism and animal models, which show immune, oxidative, mitochondrial dysregulations, are reported. Then, other biomarkers from very different systems associated with autism are reported. Finally, an attempt is made to try and integrate the available evidence, which points to a oligogenic, multifactorial aetiology that converges in an aberrant micro-organization of the cortex, with abnormal functioning of the synapses and abnormalities in very general physiological pathways (such as inflammatory, immune and redox systems).

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1. Introduction

In 1943, Leo Kanner described autism as an innate disturbance of affective contact (prior to any interpersonal contact) and, in 1944, Hans Asperger described a group of children with a psychopathic disturbance of social interaction [58]. Kanner autism was included in the chapter on Childhood Schizophrenia of the DSM-I and DSM-II in the fifties and thereafter. Some schools of psychology advanced the hypothesis that autism was a psychological reaction to very disturbed early relationships, put the mother at the core of the etiological trajectory and proposed psychological treatments with a psychoanalytic orientation [100]. Since 1980 autism has been separated from other psychotic disorders and classified in a newly created chapter called Pervasive Developmental Disorders (from DSM-III to DSM-IV-TR), and will likely be called autism spectrum disorders (ASD) in future classifications. The present concept of ASD includes individuals with early-onset reciprocal interaction and communication impairment as the sine qua non symptom and encompasses Kanner autism, Asperger syndrome, atypical autisms and a group of non-specified pervasive developmental disorders. For these children, the psychodynamic hypothesis does not seem plausible to the general academic community, is not supported by any empirical evidence and has been rejected in general terms.

On the contrary, data is progressively and robustly accumulating regarding the biological basis of autism. ASD is currently considered a group of neurodevelopmental disorders with onset very early in life and a complex, heterogeneous, multifactorial etiology. Different combinations of environmental (mainly intrauterine) and genetic risk factors and molecular/cellular signaling abnormalities are likely involved in their etiology. Rarely, a unique genetic or environmental factor seems to be the cause of a given case (such as tuberous sclerosis, fragile X or fetal valproate syndrome).

Prevalence rates of ASD have increased dramatically in the last few decades, with rates of 1/2,500 around 1980 (at the time of DSM-III, when it first appeared as a distinct entity) to rates of 1/150, which is currently the most widely used figure [123]. The increased prevalence is probably due to a combination of different factors, mainly increased recognition, increased early detection by pediatricians/teachers, and broadening of the concept from Kanner autism to extended, borderline and limited phenotypes (which includes “diagnosis accretion”, that is, subjects who would have been diagnosed with mental retardation according to earlier criteria and are now additionally diagnosed with autism). Other factors, such as advanced paternal or maternal age [65] and the potential effect of environmental toxins are currently under investigation [63].

Ample evidence is rapidly accumulating for different genetic, molecular and pathways abnormalities associated with autism. Some have stronger empirical support such as causative mechanisms in animal models, but most are merely associations at the
level of biomarkers with as yet unknown physiopathological meaning. In this review we critically summarize the recent evidence for a biological substrate of ASD, in order to review the state of the question.

2. Method

We performed a comprehensive search of the Medline database using the following syntax (neurobiology OR MRI OR metabolism OR genetics OR redox OR neurotransmitters OR neuroimaging OR biologic[Tite|Abstract]) AND (autism OR autism spectrum disorders OR pervasive development disorders OR Asperger[-Title|Abstract]). We used the following limits in the search: Editorial, Meta-Analysis, Review, Classical Article, Journal Article, English, French, German, Spanish, published in the last 5 years. This search returned 3367 citations. Two authors (M.P. and M.L.P.) then read the abstracts and limited the search to 869 papers. The main objective of this second process was to select papers focussing on autism and no other developmental disorders or specific syndromes. The abstracts were then distributed to the different authors based on their areas of expertise. After the available papers were read, we performed additional searches to examine specific issues and also reviewed relevant papers from the previous search reference lists.

3. Results

3.1. Genetic influence

Autism is a highly heritable disorder, with inherited genetic risk sets being associated with both subthreshold autistic traits and the clinical ASD phenotype in large twin studies, supporting the notion that clinical disorders exist as the quantitative extreme of a continuum [102]. As in other psychiatric disorders, common genetic variants distributed along the genome seem to increase the risk modestly, while rare genetic variants, de novo or in close ancestry, seem to increase the individual risk significantly [73]. Many genes have been now shown to carry risk for autism, with no single locus accounting for more than 1% of the cases. In addition, identical variations have been shown to carry large effects for a wide range of outcomes including ASD and other developmental disorders [119].

Autism subtypes may render different heritability patterns. The differentiation between “complex” and “essential” forms of autism is an informative broad separation for clinicians and geneticists [77]. Complex autism refers to cases with general dysmorphic features that indicate early morphogenesis anomalies; it is associated with poor prognosis and a lower male-to-female ratio, and comprises around 20–30% of autism cases. A diagnosable medical condition, cytogenetic abnormalities or single-gene defects can be found in 10–25% of cases of complex autism. Essential autism is characterized by an absence of dysmorphic features, more frequent family history of autism, is more frequently diagnosed in males and the aetiology is very rarely found [77].

3.1.1. Recurrence rates of autism

General recurrence rates of autism in the same family are in the range of 10–18% [21,101]. However, essential autism has a higher recurrence rate (up to 35%) [101]. In addition to ASD in relatives, quantitative analysis of autistic traits and language disorders shows that around 20–25% of siblings have predominantly pragmatic language deficits [21,70].

3.1.2. Twin studies

A large number of studies have used twins, as well as more distant relatives, to establish the contribution of genetic factors to the etiology of autism and ASD. The three main twin studies [8,43,103] examining the concordance of strictly defined autism between monozygotic (MZ) twins have yielded similar figures of around 60%. However, the figures change considerably when dizygotic (DZ) twins or broader phenotypes are studied. When autism-unaffected twins with developmental problems other than autism are considered “affected cases”, the rates of concordance rise to the 90s. Early twin studies reported concordance for DZ twins of 10–15%. However, the largest population-based twin study to date [43] has shown concordance rates of 21–36% in DZ twins, using DSM-IV diagnostic criteria for ASD (which are known to be associated with increased prevalence rates of ASD) [123]. Shared environment was reported in this study to account for 55 and 58% of the variance in liability, in strict autism and ASD respectively.

3.1.3. Genome-wide association (GWA) studies. Common genetic variants

GWA studies identify single-nucleotide polymorphisms (SNPs) and other common genetic variants in DNA that may be associated with a disease or trait by investigating the entire genome using an unbiased hypothesis-free search. These studies have identified more than 100 genes and 40 genomic regions as related with ASD or ASD traits, each with a weak effect. The Autism Genome Project GWA study of more than 1,000,000 SNPs in more than 2,700 families reports no single SNP association with autism or ASD [3]. Despite the lack of individual SNP associations in the threshold of \( p < 5 \times 10^{-8} \) (the threshold currently accepted as significant in genetic studies for individual SNPs), the study of the combined effect of common variants showed a significant, albeit still low, prediction of case-control status by the combined effect of the identified SNPs in this study [3]. Nevertheless, GWA studies may help identify targets for more in-depth studies of etiological mechanisms. For example, in the Autism Genome Project, the SNP with the highest uncorrected association with ASD was the rs1718101 (association found in a specific subsample), which falls in CNTNAP2, a gene located in chromosome 7 that encodes a protein of the neurexin family, thought to be involved in axonal differentiation and guidance [3].

3.1.4. Rare variants and de novo mutations

Compared with the weak association between common genetic variants and the risk of autism, rare variants occur more frequently. Cytogenetic abnormalities detectable on standard karyotyping have been calculated to occur in up to 5% of cases [77], as do single-gene mutations, while sub-microscopic deletions or duplications, called copy-number variants (CNVs), seem to be present in more than 10% of cases [77]. These CNVs are of variable clinical significance and are particularly present in cases with intellectual disability, where the rate may rise to 22% [114]. New tools for molecular genetic analyses combining comparative genomic hybridization (CGH) with array platforms show that unique and highly penetrant germline de novo mutations may play an important role in simplex families (i.e. with a single family member affected), distinct from transmitted variations that may predominate in multiplex families (multiple family members affected). The study of de novo mutations is showing evidence of converging molecular pathways affected in different samples, including formation and functioning of synapses and other developmental pathways [89,84,109]. Moreover, in syndromic autism, as in autism associated with fragile X or tuberous sclerosis syndrome, mutations affecting synaptic plasticity (via inadequate glutamate receptor-mediated protein synthesis) have been shown [7]. Although mainly unique in individual cases of ASD, some de novo mutations overlap in that they hit some genes, particularly 16p11.2, 7q11.23 and 15q11.2 [68].
3.1.5. Candidate gene studies

Candidate genes for ASD have been identified, but their contribution to pathogenesis remains unclear in many cases. Current genetic studies point towards ASD risk genes associated with proteins involved in synaptic mechanisms (including NRXNs, NLRGs, CTN3/4, CNTNTAP2 and SHANK3) [41,66,95,96]; genes associated with neuronal migration, growth and differentiation abnormalities (i.e. gene products of EN2 or MET, PTEN, TSC1/2, CNTNTAP2, FMR1) [31,97] and also genes involved in proteins related to excitatory and inhibitory neurotransmission (GABA and glutamate receptors such as GRIN2B) or membrane ion channels (SCN2A) [89,108]. Additionally, genes coding for proteins involved in cell regulation (DYRK1A) or structure (KANTAL2) [84,90], or with an action at the level of the cell nucleus (DNA binding proteins PO32 or chromatin modifiers CHD8) have been recently discovered [119,89,84]. Many of these lately discovered genes have been postulated to contribute not only to autism but also to several neurodevelopmental disorders in humans [50,14,18,104]. As an example of the genetics of syndromic autism, mutation or loss of FMRP (fragile X mental retardation protein) leads to failure of mRNA stability and dendritic targeting and also neuronal translation [27,28].

An example of how candidate genes may direct subsequent research and shed light on mechanisms underlying autistic pathology is the MET receptor tyrosine kinase gene. This gene, as well as a few others connected to the serotonin system (PIK3CG, MET, RELN), have been considered good candidate genes for ASD. The MET gene is located in the chromosome 7q31 region, which emerged from GWA studies as a candidate region for autism. The MET signal seems important in neocortical and cerebellar development, in regulation of the immune system, and in gastrointestinal repair, all processes that have manifested signs of dysfunction in autism, encompassing several of the known dysfunctions common in autism [48]. This gene has been shown to be associated with autism in family studies [17] and has been involved in the mechanisms that may lead to the pathology in autistic children with mental retardation.

3.2. Neuropathology

Abnormal minicolumnar structure in the neocortex seems to be a common finding [1]. Cerebellar abnormalities are also a common feature, and include abnormal volumes (although generally proportional to brain volume), reduced number and size of Purkinje neurons in the vermis and hemispheres and molecular defects in posterior regions [24]. Postmortem tissues provide an opportunity to study gene expression. GABA superficial expression has been shown to be decreased in the superior frontal cortex, parietal cortex, and cerebellum of subjects with autism [35]. It is important to note that most neuropathology findings come from autistic children with mental retardation and a high percentage of epilepsy [1].

3.3. Brain structure and function. Neuroimaging studies

3.3.1. Structural magnetic resonance imaging (sMRI): volumetry and morphometry studies

Early brain overgrowth is the most consistently replicated volumetric report in ASD [118]. Brains of a significant number (around 25-30%) of autistic children increase excessively in size between the first and second year of life, with a subsequent reduction or plateau, when compared with healthy controls [24]. Enlargement (coinciding with metabolic hypofunction) seems to be particularly marked in frontal regions and due for the most part to white matter (WM) increase [1,25,47]. The other area of probable precocious enlargement is the amygdala [1]; this seems to follow a different time course than in healthy controls, with respect not only to excessive early enlargement but also the subsequent plateau and absence of preadolescent enlargement seen in healthy subjects. Various possible mechanisms could underlie the enlargements observed, such as increased number, size or myelin content of neuroglia, increased elaboration of neuronal dendrites, axons or decreased pruning or inflammatory responses (described in both frontal and cerebellar regions) [30,121,23]. Other replicated findings are increased cerebellar hemisphere volume and reduced corpus callosum volume (CCV) [118,36]. More recently, additional morphometric measures focussing on the thickness, surface area, and curvature of the cortex have emerged. For instance, increased cortical thickness [32] and cortical folding [59] in parietal lobes have been reported in brains of children with ASD compared with healthy controls. These findings are more inconsistent in other brain regions.

Very few of these volumetric and morphometric abnormalities have been associated with core clinical features of ASD. One exception is caudate volume, which has been correlated with severity of repetitive behaviours [49]. This may be due to methodological and design limitations of these studies, including the use of small and heterogeneous samples with regard to age, sex, intelligence quotient cut-off, diagnostic criteria, etc., and also to the fact that ASD deficits are not always related to anatomical abnormalities, but often to functional abnormalities [99].

3.3.2. Functional MRI, DTI and neurophysiological studies. Autism as a “disconnectivity” disorder

Recent research paradigms in ASD are focussing more on impairment of specific brain networks instead of specific brain regions. In this sense, neuroimaging techniques such as functional magnetic resonance imaging (fMRI) (task-dependent, task-independent and resting-state fMRI) and diffusion tensor imaging (DTI) are being used to study functional and structural brain connectivity, respectively. fMRI studies focus on functional changes in anatomically intact brain regions, including local abnormal activation and abnormal functional connectivity between regions. DTI studies analyze the structural integrity of specific WM tracts, by measuring the diffusion of water molecules along the axons. DTI indices include, among others, fractional anisotropy (FA) and mean, axial and radial diffusivity indices, which quantify all directionality of water diffusion.

Using both techniques, a pattern of long-distance (i.e. frontal-posterior, and especially frontal-temporal [39]) “under connectivity” (i.e. less synchronously activated cortical areas and reduced FA of the WM tracts connecting those areas), together with local and short-range “over connectivity” (i.e. visual areas), have been consistently reported in brains of adult patients with ASD compared with healthy controls. Such evidence is more mixed in young patients [111,122]. This “disconnectivity” pattern would lead to a lack of effective integration of distributed brain regions and to a disruption in the modulation of brain function in relation to changing demands, and thus to ineffective complex, high-demand information processing [98]. It seems that the pattern of activation and timing or synchronization between different brain regions is the most impaired feature. Only in a few fMRI tasks, such as information processing tasks involving face and object recognition, does there seem to be an atypical location of activation [78].

DTI and fMRI findings have been associated with core clinical deficits of ASD. However, although there are several regions and networks showing abnormal patterns of activation or connectivity in patients with ASD [99] or their siblings [9] compared with healthy subjects, the individual contribution of each region and neural network to specific autistic deficits (e.g. social, language deficits and repetitive patterns of behaviours or interests) still
remains unclear, and these disconnectivity patterns have not always been correlated with the severity of the autistic phenotype [99]. One important limitation when analyzing data from neuroimaging studies is that most of the samples are restricted to high-functioning and adult autistic males [98].

Neurophysiological studies using techniques such as electroencephalography (EEG) or magnetoencephalography (MEG) have also shown this long-range brain “disconnectivity/dyssynchrony” pattern in patients with ASD [38,87,85,62].

Disconnectivity at the cellular level has been described in terms of abnormal neuronal wiring processes and abnormal synaptic functioning (supported by aberrant minicolumnar organization and the predominance of genes encoding synaptic formation and functioning within the candidate genes for autism). These aberrant neuronal processes, together with microglial activation, may lead to functional disconnection in association cortices and may, in turn, explain the lack of integration in information processing seen in autism [39].

3.3.3. Magnetic resonance spectroscopy (MRS) studies
Decreased N-acetylaspartate (NAA) in brain regions of patients with ASD compared with controls has been the most replicated finding in MRS studies [2] and has been associated with social deficits [37]. More recent studies have focused on the glutamate/glutamine (Glu/Gln) system, describing decreased Glu/Gln concentration in the right anterior cingular cortex (ACC) in patients with ASD compared with controls, together with reduced inositol concentrations in the left tempoparietal junction (TPJ) [13]. However, more MRS studies are still needed in the ASD research field.

3.4. Redox system and mitochondrial dysfunction
Recent evidence suggests that children with autism have reduced antioxidant capacity and may suffer from chronic oxidative stress [55,19]; both direct and indirect data of increased oxidative stress have been reported. Plasma reactive oxygen species (ROS) and antioxidant activity have been shown to be disturbed in autism spectrum disorders compared with healthy controls [53,116,131,94]. Oxidized glutathione, reduced cysteine and S-adenosylmethionine/S-adenosylhomocysteine ratio also support unbalanced redox equilibrium [55]. The abnormalities in methionine and glutathione metabolism found in autistic children have been shown to be shared by their parents, suggesting a genetic origin for these abnormalities [55]. Mitochondria are the main source of intracellular ROS through electron transport chain (ETC) activity, and mitochondrial dysfunction has been linked to autism [42]. ETC complexes I and II produce the free anion radical superoxide (O$_2^-$). Superoxide is converted to hydrogen peroxide (H$_2$O$_2$), which can react to generate hydroxyl radical (OH $^-$) or peroxynitrite (ONOO$^-$). Despite their known role in cell dysfunction and in aging-related neurodegenerative diseases, recent data also indicate that ROS play a physiological role in synaptic plasticity and learning and memory [74]. Their involvement in some cases of autism may be direct (as in the case of complex I disorders) [86]. In most cases, however, the involvement of redox imbalance may be a downstream effect of earlier or upstream abnormalities in other cellular processes. Causes of mitochondrial dysfunction found in ASD include lactic acidosis, carnitine deficiency, and various signs of abnormal beta-oxidation [91]. On the other hand, metabolomic studies targeting enzymes of the homocysteine (Hcy) metabolism pathway, a critical pathway in the regulation of normal redox homeostasis and cellular methylation potential, show that methylation capacity and glutathione-dependent antioxidant/detoxification capacity are both decreased in children with autism compared with controls [29]. Similarly, as mentioned above, genetic studies showing polymorphisms in the genes participating in this metabolic pathway confirm diminished methylation capacity in both autistic patients and their parents [55,54].

Ca$^{2+}$ homeostasis is a key regulator of mitochondrial function. Mitochondrial aspartate/glutamate carrier (AGC) is physiologically activated by calcium and an increase in AGC rates and Ca$^{2+}$ levels have been described in brains of patients with ASD. Abnormal neuroimmune responses may increase intracellular Ca$^{2+}$, driving oxidative stress and affecting synaptic functioning and neural connectivity [92].

3.5. Immune dysregulation
Several studies have shown how immune factors such as TNF,$\alpha$, IFN,$\gamma$, IL-1$\beta$ and IL-12 are increased in the peripheral blood of ASD patients [4,80,130]. Pro-inflammatory cytokines have also been shown to be increased in the cerebral spinal fluid (CSF) of autistic patients [121,20]. In addition, studies using protein arrays to explore the cytokine profile in postmortem brains of autistic patients showed evidence of a pro-inflammatory condition [69].

Microglia are the resident CNS immune cells, and recent studies define a role for microglia during postnatal development [110]. The hypothesis of microglia playing a role in psychiatric disorders is progressively gaining support. Their role in the progression and handling of insults to the nervous system and brain homeostasis has been demonstrated, mainly in studies using animal models [40,75]. Innate and adaptive peripheral immune abnormalities are particularly marked in low functioning children [5]. Epidemiological studies have shown a high rate of autoimmune disorders in the families of children with ASD [6,61]. Auto-antibodies against CNS proteins have also been shown to be increased in the plasma of children with ASD compared with controls, supporting the hypothesis of autoimmune mechanisms in autism [81,82,115]. There have been contradictory findings from studies of maternal serum hyperreactivity to fetal brain proteins [15]. Non-specific signs of chronic bacterial infections such as serum endotoxin levels have been shown to be significantly higher in severely affected adult autistic patients than in healthy controls, and inversely and independently correlated with socialization scores on standard instruments in patients with autism [33].

3.6. Oxytocin and other social neuropeptides
Animal models showing the relationship of oxytocin and vasopressin with social behaviour triggered the study of these neuropeptides in ASD. Specifically, the expression pattern of these peptide receptors in the brain seems to underlie the differences between two well-characterized types of rodents with extreme phenotypes in their social behaviour (the monogamous, sociable prairie vole and the promiscuous, socially aloof mountain vole) [44,129]. In subjects with ASD, low levels of oxytocin have been reported compared with controls [79], and specific SNPs of the oxytocin receptor gene have been shown to be associated with autism in different populations [52,125]. Preliminary clinical studies are showing the adjunctive effect of oxytocin in some psychotherapeutic interventions targeting social abilities in ASD patients [127].

3.7. Other biomarkers
Many findings with regard to physiological and molecular pathology have recently been reported in ASD. Most of the findings on disturbed metabolism are as yet biomarkers with unknown physiopathological significance. Apart from immune dysregulation or inflammation and oxidative stress, which are the areas where
the evidence is strongest [105], toxicant exposure and other systemic dysfunctions have been reported to be associated with ASD. Recent studies have implicated physiological and metabolic systems that transcend specific organ (brain) dysfunction [45].

Elevated platelet serotonin levels have been repeatedly found in about 30% of the children with autism [83]. The expression of 5-HT2A receptor in the duodenum of autistic children with gastrointestinal problems has also lent support to the hypothesis of serotonin dysregulation in autism [60]. In addition, elevated bufotenine (a molecule related to serotonin) levels have been found in the urine of ASD subjects [34].

Glutamate/GABA balance or excitatory/inhibitory balance is crucial for the functioning of the synapse. Multiple mechanisms may compromise the balance, some of which have been demonstrated in animal studies [76].

Impaired urinary excretion of organic acids and free amino acids, potentially indicating perturbations in different metabolic pathways, such as the tryptophan-nicotinic metabolism or the sulfur amino acid metabolism, have been shown in samples of autistic children [128]. Urine amino acid and organic acid testing is one of the per-protocol medical workups in many clinics evaluating children with autism, in order to search for treatable metabolic disorders associated with the ASD phenotype. However, the usefulness of such tests, in absence of clinical features of a metabolic disturbance is doubtful [12].

The plasma levels of multiple growth factors (GF) have been studied in ASD patients in comparison with matched controls. These include endothelial GF, platelet-derived GF, hepatocyte GF, and epidermal GF, and reduced plasma levels have been reported in different studies (generally with no clinical correlates). The effects of GF are considered to go beyond the stimulation of neural and other cell proliferation and growth, and also seem to function as immune modulators and be involved in crosstalk between the immune system and the central nervous system [51,57,88].

A significant minority of children with ASD shows functional digestive symptoms [16]. Thus, biomarkers related to digestive dysfunction have been explored. Some studies have shown an imbalance towards predominance of clostridium bacteria in the feces of children with regressive autism and also abnormal varieties of propionic acid, a short-chain fatty acid produced by these bacteria (and also a food preservative) [117,120]. Propionate has been given intraventricularly to rats, resulting in impaired metabolism of lipids and carnitines (both relevant for redox metabolism), and also autistic behaviours [120,71]. These rats also showed neuroinflammation and oxidative stress.

In 2002, Baron-Cohen reported that subjects with autism showed cognitive performance typical of an extreme male-female pattern [10]. ASD subjects showed an imbalance towards systemizing/empathizing tests compared with control subjects, the same pattern that healthy males in the normal population showed compared with females. The same investigator and others have subsequently conducted a series of studies showing increased intrauterine exposure to androgens in children performing badly on cognitive empathic ability tasks [11,64]. Epidemiological studies are now being conducted to determine if increased intrauterine androgen exposure confers a risk for ASD. In addition, conditions characterized by hyperandrogenism (such as polycystic ovary syndrome or congenital adrenal hyperplasia) have been studied, showing more autistic traits in women with these conditions and their mothers [124].

When an attempt has been made to systematically review one specific metabolic pathway, results are typically conflicting and methods very heterogeneous, complicating comparisons. Converging evidence from different research methods may help to elucidate matters. This is the case, for example, for the folate-methionine pathway [72]. In the latter case, biological validity support is given by the finding of reduced folate carrier (RFC1) G allele frequency among case mothers in a large genetic triads study [56]. Moreover, in a recent study, mothers of children with autism were less likely to have taken vitamins prior to conception. In addition, there was an interaction effect increasing the risk of autism with some variants of the MTHFR gene (coding for an enzyme that regulates folate metabolism), which is associated with reduced and inefficient folate metabolism and reduced methylation capacity [112]. Some biomarkers exemplify dynamic aspects that accompany ASD and some have been shown to be reversible in animal models, which make them particularly interesting as potentially treatable aspects of these disorders.

Attempts have been made to cluster together some of these biomarkers with other clinical features. Such an attempt was made by Sacco et al. (2010). The authors conducted a principal component analysis of more than 400 children with autism, introducing a variety of clinical and biochemical features. They describe four distinct components, namely “circadian & sensory dysfunction”, “immune dysfunction”, “neurodevelopmental delay” and “stereotypic behaviour”, together representing 74.5% of phenotypic variance of the sample. These components are claimed to be more related to disease processes than the diagnoses themselves, and to likely differ in genetic and immune underpinnings, developmental trajectories, and response to treatment [106].

3.8. Environmental factors

Plenty of potential environmental effectors have been studied with regard to their possible causal associations with autism [105]. So far, proof-of-concept evidence derives from studies that specifically link autism to exposure (thalidomide, misoprostol, valproic acid) early in pregnancy; maternal rubella infection; and the organophosphate insecticide, chlorpyrifos [67]. These environmental effectors account for a very limited number of the cases but are illustrative as examples of how external effectors (in likely interaction with genetic vulnerabilities) can initiate a pathophysiological cascade starting very early in prenatal life and ending with an autistic phenotype (sometimes with no major abnormalities in other body systems). Environmental factors such as pesticides, solvents, and so on are attractive candidates to explain part of the increase in ASD. However, it is difficult to study the effect of these compounds on autism risk. For many chemicals, air pollutants, and other toxins to which humans are now much more exposed than in the past, it is biologically plausible that they affect brain development. Some pesticides are known to impair maternal thyroid function during pregnancy; some alter the excitatory/inhibitory brain balance, and others are known to affect mitochondrial function, leading to neuroinflammation or oxidative stress [113]. In vulnerable periods of development, all or part of these mechanisms could trigger the pathophysiological pathways leading to autism. However, little is known about the thresholds of potential toxins. There are some animal models that show autistic-like behaviours after intracranial administration of environmental candidate effectors. However, exposure to any given chemical usually occurs in combination with others, making it important to study synergies and question the threshold of safe exposure. Thus the research is difficult, requires very big samples, and is still very under-developed.

3.9. Animal models

Rodents are the most common species used as an animal model for autism. At present the literature does not provide any single model that captures all three core symptoms of autism: social communication, repetitive behaviours and impairment in social
reciprocity [26]. However, several models are used for each of these core symptoms separately and provide insight into the mechanisms involved in autism physiopathology. Knock-out mice have shown the effect of single-gene abnormalities on development and behaviour. For example, mice knocked out for neuroligins show social interaction defects, memory impairment and repetitive behaviours in a review of current neuroligin mouse models [126]. In addition, a number of teratogenic agents have been given to mice, leading to behaviours that have been considered models for autistic behaviours. These teratogenic agents, which are initially foreign to the body, become part of the molecular landscape if they are able to bind to endogenous receptors or reach the intracellular space. Results are summarized in Table 1.

4. Discussion

Much data is accumulating regarding impaired biological mechanisms and their relationship with ASD. Such data comes mainly from four general approaches:

- initially, description of features associated with autism that indicated a biological substrate for the disorder; for instance, the presence of large cephalic perimeters in a percentage of cases, the existence of single-gene or single-pathway diseases with a frequent phenotype of autism (such as fragile X, tuberous sclerosis or Smitt-Lemli-Opitz syndrome), or the presence of multiple accompanying systemic disorders (such as epilepsy, sleep problems or possible gastrointestinal problems);
- research on the neurobiological basis of the core symptoms of ASD (such as social communication, social cognition, pragmatic language, stereotypies);
- study of the neurobiology of ASD as an entity or grouped into distinct phenotypes and establishment of associations between biological findings and particular phenotypes or associated features and;
- lastly, study (mainly in vitro and animal models) of the physiopathological pathways of candidate mechanisms identified in advanced data mining procedures such as GWA studies, proteomics, metabolomics and others.

The problem with the two first approaches is that their vantage point is consideration of ASD as a valid category, while its biological validity is a matter of great debate. The clinical/behavioural description of autism is increasingly considered more a common phenotypic endpoint of multiple deviant developmental pathways than a disorder or condition per se. If this is the case, the evidence derived from these two approaches can only give very limited information regarding the biology of these disorders.

Genetic studies converge in indicating a high genetic influence on autism, which is not always heritable. Complex autism with dysmorphic features is more frequently associated with an identifiable genetic abnormality than essential autism, but is less heritable. Common variants of the genome explain a small part of the variance of the autism risk. Rare variants such as CNVs explain a greater amount of genetic liability, particularly in the case of dysmorphic or intellectually disabled individuals. Many different genetic variants confer risk for autism and biological pleiotropy, with some genes conferring risk for different developmental disorders has been shown. The spatial and temporal coexpression and coregulation of different genes along brain development may partly explain why very different genes confer risk for the same outcome [119]. The most replicated findings of neuropathology studies are decreased cerebellar Purkinje neurons and cortex dysgenesis. Neurostructural findings are scarce and few have been replicated. Those that have been replicated (early brain enlargement) affect only a portion of patients with autism and certain

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<td>Pro-inflammatory status in ASD Innate and adaptive peripheral immune abnormalities and autoimmune markers in ASD and relatives</td>
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<td>Oxytocin and social peptides</td>
<td>Oxytocin receptors expression underlies social behaviour differences in animal models Low levels of oxytocin and genes coding for oxytocin are altered in autism</td>
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<td>Other biomarkers</td>
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<td>Environmental factors</td>
<td>Exposure to a number of drugs, viruses or toxins in critical periods of development increases the risk of autism The effect of other chemicals, air pollutants and toxins on brain development via impairments of excitatory/inhibitory brain balance, neuroinflammation or oxidative stress is biologically plausible</td>
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stages of development (early infancy). Either there are different structural findings for different types among the huge phenotypic heterogeneity of ASD or there is no common macroanatomic substrate for this condition. fMRI studies have demonstrated that, for specific skill deficits, there is reduced neural activity in regions that underlie the assessed functional domain. On the other hand, they have shown that distributed cortical areas are less synchronously activated in individuals with ASD during the performance of a variety of social and cognitive tasks and even at rest. These observations are consistent with long-range functional and structural connectivity in the cortex being broadly compromised in this disorder, with frontal and temporal cerebral connections being especially vulnerable [39]. New statistical modeling techniques will hopefully make it possible to model the contribution of different regions to larger functional networks and to associate specific genetic/molecular/neuropathological features with dysfunctional patterns in these networks [30].

Many of the findings regarding biomarkers come from pilot studies that are more hypothesis-generating than confirmatory. Much of the individual biomarker research comes from the same groups and most of the studies compare autistic samples with controls, without other psychiatric samples to compare the data. The findings thus are lacking in specificity to autism. Lack of replication of multiple findings in different samples or settings is a major limitation. Attempts to correlate biomarkers with clinical features have mainly failed, with a few exceptions (for example cortisol levels being partially correlated with sensory sensitivity [22]). Clustering methodologies may help associate biomarkers with clinical features, as in Sacco et al. [107]. Biomarkers are essentially dynamic and thus one way forward is not only replication by independent research groups but also a longitudinal/dynamic approach to biomarkers with repetitive analyses in the same children at different developmental points, age ranges, and genders, and in samples with different autistic phenotypes.

Altogether, immune findings support a pro-inflammatory molecular landscape in samples of ASD children, with an uncertain role in initiating or maintaining brain disease [93].

Integrating findings from different disciplines, it has been considered that aberrant pathophysiology (derived from genetic vulnerability and/or environmental triggers) could lead to a systemic (including brain) state of malfunctioning at the cellular/synaptic level responsible for the brain (somatic) and behavioural phenotype. Brain structural and functional abnormalities could then be considered a consequence and not the cause of the original deviant mechanism [46]. Excitatory/inhibitory chronic imbalance, impaired redox equilibrium, and aberrant immune/inflammatory states have been implicated. Their position in relation to the original (causative) problem is unknown. They have traditionally been placed downstream of the aberrant cascade of phenomena causing the autism phenotype (in a genetic-brain-autism behaviour approach). However, they are currently being studied as possible core mechanisms that are both a target of deleterious genetic/environmental influences and a cause of brain malfunctioning [19]. Given the interconnections among the metabolic systems involved (immune, inflammatory, oxidative, mitochondrial) any physiological abnormality in one could trigger abnormalities in the others [105]. This new approach is suggested to be a plausible explanation for electrophysiological disturbances that cause disrupted sleep or impaired sensory processing, in addition to other core symptoms of autism [46]. Savant skills or sensory hypersensitivity are other very specific features of autism that may have an explanation under the framework set by this new approach.

Data at the level of synaptic structure and functioning, in keeping with data informing about aberrant micro-organization of the cortex, abnormal neuromodulator functioning, with primary or secondary abnormal functioning of very general physiopathological pathways (such as inflammatory, immune or redox systems), could explain the very extensive and disparate manifestations characteristic of autism (from social communication to sleep or sensory regulation) [46]. Here there is a need to define molecular and protein signaling pathways that mediate not only abnormal but also normal development of language, social interaction and cognitive and motor routines [30]. Which molecular, cellular and neural-circuit-level phenotypes are more relevant for ASD is the present key challenge [119].

A coherent narrative of the neurobiology of autism is still a pending issue. Recent findings and new approaches are, however, shedding light on the issue. The conceptualization of autism as autism and the spectrum/dimensional approach are helping along the way. The deconstruction of a linear gene-brain-behaviour skeleton into multiple gene and/or environmental triggers and their interactions, which have an effect at multiple-levels (molecular, cellular, neural circuitry) leading to malfunctioning of the brain (and other systems), with chronic aberrant structural and/or dynamic modulatory states is also shedding light on the complexity and heterogeneity of autism phenotypes and the multiplicity of their outcomes.

Neurobiological findings sustain the view that the autism phenotype is the final expression of an early-onset brain conformation deviation with likely initiation in the first half of pregnancy in most cases. Evidence, however, is still patchy, and a complete theory of the key mechanisms that go from cause to outcome is still limited.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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