Involvement of myocyte enhancer factor 2c in the pathogenesis of autism spectrum disorder

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ABSTRACT

Myocyte enhancer factor 2 (MEF2), a family of transcription factor of MADS (minichromosome maintenance 1, agamous, deficiens and serum response factor)-box family needed in the growth and differentiation of a variety of human cells, such as neural, immune, endothelial, and muscles. As per existing literature, MEF2 transcription factors have also been associated with synaptic plasticity, the developmental mechanisms governing memory and learning, and several neurologic conditions, like autism spectrum disorders (ASDs). Recent genomic findings have ascertained a link between MEF2 defects, particularly in the MEF2C isoform and the ASD. In this review, we summarized a concise overview of the general regulation, structure and functional roles of the MEF2C transcription factor. We further outlined the potential role of MEF2C as a risk factor for various neurodevelopmental disorders, such as ASD, MEF2C Haploinsufficiency Syndrome and Fragile X syndrome.

1. Introduction

Neurodevelopmental disorders (NDDs) are extremely intricate brain deformities that are marked by an inability to meet normal social, cognitive, and motor developmental thresholds [1]. The term “NDD” has been extended to a diverse variety of mental illnesses involving some form of dysregulation in tightly orchestrated early embryonic events that led to brain development [2]. NDDs have a heterogeneous etiology that significantly contributes to impaired cognitive, poor communicative, and abnormal motor skills [3]. Autism spectrum disorders (ASDs), intellectual disability (ID), learning, vision, and hearing impairments, attention deficit hyperactivity disorder (ADHD), epilepsy, schizophrenia, and cerebral palsy are only a few examples of NDDs [2, 3, 4, 5, 6].

ASD is a complex neurodevelopmental condition linked with impaired cognitive abilities, poor communication skills, repetitive behaviors, and limited interests [7]. In 1943, Dr. Leo Kanner, an American psychiatrist, first published a paper on eleven of his patients at the Baltimore clinic entitled “Autistic Disturbances of Affective Contact” [8]. However, the term “autism” was first proposed in 1911 by a Swiss psychiatrist, Eugen Bleuler, who described the peculiar characteristics displayed by a schizophrenic patient [9]. The prevalence rate of ASD is about 16.8 per 10,000 (one in 59) children aged 8 years [10]. According to the World Health Organization (WHO), about 1-1.5% of children worldwide suffer from ASD [11, 12]. Besides that, around 31% of individuals with ASD have intellectual impairments [10] and 20%-37% of them had epilepsy condition [13, 14]. In general, ASDs are divided into two categories: syndromic – such as Fragile X Syndrome (FXS) [15], Rett syndrome (RS) [16] and tuberous sclerosis (TSC) [17] and non-syndromic. Furthermore, ASD has often been followed by psychological or medical conditions, including ADHD, depression, anxiety disorders, sleep disturbances, and gastrointestinal problems [18, 19, 20].

Numerous models of ASD pathogenesis and etiology have been postulated so far. It is, however, believed to be significantly linked to the interrelationships between environmental and genetic risk factors [21, 22].

The myocyte enhancer factor 2 (MEF2) transcription has been shown to influence ASD-associated gene expression and studies have also showed that autistic traits are triggered by impaired activity-dependent regulation of synaptic development [23, 24]. Such a transitory shift in the regulatory mechanism could have significantly altered the signaling of MEF2 as a potential cause [25]. The MEF2 family of transcription factors in vertebrates yields four members: MEF2A, MEF2B, MEF2C, and MEF2D. These MEF2 members are strongly conserved and critical for a number of functions in a variety of different cell types, such as cellular differentiation and extracellular stimuli response. The significance of MEF2 factors in the central nervous system (CNS) for neural cell survival, synaptic plasticity, and memory development is reinforced by the discovery that disruptions in MEF2 factors trigger heritable neurological pathologies [26, 27]. Moreover, stimuli important for neuron formation
and synapse maturation, such as growth factors and synaptic processes, may influence the regulatory mechanism of MEF2 family members [28].

Considering the importance of MEF2 in the neural system, it is of considerable interest to elucidate how MEF2 factors regulate neuronal-specific gene expression. Nonetheless, gaining a thorough understanding of MEF2 transcriptional mechanisms in the neural system is still hard to comprehend, primarily owing to the challenges of investigating gene expression mechanisms in the large and highly diverse neural cell populations, in addition to the overlaps in MEF2 expression profiles across the neuronal network.

The MEF2 proteins are essential for the optimal growth and efficient processing of the neural system. They have been found to regulate the process of neuronal differentiation [29, 30, 31, 32], neuronal migration [33], activity-dependent cell survival [34, 35, 36], dendrite formation and remodeling [37, 38, 39], and axonal guidance and pruning [40]. Gene expression studies also reveal a large number of MEF2-regulated genes involved in neural excitability, as well as synapse development and functioning [41, 42, 43]. MEF2 synapse elimination tends to happen only in developing or mature neurons. The rate of synapse elimination declines as neurons mature, but the rate of elimination remains high [44, 45]. Despite the fact that nothing much is interpreted regarding the cellular mechanisms and signaling pathways that govern synapse formation and curtailment rates during development. This is quite plausible to rely on an activity-dependent system that perceives when synaptic connections are mature and then prevents further synapse development and sustains curtailment rates [46]. MEF2 factors are triggered by neuronal depolarization and successive calcium ion (Ca^{2+}) influx, which stimulates the Ca^{2+}/Calmodulin (CaM) effectors, calcineurin, CaM kinases, and the protein phosphatase [28, 47]. The expression of a variety of proteins, including FMRP (fragile X mental retardation protein), Arc (activity-regulated cytoskeleton-associated protein), calcineurin, RNA-binding proteins, PCDH10 (protocadherin 10), mGluR1/5 (group I metabotropic glutamate receptors), NR4A1 (nuclear receptor subfamily 4 group A member 1), potentially Homer 1 and MHC1 (major histocompatibility complex class I) has been linked to MEF2-induced excitatory synapse elimination [23, 24, 42, 48, 49, 50, 51, 52, 53]. Synaptic modulation mediated by MEF2s may be synapse-specific as they function upstream of proteins, such as Arc and Npas4 (neuronal PAS domain protein 4) that can selectively regulate specific synapses within a particular cell [42, 54, 55]. The MEF2 proteins regulate the expression of multiple genes, many of which are essential for neuronal growth and differentiation, either directly or indirectly [41]. Taken together, this indicates that the reduced activity of MEF2C throughout early development has a strong impact on neural growth and development as well as neurotypical behaviors.

Human genomic experiments have highlighted the transcription factor MEF2C as a risk gene for a multitude of neurological conditions. Using human genome-wide association studies (GWAS) and genomic sequencing studies, it has been identified that MEF2C is a potential risk gene for a wide variety of mental illnesses, such as ASD [56], bipolar disorder [57, 58], major depressive disorder [59] and ADHD [60, 61]. A GWAS analysis of 74,046 people showed that the SNP (single-nucleotide polymorphism) rs1909982 close to the MEF2C region is connected with an increased risk of late-onset Alzheimer’s disease [62]. In several such studies, the effect of disease-linked SNPs on MEF2C expression or activity remains mostly unclear, but it highlights the significant characteristics of MEF2C in hale and healthy human brain. MEF2C Haplosinsufficiency Syndrome (MCHS) is a neurodevelopmental disease that is caused by microdeletions at chromosome location 5q14.3 that contain the MEF2C gene or point mutations within the MEF2C protein-coding region [63, 64, 65]. The MCHS has been associated with ASD, ID, absence of speech, and numerous motor abnormalities, including hyperactivity, and schizophrenia that are believed to be precipitated by deficits in primary phases of neuronal development [53, 64].

2. The MEF2 family of transcription factors

The MEF2 family belongs to the MADS (minichromosome maintenance 1, agamous, deficiens and serum response factor)-box evolutionary conserved family of transcription factors which are essential for cellular growth and differentiation in a wide number of tissues, including the brain [66]. It was originally discovered in muscle tissues, but it was later reported to be differentially expressed in neurons throughout the multiple brain regions [67, 68].

MEF2 is a single gene in fly (Drosophila melanogaster), worm (Caenorhabditis elegans), and yeast (Saccharomyces cerevisiae), while in vertebrates four distinct isoforms of the MEF2 family are identified, which are programmed by distinct genes and named as MEF2A, MEF2B, MEF2C and MEF2D (Figure 1) [69]. The MEF2 gene in Drosophila melanogaster is found on chromosome 2 locus. According to prior studies, MEF2A, MEF2C and MEF2D in mice are situated at chromosome location 7, 13 and 3, respectively, while MEF2B situated at chromosome 8 and it is not linked to other MEF2 genes, the intron-exon association of the MEF2B gene is identical to that of the other vertebrate MEF2 genes and the single Drosophila melanogaster MEF2 gene, indicative of the fact that these different MEF2 genes have been transformed from a universal ancestral gene. In humans, MEF2A, B, C and D are reported to be positioned at chromosome locations 15q26, 19p12, 5q14 and 1q12-q23, respectively [70, 71] (Table 1).

MEF2 proteins are associated with a number of other different factors, such as the N-terminal 56-amino acid sequence known as MADS-box, the minimal region which is responsible for sequential DNA-binding and protein dimerization. The MADS domain is a term for the earliest members of the protein family described: the yeast mating type regulator MCM1 (minichromosome maintenance 1); the plant floral determinants Agamous and Deficiens/Apetala 3; and the animal protein serum response factor (SRF) [83, 84]. Throughout many species, it is a significantly conserved structural motif that governs growth and differentiation processes. A 29-amino-acid-long MEF2 domain resides next to the MADS-box, mediating DNA binding and homo-and heterodimerization with many other MEF2 proteins [85]. The MADS-box proteins typically bind adenine(A)/thymine(T) rich DNA sequences, while MEF2 binds preferably to the consensus sequence 5′-CC(A/T)(T/A)AAATAG-3′. Both the MADS-box and the MEF2 domain are crucial for DNA binding, but neither has transcription activity of its own. MEF2 proteins have a distinct transactivation domain at the C-terminal that promotes interactions with a variety of co-factors, including co-activators, such as p300 and acetyl-transferases CBP (CREB-binding protein), or co-repressor, such as class II histone deacetylases (HDACs) and NCoR (nuclear receptor co-repressor)/SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) co-repressor complex. The C-terminus region of MEF2 is particularly subjected to alternate splicing dynamics and has a comparatively low level of amino acid similarity between the different MEF2 isoforms [86, 87, 88]. During the maturation phase of neurons in the CNS region, the four distinct isoforms of MEF2 proteins are expressed differentially in the frontal cortex, thalamus, hippocampal, cerebellum, midbrain, hindbrain, and olfactory bulb regions. MEF2A, C, and D mRNAs are abundantly expressed in the embryonic cortical neurons of rats, but MEF2B proteins are only transcribed by the MEF2C gene in the developing brain first among many other MEF2 isoforms [89, 90, 91, 92].

MEF2D isoform is expressed in proliferating glial and neuronal cells during development, i.e., expression of MEF2D isoform is enhanced in neurons following growth and differentiation and declines in matured glial cells [93]. In apoptotic cerebellar granule neurons (CGNs), all four MEF2 protein isoforms are highly expressed, but only MEF2A/D proteins have been found to be phosphorylated [34]. The MEF2C isoform is expressed at the initial stages of embryonic brain development and continues to be expressed at excessive levels in adult brains, including the...
striatum, hippocampus and cortex, indicating potential roles in embryonic and adult brain activity [67, 89]. Out of the four MEF2 proteins, MEF2C is the most predominant form of MEF2 in the developing cortex. The expression of MEF2C begins to appear on day 17 of the embryo and peaks at day 21. The level of expression of MEF2C in differentiating neurons can be detected by the presence of MEF2C in the cortical plate [94]. In previous studies, it was shown that MEF2C plays a fundamental role during the developmental phase of several lineages, including the CNS and craniofacial system [95, 96]. Several human genetic research have implicated MEF2C isoform as a crucial component in NDDs, including ASD [41, 42]. In mice, deletion of MEF2C in neural progenitor cells resulted in reduced brain masses, fewer mature neurons, and substantial behavioral deficits [33]. MEF2C is also expressed in microglia [97, 98]. Microglia is a population of tissue-resident macrophages, and are resident immune cells of the brain that control formation and pruning of synapses during early brain development [99, 100]. Furthermore, in comparison to other tissue-resident macrophages, MEF2C has been recognized as a critical transcription factor for microglia [101]. Microglia play a role in a variety of brain processes, including synapse development and elimination, longevity of oligodendrocyte precursor cells, networking of the corpus callosum, and phagocytosis of several other brain cells [102, 103, 104, 105]. Microglial cells were described as an essential players of brain growth and development [106]. Thus, disruption of these microglial cells might portray a major role in the pathology

![Figure 1. A schematic illustration of the sequence homology of the MEF2 transcription factors family from fly (Drosophila melanogaster), worm (Caenorhabditis elegans), yeast (Saccharomyces cerevisiae) and human (Homo sapiens). The percentage homology of amino acids within the three domains, namely MADS, MEF2 and TAD is standardized by human MEF2A isoform for different organisms. The N-terminal is to the left and the C-terminal to the right. Adapted from “MEF2: a central regulator of diverse developmental programs”, Potthoff MJ, Olson EN, Vol. 134, Development. 2007. p. 4131–40 [95].](image)

Table 1. Salient characteristics of the different MEF2 transcriptional factors.

| Gene Name | Gene Type | Cytogenetic Locus | Protein Name | Interacting Proteins | Major Molecular Functions | Associated Diseases | References |
|-----------|-----------|-------------------|--------------|----------------------|--------------------------|---------------------|------------|
| MEF2A     | Protein coding | 15q26.3 | Myocyte Enhancer Factor 2A | HDAC1, HDAC3, HDAC4, HDAC5, HDAC7, HDAC9, 14-3-3, TB, MAPK7, MAPK14, BPTF, CABIN1, EP300, CREBBP | • Muscle development  
• Neuronal differentiation  
• Cell growth  
• Apoptosis | Coronary artery disease 1 with Myocardial infarction (ADCAD1) | [72, 73, 74, 75, 76, 77] |
| MEF2B     | Protein coding | 19p13.11 | Myocyte Enhancer Factor 2B | CABIN1, BPTF, HDAC4, HDAC6, HDAC9, 14-3-3, EP300, CREBBP | • Development and maintenance of various tissues, such as cardiomyocytes, skeletal muscle cells, and neuronal cells | Brachydactyly, Type E1 and Mantle Cell Lymphoma. | [71, 73, 74, 75, 78] |
| MEF2C     | Protein coding | 5q14.3 | Myocyte Enhancer Factor 2C | HDAC4, HDAC5, HDAC7, 14-3-3, CABIN1, EP300, NOTCH1, GATA4, TWIST2, CREBBP, MAPK7, MAPK14, SMAD2, CDK1, SP1, HIPK2, IFRD1 | • Controls neurogenesis and myogenesis  
• Vascular development  
• Healthy neuronal growth, propagation, and electrical activity in the neocortex region  
• Development of anterior heart field and neural crest  
• Craniofacial development | ASD, ADHD, ID, Epilepsy | [27, 73, 74, 75, 79] |
| MEF2D     | Protein coding | 1q22 | Myocyte Enhancer Factor 2D | HDAC1, HDAC3, HDAC4, HDAC5, HDAC9, 14-3-3, MAPK7, MAPK14, EP300, CREBBP, SP1, CARM1, S1PR5, CASP7, BPTF, HIRA, CABIN1, 14-3-3 | • Regulation of neuronal survival and apoptosis  
• Regulate muscle and neural cell growth and differentiation | Brachydactyly, Type E1 and Migraine with or without aura | [74, 75, 80, 81, 82] |
of multiple NDDs, including ASD. In sum, this implies that MEF2C is a key element in the development and regulation of the CNS as well as plays a prominent role in several neurodevelopmental diseases.

3. Structure of MEF2C

The MEF2C gene is located at chromosome location 5q14.3 [107]. Three transcriptional initiation sites with variable 5′-UTRs are marked and the MEF2C gene comprises up to 13 exons and the primary transcript is alternatively spliced [108]. To date, a total of six human transcript variants have been annotated: NM002397, NM001131005, NM001193347, NM001193348, NM001193349, NM001193350 [65]. The MEF2C gene comprises three alternative exons: the mutually exclusive exons a1 and a2, the inclusion/skipping exon β and the 3′ splice site region γ. The mutually exclusive alternative splicing takes place in the exons a1 and a2 directly adjacent to the MEF2 domain. The a2-MEF2C isoform is expressed predominantly in striated muscle tissues, while the a1-MEF2C variant is expressed in other different tissues [109]. Exon β, positioned at TAD II (transcriptional activation domain II), is a type of cassette exon. β exon in the second transactivation domain has increased transactivation activities and is expressed in several neural tissues [109, 110]. The 3′ splice site selection-type of alternate splicing, the γ region, is situated in the terminal coding exon of the MEF2C gene. It was revealed that the γ region encodes the transcriptional repression domain (Figure 2) [108,109].

MEF2C is a 186 kb (kilobase) long gene encoding a member of the MADS-box MEF2 family of proteins [111]. The human MEF2C protein is made up of 6 domains and contains 473 amino acids, namely MADS, MEF2, HJURP-C (holliday junction recognition protein C-terminal), NLS (Nuclear localization sequence) and TAD (tRNA-specific adenosine deaminase) 1 and 2 (Figure 2). The MADS domain contains 56 amino acids at MEF2C’s N-terminus. This area is strongly conserved and contains a number of A/T base pairs. This region’s primary function is to aid dimerization, DNA binding, and co-factor interactions [84]. The MADS domain comprises 56 amino acids, the MEF2 domain begins at amino acid 57 to 86 and the HJURP-C domain contains a total of 30 amino acids, although the stereo-structure of the remainder of the domains remains unclear [112]. The MADS and the MEF2 domain are quite necessary to instigate DNA binding and dimerization, whereas other domains function as transcriptional activators [113]. Studies have confirmed that the hydrophobic furrow on the MADS-box domain of MEF2 generated by leucine66, tyrosine69, and threonine70 and delimited by helix H2 and the flexible linker between H2 and β is crucial to facilitating transcriptional co-activators or co-repressors factors like class I1a HDACs [114], Cabin1 (calcineurin binding protein 1) [115], MyoD (myoblast determination protein) [116], p300 [117] and MASTR (MEF2-activating SAP transcriptional regulator) [116]. Hydrophobic residues in such members, for example leucine in HDAC (histone deacetylase) 4 and 9, Cabin1 and a phenylalanine in myocardin-related transcription factor, MASTR induce insertion into the groove and lead to interaction with MEF2 [116]. High affinity synthetic compounds were shown to inhibit the recruitment of transcriptional factors to MEF2 [118,119]. Moreover, the HJURP-C domain, which has 30 amino acids, is located next to the MEF2 domain. TAD1 and TAD2 are transcriptional activation domains that complement the HJURP-C domain and are accountable for the activation of transcriptional processes [74, 120]. The NLS domain is positioned at the C-terminal of the MEF2C gene and regulates the translocation of proteins in the nucleus [73].

4. Functional roles of MEF2C

According to previous research, MEF2C was associated in a number of differentiation and developmental activities, such as myogenesis, neurogenesis, synaptic development, craniofacial and neural crest development, the growth of the anterior heart region, chondrocyte hypertrophy and vascularization, endothelial cell proliferation and survival [33, 95, 122], in addition, they have also been associated with the occurrence of different forms of cancer [123]. MEF2 proteins are abundant in neurons and have disparate expression profiles in different parts of the brain, with the strongest concentrations in the cerebellum, cerebral cortex and hippocampus [85, 92]. MEF2C safeguards neural cells from apoptotic cell death, implying a significant function in memory and learning.

Figure 2. A graphical illustration of the MEF2C structure alignment [A] Protein domains and their sizes. There are six domains in MEF2C: MADS, MEF2, HJURP-C, TAD1 and 2 and NLS from N-termini to C-termini. [B] Structure of conserved vertebrate exon exhibiting alternate splices with classification following Ganassi et al. (2014) [121]. Gray boxes represents alternate donor/acceptor splice site. Vertical black lines represent exon boundaries. [C] The conservation of domains is represented by color intensity. Species showing conserved regions are listed below: Homo sapiens (Hs), Danio rerio (Dr), Terebratalia transversa (Brachi), Drosophila melanogaster (Dm), Caenorhabditis elegans (Ce), Podocoryne carnea (Pc), Nemastrela vectensis (Nv).
Craniofacial impairment and even child death can result from the dysfunction of the MEF2C gene in neural crest cells [124].

In both humans and mice, high levels of MEF2C gene expression have been identified in skeletal muscles, cardiac muscles, and the brain [79, 125]. During the development of the mouse CNS, high levels of expression have been found in the embryonal cerebral cortex, amygdala, hippocampus, olfactory bulb, midbrain, and cerebellum regions, plus in the adult cerebellum, hippocampus, thalamus, frontal cortex, and dentate gyrus (Figure 3) [89]. The expression profiles and transactivation activities of alternatively spliced MEF2C transcripts differ considerably, with some have been shown to be brain-specific [79, 107, 113]. The conditional homozygous deletion of mouse MEF2C in radial glial cells during late embryogenesis period and expression of the active form of MEF2C in neurons revealed that MEF2C contributes to hippocampal-dependent memory and learning by limiting the quantity of excitatory synapses and thereby governing evoked and basal synapse transmission [126]. In particular, another group revealed that mice with a conditional MEF2C knockout in neural progenitor cells had aberrant accumulation and compression of neurons traveling to the lower tiers of the neocortex throughout the developmental process [33]. It resulted in reduced brain masses with fewer, less developed neurons later in life, resulting in irregular electrical activities in cells and tissues and acute behavior disturbances close to those observed in murine models of Rett syndrome-like altered paw-clasping and anxiety [33]. Studies have also identified that MEF2C, when activated, directs the growth of neurons from murine stem cells [29]. This clearly demonstrates the prominent characteristics of MEF2C in early neural differentiation as well as provides a relationship to Rett syndrome.

MEF2C has been described to comply with many different co-factors and to stimulate the expression of several genes, and some of those are themselves accountable for ID. Evidence has reported that fragile X mental retardation protein (FMRP) is needed to facilitate MEF2 proteins to eradicate excitatory synapses in the hippocampus neurons [127]. Expression profiles in hippocampal neurons of rats have exhibited that MEF2 also regulates multiple transcripts, such as DIA1 (diaphanous-related formin 1), PCDH10, and UBE3A (ubiquitin-protein ligase E3A) in which shortcomings are known to induce NDDs like autism, ID, and epilepsy [25, 42]. However, in spite of the potential of MEF2C as a critical activity-dependent regulator of neuronal activities, its expression profiles and functional status were only ascertained in selected regions of the brain. Genetic research findings have established critical features of MEF2 in cerebral cortex and hippocampal neurons, and the implications of MEF2 gene deletion for cerebellum development have been studied or documented by various different experiments [34, 36, 92, 128].

In the CNS, sensory stimulus triggers the stimulation of MEF2 factors and many other activity-regulated transcription factors which, consecutively, stimulate the expression of various genes necessary for development and remodeling of synapses [129]. By carrying out a number of genome-wide experiments in hippocampal regions during the development of synapses, Flavell et al., classified around 180 activity-regulated MEF2 target genes which responsible for a multitude of different characteristics of synapse activities, including inhibitory synapse development, excitatory synapse maturation and excitatory synapse strengthening and weakening [42]. A number of MEF2 target genes were shown (for e.g. igt1; leucine-rich, glioma inactivated 1, argef9; Cdc42 guanine nucleotide exchange factor (Gef) 9, kcnal1; potassium voltage-gated channel, shaker-related subfamily, member 1, ube3a; ubiquitin protein ligase E3A, slc9a6; solute carrier family 9 (sodium/-hydrogen exchanger), member 6, pcdh10; protocadherin 10, c3orf58; chromosome 3, open reading frame 58) to increase propensity to neurological diseases in human populations, such as ASD and epilepsy, implying that such disorders could be triggered at least partly by interference of activity-dependent gene mechanisms that regulate synaptic or neural circuit development [42]. Moreover, their methodology for identifying MEF2 target genes exhibited that neural activity facilitates the usage of alternative sites of polyadenylation at several MEF2 target genes, resulting in activity-dependent generation of truncated mRNAs and proteins which may represent distinct roles than their pre-existing, full-length variants [42]. In sum, their study disclosed that the activity-regulated MEF2 factors regulate maturation of synapses during brain growth and development.

**4.1. Functional role of MEF2C in neuronal differentiation**

The expression of MEF2 members typically starts when a neuron commences to differentiate, signifying that MEF2 portrays a potential
part in this process [81, 85, 89, 93]. MEF2C is the most predominant isoform of the four MEF2 proteins expressed in the developing cerebral cortex region. The expression of MEF2C is evident on embryonic day (E) 17 and peaks around E21 [36]. MEF2C was found to be predominantly expressed in the cortical plate regions though not discernible in the ventricular zones, indicating that it is specifically expressed in differentiating neurons [36]. Members of the MEF2 family perform an essential part in nerve cell differentiation, as it was exhibited to facilitate the neuronal gene expression in P19 embryonal carcinoma cells, along with the neurogenic bHLH (basic helix-loop-helix) transcription factor MASH1 (mammalian achaete scute homolog-1) [130]. MEF2C might have a neurogenic role in murine embryonal stem cells [29], and stimulate the production of neurons in hESC (human embryonic stem cell)-derived neural stem progenitor cells [30]. In a study, Mao et al. found that cells expressing the neuronal marker TUJ1 (β-tubulin type III) express MEF2C, while cells expressing the glial marker GFAP (glial fibrillary acidic protein) do not express MEF2C [36]. This indicates that MEF2C expression is strictly limited to neurons and that MEF2C is essential for neuronal differentiation. Nevertheless, possible compelling evidence suggesting that MEF2 is essential in differentiation of neuronal cells emerges from research of MEF2C-conditional knockout murine models in radial glial progenitors. Cortical layer abnormalities and deficiencies in neural cell maturation were observed in these conditional knockout models [33]. However, mice with an identical absence of MEF2C gene in premature neural progenitors have not been recognized as possessing neural maturation deficits, rather late developmental synapse deformities [126]. Although such synapse deformities may be inferior to more prominent primary deficits in neural maturation, such findings continue to be ambiguous.

Another major factor culpably involved in neuropsychiatric and neurodevelopmental conditions is disparity in the equilibrium between inhibitory and excitatory activity of neurons [131, 132]. The MEF2 members are transcribed in both inhibitory and excitatory neurons during development and adulthood [28, 33]. According to several experiments, loss of MEF2C causes an upsurge in inhibitory cortical synapse connectivity while a decline in excitatory cortical synaptic connectivity means that MEF2C acts as a master regulator of both inhibitory and excitatory synaptogenesis activities in cortical neurons [41, 133, 134]. MEF2C, a transcription factor linked to ASD, has been reported to identify early parvalbumin (PVALB) precursors from other medial ganglionic eminences (MGE)-derived interneuron types [134]. PVALB neurons are fast-spiking interneurons that express the calcium-binding protein PVALB. They are further classified into two subtypes: (1) basket and (2) chandelier cells in the cortex region [135]. PVALB neurons have become quite popular over the past few years due to their increasing role in neurodevelopmental disorders, including ASD [136]. PVALB neurons drive their activity via extensive axonal arborization, primarily inhibiting proximal dendrites and cell soma of their post-synaptic targets [137], PVALB cells are biologically engineered to provide rapid, robust and effective inhibition of their post-synaptic cells [138]. These cells have also been found to be correlated with plasticity and learning [139, 140]. Oftentimes, PVALB transcript and PV protein concentrations were found to be reduced in ASD patients’ brain. Mayer et al. reported that MEF2C-conditional deletion in inhibitory neurons leads to a particular deficiency of PVALB interneurons by P20 in cortical layers 2–6, indicating that MEF2C is an imperative element for growth and development of healthy populations [134]. In sum, these findings suggest that MEF2C is an important MEF2 factor and plays a potential role in early cortical synaptic growth, and its embryonic depletion triggers behavioral phenotypes reflective of multiple neurodevelopmental disorders, including ASD.

4.2. Functional role of MEF2C in neural circuit development

The research of MEF2 transcription factors in the nervous system has now been exaggerated to evaluate their function in the generation and development of neural circuits in vivo. In general, this entailed the alterations of MEF2-associated activities in selected regions of the brain and the recognition of changes in behavior linked to disparities in density of dendritic spines, which are neural entities that correspond with the involvement of excitatory synapses. According to a study in mice, prolonged use of cocaine suppresses MEF2A/D expression in the nucleus accumbens (NAC) region. This repression results in an upsurge of dendritic spine concentration that may possibly silence the sensitized drug responses. Moreover, upregulation of the constitutively active protein MEF2-PV16 (a fusion between MEF2 and viral transcription factor VP16) suppresses the upsurge in dendritic spine concentration and enhances the behavioral sensitivity to the drug by disrupting this reaction [141]. In sum, their findings reported that MEF2 acts as a fundamental regulator of structural synaptic plasticity.

Members of the MEF2 family were also associated with memory formation by modulating spine growth and development [75]. The analysis of negative or constitutively active MEF2 transcription factors in addition to loss-of-function studies has revealed an underlying concept of how MEF2 plays a part in memory formation. The formation of memory corresponds to inhibitory phosphorylation of MEF2A/D at S408/S444 residues that causes an increase in spine development which is typically associated with memory formation [142]. Increasing the activity of MEF2 factors by expressing MEF2-PV16 inhibits the upsurge in spines, and, subsequently, the generation of new memories. In the hippocampus, amygdala, and anterior cingulate cortex, this mechanistic pathway has been linked to spine development and memory formation [142, 143]. In addition, experiments have shown that MEF2-dependent spine regulation is largely reliant on the MEF2 modulation of its formerly discovered gene Arc [42, 142]. By increasing the endocytosis function of AMPA-type glutamate receptors (AMPARs), Arc limits their surface expression [144], resulting in reduced synapse potency and synaptic depletion, analogous to homeostatic synaptic scaling or long-term depression [145, 146]. Cole et al. observed that in mice with markedly elevated levels of MEF2 in the amygdala regions, the consequent disturbance of AMPAR-mediated endocytosis (using an inhibitory peptide) redeemed the MEF2 associated alterations in fear based memory [142]. Huber and colleagues, on the other hand, suggested a rather completely different mechanism for MEF2-mediated synaptic density modulation, though it is still associated with regulation of AMPAR expression [48]. They demonstrated that the stimulation of MEF2 in hippocampus neurons has contributed to an upsurge in the production of PCDH10, a protein that is actively involved in the ubiquitination and lysosomal targeting of the synaptic scaffold protein PSD-95 (postsynaptic density protein 95). Amplification of PCDH10-mediated PSD-95 degradation in hippocampal neurons inhibited both MEF2-mediated PSD-95 degradation and MEF2-mediated synapse removal. PSD-95 links AMPARs and other different proteins at the synapse, and lowering functional synaptic PSD-95 causes synaptic AMPARs to diffuse out of the synaptic cleft and endocytosis of AMPARs [147, 148]. Thus, this is quite plausible that MEF2 controls AMPAR expression and synaptic integrity by diverse cellular processes. Based on these findings, it was theorized that MEF2 usually impairs memory formation by accelerating the expression of Arc that afterwards curtails the expression of AMPARs, resulting in disruption and removal of synapses.

Considering the impacts on memory observed across specific brain regions, it is anticipated that memory dysfunction will also be seen in brain-specific conditional MEF2 knockout models. The hippocampus is connected with memory formation, which brain-specific deleterious regulations contribute to synaptic number regulation [28]. Brain-specific MEF2A/D deletions showed no deformity in memory formation [149]. However, multiple studies have revealed that the brain-specific MEF2C knockout disrupts hippocampus-dependent memory and learning via boosting the number and transmission of synapses [30, 126]. In a study, Barbosa et al. concluded that MEF2C promotes context-dependent fear conditioning, which is a prominent feature of hippocampus-dependent memory and learning formation during development, by repressing the amount of
excitatory synapses and thereby influencing basal and evoked synapse transmission [126]. Gain of MEF2C function restricts the number and function of synapses, thereby preserving physiologic responses, while loss of MEF2C results in disrupted physiologic regulation of synapse numbers and hampers fear conditioning [126]. Nevertheless, postnatal MEF2C deletion in the brain does not alter memory and learning, the assessment of synapse plasticity [150], asserting a distinct role of MEF2C gene in the postnatal vs. prenatal brain.

4.3. Functional role of MEF2C in neuronal survival

The majority of the preliminary studies that have investigated the functional dynamics of MEF2 in neural system explored the characteristics of MEF2's in the advancement of neuronal survival and inhibition of apoptotic death. MEF2 has been found to be vital for the survival of cerebellar granule neurons (CGNs) [36]. Apoptosis-inducing death of differentiating cells is frequently reported in the developing fetal brain [151]. Likewise, the cellular mechanism of apoptosis can also be seen in neuronal differentiating P19 cells. Thereby, P19 cells are believed to serve as a model for neuronal differentiating cells morphology, and that dysregulations in MEF2 function by dominant negative regulation could upsurge the apoptosis of differentiating P19 cells. Nonetheless, the cellular mechanism of apoptosis in P19 cells can be reinstated by the expression of the constitutively active MEF2C factor, confirming that transcriptional activity of MEF2 is deemed necessary to prevent cell death during neuronal development [32]. According to studies, when neural activities are suppressed in cultured CGNs, they typically die [36]. It has been documented that the constitutively active form of MEF2 is expressed to prevent CGNs from apoptotic death; nevertheless, the activation of the dominant negative form has escalated this process [36]. This was thought to be a mechanism that depends upon the stimulation of the P38 MAPK (mitogen-activated protein kinase) signaling. The P38 MAPK signaling was identified as a critical switch of cell death and survival in a number of different cells [152]. The P38/MEF2 signaling has been shown to be a prerequisite for Ca2+ mediated CGNs survival in a developing culture, implying that P38/MEF2 is certainly necessary in primary neurons [36]. Two critical P38 MAPK family members [1]: P38α and [2] P38β/2, are recognized to trigger MEF2 via phosphorylation of Ser/Thr residues [153, 154]. It was demonstrated that P38α is phosphorylated throughout the growth and development of nervous tissues [32]. Additionally, it was ascertained that the transfection of dominant negative P38α significantly accelerated the apoptosis in differentiating cells. However, the co-expression of the MEF2C gene dramatically salvaged these differentiating cells from apoptotic death [32]. Such findings, thus, reinforce the concept that the P38α/MEF2 signaling cascade performs a substantial part in mitigating apoptosis during differentiation of neurons.

A mechanistic pathway for neural cell death encompassing caspase-catalyzed dissociation of MEF2 proteins adversely affects the typical pro-survival activity of the P38/MEF2 signaling and leads to neuronal apoptosis [155]. Caspases are proteases which are necessary for the process of apoptosis [156]. They are formulated as inactive pro-enzymes and triggered in a proteolytic cascade after being exposed to an apoptotic signal [155]. In a study, Li et al. described the dissociation of MEF2A/D in CGNs following K+ depletion. However, their result did not validate a definite association of caspases with MEF2 factors and they were unable to locate a specific MEF2 cleavage site [34]. In another study, Okamoto et al. demonstrated that when cerebrocortical neurons are exposed to excitotoxic concentrations of NMDA (N-methyl-D-aspartate), a stimulant reported to trigger P38 signaling and caspase-related enzymes, causes caspases to cleave MEF2A, MEF2C, and MEF2D [155]. Target sites of caspase on MEF2 proteins are present in the transactivation domain with cleavage facilitating the generation of endogenous dominant-interfering forms of MEF2. Such dominant-negative MEF2 is capable of blocking intact MEF2 from activation, thus leading to neuronal apoptosis [155]. Another potential pathway for neuronal damage concerning MEF2 is the disruption of the paxinosome proliferator-activated receptor γ co-activator-1α (PGC1α) signaling and protein S-nitrosylation of MEF2C in Parkinson's disease (PD) [157]. PGC1α was considered as a substantial MEF2 effector due to its function in mitochondria and neuroprotection in PD models [158, 159]. To clarify the importance of MEF2C in PGC1α signal transduction, Ryan et al. have shown that basal and toxin-induced nitrosative stress (a nitric oxide-mediated nitrosylation of redox-sensitive thiols) can cause S-nitrosylation of MEF2C in A53T α-synuclein mutant A9 dopaminergic neurons. This reaction interrupts the MEF2C-PGC1α transcriptional pathway, leading to mitochondrial dysfunction and ultimately results in apoptosis [157]. Nitric oxide (NO) works as a signal molecule that influences a number of different targets. NO regulates diverse physiological functions inside the brain, like development of neurons, synapse plasticity, and release of neurotransmitters. However, excessive NO output in the brain is related to many acute and chronic neurodegenerative disorders, shifting the nitrosylation/denitrosylation homeostasis equilibrium towards intensified nitrosylation [160, 161]. Previous research has shown that NO can facilitate redox reactions through S-nitrosylation, demonstrating the transition of NO to a vital molecule called cyanide thiol, culminating in neurogenesis regulation and survival of neurons in multiple brain regions [162]. Recently, Ryan et al. reported that, following exposure of the recombinant MEF2C to endogenous nitric oxide S-nitrosoytyne (SNOC), MEF2C can be S-nitrosylated, and this modulation tends to occur on cysteine (Cys39) as sulfonation, leading to the suppression of MEF2C transcriptional activities and cell death by PGC1α-mediated pathway [157]. In sum, all these studies concluded that reduced MEF2 leads to the suppression of PGC1α activation, and S-nitrosylation is a potential factor in suppression of MEF2 expression. The effect of S-nitrosylation of MEF2C, on the other hand, affects not only neuronal viability in the brain during injury, such as ischemia, but also adult neurogenesis [163]. A study by Okamoto et al. revealed that redox-modification by NO interacting with several cysteine residues of MEF2 factors serves as a cellular trigger to regulate neurogenesis and apoptosis in the brain [163]. As per their findings, MEF2A stimulates the adult hippocampus neurogenesis in vivo and in vitro, but the S-nitrosylation of MEF2A suppresses this activity by inhibiting TLX (T cell leukemia homeobox) cascade [163]. In contrast, S-nitrosylation of MEF2C leads to NO-mediated apoptosis in cerebrocortical neurons during ischemic injury and in Alzheimer's disease (AD) mice models by obstructing the Bcl-xL (B-cell lymphoma-extra large) anti-apoptotic pathway [163]. Although this pathway is quite different from the MEF2C-PGC1α anti-apoptotic pathway mentioned by Ryan et al. [157]. As per these findings, S-nitrosylation of MEF2C factors serves as a redox transition switch, suppressing both neurogenesis and survival of neurons.

In addition, a number of stimuli and distortions impair the function of MEF2 in neuronal survival. Numerous kinases were defined that cause phosphorylation of MEF2 factors and restrict their activities, causing neural apoptosis. In a study, Wang et al. have demonstrated that GSK3β (glycogen synthase kinase 3 beta) causes the phosphorylation of total 3 residues in MEF2D, which suppresses the MEF2D-related activities that eventually contribute to apoptosis of CGNs [164]. Another group has demonstrated that stimulation of PKA (protein kinase A) via cAMP (cyclic adenosine monophosphate) leads to PKA-regulated inhibition of phosphorylation of MEF2D at S121/S190 residue resulting in apoptosis of hippocampal neurons [165]. Furthermore, neurotoxic stimuli may stimulate CDK5 facilitated inhibitory phosphorylation of MEF2A/D at S408 and S444 residue, respectively, ultimately resulting in apoptotic death of cortical neurons [35]. In vivo research has shown the significance of MEF2’s in the survival of neurons [149]. To more thoroughly investigate the specific and redundant characteristics of MEF2 factors in regulating survival of neurons in vivo, Akhtar et al. produced a brain-specific triple knockout (MEF2A/C/D\(^{TKO}\)) mouse model [149]. Mice with a homozygous MEF2A null mutation die prematurely during
MEF2A was then deleted explicitly throughout the CNS region via mating MEF2A<sup>loxp/loxp</sup> mice with transgenic lab mice, which express cyclic (Cre)-recombinase under the regulation of the human GFAP promoter (hGFAP-Cre), which is transcribed in neuronal progenitor cells throughout late embryogenesis [149, 167]. Similarly, hGFAP-Cre line was also used to delete MEF2C in the nervous system via mating MEF2C<sup>loxp/loxp</sup> female mice to MEF2C<sup>fl/fl</sup> heterozygous male fostering a transgene, which expresses Cre-recombinase under the influence of hGFAP-Cre [136]. MEF2A knockout and MEF2A/D double knockout mice have normal and healthy brain size and shape and display no signs of neuronal cell death, while mice with a triple deletion (MEF2A/C/D<sup>fl/fl</sup>) throughout the brain exhibit reduced brain sizes and early postnatal lethality followed by enhanced neural cell death comparative to controls [1-19]. Notably, the brain-specific MEF2C deletion solely may not induce any deformities in brain size, shape, or apoptosis, indicating that mice with mutations in either one or two of the MEF2 factors seemed to have no such complications, possibly because of the propensity of co-expressed MEF2 members to supplement one another. Furthermore, their MEF2A/C/D triple knockout mice demonstrate impairments in the measurement of hippocampus short-term plasticity. This indicates that MEF2A, C and D act superfluously in the survival of neurons, but that MEF2C is the isoform comprised in hippocampal synaptic activity [149]. Taken together, these observations contribute to an extensive elucidation of the MEF2 factors in memory and learning, and synaptic activities.

5. MEF2C as a risk factor for ASD

All MEF2 factors, particularly MEF2C, are transcribed abundantly in the neuronal cells [107]. Research has shown that the production of MEF2C is important for neural differentiation and survival, as well as in the development of synapses in the brain. However, prior reports have displayed that mice with a complete MEF2C knockout are embryonically lethal by E9.5-10 due to incomplete cardiac morphogenesis [168]. And, since the heart forms before the brain, there is evidently no brain development, resulting in embryonic lethality [168]. Thus, to study the functions of the MEF2C gene in brain development, scientists have generated brain-specific conditional knockout models to evaluate the detailed characteristics of the MEF2C gene in growth and development of the brain. For example [1]: nestin-driven Cre is used to knockout MEF2C at the neural stem/progenitor cell (NSC) stage. In this they cross those mice which are expressing the nestin-Cre transgene (n-Cre<sup>+</sup>) with mice which carry the conventional exon 2-deleted allele of MEF2C (MEF2C<sup>Δ2</sup>) to generate n-Cre<sup>+</sup>/MEF2C<sup>Δ2</sup> mice. These will then cross with the MEF2C<sup>fl/fl</sup> mice to acquire the n-Cre<sup>+</sup>/MEF2C<sup>fl/fl</sup> conditional null mice [33, 168, 169], whereas [2] GFAP and Emx1 (empty spiracles homeobox 1) are used to knockout MEF2C at a somewhat later stage [41, 126, 170]. Geneticists delineated the overlapping regions of 5q14.3q15 loci microdeletions that induce neural defects in individuals, and determined MEF2C haploinsufficiency as the significant trigger. ASD, ID, absence of speech, stereotyped behavior, epilepsy, and other motor abnormalities, including hyperactivity, are among the signs and symptoms shown by these patients [63, 79, 171, 172]. The MEF2C haploinsufficiency syndrome refers to a group of conditions accompanied by MEF2C haploinsufficiency (MCHS). MCHS accounts for a small percentage of all ASD-related cases, though certain MEF2C-mediated genes were linked with distinct ASD types. Conditional gene disruption of the MEF2C exon 2 in mice, which transcribes a significant proportion of the DNA binding domain, generates mice with multiple structural, behavioral, and synapse-related abnormalities in several neural subpopulations in the developing regions of the brain [33, 126, 173]. Tu et al. have developed MEF2C<sup>-/-</sup> (MEF2C<sup>−/-</sup>) mice to investigate the human MCHS-related ASD. In their study, it was shown that MEF2C<sup>−/-</sup> mice had synaptic and neuronal abnormalities, indicating that MEF2C haploinsufficiency plays a significant part in MCHS/ASD-like behavioral characteristics [173].

According to Z. Li et al., disruptions in MEF2 function and expression may occur very early in development, particularly in embryonal neural stem cells (NSCs). Conditional MEF2C knockout in NSCs develops certain ASD-like neurodevelopmental abnormalities [29]. In contrast, adult MEF2C conditional knockout null mice that live to adulthood exhibit features indicative of Rett syndrome [29].

Interaction of MEF2 with the other genes, such as MeCP2 (methyl CpG binding protein 2), CDKL5 (cyclin-dependent kinase-like 5) [79, 174], and UBE3A [175] is linked to Rett and Angelman syndromes, respectively. MeCP2 is a significant contributing factor to Rett syndrome when mutated and can bind to the promoter of MEF2C gene [174]. It was discovered that abnormalities in MeCP2 result in neurological symptoms that mirror Rett syndrome [174]. According to a previous study, MeCP2-null mice remain normal and healthy till 6 weeks of age. Thereafter, they acquire serious neurophysiological dysfunctions leading to death by 12 weeks of age [176]. Expression profiles of CDKL5 and MeCP2 were assessed in blood serum samples of individuals with MeCP2 mutations or 5q14.3q15 microdeletions; in all individuals, the profile of MeCP2 expression was reported to be substantially decreased, implying an analogous mechanism of MEF2C and MeCP2. Likely, the levels of CDKL5 expression have also decreased [79]. Earlier molecular experimental studies have established the involvement of CDKL5 and MeCP2 in the mutual pathway [177]. As per the co-transfection studies, CDKL5 and MeCP2 regulation are within the transcriptional control of the MEF2C gene [79]. In a study, Zweier et al. described four distinct de novo heterozygous mutations in the MEF2C gene in 4 out of 362 probands with psychological disabilities who had been monitored for dysregulations in the MEF2C gene [79]. Two of the mutations were missense mutations, and the other two were truncating mutations. Heterozygous deletions of the MEF2C gene were seen in two other patients with similar condition. In all individuals with deletions in the MEF2C gene or involving the MEF2C gene region, as well as the individuals described by Le Meur et al. [2010] [63], Guerrini et al. [2009] [178], and Engels et al. [2009] [179], blood-derived RNA results showed a substantial decrease in the amount of MEF2C isoform 2 mRNA levels, indicating haploinsufficiency. The levels of MEF2C mRNA were not reported to be lower in two individuals who had a missense mutation. Both deletion and mutation caused a significant reduction in the transcriptional function of the MEF2C gene, which might be rescued by the wild-type MEF2C gene. Eventually, all of the individuals, along with the two who had missense mutations, reported relatively low MeCP2 mRNA expression levels, and all but two individuals had reduced CDKL5 mRNA expression levels [79]. Moreover, a key finding from the Chahrouf et al. study demonstrated that MeCP2 governs the expression of several genes in the hypothalamic region, functioning not only as a transcriptional activator but also as a repressor [174]. They also stated that alterations in transcriptional activities imply that duplication of phenotype is caused because of MeCP2 gain of function instead of loss of MeCP2 function, and that RTT is mainly caused by loss of transcriptional activation instead of derepression [174]. This indicates that the gain of MeCP2 function induces considerably further activation than suppression, while the loss of MeCP2 function has the complete opposite effect. Angelman syndrome, also known as happy puppet syndrome, is a rare developmental and neurological disorder that mainly affects the nervous system. It is defined by microcephaly (shorter-than-normal head), ataxia, seizures, muscle hypotonia (decreased muscle tone) with hyper-reflexia, and motor disabilities. It is triggered by the loss of the maternal allele of the UBE3A gene in chromosome 15 locus [180], which is essential for ubiquitin-proteasome signaling and the development of synapses [181].
and copy number variations (CNVs) [182]. Some of the traits of Angelman syndrome can indeed be found on the autism spectrum, for example poor communication skills, absence of speech, attention deficits, hyperactivity, insomnia, and a deficiency in motor growth and development [183, 184]. Recently, employing a fly (Drosophila melanogaster) as a screening model for gene interactions and by consequent co-immunoprecipitation in human cell lines, Straub et al. reported a specific functional correlation between MEF2C and UBE3A that could eventually lead to the phenotypic overlap between MEF2C-related ID and Angelman syndrome [185]. Nevertheless, there is still a need for more comprehensive scientific support of UBE3A influencing MEF2C gene in an ubiquitous manner.

To specifically assess the functions of the MEF2C gene in the postnatal brain directly, Adachi et al. produced conditional MEF2C knockout mice model by means of calcium/calmodulin-dependent protein kinase II (CaMKII)-Cre93 line [150]. These conditional knockout mice have been produced by pairing floxed MEF2C (MEF2C) mice with mice transgenic for Cre-recombinase under the regulation of the CaMKII-Cre93 (CaMKII promoter), which directs the deletion of genes in anterior regions of the brain, such as the cerebral hemispheres, the thalamus, and the hypothalamus, including the hippocampus, at postnatal days 10–14 [150]. They discovered that postnatal brain deletion of the MEF2C gene ultimately led to a substantial rise in the numbers of spines in the hippocampal regions. Conditional brain-specific deletion of MEF2C in mice also exhibits variations in motor activities and abnormalities in motor coordination. Nevertheless, in mice, deletion of the MEF2C gene in the postnatal brain did not influence memory and learning function, synapse plasticity measurements, and a number of other behavioral assessments put forward to summarize the ASD-related characteristics [150]. Due to the massive frequent involvement of MEF2C gene in the negative regulation of synaptogenesis, the above observations reveal that the impact of MEF2C gene as a regulator of spine numbers is independent of embryonic and postnatal stages of growth and development. Although the functional role of the MEF2C gene as a chief regulator of memory and learning, plasticity of synapses, and behavioral assessments of ASDs relies on the expression of MEF2C throughout embryonic growth and development and is not specifically related to alterations in the numbers of spines [150]. In sum, this research gives a profound understanding into the relevance of the developmentally specific gene modulation of endogenous expression of MEF2C as a critical factor in articulating the mechanisms and functions of MEF2C in the CNS. To model human MCHS, a group of scientists developed mice with only one operational copy of the MEF2C gene, and they discovered that these mice presented increased premature death rates, had autism-like characteristics, and had decreased viability [150].

MEF2C (Me2c-Het) mice via breeding Me2c-flx mice to protamine-1 promoter (Prm)-Cre mice. Subsequently, the Prm-Cre allele was deleted from C57BL/6J wild-type mice through repeated backcrossing. Conditional Me2c-cHet mice were produced via breeding Me2c-flx mice with Eμx1-Cre, a cell type-selective Cre-expressing transgenic mice to generate Me2cflx/cHet mice [170]. In this study, authors ascertained that DNA binding–deficient Me2c-Het mice exhibited a number of MCHS-related traits like deficits in social communication and interaction, hyperactivity, monotonous behaviors, anxiety, lessened sensitivity towards painful stimuli (e.g. foot-shock), alterations in cortical gene expression, and disruptions in excitatory synapse transmission [170]. Multiple deregulated genes have been recognized in the Me2c-Het cortex, as well as extensive accumulation of autism susceptibility and excitatory neuronal genes [170]. Their results inferred that hypofunction of MEF2C during the period of growth and development induces myriad dynamic alterations in gene expression, cortical synapse transmission, and behaviors suggestive of ASD and MCHS. This research has revealed that MEF2C governs normal brain functions and development via different cell types, including excitatory neural and neuro-immune populations. However, the conditions with respect to ASD and MCHS are much more complicated. The decrease in MEF2C due to haploinsufficiency could result in a decrease in synapse number during development. And the occurrence of a one good copy of MEF2C gene on the normal allele could still lead to reduced spine numbers in mature neurons that develop under MCHS conditions. Therefore, this situation is not yet clear and further work is required.

Microglia, both developing and adult, play an essential part in the growth and development of the brain like neurogenesis, myelogenesis, synaptic phagocytosis, and synapse patterning [99, 100, 102, 186]. MEF2C has been reported to be expressed in both mouse and human microglial cells, and MEF2C members are involved in the development and regulation of microglia [98]. Microglia-enriched RNAs have been shown to be deregulated in the human cortex in idiopathic ASD individuals brain [187] and in the Me2c-Het mouse cortex [170]. Recently, a study demonstrated that hypofunction of MEF2C gene in microglial cells is enough to induce autism-like symptoms in mice and to influence cortical glutamatergic pathways [170]. In order to discover the prominent characteristics of microglia in the occurrence of MCHS-like phenotypes, the authors generated two experimental models: (1) mice heterozygous for MEF2C in Emx1-lineage cells (Mef2c-cHet[Emx1-cKO]) which account for around 85% of forebrain excitatory neurons in the cortical and hippocampal regions. Mef2c-cHet[Emx1-cKO] mice showed deregulated anxiety-like activities and male-selective upsurge in locomotor and monotonous hopping activities; however, they displayed none variations in social activities or shock sensitivity. In Mef2c-cKO[Emx1-cKO] mice, the amplitude of mEPSC (miniature excitatory postsynaptic current) in layer 2/3 pyramidal neurons was reduced, while in Me2c-cHet+[Emx1-cKO] mice, the amplitude of mEPSC was increased. This indicates that the Emx1-lineage excitatory forebrain neurons significantly support the growth of some, though not all, of the behavioral characteristics. (2) Microglia-selective Me2c-Het mice (Me2c-cHet[Emx1-cKO]) displayed social impairments in the three-chamber social interaction test. Moreover, Mef2c-cHet[Emx1-cKO] mice displayed a substantial rise in male-specific monotonous jumping, anxiety, and shock sensitivity activities. They also found a decrease in the eEPSC (evoked excitatory postsynaptic current) amplitude in the Me2c-cHet[Emx1-cKO] mice. Moreover, their findings also indicate that the lack of one MEF2C allele may not always typically generate traditional microglia instigation, but also impairment in the growth, maturation and function of microglia [170]. Future studies would therefore be necessary to ascertain the strategic roles of MEF2C in the growth and development of microglia, and whether or not MEF2C heterozygosity influences several of the described functions of microglial cells in brain growth and development.

Fragile X syndrome (FXS), also termed as Martian-Bell syndrome, is a non-Mendelian trinucleotide repeat disorder [188], FXS has been the most frequent cause of IDs and the most predominant monogenic cause of
ASD [189]. Lubs and his associates discovered FXS in 1969, but the first fragile X-linked sequence of inheritance was described in 1949 by Martian and Bell [190, 191]. The Fragile X syndrome is typically triggered via alterations in the FMR1 (Fragile X Mental Retardation 1) gene which typically entails an increase of >200 CGG repeat sequences in the 5’ UTR region of the FMR1 gene, leading to transcriptional silencing of the FMR1 gene and the lack or significant reduction of the transcribed protein (Fragile X Mental Retardation 1 protein; FMRP) [191, 192]. Studies have shown that FMRP is a significant regulator for the translation process of several mRNAs which are involved in synapse plasticity, neuronal morphology, and cognitive development, and its absence contributes to differing degrees of ID [193, 194]. Evidence shows that FMRP is needed to allow MEF2 members to remove excitatory synapses in mice’s hippocampus neurons [127]. The density of dendritic spines in the dentate gyrus region is significantly greater in FMR1 knockout mice during development than in wild-type mice, indicating a lag in the down-regulation of synapse [195]. In the hippocampal neurons of FMR1 knockout mice, though, MEF2-mediated elimination of synapses and the upsurge in synapse number because of MEF2 blockade were suppressed. These forms of deficiencies may be rescued by the acute post-synaptic FMRP expression, which demonstrates that post-synaptic communication activity among FRMP and MEF2 is disrupted in FXS [127]. A study by Zang et al. reported that in the hippocampal regions, FMRP can bi-directionally regulate the excitatory synapse activities in dendrons via linking to dendritic RNA, i.e. by fostering maturation of synapses throughout the first postnatal week 6–7, whereas disrupting synapse development during the second postnatal week 13–16 [50]. In addition, the expression of FMRP has also been regulated via MEF2 factors, which, consecutively, are attuned by neural depolarization and are stimulated following the developmental time frame [50]. In sum, this study demonstrated the potential roles of MEF2 in FXS via FMRP.

Furthermore, prior studies have demonstrated that delta (δ)-catenin, a key constituent of the cadherin–catenin cell adhesion complex, is localized to synapses and partly co-localized with synaptic proteins, implying a functional involvement at synaptic regions [196, 197]. The absence of the CTNND2 (catenin delta 2) gene, which encodes δ-catenin, was linked with the ID phenotype, indicating that therapeutic interventions that reinstate the critical functions of δ-catenin might aid the intellectual phenotypes [198]. A study by Yuan and colleagues stated that the deficiency of δ-catenin was related to intellectual disability [199]. In their study, they reported that the concentration of endogenous MEF2C is not disrupted due to the loss of δ-catenin. However, the MEF2C gene expression may facilitate the removal of excess dendritic spines observed with the loss of δ-catenin. Even in the absence of δ-catenin, the protein levels of MEF2C remain unchanged, indicating that the altered numbers of synapses found in the absence of δ-catenin may not correspond to a reduction in MEF2C levels [199]. Even though mice associated with the loss of δ-catenin display serious learning disabilities, transitory induction of MEF2C during the developmental phase would be expected to minimize some of the learning-related deficiencies [199]. In summary, their analysis has ascertained that MEF2C can facilitate the removal of an excessive number of spines produced in the absence of δ-catenin. Taken together, all the above studies on MEF2 or MEF2-regulated transcripts in synapse removal, regulation and plasticity specify that MEF2 transcription factors play a potential role in neurodevelopmental disorders.

6. Concluding remarks

Deficits in the molecular and cellular mechanisms, as well as signaling pathways that regulate neuronal and synapse development, transmission, and neural activities in the brain may eventually lead to neurodevelopmental ailments, like intellectual disability and ASDs. Therefore, to characterize the significant factors that exacerbate these brain developmental disorders, we need to search for new therapeutic targets. One such possible therapeutic target is the MEF2C gene, which has been implicated in muscle growth and development, and is ubiquitously transcribed in the developing brain regions. Several people with autism, schizophrenia, and ID have genetic mutations within or close to the MEF2C gene. MEF2, a transcription factor, functions in brain development and regulates excitatory synapses formation and removal in response to neuronal activities. MEF2C, a family member of the MEF2 family, is a transcriptional activator that binds directly to the MEF2 component. It plays an important role in various critical developmental activities, such as neurogenesis, myogenesis, synaptogenesis and hematopoiesis. The MEF2C gene is essential for normal synapse and neuron development. Studies have postulated that irregularities in MEF2C may increase susceptibility to several neurological disorders by hampering the equilibrium of inhibitory and excitatory synapses in the developing brain regions. According to recent genomic investigations, it has been ascribed that the mutation or deletion of the MEF2C gene is linked to a number of neurological conditions with ASD and ID-like characteristics. In addition, research shows that MEF2C is also correlated with other different brain-associated diseases. Previous data strongly suggests that the MEF2C gene in several brain regions responds to differing arrays of activities. The neuronal cells in the brain become more responsive as the brain matures and establishes various new connections. And here MEF2C is vital since it regulates and modulates a number of endogenous downstream factors that aid in the growth of neurons and in the formation of neuronal connections. If the neurons lose such consistency or balance, they will have an entirely abnormal set of connections. Earlier research has revealed that MEF2 factors perform a critical function in shaping and guiding the organization of brain connections at an early stage of development. Synapses between neurons are believed to form quite strenuously during brain development; however, they are chopped down during the late adolescent stage. MEF2 members, including MEF2C, tend to perform a substantial part in this critical process. Therefore, we should further investigate the molecular and cellular mechanism of the activity of MEF2 proteins in autism-like behaviors, and the impaired dysregulated genes, plus the function of the MEF2C gene in brain circuitry, which could further facilitate one of the most preponderant conjectures regarding the underlying cause of autism, which refers to a disparity in neural connectivity and/or synapse formation between brain cells. Equipped with these profound insights, it might then be possible to treat the complex symptoms of such neurodevelopmental disorders.

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