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Review Article

Is Genetics Implicated in the Causation of Autism?

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Abstract

Autism is a neurodevelopmental disorder first introduced by Kanner in 1943. Presently, a group of disorders collectively is considered to be 'Autism Spectrum Disorder' which is an outcome of complex interaction between genes and the environment.

In this investigation, authors considered the historical development of genetic study that revealed the secret of genes responsible for autism. They noted that synapse-related risk genes and susceptibility genes affecting transcription and chromatin-remodelling pathways have an important role behind the mechanism of the disease.

But there are environmental factors also. Currently 40%-80% of autism cases are related to some risk genes, but epigenetic, particularly environmental modifiers, like advanced age of father, gestational complications and infections, prenatal exposure to anticonvulsants, or paucity of oestrogen in developmental brain are also playing fundamental roles in heterogeneity of manifestations.

Till date, hundreds of genes have been recognized along with the environmental factors but how they interact to manifest the disease is still unknown due to complexities in interaction and overlap between different neuro-psychiatric disorders.

Presence of second level modifiers has made the situation more multifaceted. However, advancements in sequencing technology, analyzing software, and expansion of databases have made the study of genetic modifiers possible.

Going through the works by various scientists across the globe over the last 75 years, authors have identified a number of chromosomes, multitudes of gene loci, many genetic overlaps, and myriads of modifiers - endocrinal, environmental, and epigenetic. But, at the same time they have warned that though genes play a pivotal role, the other factors also need to be considered and studied in detail with proper attention and finally synthesis all the information reasonably to discover the root cause of autism.

Keywords: Autism; ASD; Neurodevelopmental Disorders; Neuro-psychiatric Disorders; Genetics; Epigenetics

Introduction

Autism, as first coined by Eugen Bleuler and first described in 1943 by Kanner in his detailed report with thorough patient history of 11 children showing different symptoms of autism, is a neurodevelopmental disorder with impairment of social and communicational behaviours; often narrated as 'mind blindness' in lay media.

23 years after Kanner, the first epidemiological study estimated to be 4.5 per 10,000 individuals; but increasing awareness and evolvement of Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria, drastically increased it to 1 per 59 individuals with M:F as 4-5:1 [1], but 3:1 [2]. With increase in autism diagnoses, there's a decrease in the number of children considered 'cognitively disabled' or 'learning disabled', suggesting a relabeling of children's disorders.

An Autism Spectrum Disorder (ASD) diagnosis can co-occur with numerous other conditions, due to the broad nature of these definitions; most commonly including motor abnormalities (79%), gastrointestinal problems (up to 70%), epilepsy (up to 30%), intellectual disability (45%), and sleep disorders (50–80%) [3]; even with language disorders as per the DSM-IV criteria.

According to the current DSM-5 criteria, only two core features make up an Autism Spectrum Disorder (ASD) diagnosis are persistent deficits in social communication and interaction (SCI) across multiple contexts, and restricted, repetitive patterns of behaviour (RRBs), interests, or activities [3].

Observing monozygotic concordance—prevalent in some families—effect of high paternal age, gestational comorbidities in mothers and even accusing 'refrigerator mother' [4], or relating to the contamination of vaccine with heavy metal like mercury, pathophysiology of autism has long been a wild goose to chase with in the bushes from chromosome to gene loci to epigenetics to environmental influences, so on and so forth. Plethora of overlaps among many neurodevelopmental disorders and neuro-psychiatric illnesses in childhood also makes the issue more complicated to understand. With the help of advanced tools in biotechnology, molecular biology and genetics, like micro array, array-hybridization, SNP, CNV, etc (described in the glossary), instead of unveiling the curtain off compel us to find out the actual causation of autism. In order to put many similar ailments together, an umbrella-term 'Autism Spectrum Disorder' has been coined. ASD, now recognized as a disease of complex interaction between genetics and the environment, has heritability estimating from 40% to 80% [5].

Along with extensive genetic studies revealing hundreds of genes linked to autism, epidemiological investigations have begun to illuminate the environmental factors contributing to risk, but how the two interact to contribute to ASD aetiology is still unknown.

Drastically varying phenotypes can be seen in subjects with similar pathogenic variants, as in cases of complex diseases [Cook., *et al*. 1997; Bolton., *et al*. 2001]. Factors modulating expression of other genes, i.e. genetic modifiers are likely to exist in individuals having the same pathogenic variant on opposite spectral ends.

Objective of the Study

In this perplexity, the present study is intended to move across different studies on the subject over last few decades, and to present them in an assertive way with the objective to find out a single common aetiological basis of autism.

Genetics of Autism Spectrum Disorder

Seeking for candidate ASD genes

Understanding the disease aetiology of ASD, following Kanner's classification of autism, has a paradigm shift from the initial environmental theory to the subsequent genetic theory over the observations of 50x higher incidence among the twins [Folstein., *et al*. 1977], and 60% concordance among monozygotic twins against null in dizygotics [Bailey., *et al*. 1995], risk of ASD having proportional sharing of genome with affected siblings or parents [6- 8].

Early karyotypic study focused on the specific gene loci [Gillberg., *et al*. 1985], like7q, 1p, 3q, 16p, and 15q [7,9,10], was followed by candidate approach to study specific gene families, e.g. Hox family and Wnt gene; but both turned futile [Krebs., *et al*. 2002; Lamb., *et al*. 2002; Talebizade., *et al*. 2002; Zhang., *et al*. 2002].Since 2001, there was moderate success with findings supporting reelin (RELN), aristaless related homeobox (Arx), methyl-CpG binding protein 2 (MeCP2), neuroligin 3 (NLGN3), neuroligin 4 (NLGN4), tuberous sclerosis complex 2 (TSC2), and ubiquitin protein ligase E3A (UBE3A)'s involvement in ASD aetiology [Perisco., *et al*. 2001; Strømme., *et al*. 2002; Carney., *et al*. 2003; Jamain., *et al*. 2003; Serajee *et al*.,2003; Jiang., *et al*. 2004].

High Throughput Study (HTS), in early 2000s, yielded genome-wide level studies for sequencing of involved genome and found wide variation of genomic involvements in average number of cases, except for few monogenic involvements in specific ASDs like Rett syndrome, fragile X syndrome, tuberous sclerosis, and Schuurs–Hoeijmakers syndrome [11,12].

Although myriads of genomic study identified hundreds of risk genes, yet majority of the reproducible hits were found having two functional group of proteins:

- Synapse-related risk genes, encoding cell-adhesion proteins such as neuroligins, neurexins, and cadherins; synaptic vesicle cycling proteins synapsin-1 (SYN1) and synapsin-2 (SYN2); ion transport proteins such as sodium voltage-gated channel alpha subunit 2 (SCN2A), calcium voltage-gated channel subunit alpha1 E (CACNA1E), calcium voltage-gated channel auxiliary subunit beta 2 (CACNB2), potassium voltage-gated channel subfamily Q members 3 and 5 (KCNQ3 and KCNQ5), potassium voltage-gated channel subfamily D member 2 (KCND2), glutamate receptor signalling protein SH3 and multiple ankyrin repeat domains 3 (SHANK3), synaptic Ras GTPase activating protein 1 (SYNGAP1), and gamma-aminobutyric acid type A receptor gamma3 subunit (GABARG3) [13-15]; and de novo mutation of such genes [Turner., *et al*. 2016; Turner., *et al*. 2017; Short., *et al*. 2018].

- Susceptibility genes affecting transcription and chromatin-remodelling pathways like MeCP2, UBE3A, chromodomain helicase DNA binding protein 8 (CHD8), activity dependent neuroprotector homeobox (ADNP), pogo transposable element derived with ZNF domain (POGZ), fragile X mental retardation protein (FMRP), and RNA binding forkhead box (RBFOX) genes [15,16].

ASD risk in somatic mosaicism

Post-zygotic mosaicism is often organ-specific, not ubiquitous in the whole soma, due to of 5%-7% de novo variation, which have also been reported to estimate upto 22% [17], and daily about 5 single nucleotide variants (SNV) during neurogenesis [Bae., *et al*. 2018; D'Gama., *et al*. 2018]; are mostly innocuous; but mutation in exon might have serious neurodevelopmental disorder with manifestation of ASD including Rett syndrome, tuberous sclerosis, intellectual disability, schizophrenia, and many other disorders [18,19].

Being progressed from case-based study of ASD [Oliveira., *et al*. 2003; Sauter., *et al*. 2003; Papanikolaou., *et al*. 2006; Havlovicova., *et al*. 2007; Yurov., *et al*. 2007; 64; 65; 66], through study on somatic mosaicism explaining 3%-5% cases of autism simplex [Freed., *et al*. 2016; Krupp., *et al*. 2017], to large cohort-based instrumental study (n = 5947) [17] involving Whole Exome-Sequencing (WES) yielded that critical exon-related mosaicism may result in neurodevelopmental abnormalities of either amygdala, related to difficulty in social communication [Rasia-Filho., *et al*. 2000], or cerebellum, related to gait disorder in autism [Dou., *et al*. 2017]. Freed and Pevsner through a large wave study (n = 2388) of affected families, not only identified ascertainment bias for pathogenic mosaic variations in ASD, but also confirmed implicated candidate gene *SCN2A* and some more risk genes, and also ascertained the significance of somatic mosaicism in causation of ASD [17].

Contributions of CNVs in susceptibility of ASD

Only few [20] of either inherited or de novo mutation [Thapar., *et al*. 2013] in CNV, by duplications, deletions, translocations, and inversions, even stretching several kb, [Marshall., *et al*. 2008] may result in 10% of ASD cases [Geschwind, 2011].

Studies on CNV, majorly implicating duplication of 16p11.2 and alteration in most of the 25 genes there, are related to abnormalities in neuro-development [Blaker-Lee., *et al*. 2012] and plurality in manifestations due to multigenic involvement, and KCTD 13 (potassium channel tetramerization domain containing 13) is implicated in most of the neuro-psychiatric disorders [Golzio., *et al*. 2012], might be due to aberration in synaptic transmission though Ras homolog family member A (RHOA) [Escamilla., *et al*. 2017]. Deletion in 16p11.2 region leading to altered MAP3 kinase activity results in deformed cortical cyto-architecture and reduced brain size [Pucilowska., *et al*. 2015]. Study on 16p11.2 in Drosophila using RNAi by Iyer (2018) strongly indicated that modifying interactions within CNVs result in the complex phenotypes, not involving any single gene like 16p11.2 [Iyer., *et al*. 2018].

CNVs in other disease mechanisms are less often studied due to the inadequacy of commonly affected regions. 15q11-13 and 16p11.2, the most prevalent ASD-associated CNVs, are found in roughly 1% of autism cases [Kumar., *et al*. 2008; Marshall., *et al*. 2008; Weiss., *et al*. 2008; Marshall., *et al*. 2012], and also there remain no known CNVs with complete penetrance [Marshall., *et al*. 2008].

Deletions in synaptic genes, such as SHANK3, dipeptidyl peptidase-like 10 (DPP10), neuroligins, and neurexins in autism [21], duplication of CNV in the locus containing the UBE3A gene in autistic traits in mice, and decreased glutamatergic synaptic transmission [Smith., *et al*. 2011] are also seen in some non-ASD/schizophrenic controls with dyslexia and dystaxia exhibiting same structural changes [Stefansson., *et al*. 2014].

A microarray analysis with identified CNVs in ASD-associated genes, showed a positive correlation between duplication size and autism severity, but no correlation with non-verbal IQ. CNV often

contributes to ASD risk critically with complexity, but there are highly variable phenotypes for similar structural variants in patients.

ASD under epigenetic regulation

Study with 215 candidate genes demonstrated 19.5% epigenetic modifier in causation of ASD [22]; similarly another study with risk gene also confirmed it. Phenotypic variance in ASD thus can be explained on the basis of nuclear penetrance of epigenetic modifiers. Twin study demonstrated profound epigenetic modulation of disease phenotype as in 50 pairs of monozygotic twins discordant for ASD reported numerous autism-associated differentially methylated regions, with methylation patterns at some CpG sites common to symptom groups [Wong., *et al*. 2014]. KMT2C, lysine methyltransferase 5B (KMT5B), and lysine demethylase 6B (KDM6B); chromatin remodelling proteins including MeCP2, CHD8, and POGZ; RNA-binding/splicing proteins such as FMRP and the RBFOX family, post-translational modification proteins like UBE3A, mindbomb E3 ubiquitin protein ligase 1 (MIB1); or transcription factors like ADNP and additional sex combs like 3 (ASXL3) are the genes with susceptibility loci involving methylation [16]. These are implicated in autism by altered synaptic formation. Mutations in a single epigenetic regulator modifying number of risk genes were studied in depth at 2 key susceptibility genes, MeCP2 and UBE3A. MeCP2 normally regulates synaptic function at the genomic level of GABRB3, brain derived neurotrophic factor (BDNF), distal-less homeobox 5 (DLX5), insulin like growth factor binding protein 3 (IGFBP3), cyclin dependent kinase like 1 (CDKL1), protocadherin beta 1 (PCDHB1), protocadherin 7 (PCDH7), and lin-7 homolog A (LIN7A) [23], but consistently modifies chromatin in ASD patients. Also, there is some alteration in the rate limiting regulation of Glutamergic synapse formation by MeCP2 in ASD pathology [Chao., *et al*. 2010].

UBE3A (an E3 ubiquitin protein ligase), lying in 15q11-13 and being duplicated in autism, epigenetically reduce excitatory synaptic transmission and is having positively correlated dose-dependent effect on delay of first word and psychomotor regression [Guffanti., *et al*. 2011; Smith., *et al*. 2011; Xu., *et al*. 2018]. UBE3A and one of its substrate proteasome 26S, non-ATPase 4 (Rpn10), leads to pathological alteration of dendritic fine outgrowth in autism [Hamilton., *et al*. 2012; Puram., *et al*. 2013]. Several studies on large scale have found different epigenetic regulation in ASD like alteration in histone, acetylome wide association [Sun., *et al*. 2016].

Altered methylation in the proline rich transmembrane protein 1 (*PRRT1*) 3' UTR, promoter regions of tetraspanin 32 (*TSPAN32*), and *C11orf21* in cortical tissue, and also in succinate dehydrogenase complex flavoprotein subunit A pseudogene 3 (*SDHAP3*), in cerebellar tissue [Ladd-Acosta., *et al*. 2014]; dysregulated miRNAs related to oxytocin receptor (OXTR) gene [Behnia., *et al*. 2015]. Abnormal cerebellar Purkinje growth due to epigenetic alteration in engrailed homeobox 2 (EN2) [James., *et al*. 2013]. These risk genes with epigenetic functions and their substrates are the focus of present day therapeutics, e.g. inhibition of FMRP target bromodomain containing 4 (*BRD4*) in alleviating some of the disease manifestations [Korb., *et al*. 2017].

Overlapping of ASD risk genes with other diseases

Cross-Disorder Group of the Psychiatric Genomics Consortium (PGC) in 2013 conducted a very large scale epidemiologic study with 33332 cases and 27888 controls and found multiple cross-overlaps in the risk genes, e.g. inter-alpha-trypsin inhibitor heavy chain 3 (ITIH3), arsenite methyltransferase (AS3MT), calcium voltage-gated channel subunit alpha1 C (CACNA1C), and CACNB2, amounting to genome-wide significance in several neuro-psychiatric disorders, like ASD, schizophrenia, bipolar disorder, ADHD (OR AD/HD), and major depressive disorder [24,25]; having strong correlation with structural variants in loci of dedicator of cytokinesis 8 (DOCK8) and KN motif and ankyrin repeat domains 1 (KANK1), and phenotypic variations in these disorders [Glessner., *et al*. 2017]. Schork *et al*. (2019) [26] after using GWAS to identify susceptibility loci in genes including phosphodiesterase 1A (PDE1A), protein phosphatase 1 regulatory inhibitor subunit 1C (PPP1R1C), RHOA, immunoglobulin superfamily member 11 (IGSF11), and sortilin related VPS10 domain containing receptor 3 (SORC3), recently hypothesized that a mechanism of shared risk is due to abnormal gene regulation in radial glia and interneurons during mid-gestation. Shared risk loci in FMRP targets, CHD5, CHD8, SCN2A, and neurexin 1 (NRXN1) are implicated in shared phenotypes of ASD, intellectual disability (ID), schizophrenia [Issifov., *et al*. 2014; Wang., *et al*. 2019]. Increased incidence of de novo pathogenic variants in periodic circadian regulator 1 (PER1) and lysine methyltransferase 2C (KMT2C) was found to be related to commonalities across ASD, ID, and bipolar disorder [Wang., *et al*. 2019]. Due to sharing of risk loci [Pinto., *et al*. 2010; McCarthy., *et al*. 2014], ASD and ID is found to coexist in 45% cases [3].

'Second-hit' to 23 susceptible gene loci including dopamine receptor D2 (DRD2), cholinergic receptor nicotinic alpha 7 subunit (CHRNA7), 5-hydroxytryptamine receptor 2A (HTR2A), solute carrier family 6 member 3 (SLC6A3), and tryptophan hydroxylase 2 (TPH2) in relation to dopamine and serotonin homeostasis was considered as a possible mechanism of shared features in ASD, bipolar disorder, and schizophrenia [27].

Increased prevalence of childhood autism in offspring of schizophrenic parents, and also early onset schizophrenia in autistic children as reposted by Rapoport., *et al*. (2009) and Sullivan., *et al*. (2012) [28,29], were supported by The Autism Spectrum Disorders Working Group of The Psychiatric Genomics Consortium (2017) [30] with the findings of shared risk loci including several genes involved in neurodevelopment, e.g. forkhead box P1 (FOXP1), exostosin glycosyltransferase 1 (EXT1), astrotactin 2 (ASTN2), mono-ADP ribosylase 2 (MACROD2), and histone deacetylase 4 (HDAC4).

There are high degree overlaps between autistic traits and ADHD (OR AD/HD) probrands [31], also sharing of susceptible gene loci like 7q36, 16p13, 18p11, 15q24, and 12q24 [Nijmeijer., *et al*. 2010], and sharing of gene loci for nicotinic receptor signalling pathway and cell division [Martin., *et al*. 2014].

Although Angelman disease occurs due to deletion of and autism due to duplication of ubiquitin ligase gene UBE3A, there may be coexistence of both the disorder in the same person [Peters., *et al*. 2004; Williams., *et al*. 2010; Smith., *et al*. 2011; Kalsner., *et al*. 2015; Yi., *et al*. 2015].

The shared disease mechanism is yet to be perceived due to the complexities in interaction and overlap between neuro-psychiatric disorders.

Modifying factors in ASD

Genetic factors

Despite extensive studies, including CNVs and SNPs, to find specific genetic loci behind autism nothing conclusive has yet been achieved, probably due to effect of some second modifiers either at the somatic cell levels through 'Two-hit mechanism' like retinoblastomas proposed by Knudson, or by 'two-locus model' at germline level, as in Hirschprung disease [Knudson, 1971; Fisher., *et al*. 1994; McCallion., *et al*. 2003].

Artuso., *et al*. (2011) used array-hybridization technique to study genome of 8 cases of monogenic Rett syndrome of ASD, and identified 15 "likely" and 14 "unlikely" modulators of RTT phenotypes. Although, in CNVs or epigenetic regulation in discordant monozygotic twins revealed potential alterations in methylation patterns in a case and anomalies in another at 2p25.3 region [Bruder., *et al*. 2008; Kunio., *et al*. 2013; Rio., *et al*. 2013], study with 100 twins failed to reveal discordant phenotype through CNV [Stamouli., *et al*. 2018]. Finding SHANK2 pathogenic variants in individuals with/without neuropsychiatric disease suggested the presence of additional variants in causing disease. Three cases with de novo SHANK2 mutations having deletions of CHRNA7 and cytoplasmic FMR1 interacting protein 1 (CYFIP1) – implicated in ASD – supported a "multiple-hit" model of autism [Leblond., *et al*. 2012].

An analysis revealed that 19 out of 20226 patients with CNVs in contact in 6 (CNTN6), a probable gene involved in ASD and other neurodevelopmental disorders [Repnikova., *et al*. 2019]. But, CNTN6 was found to have inconclusive role in causation of ASD. Specific 16p12.1 micro-deletion had a less severe phenotype in comparison to random second variants [Girirajan., *et al*. 2010]; and 22q11.2 deletion syndrome – all haploinsufficient for an mGluR network gene – found that 20%, co-diagnosed with autism, had second-hit pathogenic variants [Wenger., *et al*. 2016]. Bonnet-Brilhault., *et al*. (2016) assessed a family with ID and ASD due to NLGN4X pathogenic variants, and found individuals with naive ASD having second-hit variants in glycine receptor beta (GRLB) and ankyrin 3 (ANK3).

Continuing advancements in sequencing technology, analyzing software and expansion of databases is laying the foundation to noteworthy developments in the study of genetic modifiers.

Epigenetic and environmental factors

Currently 40%-80% of autism cases are related to some risk genes, but epigenetic, particularly environmental modifiers, like advanced age of father, gestational complications and infections, or prenatal exposure to anti-convulsants, are playing prime roles in heterogeneity of manifestations [32]. Among the anti-convulsants, Valproate has been studied most, and thought to be a gene modifier by inhibiting histone deacetylase to cause autism [Kataoka., *et*

al. 2013], through apoptotic cell death in the neocortex, decreased proliferation in the ganglionic eminence, increased homeobox A1 (HOXA1) expression, abnormal serotonergic differentiation via Achaete-Scute family BHLH transcription factor 1 (ASCL1) silencing, disrupted serotonin homeostasis in the amygdala, dendritic spine loss, reduced prefrontal dopaminergic activity, and disruption of the glutamatergic/GABAergic balance [33].

Impact on the transcriptome of an organism is based on epigenetic link between environmental risk factors and genetic susceptibility might be explored through more GWAS to understand the common ASD epigenomes.

Pro-inflammatory states involving cytokines and interleukins and also microglia could have some physiological and metabolic aetiogenesis of ASD [34,35]. Even dysbiosis of intestinal microbiome is also considered to alter immune function, leading to physiologic impact on learning, memory and behaviour; ultimately to ASD risk [Cao., *et al*. 2013; Bilbo., *et al*. 2015; Zhang., *et al*. 2015]. Beside cytokine related immune disruption, phosphocreatine-related mitochondrial dysfunction are having positive correlation with the severity of ASD manifestations [35]. Although causal role of such association are yet to be accertained [36].

Many of the recent studies suggest equal contribution of both genetic and non-shared environmental factors in aetiology of ASD [Roland., *et al*. 2011; Sandin., *et al*. 2014; Colvert., *et al*. 2015]. Smoking, alcohol intake, intake of valproate and SSRIs, exposure to environmental chemicals, like pesticides, metals, bisphenol A, during gestational period are considered to be major exogenous environmental factors leading to epigenetic changes through altered DNAm or DNMT expression/activity in the aetiogenesis of ASD [37-41]. But researchers found it extremely difficult in accurately estimating the levels and timings of exposure in the mother and more critically and the foetus [36]. Among the endogenous environmental factors, major contributors are considered to be paternal age, might be maternal age also, preterm birth, maternal infections and autoimmune disorders, gestational diabetes mellitus (GDM), assisted reproductive technologies (ARTs), hypoxia-related obstetrics complications. Besides this, maternal stress and maternal nutrition are also considered to be important in the aetiology of ASD. Although there are some biologically plausible explanations behind these propositions, but the role of these factors are yet to be ascertained precisely [36]. Preterm labour, multiple births are found to be associated with significant gain of methylation (GOM) in OXTR. Also some epigenetic changes are noted in OXTR, SHANK3, BCL2, apoptosis regulator (BCL2) and RORA through altered DNAm; and hence are related to ASD cases [Leavey., *et al*. 2013; Atladottir., *et al*. 2016]. ART (IVF, ICSI) are related to preterm labour, multiple births, low birth weight, and all of these factors are independently found to be related to ASD [42,43]. Loss of methylation (LOM) is found to be in increased frequency subsequent to application of ART, which goes against the proposition of increased ASD cases following ART [44,45]. Hence, contribution of ART in causation of ASD is controversial [Conti., *et al*. 2013; Schieve., *et al*. 2015]. Paternal age is related to de-novo mutations in ASD probands at an escalated number; [Sanders., *et al*. 2012] and DNAm alterations are observed both in sperms and oocytes [Ge., *et al*. 2015; Milekic., *et al*. 2015]. Hypoxia-related obstetrics complications have significant toll on ASD risks with effect estimate more than 1.4 [46]. Infections during pregnancy [Atladottir., *et al*. 2016; Brown., *et al*. 2012; Lee., *et al*. 2015] and autoimmune disorders [Chen., *et al*. 2016] are having Odds Ratios of 1.24- 1.37 and 1.34 respectively related to increased prevalence of ASD. Maternal obesity and GDM have increased epigenetic alteration of DNAm and hence are considered to be related to causation of ASD, but no convincing data are there to validate such claims [36,47,48]. Maternal stress, assessed through general and social communication scores, were found to be associated with altered DNAm of OXTR, a recurring ASD-risk gene of interest [Rijlaarsdam., *et al*. 2016]. But, specific GxE interaction failed to confirm such association [36]. Poor maternal nutrition is also considered to be of paramount importance, and it is supported by the fact that folate supplementation helps in reducing the methylation of particular DNA through OCM-SAM pathway; and thus attenuating the ASD risk by OR: 0.61 [Schmidt., *et al*. 2012; Schmidt., *et al*. 2013; Suren., *et al*. 2013].

Studies with large sample sizes are required to elicit reflection of ASD-environment association of stable epigenomes detectable in foetus and neonates, and specific GxE correlation.

Sex-linked factors

ASD is said to affect males more than females, probably due to more external manifestations in male (aggression or increased repetitive behaviour vs. depression and avoiding demands), likely

being influenced by hormones or genetics [49]. Autism in male manifests at a lower mutational burden [50]; even mothers of autistic boys have more mutation burden, particularly in 15q11-13 duplication [Cook., *et al*. 1997; Schroer., *et al*. 1998; Gurrieri., *et al*. 1999; Boyar., *et al*. 2001] related to $GABA_A$ receptor causing perturbation of GABA signalling in autism [Al-Otaish., *et al*. 2018]. Protective action of oestrogen [Macrì., *et al*. 2010; Hoffman., *et al*. 2016], male predominant expression of chromatin regulation and immune response [51], male-specific down-regulation of *MeCP2* leading to abnormal glutamate activity [Kim., *et al*. 2016] might explain male vulnerability to, if not protection in female from, autism.

Reduced aromatase CYP19A1 activity leading to less conversion of testosterone to oestrogen in adolescent brain of ASD patients [Sarachana., *et al*. 2011; Crider., *et al*. 2014] might be related to diminished RAR activity involving RAR-related orphan receptor A (RORA) gene in susceptible male with autism [Nguyen., *et al*. 2010; Sarachana., *et al*. 2011] by more sex-dependent dysregulation of RORA gene [Hu., *et al*. 2015].

Transcriptomes, with enriched ECM molecules, in female mice might have a protective effect. Sex hormone probably impart divergent modulation [52] in sex-related ASD pathology [Estes., *et al*. 2015; Koyama., *et al*. 2015; Kim., *et al*. 2017; McCarthy., *et al*. 2017; Nadeem., *et al*. 2019] through their contrasting effect on immune system [53]. Analyzing biomarkers from individuals with Asperger's syndrome, Schwarz., *et al*. (2011) found 24 male-specific and 17 female-specific hits, including many immune-related molecules. Testosterone [Hatanaka., *et al*. 2015] also affects spine density, another phenotype strongly implicated in autism [54,55].

Key neurotransmitters, implicated in ASD, like GABA, glutamate, serotonin, and BDNF are modulated by sex hormones [Kim., *et al*. 2016; Saghazade., *et al*. 2017; Al-Otaish., *et al*. 2018; Edwards., *et al*. 2018; Ferri., *et al*. 2018; Garbarino., *et al*. 2018; Zieminska., *et al*. 2018].

Whether sexually dimorphic phenotypes in ASD are modulated by sex hormones or by some other modifiers, are yet to be established through intensive studies in the field.

Conclusion

After having an intensive sojourn through all the works by various scientists across the globe over last 75 years, we have identified a number of chromosomes, multitudes of gene loci, many genetic overlaps, and myriads of modifiers— endocrinal, environmental, and epigenetic. But, all these cannot be funnelled through a single common pathway, or even a specified channel, related to the causation of autism or even ASDs. More intensive studies using more intricate tools, even yet to come out, will have to be undertaken to cognize the arena of genetic background behind ASD.

But, the studies up till now are worthy enough. Through these studies we could identify susceptible and risk genes, their metabolic implications and possible therapeutic approaches to address those, either in ameliorating the manifestations or in preventing the occurrence of autism or ASDs. A lot more studies are sought for to enrich our knowledge in pharmacogenetics related to ASD.

Last, but not the least, to visit www.thetransporters.com which helps children to recognize emotions. Baron-Cohen and his Cambridge University colleagues collaborated with Britain›s National Autistic Society and a film production company created an animated series of vehicles for children, usually fond of toy carriages. In such a manner, toy trains, trams, trucks with human faces could display various facial expressions appropriately to the situation. Watching these programs for a long time at their bedrooms, autistic children gradually got tuned up with these expressions. With such intervention over a tangible period of time, autistic children could even react to different expressions like their non-autistic peers in the schools. This implies that specially designed training programs can induce social connectivity among the autistic children. Simultaneously, it raises a pertinent question; is autism or ASD is a special type of psycho-social developmental delay? And this question evokes another possibility of explaining autism as a phenomenon of delay in mirror neuron development.

To conclude, it can be said that genetics is highly implicated in, but not solely related to the causation of autism.

Glossary

(Table 1)

Angelman syndrome	Angelman syndrome is a genetic disorder resulting in delayed development, problems with speech and balance, intellectual disability, occasional seizures, and ataxic gait. The subjects have happy and excitable personalities with frequent smiles or laughs.
Array Hybridization	Array comparative genomic hybridization (aCGH), which is also known as microarray-based comparative genomic hybridization, is a genome scale molecular cytogenetic technique for the detection of changes in the chromosomal copy number.
ASD	Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition that is associated with persistent challenges in social interactions, verbal and non-verbal communications, and restricted repetitive patterns of behaviour.
Asperger's syndrome	Asperger's syndrome (AS), also known as Asperger's, is a neurodevelopmental disorder characterized by significant difficulties in social interaction and nonverbal communication, along with restricted, repetitive patterns of behaviour.
Autism	Autism is a non-progressive, but persistent, neurological disorder, which onsets before the third year of life, and significantly affects both verbal and non-verbal communications and social interactions.
Complex genetics	A term used to explain genetic inheritance pattern resulting from the interactions among many genes and also with the environment, where the resulting phenotypes are not readily explainable by Mendelian genetics.
Concordance	The rate at which a second individual, such as the second sibling of a pair, or the second twin, has the same phenotype as the first.
Copy Number Variation (CNV)	A copy number variation refers to the variation in the number of copies of a particular gene from one individual to the next.
De novo	Anew. A biological process or entity that has begun again. De means "from" and novo means "new".
DSM	Diagnostic and Statistical Manual of Mental Disorders (DSM) developed by the American Psychiatric Association in collaboration with other groups of mental health professionals published the first edition of the DSM (DSM-I) in 1952. Six editions, including DSM IV TR, have yet been published .The DSM-5 (2013) [the roman numerals are no longer used] is the official psychiatric coding system used in the United States. The organization of disorders in DSM-5 attempts to follow the lifespan. Thus, neurodevelopmental disorders that occur in early life are listed first in the classification system, and neurocognitive disorders that occurred toward the end of the life are listed last.
Endophenotype	A heritable phenotypic feature that is closely related to a disorder, but represents a more narrow or simple component than the disorder itself. An endophenotype should be observed in first degree relatives more frequently than in the general population and may be quantitative rather than binary.
Epigenetic	Beyond genetics. Changes in DNA methylation pattern without changing the base sequences of DNA; or post-translation modifications on Histone proteins - usually methylation or acetylation- that results in chromatin compaction and hence the state of gene transcription is called epigenetic changes.
Fragile X syndrome	Fragile X syndrome (FXS) is a genetic disorder caused by mutations in the FMR1 (fragile X mental retardation 1) gene and characterized by mild-to-moderate intellectual disability. The FXS individuals do not synthesise the FMR1 protein, which is required for normal brain development. Males are more affected than the females. Despite having various degrees of ID, there may be gaze avoidance, attention deficit, shyness, social anxiety, and even fits in some cases.
GWAS	Genome-wide association studies associate specific genetic variations with particular diseases. In this method large scale scanning of the entire genomes of many different people identify genetic markers that can be used to predict the presence of a disease.
Heritability	This refers to the extent to which a particular trait is solely due to the variation in the inherited DNA sequence and not due to any contribution from the environment. A heritability of 1.0 indicates that a trait is 100% heritable.
Homeobox	A group of genes that co-ordinately regulates the development, cell-differentiation and morphogenesis of a multi-cellular organism. It was first discovered in the fruit fly Drosophila.

HTS	High throughput sequencing (HTS), also next generation sequencing (NGS), is a comprehensive term used to describe technologies that sequence multiple DNA and RNA molecules in parallel, enabling hundreds of millions of DNA and RNA molecules to be sequenced at a time in a very cost-effective manner. It is in contrast to Sanger's low throughput sequencing.
ID	Intellectual disability (ID), once called mental retardation, is characterized by below-average intelligence or mental ability and a lack of skills necessary for day-to-day living. People with intellectual disabilities can and do learn new skills, but they learn more slowly. There are varying degrees of intellectual disability and limitations in adaptive behaviour, from mild to profound.
Mendelian inheritance	A dominant or recessive pattern of inheritance first defined by Gregor Johann Mendel.
Microarray	Microarray analysis technique is a high-throughput hybridization approach capable of providing information on the genome wide transcriptome or proteome profiles.
Penetrance	For a specific genotype the extent to which a particular phenotype is observed is called penetrance. Penetrance is always relative to the phenotype in question.
Pleiotropy	When a gene influences more than one phenotype.
Polymorphism	A gene is said to be polymorphic if more than one allele occupies the same genetic locus within a population.
Proband	A person who serves as the starting point of a genetic study of a family.
Rett syndrome	Rett syndrome (RTT), also RTS, cerebroatrophic hyperammonaemia, is rare genetic mutation affecting brain development in females that typically becomes apparent after 6–18 months of age, with symptoms including impairments in language and coordination and repetitive movements, besides having slower growth, difficulty in walking, and a smaller head size.
Schuurs-Hoeijmakers syndrome	Schuurs-Hoeijmakers syndrome is a rare disease characterized by intellectual disability and dysmorphic facial features among various physical abnormalities due to PACS1 mutation.
SNP	The substitution of a single nucleotide at a specific position in the genome when present in a sufficiently large fraction of the population is known as single-nucleotide polymorphism.
SNV	A single-nucleotide variant is a variation in a single nucleotide without any limitations of frequency. SNVs may arise in somatic cells.
Somatic Mosaicism	Somatic mosaicism refers to the simultaneous occurrence of two genetically distinct populations of cells within an individual. In contrast to inherited mutations, somatic mosaic mutations may affect only a portion of the body and are not transmitted to progeny.
Tuberous sclerosis	Tuberous sclerosis is a rare disease that causes tumours, or growths, in the brain and other organs. These growths can occur in the skin, kidneys, eyes, heart, or lungs. They are usually benign (non-cancerous). Along with hypopigmented macules, facial angiofibromas, growths under or around the nails, pitted teeth, coughing or shortness of breath, symptoms of tuberous sclerosis also include mental disabilities, developmental delays and ASD.
Two-hit Mechanism	The Knudson hypothesis, also the two-hit hypothesis, is the hypothesis that most tumour suppressor genes require both alleles to be in activated, either through mutations or through epigenetic silencing, to cause phenotypic change. This hypothesis is also applied in explaining any neuro-psychiatric disorders including schizophrenia
WES	Whole genome or whole-exome sequencing refers to the sequence information of the entire genome or its protein coding regions (exome). It utilizes the high-throughput next generation sequencing technique.

Table 1

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Conflict of Interest

The authors had no monetary interest or conflict of interest while developing this review article.

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