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Characterization of *MEF2C*-Related Disorders: Genotype, Phenotype, and Gene Pathway Dysregulation

Jessica Ann Cooley Coleman
cooley8@g.clemson.edu

CHARACTERIZATION OF *MEF2C*-RELATED DISORDERS: GENOTYPE, PHENOTYPE, AND GENE PATHWAY DYSREGULATION.

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by
Jessica A. Cooley Coleman
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Accepted by:
Dr. Jane M. DeLuca, Committee Chair
Dr. Sara M. Sarasua, Committee Co-Chair
Dr. Luigi Boccuto
Dr. Steven A. Skinner
Dr. Christopher W. Cowan
Hannah Warren Moore, MS, CGC

ABSTRACT

MEF2C-related disorders are characterized by intellectual disability, developmental delay, lack of speech, seizures, stereotypic movements, hypotonia, and brain abnormalities and are caused by pathogenic alterations involving the *MEF2C* gene. Despite published cases, *MEF2C*-related disorders are difficult to recognize clinically. These studies sought to further characterize *MEF2C*-related disorders by investigating the genotypes, phenotypes, and gene functions (or dysfunctions) associated with the disorder.

Tremors have been reported in some patients with *MEF2C*-related disorders, but the concept of tremors has been complicated by vague definitions and numerous categorization methods. We performed a concept analysis following the Walker and Avant method to clarify the concept and develop an operational definition of tremors. We concluded that tremors are a movement disorder characterized by shaking motions that are involuntary, oscillatory, rhythmic, non-painful, always present although vary in severity, and can be repressed by changing posture or going into a rest position.

We then performed a systematic literature review to record the genotypes and comprehensive phenotype of *MEF2C*-related disorders reported in the literature. Forty-three articles characterizing 117 patients met the inclusion criteria. Common features included intellectual disability, developmental delay, seizures, hypotonia, absent speech, inability to walk, stereotypic movements, and MRI abnormalities. Nonclassical findings included question mark ear, jugular pit, and a unique neuroendocrine finding.

Next, we developed a survey based on validated instruments to gather developmental and clinical information from the parents of children with *MEF2C*-related

disorders. Seventy-three parents completed the survey. Limited speech, seizures, bruxism, repetitive movements, and high pain tolerance were some of the prominent features identified from the survey data. Statistical analyses showed that patients with *MEF2C* variants were similarly affected as patients with deletions and females showed higher verbal abilities. This natural history study details phenotypic and developmental information of the largest single cohort reported to date.

Lastly, we discussed current techniques used to investigate the mouse *Mef2c* gene expression and regulation in the brain. Previous unbiased RNA sequencing of whole cortex from *Mef2c* global heterozygous mice showed hundreds of dysregulated genes, particularly autism risk genes and microglial genes. The Cowan lab is currently performing single nuclei RNA sequencing (snRNAseq) to better understand the role of *Mef2c* in neurons and microglia. Techniques used include nuclei dissociation, fluorescence-activated cell sorting, library preparation and sequencing, and bioinformatic analysis of the snRNAseq data. Additional research techniques include perfusion fixation, brain extraction and slicing, and immunohistochemistry.

These studies characterize the phenotype and document the severity of the disorder. The information reported will help providers diagnose and care for patients with *MEF2C*-related disorders. Additionally, the systematic review and survey data can be useful for further genotype-phenotype correlations, as baseline data for treatment trials, and to develop future studies.

DEDICATION

First and foremost, I dedicate this work to my sweet, loving, patient husband, Antwon, who has supported me through all the ups and downs of pursuing a Ph.D. while also working full time. This work is also dedicated to my best friend Wesley, who continued to push me for greatness and inspired me to never give up. Also, thank you to all my friends for your continued support. Lastly, I would like to dedicate this work to my parents for instilling in me the importance of education and hard work. To Dad, looking down from heaven above, who always answered my inquiries with “go research it and write me a paper about it”: this is for you, my best and longest paper yet.

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Most importantly, I would like to thank the patients and families of those affected with a *MEF2C*-related disorder. Thank you for the encouragement, helping build and pilot the survey, and thanks to all for participating in the survey. This research would not be possible without your dedicated efforts.

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CHAPTER ONE

INTRODUCTION

Overview

MEF2C-related disorders, also known as *MEF2C* haploinsufficiency syndrome or chromosome 5q14.3 deletion syndrome (OMIM #613443), are characterized by intellectual disability, developmental delay, lack of speech, seizures, stereotypic movements, hypotonia, and brain abnormalities. The disorders were first associated with a loss (deletion) of a region of the long arm of chromosome 5. Occasional gains (duplications) of this region have also been reported. Early publications reported patients having deletions of various sizes in this region and one patient having a 216-kb deletion only encompassing the *MEF2C* gene (Le Meur et al., 2010). The reports indicated that the causative minimal critical region for this disorder is the *MEF2C* gene. Additionally, patients with the same phenotype have been reported to have point pathogenic variants in the *MEF2C* gene (Zweier et al., 2010), making an even stronger case that *MEF2C* is responsible.

Despite published case studies, *MEF2C*-related disorders are difficult to recognize clinically. Additionally, most manuscripts report one or only a few patients with a total of 117 patients reported to date in the literature (Cooley Coleman et al., 2021). This introduction chapter describes what is known about the *MEF2C* gene, *MEF2C*-related disorders, and methods to investigate the genotype, phenotype, and gene functions (or dysfunctions) associated with the disorder. These methods include theoretical, observational, and experimental designs including concept analyses, literature reviews,

surveys, and laboratory studies using animal models. These methods are useful not only for *MEF2C*-related disorders but also for other rare genetic disorders that have not yet been fully characterized.

MEF2C Gene

MEF2 Family

The MEF2 (myocyte enhancer factor 2) family of proteins are transcription factors within the MADS family. The MADS-box region is highly conserved across various organisms, with the name stemming from the first four identified protein members in this group: MCM1 (pheromone receptor transcription factor; yeast), AG (Agamous; *Arabidopsis)*, DEFA (Deficiens; snapdragon), and SRF (serum response factor; human) (Shore & Sharrocks, 1995). In vertebrates, there are four *MEF2* genes: *MEF2A* (chromosome 15q26.3), *MEF2B* (chromosome 19p13.11), *MEF2C* (chromosome 5q14.3), and *MEF2D* (chromosome 1q22). The MADS-box domain is located at the N-terminus of each MEF2 protein and is highly homologous to other MADS family members (including non-*MEF2* genes) across multiple organisms. In the MEF2 family, the MEF2 domain lies directly adjacent to the MADS-box domain. The MEF2 domain is a region that is only conserved within the MEF2 family (McDermott et al., 1993). After the MEF2 domain, the C-terminal of the various MEF2 members diverge. The MADS and MEF2 domains are responsible for dimerization, cofactor binding, and DNA binding while the C-terminal region is responsible for transcription regulation and nuclear localization (Assali et al., 2019).

MEF2 proteins rely on the recruitment and binding to other transcription factors to activate transcription. They form homo- and heterodimers prior to binding to DNA containing the sequence C/TTA(A/T)$_4$TAG/A (also seen as YTA(A/T)$_4$TAR in the literature) (Molkentin et al., 1996). This consensus sequence is found in control regions of genes responsible for driving tissue-specific gene expression. When studying *MEF2C* specifically, Molkentin et al. (1996) found that *MEF2C* pathogenic variants of either a deletion within the MADS or MEF2 domain failed to dimerize or bind to DNA; therefore, both the MADS and MEF2 domains are required for dimerization and DNA binding. Additionally, they found that the MADS and MEF2 domains alone were not sufficient to activate transcription: the C terminal portion was required as deletions within this portion of the protein did abolish transcriptional activation.

Pathogenic variants in *MEF2A* have been associated with coronary artery disease and myocardial infarction (L. Wang et al., 2003). Additionally, patients with congenital diaphragmatic hernia often have chromosomal abnormalities involving 15q24-q26, which includes the *MEF2A* gene (Biggio et al., 2004). *MEF2B* somatic mutations have been found in diffuse large B-cell lymphomas and follicular lymphomas, but otherwise have not been associated with any germline genetic disorders (Morin et al., 2011). *MEF2C* is the only gene in the MEF2 family that is a causative gene in a deletion syndrome: Chromosome 5q14.3 deletion syndrome. *MEF2C* alterations (point mutations and indels) have also been identified in patients with the same phenotype as those with larger chromosomal alterations (Zweier et al. 2010). Lastly, fusions involving the *MEF2D* gene

have been associated with lymphoblastic leukemia (Gu et al., 2016), and *MEF2D* overexpression has been linked to pancreatic and ovarian cancer (Li et al., 2019).

MEF2C History

MEF2C was first discovered by Leifer's team in 1993 while screening skeletal muscle cDNA libraries using a DNA probe containing the MEF2 DNA-binding domain (Leifer et al., 1993). Using this method, they isolated cDNA clones that had high homology to the MEF2 DNA-binding domain; however, the region following the MEF2 domain differed from the previously described *MEF2A* gene. The team called the gene *hMEF2C* (where *h* stands for human). Using reverse transcription PCR (RT-PCR) and Northern blotting, the team screened clones and discovered four *MEF2C* isoforms resulting from the alternative splicing of two regions (McDermott et al., 1993). Some clones found in both muscle and brain lacked a 32 amino acid region (later termed gamma, or γ). Other brain-specific clones included an 8 amino acid region (later termed beta, or β). These 8 amino acids were not found in any muscle clones. All four isoforms were shown to bind to MEF2 DNA targets using electrophoretic mobility shift assays to test the protein-DNA interactions. Additionally, the isoforms' ability to activate transcription was tested via cotransfection of HeLa cells with *hMEF2C* cDNAs and a reporter containing a promoter and MEF2 binding site to activate transcription of the *CAT* gene. All four isoforms were shown to activate transcription (Leifer et al., 1993; McDermott et al., 1993).

MEF2C Structure

The *MEF2C* gene (isoform 1, NM_002397.5) is located on chromosome 5 positions 88,717,117-88,883,184 (hg38, UCSC Genome Browser) and consists of 166,068 nucleotides (including coding regions and untranslated region (UTRs)). *MEF2C* has 11 exons, one of which is non-coding (isoform 1). Figure 1.1 shows the gene location, expression, and location of commonly reported single nucleotide polymorphisms (SNPs) per dbSNP (build 153) as displayed on the UCSC genome browser. The 10 coding exons produce a protein that is 473 amino acids long with a molecular mass of 51,221 Da (Figure 1.2).

Figure 1.1: *MEF2C* gene location and commonly reported single nucleotide polymorphisms (SNPs) per dbSNP (build 153) as displayed on the UCSC genome browser.

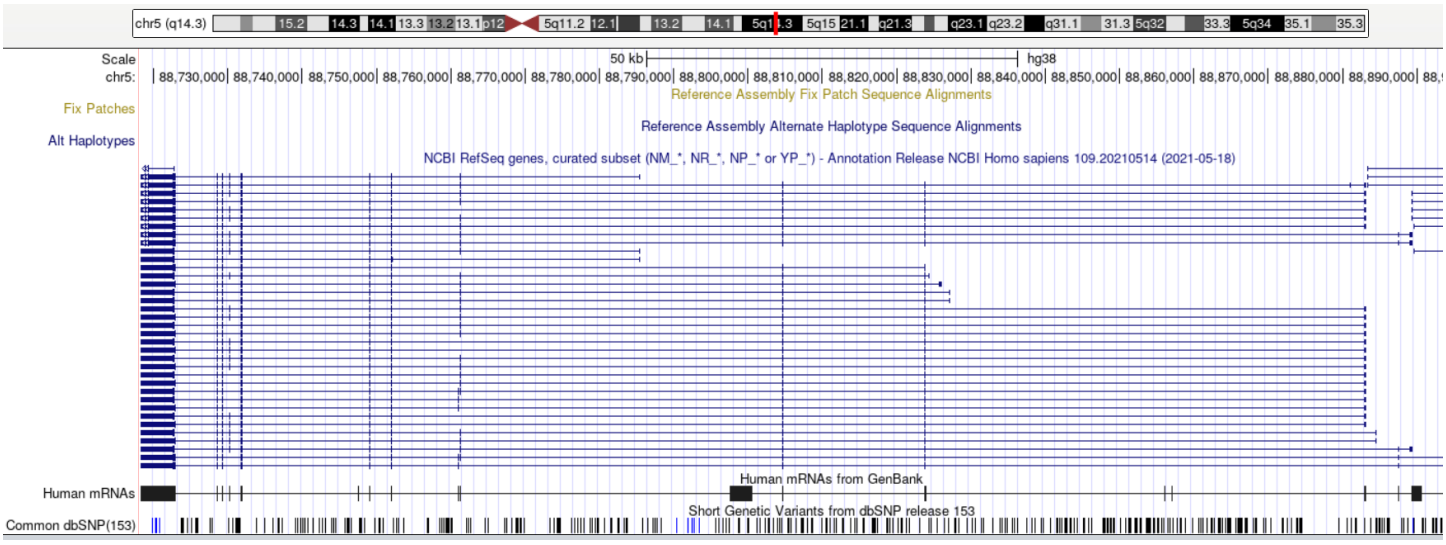
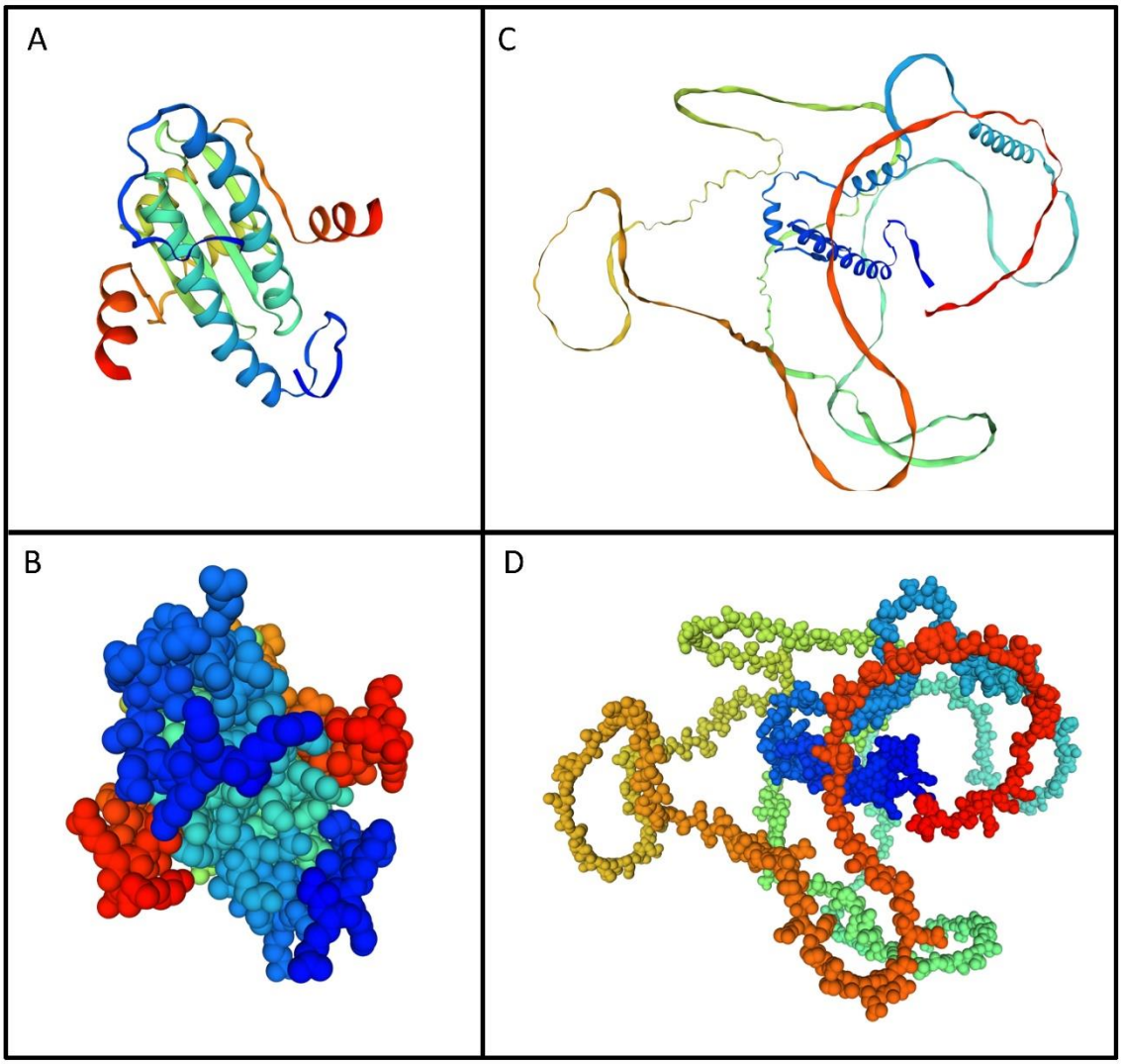


Figure 1.2:

A. MEF2C protein model depicting two MEF2C protein molecules dimerizing (homodimer) at the N terminal portion of the protein. The image shows protein regions from Glycine at amino acid position 2 (dark blue strand) through Lysine at amino acid position 91 (red strand). **B**. Surface or spacefill representation of dimer portion shown in (A). **C.** Entire MEF2C protein monomer. **D**. Surface / spacefill representation of the entire MEF2C protein monomer in the same orientation as (C).
(images from Swiss-Model, Bienert et al., 2017)



MEF2C undergoes vast alternative splicing. This process increases the diversity of mRNAs expressed from the genome, allows for tissue-specific gene variants, and has been proposed to control which target genes that *MEF2C* activates (Janson, Chen, Li, & Leifer, 2001). *MEF2C* has a total of 18 isoforms, some of which have multiple transcript variants (Table 1.1). For example, transcript variants 1, 6, and 9-11 all encode for isoform 1. These transcript variants differ at the nucleotide level (for example, transcript variants 1 and 6 differ in the 5' UTR), but they still encode the same amino acid sequence (and therefore are characterized as isoform 1). The various isoforms differ at the amino acid sequence level. Isoform 1 variant 1 is the longest *MEF2C* variant. All the transcript variants include the MADS domain (amino acids 1 to 57, MGRKKIQITRIMDERNRQVTFTKRKFGLMKKAYELSVLCDCEIALIIFNSTNKLFQY) followed by the MEF2 domain (amino acids 58 to 86, ASTDMDKVLLKYTEYNEPHESRTNSDIVE), with the exceptions of isoforms 17 (which lacks the MADs domain and most of the MEF2 domain) and isoform 18 (which lacks both domains).

Alternative splicing of *MEF2C* involves the inclusion or exclusion of the following exonic regions: mutually exclusive alpha1 or alpha2 (α1: TLRKKGLNGCDSPDPDADDSVGHSPESEDKYRKINEDIDLMISRQRLC or α2: ALNKKENKGCESPDPDSSYALTPRTEEKYKKINEEFDNMIKSHKIP), the cassette exon beta (β: SEDVDLLL), and the region called gamma (γ: ACTSTHLSQSSNLSLPSTQSLNIKSEPVSPPR) (Figure 1.3) (Zhang, Zhu, & Davie, 2015). Isoforms with α1 are found in heart tissues, while isoforms with α2 are found in

muscle tissues. The β exon is found exclusively in isoforms expressed in the brain, and the inclusion of this region has been found to enhance *MEF2C* activity (Zhang, Zhu, & Davie, 2015).

Figure 1.3: Schematic of *MEF2C* including alternatively spliced exons.

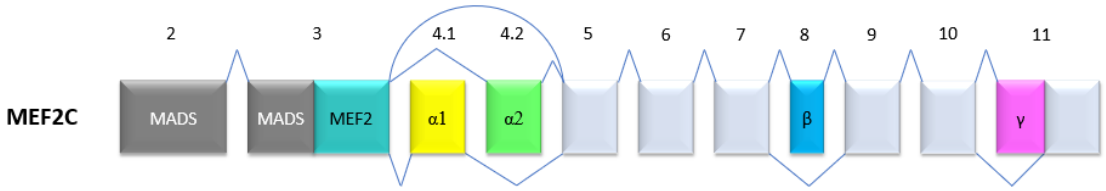


Table 1.1: Human isoforms of *MEF2C* showing which alternatively spliced exons they contain and the length of the resulting protein sequence.

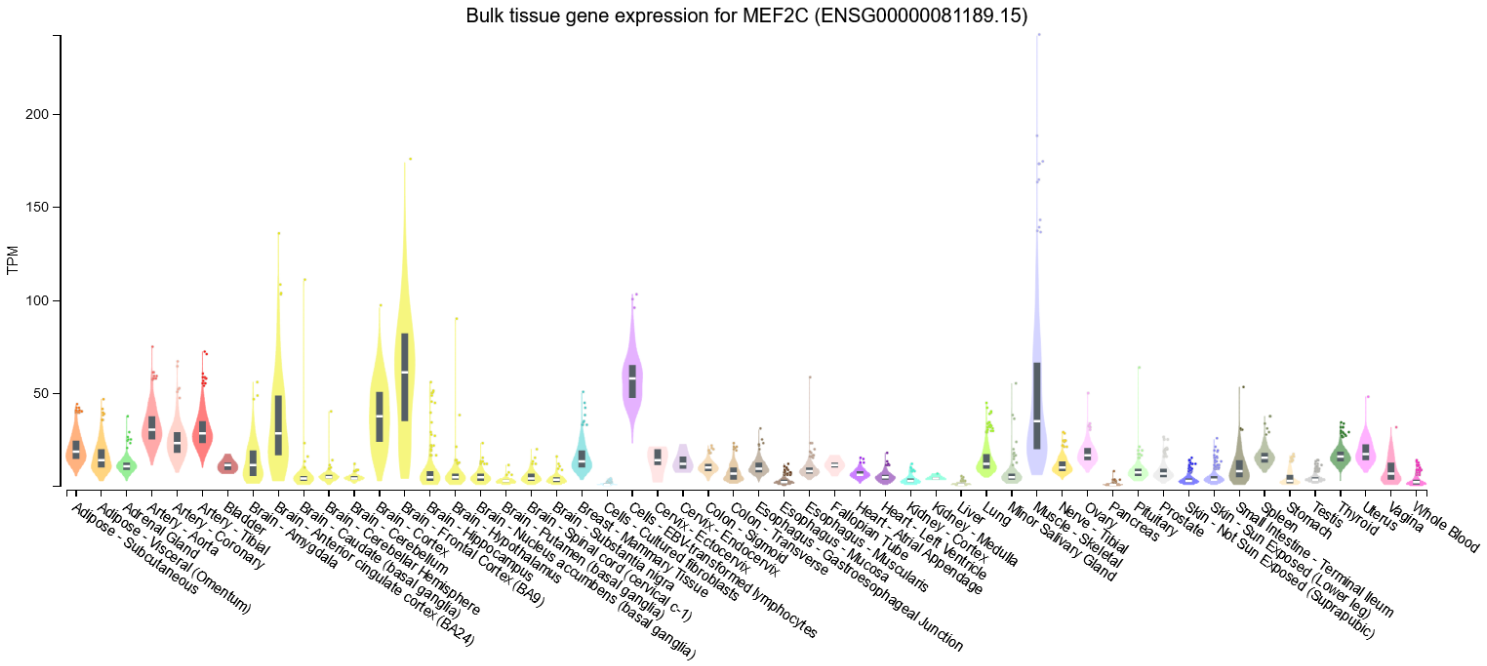
	Human Isoforms of *MEF2C*																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Exons	α1 β γ	α2 γ	α2 γ	γ	β	α1 γ	α1 β	α2	α1	α2	β γ	α1 β	α1	α2	β γ	β	β	–
# of Amino Acids	473	463	483	417	393	465	441	451	433	431	424	388	380	378	347	340	315	291

MEF2C Expression

MEF2C is expressed in multiple tissue types, with the highest levels of expression in the brain and skeletal muscle (Figure 1.4). In the brain, *MEF2C* is particularly expressed in the cerebral cortex. In cell culture experiments, *MEF2C* was not expressed

in precursor cells but was expressed in differentiating neurons leading to the hypothesis that *MEF2C* was necessary for neuronal differentiation (Mao et al., 1999). In skeletal muscle, *MEF2C* plays a role in myocyte differentiation during myogenesis and is also recruited by muscle-specific basic-helix-loop-helix (bHLH) factors to activate muscle-specific transcription (Chen et al., 2000). *MEF2C* is also expressed in the heart and may be involved in familial and sporadic congenital heart disease (Ghosh et al., 2009). In both mice and zebrafish, *Mef2c* homozygous mutants undergo embryonic death due to cardiac looping defects that prevent the right ventricle from forming (Ghosh et al., 2009; Potthoff & Olson, 2007).

Figure 1.4: MEF2C expression across various tissue types as reported in the GTEx portal.



MEF2C Protein Function

Being a transcription factor, *MEF2C* plays a role in regulating DNA transcription into RNA. Harrington et al. (2020) performed RNAseq on *Mef2c* heterozygous mice as compared to wild-type mice to identify differentially expressed genes (DEGs) resulting from the lack of *Mef2c*. A total of 490 significantly dysregulated genes were detected, many of which were excitatory neuron and microglia genes. Many of the downregulated genes were ASD-risk genes and FMRP binding genes, while microglial genes were upregulated. These results show that *Mef2c* acts as a gene-specific repressor or activator, particularly regulating microglial and neuronal genes.

MEF2C-Related Disorders and Testing Strategies

Some of the earliest cases of *MEF2C*-related disorders were of subjects sharing a similar phenotype of seizures, developmental delay, absent speech, and abnormal magnetic resonance imaging (MRI) that was attributed to a deletion of the 5q14.3-q15 region (Engels et al., 2009; Cardoso et al., 2009). At the time, *MEF2C* was not suspected as the causative gene as it was not deleted in one of the three cases described by Engels et al. and two of the three described by Cardoso et al. Shortly after these publications, seven additional patients were reported with the same phenotype (Le Meur et al., 2010). Five of these patients had deletions encompassing *MEF2C*, one patient had a duplication encompassing *MEF2C*, and the last patient had a single nonsense variant in *MEF2C*. Le Meur proposed that *MEF2C* was the causative gene and suggested that a positional effect on *MEF2C* could be responsible for the cases where *MEF2C* itself was not deleted. A

few months later, Zweier et al. (2010) reported four additional patients with point pathogenic variants in *MEF2C.* Additionally, Zweier's team performed expression studies on their patients and the three patients reported by Engels et al. This study showed that *MEF2C* expression was significantly decreased in patients with *MEF2C* truncating variants, patients with deletions encompassing *MEF2C,* and in the Engels patient who had a deletion not encompassing *MEF2C,* indicating that a positional effect was indeed likely.

Since these initial reports, a total of at least 117 patients have been reported in the literature (Cooley Coleman et al., 2021). Patients with *MEF2C*-related disorders have a phenotype of intellectual disability, developmental delay, hypotonia, absent speech, limited walking, abnormal MRI, abnormal electroencephalogram (EEG), and seizures. Dysmorphic features, including a broad forehead, downslanting palpebral fissures, large ears with prominent lobes, short philtrum, depressed nasal bridge, and tenting of the upper lip have been reported in some patients (Cooley Coleman et al., 2021). Additionally, sleep, feeding, gastrointestinal, and cardiac issues have been reported.

Testing procedures to detect *MEF2C*-related disorders typically include microarray, Sanger sequencing, and next-generation sequencing (NGS), with some patients having chromosomes and fluorescence *in situ* hybridization (FISH). Microarray technology involves fluorescently tagging patient DNA and hybridizing the DNA to probes on an array chip. An array chip may contain thousands to millions of probes to cover several genes or the entire genome. A computer records the pattern of fluorescence on the chip to determine which genomic regions are present. Patient data can be

compared to data obtained from a control subject to determine copy number variants (deletions or duplications). This assay is often a first-tier test for patients with intellectual disability and developmental delay and has diagnosed many of the patients with deletions and duplications involving *MEF2C*. Some chromosomal deletions can be seen by chromosome staining but due to the resolution, FISH or qPCR is often used to confirm *MEF2C* is included in the affected region.

Sanger sequencing is a method to determine the nucleotide sequence of a single gene. This method uses a DNA primer, DNA polymerase, normal deoxynucleotides (dNTPs), and fluorescently labeled dideoxynucleotides (ddNTPs). When ddNTPs are incorporated, the elongating DNA chain is terminated, resulting in numerous fragments of various lengths each with a ddNTP at the 3' end. The fragments undergo capillary electrophoresis where the fragments move at different speeds depending on size. A computer detects which fluorescent dye is present on the end of each fragment to determine the specific nucleotides, with software aligning the nucleotide calls to annotate the DNA strand's sequence. This method is used when the *MEF2C* gene is suspected or when researchers particularly want to study *MEF2C* and can detect single nucleotide variants and small deletions or duplications.

Next-generation sequencing also involves sequencing by termination (like Sanger); however, this method sequences millions of fragments simultaneously. Patient DNA samples undergo preparations, including tagging with a patient-specific barcode, allowing for the sequencing of multiple patients and multiple genes at once. After sequencing, the data is separated out for each patient using their known barcode. This

assay is often used when a condition that can be caused by multiple different genes is expected, or for exome or genome sequencing. Many patients with *MEF2C*-related disorders had a targeted NGS panel performed for genes associated with epilepsy (Cooley Coleman et al., 2021).

Rare Disease Research

Rare diseases are those that affect a small number of individuals as compared to the general population (*About rare diseases*, 2012). In the United States, rare diseases are defined by the Orphan Drug Act of 1983 as "any disease or condition which affects fewer than 200,000 people in the United States" (*Orphan Drug Act—Relevant Excerpts*, 2019). Other countries use different definitions; for example, countries in the European Union define rare diseases as those affecting ≤1 per 2000 persons. There is a general lack of medical awareness and knowledge on these rare diseases, which makes diagnosis difficult. One study surveyed 12,000 patients having one of eight rare diseases found that 25% waited between 5 and 30 years for the correct diagnosis, and 40% received an incorrect initial diagnosis (EURODIS, 2009). Although each disease affects a small number of people, with the roughly 7000 reported rare diseases, a large collective population is affected (*About rare diseases*, 2012). Research on rare diseases has immense impacts on those individuals, their families, and the entire rare disease community. Additionally, research helps spread knowledge of the disorder, aids in new diagnoses, and paves the way for disease management or future treatment strategies.

There are numerous methods to research rare diseases, including randomized designs (such as randomized, double-blind, placebo-controlled trials), nonrandomized controlled trials (risk-based allocation, delayed start), observational designs (pre-post studies, case reports, natural history studies), analytic methods (such as Bayesian analysis or instrumental variables), and other research designs (such as a literature review or meta-analysis) (Whicher, Philbin, & Aronson, 2018). Before treatments can be developed and tested, one must fully understand the disorder. To better understand rare disorders such as the ones associated with *MEF2C* for this dissertation, a number of different research methods were undertaken including conducting concept analysis, conducting a literature review, developing a natural history study, and using animal models.

Concept Analyses

Concept analyses are one type of theoretical-based research used to clearly define and differentiate a concept. Concept analyses are used to clarify vague, overused, or misused concepts. This analysis results in a precise, comprehensive, and standardized operational definition of the concept. There are various concept analysis methodologies described in the literature. One of the earliest contributors to the concept analysis was John Wilson, who developed an 11-step method of analysis (Wilson, 1963). These 11 steps included: 1) isolating questions of concept, 2) right answers, 3) model cases, 4) contrary cases, 5) related cases, 6) borderline cases, 7) invented cases, 8) social context, 9) underlying anxiety, 10) practical results, and 11) results in language.

Many researchers (Walker and Avant, Chinn and Kramer, and Rodgers, among others) have since developed their own methods or modified the Wilson method. The

Walker and Avant method is perhaps the most frequently used one in nursing science and is self-stated the "easiest to understand and master, especially for beginners" (Walker & Avant, 2005). Walker and Avant modified Wilson's method to have a total of eight steps, instead of eleven, while still capturing all relevant components. These steps include: 1) select a concept, 2) determine the aims and purpose of the analysis, 3) identify uses of the concept, 4) determine defining attributes, 5) identify a model case, 6) identify other cases (borderline, related, contrary, etc.), 7) identify antecedents and consequences, and 8) define empirical referents.

Concept analyses not only result in clarified terms and operational definitions but can lead to the development of tools or identification of gaps in the literature for future research. Additionally, they are an excellent exercise in critical thinking.

Systematic Literature Reviews

Literature reviews are a type of research that collects data from published scholarly work for the researcher to familiarize themselves with the topic, identify gaps in existing research, and propose new studies and methods (Purdue University, 2021). Traditional narrative literature reviews are broad in the topic and do not have a standardized methodology or search strategy (Sevetson, 2021). There are other types of literature reviews, including rapid, scoping, umbrella, meta-analysis, and systematic, with each having its own approach and purpose. Systematic literature reviews are considered the gold standard as they have a defined question to answer, must include inclusion and exclusion criteria, and follow a rigorous search, evaluation, data extraction, and analysis of the literature (Purdue University, 2021). The steps of a systematic review include

identifying the research question, defining the inclusion and exclusion criteria, performing the search, selecting studies based on the defined inclusion criteria, extracting data from those studies, performing an assessment, and presenting the results. The research question often follows a framework, such as PICO (Patient/Population problem, Intervention, Comparison or Control, Outcome), to narrow the focus and facilitate the literature search. In systematic reviews, the quality of the studies included must be assessed, and conclusions from the studies should include addressing gaps, proposing future studies, and giving recommendations for practice (Purdue University, 2021).

Natural History Study Surveys

A natural history study is an "observational study intended to track the course of the disease" (U.S. Food and Drug Administration, 2019). These studies collect demographic, genetic, and environmental information that may correlate with the disease with the goal of developing treatment. Types of natural history studies include retrospective, prospective, cross-sectional, and longitudinal (U.S. Food and Drug Administration, 2019). Retrospective studies use patient information from evaluations that have already happened, whereas prospective studies are planned for a future date. Cross-sectional studies consist of collecting data at one point in time to gather information on the disease, describe the severity of symptoms, and provide information for therapies to aid the patient population. Lastly, longitudinal studies are those in which data is collected across several time points to observe disease progression (U.S. Food and Drug Administration, 2019).

Surveys are one method used to collect patient information and can result in both quantitative data (due to the questions having either numerical or set answer choices) and qualitative data (from open-ended response questions). When developing the survey, questions should focus on a single concept and be understandable, clear, succinct, nonjudgmental, and unbiased (Burns et al., 2008). Technical jargon and double-barreled questions (single questions that ask about more than one issue) should be avoided to prevent confusion (Decarlo, 2018). Question types can include close-ended with a set of response options, open-ended, and filter questions to determine if participants should be asked additional questions. After the questions are finalized, the survey should be reviewed by experts and piloted by a small group of the target participants to obtain feedback on the questions, overall survey length, and subject matter (McInroy, 2016). Before launching the survey, it may need to be reviewed and approved by an Institutional Review Board (IRB) to ensure the rights and welfare of participants are protected throughout the research.

Animal Models

Animal models are non-human animals used for scientific research, observation, experiments, and treatment testing in place of performing these investigations on humans (Simmons, 2008). Certain research can pose a significant risk to human life. Since animals have genetic, anatomic, and physiologic similarities to humans, they can be used for research in the place of humans. According to the Model Organism Aggregated Resources for Rare Variant ExpLoration (MARRVEL), there are orthologs to the human

MEF2C gene in mice (*Mef2c*), zebrafish (*mef2cb*), drosophila (*Mef2*), and C. Elegans (*mef-2*) (J. Wang et al., 2017).

Dichoso et al. (2000) concluded that *mef-2* has a different role in *C. elegans* development and is not essential for myogenesis as compared to drosophila and vertebrates (Dichoso et al., 2000). In humans, there are four genes in the MEF2-family (A-D); however, there is only one gene, *Mef2*, in drosophila. *Mef2* expression begins early during embryogenesis in heart and muscle precursor cells. Loss-of-function *Mef2* variants result in a lack of heart and muscle differentiation in drosophila embryos (Olson et al., 1995). A recent study used RNA interference to knock down *Mef2* in the neurons of drosophila and found decreased sleep and increased night activity compared to wild-type flies (Klein et al., 2020).

Zebrafish are an excellent model for the research of human diseases. Zebrafish have external fertilization leading to transparent embryos and larvae, facilitating the observance of development (Lieschke & Currie, 2007). Interestingly, the *MEF2C* gene has been duplicated in the zebrafish genome as *mef2ca* and *mef2cb* (Adrião, Conceição, & Cancela, 2016). Both the *mef2ca* and *mef2cb* genes are expressed in several tissues, including brain, heart, vertebral column, branchial arches, muscle, kidney, mandibula, and cleithrum and operculum; however, *mef2ca* is most highly expressed in the vertebral column and *mef2cb* most highly expressed in the brain (Adrião, Conceição, & Cancela, 2016). Both *mef2cb* and *mef2cb* MEF2 domains have 100% homology compared to the human *MEF2C* MEF2 domain. Additionally, *mef2cb* MADS domain is 100% homologous to the human *MEF2C* MADS domain, whereas *mef2ca* is slightly less

similar at 98.3% homology of the human *MEF2C* MADS domain. Additionally, *mef2cb* is surrounded by many of the same genes that surround *MEF2C* in humans (including *TMEM161B, CCNH, RASA1, COX7C, EDIL3,* and *HAPLN1* downstream of *MEF2C* and *MBLAC2, POLR3G, LYSMD3, ADGRV1, ARRDC3,* and *NR2F1* upstream of *MEF2C*). Five of the genes surrounding *mef2cb* are also duplicated and surround *mef2ca* (Adrião, Conceição, & Cancela, 2016). Studies have shown that double mutant zebrafish (those lacking both *mef2ca* and *mef2cb*) lack proper cardiomyocyte differentiation and heart formation (Hinits et al., 2012). Some human patients with *MEF2C* alterations also present with cardiac findings, including ventricular septal defects (Lu et al., 2018; Qiao et al., 2017), myocardial hypertrophy (Engels et al., 2009), moderate tricuspid valve insufficiency (Cesaretti et al., 2016), and other cardiac issues.

Perhaps an even better model for human disease would be the mouse, as it is a mammal that shares a similar developmental pathway and organ systems (Why Are Mice Considered Excellent Models for Humans?, n.d.), and is more genetically similar to humans compared to other animal models, having >90% gene homology for human diseases ("A Comparison of Common Model Organisms — Part 1 - NemaMetrix," 2017). *Mef2c* homozygous null mice died in utero by embryonic day 10 due to severe heart defects: heart looping did not occur and, therefore, the right ventricle did not form (Lin et al., 1997). In order to study the role of *Mef2c* role in the developing brain, Li et al. (2008) created conditional knockout mice lacking *Mef2c* in neural progenitor cells. The mutant embryonic mice had a smaller brain size, less cortical thickness, and abnormal postmitotic neuron distribution but, overall, there was no change in cell proliferation (Li

et al., 2008). In adult mutant mice, the *Mef2c*-null neurons exhibited immature electrophysiological properties likely due to fewer synapses and postsynaptic receptors. Additionally, the *Mef2c* mutant mice exhibited behavioral phenotypes including anxiety, decreased cognitive function, and abnormal paw movement stereotypies (Li et al., 2008). The behavioral phenotypes are also seen in humans with genetic alterations involving *MEF2C,* thus providing more evidence for the use of mice as an animal model for human *MEF2C*-related disorders.

Conclusion

MEF2C-related disorders are rare neurodevelopmental disorders characterized by developmental delay, seizures, absent speech, hypotonia, and brain abnormalities. At least 117 patients have been reported worldwide; however, this disorder is difficult to diagnose clinically. Methods to research rare disease include theoretical and observational designs, such as concept analyses, literature reviews, natural history studies, and surveys, and experimental designs such as laboratory studies with animal models. In subsequent chapters, we show how these methods were used to gain further knowledge on *MEF2C*-related disorders. Specifically, we sought to elucidate the comprehensive phenotype of *MEF2C*-related disorders. In chapter 2, we use the concept analysis method to highlight, clarify, and define one of the disorder's features, tremors. In chapter 3, we perform a systematic literature review to answer the research question: "What is the comprehensive phenotype of all human patients reported with a *MEF2C*-related disorder?" In chapter 4, we further characterize the phenotype of the disorder

through a natural history study parent survey. Lastly, in chapter 5 we show how laboratory methods using *MEF2C* animal models can translate to knowledge on the human phenotype. These methods help illuminate the features of *MEF2C*-related disorders and other such rare disorders and aid in future diangosis, management, and treatment of patients.

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CHAPTER TWO:
TREMORS: A CONCEPT ANALYSIS

Title: **Tremors: A Concept Analysis**

Running Title: Tremors: A Concept Analysis

Authors: Cooley Coleman, JA[1,2], Sarasua, SM[1], Boccuto, L[1], Moore, HW[2], Skinner, SA[2], DeLuca, JM[1,2]

[1]School of Nursing, Clemson University, [2]Greenwood Genetic Center

Correspondence to: Jessica A. Cooley Coleman, cooley8@g.clemson.edu 864-941-8188

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Abstract

Aim. This article seeks to clarify and define the concept of *tremors*.

Design. The Walker & Avant (2005) concept analysis method was followed.

Methods. A search of PubMed, Academic Search Complete, CINAHL, ERIC, Google, and Google Scholar was performed.

Results. Through this process, uses of the concept were assessed including definitions and categories of tremors. Defining attributes were found to include “movement disorder”, “shaking motions”, “involuntary”, “oscillatory”, “rhythmic”, “not painful or life threatening”, “always present but variable”, and “can sometimes be repressed”. We identified two model cases and a borderline case, antecedents, consequences, and empirical referents (including measurement tools) of tremors.

Conclusion. The concept analysis process has clarified and illuminated an operational definition of tremors: that tremors are a movement disorder characterized by shaking motions that are involuntary, oscillatory, rhythmic, non-painful, always present although vary in severity, and can be repressed by changing posture or going into a rest position.

Ethics: Ethical approval was not required.

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Keywords. tremors, concept analysis, tremor management, tremor measurement, *MEF2C*, Fragile X-associated tremor/ataxia (FXTAS)

CHAPTER TWO:
TREMORS: A CONCEPT ANALYSIS

Background

Tremors are one of the most common types of movement disorders, with essential tremor (ET) being the most common of all adult movement disorders (Hess & Pullman, 2012). Tremors have been described equally in men and women and can affect a person at any age, although they are more common in adults middle-aged and older. Tremors can be a primary disorder, as seen in ET, a symptom of an underlying disorder like Parkinson disease, or they can be idiopathic (Kamble & Pal, 2018).

Of interest, tremors are present in many genetic disorders. A February 2020 search of OMIM for the term "tremor" identified 594 potential genetic conditions or genes associated with tremors (Online Mendelian Inheritance in Man, OMIM). Results at the top of the list contain the most qualities of the search term. These included hereditary ET, epilepsies, Fragile X-associated tremor/ataxia syndrome (FXTAS), Parkinson disease, and neurodegenerative conditions (Table 2.1). Tremors can be associated with metabolic conditions; examples of which include glutaric aciduria type I, Wilson disease, Niemann-Pick disease, and Krabbe disease (Online Mendelian Inheritance in Man, OMIM).

Table 2.1: Top Entries of Genetic Conditions Associated with Tremors Returned by OMIM from a 6 February 2020 search.

Result #	MIM Number	Disorder
1	#190300, %602134, %611456, #614782, #616736	TREMOR, HEREDITARY ESSENTIAL, 1, 2, 3, 4, 5; *ETM1*, *ETM2*, *ETM3*, *ETM4*, *ETM5*
2	#618524	MYOPATHY, CONGENITAL, WITH TREMOR (MYOTREM); *MYBPC1*
3	#300623	FRAGILE X TREMOR/ATAXIA SYNDROME (FXTAS); *FMR1*
4	#601068, #607876, #613608, #615127, #615400, #618074, #618075	EPILEPSY, FAMILIAL ADULT MYOCLONIC, 1, 2, 3, 4, 5, 6, 7; *FAME1*, *FAME2*, *FAME3*, *FAME4*, *FAME5*, *FAME6*, *FAME7*
5	%190310	TREMOR, NYSTAGMUS, AND DUODENAL ULCER
6	190200	TREMOR OF INTENTION, ATAXIA, AND LIPOFUSCINOSIS
7	*603967	SODIUM CHANNEL, VOLTAGE-GATED, TYPE IV, ALPHA SUBUNIT; *SCN4A*
8	#612126	GLUT1 DEFICIENCY SYNDROME 2 (GLUT1DS2); *SLC2A1*
9	#254900	EPILEPSY, PROGRESSIVE MYOCLONIC, 4, WITH OR WITHOUT RENAL FAILURE; *EPM4*
10	#607060	PARKINSON DISEASE 8, AUTOSOMAL DOMINANT (PARK8); *LRRK2*

OMIM Symbols:
#: Descriptive entry that does not represent a unique locus
%: Confirmed mendelian phenotype or phenotype locus with an unknown molecular basis
*: Gene
No symbol: Mendelian basis suspected but not confirmed

Most disorders on this list have tremors as one of many symptoms. The essential tremor disorder is different in that the only symptom is the tremor. Studies comparing monozygotic to dizygotic twins have shown that there is high genetic heritability for ET (Lorenz et al., 2004 and Tanner et al., 2001). Several genes, including *DRD3*, *FUS*, *TENM4*, *HTRA2*, *SCN4A*, *SORT1*, *SCN11A*, *NOS3*, *KCNS2*, *HAPLN4*, *USP45*, *and CACNA1G* were found to have some minor association, risk factor, or segregation in families with ET, but none are definitive. Variants found in many of these genes occur

only within certain ethnic groups (e.g. variants in *TENM4* were identified in Spanish families, but not in Chinese families). It is likely that ET is genetically heterogeneous with incomplete penetrance and is influenced by environmental and epigenetic factors. The lack of definitive causative genes is likely a result of these factors along with clinical misdiagnosis of ET (Deng, Wu, & Jankovic, 2019).

MEF2C-related disorders, also referred to as *MEF2C* haploinsufficiency syndrome, were not among the search result list in OMIM. However, an extensive review of the literature reveals cases of children with a *MEF2C*-related disorder also having tremors. One patient was reported to have a periodic tremor during infancy (Nowakowska et al., 2010) and a second patient was reported to have a hand tremor at seven years of age (Paciorkowski et al., 2013). Recently, there has been a growing interest of researching *MEF2C*-related disorders. This new connection between the disorder and tremors prompted interest in the analysis of the concept of tremors.

Although the term "tremors" may seem simple, the definition of the word is often quite vague (Tremor, 2019. In Merriam-Webster.com; Tremor, 2019. In Cambridge Dictionary; Tremor, 2019. In Lexico Oxford Dictionary), which may lead to a misunderstanding of the concept. Additionally, the concept is complicated by the various ways tremors are categorized and methods by which they are assessed clinically (Bhatia et al., 2018; Elias & Shah, 2014); therefore, it is important that researchers and healthcare providers understand how to distinguish between various tremor types, sometimes in combination with other symptoms, to properly measure, diagnose, and provide the most effective treatment to the patient.

To clarify the concept of tremors, the Walker and Avant (2005) concept analysis method was chosen due to its well-defined steps and prominent use in nursing science (Nuopponen, 2010). A concept analysis is a process in which the concept term is thoroughly explored to describe the essence and uses of the term and distinguish it from other closely related concepts (Walker & Avant, 2005). The research question undertaken with this process is: What is the conceptual and operational definition of the term tremor as it is applied in clinical practice?

Method

The Walker and Avant (2005) concept analysis method is a thorough process used to define a concept and distinguish it from other closely related concepts. This method consists of the following steps: 1) select a concept, 2) determine the aims and purpose of the analysis, 3) identify uses of the concept, 4) determine defining attributes, 5) identify a model case, 6) identify other cases (borderline, related, contrary, etc.), 7) identify antecedents and consequences, and 8) define empirical referents.

With the concept and aims identified, the next step was to identify uses of the concept. For this step, a search of the literature was performed. Walker and Avant (2005) recommends "only looking for the definitions and uses of the term", while making notes of characteristics (attributes), preceding events or incidents (antecedents), and outcomes (consequences) of the concept. The search is not for the purpose of performing a systematic literature review. The search included PubMed, Academic Search Complete, CINAHL, ERIC, Google, and Google Scholar, and used search terms "tremor", "tremors", "tremor concept", and "tremor concept analysis". These search terms were

used individually or in combination with each other. Search terms were general to entertain a broad perspective of the concept and to ensure a concept analysis did not already exist for the chosen concept. The search was limited to peer-reviewed scholarly articles published in the English language. Magazines, dissertations, and continuing education units were excluded. Additionally, the search results were limited to the past 20 years, spanning 01-01-2000 to 09-23-2019, to allow for more recent and relevant findings (Figure 2.1).

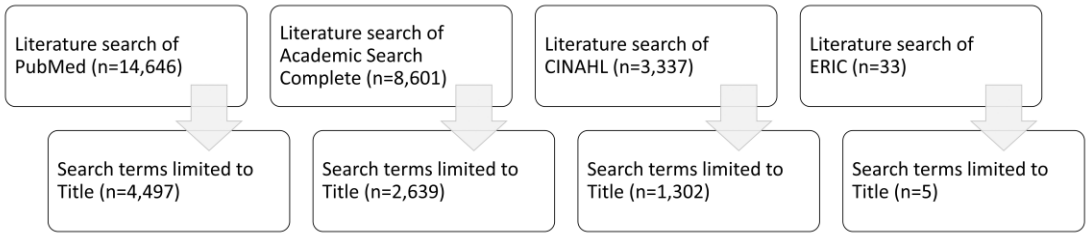

FIGURE 2.1

A literature search via PubMed, Academic Search Complete, CINAHL and ERIC was performed. Search terms used were “tremor”, “tremors”, “tremor concept”, and “tremor concept analysis”. The search was limited to peer-reviewed scholarly articles published in the English language. To allow for more recent and relevant findings, search terms were limited to the past 20 years. Next, search terms were applied specifically for the Title to narrow down results.

Of note, about one-fourth of the articles from PubMed mentioned ET and about one-eighth mentioned Parkinson in the title. Individually applying “tremor concept analysis” as the only search term within titles yielded no results in any of the searched

databases. The focus on results were limited to those featuring the biological and medical concept of tremors, and final analysis included articles, case studies, websites, and general and medical dictionaries. Definitions of "tremors" were obtained online from the Merriam-Webster Dictionary, the Cambridge Dictionary, the Lexico Oxford Dictionary, and the Mosby's Medical, Nursing, & Allied Health Dictionary.

Each step in the concept analysis process was an exercise in rigor via reading, rereading, and making critical decisions on content while avoiding topical drift (Walker & Avant, 2005). Rigor was also achieved through reflexivity by being self-aware of the content, direction, and potential biases. Additionally, this work was carefully critiqued by the coauthors who have experience in clinical genetics, qualitative research and other research methodologies. The concept analysis method consists of reviewing available literature, therefore ethical approval was not required.

Results

Aims and Purpose of Analysis

The purpose of this analysis is to clarify and develop a comprehensive operational definition of the biological and medical term and concept "tremor". Sample cases will be presented to illustrate the concept and to facilitate developing a strong operational definition. The relationship between the antecedents, defining attributes, consequences, and empirical referents of tremors can be seen in Figure 2.2, as well as thoroughly described in subsequent sections. The results of this analysis will improve knowledge and communication of the concept across many disciplines, such as education, research,

nursing, and medicine. Additionally, this information will aid healthcare providers in diagnosing and treating patients with tremors.

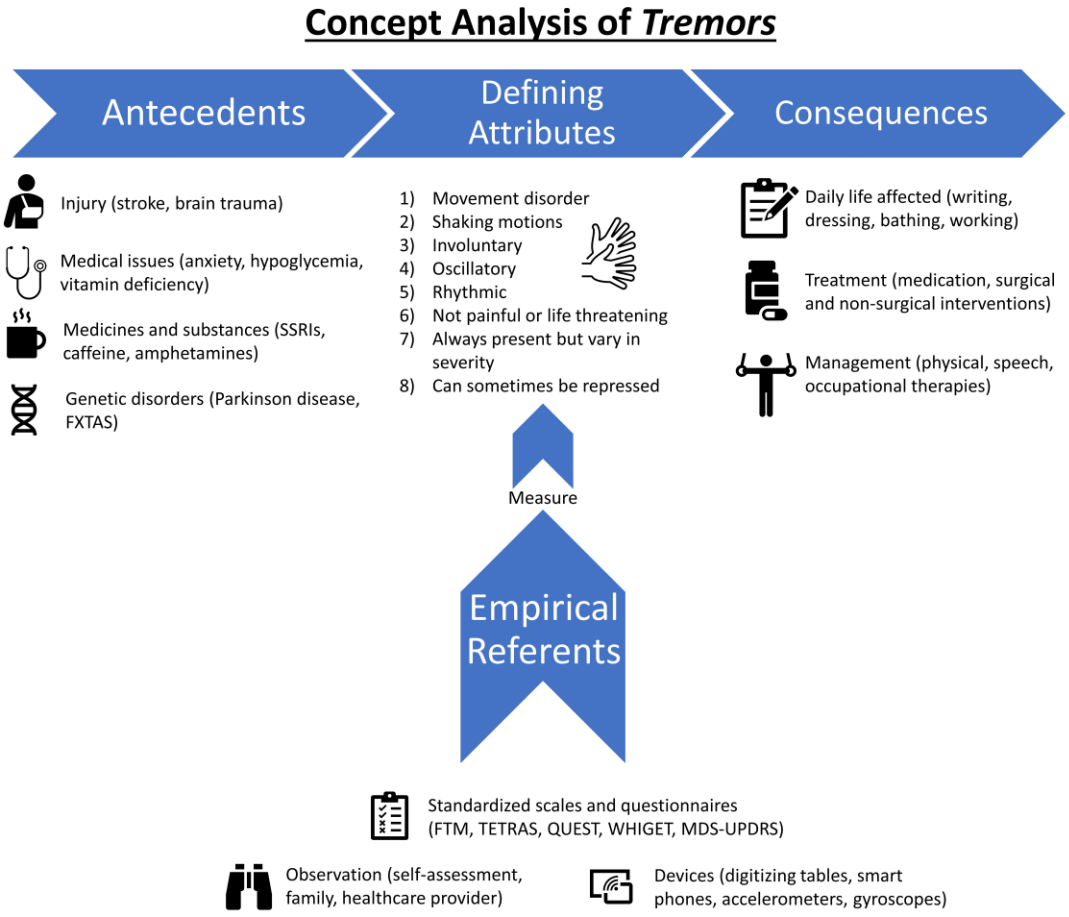

FIGURE 2.2
Schematic of the relationship between the antecedents, defining attributes, consequences, and empirical referents of tremors.

Definitions of Tremors from Dictionaries

The earliest use of the word tremor meant a feeling of terror, in line with its Latin roots, originating from the verb "*tremere*" (to tremble). In the 1600s and onward, tremor

was used to mean a shaking motion (Louis & Palmer, 2017). The Merriam-Webster Dictionary defines tremor as “1a) a trembling or shaking usually from physical weakness, emotional stress, or disease, 1b) nervous excitement; 2) a quivering or vibratory motion, especially: a discrete small movement following or preceding a major seismic event; 3a) a feeling of uncertainty or insecurity, 3b) a cause of such a feeling” (Tremor, 2019. In Merriam-Webster.com). The Cambridge Dictionary defines tremor as “1) a shaking movement in a person’s body, usually because of fright, excitement, or illness; 2) a slight earthquake (sudden, violent movement of the earth’s surface)” (Tremor, 2019. In Cambridge Dictionary). The Lexico Oxford Dictionary defines tremor as “1) an involuntary quivering movement, 1.1) a tremble or quiver in a person’s voice, 1.2) a sudden feeling of fear or excitement; 2) a slight earthquake” (Tremor, 2019. In Lexico Oxford Dictionary). As seen in definitions from various dictionaries, the word “tremor” is associated with a geological concept and as a feeling; however, these two versions of the concept will not be a focus in this analysis. Instead, we will focus on the biological and medical concept of tremors. Both the Merriam-Webster and Cambridge dictionaries include “shaking” but they differ in why a tremor takes place, except for each mentioning disease/illness. The Lexico Oxford dictionary goes a step further by clarifying these movements are “involuntary”.

Lastly, the Mosby's Medical, Nursing, & Allied Health Dictionary was consulted for a medical definition. In this dictionary, tremors are defined as “rhythmic, purposeless, quivering movements resulting from the involuntary alternating contraction and relaxation of opposing groups of skeletal muscles occurring in some elderly individuals,

certain families, and patients with various neurodegenerative disorders" (Tremors, 2001. In Mosby's Medical, Nursing, & Allied Health Dictionary).

Categories of Tremors in Literature and Practice

One common classification method is resting tremors versus action tremors (Table 2.2). Resting tremors occur in a body part that is supported against gravity with no voluntary movements taking place. Action tremors are those that take place with voluntary movements. There are further subcategories of action tremors including postural, kinetic, intention, task-specific, and isometric. Postural tremors are those that occur when a person holds a position against gravity, such as outstretching one's arms. Kinetic tremors occur during any voluntary movement. Intention tremors increase in severity as the person completes the movement. Task-specific tremors are ones that occur during specific tasks, such as writing. Lastly, isometric tremors appear after voluntary muscle contraction in an otherwise stationary body part, such as when one makes a fist (Elias & Shah, 2014).

Table 2.2: Common Classification Scheme for Tremors (Elias & Shah, 2014)

Tremor Type	**Description**
Resting	Occur in a body part that is supported against gravity with no voluntary movements taking place
Action	Takes place with voluntary movements
Postural	Occur when a person holds a position against gravity
Kinetic	Occur during any voluntary movement
Intention	Tend to increase in severity as the person completes the movement
Task-specific	Occur during specific tasks
Isometric	Occur after voluntary muscle contraction in an otherwise stationary body part

Another classification method distinguishes among physiological, exaggerated physiological, or pathological tremors (Table 2.3). Physiological tremors are present in everyone and are generally small scale and not readily detectable. These tremors are normal and occur with the transition of rest and movements of the muscles. Exaggerated, or enhanced, physiological tremors are normal tremors that worsen due to certain factors (such as age, hyperthyroidism, caffeine, stress, or anxiety) to the point of being visible. Pathological tremors are ones that impair and hinder a person's everyday life and are often a part of a disorder. The most common pathological tremors are ET and Parkinsonian tremor (Elias & Shah, 2014).

Table 2.3: Additional Classification Method for Tremors (Elias & Shah, 2014)

Tremor Type	**Description**
Physiological	Generally small-scale tremors present in most everyone but are not readily detectable
Exaggerated physiological	Physiological tremors that are worsened due to certain factors to the point of being visible
Pathological	Tremors that impair and hinder a person's everyday life and are often a part of a disorder

On other occasions, tremors are classified solely on their etiology, such as Parkinsonian tremor, or based on the anatomical origin of the tremors, such as cerebellar tremor. Others may be based on the situational occurrence of the tremor, such as primary writing tremor. It can often be difficult to distinguish between tremor conditions, and the matter can be complicated even more given the various ways to categorize tremors. The Task Force on "Tremor of the International Parkinson and Movement Disorder Society"

had published consensus criteria for tremors in 1988. They reconvened in 2018 to resolve inconsistencies and release their updated classification system. The task force proposed classification along two axes. Axis 1 included clinical characteristics and features, such as family history, age of onset, and location of the tremors in the body. Axis 2 consisted of the etiology of the tremors, such as being either acquired, genetic, or idiopathic (Bhatia et al., 2018).

Distinguishing Tremors from Other Related Disorders

Many movement disorders appear similar to tremors, but they too have their own defining attributes to differentiate them from tremors. Seizures, myoclonus, shivering, tics, and akathisia all have some overlapping features to tremors, most noticeable would be the shaking movement, but there are also clues that help distinguish them. Mostly, tremors are constant but may be so slight that one does not notice it happening. However, there are a few tremor disorders that appear intermittently, such as tremors caused by some metabolic disorders, Leigh syndrome, migraines, and dominant episodic ataxias (Torres-Russotto, 2019). Seizures may come in spells, and then the shaking disappears. During a seizure, the person may be cognitively impaired and also cannot control the seizure by simply changing their position or posture. Myoclonus movements are characterized by a "jerk-release" movement, therefore are not oscillatory. Shivering often occurs only as a single spell and can involve trunk muscles, which is not typically a feature of tremors. Tics are episodic and fast but can be voluntarily withheld by the

person at times. Akathisia consists of oscillatory movements, but they are irregular, episodic, and like tics, can be voluntarily withheld (Torres-Russotto, 2019).

Defining Attributes of Tremors

Defining attributes are the characteristics of the concept that define it and distinguish it from other concepts. Through this analysis, several defining attributes of tremors emerged. Tremors are 1) a movement disorder, characterized by 2) shaking motions that are 3) involuntary, 4) oscillatory, which is to repeat back and forth around a central point, 5) rhythmic, or having a regular pattern or motion, 6) are not painful or life threatening, and 7) the majority are always present but can vary in severity, including to the point where they do not seem noticeable by the person experiencing them; 8) lastly, tremors can sometimes be repressed by changing the body's posture, or by putting the affected body part into a rest position.

Model Case, Borderline Case, and Contrary Case

A model case is one that displays all the defining attributes and is considered a definitive example of the concept (Walker & Avant, 2005). A borderline case exhibits some but not all the defining attributes of the concept, and therefore is similar but not exactly the same. The contrary case does not exhibit any of the defining attributes, showing clearly what the concept is not. The following case reports were found in the literature and are used here to demonstrate and differentiate the concept.

Model Case 1: Essential Tremor

Hawkins-Walsh (2003) reported a 21-year-old male who saw his physician for a routine checkup. He stated he was well with no illnesses but has noticed his arms and hands were shaking quite often recently. He was unsure of exactly when the shaking started, but it has been a few years and has gotten worse lately to the point that his friends have expressed concern. He reported taking 10 mg Ritalin (methylphenidate) twice daily for attention-deficit/hyperactivity disorder, but no other medications, illegal drugs, or tobacco. He noted that alcohol consumption contributed to decreasing the shaking and reported having three to four beers a night on the weekends. He also reported drinking one to two caffeinated sodas daily. Upon physical examination, his speech was clear, there were no gait abnormalities, no clonus present, and his posture was normal, but there was shaking present upon finger-to-nose test, handwriting test, and when extending his arms against gravity. His cranial nerves and tendon reflexes were also normal. He reported no family history of Parkinson, multiple sclerosis, or seizures, but it was revealed that his father also had shaking in his hands. His father said he always thought the shaking ran in the family, indicating a larger family history (Hawkins-Walsh, 2003).

Parkinson disease was ruled out since the patient did not have any other neurological issues. The clinician tested the patient's thyrotropin levels (also known as thyroid-stimulating hormone), which came back normal. Based on the physical examination and family history, the clinician diagnosed the patient with ET. ET is the most common form of tremor and movement disorder. The upper limbs are most affected, followed by the head, lower limbs, voice, face, and trunk. ET can run in

families, indicating an autosomal dominant genetic pattern. There have been some genes linked to ET in certain populations, such as *DRD3* and *TENM4*; however, ET is very heterogeneous and many of the genes are still unknown (Online Mendelian Inheritance in Man, OMIM). ET often improves with consumption of alcohol, but it is important to note the risk of abuse if a person relies on alcohol to control the tremor, as greater amounts of alcohol will eventually be needed to achieve the same result. The patient was advised to decrease his caffeine intake and was told of potential medications that could help with ET. He was informed that Ritalin could also be aggravating the tremor. He was advised on the risks of relying on alcohol to improve his symptoms (Hawkins-Walsh, 2003). The patient was going to be able to continue his college career and said he would be sure to limit factors that would aggravate his tremors.

In conclusion, the physician was able to see the involuntary shaking in the patient's hands and arms upon physical examination. The physician would have seen that the movements were rhythmic and oscillatory. The tremors were not painful or life threatening to the patient but were always present at some level to the point that his friends had noticed. The shaking could be repressed enough to manage his academic career, but the tremors still happened quite often. It is clear to see that the patient exhibited all the defining attributes of tremors.

Model Case 2: Fragile X-associated tremor/ataxia

Another clear model case was described by Cerquera's group in their 2016 case report. A patient came to the clinic due to his disabling tremors. Upon examination, the

clinicians noted a resting tremor in his right hand, as well as rigidity, bradykinesia, or slowness of movement, and hypomimia, or reduced facial expressions. A dopamine transporter single-photon emission computerized tomography (SPECT) analysis was abnormal, showing less uptake of the injected tracer in the dopamine receptors in the brain, indicative of Parkinson disease. The patient was diagnosed with Parkinson disease. However, it was also revealed that his daughter was a premutation carrier for Fragile-X syndrome, and his grandson had a full mutation and was affected with Fragile-X syndrome. A person in the normal range would have up to 54 CGG repeats in the 5' untranslated region of the *FMR1* gene. A premutation would contain 55-200 repeats, and a full mutation is over 200 repeats (Willemsen, Levenga, & Oostra, 2011). Upon testing, it was shown that the patient had a premutation of 90 CGG repeats, which lead to the diagnosis of Fragile X-associated tremor/ataxia (FXTAS). The authors mentioned that it is possible this patient presented with parkinsonism only because of the FXTAS; however, the authors noted two other cases in the literature of patients with both Parkinson disease and FXTAS (Cerquera et al., 2016).

Upon being diagnosed with Parkinson disease, the patient was prescribed levodopa, which improved the patient's rigidity but did not have a large impact on the tremors. Over the following four years, the patient developed bilateral postural and action tremor of his hands. Additionally, his gait was affected, and he became confined to a wheelchair. The clinicians prescribed several other drugs, which he also responded to poorly (Cerquera et al., 2016). The patient opted for another form of treatment, which will be discussed in a subsequent section.

The patient has a movement disorder characterized involuntary, rhythmic, and oscillatory shaking motions. Although the tremors have affected his daily life, they were not reported to be painful or life threatening. It was not stated if the patient's tremors could be suppressed, but the tremors had increased in severity over the years. The patient's condition meets all the defining attributes of tremors.

Borderline Case: Seizures

A case report was published by Hayashi, Miura, Uzawa, Baba, and Yamamoto (2018) in which they describe a 34-year-old male with reduced vision and night blindness. The patient was being seen for a complete ophthalmic examination, including several ophthalmologic examinations, and full-field electroretinograms recordings (ERGs). During the ERG process, pupils are dilated and then electrical signals from the retina are recorded during dark and light exposure. Both dark-adapted and light-adapted ERG were performed, followed by 30 Hz light flicker light-adapted ERG (Hayashi et al., 2018).

Before transitioning to long-duration flashing ERG recordings, the patient alerted the clinician that he was developing paralysis in his upper limbs. Directly after, he started having lower limb convulsions and then lost consciousness. The patient was given an injection of diazepam, and the convulsions ceased. Later, he had magnetic resonance imaging (MRI), computed tomography of the head, and electroencephalogram examination, all of which were normal. After the ordeal, the patient mentioned that he had lost consciousness with seizures in the past. These seizures were caused by the flashing light of the ERG exam. Flickering of artificial and even natural light has been

known to induce seizures, therefore the patient was diagnosed with photosensitive epileptic seizures. The authors stress that providers should obtain a detailed seizure history about a patient before conducting ERG recordings to avoid an ordeal like this patient experienced (Hayashi et al., 2018).

The convulsions were an involuntary shaking movement disorder; however, they were not rhythmic or oscillatory and instead were very jerky movements. The flashing lights of the ERG exam led to abnormal neuronal discharges in the patient's brain, resulting in seizures and loss of consciousness. Light as a trigger and loss of consciousness are not traits that are associated with tremors. The patient's seizures only come about with certain stimuli (light), whereas tremors are usually a constant presence. The patient's condition meets the defining attributes of movement disorder, shaking motions, and involuntariness. However, oscillatory, rhythmic, not life threatening, constant presence, and ability to repress were defining attributes that were not met.

Contrary Case

Cinotti, Trovato, Fimiani, & Rubegni (2018) published a case report about a 58-year-old patient with a previous diagnosis and 20-year history of systemic lupus erythematosus. The patient came to the emergency department with multiple cutaneous hematomas that arose without any traumatic event occurring. Clinicians tested her platelet count, and the results were normal. Additionally, her lupus anticoagulant and antiphospholipid antibodies were negative. No hemorrhage was seen on abdominal ultrasound or skull computed tomography. Her partial thromboplastin time was elongated at greater than 54 seconds (normally between 20 and 34 seconds). All intrinsic factors of

coagulation (FXII, FXI, FX, FVII, FVIII) were tested. The patient's FVIII activity level was less than 1%, and a level below 50% can be indicative of hemophilia A. A Bethesda assay was performed and yielded a result of 15.2 Bethesda units (BU), whereas the normal value should be less than 0.5 BU (Cinotti et al., 2018).

The patient was diagnosed with acquired hemophilia A (AHA). Her immune system created antibodies against her own FVIII proteins, thus depleting her FVIII levels and causing the severe presentation that prompted her to go to the emergency room. The clinicians prescribed prednisolone at a dose of 1 mg/kg/day with decreasing dosage over a three-month period, and her FVIII levels returned to normal and symptoms vastly improved. The authors advise that providers consider AHA if patients with systemic lupus erythematosus also present with hematomas and prolonged partial thromboplastin time (Cinotti et al, 2018).

The patient's condition has its own set of attributes, but none match the attributes of tremors. She was not exhibiting a movement disorder and was not shaking involuntarily in a rhythmic and oscillatory fashion. It is not mentioned if the patient was having pain, but likely she was sore at the sites of the hematomas. As tremors are not painful, this is another attribute that does not match. Tremors are also not life threatening, but the patient's condition could have been if she had a traumatic event and could not stop the bleeding. Lastly, the patient's condition would not be improved simply by changing her posture or trying to prevent it. With none of the defining attributes of tremors, this is just one of the many potential examples of a contrary case to tremors.

Antecedents of Tremors

Antecedents are conditions or events that happen before the concept occurs (Walker & Avant, 2005). Antecedents of tremors include injury, genetic disorders, non-genetic medical issues, and medications or substances. Injury to the brain, such as stroke or trauma from a blow or accident, can cause a person to have tremors (Tremor Fact Sheet, NINDS, 2017). Tremors are common in patients with certain genetic disorders (Table 2.1). Some have been previously mentioned, but can include Parkinson disease, familial ET, Fragile X-associated tremor/ataxia syndrome (FXTAS), spinal muscular atrophy, spinocerebellar ataxia, as well as other perhaps less known genetic disorders such as Wilson disease, Perry syndrome, Wiedemann-Rautenstrauch syndrome, and Partington syndrome, among others (Tremors. (n.d.). In National Library of Medicine (US)). Tremors can also be caused by other non-genetic medical conditions, including anxiety, hyperthyroidism, hypoglycemia, fever, liver or kidney failure, multiple sclerosis, and vitamin E, vitamin B12, zinc, or magnesium deficiency. Lastly, tremors can be the result of certain medications or substances. Medications such as selective serotonin reuptake inhibitors (SSRIs), beta agonists, and amphetamines may have side effects of tremors (Warren, 2017). Substances such as an excess of caffeine or mercury poisoning can also cause tremors (Tremor Fact Sheet, NINDS, 2017).

Consequences of Tremors

Consequences are the events that happen after the concept has occurred (Walker & Avant, 2005). Although tremors are not life threatening, they could become so debilitating that the person's daily life is severely affected. Tremors may affect a person's

ability to feed, bathe, and dress themselves. Tremors could also affect a person's ability to write and type, which could lead to decreased job performance or termination. The tremor may be so debilitating that a caretaker is required, which would be quite an expense for the person. Tremors could also affect the person's social life as they may limit their exposure to others due to embarrassment.

Management or treatment could be a consequence of tremors. Physical, speech, and occupational therapies can help with managing tremors. Reducing external substances that cause or exaggerate tremors, such as caffeine, should be considered. Medications, including beta blockers, anti-seizure drugs, or tranquilizers can be prescribed to help with tremors. However, tranquilizers are to be used with care due to their side effects of sleepiness, poor concentration and coordination, and developing dependence. There are medications available specifically for treating tremors due to Parkinson disease. Botulinum toxin injections can also help control tremors; however, the toxin can cause muscle weakness (Tremor Fact Sheet, NINDS, 2017).

Surgical interventions may be necessary or chosen to help treat tremors. Two surgical methods include deep brain stimulation (DBS) and thalamotomy. During DBS, electrodes are surgically implanted in the brain and electrical signals are sent to the thalamus, the region of the brain responsible for involuntary body movement. A thalamotomy involves surgically destroying a small portion of the thalamus. This procedure is a last resort when medications and other treatments are not working. Thalamotomies are rarely performed today due to alternate non-surgical treatments that are available. Non-surgical interventions include radiofrequency ablation and focused

ultrasound. Radiofrequency ablation is often used to treat pain but can also treat tremors. It uses an electrical signal to heat nerve tissue, which blocks the tremor signal to the body. This method is not permanent and would have to be repeated. Focused ultrasound uses ultrasound waves guided by MRI to create a lesion in the thalamus (Tremor Fact Sheet, NINDS, 2017).

Recall the model case patient with FXTAS and Parkinson disease who developed worsening tremors over the years. The patient was not a candidate for deep brain stimulation (DBS) due to his age, cognitive impairment, and brain atrophy. Due to these issues, it was predicted that DBS would have a poor outcome and higher risk of complications. Therefore, he opted for MRI guided focused ultrasound. The patient had remarkable improvement: 83% relief of tremor severity according to two rating scales (right limbs score and Fahn-Tolosa-Marin tremor rating scale), 50% increase in motor tasks, and 40% improvement in his disability. The patient's tremor was vastly improved, and he was again able to feed himself and use utensils after having previously lost that ability (Cerquera et al., 2016).

Empirical Referents

Empirical referents are events that prove the concept occurred (Walker & Avant, 2005). The empirical referents do not measure the concept itself but identify and measure the defining attributes. A person would know the difference between normal movement and a tremor just by observation (self-assessment or observation by another person, like a family member). A healthcare provider could also be seen to confirm tremors in the

patient. Assessment may also include drawing tests, computerized tremor analysis using special devices, questionnaires, and standardized scales (Table 2.4).

Table 2.4: Empirical Referents: events that measure the tremors' defining attributes

Empirical Referents	**Types**
Transducer Devices	Accelerometers: measures tremors by linear acceleration (Elble & McNames, 2016)
	Gyroscopes: measures tremors by angular momentum to sense rotation (Elble & McNames, 2016)
	Digitizing tablets: assesses writing and drawing to measure effects of tremor (Elble & McNames, 2016)
	Smart phones: apps can measure acceleration, degree and speed of rotation (Kubben, Kuijf, Ackermans, Leentjes, & Temel, 2016)
Assessment	Self or clinical. Includes observation, writing and drawing tests.
Standardized Scales	Fahn-Tolosa-Marin Tremor Rating Scale (FTM): 5-point scale to rate tremors on severity and body part (Fahn, Tolosa, & Marin, 1988)
	The Essential Tremor Rating Assessment Scale (TETRAS): scale that assesses ET (Elble, 2016)
Questionnaires	Quality of Life in Essential Tremor Questionnaire (QUEST): questions on tremor severity, impact, perceived health and quality of life (Tröster, Pahwa, Fields, Tanner, & Lyons, 2005)
	Hand Tremor Questionnaire: questions to differentiate between ET and Parkinson Disease (Kwon et al., 2018)
PhenX Toolkit	Washington Heights-Inwood Genetic Study of Essential Tremor (WHIGET) Tremor Rating Scale: 23-item exam for the rating of tremors (Hamilton et al., 2011)
	Movement Disorder Society United Parkinson's Disease Rating Scale (MDS-UPDRS): measures the symptom severity for Parkinson Disease (Hamilton et al., 2011)

Digital tablets can be used to access writing and drawing tests instead of using the naked eye to score these tests (Elble & McNames, 2016). The frequency and amplitude of the tremors can be measured, which will also help classify what type of tremor is

occurring. For example, action and dystonic tremors often have a low frequency (4-8 Hz), physiologic and other types of action tremors may have a medium frequency (7-11 Hz), and orthostatic tremor will have a high frequency (>12 Hz) (Torres-Russotto, 2019). Transducer devices are used to measure the tremor in units of hertz (Hz). These devices are often portable and can include accelerometers, gyroscopes, digitizing tablets, and, most recently, smart phones. An accelerometer measures linear acceleration, whereas a gyroscope can sense rotation by measuring angular momentum. The use of smart phones could lead to a more rapid evaluation of the patient's tremor. TREMOR12 app was developed by Pieter L. Kubben to measure acceleration, degree of rotation, rotation speed of the tremors, and gravity to standardize. Raw data can be exported from the app for analysis (Kubben, Kuijf, Ackermans, Leentjes, & Temel, 2016).

Standardized scales, such as the Fahn-Tolosa-Marin Tremor Rating Scale (FTM) or The Essential Tremor Rating Assessment Scale (TETRAS), can be used to measure tremors. The FTM scale is a 5-point scale used to rate tremors on severity, body part, and assesses handwriting, drawing, pouring water, speaking, feeding solids and liquids, hygiene, dressing, and working (Fahn, Tolosa, & Marin, 1988). TETRAS assesses ET, especially focusing on the upper limbs which play a larger role in ET. This scale examines head, face, voice, and lower limb tremors, as well as handwriting, and standing performance, and rates each section from 0 to 4 (Elble, 2016). Differences between these two scales are that TETRAS includes a wing-beating upper limb assessment that the FTM does not include. Conversely, the FTM has a measure for rest tremor, which is omitted by the TETRAS since rest tremor is typically not a main hindrance in ET.

TETRAS may be better suited for measuring ET and severe tremors, while FTM may be better for tremor disorders that have a rest tremor component (Ondo et al., 2018).

There are also questionnaires available, such as the Quality of Life in Essential Tremor Questionnaire (QUEST) and the Hand Tremor Questionnaire. The QUEST Questionnaire has questions about tremor severity, tremor impact, and perceived health and quality of life (Tröster, Pahwa, Fields, Tanner, & Lyons, 2005). The Hand Tremor Questionnaire includes five questions in which a person with Parkinson disease would answer "yes", and seven questions in which a person with ET would answer "yes"; therefore, this scale is used to differentiate between Parkinson disease and ET (Kwon et al., 2018). The PhenX toolkit, which is a catalog of recommended measurement protocols, includes the Signs of Essential Tremors Washington Heights-Inwood Genetic Study of Essential Tremor (WHIGET) Tremor Rating Scale and Parkinsons Disease Symptoms Movement Disorder Society United Parkinson's Disease Rating Scale (MDS-UPDRS). The WHIGET tremor rating scale is a 23-item exam with items performed while seated and standing. The exam is meant to be videotaped and scored as recommended in the protocol. The MDS-UPDRS is specifically to measure severity of Parkinson disease by examining motor and non-motor exercises (Hamilton et al., 2011).

Conclusion

Tremors have been reported as a primary disorder as well as secondary symptoms of other underlying disorders, including many genetic disorders. Due to the ongoing and upcoming research on *MEF2C*-related disorders, where tremors have been occasionally reported as a symptom, the concept of tremors was chosen for this concept analysis. In

addition to clarifying the concept, an operational definition, antecedents, defining attributes, consequences, and empirical referents of the concept of tremors have emerged. The operational definition developed by this concept analysis is that tremors are a movement disorder characterized by shaking motions that are involuntary, oscillatory, rhythmic, non-painful, always present although vary in severity, and can be repressed by changing posture or going into a rest position. Additionally, two model cases, a borderline case, and a contrary case have been discussed to further illuminate and delineate the concept, and assessment tools were reviewed.

The rigorous Walker and Avant method was used to distinguish the concept of tremors, but this method has some limitations. Given the focus was on the Walker and Avant steps, information that did not fall into those specific categories could be missing. Another limitation was the number of sources returned by the literature search. Although titles were sorted and reviewed, and select sources were fully read to conduct the concept analysis steps, there is the possibility that other sources not fully read could have included helpful information for the concept analysis. Although English is considered the universal language of science, limiting the sources to English alone could be another limitation. Lastly, the concept analysis focused on the medical term of tremors, and therefore this narrower focus could be a potential limitation.

This is the first concept analysis applied to tremors. Future research could include reviewing diagnostic criteria of the empirical referents (such as the FTM or TETRAS) or performing an assessment of the knowledge and understanding of tremors in current practicing providers in order to verify the definition developed by this concept analysis.

This clarification of the concept will assist healthcare providers, researchers, and nurses in categorizing and recognizing the various types of tremors, as well as distinguishing between other closely related concepts, such as tics and seizures. This is especially important when tremors interfere with the patients' quality of life. Lastly, this information will help these professionals provide a comprehensive assessment of the type and severity of tremor, gauge the level of patient concern, and provide the best treatment and care to the patient.

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CHAPTER THREE:
COMPREHENSIVE INVESTIGATION OF THE PHENOTYPE OF MEF2C-RELATED DISORDERS IN HUMAN PATIENTS: A SYSTEMATIC REVIEW

Title: **Comprehensive Investigation Of The Phenotype Of *MEF2C*-Related Disorders In Human Patients: A Systematic Review**

Running Title: Tremors: Phenotype of *MEF2C*-Related Disorders Systematic Review

Authors: Cooley Coleman, JA[1,2], Sarasua, SM[1], Boccuto, L[1], Moore, HW[2], Skinner, SA[2], DeLuca, JM[1,2]

[1]School of Nursing, Clemson University, [2]Greenwood Genetic Center

Correspondence to: Jessica A. Cooley Coleman, cooley8@g.clemson.edu 864-941-8188

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ABSTRACT

MEF2C-related disorders (aka *MEF2C*-haploinsufficiency) are caused by variations in or involving the *MEF2C* gene and are characterized by intellectual disability, developmental delay, lack of speech, limited walking, and seizures. Despite these findings, the disorder is not easily recognized clinically. We performed a systematic review following PRISMA guidelines to assemble the most comprehensive list of patients and their phenotypes. Through searching PubMed, Web of Science, and MEDLINE, 43 articles met the inclusion criteria and were fully reviewed. One hundred and seventeen patients were identified from these publications with most having a phenotype of intellectual disability, developmental delay, seizures, hypotonia, absent speech, inability to walk, stereotypic movements, and MRI abnormalities. Non-classical findings included one patient with a question mark ear, two patients with a jugular pit, one patient with a unique neuroendocrine finding, and nine patients that did not have *MEF2C* deletions or disruptions but may be affected due to a positional effect on *MEF2C*. This systematic review characterizes the phenotype of *MEF2C*-related disorders, documents the severity of this condition, and will help providers to better diagnose and care for patients and their families. Additionally, this compiled information provides a comprehensive resource for investigators interested in pursuing specific genotype-phenotype correlations.

Keywords: *MEF2C*, *MEF2C* haploinsufficiency, phenotype, systematic review

CHAPTER THREE

COMPREHENSIVE INVESTIGATION OF THE PHENOTYPE OF MEF2C-RELATED DISORDERS IN HUMAN PATIENTS: A SYSTEMATIC REVIEW

Introduction

The *MEF2C* gene is a member of the myocyte enhancer factor 2 (MEF2) subfamily of the MADS (MCM1-agamous-deficiens-serum response factor) gene family of transcription factors. Transcription factors in the MEF2 family consist of a highly conserved N-terminal MADS-box that is adjacent to a MEF2 domain. These domains facilitate dimerization, interaction with other transcription factors, and DNA binding. *MEF2C* is particularly crucial during embryogenesis as it plays a role in myogenesis, neural crest formation, anterior heart field development, lymphoid development, neurogenesis, and synaptic formation, among other functions (Zweier et al., 2010).

Quite a few microdeletions encompassing chromosome region 5q14.3 have been reported in the literature over the past decade. Initially, some patients with similar phenotypes were reported to have microdeletions that did not include *MEF2C* (Cardoso et al., 2009; Engels et al., 2009). A year later, additional patients with deletions were reported, one of which had *MEF2C* as the only deleted gene (Le Meur et al., 2010). In the same study, a patient with a nonsense variant in *MEF2C* was reported. A few months later, another study reported two additional patients with deletions in this 5q14.3 region including the *MEF2C* gene, and four patients with point mutations in *MEF2C* (Zweier et al., 2010). This led to the determination that *MEF2C* was likely the causative gene of the phenotype in these 5q14.3 deletions.

Zweier et al. (2010) isolated RNA from blood and performed expression studies by quantitative real-time PCR on their six patients as well as the three patients reported by Engels et al. (2009), one of which had a deletion ending 329 kb upstream of *MEF2C.* Of the total nine patients, seven had *MEF2C* expression levels that were significantly decreased (five patients with microdeletions and two patients with truncating variants), one had levels that were significantly increased (a patient with a missense variant), and one had relatively normal expression levels (another patient with a missense variant). The Engels et al. patient that had a microdeletion not encompassing the *MEF2C* gene itself was among those with decreased *MEF2C* expression. It is likely that deletions distal or proximal to the *MEF2C* gene may have a positional effect that disrupts the expression of *MEF2C* (Zweier et al., 2010). However, there have been other reports of downstream deletions (1.1Mb away from *MEF2C*, Shimojima et al., 2012) and a translocation upstream of *MEF2C* (121.5kb away from *MEF2C*, Saitsu et al., 2011) that did not affect *MEF2C* gene expression. Saitsu et al. (2011) hypothesized that the expression could be tissue-specific (i.e., the developing brain), which may explain why expression was not altered in lymphoblasts in these two cases. Additional studies will need to be performed to elucidate the exact mechanism of these positional effects.

MEF2C-related disorders and haploinsufficiency are reported to have a clinical presentation of intellectual disability, developmental delay, lack of speech, limited walking, and seizures (Paciorkowski *et al*., 2014). *MEF2C*-related disorders are rare, not fully characterized, and hard to distinguish clinically. Many manuscripts report one or only a few patients. Our aim was to conduct a systematic review to assemble the most

comprehensive list of patients with a *MEF2C*-related disorder and thoroughly investigate their phenotypes. This review will further characterize the disorder, highlight the defining features, and assist healthcare providers in diagnosing and delivering the best clinical care for patients and their families.

Methods

Editorial Policies and Ethical Considerations

Ethical approval was not required as data included in this systematic review comes from peer-reviewed, published literature.

Systematic Review Protocol

We conducted a systematic literature review following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Moher, Liberati, Tetzlaff, Altman, & The PRISMA Group, 2009). The search strategy and inclusion and exclusion criteria were developed by the first author and are described below. A protocol was developed for registration to PROSPERO (supplementary document 1). The screening was performed in two stages: first on titles and abstracts and second on the full text. The PRISMA flow diagram map and Zotero Citation Manager (Version 5.0.90; Roy Rosenzweig Center for History and New Media, 2020) were used to manage the screening process and articles. Necessary data were extracted from the articles allowing final conclusions to be produced.

Systematic Review Research Question

We used the CoCoPop approach to frame our research question. The abbreviation CoCoPop stands for Condition, Context, and Population (Munn, 2018). Our research question for this systematic review was: What is the comprehensive phenotype of all human patients reported with a *MEF2C*-related disorder? The condition would be *MEF2C*-related disorders, the context would be the phenotype, and the population is human patients. This format lends itself to systematic reviews on the prevalence and/or incidence of a certain condition. Although prevalence and incidence were not addressed directly, gathering a comprehensive list of patients and their phenotypes elucidated how rare the disorder truly is.

Search Strategy

The following electronic databases were searched: Web of Science, PubMed, and MEDLINE. The search strategy included terms relating to the research question from the CoCoPop framework. Search terms were adapted for database-specific filters. Database searches were conducted using the keywords, MeSH terms, and combinations of each with specific Boolean operators as shown in Table 3.1. Other articles were selected after screening the bibliography of articles meeting the inclusion criteria.

Table 3.1: Search terms and strategy.

Concept (CoCoPop)	**Keywords**	**MeSH terms**
Co: Condition *MEF2C*-related disorder	"*MEF2C*" OR "*MEF2C*-related disorder" OR "*MEF2C* haploinsufficiency"	Haploinsufficiency (MeSH term to only be used in conjunction with "AND *MEF2C*")
Co: Context Phenotype	"phenotype" OR "present*" OR "presentation" OR	Phenotype

	"clinical presentation" OR "feature*" OR "character*"	
Pop: Population Human Patients	"human" OR "patient" OR "male" OR "female"	Humans OR Patients OR Male or Female
Overall Search		
PubMed: **((((MEF2C[Title/Abstract] OR MEF2C-related disorder[Title/Abstract] OR MEF2C haploinsufficiency[Title/Abstract] OR (MEF2C[Title/Abstract] AND** Haploinsufficiency**[MeSH Terms])) AND (phenotype OR present* OR presentation OR clinical presentation OR feature* OR character* OR phenotype[MeSH Terms])) AND (human OR patient OR male OR female OR Humans[MeSH Terms] OR Patients[MeSH Terms] OR Male[MeSH Terms] OR Female[MeSH Terms]))))**		
MEDLINE: AB (MEF2C OR "MEF2C-related disorder" OR "MEF2C haploinsufficiency" OR (MH haploinsufficiency AND MEF2C)) AND (phenotype OR present* OR presentation OR "clinical presentation" OR feature* OR character* OR MH Phenotype) AND (human OR patient OR male OR female OR MH humans OR MH patients OR MH Male OR MH Female)		
Web of Science: TOPIC: (MEF2C OR "MEF2C-related disorder" OR "MEF2C haploinsufficiency") AND TOPIC: (phenotype OR present* OR presentation OR clinical presentation OR feature* OR character*) AND TOPIC: (human OR patient OR male OR female)		

Inclusion and Exclusion Criteria

Only peer-reviewed publications in the English language were considered for inclusion. All scientific journals and article types were considered. Gray literature and dissertation material were not included. There was no restriction to publication dates: articles reviewed included those from the very first publication on the search criteria up until the search date of May 9th, 2021. Article title and abstracts were scanned for mention of phenotype information on a human patient case having a *MEF2C*-related disorder. Only articles that included phenotypic information on a human patient were considered for inclusion. Studies available in meeting abstract format only were excluded

due to lack of information. Articles focusing solely on animal or cell culture studies and lacking a human case report were excluded. Articles that met the inclusion criteria by title and abstract review were then subjected to full-text review.

Data Extraction

The first author extracted data from the articles under full-text review. A summary table was created for data extraction with the following column headers: study type, authors, year published, location published, verification of human case, number of patients, patient sex, patient age, phenotype, and clinical information reported, how phenotype was reported, variation reported, inheritance pattern, methods used to detect variation, and article citation in APA format (supplementary document 2). Special focus was given to extract all phenotype information reported. The summary table was then used to create a phenotype table (**supplementary document 3**).

Results

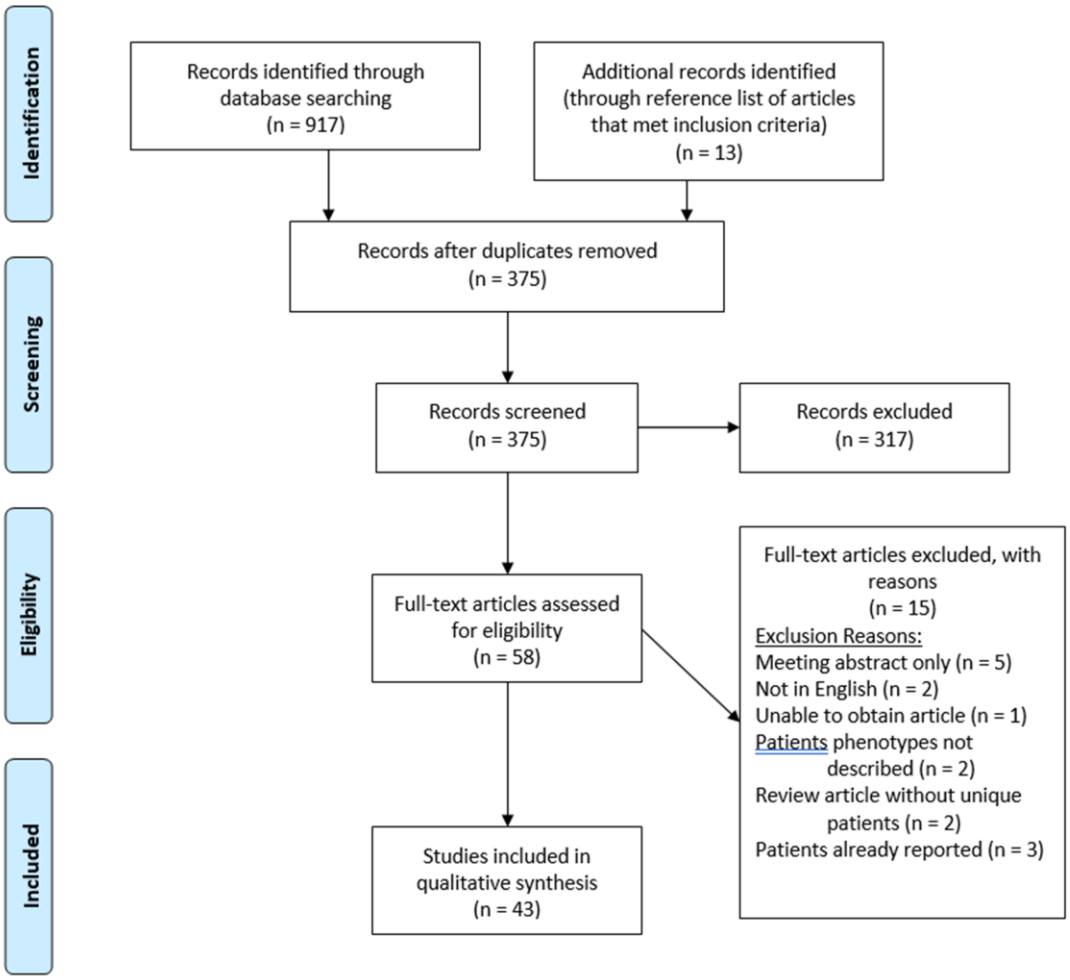
The systematic review identified 917 records using the search terms previously described. There were 542 duplicates across the three databases. An additional 13 articles were found after reviewing the bibliographies of articles meeting the inclusion criteria. After duplicates were removed, 375 records remained. The title and abstract of these articles were scanned for relevance considering the inclusion criteria. A total of 317 articles were excluded because they did not meet the inclusion criteria. After reading the remaining 58 articles, 15 were excluded. Five of these excluded records were actually meeting abstracts only. Two articles were not in the English language, one article could

not be obtained, two articles did not thoroughly describe the patient phenotype and instead focused on another subject, two articles were review articles without mention of new patients, and lastly, three articles described patients previously reported. A full summary of the PRISMA process is included in Figure 3.1. Most of the studies were case reports (67.4%). Additionally, the majority were conducted in either the US or Europe (Table 3.2).

Table 3.2: Characteristics of Included Studies

	Included Studies (N=43)	
	N	**(%)**
Study Type		
Case Report	29	(67.4%)
Cohort study	6	(14.0%)
Review	4	(9.3%)
Review with a case report	3	(7.0%)
Multicenter study	1	(2.3%)
Location of Study		
US	7	(16.3%)
France	6	(14.0%)
China	5	(11.6%)
Italy	5	(11.6%)
Germany	3	(7.0%)
Japan	4	(9.3%)
UK	2	(4.7%)
Portugal	2	(4.7%)
Canada	1	(2.3%)
Cyprus	1	(2.3%)
Ireland	1	(2.3%)
Mexico	1	(2.3%)
Norway	1	(2.3%)
Poland	1	(2.3%)
South Korea	1	(2.3%)
Spain	1	(2.3%)
Multicenter study (Italy, Demark, UK)	1	(2.3%)

FIGURE 3.1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram



Demographic Information and Variant Types

A total of 117 patients with a *MEF2C*-related disorder were identified in our systematic literature search (supplementary document 3). There were 59 females (50.4%), 56 males (47.9%), and 2 (1.7%) patients with an unknown gender in the cohort.

The average age was 8.52 years (SD 9.33 years). Two fetuses were terminated at 20 weeks gestation after considering ultrasound and magnetic resonance imagining abnormalities. The youngest living patient was five months old and the oldest 52 years old (Table 3.3).

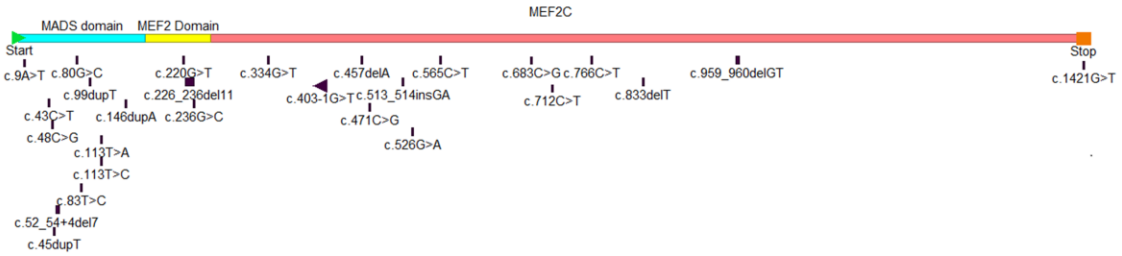
Table 3.3: Demographic Information and Variant Types from Patients with Reported *MEF2C*-Related Disorders

Gender	**No. (%)**
Female	59 (50.4%)
Male	56 (47.9%)
Unknown	2 (1.7%)
Age Group	**No. (%)**
Fetus (Fetus)	2 (1.7%)
Newborn (Birth to 1 month)	0 (0.0%)
Infant (>1 month to < 24 months)	20 (17.1%)
Preschool (2 years to < 6 years)	31 (26.5%)
Child (6 years to < 13 years)	40 (34.2%)
Adolescent (13 years to < 19 years)	14 (12.0%)
Adult (19 years to < 45 years)	7 (6.0%)
Middle age (45 years to < 65 years)	3 (2.6%)
Type	**No. (%)**
MEF2C affected/altered/disrupted	108 (92.3%)
Possible Positional Regulatory Effect	9 (7.7%)
Type	**No. (%)**
Deletion	58 (59.8%)
Translocation	6 (5.1%)
Deletion with Translocation	1 (0.9%)
Insertion	1 (0.9%)
Duplication	3 (2.6%)
Point Variant (Missense, Nonsense, Frameshift)	35 (29.9%)
Nonsense	8/35 (22.9%)
Missense	16/35 (45.7%)
Frameshift	8/35 (22.9%)
Stop Loss	1/35 (2.9%)
Splicing	2/35 (5.7%)
Not provided	1 (0.9%)

Over half of the patients (59.8%) presented with deletions encompassing part or the entire *MEF2C* gene, or with a deleted region near *MEF2C* that may cause a positional regulatory effect disrupting expression of *MEF2C*. The second most common group of variants were point mutations, including missense, nonsense, splicing, and frameshift variants. Insertions, duplications, and translocations were also reported, although not as often. The alteration types for reported patients can be found in Table 3.3. Variant locations can be found in Figure 3.2.

FIGURE 3.2: Variant locations from patients with reported MEF2C-related disorders. (a) Locations of point variants (nonsense, missense, frameshift, splicing, stop loss) across the MEF2C coding region. (b) Map of microdeletions and duplications involving or associated with MEF2C, using UCSC hg18 genome build. Black = deletion; blue = duplication; pink = MEF2C not involved, possible regulatory positional effect; pink and gray stripes = deleted region (MEF2C not involved) compounded with a translocation in the patient.

A


MEF2C
MADS domain
MEF2 Domain
Start
Stop
c.9A>T
c.80G>C
c.99dupT
c.43C>T
c.146dupA
c.48C>G
c.113T>A
c.113T>C
c.83T>C
c.52_54+4del7
c.45dupT
c.220G>T
c.226_236del11
c.236G>C
c.334G>T
c.457delA
c.403-1G>T
c.513_514insGA
c.471C>G
c.526G>A
c.565C>T
c.683C>G
c.712C>T
c.766C>T
c.833delT
c.959_960delGT
c.1421G>T

B


chr5 (q12.3-q22.3)
Scale
chr5:
70,000,000
75,000,000
80,000,000
85,000,000
90,000,000
95,000,000
100,000,000
105,000,000
110,000,000
115,000,000
10 Mb
hg18
MEF2C CNVs
RefSeq Genes

Common Symptoms

The majority of patients presented with features typically described for *MEF2C*-related disorders. For articles reporting the following information, patients presented with intellectual disability (97.6%), developmental delay (99.0%), hypotonia (98.3%), absent speech (92.9%), and seizures and spasms (87.3%) (Table 3.4). Of patients three years of age and older, only five were able to speak several words (7.1%); however, their language skills were severely delayed. Speech was absent in the remaining patients over three years of age, but some patients did know a few words, or were able to babble, have vocalizations, mimic sounds, and use body language. Seizure types included fever-induced (or febrile), infantile spasms, generalized tonic-clonic, myoclonic, and focal. Thirty-nine patients presented with multiple seizure types. The two most common seizure types reported were febrile (31/89, 34.8%) and myoclonic (30/89, 33.7%). Tonic-clonic and spasms were both present in 17 of 89 patients (19.1%), followed by focal seizures in 14 patients (15.7%). Less prevalent were absence (5.6%), afebrile (3.4%), and atonic (2.2%). Seizure type was broadly characterized as “epilepsy” or “generalized” in 13 patients (14.6%), and “unspecified” in 5 patients (5.6%). Seizures typically had an infantile onset of less than one year of age (61.6%), and 87.7% had an onset under 2 years of age. Many patients were not able to walk independently (N=31, 56.4%). These 31 patients were all over 18 months of age, with the youngest being 20 months and the oldest 46 years. Additionally, two patients were reported to have spastic quadriplegia, one of which had hypotonia during the early infantile period (Saitsu et al., 2011; Shimojima et al., 2012). Stereotypic movements, including hand flapping, hand

mouthing, hand clapping, hand biting, hand washing, grasping the midline, and head banging, were reported in 83.6% of patients.

Table 3.4: Phenotypes Found in Patients with Reported *MEF2C*-Related Disorder
Not all phenotypes were reported for all patients and thus sample size varies.

Type	No. (%)
Developmental delay	96/97 (99.0%)
Seizures	89/102 (87.3%)
Intellectual disability	83/85 (97.6%)
Hypotonia	58/59 (98.3%)
Absent speech (age > 3 years)	65/70 (92.9%)
Social and behavioral issues	62/71 (87.3%)
Dysmorphic features	68/69 (98.6%)
Stereotypic movements	46/55 (83.6%)
Abnormal MRI	58/86 (67.4%)
Feeding and digestion issues	35/36 (97.2%)
Abnormal EEG	50/73 (68.5%)
Inability to walk (age > 18 months)	31/55 (56.4%)
Vision issues	24/24 (100.0%)
Sleeping issues	20/28 (71.4%)
Cardiac issues	17/17 (100.0%)

Physical Features

Head circumference information was reported for 67 patients, of which 16 patients had a head circumference size consistent with microcephaly (23.9%). Only two patients were reported to have macrocephaly (3.0%) (Cardoso et al., 2009; Mikhail et al., 2011). Dysmorphic features when reported were typically mild and included a broad forehead, down-slanting palpebral fissures, large ears, prominent ear lobes, short philtrum, depressed nasal bridge, and tenting of the upper lip. One patient presented with a question mark ear but had normal ear canals (Gordon et al., 2018). Two patients

presented with a jugular pit (Al-Shehhi et al., 2016; Berland & Houge, 2010). Two patients presented with capillary malformation-arteriovenous malformation (CM-AVM) syndrome in addition to features of the *MEF2C*-related disorders (Carr et al., 2011; Ilari et al., 2016). CM-AVM is characterized by small pink round or oval-shaped vascular lesions, many with telangiectatic vessels in the center. One of the patients had 17 typical CMs on her head, trunk, and extremities, as well as two irregular CMs on the popliteal fossa and upper left posterior thigh. The patient did not present with any AVMs or arteriovenous fistulas on cranial MRI (Carr et al., 2011). The second patient had CMs on the trunk and extremities as well, including the right arm and thorax. This patient had two reported AVMs, one on the right frontal area and the second on the basilar artery. This syndrome is typically caused by variations in *RASA1*, a gene in close proximity to *MEF2C*. For the two patients that presented with these features, each had one deletion that included both the *RASA1* and *MEF2C* genes. Two additional patients with deletions encompassing both *MEF2C* and *RASA1* presented with hemangiomas (Vrečar et al., 2017). Another patient with a *MEF2C* plus *RASA1* deletion presented with characteristic capillary malformation of the skin and atrophic skin adjacent to the suprasternal notch (Paciorkowski et al., 2013).

MRI and EEG

Abnormal electroencephalograms (EEGs) were reported in 68.5% of patients and findings included hypsarrhythmia, high voltage spike, poly-spike, and slow waves, focal or multifocal bilateral spikes, and a generalized epileptiform pattern. Abnormal MRI findings were reported in 67.4% of cases, typically including abnormalities of the corpus

callosum (thinning, shortening, hypoplasia, aplasia, partial agenesis, thickening) (Carr et al., 2011; Ilari et al, 2016; Engels et al., 2009; Toral-López et al., 2012; Raviglione et al., 2021; Saitsu et al., 2011; Shimojima et al., 2012; Vrečar et al., 2017; Yang et al., 2015; Al-Shehhi et al., 2016; Paciorkowski et al., 2013; Cesaretti et al., 2016; Nowakowska et al., 2010). Abnormalities of the white matter (delayed myelination, reduced volume) were not uncommon (Engels et al., 2009; Novara et al., 2010; Raviglione et al., 2021; Saitsu et al., 2011; Shimojima et al., 2012; Vrečar et al., 2017; Paciorkowski et al., 2013; Shim et al., 2015; Borlot et al., 2019; Zweier et al., 2010; Nowakowska et al., 2010; Sobreira et al., 2009). Other findings included simplified gyri (Carr et al., 2011; Hotz et al., 2013), aplasia of the cerebellar vermis, moderate atrophy of supra- and infratentorial region, and prominence of arachnoid spaces (Engels et al., 2009), leukomalacia (Novara et al., 2010; Floris et al., 2007), ventriculomegaly (Engels et al, 2009; Toral-López et al., 2012; Raviglione et al., 2021; Shimojima et al., 2012; Vrečar et al., 2017; Novara et al., 2013; Hotz et al., 2013; Zweier et al., 2010; Cesaretti et al., 2016; Nowakowska et al., 2010), Dandy-Walker malformation (Toral-López et al., 2012), reduced brainstem volume (Shimojima et al., 2012; Hotz et al., 2013), cortical atrophy (Vrečar et al., 2017; Toral-López et al., 2012; Paciorkowski et al., 2013), cerebellar vermis hypoplasia (Paciorkowski et al., 2013; Raviglione et al., 2021), small forebrain and frontal lobes (Hotz et al., 2013), periventricular heterotopia (Cardoso et al., 2009), abnormalities in the posterior fossa including Chiari Type 1 malformation, enlarged cisterna magna, and hippocampal abnormalities (Raviglione et al., 2021), and cysts (septum pellucidum, pineal) (Yang et al., 2015; Wang et al., 2018; Nowakowska et al., 2010).

Social, Behavioral and Sleep Issues

Autistic traits or behaviors were reported in 24 patients (Berland & Houge, 2010; Boutry-Kryza et al., 2015; Floris et al., 2007; Hotz et al., 2013; Nowakowska et al., 2010; Raviglione et al., 2021; Schluth-Bolard et al., 2019; Vidal et al., 2019; Vrečar et al., 2017; Wang et al., 2018; Zweier et al., 2010). Additionally, other social and behavioral issues were reported. Most patients displayed a lack of social smile and interest in surroundings, or limited social interactions (Engels et al., 2009; Ilari et al., 2016; Novara et al., 2010; Rocha et al., 2016; Shim et al., 2015; Wang et al., 2018) and poor eye contact (Berland & Houge, 2010; Bienvenu et al., 2013; Gordon et al., 2018; Le Meur et al., 2010; Novara et al., 2010; Paciorkowski et al., 2013; Rocha et al., 2016; Wang et al., 2018; Yang et al., 2015). Some patients had a lack of social interaction (Ilari et al., 2016; Nowakowska et al., 2010; Vrečar et al., 2017), whereas a few were reported to enjoy human contact, especially with other children (Vrečar et al., 2017). Many patients were described as having a generally happy disposition (Berland & Houge, 2010; Bienvenu et al., 2013; Paciorkowski et al., 2013; Raviglione et al., 2021). Only a few patients were reported to have negative behaviors, including obsessive behaviors, severe attention deficit hyperactivity disorder and aggressive behaviors (Sobreira et al., 2009), agitation and self-mutilation (Paciorkowski et al., 2013), and self-biting (Rocha et al., 2016). A few patients were noted to easily startle with loud noises (Berland & Houge, 2010; Borlot et al., 2019; Nowakowska et al., 2010; Tanteles et al., 2015). Lastly, some patients had fascinations with random items and events, including running water or water in general,

bright objects, and opening and closing doors (Berland & Houge, 2010; Gordon et al., 2018; Tanteles et al., 2015; Vrečar et al., 2017).

Sleep issues were reported in 41.4% of patients and included sleeping a lot with short awakening stages, sleep disturbance, and irregular sleep initiation and maintenance (Engels et al., 2009; Hotz et al., 2013; Le Meur et al., 2010; Paciorkowski et al., 2013; Vrečar et al., 2017; Wang et al., 2018; Yang et al., 2015; Zweier et al., 2010).

Feeding and Gastrointestinal Issues

Feeding and digestion issues were common and included constipation, feeding difficulties, poor sucking as an infant, frequent vomiting, inability to feed self, needing puree foods only, gastrostomy tube fed, slow gastric emptying, dysphagia, episodes of appetite loss, and gastroesophageal reflux disease (GERD) (Al-Shehhi et al., 2016; Bienvenu et al., 2013; Engels et al., 2009; Gordon et al., 2018; Le Meur et al., 2010; Novara et al., 2013; Nowakowska et al., 2010; Paciorkowski et al., 2013; Saitsu et al., 2011; Sakai et al., 2013; Schluth-Bolard et al., 2019; Shimojima et al., 2012; Vrečar et al., 2017; Wang et al., 2018; Zweier et al., 2010).

Ophthalmological Issues

Eye concerns included bilateral optic atrophy and hyperopia (Engels et al., 2009; Novara et al., 2013; Zweier et al., 2010), strabismus (Berland & Houge, 2010; Bienvenu et al., 2013; Engels et al., 2009; Novara et al., 2010; Zweier et al., 2010) myopia (Schluth-Bolard et al., 2019; Vrečar et al., 2017), bilateral esotropia (Marashly et al., 2010; Nowakowska et al., 2010; Shim et al., 2015), nystagmus (Berland & Houge, 2010;

Zweier et al., 2010), bilateral ptosis (Nowakowska et al., 2010), coloboma of the iris in two patients (Cardoso et al., 2009; Sobreira et al., 2009), and cortical blindness in one patient (Le Meur et al., 2010).

Cardiac Phenotype

Cardiac issues have not typically been associated with *MEF2C*-haploinsufficiency. However, cardiac issues could be expected due to the role of *MEF2C* in myogenesis and heart development. Cardiac issues were reported in 17 patients in total. Cardiac phenotypes included concentric myocardial hypertrophy, patent foramen ovale, patent ductus arteriosus, abnormal fetal cardiac rhythm, bi-ventricular hypertrophy, moderate tricuspid valve insufficiency, moderate bilateral ventricular valve insufficiency, and murmur. Nine patients were reported with cardiac phenotypes in addition to other features commonly found in *MEF2C*-related disorders (Cesaretti et al., 2016; Engels et al., 2009; Le Meur et al., 2010; Novara et al, 2013; Nowakowska et al., 2010; Stoll et al., 1980; Vrečar et al., 2017). Three articles focused solely on cardiac studies and did not report any non-cardiac phenotypes in those 10 patients (Lu et al., 2018; Yuan et al., 2017; Qiao et al., 2017).

Lu et al. (2018) performed Sanger sequencing of the *MEF2C* gene on a cohort of 186 unrelated patients with congenital heart defects and 300 healthy matched controls. One patient who had a family history of ventricular septal defect (VSD) and double outlet right ventricle (DORV) was identified with a heterozygous missense variant (c.43C>T; p.Arg15Cys) in *MEF2C.* This variant was not present in any of the 300 controls. Family studies revealed that the variant was paternally inherited and that the proband's uncle also

carried the variant. All three individuals carried the missense change and had the phenotype of VSD and DORV. The proband's grandfather was deceased but shared the phenotype so may also have carried the variant as well. No other phenotypic information was reported apart from the cardiac phenotype.

Yuan et al. (2017) also performed Sanger sequencing on a cohort to identify *MEF2C* variants associated with dilated cardiomyopathy (DCM). There were 172 unrelated individuals with DCM and 300 healthy controls sequenced. A heterozygous nonsense variant (c.471C>G; p.Tyr157Ter) was detected in a patient with a positive family history and phenotype of adult-onset DCM. The patient's daughter and brother both carried the variant. The daughter shared the phenotype of DCM, and the patient's brother had a phenotype of DCM and ventricular septal defect (VSD). These patients were also reported to have intellectual disability, childhood epilepsy, stereotypic movements, and absent speech. These features overlap with the traditionally reported phenotype of *MEF2C*-related disorders and haploinsufficiency.

Lastly, Qiao et al. (2017) performed Sanger sequencing on a cohort of 200 unrelated patients with a congenital heart defect and 300 healthy controls. A heterozygous missense variant (c.113T>C; p.Leu38Pro) was identified in a one-year-old male with patent ductus arteriosus (PDA) and ventricular septal defect (VSD). The patient's father, uncle, and female first-cousin all carried the variant and shared a similar cardiac phenotype. All family members had PDA. The proband's father shared the same phenotype of PDA and VSD. The proband's uncle had pulmonary stenosis (PS) in addition to PDA. The proband's cousin was only reported to have PDA. The proband's

grandfather was reported to have all three cardiac features (PDA, VSD, and PS); however, the grandfather was deceased therefore carrier status could not be assessed. The father and uncle were also reported to have intellectual disability, stereotypic movements, and paroxysmal epilepsy.

Non-classical Findings

There were a number of patients in the literature with either non-classical symptoms or unique pathogenesis. As previously mentioned, one patient presented with a question mark ear (Gordon et al., 2018) and two patients presented with a jugular pit (Al-Shehhi et al., 2016; Berland & Houge, 2010). One other patient was reported to have mild to moderate hypoglycemia, with a blood glucose level not exceeding 90 mg/dl even after a meal (Sakai et al., 2013). This is perhaps the only reported neuroendocrine phenotype related to deletions in the 5q14.3 region that included *MEF2C*. However, this phenotype could be present but unrecognized in additional patients due to the severity of the other features (i.e., intellectual disability and seizures). This patient had a normal hypothalamus by MRI; therefore, the deficits likely occur within the hypothalamic signaling pathway. Other genes within this patient's deletion were not expected to be expressed in the endocrine system, therefore were deemed not the likely cause of the neuroendocrine phenotype leaving the authors to suspect *MEF2C*. The authors performed expression studies in the mouse brain and found *MEF2C* was highly expressed in neuropeptide Y (NPY)-positive hypothalamic interneurons. Conversely, NPY-positive neurons had lower expression of *MECP2*, the gene associated with Rett syndrome. Further analysis showed *MECP2* is involved in the repression of *MEF2C* and *NPY*. The

common pathway of *MEF2C* and *MECP2* could explain the phenotypic similarities between *MEF2C*-related disorders and Rett syndrome.

Nine patients who did not have a deleted or disrupted *MEF2C* gene yet presented with a similar phenotype as the other diagnosed *MEF2C* patients (Boutry-Kryza et al., 2015; Cardoso et al., 2009; Engels et al., 2009; Floris et al., 2007; Marashly et al., 2010; Saitsu et al., 2011; Shimojima et al., 2012; Sobreira et al., 2009; Yauy et al., 2019). It was hypothesized that there may be a regulatory positional effect for copy number variations with a breakpoint on either side of the *MEF2C* gene. Of these nine, six had deletions that did not encompass *MEF2C* and three were translocations that did not disrupt *MEF2C*. In the patient reported by Engels et al. (2009), *MEF2C* expression levels were confirmed to be decreased in an RNA study in collaboration with Zweier et al. (2010). One patient with a balanced translocation actually had *MEF2C* overexpression (Yauy et al., 2019). Two patients had normal *MEF2C* expression levels by lymphoblast RNA testing, one of which had a deletion and the other a translocation (Saitsu et al., 2011; Shimojima et al., 2012). This could be explained by tissue-specific expression where the sample type tested had normal *MEF2C* expression, but tissue from another location (i.e., the brain), if tested, may actually have decreased expression. The remaining five patients had no mention of expression levels but could still fall within the category of patients affected due to the positional effect of their deletion to *MEF2C*.

Discussion

We performed a systematic review to assemble the most comprehensive list of patients with a *MEF2C*-related disorder along with their phenotypes. One hundred and

seventeen patients were identified with a *MEF2C*-related disorder and the phenotypes reported included intellectual disability, developmental delay, seizures, hypotonia, absent speech, inability to walk, stereotypic movements, and MRI abnormalities. Additional features detected were jugular pit, cardiac issues, and a neuroendocrine phenotype of hypoglycemia. Although the patients shared many of the same features, differences between patient phenotypes could be explained by the difference in the type of variants (point mutations rather than chromosomal rearrangements), variant locations within the *MEF2C* gene, or deletion sizes and whether additional genes were involved in the deletion along with *MEF2C*. Genotype-phenotype correlation analysis may provide some insights into the clinical variability across individuals with MEF2C-related disorders. Other divergencies between the phenotypes reported in the articles could be due to the purpose of the study. Authors may have focused on only one feature for their study (e.g., epilepsy), thereby limiting the phenotypic information presented for other features. For example, of the six cohort studies, three focused on the cardiac phenotype, one on infantile spasms, one on developmental disorders, and one on intellectual disability. In contrast, twenty-nine articles (67.4%) were case reports in which more general phenotypic information was presented.

Nine patients were reported to have chromosomal rearrangements not encompassing or disrupting the *MEF2C* gene; however, these patients still exhibited a similar phenotype to the other reported patients. This could be explained by a possible positional regulatory effect. Six patients had no expression studies performed, two patients had normal *MEF2C* expression, and one patient had decreased *MEF2C*

expression. Further studies will be needed to understand this positional effect and determine if expression could be tissue-specific.

Several clinical implications can be deduced given the results of this literature review. Early referral for therapies (such as physical, occupational, and speech) is recommended. Patients should undergo a full neurological evaluation including an EEG and brain MRI if concerning neurological symptoms arise. If seizures, constipation, or gastroesophageal reflux are occurring, treatment should be as per standard care. Also recommended is an evaluation with a developmental specialist to screen for ASD and behavioral issues, such as ADHD and anxiety. Given the cardiac findings from this review, a cardiac evaluation with an echocardiogram and EKG is recommended. Lastly, the *MEF2C* gene should be included in all Next Generation Sequencing (NGS) epilepsy/seizure panels.

There are some limitations to this study. Despite the rigorous method and two independent article reviewers, relevant articles matching the inclusion criteria might have been missed. During the review, two articles were excluded as they were not in English and one other article could not be obtained. Additionally, we only searched three major databases indexing biomedical literature; therefore, any articles matching the inclusion criteria in other databases were not included. A final limitation arises from using the systematic review method where the data of this study relies on the information each article contained. The articles may have focused only on specific clinical features without reporting other potentially relevant information. As our study was a review of the literature, we were not able to pursue additional patient information to fill the gaps. Thus,

the sample size for each feature assessed varied. Future studies could involve contacting the authors of the 43 manuscripts included in this study to gather the same clinical information across all reported patients.

This review characterizes the phenotype of *MEF2C*-related disorders and documents the severity of this condition, which can aid healthcare providers in diagnosing patients and delivering the best care possible to current patients and their families. Detailed information on the 117 patients is provided in the supplemental table which may be a valuable resource for investigators interested in pursuing specific genotype-phenotype correlations.

Author Contributions

The authors have contributed to the manuscript as follows: JACC created the search strategy and keywords and performed the literature search. Article titles and abstracts returned by the search were independently screened by both JACC and JMD. SMS was available as a third reviewer in case of any disagreements for the inclusion or exclusion of articles. JACC took the lead in data extraction, analysis, and drafting the manuscript. All authors contributed by critically revising the manuscript and have given approval of the final version to be published.

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CHAPTER FOUR
CLINICAL FINDINGS FROM THE LANDMARK MEF2C-RELATED DISORDERS NATURAL HISTORY STUDY

Title: Clinical Findings from the Landmark *MEF2C*-Related Disorders Natural History Study
Running Title: *MEF2C*-Related Disorders Natural History Study Findings

Authors: Jessica A. Cooley Coleman[1, 2], Sara M. Sarasua[1], Hannah Warren Moore[2], Luigi Boccuto[1], Christopher W. Cowan[3], Steven A. Skinner[2], Jane M. DeLuca[1, 2]
Correspondence to: Jessica A. Cooley Coleman, 106 Gregor Mendel Cir. Greenwood, SC 29646. cooley8@g.clemson.edu
[1]School of Nursing, Clemson University, [2]Greenwood Genetic Center, [3]Department of Neuroscience, Medical University of South Carolina

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Conflict of Interest
The authors have no conflict of interest to declare.

Data Availability
The data that supports the findings of this study are available within the article and in the supplementary material. Raw data and the survey instrument may be available upon request.

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Ethics Declaration
The study was approved by the Self Regional Healthcare IRB (Pro00091979). No personally identifiable information was collected.

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ABSTRACT

Introduction
MEF2C-related disorders are characterized by developmental and cognitive delay, limited language and walking, hypotonia, and seizures. A recent systematic review identified 117 patients with *MEF2C*-related disorders across 43 studies. Despite these reports, the disorder is not easily recognized and assessments are hampered by small sample sizes. Our objective was to gather developmental and clinical information on a large number of patients.

Methods
We developed a survey based on validated instruments and subject area experts to gather information from parents of children with this condition. No personal identifiers were collected. Surveys and data were collected via REDCap and analyzed using Excel and SAS v9.4.

Results
Seventy-three parents completed the survey, with 39.7% reporting a *MEF2C* variant and 54.8% reporting a deletion involving *MEF2C*. Limited speech (82.1%), seizures (86.3%), bruxism (87.7%), repetitive movements (94.5%), and high pain tolerance (79.5%) were some of the prominent features. Patients with *MEF2C* variants were similarly affected as those with deletions. Female subjects showed higher verbal abilities.

Conclusion
This is the largest natural history study to date and establishes a comprehensive review of developmental and clinical features for *MEF2C*-related disorders. This data can help providers diagnose patients and form the basis for longitudinal or genotype-phenotype studies.

Keywords: *MEF2C*, *MEF2C*-Related Disorders, natural history study, parent survey, neurodevelopmental, social media research

CHAPTER FOUR

CLINICAL FINDINGS FROM THE LANDMARK MEF2C-RELATED DISORDERS NATURAL HISTORY STUDY

INTRODUCTION

MEF2C-related disorders, also known as *MEF2C* haploinsufficiency syndrome or 5q14.3 microdeletion syndrome (OMIM #613443), are neurodevelopmental disorders characterized by developmental delay, intellectual disability, lack of verbal language, limited walking, hypotonia, and seizures[1]. Originally, patients with this phenotype were found to have microdeletions of the 5q14.3 region, with most including the *MEF2C* gene (OMIM *600662). Eventually, *MEF2C* was identified as the causative gene after patients were reported with microdeletions only encompassing *MEF2C*[2,3] as well as another patient with a nonsense variant in *MEF2C*[4]. There have also been some cases reported of patients with a similar phenotype that had microdeletions in the proximal or distal region closely surrounding but not including the *MEF2C* gene[5,6]. It is hypothesized that these deletions may disrupt the regulation and expression of *MEF2C*, and therefore cause the same phenotype. Interestingly, some patients with *MEF2C* variants and microdeletions not only had diminished *MEF2C* expression but also diminished *CDKL5* and *MECP2* expression, indicating a shared molecular pathway[7]. Although the phenotype has some overlap to Rett syndrome, patients do not typically have regression and would not meet current criteria for the diagnosis of Rett syndrome[8].

A recent systematic review of the literature revealed 43 manuscripts describing 117 patients with a *MEF2C*-related disorder reported to date[9]. Most publications report

only one or a few patients, with the largest cohort being 17 new patients in one publication[10]. Despite the phenotypic information provided, the disorder is not easily recognized clinically. Additionally, the disorder has only been described for just over a decade, a much shorter time than other similar, but well-characterized, neurodevelopmental disorders, such as Rett syndrome, prompting the need to further characterize the disorder. We conducted a natural history study in the form of a parent survey to gather additional data and improve the clinical description of the disorder. This is the largest cohort to date containing parent-reported phenotype information about *MEF2C*-related disorders. The information revealed by the survey further characterizes the disorder, aids providers in recognizing, diagnosing, and treating patients, and illuminates features not previously reported.

METHODS

Ethical Compliance

The study was approved by the Self Regional Healthcare IRB (Pro00091979). No personally identifiable information was collected. IRB approval was shared with the Clemson University IRB. No additional IRB approval was required by Clemson University.

Survey Development

Survey development commenced in January 2019. The Rett Syndrome Natural History Study[11,12] and the Fragile X Online Registry with Accessible Research Database (FORWARD)[13] surveys were used as guides to help develop appropriate survey

questions. The draft of the survey was piloted by four parents of children with *MEF2C*-related disorder. These parents were asked for feedback and any additional question suggestions. The final survey contains 81 questions on demographic information, developmental history, medical issues and symptoms. The survey questions were vetted by a team of clinical and research experts from the Greenwood Genetic Center (GGC), Clemson University, and the Medical University of South Carolina (MUSC). The final version was then loaded into REDCap (Research Electronic Data Capture)[14] for online survey distribution. The questionnaire may be made available upon request.

Recruitment

The survey was opened for online data collection in January 2020. Any patient with a previously reported *MEF2C* alteration (variant, deletion, duplication) met the criteria for this study. The research team had a goal of 50 survey responses. Parents, relatives, and guardians or caregivers of a child with a *MEF2C*-related disorder were made aware of the survey via an IRB-approved advertising script posted to the Facebook support group "MEF2C Medical Personnel and Families". As of August 4th, 2021, the Facebook group had over 350 worldwide members, including medical personnel, parents, and family. A reminder post was put on the Facebook support group twice, each about two months apart from the last post, for a total of three advertising posts. Additionally, two parents shared the advertising script and survey link to the parents-only Facebook group "MEF2C Parent Support Group" on behalf of the research team. Although the survey remained anonymous, informed consent was obtained electronically by each parent prior to starting the survey. The survey was closed in June 2020.

Data Analysis

Survey results were exported from REDCap into an Excel file. Descriptive statistical analysis, including percentages, means, and standard deviations (SDs) were performed using both Excel and SAS v9.4. Categorical analyses (between alteration type or gender, and anxiety, hyperactivity, seizures, abnormal MRI, use of words for communication, and walking) were assessed with chi-square tests or, when cell counts were small, Fisher's Exact test. Ordinal analyses (between age group and anxiety, hyperactivity, seizures, abnormal MRI, use of words for communication, and walking) were assessed using the Cochran–Armitage trend test. For tests of association, alteration type was divided into two categories of variant (SNV / point mutation / INDEL) or deletion (large deletion / CNV). There were no participants reporting a large duplication. Patients with an uncertain type of pathogenic alteration were excluded from the analysis. Gender was male or female, and age group consisted of infant (9 months to <24 months), preschool (2 years to <6 years), child (6 years to <13 years), adolescent (13 years to <19 years), and adult (19 years to <45 years). The dichotomous choice for the use of words for communication, anxiety, hyperactivity, seizures, abnormal MRI, and walking was either yes or no. Missing data were omitted from the analysis. Chi-square test, Fisher's Exact test, and Cochran–Armitage trend test were carried out using SAS v9.4. A *P*-value <0.05 was considered statistically significant.

RESULTS

Study Population

A total of 108 survey records were available in REDCap. There were 35 incomplete records of which the majority had only answered one question before closing the survey. Only three of the incomplete records were at least 50% completed. These incomplete records were excluded and data analysis proceeded only on the 73 complete survey responses. All 73 completed responses (100%) were submitted by a parent who had a child with a *MEF2C*-related disorder (versus relative or guardian/caregiver).

Of the 73 parent-completed survey results, 35 reported having a female child (48%) and 38 reported having a male child (52%) with a *MEF2C*-related disorder. The majority of children (91.7%) were reported to be of White race and not of Hispanic, Latino, or Spanish ethnicity. Mother's age at the child's birth ranged from 20 to 41 years of age (mean 31.8 years, SD = 5.12). The children's current age at the time of the survey ranged from 9 months to 38 years (mean 8.12 years, SD = 7.21). BMI was calculated based on parent-reported height and weight, and 46.6% fell within the normal / healthy weight category (Table 1). Nearly 33% (22/67) had short stature, with a height falling below the third percentile compared to individuals of the same sex and age in the general population.

Of the 73 patients, 29 (39.7%) reported a *MEF2C* variant (point mutation or INDEL), 40 (54.8%) reported a deletion involving the *MEF2C* gene, and 4 (5.5%) were uncertain of the pathogenic alteration at the time of taking the survey. There were no reported large duplications and only one small duplication (6 base pairs) in the INDEL category. About 33% of parents provided the specific variant nomenclature or deletion coordinates (16 variant and 8 deletion). Of the variants reported, seven fell within the

MADS domain, one was in the MEF2 domain, and the remaining eight variants were downstream of these two domains. Reported deletions ranged in size from 217KB to 8MB, including anywhere from one or a few exons to the entire gene being deleted. Other parents gave a description of what they remembered, such as "location of stop codon is halfway, not at the end of the gene" or "217k deletion of 5q14.3".

Table 4.1: Demographic, physical, and genetic information reported by parents regarding their child with *MEF2C*-related disorder.

	Totals (N=73)
Child's Gender	
Female	35 (47.9%)
Male	38 (52.1%)
Ethnicity	
Hispanic, Latino, or Spanish origin	6 (8.2%)
Not Hispanic, Latino, or Spanish origin	63 (86.3%)
Unknown	4 (5.5%)
Race	
White or Caucasian	67 (91.7%)
Black or African American	3 (4.1%)
Asian	1 (1.4%)
American Indian or Alaskan Native	1 (1.4%)
Unknown	1 (1.4%)
Mother's Age When Child Was Born	
Average	31.8 yr (SD 5.12 yr)
Range	20-41 yr
Father's Age When Child Was Born	
Average	33.6 yr (SD 7.07 yr)
Range	21-57 yr
Child's Birth Weight	
Extremely low birth weight (less than 0.992kg)	1 (1.4%)
Very low birth weight (between 0.993kg and 1.616kg)	0 (0%)
Low birth weight (between 1.617kg and 2.495kg)	13 (17.8%)
Normal birth weight (between 2.496kg and 3.997kg)	57 (78.1%)
High birth weight (greater than 3.997kg)	2 (2.7%)
Child's Current Age	

Infant (9 months to < 24 months)	8 (11.0%)
Preschool (2 years to < 6 years)	36 (49.3%)
Child (6 years to < 13 years)	11 (15.1%)
Adolescent (13 years to < 19 years)	10 (13.7%)
Adult (19 years to < 45 years)	8 (11.0%)
Average	8.12 yr (SD 7.21 yr)
Range	9 mo – 38 yr
Child's Current BMI	**N=58**
Underweight (Child and Teen: less than 5th percentile; Adult: BMI below 18.5)	18 (31.0%)
Normal / Healthy Weight (Child and Teen: 5th to less than 85th percentile; Adult: BMI of 18.5 to 24.9)	27 (46.6%)
Overweight (Child and Teen: 85th to less than 95th percentile; Adult: BMI of 25.0 to 29.9)	6 (10.3%)
Obese (Child and Teen: 95th percentile or greater; Adult: BMI of 30.0 or greater)	7 (12.1%)
Genetic Alteration	
MEF2C variant (point mutation / INDEL)	29 (39.7%)
Deletion involving the *MEF2C* gene	40 (54.8%)
Uncertain	4 (5.5%)

Note: SD = Standard Deviation

Maternal Pregnancy History

Twenty-five parents (34.2%) reported pregnancy exposures, which included tobacco (8.2%), secondhand smoke (8.2%), alcohol (5.5%), chemicals (1.4%), prescription medicines (12.3%), and other (9.6%; Table S1). Of these exposures, only tobacco use was higher, albeit only slightly, as compared to the 7.2% in the general population that reported smoking during pregnancy[15]. Thirty parents (41.1%) reported pregnancy complications, including premature labor (8.2%), preeclampsia (5.5%), low

amniotic fluid (1.4%), gestational diabetes (4.1%), illness (5.5%), and other (26.0%; Table S1). These percentages were less than or in range with percentages seen in the general population. Thirty-five parents (47.9%) reported birth complications, including breech position (8.2%), failure to progress (11.0%), fetal meconium aspiration (5.5%), fetal distress (19.2%), and other (21.9%; Table S1). Of note, the percentage of breech position and fetal distress were higher in our cohort as compared to the general population (3-4% and about 4%, respectively, in the general population)[16,17]. Fifty-five (75.3%) mothers carried their child to full term (delivery between 38-42 weeks), whereas the remaining 18 (24.7%) reported a gestational age of before 38 weeks.

Early Development

Most children learned to roll over (90.4%), with this activity first occurring between 3 months of age and 10 years (mean 1.43 years, SD 1.57 years). Most children also learned to sit up (80.8%), with the first occurrence ranging between 6 months and 12 years (mean of 2.17 years, SD 2.15 years), 61.6% learned to crawl, ranging between 1 year and 16 years (mean of 2.55 years, SD 2.50 years), 50.7% of the children over 18 months of age had learned to walk, with first occurrence ranging between 1.33 and 6 years (mean of 3.15 years, SD 1.27 years).

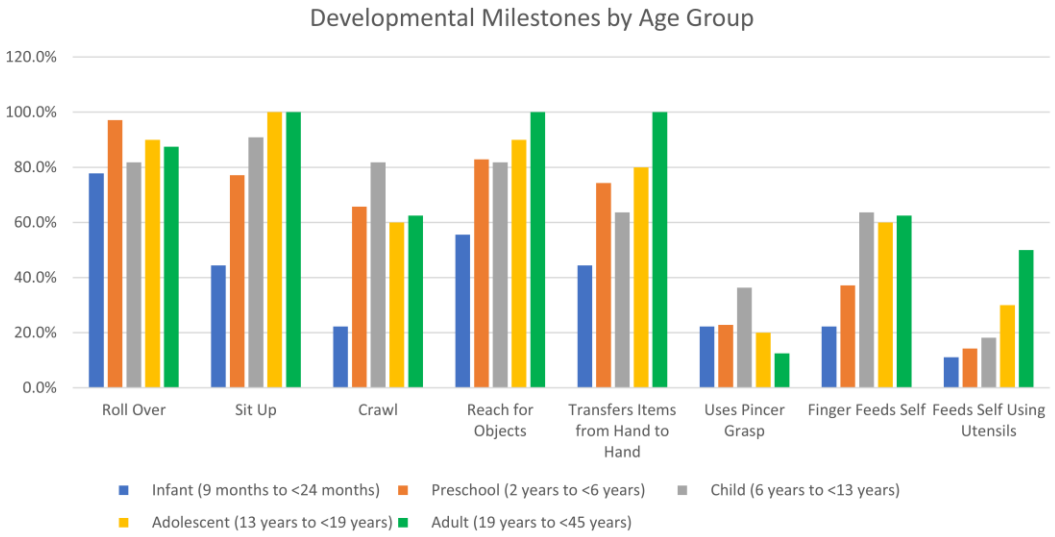
By the time of the survey, most children learned some useful hand functions; 82.2% learned to reach for objects with first occurrence ranging between 2 months and 14 years (mean 2.04 years of age, SD 2.37 years), 72.6% learned to transfer items from hand to hand with first occurrence between 6 months and 11 years (mean 2.31 years, SD

2.13 years), 23.3% developed a pincer grasp with first occurrence between 9 months and 6 years of age (mean 3.25 years, SD 1.70 years), and 45.2% were able to finger feed themselves with first occurrence between 1 and 8 years of age (mean 2.69 years, SD 1.70 years). Lastly, 21.7% of the children over 18 months of age were able to feed themselves with utensils with first occurrence between 20 months and 14 years of age (mean 5.98 years, SD 4.31 years) (Table S2; Figure 1).

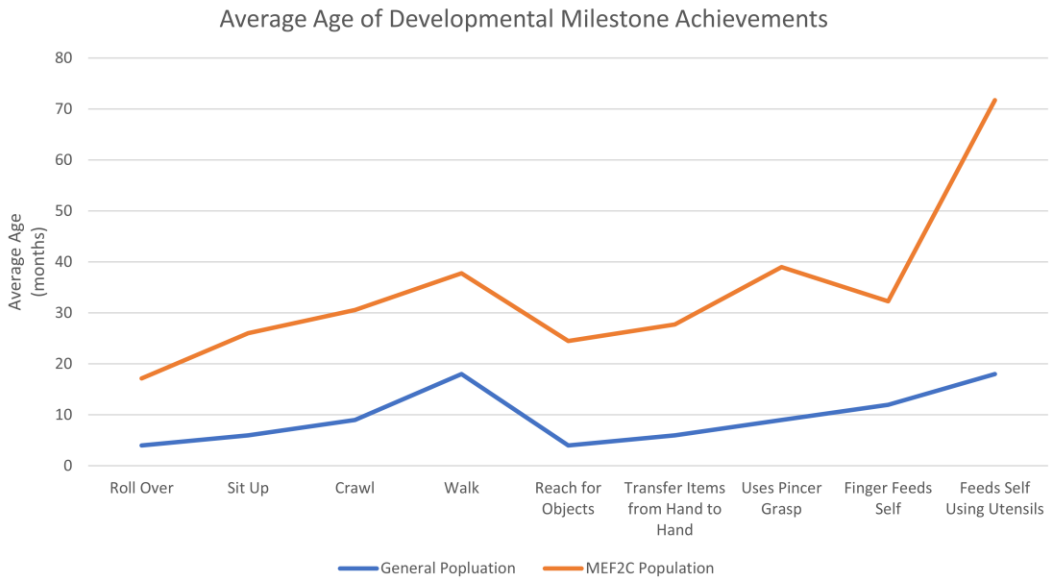
Only one child (1.4%) was reported to be both bowel and urine trained, and seven participants (9.6%) were time trained. The remaining 65 (89.0%) were not toilet trained.

Figure 4.1: Developmental milestones. **A**: Percentages by age group. **B**: Average age the milestone was achieved in this patient cohort with *MEF2C*-related disorders as compared to the general population[18].

A


Developmental Milestones by Age Group
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
Roll Over
Sit Up
Crawl
Reach for Objects
Transfers Items from Hand to Hand
Uses Pincer Grasp
Finger Feeds Self
Feeds Self Using Utensils
Infant (9 months to <24 months)
Preschool (2 years to <6 years)
Child (6 years to <13 years)
Adolescent (13 years to <19 years)
Adult (19 years to <45 years)

B


Average Age of Developmental Milestone Achievements
Average Age (months)
80
70
60
50
40
30
20
10
0
Roll Over
Sit Up
Crawl
Walk
Reach for Objects
Transfer Items from Hand to Hand
Uses Pincer Grasp
Finger Feeds Self
Feeds Self Using Utensils
General Popluation
MEF2C Population

Communication Skills

Of 29 children aged six years and older, 26 (89.7%) were reported to have intellectual disability. In addition, most were reported to have limited language, with 89.2% of children over two years of age lacking any spoken words (Table 2). When assessing children over five years of age, the majority (82.1%) lacked any spoken words. Overall, only eight children were reported to use at least a small number of words for communication, one of whom was able to use a series of single words or two-word combinations meaningfully, and one was able to use phrases or sentences of three words or more.

There was not a significant difference between alteration type (p=0.1194), while there was a significant difference between gender (p=0.0033) and age groups (p=0.0416) showing that females and older subjects were more likely to use words to communicate (Figure 2, Table S3). Interestingly, all eight patients able to use words to communicate were female with their current ages ranging from infancy (<24 months) to adulthood. Alternate speech methods used included signing (19.2%), picture exchange communication system (PECS) or equivalent (26.0%), apps on an iPad/iPhone, smartphone, or tablet (12.3%), and augmentative communication device (16.4%), with some patients (18 of 71, or 25.4%) using more than one type. Nearly 18% pointed, 30.1% used gestures or waves, and 38.4% were reported to follow one-step or simple commands. Of those over two years of age, 25 (39.1%) were nonverbal and not using signs. Additionally, 16 of these 25 did not report using any alternate communication methods.

Table 4.2: Child's developmental, language, and motor milestones as reported in the survey.

Most Recent Milestone	**Totals No. (%)**
Developmental	**N=73**
Roll over	66 (90.4%)
Sit up	59 (80.8%)
Crawl	45 (61.6%)
Reach for objects	60 (82.2%)
Transfer items from hand to hand	53 (72.6%)
Pincer grasp	17 (23.3%)
Finger feed self	33 (45.2%)
Feed self using utensils (>18 months of age)	15 (21.7%)
Toileting	**N=73**
Both bowel and urine trained	1 (1.4%)
Bowel or urine trained only	0 (0.0%)
Time trained	7 (9.6%)
Not toilet trained	65 (89.0%)
Language	**N=73**
Nonverbal/no signs	26 (35.6%)
Nonverbal but using signing in a meaningful way	6 (8.2%)
Babbling/vocalizations	33 (45.2%)
A small number of words or signs for minimal communication	6 (8.2%)
Series of single words or 2-word combinations used meaningfully	1 (1.4%)
Phrases/sentences of 3 words or more	1 (1.4%)
Alternate Communication Methods	**N=71**[†]
Signing	14 (19.2%)
Picture exchange communication system (PECS) or equivalent	19 (26.0%)
Apps on an iPad/iPhone, smart phone, or tablet	9 (12.3%)
Augmentative communication device	12 (16.4%)
Other (hand leading, singing nursery rhymes, and vocalizations for agreement, annoyance, and attention)	4 (5.5%)
None of the above	36 (49.3%)
Motor Abilities	**N=73**

Unable to Roll	1 (1.4%)
Rolls	4 (5.5%)
Sits with Support	8 (11.0%)
Sits Unaided	9 (12.3%)
Crawls	4 (5.5%)
Stands with Support	7 (9.6%)
Stands Unaided	0 (0.0%)
Walks with Support	12 (16.4%)
Walks Unaided	22 (30.1%)
Runs Unaided	6 (8.2%)

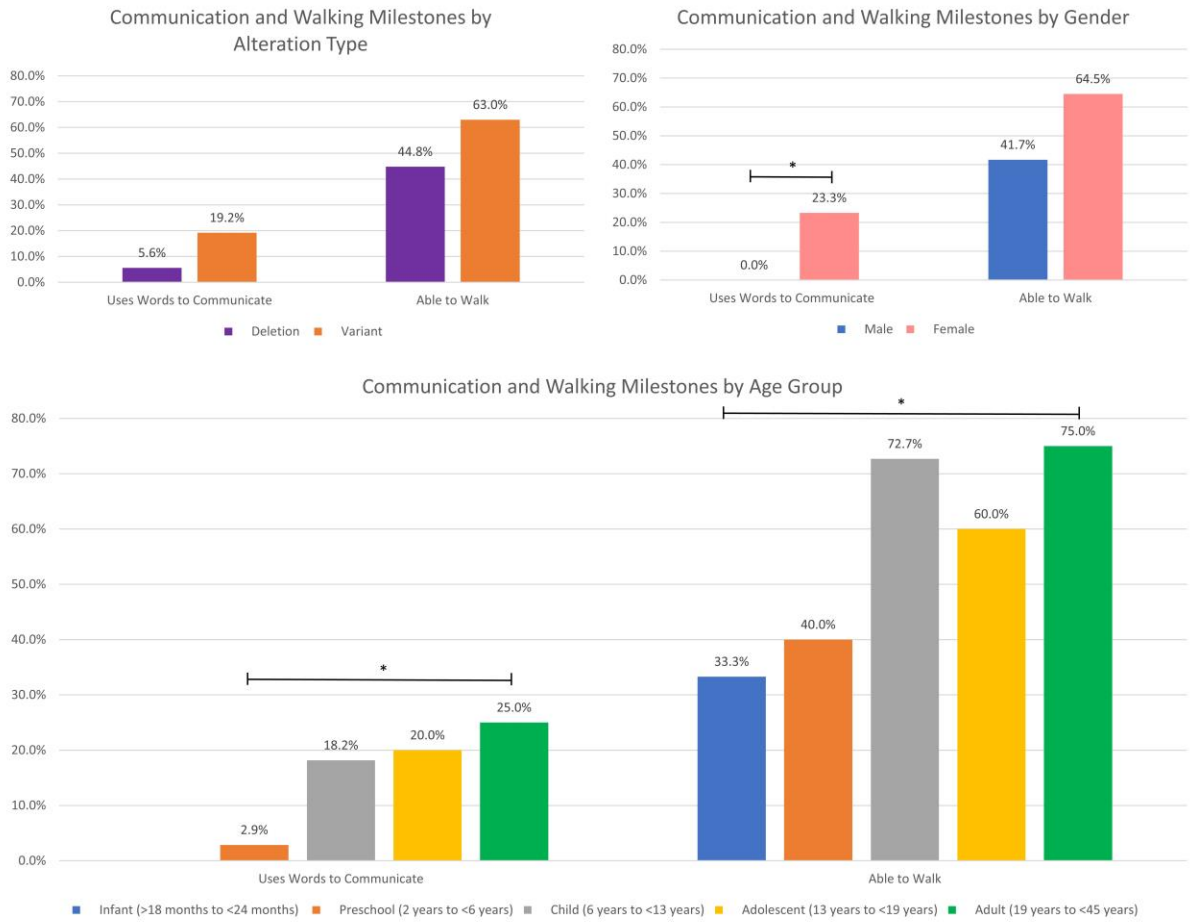
†: A total of 71 parents answered this question with 18 using more than one type of alternate speech method; therefore, the total counts and percentages look to exceed 71/100%.

Motor Milestones

Assessing the highest motor milestone obtained, 40.5% of children over 18 months of age were able to run or walk without support, 17.4% were able to walk with support, and the remaining 42.0% were unable to walk (Table 2). A higher percentage of females (57.1%) compared to males (39.5%) had learned to walk; however, the difference between males and females learning to walk was not significant (p=0.0867). Similarly, a higher percentage of patients with variants (58.6%) compared to those with large deletions (42.5%) had learned to walk but the difference was also not significant (p=0.2083). There was a significant association between being able to walk and age group (p=0.0483) (Table S4). This is expected, as walking is a milestone met with increasing age. With each age group, the percentage of those able to walk generally increased, with 75% of those in the adult group being able to walk (Figure 2).

Of those six who were able to run unaided, 50% were unsteady when walking. Of the 22 who were able to walk unaided, 95.5% were reported to be unsteady when walking. Of the 12 who were able to walk with support, 100% were reported to be unsteady when walking. Most had seemingly low muscle tone (72.6%), whereas 19.2% reported normal muscle tone, and 8.2% reported increased muscle tone.

Figure 4.2: Communication and Walking Milestones by Alteration Type, Gender, and Age Group. * Significant at $p<0.05$



Social Characteristics

Fifty of the children (68.5%) were reported to like giving affection, and 58 liked receiving affection (79.5%). The majority (71.2%) could recognize family members. Forty of the children (54.8%) reported to typically resist holding someone's hand. Fifty-three (79.1%) were reported to have a reduced concern with an environmental threat (i.e.: walks off, explores, lack of "stranger danger") and 34 (46.6%) actively sought social interaction. Poor eye contact and attention problems were reported in over half (60.3% and 70.4% respectively); however, hyperactivity and anxiety were not as common (37.5% and 17.1% respectively). For hyperactivity and anxiety, there was not a significant difference in gender (p=0.9515; p=0.3936), alteration type (p=0.0807; p=0.3936), or age group (p=0.5971; p=0.6655) (Table S5). Nearly one-fourth (25.7%) reported that their child had been diagnosed with autism spectrum disorder.

Sensory Systems

Forty-four (61.1%) reported vision impairments, which included myopia (27.3%), hyperopia (29.5%), problems with depth perception (38.6%), cortical visual impairment (38.6%), strabismus (47.7%), and other issues (15.9%: esotropia, nystagmus, astigmatism). Hearing impairments were less common (8.3%), and included bilateral sensorineural hearing loss, deafness in one ear, mild to moderate loss of certain tones, and moderate mixed hearing loss. Additionally, 61.6% reported sensitivity to loud noises. Few reported sensitivity to clothing textures (6.8%). Food textures sensitivities were slightly more common (36.1%), with those parents noting the child had issues chewing

and swallowing, and therefore preferred soft or pureed foods. Many reported sensitivity to heat (27.4%), cold (4.1%), or both (23.3%). Lastly, 58 (79.5%) reported a high pain tolerance.

Other System Symptoms

Many parents reported their child has trouble falling asleep (42.5%) and staying asleep (49.3%). Sleep medications were reported by 38.4% and included melatonin, Zonegran, Cicardin, Clonidine, Gabapentin, Trazadone, Cyproheptadine, in addition to essential oils and CBD and CBN oil. Medical conditions, digestion issues, immunological, and neuropsychological issues are reported in Table 3 and Table S1. Two parents reported that their children are 100% fed via gastrostomy tube.

Puberty typically occurs between 11-14 years of age[18]. Nineteen (26.0%) parents reported their child had gone through puberty; seven (36.8%) started puberty before 11 years of age, 10 (52.6%) started puberty between the typical ages of 11-14 years of age, and 1 (5.3%) started puberty after the age of 14. Of those who had not yet started puberty, the majority (96.3%) were under the age of 11, one patient (1.85%) was within the 11–14-year range, and one patient (1.85%) was over the 11-14-year.

Immunological issues are reported in Table 3. “Other” frequent illnesses that the parents described included respiratory infections, tonsillitis, frequent colds and pneumonia, and chronic ear infections. Interestingly, a few parents reported some improvements in developmental skills when the child has a fever (16.4%).

Seizures were reported by 63 parents (86.3%); there was not a significant difference between alteration type (p=0.3928), gender (p=0.4114), or age group (p=0.8165) for having seizures (Table S6). Seizure types included generalized (25.8%), partial (8.1%), febrile (33.9%), and other (27.4%; multiple seizure types, absence, atonic, myoclonic seizures, atypical complex febrile, infantile spasms, and generalized tonic-clonic). The onset of seizures ranged from the postnatal period up to 9 years of age. The average onset age of seizures was 1.08 years old (SD 1.28 years). Many parents reported that their child's seizures were under control, and they were no longer having seizures occurring regularly as of the time of the survey (44.4%). For those having seizures currently, 10 (16.4%) reported their child has more than one seizure a day, seven (11.5%) reported daily seizures, one (1.6%) reported weekly seizures, two (3.3%) reported monthly seizures, and 13 (21.3%) reported seizures less than monthly. Thirty-eight parents (61.3%) reported their child takes medication for seizures and 37 of these parents (97.4%) reported the medications helped. Nineteen of the 38 (50%) reported the use of multiple seizure medications. Many (20/38, 52.6%) reported using Keppra (levetiracetam). Other commonly used seizure medications are reported in Table S1. Two parents noted that the ketogenic diet has helped with their child's seizures. Types and frequencies of certain neuropsychological issues are reported in Table 3.

Table 4.3: Symptoms (including medical, digestive, immunological, and neuropsychological) as reported by the parents about their child with *MEF2C*-related disorder.

Symptoms Reported	Totals (N=73) No. (%)
Sleep Issues	
Trouble falling asleep	31 (42.5%)
Trouble staying asleep	36 (49.3%)
Medical Conditions	
Diabetes	0 (0.0%)
Congenital Heart Defect	5 (6.8%)
Asthma or Other Respiratory Issues	8 (11.0%)
Thyroid Problems	1 (1.4%)
Sleep Apnea	4 (5.5%)
Other	24 (32.9%)
None	41 (56.2%)
Digestion Issues	
Diarrhea	10 (13.7%)
Constipation	52 (71.2%)
Reflux	30 (41.1%)
Gall Bladder Dysfunction	0 (0.0%)
Abdominal Distention/ Bloating	10 (13.7%)
Other	9 (12.3%)
None	11 (15.1%)
Recurrent Immune-related Problems or Frequent Illness	**31 (42.5%)**
Frequent Illnesses	26/31 (83.9%)
Frequent Fevers	13/31 (41.9%)
Severe Allergic Reactions	3/31 (9.7%)
Joint Inflammation	0/31 (0.0%)
Skin Issues (such as eczema)	9/31 (29.0%)
Other	6/31 (19.4%)
Seizures	**63 (86.3%)**
Generalized	16/63 (25.4%)
Partial	5/63 (7.9%)
Febrile	21/63 (33.3%)
Other	17/63 (27.0%)
Unknown	3/63 (4.8%)
Not Answered	1/63 (1.6%)

Puberty	**19 (26.0%)**
Scoliosis	**9 (12.3%)**
Hyper-flexibility of fingers, hips, joints, etc	**52 (71.2%)**
Regressions in Development	**25 (34.2%)**
Neuropsychological	
Tremors	22 (30.1%)
Hyperventilation	22 (30.1%)
Breath Holding	25/72 (34.7%)
Aerophagia	19/72 (26.4%)
Food Pocketing	27/72 (37.5%)
Chewing or Swallowing Problems	48 (65.8%)
Bruxism	64 (87.7%)
Repetitive Hand Movements	69 (94.5%)
Obsessive Fascination with Water	50/72 (69.4%)

Previous Imaging Reported

Most patients (69/72) previously had a brain MRI (95.8%) with 40 (58.8%) having abnormal results. These abnormal results included thinning of the corpus callosum, partial agenesis of the corpus callosum, enlarged ventricles, cerebral atrophy, suggestive Chiari malformation, dysmorphic basal ganglia, flattening of the pons, myelination delay, white matter atrophy, Blake's Pouch cyst, grey matter heterotopia, right amygdala lesion, cortical dysplasia, asymmetrical hippocampi, and excess fluid in the frontal lobe. There was not a significant difference in gender (p=0.5411), alteration type (p=0.5951), or age group (p=0.0669) for having an abnormal MRI (Table S7). Interestingly, 36 of 40 reported both abnormal MRI results and seizures.

DISCUSSION

We presented phenotypic data collected from the parents of 73 patients with a *MEF2C*-related disorder, making this the largest study to date. Both children and adults were represented in the cohort. The most prominent features were limited speech (82.1% of children over the age of five not using words for communication), seizures (86.3%), bruxism (87.7%), repetitive hand movements (94.5%), and high pain tolerance (79.5%). Only eight patients (11.0%) were reported to use a small number of words, or a combination of words or phrases, to communicate, all of whom were female. Additionally, we found communication to be significantly associated with gender (p=0.0033) and age group (p=0.0416), with females and older subjects more likely to use words to communicate. Nearly 51% of children over 18 months of age were able to walk; the percentage generally increased with age, with a significant correlation between age group and the ability to walk (p=0.0483). Most patients were able to reach for objects and transfer them from hand to hand, but more fine motor skills (such as pincer grasping and using utensils to feed oneself) were less common.

Many of these features were also the most prevalent found in a systematic review that compiled information on 117 patients reported in the literature[9]. Similar to the results of our survey, phenotypic information on these 117 patients in the literature included limited speech in 92.9%, seizures in 87.3%, and stereotypic movements in 83.6% of patients. Our survey revealed an abnormal MRI in 54.8% of patients, while the systematic review revealed this feature in 67.4%. For a final comparison, our survey revealed 59.4% of children over 18 months of age were unable to walk without support,

while the systematic review revealed 56.4% over the age of 18 months were unable to walk.

Early studies revealed that *MEF2C* is highly expressed in neurons and plays a role in neuronal differentiation[19,20]. Correlating to the neuron expression, many symptoms in patients are neurological, including abnormal MRI findings, seizures, speech and motor impairments, high pain tolerance, and hand stereotypies. Additionally, MEF2C is also expressed in muscle[21], which may relate to the phenotypes of hypotonia, gastrointestinal issues such as constipation, and walking. Of note, *Mef2c* heterozygous mice serve as a valid animal model for *MEF2C*-related disorders as the mice display phenotypic similarities to patients including social and communication impairments, repetitive behaviors, and increased pain tolerance[22]. In an RNA-seq experiment on cortical tissue, Harrington et al. (2020) found that hundreds of genes were dysregulated in the *Mef2c* heterozygous mice as compared to wildtype. Many of the upregulated genes were microglial genes, while a large portion of downregulated genes were autism risk-linked genes. *MECP2*, the gene responsible for Rett syndrome, was previously found to be downregulated in patients with *MEF2C* deletions, truncating mutations, and missense variants, indicating a common pathway between the two genes[7]. This may also explain the phenotypic similarities between Rett syndrome and *MEF2C*-related disorders, including seizures, intellectual disability, developmental delay, and stereotypic movements. However, regression of skills is a requirement for the diagnosis of Rett syndrome[8], whereas regression is not seen in all patients with *MEF2C*-related disorders (34.2% of parents reported developmental regression).

We developed a survey to further characterize *MEF2C*-related disorders. Our survey was based upon well-regarded, validated instruments for Rett syndrome (a condition in the differential diagnosis for *MEF2C*-related disorders) and fragile X syndrome. The survey was vetted by experienced clinical geneticists and other genetics providers and pilot tested by families who have a child with a *MEF2C*-related disorder. This study is responsive to the requests of families and the research community. This survey was made available to two Facebook groups, reaching large numbers of families with multiple reminders. There was an exceptional response rate, exceeding the goal of 50 with a total of 73 complete responses. This study provided parents the opportunity to participate across the world without requiring onerous travel and was successful in obtaining comprehensive information on the largest group of patients to date. The use of Facebook to conduct research has been established as a time- and cost-effective means of recruiting hard-to-reach populations[23,24]. Additionally, using Facebook for recruitment has facilitated research for our team and others[25] in the era of COVID-19 when in-person evaluations were not feasible.

There are limitations to our study. First, the prevalence of *MEF2C*-related disorders is yet to be determined. Although the Facebook group where our study was advertised contains hundreds of members, it consists of family members and medical professionals. There is another *MEF2C* Facebook group in which only parents have membership and access. Therefore, our study may have missed potential participants by not being able to routinely advertise in the parents-only group as often as we did in the family members and medical professionals group. Second, by advertising the survey

through Facebook, participants from across the world were given the opportunity to respond; however, the survey was in English and required Internet access. It may have been difficult for participants to translate if English was not their first language. At least one parent responded in a different language for the open-ended questions responses, which had to be translated back to English for analysis. Third, the participants may have given certain information from memory (such as variant type and nomenclature as well as early developmental milestones). Future studies may benefit from including instructions prompting the participants to gather their genetic reports for reference prior to beginning the study. Lastly, the recent systematic literature review[9] illuminated cardiac issues that have not typically been associated with *MEF2C*-related disorders, and of note, *Mef2c* total knockout mice are embryonic lethal due to heart formation defects[26]. The parent survey was developed prior to the publication of the systematic review; therefore, detailed cardiac-related questions were not considered for inclusion in the survey.

The information collected during this study is a valuable resource to many. Healthcare providers can use the results to learn more about *MEF2C*-related disorders, allowing better diagnosis and care for the patients and families. Families can use this data to obtain answers and see how their child compares or falls within the 73-patient cohort. Lastly, researchers may be able to use this data to pursue specific genotype-phenotype relationships, use it as baseline data for comparison for treatment trials, and for the development of future patient-centered studies.

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CHAPTER FIVE

CURRENT TECHNIQUES TO INVESTIGATE THE MOUSE *Mef2c* GENE

Abstract

This chapter describes the laboratory techniques used by the author (Jessica Cooley Coleman) in Dr. Christopher Cowan's laboratory at the Medical University of South Carolina (MUSC) to investigate *Mef2c* expression and gene regulation in the mouse brain. The author performed nuclei dissociation, bioinformatics analysis of single nuclei RNAseq data, perfusion fixation, brain extraction, brain slicing by microtome, and immunohistochemistry. Following nuclei dissociation, Fluorescence-Activated Cell Sorting (FACS) was performed by Cowan laboratory graduate students. The dissociated nuclei were given to the MUSC Translation Science Lab (outside of the Cowan laboratory) for library preparation with the 10X Genomics Chromium Single Cell 3' Reagent Kit. The resulting libraries are sent to a core laboratory for Illumina sequencing. All of these aforementioned techniques (whether performed by the author or not) are discussed in this manuscript. This information may be helpful to future researchers in using and understanding the techniques.

Introduction

MEF2C (myocyte enhancer factor 2C) is a transcription factor that is highly expressed in the nervous, muscular, and immune systems. In the brain, it is expressed in both excitatory and inhibitory neurons and microglia, and plays a role in neurogenesis, synaptic formation, and remodeling (Assali et al., 2019). Pathogenic variants and macro-

and microdeletions involving *MEF2C* are associated with *MEF2C*-related disorders, also known as *MEF2C* haploinsufficiency syndrome. *MEF2C*-related disorders are characterized by intellectual disability, developmental delay, lack of speech, seizures, hypotonia, brain abnormalities, stereotypic movements, and limited walking. *Mef2c* global heterozygous mice (lacking one copy *Mef2c* exon 2 globally across all tissues) and conditional heterozygous mice (lacking one copy of *Mef2C* only in a certain tissue type) also exhibit repetitive behaviors and social deficits, reduced ultrasonic vocalizations, reduced sensory sensitivity (pain and hearing), abnormal sleep, and altered approach/avoidance behavior; therefore, these mice can serve as a face- and construct-valid animal model for the human syndrome.

Harrington, Bridges, et al. (2020) performed unbiased RNA-sequencing on whole cortex from *Mef2c* global heterozygous mice compared to control mice and found 490 genes that were significantly dysregulated, including microglial genes and autism spectrum disorder risk genes. The authors also analyzed single-cell (sc) RNA-seq data and ChIP-Seq data and found differentially expressed genes associated with excitatory neurons and microglia. The scRNA-seq data showed an increase in expression of genes associated with embryonic and immature microglia, suggesting delayed microglial maturation in *Mef2c* heterozygous mice.

Of note, single nuclei (sn)RNA-seq has several advantages over scRNA-seq, including reduced dissociation bias, reduced dissociation stress response, and the ability to use frozen samples (Wu et al., 2019). To better understand the role that *MEF2C* plays in microglial maturation and neurons (specifically GABAergic subtype), we are currently

performing nuclei dissociation from the prefrontal cortex for snRNA-seq. The laboratory methods and bioinformatic analyses associated with snRNA-seq are discussed in this manuscript. Additionally, we discuss other current techniques used to investigate the mouse *Mef2c* gene in order to glean insight into the human disorder.

Table 5.1: Key Terms Defined

Key Terms	
Barcode	Short nucleotide sequence used to tag each cell or nuclei's transcriptome (in the case of RNA sequencing)
Gel Beads-in-Emulsions (GEMs)	Nanoliter-sized droplet containing a single cell or nuclei, a unique barcode, reagents, and partitioning oil.
Hemocytometer	Counting chamber device.
Immunohistochemistry (IHC)	Method that uses antibodies to detect antigens in a tissue sample.
Next Generation Sequencing (NGS)	Massively parallel sequencing.
NGS Library	Collection of similar sized DNA or cDNA fragments with adaptors added ready for next generation sequencing.
Nuclei Dissociation	Separation or isolation of nuclei from cells within a tissue sample.
Single Cell RNA Sequencing	Methodology to assess gene expression of messenger RNA from isolated whole cells.
Single Nuclei RNA Sequencing	Methodology to assess gene expression of messenger RNA from isolated nuclei.

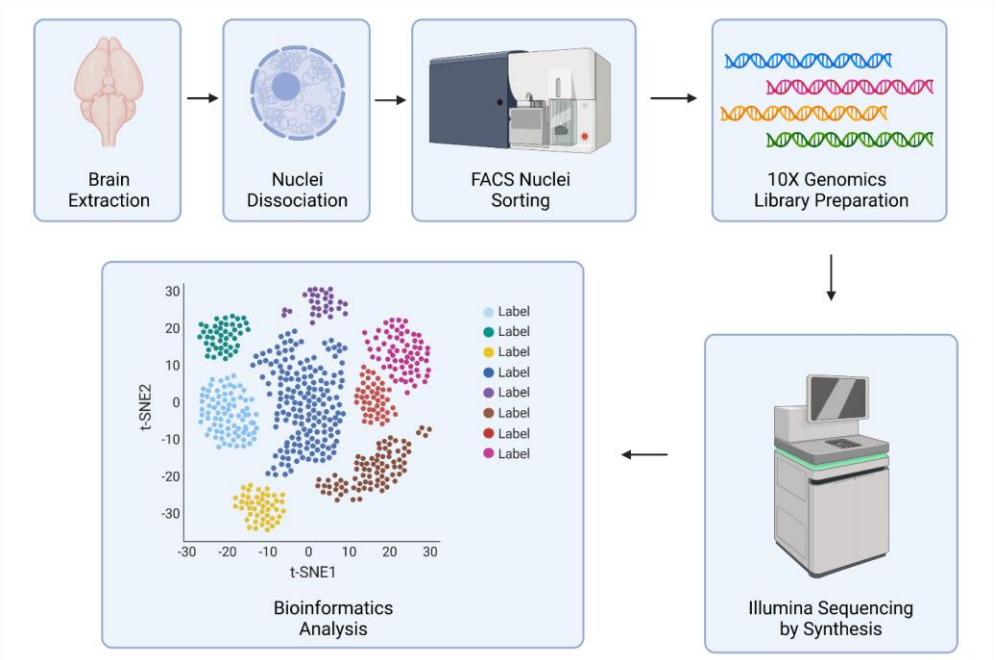
Institutional Animal Care and Use Committee Approval

All animal use was approved and done in accordance with the Medical University of South Carolina Institutional Animal Care and Use Committee (IACUC) and National Institute of Health (NIH) guidelines.

Laboratory Techniques and Analyses

Before preparing libraries for single nuclei RNA sequencing, the brain is extracted and the nuclei must be dissociated, or separated, from the cells within the tissue sample. The dissociated nuclei can undergo Fluorescence-Activated Cell Sorting (FACS) to gather a highly purified high-quality sample. The nuclei then undergo library preparation with 10X Genomics Chromium Single Cell kit. The final libraries are sequenced on an Illumina instrument, such as the NovaSeq. Finally, the data can be bioinformatically analyzed to assess gene expression differences not only between cell types but also between control groups (such as wildtype versus *Mef2c* global heterozygous mice). Additional techniques performed in the Cowan laboratory include perfusion fixation, sectioning of the brain using the microtome, and immunohistochemistry.

Figure 5.1: Single Nuclei RNAseq Workflow. Created with BioRender.com.



Nuclei Dissociation

For the nuclei dissociation, Brandon W. Hughes in the Cowan laboratory modified a protocol from the Day laboratory at the University of Alabama (Hughes, 2021). At the desired timepoint or age, the mouse is decapitated, and the brain rapidly extracted. Live decapitation is necessary as anesthetics will alter gene expression, rendering downstream analyses unreliable. The brain is briefly submerged in a nutrient medium containing RNase inhibitor then sliced in a brain block to obtain 1mm slices. The prefrontal cortex (PFC) and other brain regions of interest are micro-dissected out, placed into 1.5mL tubes, and flash frozen on dry ice. Samples are then frozen at -80˚C until the dissociation procedure is started.

For the nuclei dissociation, the frozen brain samples are thawed on wet ice then placed on a glass Petri lid. The tissue is chopped orthogonally 60-100x to break the tissue into smaller pieces. The chopped tissue is added to a 15mL tube with a chilled lysis buffer to break the cell membrane. The lysis buffer component concentrations and incubation time lyses the cell membrane but does not affect the nuclear membrane. After lysis, the tissue pieces are triturated, or broken into smaller pieces, by pipette mixing with different sized fire-polished Pasteur pipettes, starting with the largest to smallest diameter. Then, the tissue is passed through a 40µm filter to remove cell debris. The nuclei are washed with a phosphate-buffered saline mixture, then resuspended with the same buffer mixture.

This final sample can be stained with 7-aminoactinomycin D (7AAD), a fluorescent solution that intercalates in DNA, which allows the nuclei to be easily viewed

and counted on a hemocytometer. 7AAD typically will not stain live cells but is able to intercalate with DNA in dissociated nuclei. Fluorescence-activated cell sorting (FACS) can be performed on the sample to further isolate high-quality nuclei, which are needed before proceeding with single nuclei RNA sequencing. When viewed in a hemocytometer under the microscope, high quality nuclei will have a well-defined intact nuclear membrane.

Fluorescence-Activated Cell Sorting (FACS)

Fluorescence-Activated Cell Sorting (FACS) is a technique to sort cells based on fluorescence from staining, size, and granularity and yields a highly purified sample (Basu et al., 2010). This method can also be used for nuclei sorting. Typically, the cells are tagged with a fluorescently labelled antibody specific to a cell surface protein (Alexa Fluor 488-conjugated anti-NeuN for single nuclei). Using high sensitivity flow cytometry (such as the BD fascaria III sorter), the solution of cells or nuclei is passed as a droplet stream in front of a fluorescence-detecting laser. When the specified fluorescence is detected, the machine applies a charge to that droplet allowing it to be electrostatically deflected and thus separated from non-charged droplets.

After FACS, the nuclei are placed in a saline solution. Therefore, an additional step is needed to resupply the correct buffer. The nuclei are rinsed, pelleted, and again resuspended in the same phosphate buffered saline mixture from the dissociation protocol. The hemocytometer step is repeated to view the post-FACS sorted sample. The final sample should have 1500 nuclei per µL and may require diluting the sample to the correct concentration. At this point, the sample is ready for 10x Genomics Chromium

Single Cell library preparation, then sent for sequencing on an Illumina NovaSeq 6000 at a core laboratory.

Single Nuclei RNA Sequencing

10X Genomics Chromium Single Cell Library Preparation

After quality nuclei are dissociated, libraries are prepared for single nuclei RNA sequencing using the 10X Genomics Chromium Single Cell 3' Reagent Kit. Although this step is not performed within the Cowan laboratory, it is helpful to understand the complete process of snRNAseq from dissociation to final data output. For library preparation, the first step of the 10X Chromium single-cell method is to generate Gel Beads-in-Emulsions (GEMs) and barcode each individual cell or nuclei (10X Genomics, 2019). A pool of roughly 3.5 million unique barcodes (16 nucleotide sequences), the cell or nuclei solution, reagents, and portioning oil are loaded onto the Chromium Next GEM Chip G, which uses microfluidics at the nano-liter level to stream and combine one individual cell and one individual unique barcode, creating GEMs (Figure 5.1). Further, to ensure single-cell resolution, the cell solution is diluted so that most GEMs actually contain no cell, and the remaining GEMs only contain one cell. The gel beads contain primers consisting of an Illumina read 1 sequencing primer, the 16-nucleotide 10X Barcode, a 12-nucleotide unique molecular identifier (UMI), and a 30-nucleotide poly(dT) sequence. The poly(dT) is complementary to the poly-A tail of messenger RNA (mRNA).

Figure 5.2: Chromium Next GEM Chip G. Oil, cells combined with reagents, and beads are loaded onto Chromium Next GEM Chip G. Within the GemCode platform, barcoded gel beads are combined with cells and reagents to form GEMs. Reverse Transcription PCR takes place inside each GEM, then cDNA is purified to undergo library preparation steps.

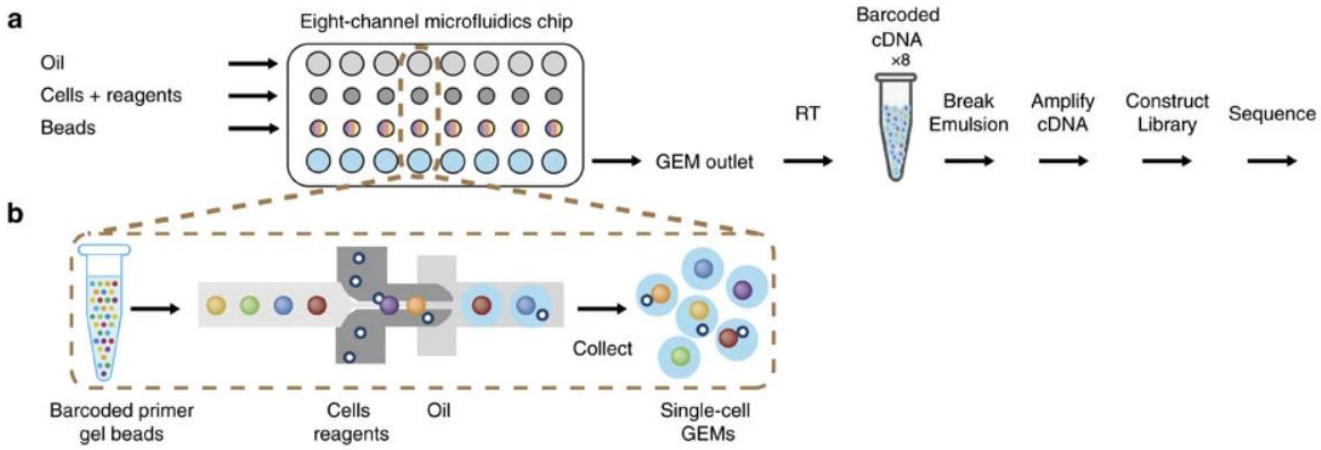
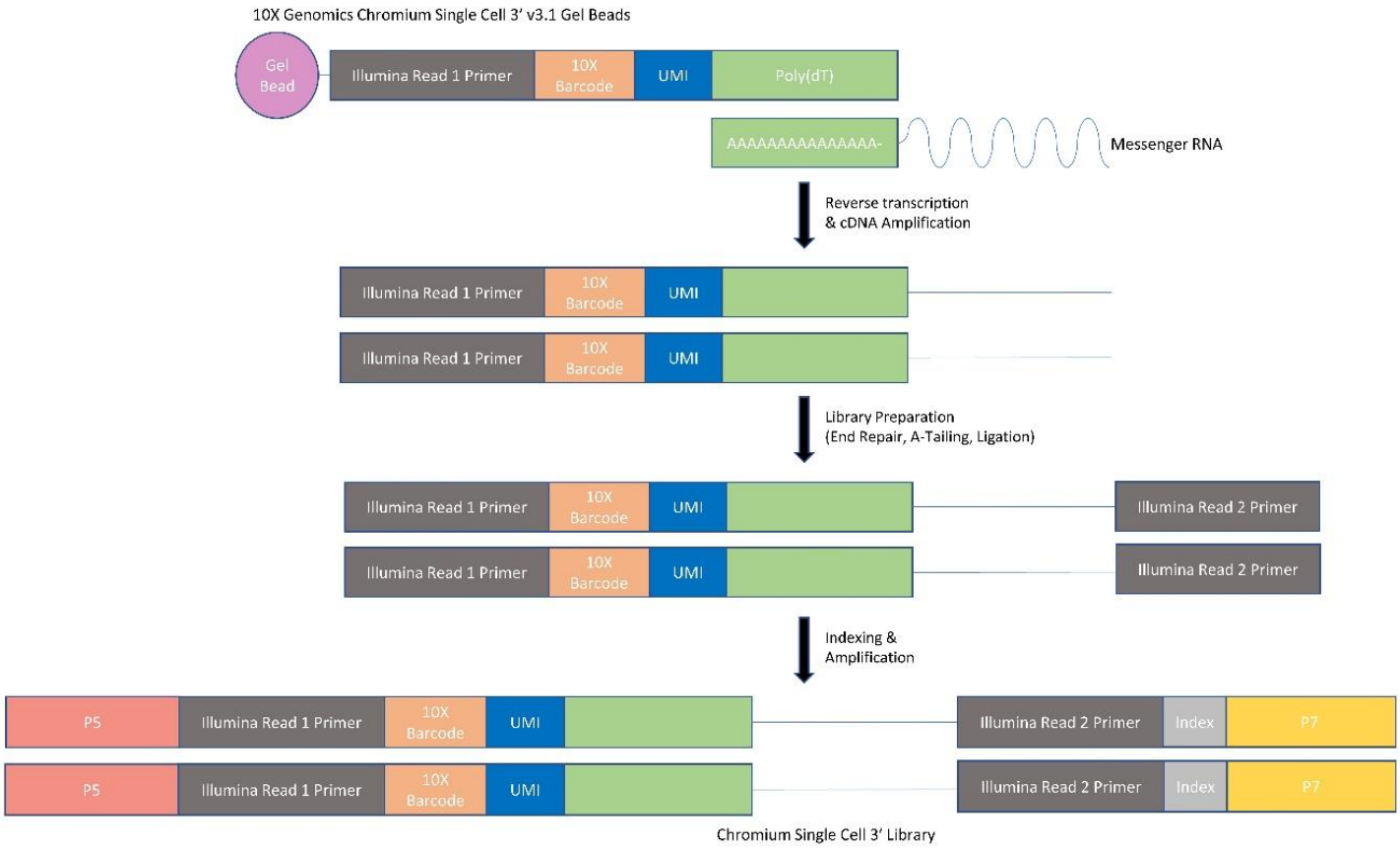


Figure from Zheng et al., 2007. Reproduced with permission from Springer Nature via the Creative Commons Attribution 4.0 International License https://creativecommons.org/licenses/by/4.0/.

Next, the primers are released inside the GEM and a reverse transcription (RT) master mix is added to convert the mRNA into barcoded cDNA (10X Genomics, 2019) (Figure 5.2). Then, the GEMs themselves are broken to release the cDNA, which gets amplified, enzymatically fragmented to a smaller size, and ligated with the Illumina TruSeq Read 1 primer. The fragments undergo end repair to fill in fragment 5' and 3' overhangs and addition of an A-tail. Then, P5, P7, a sample index, and the Illumina TruSeq Read 2 primer are ligated to the fragments. The P5 and P7 adapters are complementary to adaptors in the Illumina sequencing kit for the sequencer instrument. Lastly, there is a final PCR amplification step, resulting in the final Chromium Single Cell 3' libraries.

Figure 5.3: 10X Genomics Chromium Single Cell 3' Library Preparation Technique.



Illumina Sequencing by Synthesis

After library preparation, the libraries can be pooled, denatured, and loaded onto the NovaSeq 6000 (or other specified Illumina sequencing platform). The first step on the instrument is cluster generation (Illumina, 2017). The library fragments bind to the flow cell oligo lawn, which consists of P5 and P7 oligos complementary to the ones incorporated into the sample libraries. Each fragment is amplified into a cluster via bridge amplification. The reverse strands are cleaved so sequencing by synthesis (SBS) can occur on the forward strand. The instrument releases all four uniquely-fluorescent-tagged

bases (A, T, C, G) simultaneously and when the correct base incorporates into the growing strand, fluorescence is released and detected by the instrument. This process continues until the entire complementary strand has been synthesized, or the specified number of sequencing cycles met, and each incorporated base has been recorded by the instrument. After sequencing the forward strand, bridge amplification occurs once more to regenerate the reverse strand. The forward strand is this time cleaved for SBS to occur on the reverse strand.

Single Nuclei RNA Sequencing Bioinformatics Analysis

The raw data generated by the Illumina sequencer undergoes the Cell Ranger analysis pipeline to transform the raw data into workable data. The first step in this pipeline is demultiplexing using the P7 indices to convert raw base calls into reads (*What Is Cell Ranger?,* 2020). Next, reads are aligned to the mouse GRCm38/mm10 reference genome. The reads are further demultiplexed using sample-specific indices that were added during library preparation, which separates the data by library (which may represent individual mice with their specific test conditions). The output file generated is a feature-barcode matrix (where the features consist of data from the various genes, separated out by sample-specific barcode).

This output file is then loaded into RStudio v4.0.2 for secondary analysis using the Seurat 4.0.6 toolkit (*Seurat – Guided Clustering Tool*, 2022). Next, the data must undergo a pre-processing workflow, including QC steps to filter out low-quality cells (having less than 200 genes, as low-quality cells often have a very low gene count), cell

duplets and multiplets (GEMs that contained more than one cell, which bioinformatically show extremely high gene count (value of over 2,500)), and dying cells (which show >5% of mitochondrial gene counts). The quality reads remaining then undergo a normalization step (such as using the "LogNormalize") to correct for cells having different sequencing depths from one another thus ensuring accurate comparisons between cells. The next step is to identify highly variable features, or genes that are highly expressed in some cells and lowly expressed in others, allowing the various cell types within each test group to be separated in subsequent steps. This coding uses a statistical calculation to distinguish the biological signal from technical noise (*Seurat – Guided Clustering Tool*, 2022). Then, the data must be scaled using a linear transformation to prevent highly expressed genes from dominating the downstream analysis, giving equal chance to genes with lower expression. Alternatively, the SCT normalization method can be used, which combines normalization, identifying highly variable features, and scaling.

Using the variable features, Principal Component Analysis (PCA) is performed to determine the dimensionality and see the variation and patterns within the data set. For N number of cells, there are N-directions of variation, called principle components (PCs). The PC with the highest variation is PC1, the second highest is PC2, and so forth. Given the vast number of PCs in these datasets, the bioinformatician must figure out how many PCs to take into consideration for analysis. The JackStrawPlot function is used to visualize the p-values of each PC, allowing us to select significant PCs (those having low p-values).

The cells can finally be clustered using the "FindNeighbors" and "FindClusters" codes and inputting the previously determined dimensionality of the dataset (i.e., the first 10 PCs) and a resolution parameter (typically in the range of 0.4-1.2). A higher resolution results in a greater number of clusters. Uniform Manifold Approximation and Projection (UMAP), a non-linear dimensional reduction technique, is used to construct a low-dimensional graph for visualization of the data. The resulting graph shows color-coded clusters of cells, with cells within each cluster coming from the same cell type. Seurat coding can also find cell-specific gene markers that define each cluster, allowing the user to label each cluster by the cell type name. For example, "cluster 10" may have a high expression of *C1QA*, a microglial marker. Therefore, "cluster 10" could be renamed as "microglia".

Further coding can be input to find differential gene expression between study groups (such as wildtype versus *Mef2c* heterozygous mice). The researcher may choose to focus solely on one cluster / cell type (such as microglia) and see differential gene expression within that cell type between the study groups. For example, certain microglial genes may be upregulated in *Mef2c* heterozygous mice as compared to wild-type mice, suggesting delayed microglial maturation. This pattern was noted on previous unbiased RNAseq data (Harrington, Bridges, et al., 2020) and will be investigated further using single nuclei RNAseq.

Tissue Fixation Methods for Mice

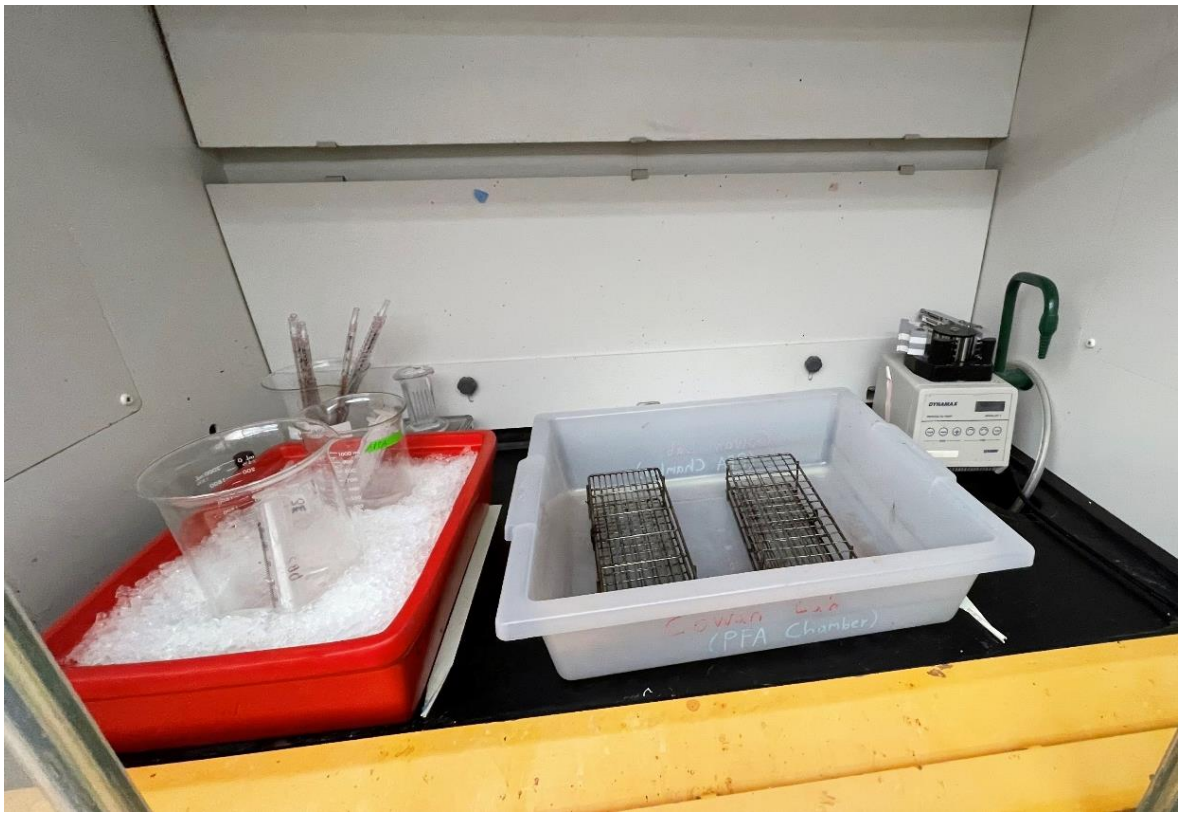
Whole-body perfusion fixation of mice can be used to preserve tissue throughout the entire body, after which the fixed tissue of interest (such as the brain) can be extracted for downstream assays. This perfusion fixation method uses the circulatory system of the mouse to deliver fixatives, penetrating every region of the body at a quick and steady rate (Gage, Kipke, & Shain, 2012). This method is beneficial for larger specimens as opposed to immersion fixation, which would not reach all regions of the tissue in time before the biological responses to hypoxia commence.

First, the mice are weighed to determine the appropriate amount of anesthesia (a mixture of ketamine at 100 mg/mL and xylazine at 20 mg/mL) to administer by intraperitoneal injection. Before proceeding with perfusion, the mice should be thoroughly checked for toe-pinch pain reflex. Once fully sedated (unresponsive to toe-pinch), the mouse is placed belly side up on a work block and the forepaws and hindpaws are taped to the side. The work block is placed in a collection bin inside a chemical fume hood (Figure 5.3). Using forceps, the skin on the stomach above the xiphoid process is pulled up and a cut is made laterally using scissors. The next cut is through the diaphragm, avoiding cutting any organs, then cut upwards through the ribs on both sides. The resulting flap is clamped above the head to expose the liver and heart.

Next, a butterfly needle attached via tubing to a perfusion pump is inserted in the left ventricle next to the apex. An incision is made in the right atrium to allow the blood and perfusion buffers to drain. The pump is turned on and the valve switched on to allow 1X Phosphate Buffered Saline (PBS) perfusate to flow through the mouse. 1X PBS is

used to flush the tissue and prevent fixing erythrocytes in place which would block access to smaller vessels. The liver will turn white as blood is replaced with 1X PBS. After about four minutes, the 1X PBS valve is closed and the 1.5% paraformaldehyde (PFA) valve is opened with perfusion occurring for another four minutes. After completion, the desired fixed tissues can be collected and stored.

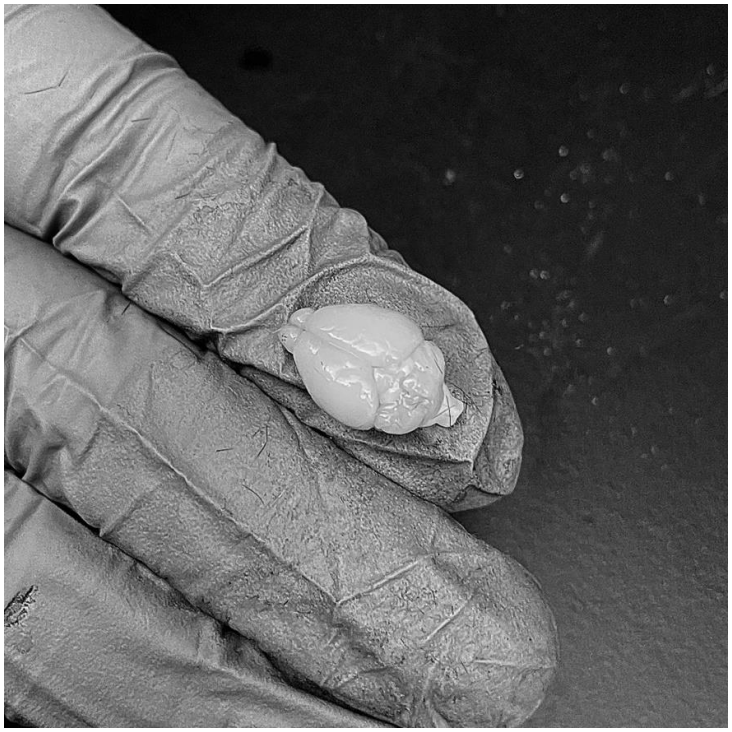
Figure 5.4: Perfusion setup inside chemical fume hood, prior to attaching the lines and handling the mice. Setup includes an ice bath with beakers to hold 1X PBS and 1.5% PFA, collection bin in the middle to collect draining fluids during the procedure, racks within the collection bin to act as a platform to hold the working block and mouse, and perfusion pump and lines in the back right corner.



Extraction of the Brain

For an extraction of either a fresh or fixed brain, the mouse is first decapitated. The excess muscle is trimmed to help expose the back of the skull. Next, the skin is cut down the midline to expose the top of the skull. Scissors are inserted through the foramen magnum and carefully cut upwards through the skull to avoid damaging the brain underneath. The scissors are gently pushed slightly behind the eyes and the blades slowly opened to fully open the skull and expose the brain. The pieces of skull are pulled aside, and forceps are slid under the brain to gently remove it from the cranium (Figure 5.4). The brain is post-fixated in 1.5% PFA for one hour then transferred the brain into 1X PBS + NaN_3 for long-term storage.

Figure 5.5: Mouse brain extracted after perfusion fixation. Photo Credit: Jessica Cooley Coleman.

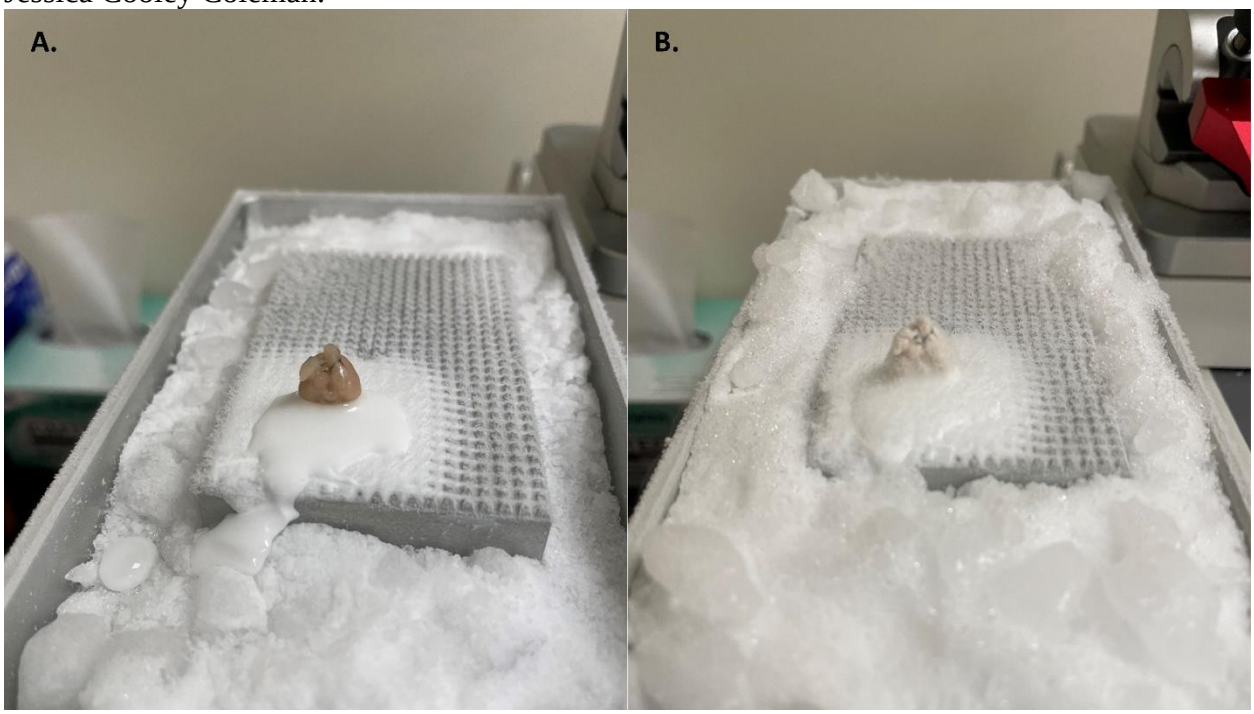


Brain Sectioning using Microtome

To section the fixed brain for downstream immunohistochemistry (IHC), the microtome can be used. The microtome is an instrument containing a sharp knife that is manually drawn across frozen tissue to cut the tissue into thin sections (Figure 5.5). The desired thickness of each slice can be set, and after each pass of the knife the instrument mechanically lowers the knife by the preset amount allowing uniformly cut sections. First, the top row of a 24-well culture plate is filled with 1mL of 1X PBS NaN_3 for post-slicing storage. Next, the brain mounting platform is leveled, and dry ice is carefully added into the surrounding trough using a spoon. The bottom portion of the fixed brain (occipital lobe, or caudal section) is cut slightly with a razor blade to make a flat surface for mounting onto the microtome platform. OCT (optimal cutting temperature compound) is applied to the platform, and the brain must be quickly positioned in the liquid before it freezes. The OCT and the brain must freeze completely (turn white) before proceeding with sectioning (Figure 5.6).

Figure 5.6: Microtome instrument (prior to installing the platform and knife). Photo Credit: Jessica Cooley Coleman.

Figure 5.7: A) Mounted fixed brain on the Microtome platform using OCT. **B)** The brain was allowed to freeze completely before proceeding with sectioning. Photo Credit: Jessica Cooley Coleman.



After the brain is completely frozen on the platform, the knife is installed and you can proceed with making slices. A thin paintbrush is used to collect each brain slice and alternating placing them across in the top row (6 wells) of the prefilled 24-well culture dish. The dry ice should be replenished as necessary during slicing to keep the brain frozen. Slicing continues until all desired brain regions are collected (Figure 5.7). The brain slices can be stored in the refrigerator until IHC or other assays are performed.

Figure 5.8: Brain during microtome slicing. Photo Credit: Jessica Cooley Coleman.

Immunohistochemistry

Immunohistochemistry is a technique to detect antigens present in sections of tissue. To perform IHC, the brain sections are first washed with 1X PBS and then incubated in a blocking solution consisting of serum (normal goat serum and normal donkey serum) and proteins (bovine serum albumin, or BSA) to bind to reactive sites, thus helping prevent non-specific antibody binding in subsequent steps. Next, the sections are incubated overnight in a primary antibody solution specific to the antigen or protein of interest (such as NeuN for neurons and Iba1 for microglia). After the overnight incubation, the slices are washed then incubated with a fluorescently tagged secondary antibody (such as Alexa Fluor 488 donkey anti-mouse). This secondary antibody is not protein specific, and instead interacts with the primary antibody and delivers fluorescence

to later image the cells. The slices are washed again and Hoechst or DAPI (4′,6-diamidino-2-phenylindole) stain is added to stain the nucleus. This step ensures that cells are stained, as now the cell membrane and nucleus are individually stained and both can be viewed to help differentiate from potential debris. Finally, the slices are positioned onto microscope slides, a few drops of ProLong Gold Antifade Mountant with additional DAPI is placed on the slices, and a coverslip is placed on top (Figure 5.9). The slide is allowed to cure for 24 hours then can be imaged. The fluorescence can be detected on a light microscope to visualize the intended target (neurons) within the tissue sample (Figure 5.10).

Figure 5.9: Brain slices arranged on a slide prior to addition of mountant. Photo Credit: Jessica Cooley Coleman.

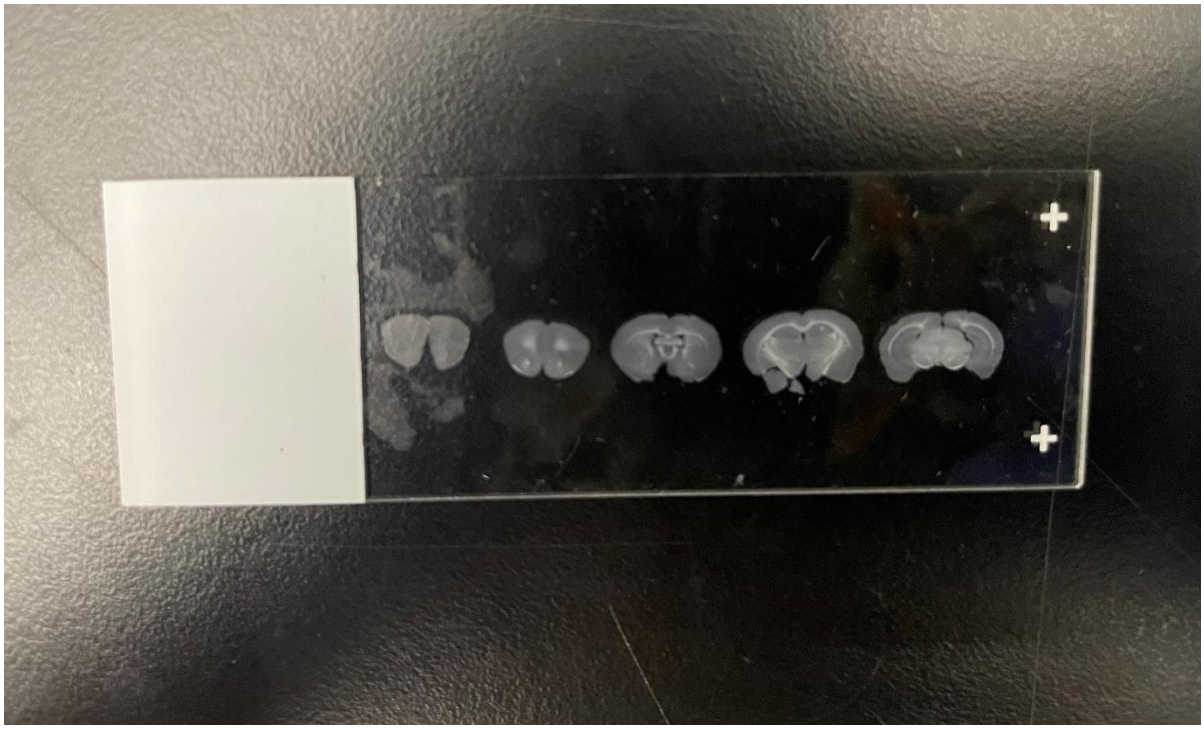
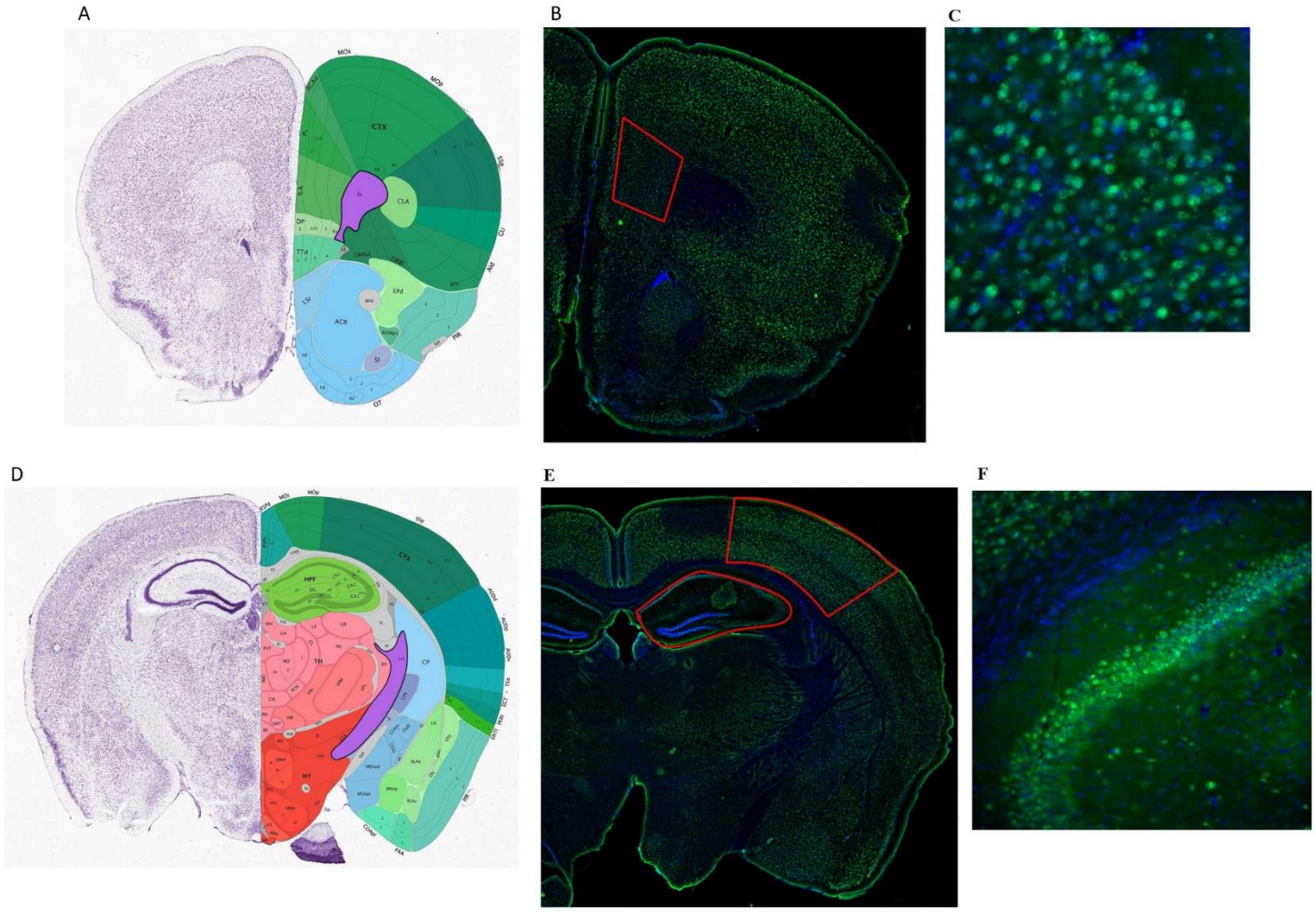


Figure 5.10: Final images from immunohistochemistry of a wildtype mouse brain stained with DAPI for cells (blue) and NeuN for neurons (green). **A:** Nissl (left) and anatomical annotations (right) from the Allen Mouse Brain Atlas and Allen Reference Atlas - Mouse Brain, at the same slice position as B. **B:** Brain section with prefrontal cortex (red box) at 5X. **C:** Prefrontal cortex at 40X. **D:** Nissl (left) and anatomical annotations (right) from the Allen Mouse Brain Atlas and Allen Reference Atlas - Mouse Brain, at the same slice position as E. **E:** Brain section with somatosensory cortex (upper red box) and hippocampus (middle red circle) at 5X. **F:** Somatosensory cortex and hippocampus at 20X.



Conclusion

Mef2c global and conditional heterozygous mice share phenotypic similarities with human patients affected with *MEF2C*-related disorders, including repetitive behaviors and social deficits. These similarities make the mouse an excellent animal model to study the gene and the associated disorder. Nuclei dissociation with purification by FACS, single nuclei RNAseq, whole mice body perfusion, fresh and fixed brain extraction and slicing, and immunohistochemistry are some of the many current techniques used to research *Mef2c* in mice. Performing single nuclei RNAseq can help elucidate *MEF2C's* role in the development and maturation of neurons, microglia, and other cell types, and reveal gene dysregulation between wildtype and *Mef2c* heterozygous mice.

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CHAPTER SIX

CONCLUSIONS

MEF2C-related disorders are neurodevelopmental disorders characterized by intellectual disability, developmental delay, lack of speech, seizures, hypotonia, and brain abnormalities. This disorder is rare with only 117 patients reported in the literature to date, making the disorder difficult to recognize clinically. This research sought to thoroughly describe the genotypes leading to *MEF2C*-related disorders, elucidate the phenotypic features, and assess *MEF2C*'s role in gene regulation. The purpose of this work is to advance what is known about *MEF2C*-related disorders with the goal of improving diagnosis, patient care, and future development of treatments.

First, the *MEF2C* gene was described (Chapter 1), including its history and discovery. The transcription factor *MEF2C* contains the highly conserved MADS domain followed by the MEF2 domain (conserved across only the MEF2 family), which are responsible for dimerization, cofactor binding, and DNA binding. We have described in detail the structure of *MEF2C,* including exact amino acids encompassing the MADS and MEF2 domains, total number of nucleotides, number of exons, and number of different isoforms due to differential splicing found in the human body. We also covered the history of *MEF2C*-related disorders. Additionally, we discussed methodologies for rare disease research, specifically including concept analyses, systematic literature reviews, natural history study surveys, and animal model studies (all of which are used in subsequent chapters).

In Chapter 2, we performed a concept analysis of tremors following the Walker & Avant method (Walker & Avant, 2005). According to a February 2020 search, tremors are associated with 594 potential genetic conditions and genes. *MEF2C*-related disorders were not among this list; however, the literature reported at least two patients with *MEF2C*-related disorders having tremors, one of whom had a periodic tremor in infancy and the other had a childhood hand tremor. The concept of tremors has been complicated by vague definitions and numerous categorization methods; therefore, we chose to perform a concept analysis to clarify the concept and develop an operational definition of tremors. Using the Walker and Avant method involved determining the aims of the analysis and uses of the concept, defining attributes, highlighting a model case and other cases, identifying the antecedents and consequences, and defining empirical referents. This process allowed us to develop an operational definition that tremors are a movement disorder characterized by shaking motions that are involuntary, oscillatory, rhythmic, non-painful, always present although variable in severity, and can be repressed by changing posture or going into a rest position. This concept analysis will assist providers, nurses, and researchers to correctly recognize and categorize tremors and provide the best treatment and care to their patients. This concept analysis was peer reviewed and published *Nursing Open* in 2021 (Cooley Coleman et al., 2021a).

To further investigate the symptoms, features, and overall phenotype of *MEF2C*-related disorders, we performed a systematic literature review (Chapter 3) to answer the research question: What is the comprehensive phenotype of all human patients reported in the literature with a *MEF2C*-related disorder? We derived keywords and MeSH terms

from the research question to search Web of Science, PubMed, and MEDLINE for articles meeting our inclusion criteria. A total of 43 articles met the inclusion criteria and were fully reviewed, revealing phenotypic information on 117 patients with *MEF2C*-related disorders. Most patients had features including intellectual disability, developmental delay, seizures, hypotonia, absent speech, inability to walk, stereotypic movements, and MRI abnormalities. We also found cardiac issues to be of higher prevalence than previously appreciated. Non-classical features included a question mark ear, jugular pit, and a unique neuroendocrine finding. Additionally, we found nine patients with the phenotype of *MEF2C*-related disorders who had deletions not containing *MEF2C*, revealing a potential positional effect. This systematic review further characterizes the disorder, providing information that healthcare providers can use to better diagnose and care for patients. This review was published in the *American Journal of Medical Genetics, Part A* in 2021 (Cooley Coleman et al., 2021b).

Next, we developed a natural history study in the format of a parent survey to gather additional developmental and clinical information on a large single cohort of patients with *MEF2C*-Related Disorders (Chapter 4). A total of 73 parents completed the survey. Limited speech (82.1%), seizures (86.3%), bruxism (87.7%), repetitive movements (94.5%), and high pain tolerance (79.5%) were some of the prominent features. Additionally, these features and percentages were closely aligned with those revealed by the literature review. A total of 39.7% of parents reported a *MEF2C* variant and 54.8% reported a deletion involving *MEF2C*. Statistical analyses showed patients with *MEF2C* variants were similarly affected as those with deletions, and females

showed higher verbal abilities. This study obtained comprehensive phenotypic information on the largest single cohort of patients with a *MEF2C*-related disorder. The information provided by the study can be useful to healthcare providers in diagnosing and caring for patients and can also be a valuable resource for researchers performing additional analysis (such as genotype-phenotype correlations) or developing further studies. This study was accepted for publication in *Molecular Genetics & Genomic Medicine* (Cooley Coleman et al., 2022).

Finally, we used the mouse as an animal model to investigate *MEF2C's* role in expression and gene regulation in the brain. Previous unbiased RNA sequencing showed a dysregulation of genes associated with microglia, excitatory neurons, and autism spectrum disorder risk genes in *Mef2c* global heterozygous mice compared to control mice. To further investigate the role of *MEF2C* within microglia and GABAergic subtype neurons, we decided to pursue single nuclei RNA sequencing (snRNAseq). The workflow entails performing nuclei dissociation on dissected sections of the brain (particularly prefrontal cortex), purifying the nuclei using Fluorescence-Activated Cell Sorting (FACS), and sending the sample off for library preparation and single nuclei RNA sequencing. When the data is returned by the sequencing core lab, bioinformatic analysis is performed to cluster the data into cell types in order to investigate differential gene expression within microglia and GABAergic neurons. This study is still ongoing, and results will be a part of a larger publication in the future. We also learned other current laboratory techniques including perfusion fixation, brain extraction and slicing, and immunohistochemistry.

Throughout this research, we have expanded on the phenotype through a concept analysis, systematic literature review, and natural history study parent survey. We have also thoroughly cataloged the pathogenic alterations (genotype) of patients having *MEF2C*-related disorders reported in the literature. The data from both our literature review and parent survey can be useful for future genotype-phenotype correlation studies. Of the 43 manuscripts identified in the literature review, some manuscripts focused on a specific feature (i.e. cardiac issues); therefore, some features or symptoms may not have been reported, which changed our N for each feature (as we could not assume the patient lacked that symptom just because it was not mentioned). One future direction could entail contacting the authors of these 43 manuscripts to gather the same clinical information across all reported patients. This would allow a more accurate assessment of the phenotype, prevalence of each feature, and allow for more statistical analyses.

Other future directions could include initiating a clinical longitudinal study of individuals with *MEF2C*-related disorders. Our survey could work as a baseline for such a study, with a similar survey being sent out at another time frame (i.e. 5 years later) to measure any changes over time. It would also be beneficial to have additional information on adults with a *MEF2C*-related disorder, as most individuals from the systematic review and survey were in the childhood range. A longitudinal study of individuals would allow capturing information of current patients as adults in the future. The longitudinal study could be based on more parent surveys, or the patients could be seen clinically (in person or via telemedicine) to allow for gathering objective information by a healthcare provider. Another future project could entail performing a

quality of life assessment for individuals with a *MEF2C*-related disorder. A *MEF2C*-related disorders online patient registry could be beneficial in housing the information obtained from these studies. Lastly, additional functional studies on *MEF2C*, such as those being performed by the Cowan laboratory, will advance the knowledge about *MEF2C* and *MEF2C*-related disorders. Ultimately, it is our hope that this research and future research studies will advance our knowledge, guide treatment development, and help improve the lives of people with *MEF2C*-related disorders.

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APPENDICES

Appendix A

Abbreviations

7AAD: 7-aminoactinomycin D

ADHD: Attention deficit hyperactivity disorder

AHA: Acquired hemophilia A

ASD: Autism spectrum disorder

bHLH: Basic-helix-loop-helix

BSA: Bovine serum albumin

BU: Bethesda units

CNV: Copy number variation

CoCoPop: Condition, Context, and Population

DAPI: 4′,6-diamidino-2-phenylindole

DBS: Deep brain stimulation

DCM: Dilated cardiomyopathy

ddNTPs: Dideoxynucleotides

DNA: Deoxyribonucleic acid

dNTPs: Deoxynucleotides

DORV: Double outlet right ventricle

EEG: Electroencephalogram

ERG: Electroretinograms recording

ET: Essential tremor

FACS: Fluorescence-Activated Cell Sorting

FISH: Fluorescence in situ hybridization

FORWARD: Fragile X Online Registry with Accessible Research Database

FTM: Fahn-Tolosa-Marin Tremor Rating Scale

FXTAS: Fragile X-associated tremor/ataxia syndrome

GEMs: Gel beads-in-emulsions

GERD: Gastroesophageal reflux disease

GGC: Greenwood Genetic Center

IACUC: Institutional Animal Care and Use Committee

IHC: Immunohistochemsitry

IRB: Institutional Review Board

MADS-box region: from the first four protein member identified in this group, MCM1, AG, DEFA, and SRF

MARRVEL: Model Organism Aggregated Resources for Rare Variant ExpLoration

MCHS: *MEF2C* Haploinsufficiency Syndrome

MDS-UPDRS: Movement Disorder Society United Parkinson's Disease Rating Scale

MEF2: MADS box transcription enhancer 2

MEF2A: MADS box transcription enhancer 2A

MEF2B: MADS box transcription enhancer 2B

MEF2C: MADS box transcription enhancer 2C

MEF2D: MADS box transcription enhancer 2D

MRI: Magnetic resonance imaging

mRNA: Messenger RNA

MUSC: Medical University of South Carolina

NGS: Next generation sequencing

NIH: National Institute of Health

NPY: Neuropeptide Y

OCT: Optimal cutting temperature compound

OHRP: Office for Human Research Protections

OMIM: Online Mendelian Inheritance in Man

PBS: Phosphate buffered saline

PCA: Principal component analysis

PCs: Principal components

PDA: Patent ductus arteriosus

PECS: Picture exchange communication system

PFA: Paraformaldehyde

PFC: Prefrontal cortex

PICO: Patient/Population problem, Intervention, Comparison or Control, Outcome

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PS: Pulmonary stenosis

QUEST: Quality of Life in Essential Tremor Questionnaire

REDCap: Research Electronic Data Capture

RNA: Ribonucleic acid

RT: Reverse transcription

SBS: Sequencing by synthesis

scRNAseq: Single cell RNA sequencing

SD: Standard deviation

SNP: Single nucleotide polymorphism

snRNAseq: Single nuclei RNA sequencing

SNV: Single nucleotide variant

SPECT: Single-photon emission computerized tomography

SSRIs: Selective serotonin reuptake inhibitors

TETRAS: The Essential Tremor Rating Assessment Scale

UMAP: Uniform manifold approximation and projection

UMI: Unique molecular identifier

UTRs: Untranslated region

VSD: Ventricular septal defect

WHIGET: Washington Heights-Inwood Genetic Study of Essential Tremor

Appendix B

Supplemental PROSPERO Systematic Literature Review Protocol

Comprehensive Investigation of the Phenotype of *MEF2C*-Related Disorders in Human Patients: A Systematic Review.

Jessica A. Cooley Coleman

Citation

Cooley Coleman, Jessica A.. Comprehensive Investigation of the Phenotype of MEF2C-Related Disorders in Human Patients: A Systematic Review. PROSPERO 2021 CRD42021238965
Available from:
https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021238965

Review question

What is the comprehensive phenotype of human patients with *MEF2C*-related disorder?

Searches

The following electronic databases will be searched: Web of Science, PubMed, and MEDLINE.
The search strategy will include only terms relating to the framework. The search terms will be adapted for database-specific filters. The search will only include peer-reviewed publications. There will be no restriction to publication dates. Only articles in the English language will be included.

Concept (CoCoPop)	**Keywords**	**MeSH terms**
Co: Condition *MEF2C*-related disorder	"MEF2C" OR "*MEF2C*-related disorder" OR "*MEF2C* haploinsufficiency"	Haploinsufficiency (MeSH term to only be used in conjunction with "AND MEF2C")
Co: Context Phenotype	"phenotype" OR "present*" OR "presentation" OR "clinical presentation" OR "feature*" OR "character*"	Phenotype
Pop: Population Human Patients	"human" OR "patient" OR "male" OR "female"	Humans OR Patients OR Male or Female

PubMed:

((((MEF2C[Title/Abstract] OR MEF2C-related disorder[Title/Abstract] OR MEF2C haploinsufficiency[Title/Abstract] OR (MEF2C[Title/Abstract] AND Haploinsufficiency**[MeSH Terms])) AND (phenotype OR present* OR presentation OR clinical presentation OR feature* OR character* OR phenotype[MeSH**

Terms])) AND (human OR patient OR male OR female OR Humans[MeSH Terms] OR Patients[MeSH Terms] OR Male[MeSH Terms] OR Female[MeSH Terms]))))

MEDLINE:
AB (MEF2C OR "MEF2C-related disorder" OR "MEF2C haploinsufficiency" OR (MH haploinsufficiency AND MEF2C)) AND (phenotype OR present* OR presentation OR "clinical presentation" OR feature* OR character* OR MH Phenotype) AND (human OR patient OR male OR female OR MH humans OR MH patients OR MH Male OR MH Female)

Web of Science:
TOPIC: (MEF2C OR "MEF2C-related disorder" OR "MEF2C haploinsufficiency") AND TOPIC: (phenotype OR present* OR presentation OR clinical presentation OR feature* OR character*) AND TOPIC: (human OR patient OR male OR female)

Types of study to be included
Any study type that includes phenotypic information on human cases of *MEF2C*-related disorder will be included for review. Cell or animal studies will be excluded.

Condition or domain being studied
MEF2C-related disorders, also referred to as *MEF2C* haploinsufficiency disorder.

Participants/population
Individuals of any age with a diagnosis *MEF2C*-related disorders, also referred to as *MEF2C* haploinsufficiency disorder confirmed by genetic testing.

Intervention(s), exposure(s)
Phenotype of individuals with a *MEF2C*-related disorder.

Comparator(s)/control
No control conditions are required.

Main outcome(s)
To compile an up-to-date list of reported patients and their phenotypes in order to further characterize the phenotype of the disorder.

Additional outcome(s)
None.

Data extraction (selection and coding)
Search results from the three databases will be saved into one library using the reference manager Zotero. Duplicate records will be removed.

Titles and/or abstracts of studies retrieved will be screened by two review authors to identify articles that may meet the inclusion criteria. For the articles that pass the title/abstract review, the full text will be retrieved and independently assessed by two review team members. Any disagreement between the two reviewers will be resolved through discussion with a third reviewer.

Data extracted from the articles will include the study design, study population, population demographics, population phenotypic information, as well as any other useful information pertaining to the patient's disorder.

Risk of bias (quality) assessment
Two review authors will independently assess the articles, thus decreasing the risk of bias of the articles included in the study.

Strategy for data synthesis
A qualitative synthesis of the phenotypic findings from the included studies will comprise the data synthesis.

Analysis of subgroups or subsets
None.

Contact details for further information
Jessica A. Cooley Coleman
cooley8@g.clemson.edu

Organizational affiliation of the review
Clemson University, Greenwood Genetic Center.

Review team members and their organizational affiliations
Jessica A. Cooley Coleman, MB(ASCP)^{CM}, Doctoral Student, School of Nursing, Clemson University
Sara M. Sarasua, PhD, MSPH, Assistant Professor, School of Nursing, Clemson University
Luigi Bocutto, MD, Lecturer, School of Nursing, Clemson University
Hannah Warren Moore, MS, CGC, Clinical Genetic Counselor, Greenwood Genetic Center
Steven Skinner, MD, Director, Greenwood Genetic Center
Jane M. DeLuca PhD, RN, CPNP, Associate Professor, School of Nursing, Clemson University

Type and method of review
Systematic review

Anticipated or actual start date

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English

Country
United States of America

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Review Ongoing

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MEF2C, *MEF2C*-related disorders, *MEF2C* haploinsufficiency

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23 February 2021

Stage of review at time of this submission
Review Ongoing

Appendix C

Supplemental Literature Review Extraction Table

(starts on following page)

	A	B	C	D	E	F	G	H	I	J
1		**Study Type**	**Authors**	**Year Published**	**Locations Published**	**Verification of Human Case**	**Number of Patients**	**Patient Sex**	**Patient Age**	**Phenotype and Clinical Information Reported**
2	1	case report	Ilari, Agosta, & Bacino	2015	Texas, US	human	1	F	9yr	ID, lack of speech, no social smile, no social communication, delayed milestones (head control at 12mo, sitting at 2yr but wasn't controlled until 5 years), hypotonia in the trunk, unable to stand on her own, unable to walk, repetitive hand movements (flapping, clapping, hand washing, hand-to-mouth movements), no purposeful hand movement, febrile seizures, complex partial seizures, dysmorphic (broad nose, deep nasal bridge, short philtrum), mouth open, abnormal MRI (distal corpus callosum thinning, two arteriovenous malformations), small pink rounded or oval- shaped vascular lesions (many with telangiectatic vessels in center). Capillary
3	2	case report	Carr, Zimmerman, Martin, Vikkula, Byrd, & Abdul-Rahman	2011	Tennessee, US	human	1	M	16yr (last age mentioned)	motor delay, hypotonia from 6mo, axial then later global hypotonia and 2+ reflexes at 10mo, sitting unsupported at 2.5yr, not able to walk, no speech (uttered noises), stereotypic hand movements (hand flapping), persistent bruxism, myoclonic seizures, open mouth, multiple skin legions by age 3 yr consistent with CMs with telangiectatic vessels, abnormal MRI (thickened anterior corpus callosum and simplified gyral with gyral thickening), hypopigmentation consistent with vitiligo from age 13 (not associated RASA1, coincidental finding), reflexes 3+ at 14yrs, dysmorphic features (prominent forehead, bitemporal narrowing, hypoplastic orbital ridges, downslanting palpebral fissures, sparse bilateral medial eyebrows)
4	3	cohort and functional study	Lu, Wang, Wang, Liu, & Yang	2018	Shanghai, China	human	1proband, 3 affected family members (father, uncle, grandfather)	M	30yr	VSD ventricular septal defect, DORV double outlet right ventricle
5								M	27yr	VSD ventricular septal defect, DORV double outlet right ventricle
6								F	1yr	VSD ventricular septal defect, DORV double outlet right ventricle
7	4	case reports	Engels et al.	2009	Germany	human	3	F	6yr	muscular hypotonia and tachydyspnoea, infantile spasms, abnormal MRI (aplasia of cerebellar vermis and posterior corpus callosum, multiple plexus cysts, enlarged occipital horns of lateral ventricles), hypsarrhthmia, concentric myocardial hypertrophy, incomplete closure of thoracic vertebral arches Th2-Th10. chronica constipation, increased sweating, bilateral optic atrophy, truncal hypotonia, bilateral pes equines, psychomotor delay, puree fed only, frequent upper respiratory tract infections, no facial dysmorphism but large ears and broad eyebrows, bilateral transverse palmar creases, cafe-au-lait
8								F	8yr	feeding difficulties, failure to thrive, frequent vomiting, developmental delay (social smile at 5mo), truncal hypotonia, babbling at 7yrs, not able to sit independently at 7 yrs, febrile seizures, visual preoccupation with stripes, brachycephaly with low anterior hairline, dysmorphic features (downslanting palpebral fissures, philtral haemangioma), abnormal MRI (prominence o farachnoid spaces in perivascular areas), myoclonic jerks, EEG (high amplitude prominent rhythmical activity in temporal regions with generalized burst of spike and slow
9								F	6yr 9mo	slept a lot, sensitive to noise, psychomotor delay, severe muscular hypotonia, developmental delay (head control later than 1 yr, sitting unsupported at 2.5yr, no walking as of 7yrs), uses syllables (mamama, papapa at 3yrs) and syllables in a directed fashion at 6yr, could express basic needs with electronic speaking aid, hyperopia, strabsimus, EEG (short focal seizures accompanied by atypical absences), MRI abnormal (moderate atrophy of supra- and infratentorial region, slighly enlarged ventricular system, and unspecific leucoencephalopathy), atypical absences at 4yr9mo, one grand mal seizure at 5yr3mo, MR, limited social interactions, could not roll or move without support, mild dysmorphisms (simple ears, slighly narrowed supraorbital region, slightly upslanting palpebral fissures), open mouth, hypersalivated,

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
2	neurology clinic 1st, department of molecular and human genetics 2nd	loss 5q14.3 (hg 19n arr 5q14.3 [86185831–88909378]) with a minimum size of 2.724Mb encompassing MIR4280, RASA1, CCNH, TMEM161B, LOC645323, MIR9-2, and MEF2C chr5:86221587-88945134 (hg18)	unknown	Chromosomal Microarray Analysis–HR (V8.1 OLIGO clinical genomic microarray)	Ilari, R., Agosta, G., & Bacino, C. (2016). 5q14.3 deletion neurocutaneous syndrome: Contiguous gene syndrome caused by simultaneous deletion of RASA1 and MEF2C: A progressive disease. American Journal of Medical Genetics. Part A, 170(3), 688–693. https://doi.org/10.1002/ajmg.a.37472
3	clinical assessment	~3.1Mb interstitial deletion of 5q14.3 was identified spanning from 85,208,054 to 88,290,255 bp encompassed five known genes: COX7C, RASA1, CCNH, TMEM161B, and MEF2C chr5:85208054-88290255 (hg18)	de novo	Whole-genome cytogenetic array comparative genomic hybridization (aCGH) analysis using a custom-designed 44K oligonucleotide array with a backbone resolution of ~250 kb	Carr, C. W., Zimmerman, H. H., Martin, C. L., Vikkula, M., Byrd, A. C., & Abdul-Rahman, O. A. (2011). 5q14.3 neurocutaneous syndrome: A novel contiguous gene syndrome caused by simultaneous deletion of RASA1 and MEF2C. American Journal of Medical Genetics. Part A, 155A(7), 1640–1645. https://doi.org/10.1002/ajmg.a.34059
4 5 6	clinical assessment (family history)	c.43C>T;p.Arg15Cys	Likely paternal, father deceased (not tested, but has phenotype) paternal	Sanger sequencing	Lu, C.-X., Wang, W., Wang, Q., Liu, X.-Y., & Yang, Y.-Q. (2018). A Novel MEF2C Loss-of-Function Mutation Associated with Congenital Double Outlet Right Ventricle. Pediatric Cardiology, 39(4), 794–804. https://doi.org/10.1007/s00246-018-1822-y
7	clinical assessment	5.69Mb deletion of 5q14.3-q15 containing 234 SNP probes (start SNP: rs10514301, genomic position/NCBI assembly 36: bp 87975410; end SNP rs9314105, bp 93668872), 5q14.3q15(rs10514301-rs9314105) x1 dn. chr5:87975410-93668872 (hg18)	de novo	GeneChip Human Mapping 100K SNP array (Affymetrix)	Engels, H., Wohlleber, E., Zink, A., Hoyer, J., Ludwig, K. U., Brockschmidt, F. F., Wieczorek, D., Moog, U., Hellmann-Mersch, B., Weber, R. G., Willatt, L., Kreiss-Nachtsheim, M., Firth, H. V., & Rauch, A. (2009). A novel microdeletion syndrome involving 5q14.3-q15: Clinical and molecular cytogenetic characterization of three patients. European Journal of Human Genetics: EJHG, 17(12), 1592–1599. https://doi.org/10.1038/ejhg.2009.90
8	clinical assessment	3.93Mb and the patient's karyotype is arr cgh 5q14.3(RP11-291O24-RP11-62E10 x 1 dn. (including MEF2C and RASA1) chr5:86206067-90139366 (hg18)	de novo	BAC array analysis using the Sanger 1Mb array	
9	clinical assessment	3.574Mb heterozygous deletion, arr cgh 5q14.3q15 (rs10223241-rs17664587) x 1 dn. **MEF2C not deleted** (del is slightly downstream of MEF2C) chr5:88448144-92022455 (hg18)	de novo	Illumina Sentrix HumanHap 550-Duo v3Beadchip	

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
10	5	case report	Toral-Lopex et al.	2012	Mexico	human	1	F	3yr	ID, muscular hypotonia, developmental delay (head control at 1yr3mo), could no sit or walk unsupported, no language, not receptive to language either, seizures at 3mo and 1yr, dysmorphic features (occipital plagiocephaly, large and lowset ears, narrow forehead, depressed nasal bridge, flat facial profile, synophrys, narrow palpebral fissures, right eye esotropia, short nose and philtrum, downturned corners of mouth, small mandible), short neck, prominent anterior chest, aberrant right palmar creases, bilateral fifth finger clinodactyly, abnormal MRI (left-sided cerebral hemiatrophy, fronto-temporal cortical atrophy, dandy-walker malformation, partial agensis of corpus callosum and cerebellum, ventriculomegaly, abnormal cortical hyration)
11	6	case report	Mikhail et al.	2011	AL, US	human	8 total (but only 1 with MEF2C deleted)	M	2.5yr	global developmental delay, language affected (expressive and receptive)- uses no words and doesn't follow spoken commands, cannot walk, relative macrocephaly, dysmoprhic features (epicanthic folds depressed nasal bridge, slighly posteriorly rotated ears) hyperkinesis with constant movements of hands and feet,
12	7	case report	Saitsu et al.	2011	Japan	human	1	M	7yr	poor visual contact an dnystagmus at 3mo, upward gazing, tonic seizures of lower extremeties followed by generalized clonic seizures at 3mo, hypsarrhythmia when asleep, low perfusion at right frontal areawith cerebral blood flow exam, MRI abnormal (reduced volume of white matter, hypoplastic corpus callosum especially in genu and splenium), ID, DD, spastic quadriplegia, NO hypotonia, could not walk or speak, poor eye contact, can't sit alone or roll over, gastroesophageal reflux and tube fed, NO steroetypic movements, deformity of trunk and extremities, dysmorphic (infancy: square face with short palpebral fissures, short depressed nose with anteverted nostrils, tented vermilion of upper lip, protruded tongue. Childhood: face became round and flat), encephalopathy
13	8	case report	Shimojima et al.	2012	Japan	human	1	M	1yr 8mo	generalized hypotonia at birth, feeding difficulty, repsiratory distress due to dysphagia and airway narrowing, opishotonic posture, truncal hypertonia, DD (head control at 7mo, visual fixation and social smile at 1yr), epileptic seizures (started at 4mo) characterized by spasms, drops, abductions of arms and eye rolling, hypsarrhythmia, abnormal MRI (reduced volume of the frontal lobe, hypoplastic corpus callosum, dilatation of the lateral cerebral ventricles, reduced white matter especially in frontal and anterior temporal lobes, remarkable dilatation of the lateral ventricles especially occipital and inferior horns showing colpocephalic appearance, severe dysgenesis of corpus callosum, ventral fornix hyperplastic, brainstem volume reduced, upper cerebellar peduncles were hypoplastic), microcephaly, cannot sit or roll over, no meaningful speech, dysmorphic (flat occiput, hypertelorism, depressed nasal bridge, small nose, low set ears, micrognathia, short tapering fingers, single transverse palmar creases in both hands), deep tendon refelxes hyperactive, spastic quadriplegia

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
10	clinical assessment	1) 46,XX,t(2;5) (q13;q14), 2) 1.59 Mb deletion on chromosome 5q14.3 (87.550–89.140 kb) which involved TMEM161B, LOC100505894, LOC645323, MIR9-2, LOC100505949 and MEF2C genes. And 600 kb deletion on 2q13 (111.720–112.320 kb) involving the RPL5P9, ACOXL, FLJ44006, BCL2L11, LOC100505634, LOC100128130, and RPS14P4 genes The final karyotype was 46,XX,t(2;5)(q13;q14.3), arr 5q14.3(87,542,893-89,148,294)x1 dn, 2q13 (111,720,074-112,320,297)x1 dn.	de novo	1) Chromosomal karyotyping confirmed by FISH 2) GeneChip Human Mapping 250K Nsp Array (Affymetrix)	Toral-López, J., Buentello-Volante, B., Balderas-Minor, M. M., Amezcua-Herrera, C., Valdes-Miranda, J. M., González-Huerta, L. M., Gudiño, M., Cuevas-Covarrubias, S. A., & Zenteno, J. C. (2012). An intellectually disabled patient with the 5q14.3q15 microdeletion syndrome associated with an apparently de novo t(2;5)(q13;q14). American Journal of Medical Genetics. Part A, 158A(4), 942–946. https://doi.org/10.1002/ajmg.a.35262
11	clinical assessment	~412 kb deletion at 5q14.3 with breakpoints at genomic positions 88,205,506 and 88,618,256 bp, which encompasses the promoter region and the first three exons of the MEF2C gene chr5:88205506-88618256 (hg18)	de novo	High-resolution whole-genome Array CGH using 4x44k and/or 2x105k Agilent oligo-arrays. Confirmed by FISH	Mikhail, F. M., Lose, E. J., Robin, N. H., Descartes, M. D., Rutledge, K. D., Rutledge, S. L., Korf, B. R., & Carroll, A. J. (2011). Clinically relevant single gene or intragenic deletions encompassing critical neurodevelopmental genes in patients with developmental delay, mental retardation, and/or autism spectrum disorders. American Journal of Medical Genetics. Part A, 155A(10), 2386–2396. https://doi.org/10.1002/ajmg.a.34177
12	clinical assessment	balanced translocation, t(5;15)(q13.3;q26.1) Upstream of MEF2C	de novo	Cytogenetics Whole-Genome 2.7M Array (Affymetrix). Breakpoint analyzed by Southern then Gel extraction. Breakpoint junction amplified and Sanger sequenced. Also FISH.	Saitsu, H., Igarashi, N., Kato, M., Okada, I., Kosho, T., Shimokawa, O., Sasaki, Y., Nishiyama, K., Tsurusaki, Y., Doi, H., Miyake, N., Harada, N., Hayasaka, K., & Matasumoto, N. (2011). De novo 5q14.3 translocation 121.5-kb upstream of MEF2C in a patient with severe intellectual disability and early-onset epileptic encephalopathy. American Journal of Medical Genetics. Part A, 155A(11), 2879–2884. https://doi.org/10.1002/ajmg.a.34289
13	clinical assessment	loss of 3.4-Mb indicating arr 5q14.3(83,468,682–86,939,957 x 1, including 6 genes EDIL3, NBPF22F, COX7C, FLJ11292, RASA1, CCNH. ish del(5)(q14.3q14.3)(RP11-94J21p,RP11-111M24 ,RP11-117A24p). chr5:83468682-86939957 (hg18) Downstream of MEF2C	de novo	Agilent 44K oligonucleotide microarray aCGH. Confirmed by FISH	Shimojima, K., Okumura, A., Mori, H., Abe, S., Ikeno, M., Shimizu, T., & Yamamoto, T. (2012). De novo microdeletion of 5q14.3 excluding MEF2C in a patient with infantile spasms, microcephaly, and agenesis of the corpus callosum. American Journal of Medical Genetics. Part A, 158A(9), 2272–2276. https://doi.org/10.1002/ajmg.a.35490

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
14	9	case report	Yauy et al.	2019	France	human	1	F	9yr	global DD since 2yr, sat at 10mo and learned to walk at 22mo, ADHD at 9 yrs, no autistic or stereotypic features, had febrile seizures, dysmorphic (spread eyebrows, protruding ears with simplified helices and abnormal dermatoglypcis), bilateral fifth finger clinodactyly, normal MRI and EED
15								F	10yr 7mo	global DD, absent speech, gross motor delay, sat independenlty at 18mo, not able to walk, myclonic epilepsy starting less than 1 yr old, sterotypic movements including hand flapping, does have purposeful hand movement, ceiling gazing, bruxism, hand mouthing, head nodding, makes eye contact, has sleeping problems, obsessive, specific eating pattern, likes running water, plays along with simple activies (not very social with others), mild myopia episodic breathing abnormalities starting at 2wk, hypotonia progressed to spasticity by age 7yr, reduced reflexes, abnormal MRI (thick corpus callosum), dysmorphic (broad forehead, down turned corners of mouth, prominent philtral pillars, short columella, depressed nasal bridge, epicanthic folds, hypertelorism, large mouth/lips), recurrent infections, severe GERD, constipation, abnormal EEG (high amplitude spike and slow wave complexes bilaterally with slight right sided predominance), pigmentation (pale blue eyes, hemangiomas > RASA1), cold hands and feet, floppy larynx
16								F	6yr 6mo	global DD, absent speech, gross motor delay, sat independently at 12-14mo, walked independently at 2yr, generalized tonic-clonic seizures started as febrile in infancy, stereotypic movements, has some purposeful hand use, bruxism, poor eye contact, no sleeping issues, autistic traits, likes music, light, and water, plays alone, tolerates hugs, registered blind, hypotonia, hypermobility, abnormal MRI (frontal corical atrophy and moderate ventriculomagaly), dysmorphic features (broad forehead, prominent philtral pillars, short columella, tented upper lip), recurrent infections, Patent ductus arteriosus (PDA) closed with a coil, PFO (persistent foramen ovale) (cardiac issues could be due to mother taking valproate during pregnancy), pigmentation (hemangiomas > large capillary nevus of the lower limb but no RASA1 del, pale blue eyes), duplex left kidney, broad based and unstable gait association of MEF2C exon 1 to 3 deletion with vascular malformations and a newgenotype–phenotype correlation (also seen in patient by Tanteles)

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
14	clinical assessment	balanced reciprocal translocation 46,XX,t(3;5)(p26.3;q14.3)dn. Breakpoints are chr3:920,589 and chr5:88,347,198 with the presence of a micro-homology of 3 nucleotides (TGC). Upstream of MEF2C	de novo	Chromosomes confirmed by FISH. Then microarray normal. Did array painting and LR PCR to map the breakpoints.	Yauy, K., Schneider, A., Ng, B. L., Gaillard, J.-B., Sati, S., Coubes, C., Wells, C., Tournaire, M., Guignard, T., Bouret, P., Geneviève, D., Puechberty, J., Pellestor, F., & Gatinois, V. (2019). Disruption of chromatin organisation causes MEF2C gene overexpression in intellectual disability: A case report. BMC Medical Genomics, 12(1), 116. https://doi.org/10.1186/s12920-019-0558-8
15	clinical assessment	het del Chr5:85,748,110 - 91,307,813 including MEF2C, RASA1, ARRDC3, CCNH, CETN3, COX7C, GPR98 chr5:85748110-91307813 (hg18)	de novo	OGT (Oxford Gene Technology) chromosomal microarray	
16	clinical assessment	het del Chr5:88,098,253-88,592,348 including MEF2C exons 1–3 chr5:88098253-88592348 (hg18)	de novo	OGT (Oxford Gene Technology) chromosomal microarray	

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
17	10	Review	Vrecar et al	2017	UK	human	6	F	9yr	global DD, no words by age 40mo, few words by age 9yrs, gross motor delay, sat independently at 12mo, walked independently at 3yr 6mo, generalized seizures started as febrile at less than 1yr of age, stereotypic movements, hand flapping, clasping in midline, screw paper up, has some purposeful hand use, bruxism, hand mouthing, tongue thrusting, makes eye contact with people she knows but won't look at strangers, has sleeping problems, generally happy, laughing, short attention spand, overfills mouth when eating, loves water, enjoys being around children, smiles at people she knows, doesn't like being touched, episodic breathing abnormalities starting at age 3yr, hypotonia, abnormal MRI (small corpus callosum, possible white matter abnormality in occipital lobes), abnormal EEG (dysrythmic background with high voltage poly spike wave bursts- centrencephalic neuronal hyperexcitability), scaphocephaly, dysmorphic features (broad forehead, down turned corners of the mouth, prominent philtral pillars, short columella, tented upper lip, depressed nasal bridge, large mouth/lips), feeding difficulties, pigmentaiton (pale blue eyes), drooling, poor coordination, wide-based gait
18								M	2yr 6mo	global DD, absent speech, gross motor delay, sat independently at 10-11mo, walked independently at 2yr 6mo, generalized seizures and absences, started as febrile at 15mo, stereotypic movements, has some purposeful hand use, hand mouthing, sleeping issues, overfills mouth when self-feeding, responsive to familiar adults, possible autism, hypotonia, brisk reflexes, abnormal MRI (small splenium of corpus callosum, mild ventriculomegaly), dysmorphic (broad forehead, prominent philtral pillars, short columella, tented upper lip, depressed nasal bridge, large mouth/lips), severe GERD, constipation as a baby
19								F	3yr 1 mo	global DD, absent speech, gross motor delay, sat independently at 12mo, not able to walk, febrile and afebrile seizures stared at less than 1yr of age, stereotypic movements but not repetitive hand movements, has some purposeful hand use, bruxism, rocks in her chair, transient eye contact, sleeping issues, happy, loves human contact and interaction, mild myopia, hypotonia, hypermobility, normal MRI, abnormal EEG (bilateral temporal slow waves and bilateral parietal spike waves), mild posterior plagiocephaly, dysmorphic features (broad forehead, Down turned corners of the mouth, Prominent philtral pillars, Short columella, slightly tented upper lip, Depressed nasal bridge), recurrent infections, severe GERD, constipation, pigmentation = pale blue eyes, cleft palate
20								F	3yr	global DD, good understanding and used 15 words by age 3yrs, gross motor delay, walked independently at 2yr 2mo, not able to sit independently, no seizures, stereotypic movements, hand wringing, has some purposeful hand use, hand wringing, excitable personality, hypotonia, hypermobility, dysmorphic features (broad forehead, Prominent philtral pillars, Short columella, Epicanthic folds, large mouth/lips), feeding difficulties, drooling, ataxic, walking with support at age 3yr

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
17	clinical assessment	het del Chr5: 88,034,622-88,164,453 including MEF2C exons 2-10 chr5:88034622-88164453 (hg18)	de novo	SNP6.0 array	Vrečar, I., Innes, J., Jones, E. A., Kingston, H., Reardon, W., Kerr, B., Clayton-Smith, J., & Douzgou, S. (2017). Further Clinical Delineation of the MEF2C Haploinsufficiency Syndrome: Report on New Cases and Literature Review of Severe Neurodevelopmental Disorders Presenting with Seizures, Absent Speech, and Involuntary Movements. Journal of Pediatric Genetics, 6(3), 129–141. https://doi.org/10.1055/s-0037-1601335
18	clinical assessment	het del Chr5:88,193,289-88,450,318 including MEF2C exon 1 chr5:88193289-88450318 (hg18)	de novo	OGT (Oxford Gene Technology) chromosomal microarray	
19	clinical assessment	c.220G>T; p.Glu74Ter (heterozygous)	de novo	NGS on severe infantile epilepsy gene panel	
20	clinical assessment	het del MEF2C exons 1-2 chr5:88136171-88155361 (hg18)	de novo	MLPA	

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
21	11	case report	Marashly et al.	2010	Louisiana, US	human	1	M	14mo	bilateral esotropia, motor and language milestones delayed, seizures began at 6mo, hypotelorism, slighlty upslanted palpebral fissures, long lashes, exaggerated bow on the upper lip, short upturned nose, ear lobes uplifted, EEG (paroxysms of high voltage spike polyspike and slow wave discarges diffusely with multifocal spike and polyspike discahrages most prominent in the posterior quadrants, hypsarrythmia), MRI normal
22	12	case report	Yang et al.	2014	Changsha, China	human	1	M	(deceased at) 5mo	dysmorphic facies (narrow prominent forehead, mildly upslanting palpebral fissures, widely spaced eyes, depressed nasal bridge with anteverted nares, long philtrum with deep groove, prominent cupid bow of the upper lip vermilion, hypotonic mouth, micrognathia, cavate auricular lobule), febrile seizures and 30 days old, fever for 15 days, frequent crying, disturbed sleep, poor eye contact, abnormal MRI (agensis of corpus callosum, cyst of pellucid septal cave), adenoidal hypertrophy, grand mal seizures, death from respiratory failure > SUDEP (6 hr after last seizure)
23	13	case report	Tonk et al.	2011	Texas, US	human	1	F	18yr	milestones at the upper range of normal (sitting at 7 months, walking at 14 months, single words at 10–12 months, 3-word phrases at age 4 years), early hypotonia, dysmorphic features (narrowing at the temples, lateral extension of the superior ear helices, U-shaped upper lip vermilion), febrile seizures, myoclonic epilepsy, progressed in regular classes with some special ed and tutoring IQ of 69 slightly lower scores in language, EEG showed focal activity after grand mal seizure, MRI normal, no sleep gastrointestinal or skin problems
24	14	case report	Al-Shehhi et al.	2016	Ireland	human	1	M	22mo	thalamic vein abnormality by antenatal ultrasound, feeding difficulties, hypotonic, delayed, abnormal MRI (large left thalmostriate vein, absence of posterior aspect and adjacent body of corpus callosum), seizures started at 15mo, EEG (multifocal bisynchronous high voltage bitemporal spike waves), seizures evolved to bilateral refractory myoclonic jerks, not able to sit at 22mo, didn't fix with his eyes, never babbled at 22mo, had stereotypic hand movemnts, axial and peripheral hypotonia, dysmorphic (prominent forehead, open mouth appearnace), jugular pit in his suprasternal notch
25	15	case report	Berland & Houge	2010	Norway	human	1	F	11yr	lack of eye contact at 6wk, strabismus, intermittent nystagmus, mild hypotonia at 4mo, febrile tonic-clonic seizures between 1-7yrs, atypical seizures with myoclonic jerks, EEG (generlized epileptiform pattern), psychomotor developmental delay, sat at age 3yr, crawled and walked with support at 4yr, walk unaided at 11yr, no verbal language, mimics sounds, makes use of body language, receptive language better than expressive, can follow instructions, dysmoprhic (long upslanted palpebral fissures, everted lower lids, wide forehead, mild brachycephaly, short and wide philtrum with an everted upper lip, short and broad chin), mild clinodactyly and short and narrow feet, poor eye contact, stereotypic movements, jugular fossa pit, happy and joyful, no panic attacks but easily scared of loud sounds, autistic features, fascinated by water and bright objects at younger age, had typical hand washing sterotypies when young and now flipping stereotypies (flipping corners of a page or carpet), puberty occured early, jugular pit

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
21	clinical assessment	arr cgh 5q14.3(85114182-88569430)x1 chr5:85114182-88569430 (hg18)	mother WT; father unknown	chromsomal microarray	Marashly, A., Riel-Romero, R. M. S., Ursin, S., & Ghawi, H. (2010). Infantile spasms associated with 5q14.3 deletion. The Journal of the Louisiana State Medical Society: Official Organ of the Louisiana State Medical Society, 162(4), 223–226.
22	clinical assessment	21.02Mb deletion in the 5q14.3q21.3 band region (88,047,621–109,072,596) The co-disruption of MEF2C and EFNA5 is hypothesized to lead to a severe form of neurological malformation and SUDEP in the male described in this study. chr5:88047621-109072596 (hg18)	de novo (paternal allele was the mutated one)	First chromosomes showed 46,XY,del (5)(q14?) dn. Then, Agilent's 4x180 K commercial arrays that contain 60-mer oligonucleotide probes	Yang, Y., Yao, X., Guo, J., Zhao, R., He, X., Zhao, L., Tu, M., & Zhu, Y. (2015). Interstitial deletion 5q14.3q21.3 associated with lethal epilepsy. American Journal of Medical Genetics. Part A, 167A(4), 866–871. https://doi.org/10.1002/ajmg.a.36991
23	clinical assessment	del 5(q14.3q21.3) by karyotype, minimal DNA deletion of 21.08Mb (arr chr5:83,592,798-104,671,993 X1) encompassed at least 50 genes including MEF2C chr5:83592798-104671993 (hg18)	unknown	chromsomes, then array CGH	Tonk, V., Kyhm, J. H., Gibson, C. E., & Wilson, G. N. (2011). Interstitial deletion 5q14.3q21.3 with MEF2C haploinsufficiency and mild phenotype: When more is less. American Journal of Medical Genetics. Part A, 155A(6), 1437–1441. https://doi.org/10.1002/ajmg.a.34012
24	clinical assessment	del 3.1 Mb and included MEF2C as well as RASA1 chr5:86335756-89235756 (hg18)	unknown	array CGH 8x60K	Al-Shehhi, M., Betts, D., Mc Ardle, L., Donoghue, V., & Reardon, W. (2016). Jugular pit associated with 5q14.3 deletion incorporating the MEF2C locus: A recurrent clinical finding. Clinical Dysmorphology, 25(1), 23–26. https://doi.org/10.1097/MCD.0000000000000102
25	clinical assessment	1.15Mbdeletion, karyotype was 46,XX.arr 5q14.3(87449860-88600147)x1 two genes deleted, MEF2C and TMEM161B chr5:87449860-88600147 (hg18)	de novo	Affymetrix Genome-Wide Human SNP Array 6.0	Berland, S., & Houge, G. (2010). Late-onset gain of skills and peculiar jugular pit in an 11-year-old girl with 5q14.3 microdeletion including MEF2C. Clinical Dysmorphology, 19(4), 222–224. https://doi.org/10.1097/MCD.0b013e32833dc589

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
26	16	case report	Novara et al.	2013	Italy	human	2	F	6yr	poor sucking in neonate period, delayed milestones, impairment of language an dlocomotor performances, anxiety, maxillofacial asymmetry due to ocular dimension difference and eyes frontally misaligned, febrile seizure at 2yr, motor clumsiness, mild language disorder, severe hypermetropia, relative microcephaly, abnormal MRI (mild enlargement of lateral ventricles with mild asymmetry), EEG not diagnostic but marked by high number of rapid rhythmic components.
27								F	1yr	Poor sucking and fialure to thrive, delayed milestones, generalized hypotonia, microcephaly with metopic prominence and dysmorphic features (wide and flat nasal root, smooth filtrum, microretrognathia, clinodactyly of fourth and fifth toes), MRI and EEG normal, patent foramen ovale, persistent aseptic fever at 1yr,
28	17	case report	Le Meur et al.	2010	France	human	7	F	4yr 9mo latest mention	ID, single episode of cyanosis with eye revulsion at 3 days of age, frequenct crying, sleep disturbance, hypotonia, poor visual contact at 3mo, from 4mo had myoclonic jerks of upper limbs then brief episodes of eye revulsion concomitant with jerks, epilepsy at 7mo characerized by several bilateral isolated spasms and frequent synchronous myoclonus with abnormal and slow background EEG pattern, severe DD, sat unaided and able to crawl, transient eye contact, speech absent, stereotypic hand movements, rocking her head and rubbing her chin with hands
29								F	9mo latest mention	ID, tonico-clonic seizures since day 1 of life, EEG (frequent bursts with no basic rhythm and very unstructured pattern), severe hypotonia, poor eye contact, awakening stages short
30								M	18mo latest mention	ID, abnormal foetal cardiac rhythm, head circumference small at birth, severe hypotonia, absent eye contact, cortical blindness, EEG (slow basic rhythm with infraclinical temporoparietal paroxystic discharges), insufficient weight gain by 18mo, gastrostomy tube, hypotonia,
31								M	3yr	ID, failure to thrive, severe hypotonia led to several neurological investigations by 4mo, eye contact difficult to obtain, no seizures, EEG normal, sat unaided at 18mo, crawled at 2yr, could stand and cruise along furniture and manipulate tyoues at 3yrs, absent speech, eye contact transient, repetitive hand flapping and clapping
32								F	7yr latest mention	ID, growth paramtere -2SD at birth, hypotonia, DD, tonico-clonic febrile seizures at 3 yrs, unable to walk and no language at 7yrs, repetitive hand washing and hand-to-mouth movement,
33								M	6yr latest mention	ID, mild global DD, able to walk unaided since 2 yrs, MRI and EEG normal, microcephaly at 6yrs, ID (IQ between 50-60), speech delayed but understandable, not able to pronounce short sentences. Normal eye contact, behavior and social skills. Special education required.
34								F	7yr latest mention	ID, difficulties in breastfeeding, regressed after age 5mo and lost previously acquired skills, unable to use her hands purposefully, failed to acquire vocalization with intonation, walked unaided at 3yr, behavioral disorders, decreased eye contact, lack of emotional reciprocity, lack of interest in her surroundings, hand and hand-mouth stereotypic movments, feeding difficulites and 5mo and onward, generalized tonico-clonic seizures at 9mo, mentally impaired poor eye contact and no speech at 7yrs, unstable wide-based gait

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
26	clinical assessment	duplication of 5.5 Mb [arr 5q14.3(85,598,295-91,182,469)x3] 14 genes included MEF2C chr5:85634051-91218225 (hg18)	de novo	Array-CGH analysis was performed using the Agilent array 180K. Also did qPCR, genotyping, cDNA expression analysis, and FISH afterwards.	Novara, F., Rizzo, A., Bedini, G., Girgenti, V., Esposito, S., Pantaleoni, C., Ciccone, R., Sciacca, F. L., Achille, V., Della Mina, E., Gana, S., Zuffardi, O., & Estienne, M. (2013). MEF2C deletions and mutations versus duplications: A clinical comparison. European Journal of Medical Genetics, 56(5), 260–265. https://doi.org/10.1016/j.ejmg.2013.01.011
27	clinical assessment	duplication of 5.2 Mb [arr 5q14.3(87,356,360-92,591,506)x3] 10 genes including MEF2C and RASA1 chr5:87392116-92617262 (hg18)	de novo		
28	clinical assessment	del 2.68 Mb (full MEF2C gene) chr5:86939816-89709694 (hg18)	de novo	Karyotype first. Then array CGH (Human Genome CGH microarray 44B kit Agilent or 244B kit Agilent). Confirmed by: FISH (case 1, 2, 3, 5, 6), QMPSF quantitative multiplex PCR of short fluroescent fragments (case 1), MP-LC multiplex PCR/Liquid chromatography (case 5 and 6), qPCR (case 4) sequencing (case 7)	Le Meur, N., Holder-Espinasse, M., Jaillard, S., Goldenberg, A., Joriot, S., Amati-Bonneau, P., Guichet, A., Barth, M., Charollais, A., Journel, H., Auvin, S., Boucher, C., Kerckaert, J.-P., David, V., Manouvrier-Hanu, S., Saugier-Veber, P., Frébourg, T., Dubourg, C., Andrieux, J., & Bonneau, D. (2010). MEF2C haploinsufficiency caused by either microdeletion of the 5q14.3 region or mutation is responsible for severe mental retardation with stereotypic movements, epilepsy and/or cerebral malformations. Journal of Medical Genetics, 47(1), 22–29. https://doi.org/10.1136/jmg.2009.069732
29		del 3.5 Mb, (full MEF2C gene) chr5:87770283-91730827 (hg18)	de novo		
30		del 8.8 Mb, (full MEF2C gene) chr5:86142271-95494937 (hg18)	de novo		
31		del 1.57 Mb, (del MEF2C exon 1) chr5:88221326-89966438 (hg18)	de novo		
32		del 216 Kb, (full MEF2C gene) chr5:87770283-88629033 (hg18)	de novo		
33		5q14 duplication which size was estimated to 4.6 Mb chr5:85951601-90731163 (hg18)	de novo		
34		NM_002397.2:c.683C>G; p.Ser228X	de novo		

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
35								M	13yr	IS09-024 Dystonia, stereotypies (hand flapping), nonambulatory, ISS/6 months, GERD, Averbal, no meaningful communication, Generally happy, with inappropriate laughter, High pain tolerance, poor eye contact, normal sleep, global DD, EEG (spike-wave associated with epileptic spasm)
36								F	11mo	LR11-305 Dystonia, Myoclonic/11 months, Feeding difficulties in infancy, babbles, no visual fixation, global DD,
37								M	5yr 3 mo	LR11-306 Stereotypies (hand flapping, chin rubbing), nonambulatory, epilepsy Type unknown/onset after 1 year, Averbal, no meaningful communication, Diminished responses with others, normal sleep,
38								F	13yr	LR11-307 Stereotypies (hand-flapping, rocking) abnormal gait with pes planus and valgus deformity, Myoclonic at 4 months/ISS by 9 months, Hyperventilation/hypoventilation, constipation, Averbal, no meaningful communication, Easily agitated, with self-mutilating behaviors, High pain tolerance, Poor attention, inconsistent eye contact, no pointing, Disrupted sleep, global DD, MRI (dysmorphic corpus callosum and mild cerebellar vermis hypoplasia)
39								M	46yr	LR11-308 hypokinetic spasticity, nonambulatory, No epilepsy, Averbal, no meaningful communication, Generally happy, Poor eye contact,
40								F	17mo	LR11-309 Hyperkinesis, bruxism, does not roll or lift head, Myoclonic and generalized/13 months, Averbal vocalizations only, no meaningful communication, Poor visual tracking, global DD, slight tenting of upper lip
41								F	22mo	LR11-310 Stereotypies (hand-wringing), began walking at 22 months, Myoclonic and atonic/18 months, global DD, MRI (mild thinning of the cortical white matter of T2 axial), EEG (multifocal epileptiform activity and poorly developed anterior-posterior gradient)
42								F	5yr 5mo	LR11-312 Hyperkinesis, stereotypies (rocking, side-to-side head movements), Myoclonic and ISS/3 months, Slow gastric emptying, GERD, constipation, Averbal, no meaningful communication, Generally happy, Poor visual awareness, limited engagement, Irregular sleep initiation and maintenance, global DD, MRI (frontal bossing an dbrachycephaly, mild cortical atrophy and thinning of the white matter
43								M	6mo	LR11-325 Hyperkinesis, ISS/4 months, global DD, normal brain MRI, tenting of upper lip
44	18	case report and review	Paciorkowski et al.	2013	NY, US	human	16	M	6yr	LR11-387 Hyperkinesis, dystonia, can take steps with gait trainer, No epilepsy, febrile seizures, Severe GERD and dysphagia, constipation Averbal, no meaningful communication, Generally happy, occasional inappropriate laughter, High pain tolerance, Poor visual tracking, Occasionally irregular sleep maintenance, global DD,
45								F	5yr 6mo	LR11-388 Hyperkinesis, stereotypies (hand flapping, head shaking), Myoclonic/2 years, Mild GERD and dysphagia, constipation, Averbal, no meaningful communication, Generally happy, occasional inappropriate laughter, High pain tolerance, Poor visual tracking, does not appear to distinguish individuals, normal sleep, global DD,
46								M	7yr	LR12-013 Hyperkinesis, stereotypies (arm flapping, bruxism), No epilepsy, febrile seizures, Breath-holding behavior Severe GERD in infancy, Averbal, no meaningful communication, Generally happy, with inappropriate laughter, High pain tolerance, Poor eye contact in early childhood, but improving, no reciprocal play, Normal sleep, global DD,

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
35		del 3.6Mb chr5:85855118-89474751 (hg18)		Affymetrix SNP array	
36		del 5.11Mb chr5:85684257-90798560 (hg18)		on a custom 105K-feature whole genomic microarray	
37		del 1.0Mb chr5:88018766-89063989 (hg18)		on a custom 105K-feature whole genomic microarray (Agilent)	
38		del 1.38Mb chr5:87905325-89289023 (hg18)		on a custom 105K-feature whole genomic microarray (Agilent)	
39		del 3.02Mb chr5:87591751-90619421 (hg18)		on a custom 105K-feature whole genomic microarray	
40		del 0.32Mb chr5:87905325-88220403 (hg18)		on a custom 105K-feature whole genomic microarray (Agilent)	
41		c833delT;Leu278Ter		not said	
42		del 1.95Mb chr5:87566009-89505509 (hg18)		microarray Illumina BeadChip 6.0, confirmed by FISH	
43		del 6.0Mb chr5:87719139-93736389 (hg18)		Affymetrix Whole-Genome 2.7M SNP array	
44	clinical assessment	del 11.6Mb chr5:81657245-93240731 (hg18)	unknown	Illumina HumanQuad610 BeadChip SNP array, confirmed by FISH	Paciorkowski, A. R., Traylor, R. N., Rosenfeld, J. A., Hoover, J. M., Harris, C. J., Winter, S., Lacassie, Y., Bialer, M., Lamb, A. N., Schultz, R. A., Berry-Kravis, E., Porter, B. E., Falk, M., Venkat, A., Vanzo, R. J., Cohen, J. S., Fatemi, A., Dobyns, W. B., Shaffer, L. G., ... Marsh, E. D. (2013). MEF2C Haploinsufficiency features consistent hyperkinesis, variable epilepsy, and has a role in dorsal and ventral neuronal developmental pathways. Neurogenetics, 14(2), 99–111. https://doi.org/10.1007/s10048-013-0356-y
45		del 5.4Mb chr5:88185348-93546896 (hg18)		GeneDx "GenomeDx" v1.0 oligo array, confirmed by qPCR	
46		del 0.41Mb chr5:88177038-88592311 (hg18)		44K oligo array, confirmed by FISH	

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
47								M	30mo	LR12-021 Repetitive back arching, stereotypies (hand batting, head shaking, bruxism), No epilepsy, Averbal, some babbling Generally happy, with inappropriate laughter, easily excitable, High pain tolerance, Inconsistent eye contact, no reciprocal play, sleep very disrupted in infancy now improving, global DD,
48								F	7yr	LR12-022 Hand tremor, stereotypies (hand flapping, waving hands in front of face, bruxism), Single generalized seizure, no epilepsy, Severe GERD, in infancy, Has 10 consistent words, some jargon, Generally happy, with some inappropriate laughter, High pain tolerance, Eye contact emerged at 3 years, some reciprocal interactions, Normal sleep, global DD,
49								M	6yr	LR12-031 Hyperkinesis, back arching, stereotypies (waving hands in front of eyes), Myoclonic/6 months, GERD in infancy, treated with medication and now resolved, Averbal, some vocalizations, Irritable until 2.5 years; now generally happy with Inappropriate nocturnal laughter, inappropriate pain response (laughs with vaccinations), Poor visual fixation and attention, avoided eye contact until age 3 years, Difficult sleep onset and maintenance, characteristic capillary malformation of the skin and atrophic skin adjacent to the suprasternal notch (RASA1 deleted too), global DD
50								M	21mo	LR12-275 Hyperkinesis, stereotypies (head shaking, leg kicking), No epilepsy, Some babbling, Generally happy, High pain tolerance, Poor eye contact, poor visual tracking, Difficulty with sleep onset, global
51	19	Review	Rocha et al.	2016	Portugal	human	1	M	10yr	psychomotor delay starting at 11mo, severe generalized hypotonia with absence of axial control, poor eye contact and lack of interest in surroundings (sounds, lights, faces), strabismus convergens of left eye, inability to manipulate objects, hand stereotypies, could follow faces and hold objects with mouthing behaviors at 20mo, hand to mouth including biting self, epileptic seizures at 26mo characterized by psychomotor arrest or sudden drops of head later with myoclonic seizures, EEG (abnormal and slow background patter, focal right frontal hemispheric epileptic discharges with frequent generaliztion), MRI (slight increase in periventricular white matter signal and global enlarged cerebrospinal fluid spaces including cortical sulcus), ID, not walking or talking, hyperkinesis, facial dysmorphic (broad forehead, strabsimus, large ears, flat nasal root, tented upper lip, everted lower lip, widely spaced teeth)
52	20	original article? Cohort study	Yuan et al.	2017	China	human	3	M	52yr	adult-onset dilated cardiomyopathy (DCM), intellectual disability with inability to speak, epilepsy and stereotypic movements (all of which when they were children) (proband)
53								M	49yr	adult-onset dilated cardiomyopathy (DCM), ventricular septal defect (VSD), intellectual disability with inability to speak, epilepsy and stereotypic movements (all of which when they were children) (brother)
54								F	26yr	adult-onset dilated cardiomyopathy (DCM), intellectual disability with inability to speak, epilepsy and stereotypic movements (all of which when they were children) (daughter)

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
47		del 0.05Mb chr5:88051970-88104535 (hg18)		244K oligo array, confirmed by FISH	
48		del 0.30Mb chr5:88167504-88472051 (hg18)		180K oligo array, confirmed by FISH	
49		del 5.2Mb chr5:84520000-89800000 (hg18)		custom Agilent oligo array with 40K features, confirmed by FISH	
50		del 2.0Mb chr5:86972414-88928741 (hg18)		44K oligo array, confirmed by FISH	
51	Neuropediatric outpatient clinic	MEF2C, c.9A>T; p.Arg3Ser (heterozygous)	de novo	NGS epileptic encephalopathies panel	Rocha, H., Sampaio, M., Rocha, R., Fernandes, S., & Leão, M. (2016). MEF2C haploinsufficiency syndrome: Report of a new MEF2C mutation and review. European Journal of Medical Genetics, 59(9), 478–482. https://doi.org/10.1016/j.ejmg.2016.05.017
52	clinical assessment	c.471C>G;p.Tyr157Ter (heterozygous)	likely paternally inherited but father deceased. Brother and daughter also have variant	Sanger sequencing	Yuan, F., Qiu, Z.-H., Wang, X.-H., Sun, Y.-M., Wang, J., Li, R.-G., Liu, H., Zhang, M., Shi, H.-Y., Zhao, L., Jiang, W.-F., Liu, X., Qiu, X.-B., Qu, X.-K., & Yang, Y.-Q. (2018). MEF2C loss-of-function mutation associated with familial dilated cardiomyopathy. Clinical Chemistry and Laboratory Medicine, 56(3), 502–511. https://doi.org/10.1515/cclm-2017-0461
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	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
55	21	original article? Cohort study	Qiao et al.	2017	China	human	4	M	1yr	patent ductus arteriosus (PDA), ventricular septal defect (VSD), and family history of CHD (proband)
56								M	26yr	stereotypic movements, ID, and paroxysmal epilepsy, patent ductus arteriosus (PDA), ventricular septal defect (VSD), Congenital heart disease (CHD)
57								M	32yr	stereotypic movements, ID, and paroxysmal epilepsy, patent ductus arteriosus (PDA), pulmonary stenosis, Congenital heart disease (CHD)
58								F	5yr	patent ductus arteriosus (PDA)
59	22	case report	Shim et al.	2015	South Korea	human	1	F	6yr 7mo	head control in erect position at 5mo, side-rolling at 7mo, mild DD early on, global DD at clinical assessment (including gross and fine motor, cognition, speech, social behavior), poor hand-eye coordination, paid little attention to stimuli including calling her name, couldn't recognize family members, no meaninful language but had some vocalization, not toilet trained, trunk hypotonia with mild cervicothoracic scoliosis, bilateral coxa valga, and pes planus, febrile convulsions with parital seizures confirmed by EEG, MRI (delayed myelination), bilateral esotropia (corrective surgery at 8mo), only
60	23	Review	Borlot et al.	2019	Canada	human	1	M	2yr	atypical febrile seizures with respiratory tract infection at 7mo consisting of focal motor seizures with unilateral but alternating left and right sided clonic activity, generalized myoclonias, and impaired awareness. Global DD, not able to sit or roll over, non-verbal (not able to imitate or babble), axial hypotonia, MRI (small areas of non-specific T2 white matter hyperintensity in parietal lobes), startles easily to loud noises or sudden sensory stimulation, EEG (7mo, high voltage gerneralized spike-and-waves ad polyspikes with alternating right and left frontal predominance. 12 and 15mo follow ups, independent bilateral multifocal spike-and-slow wave discharges, high voltage generalized polyspikes/spike-and-slow waves), low axial tone, able to sit and stand but cannot walk, no hand preference or pincer grasp, no
61	24	case report	Hotz et al.	2013	Germany	human	2	M	2yr	flattish profile, microcephaly, hydrocephalus and wide colon convolutions by ultrasound. dysmorphic features (broad and short forehead, a broad nasal bridge, upslanting palpebral fissures, and small eyes). severe psychomotor retardation, no head control, could not roll onto his side, could not sit independently, could not visually track or fix or grasp objects, muscular hypertonia, tendency towards opisthotonic body movements, sleep disturbances, EEG (nonspecific abnormalities), seizure first at 13mo, inverted mamillae, small sacral dermal appendage, cafe au lait spot on back. MRI (small forebrain with especially small frontal lobes, partial agenesis of corpus callosum, simplified gyral patteran and shallow sulci of anterior basal frontal lobes and anterior temporal lobes, bilateral enlargement of the posterior horns resulting in colpocephaly. Slim brainstem and enlarged cisterna magna, malrotated hippocampi)
62								M	4yr	Psychomotor retardation first at age 6–7 mo, myoclonic episodes of the arms with a fixed upwards stare starting at 10mo, truncal hypotonia, myoclonic epilepsy, strabismus divergens without dysmorphic features other than a broad forehead and downslanting palpebral fissures. EEG (highly pathological revealing myoclonic epilepsy), sat undaided at 18mo, able to crawl and stand, walked with aid, autistic behavior, no language skills, MRI (slightly reduced volume of the frontal lobes, with slightly broadened gyri and widened subarachnoid space around the anterior frontal lobes.)

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
55	Clinical Assessment	c.113T>C;p.Leu38Pro (heterozygous)	paternal	Sanger sequencing	Qiao, X.-H., Wang, F., Zhang, X.-L., Huang, R.-T., Xue, S., Wang, J., Qiu, X.-B., Liu, X.-Y., & Yang, Y.-Q. (2017). MEF2C loss-of-function mutation contributes to congenital heart defects. International Journal of Medical Sciences, 14(11), 1143–1153. https://doi.org/10.7150/ijms.21353
56			likely paternally inherited but father		
57			likely paternally inherited but father		
58			paternal		
59	Clinical Assessment	1332.682 kb in size, starting from 88031637 and ending at 89364319 46,XX.arr 5q14.3(88031637-89364319)x1 dn only gene deleted was MEF2C chr5:88031637-89364319 (hg18)	de novo	array comparative genomic hybridization (CGH)	Shim, J. S., Min, K., Lee, S. H., Park, J. E., Park, S. H., Kim, M., & Shim, S. H. (2015). MEF2C-Related 5q14.3 Microdeletion Syndrome Detected by Array CGH: A Case Report. Annals of Rehabilitation Medicine, 39(3), 482–487. https://doi.org/10.5535/arm.2015.39.3.482
60	Clinical Assessment	c.236G>C; p.Arg79Pro	de novo	WES	Borlot, F., Whitney, R., Cohn, R. D., & Weiss, S. K. (2019). MEF2C-related epilepsy: Delineating the phenotypic spectrum from a novel mutation and literature review. Seizure, 67, 86–90. https://doi.org/10.1016/j.seizure.2019.03.015
61	clinical assessment	4.1 Mb heterozygous deletion (87,646,931–91,754,042) included MEF2C and ARRDC3 chr5:87646931-91754042 (hg18)	de novo	ArrayCGH 105 K-Chip Agilent, confirmed by FISH, qPCR, and Sanger	Hotz, A., Hellenbroich, Y., Sperner, J., Linder-Lucht, M., Tacke, U., Walter, C., Caliebe, A., Nagel, I., Saunders, D. E., Wolff, G., Martin, P., & Morris-Rosendahl, D. J. (2013). Microdeletion 5q14.3 and anomalies of brain development. American Journal of Medical Genetics. Part A, 161A(9), 2124–2133. https://doi.org/10.1002/ajmg.a.36020
62	clinical assessment	1.7 Mb heterozygous deletion by BAC array 1.9Mb at minimum by qPCR included MEF2C and disrupted GPR98 chr5:87946507-89968372 (hg18)	de novo	BAC Array Cytochip v2 (BlueGnome), confirmed by FISH, qPCR, and Sanger	

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
63	25	cohort study	Boutry-Kryza et al.	2015	France	human	1 with MEF2C-related	F	4yr	spasms started at 4mo, Hypsarrhythmia, Autistic features, stereotypes, severe cognitive impairment
64	26	cohort with case reports	Zweier et al.	2010	Germany	human	9	F	2yr 2mo	severe MR, hypotonia, febrile seizures starting at 1yr, strabism, MRI anomalies (mildly enlarged extracerebral CSF space, two unspecific white matter lesions in the internal capsule/insular and the parietodorsal regions), not able to walk, no speech, dysmorphic features (large ears, broad forehead, prominent ear lobe, mild upslanting palpebral fissures, widely spaced teeth, tented upper lip),
65								F	3yr	severe MR, hypotonia, seizures first at 10mo, strabism, MRI anomalies (generalized lack of white matter bulk and delay in myelin maturation), not able to walk, no speech, dysmorphic features (large ears, broad forehead, prominent ear lobe, mild upslanting palpebral fissures, widely spaced teeth, cupid bowed upper lip), high glycine in
66								M	14yr	severe MR, autistic features, hypertonia, complex partial seizures starting at 10mo, MRI anomalies (mildly enlarged ventricles), walked at 2yr 8mo, no speech, dysmorphic features (large ears, broad forehead, prominent ear lobe, mild upslanting palpebral fissures, tented upper lip in infancy, now cupid bowed upper lip), hypermetropia, normal puberty, needs feeding, daytime continence
67								F	7yr	severe MR, hypotonia, seizures first started at 3-6mo, strabism, not able to walk, no speech, dysmorphic features (broad forehead, prominent ear lobe, widely spaced teeth, tented uppe rlip), heterochromasia, high pain tolerance, sleeping problemsn, joint
68								F	10yr 5mo	severe MR, hypotonia, seizures first started at 6mo, strabism, MRI anomalies (mild under myelinisation of insular cortices bilaterally), walked with support at 8yr, episodic hyperventilation, no speech, dysmorphic features (large ears, broad forehead, fleshy prominent ear lobe, downslanting palpebral fissures, crowded teeth, full upper lip), nails grow quickly, thick hair
69								M	3yr	severe MR, autistic features, hypotonia, spasms, myoclonic events starting at 3mo, stereotypic hand movements, strabism, MRI anomalies (mild under myelinisation), not able to walk, no speech, dysmorphic feature (large ears, broad forehead, prominent ear lob, slighly cupid bowed upper lip), nystagmus
70	27	case report	Sakai et al.	2013	Japan	human	1	M	14yr	severe ID, epileptic seizures, infantile spasms started at 3mo of age, severe DD, episodes of appetite loss, mild to moderate hypoglycemia, hypothermia, MRI (presence of ischemic lesions and structural anomalies of the hypothalamus), dysmoprhic features (broad forehead, hypertelomeric, down-slanted palpebral fissures, backward-positioned low-set ears, and upward-protruding, cupid-like lips.). Interesting neuroendocrine phenotype that hasn't been seen yet with these deletions. hormonal loading tests showed he had a central insufficiency in GH production against hypoglycemic conditions.

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
63	clinical assessment	arr5q14.3q15(890,687,77-923,160,85) x 1 3.2 Mb deletion 1 Mb upstream to the MEF2C gene chr5:89104533-92341841 (hg18)	unknown	array CGH	Boutry-Kryza, N., Labalme, A., Ville, D., de Bellescize, J., Touraine, R., Prieur, F., Dimassi, S., Poulat, A.-L., Till, M., Rossi, M., Bourel-Ponchel, E., Delignières, A., Le Moing, A.-G., Rivier, C., des Portes, V., Edery, P., Calender, A., Sanlaville, D., & Lesca, G. (2015). Molecular characterization of a cohort of 73 patients with infantile spasms syndrome. European Journal of Medical Genetics, 58(2), 51–58. https://doi.org/10.1016/j.ejmg.2014.11.007
64	clinical assessment	2.4Mb deletion including exons 5-11 of MEF2C chr5:85447085-88099696 (hg18)	de novo	high-resolution Genome-Wide Human SNP Array 6.0 (Affymetrix), MLPA	Zweier, M., Gregor, A., Zweier, C., Engels, H., Sticht, H., Wohlleber, E., Bijlsma, E. K., Holder, S. E., Zenker, M., Rossier, E., Grasshoff, U., Johnson, D. S., Robertson, L., Firth, H. V., Cornelia Kraus, Ekici, A. B., Reis, A., & Rauch, A. (2010). Mutations in MEF2C from the 5q14.3q15 microdeletion syndrome region are a frequent cause of severe mental retardation and diminish MECP2 and CDKL5 expression. Human Mutation, 31(6), 722–733. https://doi.org/10.1002/humu.21253
65		c.113T>A;p.Leu38Gln (heterozygous)	de novo	Sanger sequencing	
66		c.99dupT;p.Glu34X (heterozygous)	de novo		
67		c.226_236delCATGAGAGCCG; p.H76DfsTer15	de novo		
68		c.80G>C;p.Gly27Ala (heterozygous)	de novo		
69		g.(87,397,069_87,400,499)_(88,895,460_88,896,692)del 1.5Mb deletion including MEF2C and two other genes	mother WT; father unknown	high-resolution Genome-Wide Human SNP Array 6.0 (Affymetrix)	
70	clinical assessment	7.4Mb deletion chr5:82413149e898245489 including MEF2C and centromeric deviation of the deleted region also other genes deleted but no hypoglycemia or other neuroendocrine phenotypes were not described in those genes chr5:82448905-89860304 (hg18)	unknown	Microarray-comparative genome hybridization (CGH)	Sakai, Y., Ohkubo, K., Matsushita, Y., Akamine, S., Ishizaki, Y., Torisu, H., Ihara, K., Sanefuji, M., Kim, M.-S., Lee, K.-U., Shaw, C. A., Lim, J., Nakabeppu, Y., & Hara, T. (2013). Neuroendocrine phenotypes in a boy with 5q14 deletion syndrome implicate the regulatory roles of myocyte-specific enhancer factor 2C in the postnatal hypothalamus. European Journal of Medical Genetics, 56(9), 475–483. https://doi.org/10.1016/j.ejmg.2013.06.009

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
71	28	case report	Wang et al.	2018	China	human	5	F	5yr 9mo	hypotonia, psychomotor milestones delayed, raised head at 8mo, sat alone at 1yr, currently unable to walk unaided, poor eye contact, hand clapping and wringing, bruxism at 1yr, deterioration of hand skills, epileptic attack at 20mo, EEG (spike-slow waves at righ tmedial and posterior temporal, with generalization), MRI (enlargement of frontal subarachnoid space), hypalgesia
72								F	2.5yr	hypotonia, ID, profound psychomotor retardation, head control at 5mo, sat alone at 8mo, unable to walk independently, no speech, stereotypic hand movents, bruxism at 2yr, no seizure but had epileptic discharge on EEG, MRI (high T1 and T2 signal at posterior horn of bilateral ventricle), poor hand skills
73								F	23mo	hypotonia, feeding difficulties at 3mo, febrile conversions at 9mo but not epilepsy, significant milestone delay, could sit alone at 1yr, walk at 23mo with abnormal gait, unmeaningful language at 12mo, poor eye contact, stereotypic actions, breathing disturbances, sleep abnormalities, recurrent respiratory infections at 1yr 10mo, irritability, poor hand skills
74								M	7yr 8mo	only somewhat delayed motor developmental milestones, raising head at 1yr, sitting alone at 1yr 2mo, walking at 1.5yr, lack of speech with no single words, little interest in others, lacked eye contact, febrile seizures at 1yr, turned to afebrile at 2yr, partial seizures, EEG (multi spike and slow waves at right occipital region, with slow rhythm on the background), MRI normals at 3yr
75								M	6yr 4mo	DD, raising head at 7mo, sitting alone at 1yr, walking at 2yr, language delay could speak only a few words, autistic behavior, repitive hand movements, no eye contact, no interest in others, febrile seizures at 8mo, MRI (long T1 and T2 signal around bilateral ventricle and a septum pellucidum cyst), EEG normal at 4yr
76	29	Review with a case report	Tanteles et al.	2015	Cyprus	human	1	M	14yr	sister had mitral valve prolapse, jerking episodes involving his feet while sleep at 8mo but it resolved after a month, gross development delay, sat unaided at 8mo, walked independently at 2.5yr, no speech, severe DD, not potty trained, could walk but had wide gait, can't run, no seizures, stereotypic movements includind hand biting and hand falpping, head banging, scared of loud noises, left-sided Perthese disease, myopia, bilateral inguinal herniae which was repaired, normal sleep and breathing, enjoyed water, dysmorphic (broad forehead, cupid's bow upper lip), dupernumerary nipple, two small hyperpigmented and one hypopigmented macule on chest, swallow broad jugula rpit with overlying cutaneous capillary malformation, toes
77								M	7yr	coloboma of the left iris at birth, Generalized hypotonia and DD at 2yr, minor cranial and facial dysmorphic featuers (high forehead, hypertelorism, high arched eyebrows, mild downward slanting of the palpebral fissures, depressed nasal bridge, thick columella, and a flat long philtrum), left eye exotropia, walked independenlty at 5yr, hypotonic, no language skills, febrile seizures at 1yr, generalized tonic-clonic seizures at 6yr, EEG normal, MRI (bilateral Periventricular heterotopia involving temporal and frontal horns)

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
71	clinical assessment	c.48C>G;p.Asn16Lys	de novo	Targeted NGS panel	Wang, J., Zhang, Q., Chen, Y., Yu, S., Wu, X., Bao, X., & Wen, Y. (2018). Novel MEF2C point mutations in Chinese patients with Rett (-like) syndrome or non-syndromic intellectual disability: Insights into genotype-phenotype correlation. BMC Medical Genetics, 19(1), 191. https://doi.org/10.1186/s12881-018-0699-1
72		c.565C>T;p.Arg189Ter	mother WT; father unknown		
73		c.334G>T;p.Glu112Ter	father WT; mother unknown		
74		c.403-1G>T	de novo		
75		c.766C>T;p.Arg256Ter	de novo		
76	clinical assessment	minimal deletion size of 147kb maximal deletion size of 167kb MEF2C exon 1-3 deleted chr5:88155310-88302622 (hg18)	de novo	array CGH Cytochip ISCA array confirmed by qRT-PCR	Tanteles, G. A., Alexandrou, A., Evangelidou, P., Gavatha, M., Anastasiadou, V., & Sismani, C. (2015). Partial MEF2C deletion in a Cypriot patient with severe intellectual disability and a jugular fossa malformation: Review of the literature. American Journal of Medical Genetics. Part A, 167A(3), 664–669. https://doi.org/10.1002/ajmg.a.36945
77	clinical assessment	46, XY, der(5) del(5) (q14;q21) t(1;5) (q31;q14) karyotype breakpoint on 5q was 17Mb region with 88,945,075-134 bp being the first oligomer deleted, and 105,929,496-555 bp the first oligomer present Didn't include MEF2C, just upstream of it	de novo	chromsomes, then FISH and array comparative genomic hybridization (CGH)	

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
78	30	case report	Cardoso et al.	2009	Italy	human	3	F	5yr	pes talus at birth, infantile spasms at 9mo, EEG (poorly organized background activity and multifocal epilptiform discharges), epileptic spasms still present at 3yr, severe DD, no speech, minor dysmorphic (high forehead, frontal bossing, hypertelorism, anteverted nostrils, high arched eyebrows, depressed nasal bridge, thick columella, long philtrum, thin lips, and micrognathia), cognitive developmental impairment and no language still at 5yr, MRI (bilateral PH involving the temproal and occipital horns)
79								M	5yr	right postaxial polydactyly of his toes at birth, triangular shaped head, poor truncal tone with variable tone of the limbs, episodes of unresponsiveness lasting 10–20 seconds occurred many times a day from 8mo, isolated myoclonic jerks at 18mo, EEG (showed bursts of multifocal and bilaterally synchronous epileptiform activity), walked at 3yr, no speech and delayed at 5yr, macrocephaly, MRI (bilateral PH, involving the temporal and occipital horns. under rotated hippocampi, more severely on the right and irregular thickening and folding of the cortex in the posterior perisylvian regions, consistent with polymicrogyria)
80	31	case report	Cesaretti et al.	2016	Italy	human	2, monochorionic twins	unknown	20 weeks gestation	ultrasoud showed difference between the two fetuses in crown-rump length of 22%, low nuchal translucency in both twins, normal amniotic fluid and baldder filling, heart involvement (bi-ventricular hypertrophy and moderate tricuspid valve insufficiency), moderate bilateral ventricular valve insufficiency, short corpus callosum (10mm of anterior-posterior diameter), partial agensis of corpus callosum. Preggnacy was terminated. Autopsy found short and thin corpus callosum with rudimentary genu deteached from the corpus callosum
81								unknown		ultrasoud showed difference between the two fetuses in crown-rump length of 22%, low nuchal translucency in both twins, abdominal circumference was <5th centile, had oligohydramnios and small bladder, bilateral mild veentriculomegaly with width of posterior horns of 11mm. Short corpus callosum (13mm of anterior-posterior diameter), bilateral mild ventriculomegaly. partial agensis of corpus callosum. Preggnacy was terminated. Autopsy found short and thin corpus callosum with no detectable region of genu

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
78	clinical assessment	8.4 Mb with 87,086,298-357 bp being the first oligomer deleted, and 95,538,640-699 bp the first oligomer present included MEF2C chr5:87086298-95538640 (hg18)	de novo	array CGH	Cardoso, C., Boys, A., Parrini, E., Mignon-Ravix, C., McMahon, J. M., Khantane, S., Bertini, E., Pallesi, E., Missirian, C., Zuffardi, O., Novara, F., Villard, L., Giglio, S., Chabrol, B., Slater, H. R., Moncla, A., Scheffer, I. E., & Guerrini, R. (2009). Periventricular heterotopia, mental retardation, and epilepsy associated with 5q14.3-q15 deletion. Neurology, 72(9), 784–792. https://doi.org/10.1212/01.wnl.0000336339.08878.2d
79	clinical assessment	46,XY, del(5)(q14.2q15) 6.3 Mb between 88,641,401 bp and 94,876,462 bp Does not include MEF2C (gene is at 88048690-88220948 in the build they used) del is upstream of MEF2C chr5:88641401-94876462 (hg18)	de novo	chromosomes, then high density synthetic oligonucleotide array	
80	clinical assessement, ultrasound and autopsy	4.6Mb on the long arm of chromosome 5 [arr 5q14.3 (86,129,664-90,762,803)x3] including MEF2C, RASA1, and GPR98 chr5:86165420-90798559 (hg18)	de novo	Agilent Human Genome CGH Microarray ISCA 4 x180K + SNP format on amniocentesis	Cesaretti, C., Spaccini, L., Righini, A., Parazzini, C., Conte, G., Crosti, F., Redaelli, S., Bulfamante, G., Avagliano, L., & Rustico, M. (2016). Prenatal detection of 5q14.3 duplication including MEF2C and brain phenotype. American Journal of Medical Genetics. Part A, 170A(5), 1352–1357. https://doi.org/10.1002/ajmg.a.37594
81	clinical assessement, ultrasound and autopsy	4.6Mb on the long arm of chromosome 5 [arr 5q14.3 (86,129,664-90,762,803)x3] including MEF2C, RASA1, and GPR98 chr5:86165420-90798559 (hg18)	de novo		

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
82	32	case report	Novara et al.	2010	Italy	human	2	M	14yr	Mother and cousin have epilepsy, absent eye contact and social smile at 3mo, hypotonia and irritable behavior, MRI (cystic lesion and leucoencephalopathy in left frontal region, likely due to perinatal hemorrhage), psychomotor delay, sitting with little support at 2yr, MRI at 2 yr showed periventricular leucomalacia and atrophy of frontal cortex at left side, not able to walk, severe axial hypotonia, epilepsy in firs tyear of life, initially myoclonic jerks later evolving to infnatile spasms with continuous epileptic activity bi-posterior with no basic rhythm on EEG. absent speech, regression to need wheelchair, cerebral palsy with severe axial hypotonia and compensatory peripheral hypertonia, external strabismus, no stereotypic movements, mild dysmorphisms (prominent ear lobes, short prominent philtrum with a cupids bow and macrodontia)
83								M	3yr 10mo	lack of reactivity, severe hypontonia, dystonic motor activity, absent head control, ppor visual tracking at 4mo. Psychomotor delay and myoclonus at 5mo. EEG (slow background activity with theta waves degraded over the central regions of the two hemispheres and degraded diffuse discharges, sometimes with episodes of rhythmic sharp wave activity), occipital plagiocephaly, hypertelorism, flattened nasal bridge, small an dhook nose, ogival palate, low set and dysmorphic ears. myopia with alternating esotropia. MRI (moderate dilatatio of lateral ventricles and hypoplasia of the corups callosum with abnormal aspect of the splenius), cognitive deficity, behavioral stereotypies, axial hypotonia, increased muscle tone in lower limbs with dystonic-dyskinetic movements, could fix and follow objects, absent language.
84	33	short communicaiton / case report	Bienvenu et al.	2012	Portugal	human	1	F	8yr 2mo	ID, severe DD, no speech, dysmorphic features (large eyebrows, open mouth with thick everted lower lip, and anteverted nares), severe feeding difficulties due to marked hypotonia, poor eye contact, strabismus, delayed motor milestones (walked independently at 4 yr), unstable wide baised gait, single episode of myoclonic febrile seizures at 18mo, happy behavior, hand stereotypies, hand mouthing, EEG (low generalized spike and wave, sometimes massive myoclonies sometimes followed by spikes bifrontal slow waves), MRI normal
85								M	3yr	Periodic tremor and abnormal motor pattern with mirror movement of upper limbs in infancy. Severe psychomotor delay with absent speech, hypotonia, bruxism, epilepsy, and autistic behavior at 2yr. mild dysmorphic features (frontal bossing, mild bilateral epicanthus, a broad nose, and full lips, open mouth). MRI (mild thinning of the corpus callosum and delay of white matter myelination in the occipital lobes). EEG (abnormal sleep architecture and generalized discharges localized to the posterior regions.), Began to walk at 3yr but shaky, wide-based stance and gait
86								F	30mo	severe DD, She rolled over and could replace her pacifier into her mouth but could not sit unsupported, extremely hypotonic, hypoteloric, poor visual tracking, little social interaction, generalized seizures at 15 mo, now has episodes of startling, opisthotonic posturing, marked truncal hypotonia with more increased tone distally with scissoring, Reflexes are brisk and toes are downgoing. period of failure to thrive with no weight gain prompted G-tube placement, MRI (colpocephaly and an incidental pineal cyst, Ventricles were borderline large.)

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
82	clinical assessement	318357 bp (87,978,527–88,296,884) harboring only MEF2C chr5:88014283-88332640 (hg18)	mother WT; father unknown	Chromsomes and Agilent array 105 K array	Novara, F., Beri, S., Giorda, R., Ortibus, E., Nageshappa, S., Darra, F., Dalla Bernardina, B., Zuffardi, O., & Van Esch, H. (2010). Refining the phenotype associated with MEF2C haploinsufficiency. Clinical Genetics, 78(5), 471–477. https://doi.org/10.1111/j.1399-0004.2010.01413.x
83	clinical assessement	deletion of 1140131 bp (87,234,127–88,374,258). including MEF2C and TMEM161B. chr5:87269883-88410014 (hg18)	de novo		
84	clinical assessment	c.457delA; p.Asn153ThrfsTer33	de novo	Sanger sequencing	Bienvenu, T., Diebold, B., Chelly, J., & Isidor, B. (2013). Refining the phenotype associated with MEF2C point mutations. Neurogenetics, 14(1), 71–75. https://doi.org/10.1007/s10048-012-0344-7
85	clinical assessment	about 140 kb deletion encompassing the first three exons of MEF2C chr5:88104594-88252348 (hg18)	de novo	custom-designed 105 K oligonucleotide V7.4 array CGH	
86	clinical assessment	About 1,8Mb deletion was identified which encompassed two genes: TMEM161B and MEF2C chr5:87086357-88912534 (hg18)	de novo	custom-designed 105 K oligonucleotide V7.4 array CGH	

	A	B	C	D	E	F	G	H	I	J
1		**Study Type**	**Authors**	**Year Published**	**Locations Published**	**Verification of Human Case**	**Number of Patients**	**Patient Sex**	**Patient Age**	**Phenotype and Clinical Information Reported**
87	34	case report	Nowakowska et al.	2019	Poland	human	4	F	34mo	two bilateral tonic–clonic seizures with fever at 14mo, extensor myoclonus on awakening. EEG at 15 mo (multiple, generalized spike and poly-spike, and slow wave discharges), EEG at 19 mo (generalized semi-rhythmic bursts of polyspike and wave activity), failure to thrive, mild bilateral ptosis, a thin nose, asymmetric ears, a short philtrum, micrognathia, and a pectus excavatum, bilateral esotropia, truncal hypotonia, could roll over, could not sit unsupported, or crawl, would not bear weight on her legs and she showed scissoring of the legs when held, rigid posture, limited fine motor coordination, some vocalizations but no speech, G-tube, bruxism, constipation, heart murmur but normal echocardiogram, MRI (thinning of the corpus callosum, most prominent in the splenium, and mild global white matter loss, but no periventircular heterotopias)
88								F	18mo	DD and seizures, infantile seizures at 3-4mo, generalized tonic–clonic seizures once a month and infantile spasms weekly despite medicine, quick jerking movements, delay in milestones, babbles but no speech, significant head lag unless she's sitting supported, will not reach, sleeps well, no temperment problem, can roll one way, minor dysmorphic features (brachycephaly, a wide nasal bridge, down-turned corners of her mouth with a cupidbow upper lip), 10-25th percentile for weight, microcephaly, upper extremity and truncal hypotonia with increased tone in her lower extremities, MRI (microcephaly, a shorter than expected corpus callosum, prominent lateral, third, and fourth ventricles, slightly wide sylvian fissures, and small frontal lobes with a paucity of the cerebral gyri. Focal increased T2 signal was detected within the globus pallidi. The gray–white matter interface within the temporal lobes appeared ill defined, suggesting either delayed myelination or cortical dysplasia.), PET scanning (hypermetabolism in the right cerebellum with hypometabolism in the left hemisphere and was diffusely suggestive of a cortical dysplasia), EEG (frequent and spike and wave activity in the left temporal-occipital and left central temporal regions as well as spikes in the right occipital area.)
89	35	case report	Gordon et al.	2017	France	human	1	M	2.5yr	axial hypotonia with little spontaneous movements and severe motor milestone delay, sit unsupported but could not stand or walk unsupported. He grabbed objects with all fingers and could not eat unassisted, limited communication, poor eye contact, no words, unable to mimic or play symbolic games, fascinated by opening and closing doors, stereotypic hand movements with grasping at the midline and flapping, right question mark ear (QME), dysplastic left ear with normal ear canals and a normal oral cavity, hooked first toes, MRI normal, no seizures, global hypotonia with kyphosis at sitting position

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
87	clinical assessment	About 2.4Mb deletion chr5:87807115-90158137 (hg18)	de novo	V7.2 OLIGO microarray (Agilent)	Nowakowska, B. A., Obersztyn, E., Szymańska, K., Bekiesińska-Figatowska, M., Xia, Z., Ricks, C. B., Bocian, E., Stockton, D. W., Szczałuba, K., Nawara, M., Patel, A., Scott, D. A., Cheung, S. W., Bohan, T. P., & Stankiewicz, P. (2010). Severe mental retardation, seizures, and hypotonia due to deletions of MEF2C. American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics, 153B(5), 1042–1051. https://doi.org/10.1002/ajmg.b.31071
88	clinical assessment	About 5.7Mb deletion encompassing six genes: EDIL3, COX7C, RASA1, CCNH, TMEM161B, and MEF2C chr5:83139263-88799227 (hg18)	de novo	custom-designed 105 K oligonucleotide V7.4 array CGH	
89	clinical assessment	c.146dupA;p.Asn49LysfsTer29	unknown	NGS targeted panel, confrimed by Sanger	Gordon, C. T., Tessier, A., Demir, Z., Goldenberg, A., Oufadem, M., Voisin, N., Pingault, V., Bienvenu, T., Lyonnet, S., de Pontual, L., & Amiel, J. (2018). The association of severe encephalopathy and question mark ear is highly suggestive of loss of MEF2C function. Clinical Genetics, 93(2), 356–359. https://doi.org/10.1111/cge.13046

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
30	36	review with cases	Vidal et al.	2019	Spain	human	4	F	24yr	MRI normal, hypotonia, psychomotor delay, walked with help at 6yr, has a few words, profound ID, autistic features, epilepsy with no refractoriness that was controlled by anti-epileptic drugs, walk with support but unstable wide-based gait, Hand stereotypies, uses a few words
31								F	6yr	hypotonia, psychomotor delay, can walk with help, has a few words, profound ID, epilepsy with no refractoriness that was controlled by anti-epileptic drugs, independent walking Hand stereotypies, uses a few words
32								F	8yr	MRI normal, hypotonia, psychomotor delay, walked with help at 1y2mo, no speech, profound ID, autistic features, epilepsy with no refractoriness that was controlled by anti-epileptic drugs, walk with support but unstable wide-based gait, Hand stereotypies, no speech
33								F	18yr	hypotonia, psychomotor delay, walked aided at 3yr, no speech, profound ID, seizure free, independent walking, Hand stereotypies
34	37	cohort study	Schluth-Bolard et al.	2019	France	human	3	M	9yr	EBI/0401 ID, IUGR, post-natal growth retardation, microcephaly, sleeping and feedings difficulties, epilepsy (hyperthermic seizures), choreic and dystonic abnormal movements, stabismus
35								F	5yr	OLI/2202 Severe ID, absent speech, sterotypy, epileptic encephlopathy, constipation, myopia
36								F	11yr	MDI/2203 Severe ID (walking at 9 years old), absent speech, autistic spectrum disorder, epilepsy, facial dysmorphism, constipation
37	38	case study, short communication	Ohdo et al.	1982	Japan	human	1	F	7mo	DD, short neck, reduced weight gain, coarse and abundant hair, narrow forehead with hypertrichosis, flat occiput, hypertelorism, short nose with anteverted nostrils, a large philtrum with a deep groove, cleft palate, retromicrognathia, simply formed auricle on the right, imperforate anus with rectoperineal fistula, camptodactyly of the right third finger and left second finger, and bilateral pes adductus. single transverse flexion crease on her left palm with a transitional crease on her right palm.

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
30	clinical assessment	c.48C>G;p.Asn16Lys	de novo	NGS targeted panel	Vidal, S., Brandi, N., Pacheco, P., Maynou, J., Fernandez, G., Xiol, C., Pascual-Alonso, A., Pineda, M., Rett Working Group, & Armstrong, J. (2019). The most recurrent monogenic disorders that overlap with the phenotype of Rett syndrome. European Journal of Paediatric Neurology: EJPN: Official Journal of the European Paediatric Neurology Society, 23(4), 609–620. https://doi.org/10.1016/j.ejpn.2019.04.006
31		c.513_514insGA;p.Leu172AspfsTer16	de novo		
32		c.959_960delGT;p.Glu320AspfsTer7	de novo		
33		c.1421G>T;p.Ter473Leu	de novo		
34	clinical assessment	46,XY,ins(5)(q15q23.3q34) by array NGS revised: ins(5)(q14.2q23.2q34)	de novo	microarray 1st detected apparently balanced chromosomal rearrangements, then WGS	Schluth-Bolard, C., Diguet, F., Chatron, N., Rollat-Farnier, P.-A., Bardel, C., Afenjar, A., Amblard, F., Amiel, J., Blesson, S., Callier, P., Capri, Y., Collignon, P., Cordier, M.-P., Coubes, C., Demeer, B., Chaussenot, A., Demurger, F., Devillard, F., Doco-Fenzy, M., … Sanlaville, D. (2019). Whole genome paired-end sequencing elucidates functional and phenotypic consequences of balanced chromosomal rearrangement in patients with developmental disorders. Journal of Medical Genetics, 56(8), 526–535. https://doi.org/10.1136/jmedgenet-2018-105778
35		NGS revised: t(1;14)(q32.1;q21.3) insertion of chromosomal fragments of 153 and 736 kb from the 5q14.3 region to the breakpoint of derivative 1 and to the breakpoint of derivative 14, respectively. MEF2C put on chr1	de novo		
36		NGS revised: t(1;3;5)(p22.2;p24.3;q33.2)	de novo		
37	clinical assessment	46,XX,del(5)(q13q22) chr5:66465756-115078697 (hg18)	de novo	chromosomes	Ohdo S, Madokoro H, Hayakawa K. 1982. Interstitial deletion of the long arm of chromosome 5: 46,XX,del(5)(q13q22). J Med Genet 19:479.

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
98	39	case report	Stoll et al.	1980	France	human	1	M	6mo	DD, a small and narrow forehead, a small, broad, upturned nose, a flat nasal bridge, hypertelorism, upward curving eyelashes, a large prominent metopic suture, a triangular shaped mouth, a large philtrum with a deep groove, retromicrognathia, large ears, short neck, short upper limbs, syndactyly of the big toe and the 3rd and 4th toes, and clinodactyly of the 5th finger. 7 whorls and 3 ulnar loops on 2nd and 3rd finger on right hand adn 2nd finger on left. A cardiac murmur was also heard.
99	40	case report	Sobreira et al.	2009	MD, USA	human	1	M	11yr	intellectual disability, severe attention deficit hyperactivity disorder, aggressive and stereotyped behaviors, iris coloboma, short stature, high frequency hearing loss, dental anomaly, and dysmorphic facial features (down-slanting palpebral fissures, bilateral iris coloboma with small optic nerves, cup-shaped ears, misplacement of frontal/lateral incisors, brachydactyly of hands, bilateral clinodactyly of the fifth fingers, and small feet), MRI (mild delay in myelination but no structural anomaly)
100	41	case report	Floris et al.	2007	Italy	human	1	M	5.5yr	epilepsy, mother had complicated pregnancy (funiculuar knot in 3rd month, oligohydramnio since 5th month, fetal growth retardation since 32nd week), low birth weight, brain ultrasonography (calcifications in thalamus and nucleus dentatus bilaterally), delayed motor development, ID, severe speech delay, short attention span, autism, wide-based gait, right hemiparesis, stereotypic hand movements, no social interest, brain MRI (periventricular leukomalacia more prevalent in left cerebral hemisphere), EEG (multifocal, parossistic an dpolymorphic anomalies, especially in anterior cerebral area).
101	42	review	Ramji et al.	2020	UK	human	1	F	23mo	Pregnancy complicated by probable maternal hemolysis, Elevated Liver enzymes, and Low Platelet count syn-drome (HELPP). A squint, delayed visual and motor development were noted at 4 months of age. Generalized seizures at 9 months of age, macrocephaly and facial dysmorphism. MRI characteristic features of AED with bilateral symmetrical diffusion restriction in the cerebral white matter andstriking T2 hyperintensity in the juxtacortical U-fibres. Neurological decline.
102								M	17yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, neither febrile nor epileptic seizures, MRI Altered venous drainage in right cerebellar hemisphere and in the parieto-occipital regions, Cavum vergae, Cavum septi pellucidi, mild posterior CC thinning. EEG background diffuse excess of fast activity. Absent speech, autistic features.
103								M	12 yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, epileptic encephalopathy with epileptic spasms, myoclonic seizures, generalized bilateral tonic-clonic seizure (2mo), EEG Generalized polyspike wave complexes and bilateral asynchronous epileptiform discharges predominant in posterior/temporal regions during sleep, Rhythmic theta activity during wakefulness. MRI Hypoplastic CC, delayed myelination.

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
98	clinical assessment	46,XY,del(5)(q13q15) chr5:66465756-98117892 (hg18)	de novo	chromosomes	Stoll, C., Levy, J., & Roth, M. P. (1980). Interstitial deletion of the long arm of chromosome 5 in a deformed boy: 46,XY,del(5)(q13q15). Journal of Medical Genetics, 17(6), 486–487.
99	clinical assessment	7.4Mb deletion (90,787,099–98,232,469 bp) NOT including MEF2C, just upstream of it chr5:90787099-98232469 (hg18)	unknown	Illumina 610,000 SNP array platform, confirmed by chromosomes and FISH	Sobreira N, Walsh MF, Batista D, Wang T. 2009. Interstitial deletion 5q14.3–q21 associated with iris coloboma, hearing loss, dental anomaly, moderate intellectual disability, and attention deficit and hyperactivity disorder. Am J Med Genet Part A 149A:2581–2583.
100	clinical assessment	balanced translocation, breakpoint 500kb upstream of MEF2C t(5;8)(q14.3;q23.3)	de novo	Chromosome anlaysis confirmed by FISH	Floris, C., Rassu, S., Boccone, L., Gasperini, D., Cao, A., & Crisponi, L. (2008). Two patients with balanced translocations and autistic disorder: CSMD3 as a candidate gene for autism found in their common 8q23 breakpoint area. European Journal of Human Genetics : EJHG, 16(6), 696–704. https://doi.org/10.1038/ejhg.2008.7
101	clinical assessment	not reported	unknown	unknown	Ramji, S., McCullagh, G., Ram, D., Vassallo, G., & Pavaine, J. (2020). T2-highlighted U-fibres and rapid parenchymal volume loss in AESD: An under-recognised subtype of paediatric acute encephalopathy syndromes. Journal of neuroradiology = Journal de neuroradiologie, 47(6), 458–463. https://doi.org/10.1016/j.neurad.2019.09.003
102	clinical assessment	chr 5q14.3q15 (85045530_96578026)x1, 11.5 Mb	unknown		
103	clinical assessment	chr 5q14.3 (85381873_ 90368235)x1, 5 Mb	unknown		

	A	B	C	D	E	F	G	H	I	J
1		Study Type	Authors	Year Published	Locations Published	Verification of Human Case	Number of Patients	Patient Sex	Patient Age	Phenotype and Clinical Information Reported
104								F	1yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, generalized epilepsy, epileptic encephalopathy with epileptic spasms, generalized bilateral tonic-clonic seizure, focal motor seizure (7mo). MRI small focal white matter alterations in the right mesial temporal region and both occipital lobes, Hypoplastic CC, alterations of white matter mesial temporal right, delayed myelination, frontal lobe atrophy, mild lateral ventricles enlargement. EEG Focal or multifocal
105								M	3yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, "generalized myoclonic epilepsy" spectrum, generalized epilepsy, myoclonic seizures, absence seizures (6mo). EEG Diffuse discharge of spikes and poly-spikes and waves. Abnormal sleep pattern. At wake spikes and waves related to eyelid myoclonias, impairment of awareness, jerks at arms. in wakefulness spikes and waves complex in frontotemporal regions (right>left), slowing background. MRI cerebellar vermis hypoplasia, IV ventricle-lateral ventricles enlargment, Hippocampal abnormalities, cavum vergae, cavum septipellucidi, empty sella. Periventricular white matter abnormalities, Hypoplastic CC, Delayed myelination. Absent speech.
106								M	4yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, epileptic encephalopathy with epileptic spasms, generalized epilepsy, Febrile Seizures, myoclonic seizures, generalized bilateral tonic-clonic seizure, Absence Seizures, spasms (3mo). EEG Focal or multifocal spikes, high amplitude spike/poly spike and slow wave complexes, hypsarrhythmia, slowing background. Absent speech, autistic features. MRI Hypoplastic CC, delayed myelination, hypoplasia cerebellar vermis
107								M	9yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, "generalized myoclonic epilepsy" spectrum, Myoclonic seizures, focal motor seizure with impairment of awareness, ATONIC (14mo). EEG Bilateral temporooccipital spikes and spike-waves, slowing background. Absent speech, autistic features, happy demeanor. MRI not available.
108								M	7yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, focal epilepsies Febrile Seizure, focal motor seizure, focal seizure with impairment of awareness (7mo, 18mo), EEG Bisynchronous high voltage generalized slow spike and wave complexes, more evident in frontal regions, increased in sleep, slowing background. MRI normal. Absent speech, autistic features.
109	43	multicenter study	Raviglione et al.	2021	Italy, Denmark, UK	human	17 new (25 total)	F	8yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, focal epilepsies, unilateral myoclonic seizures (1yr). MRI Frontal cortical atrophy and enlarged cisterna magna, partial agenesis CC, enlarged LV. EEG Diffuse delta activity and bisynchronous high-voltage generalized slow spike and wave complexes, more evident in frontal regions right>left, slowing background. Absent speech.

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
104	clinical assessment	chr 5q14.3 (87050542_91327145)x1, 4.3 Mb	de novo		
105	clinical assessment	chr 5q14.3 (85440219_89051857)x1, 3.6 Mb	unknown		
106	clinical assessment	chr 5q14.3 (87928008_89930741), 2 Mb	unknown		
107	clinical assessment	chr 5q14.3 (86487715_88232646)x1, 1.7 Mb	de novo		
108	clinical assessment	chr 5q14.3 (88169950_88691724)x1,bb - dn	de novo		
109	clinical assessment	chr 5q14.3 (88086124_88439907)x1, 354 Kb	unknown	Of 25 total patients: array-CGH in 17 patients, MEF2C single gene sequencing in two patients, and targeted re-sequencing through next-generation sequencing (NGS) multi-gene	Raviglione, F., Douzgou, S., Scala, M., Mingarelli, A., D'Arrigo, S., Freri, E., Darra, F., Giglio, S., Bonaglia, M. C., Pantaleoni, C., Mastrangelo, M., Epifanio, R., Elia, M., Saletti, V., Morlino, S., Vari, M. S., De Liso, P., Pavaine, J., Spaccini, L., Cattaneo, E., ... Striano, P. (2021). Electroclinical features of MEF2C haploinsufficiency-related epilepsy: A multicenter European study. Seizure, 88, 60–72. Advance online publication. https://doi.org/10.1016/j.seizure.2021.03.025

	A	B	C	D	E	F	G	H	I	J
1		**Study Type**	**Authors**	**Year Published**	**Locations Published**	**Verification of Human Case**	**Number of Patients**	**Patient Sex**	**Patient Age**	**Phenotype and Clinical Information Reported**
110	43	study	Haviglione et al.	2021	UK	human	17 new (25 total)	F	9yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, neither febrile nor epileptic seizures, No EEG and background normal, MRI Not Avaliable, No abnormal behavior findings noted.
111								F	18yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, isolated febrile seizures Complex Febrile Seizures (7mo). EEG Diffuse frontal dominant discharges of high voltage slow waves, slowing background. MRI delayed myelination. Absent speech, autistic features, happy demeanor.
112								M	11yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, focal epilepsies Complex Febrile Seizures (1yr) focal motor seizure with impairment of awareness (2yr). EEG Slowing of background activity (theta and/or delta waves in parieta and occipital regions), focal or multifocal spikes, increase incidence of focal or multifocal spikes during sleep, slowing background. MRI Abnormalities in the posterior fossa included Chiari Type 1 malformation. Absent speech, autistic features.
113								M	10yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, generalized epilepsy in the GEFS+ spectrum showing bilateral tonic-clonic seizures often induced by fever, Focal Motor Seizures (2yr) generalized bilateral tonic-clonic seizure (3yr). EEG Frontal spikes discharges; centro temporal spikes, bilateral asynchronous > right, irregular organization of activity during sleep. MRI Non specific hyperintensity spot in frontal white matter, abnormal venous drainage in right parietal-occipital regions. Absent speech, autistic features.
114								M	7yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, generalized epilipsy, focal epilepsies, Febrile Seizures, Focal Motor Seizures (9mo), generalized bilateral tonic-clonic seizure Epileptic Status (3yr). EEG Theta slow waves, then diffuse and high voltage waves. multifocal bilateral spikes, slowing background. MRI decrease in white matter volume in temporal-parietal-occipital regions, Very thin CC, mild dilatation LV with dilated frontal horns, hippocampal abnormalities, reduction of white matter thickness in temporal-parietaloccipital regions. Absent speech, autistic features, happy demeanor.
115								F	8yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, isolated febrile seizures Complex Febrile Seizure (15mo). EEG Multifocal asynchronous bilateral spikes, diffuse discharges of spike and waves, more evident during sleep, slowing background activity, slowing background. MRI hypoplastic CC. Absent speech, autistic features.

	K	L	M	N	O	P
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**	
110	clinical assessment	chr 5q14.3 (88193092_88450493)x1, 257 Kb (also has chr 22q13.2 (43415399-43577990)x3, mat)	de novo	patients, and targeted re-sequencing through next-generation sequencing (NGS) multi-gene panel in six subjects.	J., Spaccini, L., Cattaneo, E., ... Striano, P. (2021). Electroclinical features of MEF2C haploinsufficiency-related epilepsy: A multicenter European study. Seizure, 88, 60–72. Advance online publication. https://doi.org/10.1016/j.seizure.2021.03.025	
111	clinical assessment	chr 5q14.3 (88149592_88348206)x1, 198.6 Kb	unknown			
112	clinical assessment	chr 5q14.3 (88149592_88348206)x1, 198.6 Kb	de novo			
113	clinical assessment	chr 5q14.3 (88119525_88193351)x1, 74 kb	de novo			
114	clinical assessment	c.52_54+4delCAGGTGA	de novo			
115	clinical assessment	c.83T>C, p.Leu28Ser	de novo			

	A	B	C	D	E	F	G	H	I	J
1		**Study Type**	**Authors**	**Year Published**	**Locations Published**	**Verification of Human Case**	**Number of Patients**	**Patient Sex**	**Patient Age**	**Phenotype and Clinical Information Reported**
116								F	10yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, neither febrile nor epileptic seizures. EEG Dysrytmic backgroud activity; generalized theta slow waves, slowing backgorund. MRI normal. No abnormal behavioral features noted.
117								F	22yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, focal epilepsies, unilateral myoclonic seizures, Focal Motor Seizures, Absence Seizure (8mo). EEG Multifocal spike and slow waves predominant in the occipital tregions at 3 years of age, than in the centro-parietal regions at 10 years of age, slowing background. MRI normal. Autistic features, happy demeanor.
118								M	2yr	developmental delay resulting in moderate to severe intellectual disability, autistic behavior/autistic spectrum disorder, and distinctive dysmorphic features, "generalized myoclonic epilepsy" spectrum, Focal Epilepsy, Focal Motor Seizures (10mo) Myoclonic Seizures (15mo). EEG Focal spikes (parietal) activation of spikes and waves during sleep, slowing background. MRI hypoplastic CC. Autistic features.

	K	L	M	N	O
1	**How phenotype was reported**	**Variation Reported**	**Inheritance Pattern**	**Method Used to Detect Variant**	**Article Citation**
116	clinical assessment	c.526G>A, p.Gly176Ser	unknown		
117	clinical assessment	c.45dupT, p.Asn16Ter (rs1554150552)	unknown		
118	clinical assessment	c.712C>T, p.Arg238Ter	de novo		

Appendix D

Supplemental Literature Review Full Phenotypes Table

(starts on following page)

	Patient	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Ilari et al., 2016)	P1	Deletion of ~2.724Mb	N/A	Unknown	+	+ (head control at 12mo, sat independently at 2yr)	+	N/A	-	-	+ (6 mo)	febrile, complex partial	+ (hand washing, flapping, clapping, hand-to-mouth)	+ (broad nose, deep nasal bridge, short philtrum)
(Carr et al., 2011)	P2	Deletion of ~3.1Mb	N/A	de novo	+	+ (sat independently at 2.5yr)	+	-	-	-	+	myoclonic	+ (hand flapping)	+ (prominent forehead, bitemporal narrowing, hypoplastic orbital ridges, downslanting palpebral fissures, sparse bilateral medial [illegible])
(Lu et al., 2018)	P3	Missense Variant	c.43C>T het p.Arg15Cys	Paternal	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
(Lu et al., 2018)	P4	Missense Variant	c.43C>T het p.Arg15Cys	Paternal	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
(Lu et al., 2018)	P5	Missense Variant	c.43C>T het p.Arg15Cys	Paternal	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
(Engels et al., 2009)	P6	Deletion of ~5.69Mb	N/A	de novo	+	+	+	-	-	-	+	infantile spasms	N/A	- (only large ears and broad eyebrows)
(Engels et al., 2009)	P7	Deletion of ~3.33Mb	N/A	de novo	+	+ (unable to sit independently at 7yrs)	+	-	- (babbling at 7yr)	-	+	febrile, myoclonic jerks	N/A	+ (downslanting palpebral fissures, philtral haemangioma, brachycephaly with low anterior hairline)
(Engels et al., 2009)	P8	Deletion of ~3.574Mb	N/A	de novo	+	+ (head control later than 1 yr, sitting unsupported at 2.5yr)	+	-	- (uses syllables, can use electronic speaking aid in directed fashion)	-	+ (4yr 9mo)	atypical absences, one grand mal seizure at 5yr 3mo	+ (dyskinetic)	+ mild dysmorphisms (simple ears, slighly narrowed supraorbital region, slightly upslanting palpebral fissures)
(Toral-López et al., 2012)	P9	Deletion of ~1.59Mb (patient also has a 600kb deletion of 2q13)	N/A	de novo	+	+ (head control at 1yr3mo, unable to sit unsupported)	+	N/A	-	-	+ (3 mo)	unspecified seizures	N/A	+ (occipital plagiocephaly, large and lowset ears, narrow forehead, depressed nasal bridge, flat facial profile, synophrys, narrow palpebral fissures, right eye esotropia, short nose and philtrum, downturned corners of mouth, small mandible, short neck, prominent anterior chest, aberrant right palmar creases, bilateral fifth finger clinodactyly)
(Mikhail et al., 2011)	P10	Deletion of ~412Kb	N/A	de novo	N/A	+	N/A	- (macrocephaly)	-	-	-	N/A	+ (hands and feet)	+ (epicanthic folds depressed nasal bridge, slighly posteriorly rotated ears)

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Ilari et al., 2016)	P1	+ (distal corpus callosum thinning, two arteriovenous malformations)	N/A	+ (no social smile, no social communication)	N/A	N/A	N/A	N/A	open mouth, small pink rounded or oval-shaped vascular lesions (many with telangiectatic vessels in center), Capillary Malformation-Arteriovenous Malformation	RASA1, MEF2C
(Carr et al., 2011)	P2	+ (thickened anterior corpus callosum and simplified gyral with gyral thickening)	No seizure activity	N/A	N/A	N/A	N/A	N/A	open mouth, bruxism, hypopigmentation consistent with vitiligo, multiple skin legions by consistent with Capillary Malformation-Arteriovenous Malformation with telangiectatic vessels	RASA1, MEF2C
(Lu et al., 2018)	P3	N/A	N/A	N/A	N/A	+ (ventricular septal defect, double outlet right ventricle)	N/A	N/A	N/A	MEF2C
(Lu et al., 2018)	P4	N/A	N/A	N/A	N/A	+ (ventricular septal defect, double outlet right ventricle)	N/A	N/A	N/A	MEF2C
(Lu et al., 2018)	P5	N/A	N/A	N/A	N/A	+ (ventricular septal defect, double outlet right ventricle)	N/A	N/A	N/A	MEF2C
(Engels et al., 2009)	P6	+ (aplasia of cerebellar vermis and posterior corpus callosum, multiple plexus cysts, enlarged occipital horns of lateral ventricles)	+ (hypsarrhthmia)	N/A	+ (feeding difficulties (puree fed only), chronic constipation)	+ (concentric myocardial hypertrophy)	+ (bilateral optic atrophy)	N/A	tachydyspnoea, bilateral transverse palmar creases, cafe-au-lait spots, incomplete closure of thoracic vertebral arches Th2-Th10, increased sweating, bilateral pes equinus, frequent upper respiratory tract infections	MEF2C
(Engels et al., 2009)	P7	+ (prominence of arachnoid spaces in perivascular areas)	+ (high amplitude prominent rhythmical activity in temporal regions with generalized burst of spike and slow waves)	N/A	+ (feeding difficulties, frequenct vomiting)	N/A	N/A	N/A	failure to thrive, visual preoccupation with stripes	RASA1, MEF2C
(Engels et al., 2009)	P8	+ (moderate atrophy of supra- and infratentorial region, slighly enlarged ventricular system, and unspecific leucoencephalopathy)	+ (short focal seizures accompanied by atypical absences)	+ (limited social interactions)	+ (feeding difficulties (puree fed)	N/A	+ (hyperopia, strabsimus)	+ (slept a lot)	open mouth, hypersalivated, bruxism, sensitive to noise	(MEF2C not included)
(Toral-López et al., 2012)	P9	+ (left-sided cerebral hemistrophy, fronto-temporal cortical atrophy, dandy-walker malformation, partial agensis of corpus callosum and cerebellum, ventriculomegaly, abnormal cortical hyration)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	MEF2C
(Mikhail et al., 2011)	P10	-	N/A	-	N/A	N/A	N/A	N/A	hyperkinesis with constant movements of hands and feet, relative macrocephaly	MEF2C (promotor and exons 1-3)

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Saitsu et al., 2011)	P11	M	7yr	Balanced translocation t(5;15)(q13.3;q26.1)	N/A	de novo	+	+ (unable to sit unsupported)	-	+	-	-	+ (3 mo)	tonic seizures of lower extremeties followed by generalized clonic seizures	-	+ (infancy: square face with short palpebral fissures, short depressed nose with anteverted nostrils, tented vermilion of upper lip, protruded tongue. Childhood: face became round and flat. Deformity of trunk and extremities)
(Shimojima et al., 2012)	P12	M	1yr 8mo	Deletion of ~3.4Mb	N/A	de novo	+	+ (head control at 7mo, visual fixation and social smile at 1yr, unable to sit or roll over)	+	+	-	-	+ (4 mo)	epilepsy characterized by spasms, drops, abductions of arms and eye rolling	N/A	+ (flat occiput, hypertelorism, depressed nasal bridge, small nose, low set ears, micrognathia, short tapering fingers, single transverse palmar creases in both hands)
(Yauy et al., 2013)	P13	F	3yr	Balanced translocation t(3;5)(p26.3;q14.3)dn	N/A	de novo	+	+ (sat independently at 10 mo)	N/A	N/A	-	+ (22 mo)	+ (N/A)	febrile	-	+ (spread eyebrows, protruding ears with simplified helices and abnormal dermatoglypcis, bilateral fifth finger clinodactyly)
(Vreeburg et al., 2017)	P14	F	10yr 7mo	Deletion of ~5.6Mb	N/A	de novo	+	+ (sat independently at 18 mo)	+ (progressed to spasticity)	-	-	-	+ (less than 1 yr)	myoclonic epilepsy	+ (hand flapping, hand mouthing, head nodding)	+ (broad forehead, down turned corners of mouth, prominent philtral pillars, short columella, depressed nasal bridge, epicanthic folds, hypertelorism, large mouth/lips)
(Vreeburg et al., 2017)	P15	F	6yr 6mo	Deletion of ~494Kb	N/A	de novo	+	+ (sat independently at 12-14 mo)	+	-	-	+ (2 yr, broad based and unstable gait)	+ (infancy)	febrile then generalized tonic-clonic	+	+ (broad forehead, prominent philtral pillars, short columella, tented upper lip)
(Vreeburg et al., 2017)	P16	F	3yr	Deletion of ~130Kb	N/A	de novo	+	+ (sat independently at 12 mo)	+	-	+ (a few words)	+ (3yr 6mo, wide-based gait)	+ (less than 1 yr)	febrile then generalized	+ (hand flapping, clasping in midline, screw paper up)	+ (broad forehead, down turned corners of the mouth, prominent philtral pillars, short columella, tented upper lip, depressed nasal bridge, large mouth/lips)
(Vreeburg et al., 2017)	P17	M	2yr 6mo	Deletion of ~257Kb	N/A	de novo	+	+ (sat independently at 10-11 mo)	+	-	-	+ (2yr 6mo)	+ (15 mo)	febrile then generalized seizures and absences	N/A	+ (broad forehead, prominent philtral pillars, short columella, tented upper lip, depressed nasal bridge, large mouth/lips)

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Saitsu et al., 2011)	P11	+ (reduced volume of white matter, hypoplastic corpus callosum especially in genu and splenium)	+ (hypsarrhythmia when asleep)	+ (poor visual contact)	+ (gastroesophageal reflux and tube fed)	N/A	N/A	N/A	nystagmus, upward gazing, spastic quadriplegia, encephalopathy, low perfusion at right frontal area with cerebral blood flow exam	breakpoint upstream of MEF2C
(Shimojima et al., 2012)	P12	+ (reduced volume of the frontal lobe, hypoplastic corpus callosum, dilatation of the lateral cerebral ventricles, reduced white matter especially in frontal and anterior temporal lobes, remarkable dilatation of the lateral ventricles especially occipital and inferior horns showing colpocephalic appearance, severe dysgenesis of corpus callosum, ventral fornix hyperplastic, brainstem volume reduced, upper cerebellar peduncles were hypoplastic)	+ (atypical hypsarrhythmia)	N/A	+ (feeding difficulties)	N/A	N/A	N/A	repsiratory distress due to dysphagia and airway narrowing, opisthotonic posture, hypsarrhythmia, deep tendon refelxes hyperactive, spastic quadriplegia	RASA1. (MEF2C not included)
(Yauy et al., 2013)	P13	-	-	N/A	N/A	N/A	N/A	N/A	ADHD	breakpoint upstream of *MEF2C*
(Vrečar et al., 2017)	P14	+ (thick corpus callosum)	+ (high amplitude spike and slow wave complexes bilaterally with slight right sided predominance)	+ (Not very social with others, obsessive)	+ (Severe GERD, constipation)	N/A	+ (Mild myopia)	+	ceiling gazing, bruxism, specific eating pattern, likes running water, plays along with simple activies, episodic breathing abnormalities starting at 2wk, reduced reflexes, recurrent infections, pigmentation (hemangiomas), cold hands and feet, floppy larynx	MEF2C, RASA1
(Vrečar et al., 2017)	P15	+ (frontal cortical atrophy and moderate ventriculomegaly)	N/A	+ (Autistic traits, plays alone, tolerates hugs, poor eye contact)	-	+ (Patent ductus arteriosus (PDA) closed with a coil, PFO (persistent foramen ovale))	+ (Registered blind)	-	bruxism, hypermobility, recurrent infections, pigmentation (hemangiomas, large capillary nevus of the lower limb), duplex left kidney, likes music, light, and water	MEF2C Exons 1-3
(Vrečar et al., 2017)	P16	+ (small corpus callosum, possible white matter abnormality in occipital lobes)	+ (dysrythmic background with high voltage poly spike wave bursts-centrencephalic neuronal hyperexcitability	+ (Eye contact with people she knows but won't look at strangers but enjoys being around children, generally happy, laughing, short attention span,	+ (Feeding difficulties and overfills mouth when eating)	N/A	N/A	+	bruxism, hand mouthing, tongue thrusting, loves water, episodic breathing abnormalities starting at age 3yr, scaphocephaly, drooling, poor coordination	MEF2C Exons 2-10
(Vrečar et al., 2017)	P17	+ (small splenium of corpus callosum, mild ventriculomegaly)	N/A	+ (Responsive to familiar adults, possible autism)	+ (Severe GERD, constipation)	N/A	N/A	+	has some purposeful hand use, hand mouthing, overfills mouth when self-feeding, brisk reflexes	MEF2C Exon 1

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Vreteř et al., 2017)	P18	F	3yr 1 mo	Missense Variant	c.220G>T het p.Glu74*	de novo	+	+ (sat independently at 12 mo)	+	-	-	-	+ (less than 1 yr)	febrile and afebrile seizures	+ (not of the hands)	+ (broad forehead, down turned corners of the mouth, prominent philtral pillars, Short columella, slightly tented upper lip, depressed nasal bridge, cleft palate, mild posterior plagiocephaly)
(Vreteř et al., 2017)	P19	F	3yr	Deletion of at least 19Kb	N/A	de novo	-	+ (not able to sit independently)	+	-	+ (understands and uses 15 words)	+ (2yr 2mo, needs support)	-	N/A	+ (hand wringing)	+ (broad forehead, Prominent philtral pillars, Short columella, Epicanthic folds, large mouth/lips)
(Marashly et al., 2010)	P20	M	14mo	Deletion of ~3.5Mb	N/A	mother WT; father unknown	N/A	+	N/A	N/A	N/A	N/A	+ (6 mo)	infantile spasms, characterized drop of the head, abductions of arms and eye rolling	N/A	+ (hypotelorism, slighlty upslanted palpebral fissures, long lashes, exaggerated bow on the upper lip, short upturned nose, ear lobes uplifted)
(Yang et al., 2015)	P21	M	(deceased at) 5mo	Deletion of ~21.02Mb	N/A	de novo	N/A	N/A	+	N/A	N/A	N/A	+ (30 days)	febrile, grand mal	N/A	+ (narrow prominent forehead, mildly upslanting palpebral fissures, widely spaced eyes, depressed nasal bridge with anteverted nares, long philtrum with deep groove, prominent cupid bow of the upper lip vermilion, hypotonic mouth, micrognathia, cavate auricular lobule)
(Tonk et al., 2011)	P22	F	18yr	Deletion of at least 21.08Mb	N/A	Unknown	+ (IQ of 63, progressed in regular classes with some special ed and tutoring)	- milestones at the upper range of normal (sitting at 7 months)	+	-	+ (single words at 10–12 months, 3-word phrases at age 4 years)	+ (14 mo)	+ (less than 18 mo)	febrile, myoclonic epilepsy, grand mal	N/A	+ (narrowing at the temples, lateral extension of the superior ear helices, U-shaped upper lip vermilion)
(Al-Shehhi et al., 2016)	P23	M	22mo	Deletion of ~3.1Mb	N/A	Unknown	N/A	+ (unable to sit independently at 22 mo)	+	N/A	-	-	+(15 mo)	evolved to bilateral refractory myoclonic jerks,	+ (hand movements)	+ (prominent forehead, open mouth appearnace)

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Vrečar et al., 2017)	P18	-	+ (bilateral temporal slow waves and bilateral parietal spike waves)	+ (Transient eye contact, happy, loves human contact and interaction)	+ (Severe GERD, constipation)	N/A	+ (Mild myopia)	+	has some purposeful hand use, bruxism, rocks in her chair, hypermobility, recurrent infections	MEF2C
(Vrečar et al., 2017)	P19	N/A	N/A	+ (Excitable personality)	+ (Feeding difficulties)	N/A	N/A	N/A	has some purposeful hand use, hypermobility, drooling, ataxic movements	MEF2C exons 1-2
Marashly et al., 2010)	P20	-	+ (paroxysms of high voltage spike polyspike and slow wave discarges diffusely with multifocal spike and polyspike discahrages most prominent in the posterior quadrants, hypsarrythmia)	N/A	N/A	N/A	+ (Bilateral esotropia)	N/A	N/A	(MEF2C not included)
(Yang et al., 2015)	P21	+ (agensis of corpus callosum, cyst of pellucid septal cave)	N/A	+ (Frequent crying, poor eye contact)	N/A	N/A	N/A	+	adenoidal hypertrophy, death from respiratory failure > SUDEP (6 hr after last seizure)	MEF2C, EFNA5
(Tonk et al., 2011)	P22	-	+ (focal activity after grand mal seizure)	- (Appropriate social skills)	N/A	N/A	N/A	-	N/A	MEF2C
al-Shehhi et al., 2016)	P23	+ (large left thalmostriate vein, absence of posterior aspect and adjacent body of corpus callosum)	+ (multifocal bisynchronous high voltage bitemporal spike waves)	N/A	+ (Feeding difficulties)	N/A	N/A	N/A	thalamic vein abnormality by antenatal ultrasound, didn't fix with his eyes, dysmorphic, jugular pit in his suprasternal notch	MEF2C, RASA1

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Berland & Houge, 2010)	P24	F	11yr	Deletion of ~1.15Mb	N/A	de novo	+	+ (sat independently at 3 yr, crawled and walked with support at 4 yr)	+	+	- (mimics sounds, makes use of body language, receptive language better than expressive, can follow instructions)	+ (11 yr)	+ (1 yr)	febrile tonic-clonic, atypical seizures with myoclonic jerks	+ (hand washing when younger, now flipping stereotypies such as flipping corners of a page or carpet)	+ (long upslanted palpebral fissures, everted lower lids, wide forehead, mild brachycephaly, short and wide philtrum with an everted upper lip, short and broad chin, mild clinodactyly and short and narrow feet)
(Novara et al., 2013)	P25	F	6yr	Duplication of ~5.5Mb	N/A	de novo	+	+	N/A	+	+ impairment of language, mild language disorder	N/A	+ (2 yr)	febrile	N/A	+ (maxillofacial asymmetry due to ocular dimension difference and eyes frontally misaligned)
(Novara et al., 2013)	P26	F	1yr	Duplication of ~5.2Mb	N/A	de novo	N/A	+	+	+	N/A	N/A	N/A	N/A	N/A	+ (wide and flat nasal root, smooth filtrum, microretrognathia, clinodactyly of fourth and fifth toes)
(Le Meur et al., 2010)	P27	F	4yr 3mo	Deletion of ~2.68Mb	N/A	de novo	+	+ (sat unaided and able to crawl)	+	-	-	N/A	+ (7 mo)	from 4 mo had myoclonic jerks of upper limbs then brief episodes of eye revulsion concomitant with jerks, epilepsy	-	N/A
(Le Meur et al., 2010)	P28	F	9mo	Deletion of ~3.5Mb	N/A	de novo	+	N/A	+	+	N/A	N/A	+ (1 day)	tonico-clonic seizures since day	N/A	N/A
(Le Meur et al., 2010)	P29	M	18mo	Deletion of ~8.8Mb	N/A	de novo	+	N/A	+	+	N/A	N/A	N/A	N/A	N/A	N/A
(Le Meur et al., 2010)	P30	M	3yr	Deletion of ~1.57Mb	N/A	de novo	+	+ (sat unaided at 18 mo, crawled at 2 yr, stand and cruise along furniture and manipulate toys at 3 yr)	+	-	-	N/A	-	N/A	+ (hand flapping and clapping)	N/A
(Le Meur et al., 2010)	P31	F	7yr	Deletion of ~216Kb	N/A	de novo	+	+	+	+	-	-	+ (3 yr)	tonic-clonic febrile seizures	+ (hand washing and hand-to-mouth movement)	N/A
(Le Meur et al., 2010)	P32	M	6yr	Duplication of ~4.6Mb	N/A	de novo	+ (IQ between 50-60)	+	N/A	+	+ (delayed but understandable, not able to pronounce short sentences)	+ (2 yr)	N/A	N/A	N/A	N/A

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Berland & Houge, 2010)	P24	N/A	+ (generlized epileptiform pattern)	+ (Poor eye contact, happy and joyful, no panic attacks but easily scared of loud sounds, autistic features)	N/A	N/A	'+ (strabismus, intermittent nystagmus)	N/A	jugular fossa pit, fascinated by water and bright objects at younger age, puberty occured early	MEF2C, TMEM161B
(Novara et al., 2013)	P25	+ (mild enlargement of lateral ventricles with mild asymmetry)	- (however, marked by high number of rapid rhythmic [illegible])	N/A	+ (Poor sucking in neonate period)	N/A	+ (severe hypermetropia)	N/A	motor clumsiness	MEF2C
(Novara et al., 2013)	P26	-	-	N/A	+ (Poor sucking)	+ (Patent foramen ovale)	N/A	N/A	fialure to thrive, microcephaly with metopic prominence, persistent aseptic fever at 1yr	MEF2C, RASA1
(Le Meur et al., 2010)	P27	N/A	+ (several bilateral isolated spasms and frequent synchronous myoclonus with abnormal and slow background pattern)	+ (frequenct crying, poor visual contact)	N/A	N/A	N/A	+	single episode of cyanosis with eye revulsion at 3 days of age, rocking her head and rubbing her chin with hands	MEF2C
(Le Meur et al., 2010)	P28	N/A	+ (requent bursts with no basic rhythm and very unstructured pattern)	N/A	N/A	N/A	N/A	+ (awakening stages short)	poor eye contact	MEF2C
(Le Meur et al., 2010)	P29	N/A	+ (slow basic rhythm with infraclinical temporoparietal paroxystic discharges)	+ (Transient eye contact)	+ (insufficient weight gain by 18mo, gastrostomy tube)	+ (abnormal foetal cardiac rhythm)	+ (cortical blindness)	+ (sleep disturbance)	head circumference small at birth	MEF2C
(Le Meur et al., 2010)	P30	N/A	-	+ (Eye contact difficult to obtain and transient)	N/A	N/A	N/A	N/A	failure to thrive	MEF2C Exon 1
(Le Meur et al., 2010)	P31	N/A	N/A	N/A	N/A	N/A	N/A	N/A	growth paramtere -2SD at birth, hyperventilation	MEF2C
(Le Meur et al., 2010)	P32	-	-	- (Normal eye contact, behavior and social skills.)	N/A	N/A	N/A	N/A	special education required.	MEF2C

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Le Meur et al., 2010)	P33	F	7yr	Nonsense Variant	c.683C>G het p.Ser228*	de novo	+	+	N/A	-	-	+ (3 yr, unstable wide-based gait)	+ (9 mo)	generalized tonic-clonic seizures	+ (hand and hand-mouth stereotypic movments)	N/A
(Paciorkowski et al., 2013)	P34	M	13yr	Deletion of ~3.6Mb	N/A	Unknown	+	+	+	-	-	-	+ (6 mo)	infantile spasms	+ (hand flapping)	N/A
(Paciorkowski et al., 2013)	P35	F	11mo	Deletion of ~5.11Mb	N/A	Unknown	N/A	+	+	-	- (babbles)	N/A	+ (11 mo)	myoclonic	-	N/A
(Paciorkowski et al., 2013)	P36	M	5yr 3 mo	Deletion of ~1.0Mb	N/A	Unknown	+	+	+	-	-	-	+ (after 1 yr)	epilepsy type unknown	+ (hand flapping, chin rubbing)	N/A
(Paciorkowski et al., 2013)	P37	F	13yr	Deletion of ~1.38Mb	N/A	Unknown	+	+	N/A	-	-	+ (N/A, abnormal gait)	+ (4 mo)	Myoclonic at 4 months, infantile spasms at 9 months	+ (hand-flapping, rocking)	N/A
(Paciorkowski et al., 2013)	P38	M	46yr	Deletion of ~3.02Mb	N/A	Unknown	+	+	+	-	-	-	-	-	-	N/A
(Paciorkowski et al., 2013)	P39	F	17mo	Deletion of ~320Kb	N/A	Unknown	N/A	+	+ (does not roll or lift head)	-	-	N/A	+ (13 mo)	Myoclonic and generalized	-	+ (slight tenting of upper lip)
(Paciorkowski et al., 2013)	P40	F	22mo	Frameshift Variant	c.833delT het	Unknown	N/A	+	N/A	-	-	+ (22 mo)	+ (18 mo)	Myoclonic and atonic	+ (hand-wringing)	N/A
(Paciorkowski et al., 2013)	P41	F	5yr 5mo	Deletion of ~1.35Mb	N/A	Unknown	+	+	+	-	-	N/A	+ (3 mo)	Myoclonic and infantile spasms	+ (rocking, side-to-side head movements)	N/A
(Paciorkowski et al., 2013)	P42	M	6mo	Deletion of ~6.0Mb	N/A	Unknown	N/A	+	+	-	-	N/A	+ (4 mo)	Infantile spasms	-	+ (tenting of upper lip)
(Paciorkowski et al., 2013)	P43	M	6yr	Deletion of ~11.6Mb	N/A	Unknown	+	+	N/A	-	-	- (can take steps with gait trainer)	+ (N/A)	febrile	-	N/A
(Paciorkowski et al., 2013)	P44	F	5yr 6mo	Deletion of ~5.4Mb	N/A	Unknown	+	+	N/A	-	-	N/A	+ (2 yr)	myoclonic	+ (hand flapping, head shaking)	N/A

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Le Meur et al., 2010)	P33	N/A	N/A	+ (Behavioral disorders, decreased eye contact, lack of emotional reciprocity, lack of interest in her surroundings)	+ (Difficulties in breastfeeding and feeding at 5 mo and onward)	N/A	N/A	N/A	regressed after age 5mo and lost previously acquired skills, unable to use hands purposefully	MEF2C only
(Paciorkowski et al., 2013)	P34	-	+ (spike-wave associated with epileptic spasm)	+ Generally happy, with inappropriate laughter, poor eye contact	+ (GERD)	N/A	N/A	-	High pain tolerance, dystonia	MEF2C
(Paciorkowski et al., 2013)	P35	-	-	N/A	+ (Feeding difficulties in	N/A	N/A	N/A	Dystonia, no visual fixation	MEF2C
(Paciorkowski et al., 2013)	P36	-	-	+ (Diminished responses to others)	N/A	N/A	N/A	-	N/A	MEF2C
(Paciorkowski et al., 2013)	P37	+ (dysmorphic corpus callosum and mild cerebellar vermis hypoplasia)	-	+ (Easily agitated, with self-mutilating behaviors, poor attention, inconsistent eye contact)	+ (constipation)	N/A	N/A	+ (Disrupted sleep)	pes planus and valgus deformity, hyperventilation/ hypoventilation, high pain tolerance	MEF2C
(Paciorkowski et al., 2013)	P38	-	-	+ (Generally happy, poor eye contact)	+ (constipation)	N/A	N/A	N/A	hypokinetic spasticity	MEF2C
(Paciorkowski et al., 2013)	P39	-	-	+ (Poor visual tracking)	N/A	N/A	N/A	N/A	hyperkinesis, bruxism,	MEF2C
(Paciorkowski et al., 2013)	P40	+ (mild thinning of the cortical white matter of T2 axial)	+ (multifocal epileptiform activity and poorly developed anterior-posterior gradient)	N/A	N/A	N/A	N/A	N/A	N/A	MEF2C only
(Paciorkowski et al., 2013)	P41	+ (frontal bossing and brachycephaly, mild cortical atrophy and thinning of the white matter on T2 axial)	-	+ (generally happy, poor visual awareness, limited engagement)	+ (slow gastric emptying, GERD, constipation)	N/A	N/A	+ (irregular sleep initiation and maintenance)	hyperkinesis	MEF2C
(Paciorkowski et al., 2013)	P42	-	-	N/A	N/A	N/A	N/A	N/A	hyperkinesis	MEF2C
(Paciorkowski et al., 2013)	P43	-	-	+ (generally happy, occasional inappropriate laughter)	+ (severe GERD and dysphagia, constipation)	N/A	N/A	+ (occasionall y irregular sleep maintenance	hyperkinesis, dystonia, high pain tolerance, poor visual tracking,	MEF2C
(Paciorkowski et al., 2013)	P44	-	-	+ (generally happy, occasional inappropriate laughter, does not appear to distinguish individuals)	+ (mild GERD and dysphagia, constipation)	N/A	N/A	-	hyperkinesis, high pain tolerance, poor visual tracking	MEF2C

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Paciorkowski et al., 2013)	P45	M	7yr	Deletion of ~410Kb	N/A	Unknown	+	+	N/A	-	-	N/A	+ (N/A)	febrile	+ (arm flapping)	N/A
(Paciorkowski et al., 2013)	P46	M	30mo	Deletion of ~50Kb	N/A	Unknown	N/A	+	N/A	-	- (some babbling)	N/A	-	-	+ (hand batting, head shaking)	N/A
(Paciorkowski et al., 2013)	P47	F	7yr	Deletion of ~300Kb	N/A	Unknown	+	+	N/A	-	- (has 10 words)	N/A	+ (N/A)	Single generalized seizure	+ (hand flapping, waving hands in front of face)	N/A
(Paciorkowski et al., 2013)	P48	M	6yr	Deletion of ~5.2Mb	N/A	Unknown	+	+	N/A	-	- (some vocalizations)	N/A	+ (6 mo)	myoclonic	+ (waving hands in front of eyes)	N/A
(Paciorkowski et al., 2013)	P49	M	21mo	Deletion of ~2.0Mb	N/A	Unknown	N/A	+	N/A	-	- (some babbling)	N/A	-	-	+ (head shaking, leg kicking)	N/A
(Rocha et al., 2016)	P50	M	10yr	Missense Variant	c.9A>T het p.Arg3Ser	de novo	+	+ (inability to manipulate objects)	+	-	-	-	+ (26 mo)	epileptic seizures characterized by psychomotor arrest or sudden drops of head later with myoclonic seizures	+ (hand stereotypies)	+ (broad forehead, strabismus, large ears, flat nasal root, tented upper lip, everted lower lip, widely spaced teeth)
(Yuan et al., 2018)	P51	M	52yr	Nonsense Variant	c.471C>T het p.Tyr157*	Unknown (likely paternal due to pedigree)	+	N/A	N/A	N/A	-	N/A	+ (child)	epilepsy	+	N/A
(Yuan et al., 2018)	P52	M	43yr	Nonsense Variant	c.471C>T het p.Tyr157*	Unknown (likely paternal due to pedigree)	+	N/A	N/A	N/A	-	N/A	+ (child)	epilepsy	+	N/A

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Paciorkowski et al., 2013)	P45	-	-	+ (generally happy, with inappropriate laughter, poor eye contact in early childhood, but improving, no reciprocal play)	+ (severe GERD, in infancy)	N/A	N/A	-	hyperkinesis, bruxism, febrile seizures, breath-holding behavior, high pain tolerance	MEF2C
(Paciorkowski et al., 2013)	P46	-	-	+ (generally happy, with inappropriate laughter, easily excitable, inconsistent eye contact, no reciprocal play)	N/A	N/A	N/A	+ (sleep very disrupted in infancy now improving)	high pain tolerance, repetitive back arching, bruxism	MEF2C
(Paciorkowski et al., 2013)	P47	-	-	+ (eye contact emerged at 3 years, some reciprocal interactions, generally happy, with some inappropriate laughter)	+ (severe GERD, in infancy)	N/A	N/A	-	hand tremor, bruxism, high pain tolerance	MEF2C
(Paciorkowski et al., 2013)	P48	-	-	+ (irritable until 2.5 years; now generally happy with inappropriate nocturnal laughter, inappropriate pain response (laughs with vaccinations), poor visual fixation and attention, avoided eye contact until age 3 years)	+ (GERD in infancy, treated with medication and now resolved)	N/A	N/A	+ (difficult sleep onset and maintenance)	hyperkinesis, back arching, characteristic capillary malformation of the skin and atrophic skin adjacent to the suprasternal notch	MEF2C
(Paciorkowski et al., 2013)	P49	-	-	+ (generally happy, poor eye contact and visual tracking)	N/A	N/A	N/A	+ (difficulty with sleep onset)	hyperkinesis, high pain tolerance	MEF2C
(Rocha et al., 2016)	P50	+ (slight increase in periventricular white matter signal and global enlarged cerebrospinal fluid spaces including cortical sulcus)	+ (abnormal and slow background patter, focal right frontal hemispheric epileptic discharges with frequent generalization)	+ (poor eye contact and lack of interest in surroundings (sounds, lights, faces), hand to mouth including biting self)	N/A	N/A	N/A	N/A	strabismus convergens of left eye, hyperkinesis,	MEF2C only
(Yuan et al., 2018)	P51	N/A	N/A	N/A	N/A	+ (adult-onset dilated cardiomyopathy (DCM))	N/A	N/A	N/A	MEF2C only
(Yuan et al., 2018)	P52	N/A	N/A	N/A	N/A	+ (adult-onset dilated cardiomyopathy (DCM), ventricular septal defect (VSD))	N/A	N/A	N/A	MEF2C only

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features	
(Yuan et al., 2018)	P53	F	26yr	Nonsense Variant	c.471C>T het p.Tyr157*	Unknown (likely paternal due to pedigree)	+	N/A	N/A	N/A	-	N/A	+ (child)	epilepsy	+	N/A	
(Qiao et al., 2017)	P54	M	1yr	Missense Variant	c.113T>C het p.Leu38Pro	Paternal	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
(Qiao et al., 2017)	P55	M	26yr	Missense Variant	c.113T>C het p.Leu38Pro	Unknown (likely paternal due to pedigree)	+	N/A	N/A	N/A	N/A	N/A	+	paroxysmal epilepsy	+	N/A	
(Qiao et al., 2017)	P56	M	32yr	Missense Variant	c.113T>C het p.Leu38Pro	Unknown (likely paternal due to pedigree)	+	N/A	N/A	N/A	N/A	N/A	+	paroxysmal epilepsy	+	N/A	
(Qiao et al., 2017)	P57	F	5yr	Missense Variant	c.113T>C het p.Leu38Pro	Paternal	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
(Shim et al., 2015)	P58	F	6yr 7mo	Deletion of ~1.33Mb	N/A	de novo	+	+ (mild, head control at 5 mo, side-rolling at 7 mo)	+	N/A	- (vocalizations)	N/A	+ (N/A)	febrile convulsions with partial seizure	N/A	+ (broad frorehead and midly depressed nasal bridge)	
(Borlot et al., 2019)	P59	M	2yr	Missense Variant	c.236G>C het p.Arg79Pro	de novo	N/A	+ (not able to sit or roll over)	+	-	- (not able to imitate or babble)	-	+ (7 mo)	atypical febrile seizures with respiratory tract infection at 7mo consisting of focal motor seizures with unilateral but alternating left and right sided clonic activity, generalized myoclonias	N/A	N/A	(s w

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Yuan et al., 2018)	P53	N/A	N/A	N/A	N/A	+ (adult-onset dilated cardiomyopathy (DCM))	N/A	N/A	N/A	MEF2C only
(Qiao et al., 2017)	P54	N/A	N/A	N/A	N/A	+ (patent ductus arteriosus (PDA), ventricular septal defect (VSD), and family history of CHD)	N/A	N/A	N/A	MEF2C only
(Qiao et al., 2017)	P55	N/A	N/A	N/A	N/A	+ (patent ductus arteriosus (PDA), ventricular septal defect (VSD), Congenital heart disease (CHD))	N/A	N/A	N/A	MEF2C only
(Qiao et al., 2017)	P56	N/A	N/A	N/A	N/A	+ (patent ductus arteriosus (PDA), pulmonary stenosis, Congenital heart disease (CHD))	N/A	N/A	N/A	MEF2C only
(Qiao et al., 2017)	P57	N/A	N/A	N/A	N/A	+ (patent ductus arteriosus (PDA))	N/A	N/A	N/A	MEF2C only
(Shim et al., 2015)	P58	+ (delayed myelination)	+	+ (paid little attention to stimuli including calling her name, couldn't recognize family members)	N/A	N/A	+ (bilateral esotropia (corrective surgery at 8mo))	N/A	poor hand-eye coordination, not toilet trained, mild cervicothoracic scoliosis, bilateral coxa valga, and pes planus	MEF2C only
(Borlot et al., 2013)	P59	+ (small areas of non-specific T2 white matter hyperintensity in parietal lobes)	+ (7mo, high voltage generalized spike-and-waves ad polyspikes with alternating right and left frontal predominance. 12 and 15mo follow ups, independent bilateral multifocal spike-and-slow wave discharges, high voltage generalized polyspikes/spike-and-slow waves)	+ (impaired awareness, startles easily to loud noises or sudden sensory stimulation)	N/A	N/A	N/A	N/A	N/A	MEF2C only

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Hotz et al., 2013)	P60	M	2yr	Deletion of ~4.1Mb	N/A	de novo	N/A	+ (unable to sit or roll and no head control)	+	+	N/A	N/A	+ (13 mo)	N/A	N/A	+ (broad and short forehead, a broad nasal bridge, upslanting palpebral fissures, and small eyes)
(Hotz et al., 2013)	P61	M	4yr	Deletion of ~1.7Mb	N/A	de novo	+	+ (sat undaided at 18mo, able to crawl and stand)	+	-	-	- (can only walk with aid)	+ (10 mo)	myoclonic epilepsy	N/A	+ (broad forehead and downslanting palpebral fissures)
(Boutry-Kryza et al., 2015)	P62	F	4yr	Deletion of ~3.2Mb	N/A	Unknown	+	+	N/A	-	N/A	N/A	+ (4 mo)	infantile spasms	+	N/A
(Zweier et al., 2010)	P63	F	2yr 2mo	Deletion of ~2.4Mb	N/A	de novo	+	+	+	-	-	-	+ (1 yr)	febrile	N/A	+ (large ears, broad forehead, prominent ear lobe, mild upslanting palpebral fissures, widely spaced teeth, tented upper lip)
(Zweier et al., 2010)	P64	F	3yr	Missense Variant	c.113T>A het p.Leu38Gln	de novo	+	+	+	-	-	-	+ (10 mo)	N/A	N/A	+ (large ears, broad forehead, prominent ear lobe, mild upslanting palpebral fissures, widely spaced teeth, cupid bowed upper lip)
(Zweier et al., 2010)	P65	M	14yr	Frameshift Variant	c.33dupT het p.E34X	de novo	+	+	+	-	-	+ (2 yr 8 mo)	+ (10 mo)	complex partial	N/A	+ (large ears, broad forehead, prominent ear lobe, mild upslanting palpebral fissures, tented upper lip in infancy, now cupid bowed upper lip)
(Zweier et al., 2010)	P66	F	7yr	Frameshift Variant	c.226_236delCATGAGAGCCG del p.His76AspfsTer15	de novo	+	+	+	-	-	-	+ (3-6 mo)	N/A	N/A	+ (broad forehead, prominent ear lobe, widely spaced teeth, tented upper lip)
(Zweier et al., 2010)	P67	F	10yr 5mo	Missense Variant	c.80G>C het p.Gly27Ala	de novo	+	+	+	-	-	- (walked with support at 8 yr)	+ (6 mo)	N/A	N/A	+ (large ears, broad forehead, fleshy prominent ear lobe, downslanting palpebral fissures, crowded teeth, full upper lip)
(Zweier et al., 2010)	P68	M	3yr	Deletion of ~1.5Mb	N/A	mother WT; father unknown	+	N/A	+	-	-	-	+ (3 mo)	spasms, myoclonic	+ (hand stereotypies)	+ (large ears, broad forehead, prominent ear lob, slighly cupid bowed upper lip)
(Sakai et al., 2013)	P69	M	14yr	Deletion of ~7.4Mb	N/A	Unknown	+	+	N/A	N/A	N/A	N/A	+ (3 mo)	spasms, epilepsy	N/A	+ (broad forehead, hypertelomeric, down-slanted palpebral fissures, backward-positioned low-set ears, and upward-protruding, cupid-like lips)

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Hotz et al., 2013)	P60	+ (small forebrain with especially small frontal lobes, partial agenesis of corpus callosum, simplified gyral patteran and shallow sulci of anterior basal frontal lobes and anterior temporal lobes, bilateral enlargement of the posterior horns resulting in colpocephaly. Slim brainstem and enlarged cisterna magna, malrotated hippocampi)	+ (nonspecific abnormalities)	N/A	N/A	N/A	N/A	+	hydrocephalus and wide colon convolutions by ultrasound, could not visually track or fix or grasp objects, tendency towards opisthotonic body movements, inverted mamillae, small sacral dermal appendage, cafe au lait spot on back.	MEF2C and ARRDC3
(Hotz et al., 2013)	P61	+ (slightly reduced volume of the frontal lobes, with slightly broadened gyri and widened subarachnoid space around the anterior frontal lobes.)	+ (highly pathological revealing myoclonic epilepsy)	+ (autistic behavior)	N/A	N/A	N/A	N/A	strabismus divergens	MEF2C and disrupted GPR98
(Boutry-Kryza et al., 2015)	P62	N/A	N/A	+ (autistic behavior)	N/A	N/A	N/A	N/A	Hypsarrhythmia	(not including MEF2C)
(Zweier et al., 2010)	P63	+ (mildly enlarged extracerebral CSF space, two unspecific white matter lesions in the internal capsule/insular and the parietodorsal regions)	N/A	N/A	N/A	N/A	+ (strabismus)	+	dystonia	MEF2C Exons 5-11
(Zweier et al., 2010)	P64	+ (generalized lack of white matter bulk and delay in myelin maturation)	N/A	N/A	N/A	N/A	+ (strabismus)	N/A	high glycine in urine	MEF2C only
(Zweier et al., 2010)	P65	+ (mildly enlarged ventricles)	N/A	+ (autistic behavior)	+ (needs feeding)	N/A	+ (hypermetropia)	N/A	daytime continence	MEF2C only
(Zweier et al., 2010)	P66	N/A	N/A	N/A	N/A	N/A	+ (strabismus)	+	heterochromasia, high pain tolerance, joint hyperlaxity	MEF2C only
(Zweier et al., 2010)	P67	+ (mild under myelinisation of insular cortices bilaterally)	N/A	N/A	N/A	N/A	+ (strabismus)	N/A	episodic hyperventilation, nails grow quickly, thick hair	MEF2C only
(Zweier et al., 2010)	P68	+ (mild under myelinisation)	N/A	+ (autistic behavior)	N/A	N/A	+ (strabismus, nystagmus)	N/A	N/A	MEF2C
(Sakai et al., 2013)	P69	+ (presence of ischemic lesions and structural anomalies of the hypothalamus)	N/A	N/A	+ (episodes of appetite loss)	N/A	N/A	N/A	mild to moderate hypoglycemia, hypothermia	MEF2C

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Wang et al., 2018)	P70	F	5yr 9mo	Missense Variant	c.48C>G het p.Asn16Lys	de novo	N/A	+ (raised head at 8mo, sat alone at 1yr)	+	N/A	N/A	-	+ (20 mo)	epileptic attack	+ (hand clapping and wringing)	N/A
(Wang et al., 2018)	P71	F	2.5yr	Nonsense Variant	c.565C>T het p.Arg189*	mother WT; father unknown	+	+	+	-	-	-	-	N/A	+ (hand stereotypies)	N/A
(Wang et al., 2018)	P72	F	23mo	Nonsense Variant	c.334G>T het p.Glu112*	father WT; mother unknown	N/A	+	+	-	-	+ (23 mo, abnormal gait)	+ (9 mo)	febrile convulsions	+	N/A
(Wang et al., 2018)	P73	M	7yr 8mo	Splicing Variant	c.403-1G>T	de novo	+	+ (raised head at 1 yr, sat alone at 1 yr 2 mo)	N/A	N/A	-	+ (1.5 yr)	+ (1 yr)	febrile seizures at 1 yr, turned to afebrile at 2 yr, partial seizures	N/A	N/A
(Wang et al., 2018)	P74	M	6yr 4mo	Nonsense Variant	c.766C>T het p.Arg256*	de novo	+	+ (raised head at 7 mo, sat alone at 1 yr)	N/A	-	- (only a few words)	+ (2 yr)	+ (8 mo)	febrile	+ (hand stereotypies)	N/A
(Tanteles et al., 2015)	P75	M	14yr	Deletion of ~147Kb	N/A	de novo	+	+ (sat unaided at 8 mo)	N/A	N/A	-	+ (2.5 yr, wide gait)	-	N/A	+ (hand biting and hand flapping, head banging)	+ (broad forehead, cupid's bow upper lip, toes curled and broad, dupernumerary nipple, bilateral inguinal hernias which was repaired)
(Cardoso et al., 2009)	P76	M	7yr	Translocation der(5) del(5) (q14;q21) t(1;5) (q31;q14)	N/A	de novo	+	+	+	N/A	-	+ (5 yr)	+ (1 yr)	febrile then generalized tonic-clonic seizures at 6yr	N/A	+ (high forehead, hypertelorism, high arched eyebrows, mild downward slanting of the palpebral fissures, depressed nasal bridge, thick columella, and a flat long philtrum)
(Cardoso et al., 2009)	P77	F	5yr	Deletion of ~8.4Mb	N/A	de novo	+	+	N/A	N/A	-	N/A	+ (9 mo)	infantile spasms	N/A	+ (high forehead, frontal bossing, hypertelorism, anteverted nostrils, high arched eyebrows, depressed nasal bridge, thick columella, long philtrum, thin lips, and micrognathia)
(Cardoso et al., 2009)	P78	M	5yr	Deletion of ~6.3Mb	N/A	de novo	-	+	+	N/A	-	+ (3 yr)	+ (18 mo)	myotonic jerks	N/A	+ (right postaxial polydactyly of his toes at birth, triangular shaped head)

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Wang et al., 2018)	P70	+ (enlargement of frontal subarachnoid space)	+ (spike-slow waves at righ tmedial and posterior temporal, with generalization)	+ (poor eye contact)	N/A	N/A	N/A	N/A	bruxism, deterioration of hand skills, hypalgesia	MEF2C only
(Wang et al., 2018)	P71	+ (high T1 and T2 signal at posterior horn of bilateral ventricle)	+ (epileptic discharge although not had seizures)	N/A	N/A	N/A	N/A	N/A	bruxism, poor hand skills	MEF2C only
(Wang et al., 2018)	P72	N/A	N/A	N/A	+ (feeding difficulties)	N/A	N/A	+	poor eye contact, breathing disturbances, recurrent respiratory infections at 1yr 10mo, irritability, poor hand skills	MEF2C only
(Wang et al., 2018)	P73	-	+ (multi spike and slow waves at right occipital region, with slow rhythm on the background)	+ (little interest in others, lacked eye contact)	N/A	N/A	N/A	N/A	N/A	MEF2C only
(Wang et al., 2018)	P74	+ (long T1 and T2 signal around bilateral ventricle and a septum pellucidum cyst)	-	+ (no eye contact, no interest in others, autistic behavior)	N/A	N/A	N/A	N/A	N/A	MEF2C only
(Tantcles et al., 2015)	P75	N/A	N/A	+ (scared of loud noises)	N/A	N/A	+ (myopia)	-	sister had mitral valve prolapse, jerking episodes involving his feet while sleep at 8mo but it resolved after a month, not potty trained, left-sided Perthese disease, enjoyed water, two small hyperpigmented and one hypopigmented macule on chest, swallow broad jugula rpit with overlying cutaneous capillary malformation	MEF2C Exons 1-3
(Cardoso et al., 2003)	P76	+ (bilateral Periventricular heterotopia involving temporal and frontal horns)	-	N/A	N/A	N/A	+ (coloboma of the left iris at birth, left eye exotropia)	N/A	N/A	(MEF2C not included)
(Cardoso et al., 2003)	P77	+ (bilateral PH involving the temproal and occipital horns)	+ (poorly organized background activity and multifocal epilptiform discharges)	N/A	N/A	N/A	N/A	N/A	pes talus at birth	MEF2C
(Cardoso et al., 2003)	P78	+ (bilateral PH, involving the temporal and occipital horns. under rotated hippocampi, more severely on the right and irregular thickening and folding of the cortex in the posterior perisylvian regions, consistent with polymicrogyria)	+ (showed bursts of multifocal and bilaterally synchronous epileptiform activity)	N/A	N/A	N/A	N/A	N/A	episodes of unresponsiveness lasting 10-20 seconds which occurred many times a day from 8mo, macrocephaly	(MEF2C not included)

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Cesaretti et al., 2016)	P79	unknown	20 weeks gestation	Deletion of ~4.6Mb	N/A	de novo	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
(Cesaretti et al., 2016)	P80	unknown	20 weeks gestation	Deletion of ~4.6Mb	N/A	de novo	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
(Novara et al., 2010)	P81	M	14yr	Deletion of ~318Kb	N/A	mother WT; father unknown	+	+ (sitting with little support at 2yr)	+	-	-	-	+ (less than 1 yr)	epilepsy, infantile spasms, myoclonic jerks	-	+ (prominent ear lobes, short prominent philtrum with a cupids bow and macrodontia)
(Novara et al., 2010)	P82	M	3yr 10mo	Deletion of ~1.1Mb	N/A	de novo	+	+ (absent head control)	+	-	-	N/A	+(5 mo)	myoclonus	+	+ (occipital plagiocephaly, hypertelorism, flattened nasal bridge, small and hook nose, ogival palate, low set and dysmorphic ears)
(Bienvenu et al., 2013)	P83	F	8yr 2mo	Frameshift Variant	c.457delA het p.Asn153ThrfsX33	de novo	+	+	+	-	-	+ (4 yr, unstable wide based gait)	+ (18 mo)	single episode of myoclonic febrile seizures at 18mo	+ (hand mouthing)	+ (large eyebrows, open mouth with thick everted lower lip, and anteverted nares)
(Nowakowska et al., 2010)	P84	M	3yr	Deletion of ~140Kb	N/A	de novo	N/A	+	+	-	-	+ (3 yr, but unstable wide-based gait)	+ (N/A)	epilepsy	N/A	+ (frontal bossing, mild bilateral epicanthus, a broad nose, and full lips, open mouth)

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Cesaretti et al., 2016)	P79	+ (By ultrasound: short corpus callosum (10mm of anterior-posterior diameter), partial agensis of corpus callosum. Autopsy found short and thin corpus callosum with rudimentary genu deteached from the corpus callosum.)	N/A	N/A	N/A	+ (bi-ventricular hypertrophy and moderate tricuspid valve insufficiency, moderate bilateral ventricular valve insufficiency)	N/A	N/A	low nuchal translucency, pregnancy was terminated	MEF2C, RASA1, GPR98
(Cesaretti et al., 2016)	P80	+ (By ultrasound: bilateral mild veentriculomegaly with width of posterior horns of 11mm. Short corpus callosum (13mm of anterior-posterior diameter), bilateral mild ventriculomegaly. partial agensis of corpus callosum. Autopsy found short and thin corpus callosum with no detectable region of genu.)	N/A	N/A	N/A	N/A	N/A	N/A	low nuchal translucency, pregnancy was terminated, abdominal circumference was <5th centile, had oligohydramnios and small bladder	MEF2C, RASA1, GPR98
(Novara et al., 2010)	P81	+ (cystic lesion and leucoencephalopathy in left frontal region, likely due to perinatal hemorrhage, periventricular leucomalacia and atrophy of frontal cortex at left side)	+ (continuous epileptic activity bi-posterior with no basic rhythm)	+ (absent eye contact and social smile at 3mo, irritable behavior)	N/A	N/A	+ (external strabismus)	N/A	Mother and cousin have epilepsy, regression to need wheelchair, cerebral palsy with severe axial hypotonia and compensatory peripheral hypertonia	MEF2C only
(Novara et al., 2010)	P82	+ (moderate dilatatio of lateral ventricles and hypoplasia of the corupe callosum with abnormal aspect of the splenius)	+ (slow background activity with theta waves degraded over the central regions of the two hemispheres and degraded diffuse discharges, sometimes with episodes of rhythmic sharp wave activity)	+ (lack of reactivity, poor visual tracking at 4 mo, could fix and follow objects)	N/A	N/A	+ (myopia with alternating esotropia)	N/A	increased muscle tone in lower limbs with dystonic-dyskinetic movements	MEF2C, TMEM161B
(Bienvenu et al., 2013)	P83	-	+ (low generalized spike and wave, sometimes massive myoclonies sometimes followed by spikes bifrontal slow waves)	+ (poor eye contact, happy behavior)	+ (severe feeding difficulties)	N/A	;+ (strabismus)	N/A	N/A	MEF2C only
(Nowakowska et al., 2010)	P84	+ (mild thinning of the corpus callosum and delay of white matter myelination in the occipital lobes)	+ (abnormal sleep architecture and generalized discharges localized to the posterior regions)	+ (autistic behaviors)	N/A	N/A	N/A	N/A	Periodic tremor and abnormal motor pattern with mirror movement of upper limbs in infancy, bruxism	MEF2C Exons 1-3

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features	
(Nowakowska et al., 2010)	P85	F	30mo	Deletion of ~1.8Mb	N/A	de novo	N/A	+	+	-	N/A	N/A	+ (15 mo)	generalized	N/A	N/A	(
(Nowakowska et al., 2010)	P86	F	34mo	Deletion of ~2.4Mb	N/A	de novo	N/A	+	+	+	- (some vocalizations)	N/A	+ (14 mo)	two bilateral tonic-clonic seizures with fever, extensor myoclonus on awakening	N/A	+ (thin nose, asymmetric ears, a short philtrum, micrognathia, and a pectus excavatum)	
(Nowakowska et al., 2010)	P87	F	18mo	Deletion of ~5.7Mb	N/A	de novo	N/A	+	+	+	- (babbles)	N/A	+ (3-4 mo)	infantile seizures, generalized tonic-clonic seizures once a month and infantile spasms weekly despite medicine	N/A	+ (brachycephaly, a wide nasal bridge, down-turned corners of her mouth with a cupidbow upper lip)	
(Gordon et al., 2018)	P88	M	2.5yr	Frameshift Variant	c.146dup het p.Asn49Lysfs*23	Unknown	N/A	+	+	-	-	-	-	N/A	+ (hand stereotypies, grasping at the midline and flapping)	+ (right question mark ear (QME), dysplastic left ear with normal ear canals and a normal oral cavity, hooked first toes)	
(Vidal et al., 2019)	P89	F	24yr	Missense Variant	c.48C>G het p.Asn16Lys	de novo	+	+	+	N/A	- (only a few words)	- (walk with support but unstable wide-based gait)	+ (N/A)	epilepsy	+ (hand stereotypies)	N/A	
(Vidal et al., 2019)	P90	F	6yr	Frameshift Variant	c.513_514insGA het p.Leu172Aspfs*16	de novo	+	+	+	N/A	- (only a few words)	+ (N/A)	+ (N/A)	epilepsy	+ (hand stereotypies)	N/A	
(Vidal et al., 2019)	P91	F	8yr	Frameshift Variant	c.959_960delGT het p.Gly320Aspfs*7	de novo	+	+	+	N/A	-	- (needs support and has unstable wide-based gait)	+ (N/A)	epilepsy	+ (hand stereotypies)	N/A	

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Nowakowska et al., 2010)	P85	+ (colpocephaly and an incidental pineal cyst, Ventricles were borderline large)	N/A	+ (poor visual tracking, little social interaction, now has episodes of startling)	+ (period of failure to thrive with no weight gain prompted G-tube placement)	N/A	N/A	N/A	hypoteloric, opisthotonic posturing, reflexes are brisk and toes are downgoing	TMEM161B and MEF2C
(Nowakowska et al., 2010)	P86	+ (thinning of the corpus callosum, most prominent in the splenium, and mild global white matter loss, but no periventricular heterotopias)	(multiple, generalized spike and poly-spike, and slow wave discharges at 15 mo., generalized semi-rhythmic bursts of polyspike and wave activity at 19 mo)	N/A	+ (G-tube fed, constipation)	+ (heart murmur but normal echocardiogram)	+ (bilateral esotropia, mild bilateral ptosis)	N/A	failure to thrive, rigid posture, bruxism	MEF2C
(Nowakowska et al., 2010)	P87	+ (a shorter than expected corpus callosum, prominent lateral, third, and fourth ventricles, slightly wide sylvian fissures, and small frontal lobes with a paucity of the cerebral gyri. Focal increased T2 signal was detected within the globus pallidi. The gray–white matter interface within the temporal lobes appeared ill defined, suggesting either delayed myelination or cortical dysplasia) PET scanning (hypermetabolism in the right cerebellum with hypometabolism in the left hemisphere and was diffusely suggestive of a cortical dysplasia)	+ (frequent and spike and wave activity in the left temporal-occipital and left central temporal regions as well as spikes in the right occipital area)	+ (no temperment problem)	N/A	N/A	N/A	N/A	quick jerking movements, 10-25th percentile for weight, upper extremity and truncal hypotonia with increased tone in her lower extremities	MEF2C
(Gordon et al., 2018)	P88	-	N/A	+ (poor eye contact, unable to mimic or play symbolic games)	+ (cannot eat unassisted)	N/A	N/A	N/A	fascinated by opening and closing doors, kyphosis at sitting position	MEF2C only
(Vidal et al., 2019)	P89	-	N/A	+ (Autistic features)	N/A	N/A	N/A	N/A	N/A	MEF2C only
(Vidal et al., 2019)	P90	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	MEF2C only
(Vidal et al., 2019)	P91	-	N/A	+ (Autistic features)	N/A	N/A	N/A	N/A	N/A	MEF2C only

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Vidal et al., 2013)	P32	F	18yr	Stop loss	c.1421G>T p.*473Lnext*58	de novo	+	+	+	N/A	-	+ (walked aided at 3 yr, eventually independent)	-	N/A	+ (hand stereotypies)	N/A
(Schluth-Bolard et al., 2013)	P33	M	3yr	Insertion of ~ ins(5)(q14.2q 23.2q34)	N/A	de novo	+	N/A	N/A	+	N/A	N/A	+ (N/A)	epilepsy, hyperthermi c seizures	N/A	N/A
(Schluth-Bolard et al., 2013)	P34	F	5yr	Translocation t(1;14)(q32.1; q21.3) with 5q14.3 region (MEF2C) put on chr1	N/A	de novo	+	N/A	N/A	N/A	-	N/A	+ (N/A)	epilepsy	+	N/A
(Schluth-Bolard et al., 2013)	P35	F	11yr	Translocation t(1;3;5)(p22.2 ;p24.3;q33.2)	N/A	de novo	+	+	N/A	N/A	-	+ (3 yr)	+ (N/A)	epilepsy	N/A	+
(Ohdo et al., 1982)	P36	F	7mo	Deletion of ~48Mb del(5)(q13q2 2)	N/A	de novo	N/A	+	N/A	+	N/A	N/A	N/A	N/A	N/A	+ (short neck, reduced weight gain, coarse and abundant hair, narrow forehead with hypertrichosis, flat occiput, hypertelorism, short nose with anteverted nostrils, a large philtrum with a deep groove, cleft palate, retromicrognathia, simply formed auricle on the right, imperforate anus with rectoperineal fistula, camptodactyly of the right third finger and left second finger, and bilateral pes adductus. single transverse flexion crease on her left palm with a transitional crease on her right palm.)
(Stoll et al., 1980)	P37	M	6mo	Deletion of ~31Mb del(5)(q13q15)	N/A	de novo	N/A	+	N/A	+	N/A	N/A	N/A	N/A	N/A	+ (small and narrow forehead, a small, broad, upturned nose, a flat nasal bridge, hypertelorism, upward curving eyelashes, a large prominent metopic suture, a triangular shaped mouth, a large philtrum with a deep groove, retromicrognathia, large ears, short neck, short upper limbs, syndactyly of the big toe and the 3rd and 4th toes, and clinodactyly of the 5th finger)
(Sobreira et al., 2003)	P38	M	11yr	Deletion of ~7.4Mb	N/A	Unknown	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	+	+ (down-slanting palpebral fissures, cup-shaped ears, misplacement of frontal/lateral incisors, brachydactyly of hands, bilateral clinodactyly of the fifth fingers, and small feet),

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Vidal et al., 2013)	P92	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	MEF2C only
(Schluth-Bolard et al., 2013)	P93	N/A	N/A	N/A	+ (IUGR, post-natal growth retardation)	N/A	N/A	+	choreic and dystonic abnormal movements, stabismus	MEF2C
(Schluth-Bolard et al., 2013)	P94	N/A	N/A	N/A	+ (constipation)	N/A	+ (myopia)	N/A	N/A	MEF2C
(Schluth-Bolard et al., 2013)	P95	N/A	N/A	+ (autistic spectrum disorder)	+ (constipation)	N/A	N/A	N/A	N/A	MEF2C
(Ohdo et al., 1982)	P96	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	MEF2C
(Stoll et al., 1980)	P97	N/A	N/A	N/A	N/A	+ (murmur)	N/A	N/A	N/A	MEF2C
(Sobreira et al., 2003)	P98	+ (mild delay in myelination but no structural anomaly)	N/A	+ (severe attention deficit hyperactivity disorder, aggressive behaviors)	N/A	N/A	+ (bilateral iris coloboma with small optic nerves)	N/A	short stature, high frequency hearing loss, dental anomaly	(MEF2C not included)

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Floris et al., 2007)	P99	M	5.5yr	balanced translocation, t(5;8)(q14.3;q23.3)	N/A	de novo	+	+	N/A	N/A	-	+ (N/A)	+ (N/A)	Epilepsy	+	N/A
(Ramji et al., 2020)	P100	F	23mo	N/A	N/A	N/A	N/A	N/A	N/A	+	N/A	N/A	+ (9mo)	Generalized seizures.	N/A	+
(Raviglione et al., 2021)	P101	M	17yr	Deletion of ~11.5Mb	N/A	unknown	+	+	N/A	N/A	-	N/A	-	N/A	N/A	+
(Raviglione et al., 2021)	P102	M	12yr	Deletion of ~5Mb	N/A	unknown	+	+	N/A	N/A	N/A	N/A	+ (2mo)	Epileptic encephalopathy with epileptic spasms, myoclonic seizures, generalized bilateral tonic-clonic seizure.	N/A	+
(Raviglione et al., 2021)	P103	F	1yr	Deletion of ~4.3Mb	N/A	de novo	+	+	N/A	N/A	N/A	N/A	+ (7mo)	Generalized epilepsy, epileptic encephalopathy with epileptic spasms, generalized bilateral tonic-clonic seizure, focal motor seizure.	N/A	+

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Floris et al., 2007)	P33	+ (periventricular leukomalacia more prevalent in left cerebral hemisphere)	+ (multifocal, parossistic an dpolymorphic anomalies, especially in anterior cerebral area)	+ (no social interest, short attention span, autism)	N/A	N/A	N/A	N/A	mother had complicated pregnancy (funiculuar knot in 3rd month, oligohydramnio since 5th month, fetal growth retardation since 32nd week), wide-based gait, right hemiparesis, neonatal brain ultrasonography (calcifications in thalamus and nucleus dentatus bilaterally)	breakpoint upstream of *MEF2C*
(Ramji et al., 2020)	P100	+ (Features of AED with bilateral symmetrical diffusion restriction in the cerebral white matter andstriking T2 hyperintensity in the juxtacortical U-fibres.0	N/A	N/A	N/A	N/A	+ (Squint and delayed visual development)	N/A	Pregnancy complicated by probable maternal hemolysis, elevated liver enzymes, and low platelet count syn-drome (HELPP).	*MEF2C*
(Raviglione et al., 2021)	P101	+ (Altered venous drainage in right cerebellar hemisphere and in the parieto-occipital regions, cavum vergae, cavum septi pellucidi, mild posterior corpus callosum thinning.)	+ (Background diffuse excess of fast activity.)	+ (Autistic features)	N/A	N/A	N/A	N/A	N/A	*MEF2C, COX7C, RASA1, CCNH, LINC00461, MIR9-2, CETN3, POLR3G, ADGRV1, LUC7L1, ARRDC3, NR2F1, KIAA0825, SLF1, INCTP1, TTC37, ARSK, SPATA5, RHOBTB3, GLRX, ELL2, PCSK1, CAST, ERAP1, ERAP2, LNPEP, LIX1, RIOK2*
(Raviglione et al., 2021)	P102	+ (Hypoplastic corpus callosum, delayed myelination.)	+ (Generalized polyspike wave complexes and bilateral asynchronous epileptiform discharges predominant in posterior/tempo ral regions during sleep, Rhythmic theta activity during wakefulness.)	-	N/A	N/A	N/A	N/A	N/A	*MEF2C, COX7C, RASA1, CCNH, LINC00461, MIR9-2, CETN3, POLR3G, ADGRV1* (partially involved)
(Raviglione et al., 2021)	P103	+ (Small focal white matter alterations in the right mesial temporal region and both occipital lobes, hypoplastic corpus callosum, alterations of white matter mesial temporal right, delayed myelination, frontal lobe atrophy, mild lateral ventricles enlargement.)	+ (Focal or multifocal spikes, hypsarrhythmia, slowing background.)	-	N/A	N/A	N/A	N/A	N/A	*MEF2C, LINC00461, MIR9-2, CETN3, POLR3G, ADGRV1, LUC7L1, ARRDC3*

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Raviglione et al., 2021)	P104	F	3yr	Deletion of ~3.6Mb	N/A	unknown	+	+	N/A	N/A	-	N/A	+ (6mo)	"Generalized myoclonic epilepsy" spectrum, generalized epilepsy, myoclonic seizures, absence seizures.	N/A	+
(Raviglione et al., 2021)	P105	M	4yr	Deletion of ~2Mb	N/A	unknown	+	+	N/A	N/A	-	N/A	+ (3mo)	Febrile Seizures, myoclonic seizures, generalized bilateral tonic-clonic seizure, absence seizures, spasms.	N/A	+
(Raviglione et al., 2021)	P106	M	9yr	Deletion of ~1.7Mb	N/A	de novo	+	+	N/A	N/A	-	N/A	+ (14mo)	"Generalized myoclonic epilepsy" spectrum, Myoclonic seizures, focal motor seizure with impairment of awareness, atonic.	N/A	+
(Raviglione et al., 2021)	P107	M	7yr	Deletion of ~522Kb	N/A	de novo	+	+	N/A	N/A	-	N/A	+ (7mo, 18mo)	Febrile Seizure, focal motor seizure, focal seizure with impairment of awareness (7mo, 18mo).	N/A	+

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Raviglione et al., 2021)	P104	+ (Cerebellar vermis hypoplasia, IV ventricle-lateral ventricles enlargment, hippocampal abnormalities, cavum vergae, cavum septipellucidi, empty sella. periventricular white matter abnormalities, hypoplastic corpus callosum, delayed myelination.)	+ (Diffuse discharge of spikes and poly-spikes and waves. Abnormal sleep pattern. At wake spikes and waves related to eyelid myoclonias, impairment of awareness, jerks at arms. in wakefulness spikes and waves complex in frontotemporal regions (right>left), slowing background.)	-	N/A	N/A	N/A	N/A	N/A	*MEF2C, COX7C, RASA1, CCNH, LINC00461, MIR9-2*
(Raviglione et al., 2021)	P105	+ (Hypoplastic corpus callosum, delayed myelination, hypoplasia cerebellar vermis.)	+ (Focal or multifocal spikes, high amplitude spike/poly spike and slow wave complexes, hypsarrhythmia, slowing background.)	+ (Autistic features)	N/A	N/A	N/A	N/A	N/A	*MEF2C, LINC00461, MIR9-2, CETN3, POLR3G, ADGRV1* (partially involved)
(Raviglione et al., 2021)	P106	N/A	+ (Bilateral temporooccipital spikes and spike-waves, slowing background.)	+ (Autistic features, happy demeanor.)	N/A	N/A	N/A	N/A	N/A	*MEF2C, RASA1, CCNH, LINC00461, MIR9-2*
(Raviglione et al., 2021)	P107	-	+ (Bisynchronous high voltage generalized slow spike and wave complexes, more evident in frontal regions, increased in sleep, slowing background.)	+ (Autistic features)	N/A	N/A	N/A	N/A	N/A	*MEF2C* exons 1-2

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Raviglione et al., 2021)	P108	F	8yr	Deletion of ~354Kb	N/A	unknown	+	+	N/A	N/A	-	N/A	+ (1yr)	Focal epilepsies, unilateral myoclonic seizures.	N/A	+
(Raviglione et al., 2021)	P109	F	3yr	Deletion of ~257Kb	N/A	de novo	+	+	N/A	N/A	N/A	N/A	-	N/A	N/A	+
(Raviglione et al., 2021)	P110	F	18yr	Deletion of ~138.6Kb	N/A	unknown	+	+	N/A	N/A	-	N/A	+ (7mo)	Complex Febrile Seizures	N/A	+
(Raviglione et al., 2021)	P111	M	11yr	Deletion of ~97Kb	N/A	de novo	+	+	N/A	N/A	-	N/A	+ (1yr, 2yr)	Complex febrile seizures (1yr) focal motor seizure with impairment of awareness (2yr).	N/A	+
(Raviglione et al., 2021)	P112	M	10yr	Deletion of ~74Kb	N/A	de novo	+	+	N/A	N/A	-	N/A	+ (2yr, 3yr)	Generalized epilepsy in the GEFS+ spectrum showing bilateral tonic-clonic seizures often induced by fever, Focal Motor Seizures (2yr) generalized bilateral tonic-clonic seizure (3yr).	N/A	+

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Raviglione et al., 2021)	P108	+ (Frontal cortical atrophy and enlarged cisterna magna, partial agenesis corpus callosum, enlarged lateral ventricles.)	+ (Diffuse delta activity and bisynchronous high-voltage generalized slow spike and wave complexes, more evident in frontal regions right>left, slowing background.)	-	N/A	N/A	N/A	N/A	N/A	*MEF2C* exons 1-4
(Raviglione et al., 2021)	P109	N/A	-	-	N/A	N/A	N/A	N/A	N/A	*MEF2C* exon 1
(Raviglione et al., 2021)	P110	+ (Delayed myelination.)	+ (Diffuse frontal dominant discharges of high voltage slow waves, slowing background.)	+ (Autistic features, happy demeanor.)	N/A	N/A	N/A	N/A	N/A	*MEF2C* exons 1-2
(Raviglione et al., 2021)	P111	+ (Abnormalities in the posterior fossa included Chiari Type 1 malformation.)	+ (Slowing of background activity (theta and/or delta waves in parieta and occipital regions), focal or multifocal spikes, increase incidence of focal or multifocal spikes during sleep, slowing background.)	+ (Autistic features)	N/A	N/A	N/A	N/A	N/A	*MEF2C* exons 1-3
(Raviglione et al., 2021)	P112	+ (Non specific hyperintensity spot in frontal white matter, abnormal venous drainage in right parietal-occipital regions.)	+ (Frontal spikes discharges; centro temporal spikes, bilateral asynchronous > right, irregular organization of activity during sleep.)	+ (Autistic features)	N/A	N/A	N/A	N/A	N/A	*MEF2C* exon 2 and part of exon 3

	Patient	Sex	Age	Variation Type	Variation	Inheritance Pattern	ID	DD	Hypotonia	Microcephaly	Speech	Independent Walking / Age	Seizures / Age	Seizure Type	Stereotypic Movements	Dysmorphic Features
(Raviglione et al., 2021)	P113	M	7yr	Splicing Variant	c.52_54+4delCAGGTGA	de novo	+	+	N/A	N/A	-	N/A	+ (3mo, 3yr)	Generalized epilipsy, focal epilepsies, febrile seizures, focal motor seizures (3mo), generalized bilateral tonic-clonic seizure epileptic status (3yr).	N/A	+
(Raviglione et al., 2021)	P114	F	8yr	Missense Variant	c.83T>C, p.Leu28Ser	de novo	+	+	N/A	N/A	-	N/A	+ (15mo)	Complex Febrile Seizure	N/A	+
(Raviglione et al., 2021)	P115	F	10yr	Missense Variant	c.526G>A, p.Gly176Ser	unknown	+	+	N/A	N/A	N/A	N/A	-	N/A	N/A	+
(Raviglione et al., 2021)	P116	F	22yr	Frameshift Variant	c.45dupT, p.Asn16Ter (rs1554150552)	unknown	+	+	N/A	N/A	N/A	N/A	+ (8mo)	Focal epilepsies, unilateral myoclonic seizures, focal motor seizures, absence seizures.	N/A	+
(Raviglione et al., 2021)	P117	M	2yr	Nonsense Variant	c.712C>T, p.Arg238Ter	de novo	+	+	N/A	N/A	N/A	N/A	+ (10mo, 15mo)	"Generalized myoclonic epilepsy" spectrum, Focal Epilepsy, Focal Motor Seizures (10mo) Myoclonic Seizures (15mo).	N/A	+

*Affected genes are not the complete list of genes deleted, inserted, duplicated, or translocated. Only MEF2C and genes specifically mentioned by the authors were included in this column.
N/A = not applicable, data not available for this particular feature

	Patient	Abnormal MRI	Abnormal EEG	Social and Behavioral Issues	Feeding and Digestion Issues	Cardiac Issues	Vision Issues	Sleeping Issues	Other	MEF2C Affected? Other Relevent Genes?*
(Raviglione et al., 2021)	P113	+ (Decrease in white matter volume in temporal-parietal-occipital regions, very thin corpus callosum, mild dilatation of the lateral ventricles with dilated frontal horns, hippocampal abnormalities, reduction of white matter thickness in temporal-parietaloccipital regions.)	+ (Theta slow waves, then diffuse and high voltage waves. multifocal bilateral spikes, slowing background.)	+ (Autistic features, happy demeanor.)	N/A	N/A	N/A	N/A	N/A	*MEF2C*
(Raviglione et al., 2021)	P114	+ (Hypoplastic corpus callosum)	+ (Multifocal asynchronous bilateral spikes, diffuse discharges of spike and waves, more evident during sleep, slowing background activity, slowing background.)	+ (Autistic features)	N/A	N/A	N/A	N/A	N/A	*MEF2C*
(Raviglione et al., 2021)	P115	-	+ (Dysrytmic backgroud activity; generalized theta slow waves, slowing backgorund.)	-	N/A	N/A	N/A	N/A	N/A	*MEF2C*
(Raviglione et al., 2021)	P116	-	+ (Multifocal spike and slow waves predominant in the occipital tregions at 3 years of age, than in the centro-parietal regions at 10 years of age, slowing background.)	+ (Autistic features, happy demeanor.)	N/A	N/A	N/A	N/A	N/A	*MEF2C*
(Raviglione et al., 2021)	P117	+ (Hypoplastic corpus callosum)	+ (Focal spikes (parietal) activation of spikes and waves during sleep, slowing background.)	+ (Autistic features.)	N/A	N/A	N/A	N/A	N/A	*MEF2C*

Appendix E

Supplemental Tables for "Clinical Findings from the Landmark *MEF2C*-Related Disorders Natural History Study"

Authors: Jessica A. Cooley Coleman[1, 2], Sara M. Sarasua[1], Hannah Warren Moore[2], Luigi Boccuto[1], Christopher W. Cowan[3], Steven A. Skinner[2], Jane M. DeLuca[1, 2]
[1]School of Nursing, Clemson University, [2]Greenwood Genetic Center, [3]Department of Neuroscience, Medical University of South Carolina
Clinical Genetics, 2021.

Table S1: Overall Responses to the MEF2C Natural History Study

	Totals (N=73)
Who is Completing the Survey	
Parent	**73 (100%)**
Child's Gender	
Female	35 (47.9%)
Male	38 (52.1%)
Ethnicity	
Hispanic, Latino, or Spanish origin	6 (8.2%)
Not Hispanic, Latino, or Spanish origin	63 (86.3%)
Unknown	4 (5.5%)
Race	
White or Caucasian	67 (91.7%)
Black or African American	3 (4.1%)
Asian	1 (1.4%)
American Indian or Alaskan Native	1 (1.4%)
Unknown	1 (1.4%)
Child's Current Age	
Infant (9 months to < 24 months)	8 (11.0%)
Preschool (2 years to < 6 years)	36 (49.3%)
Child (6 years to < 13 years)	11 (15.1%)
Adolescent (13 years to < 19 years)	10 (13.7%)
Adult (19 years to < 45 years)	8 (11.0%)
Average	8.12 yr (SD 7.21 yr)
Range	9 mo – 38 yr
Child's Current Weight	
Average	25.4 kg
Range	8.8 – 96.2 kg
Child's Current Height	

Average	1.17 m
Range	0.71 – 1.75 m
Gestational Age	
Before 38 weeks	18 (24.7%)
38-42 weeks	55 (75.3%)
After 42 weeks	0 (0%)
Birth Weight	
Extremely low birth weight (less than 0.992kg)	1 (1.4%)
Very low birth weight (between 0.993kg and 1.616kg)	0 (0%)
Low birth weight (between 1.617kg and 2.495kg)	13 (17.8%)
Normal birth weight (between 2.496kg and 3.997kg)	57 (78.1%)
High birth weight (greater than 3.997kg)	2 (2.7%)
Mother's Age When Child Was Born	
Average	31.8 yr (SD 5.12 yr)
Range	20-41 yr
Father's Age When Child Was Born	
Average	33.6 yr (SD 7.07 yr)
Range	21-57 yr
Pregnancy Exposures	**25 (34.2%)**
Tobacco	6 (8.2%)
Secondhand Smoke	6 (8.2%)
Alcohol	4 (5.5%)
Chemicals	1 (1.4%)
Prescription Medicine (zofran, ranitidine, sertraline, levothyroxine, antibiotics, nifedipine, oxycontin, amoxicillin, lovenox)	9 (12.3%)
Unknown	1 (1.4%)
Other (Linoleum glue, lawn pesticides, hair chemicals, fast food smoke, laboratory chemicals, waste incineration, progesterone suppositories, Wifi)	7 (9.6%)
Not answered	4 (5.5%)
Pregnancy Complications	**30 (41.1%)**
Premature labor	6 (8.2%)
Preeclampsia	4 (5.5%)
Low amniotic fluid	1 (1.4%)
Gestational diabetes	3 (4.1%)
Placenta Previa	0 (0.0%)
Illness/ Infection	4 (5.5%)
Unknown	2 (2.7%)
Other	19 (26.0%)

(intrauterine growth restriction (IUGR), vaginal bleeding, loss of twin, dilated fetal kidneys, hypertension, polyhydramnios, maternal wrist fracture, subchorionic hemorrhage, cerebral abnormalities, preterm contractions, single umbilical artery, fetal intestine cyst, nuchal fold, breech)	
Not answered	2 (2.7%)
Birth Complications	**35 (47.9%)**
Breech position	6 (8.2%)
Failure to Progress	8 (11.0%)
Fetal meconium aspiration	4 (5.5%)
Fetal Distress	14 (19.2%)
Unknown	1 (1.4%)
Other (Oxygen deprivation, forceps delivery, vacuum delivery, long labor, cesarean, neonatal jaundice, maternal hemorrhaging, external cephalic version, reduced/absent fetal movement, probe to find heartbeat, absent dropping, resuscitation, fetal ejection reflex, retained placenta, cervix dilation failure, hyperbilirubinemia)	16 (21.9%)
Not answered	2 (2.7%)
Developmental	**N=73**
Roll over	66 (90.4%)
Sit up	59 (80.8%)
Crawl	45 (61.6%)
Reach for objects	60 (82.2%)
Transfer items from hand to hand	53 (72.6%)
Pincer grasp	17 (23.3%)
Finger feed self	33 (45.2%)
Feed self using utensils (>18 months of age)	15 (21.7%)
Gestures or waves	22 (30.1%)
Points for wants	13 (17.8%)
Follows commands	28 (38.4%)
Diagnosed with intellectual disability	54 (74.0%)
Language	**N=73**
Nonverbal/ no signs	26 (35.6)
Nonverbal but using signing in a meaningful way	6 (8.2)
Babbling/vocalizations	33 (45.2)
A small number of words or signs for minimal communication	6 (8.2)
Series of single words or 2-word combinations used meaningfully	1 (1.4)
Phrases/sentences of 3 words or more	1 (1.4)
Alternate Speech Methods	**N=71***

Signing	14 (19.2%)
Picture exchange communication system (PECS) or equivalent	19 (26.0%)
Apps on an iPad/iPhone, smart phone, or tablet	9 (12.3%)
Augmentative communication device	12 (16.4%)
Other (hand leading, singing nursery rhymes, and vocalizations for agreement, annoyance, and attention)	4 (5.5%)
None of the above	36 (49.3%)
Motor	**N=73**
Runs Unaided	6 (8.2)
Walks Unaided	22 (30.1)
Walks with Support	12 (16.4)
Stands Unaided	0 (0.0)
Stands with Support	7 (9.6)
Crawls	4 (5.5)
Sits Unaided	9 (12.3)
Sits with Support	8 (11.0)
Rolls	4 (5.5)
Unable to Roll	1 (1.4)
If walking, walking unsteady	**N=49**
Yes	40 (81.6%)
Muscle Tone	**N=73**
Normal	14 (19.2%)
Low muscle tone	53 (72.6%)
Increased muscle tone	6 (8.2%)
Toilet trained	**N=73**
Bowel and urine	1 (1.4%)
Bowel only	0 (0.0%)
Urine only	0 (0.0%)
Time trained only	7 (9.6%)
No	65 (89.0%)
Social	
Likes giving affection	50 (68.5%)
Likes receiving affection	58 (79.5%)
Resists holding hands	40 (54.8%)
Reduced concern with environmental threat	53/67 (79.1%)
Seek social interaction	34 (46.6%)
Recognizes family	52 (71.2%)
Poor eye contact	44 (60.3%)
Attention problems	50/71 (70.4%)
Hyperactivity	27/72 (37.5%)

Anxiety	12/70 (17.1%)
Diagnosed with autism	18/70 (25.7%)
Sensory Systems	
Vision impairments	44/72 (61.1%)
Hearing impairments (bilateral sensorineural hearing loss, deafness in one ear, mild to moderate loss of certain tones, moderate mixed hearing loss)	6/72 (8.3%)
Sensitive to loud noises	45 (61.6%)
High pain tolerance	58 (79.5%)
Sensitivity to clothing textures	5 (6.8%)
Issues with food textures	26/71 (36.1%)
Vision Impairment Types	**N=44**
Myopia	12 (27.3%)
Hyperopia	13 (29.5%)
Problems with depth perception	17 (38.6%)
Cortical visual impairment	17 (38.6%)
Strabismus	21 (47.7%)
Other (esotropia, nystagmus, astigmatism, or wrote that they were unsure of their child's potential vision impairment)	7 (15.9%)
Temperature Sensitivity	**N=73**
Yes, to heat	20 (27.4%)
Yes, to cold	3 (4.1%)
Yes, both heat and cold	17 (23.3%)
No	33 (45.2%)
Sleep Issues	**N=73**
Falling asleep: yes, currently	31 (42.5%)
Falling asleep: yes, previously but no longer an issue	23 (31.5%)
Staying asleep: yes, currently	36 (49.3%)
Staying asleep: yes, previously but no longer an issue	20 (27.4%)
Take medications to help with sleeping	28 (38.4%)
Medicines: melatonin, Zonegran, Cicardin, Clonidine, Gabapentin, Trazadone, Cyproheptadine, in addition to essential oils and CBD and CBN oil.	
Medical Conditions	**N=73**
Diabetes	0 (0.0%)
Congenital heart defect	5 (6.8%)
Asthma or other respiratory issues	8 (11.0%)
Thyroid problems	1 (1.4%)
Sleep apnea	4 (5.5%)
Other	24 (32.9%)

(hypoglycemia, hip dysplasia, laryngomalacia, tracheomalacia, eosinophilic esophagitis due to allergy, dermatitis, atrial septal defect, ventricular septal defect, hypotonia, pre-osteoporosis, pectus excavatum, congenital diaphragmatic hernia, and undescended testicles)	
None	41 (56.2%)
Digestion Issues	**N=73**
Diarrhea	10 (13.7%)
Constipation	52 (71.2%)
Reflux	30 (41.1%)
Gall bladder dysfunction	0 (0.0%)
Abdominal distention/ bloating	10 (13.7%)
Other (potential undiagnosed reflux, milk protein intolerance, extreme slow intestinal motility, and food intolerances that cause painful bloating and gas)	9 (12.3%)
None	11 (15.1%)
Health Related	**N=73**
Scoliosis	9 (12.3%)
Hyper flexibility	52 (71.2%)
Regressions	25 (34.2%)
Puberty	19 (26.0%)
Frequent illnesses	31 (42.5%)
Improvement in skills with a fever	12 (16.4%)
Seizures	63 (86.3%)
Taking medications for seizures	38/62 (61.3%)
Seizure medications helped	37/38 (97.4%)
Neuropsychological	
Tremors	22 (30.1%)
Hyperventilation	22 (30.1%)
Breath holding	25/72 (34.7%)
Swallowing air	19/72 (26.4%)
Food pocketing	27/72 (37.5%)
Problems with chewing and swallowing	48 (65.8%)
Teeth grinding	64 (87.7%)
Repetitive hand movements	69 (94.5%)
Fascination with water	50/72 (69.4%)
Recurrent Immune-related Problems or Frequent Illness	**N=31**
Frequent illnesses	26 (83.9%)
Frequent fevers	13 (41.9%)
Severe allergic reactions	3 (9.7%)

Joint inflammation	0 (0.0%)
Skin issues (such as eczema)	9 (29.0%)
Other (respiratory infections, tonsilitis, frequent colds and pneumonia, and chronic ear infections)	6 (19.4%)
Seizure Type	**N=62**
Generalized	16 (25.8%)
Partial	5 (8.1%)
Febrile	21 (33.9%)
Other (generalized tonic-clonic, absence, drop or atonic, myoclonic seizures and jerks, atypical complex febrile, infantile spasms)	17 (27.4%)
Unknown	3 (4.8%)
Seizure occurrence	**N=61**
More than one a day	10 (16.4%)
Daily	7 (11.5%)
Weekly	1 (1.6%)
Monthly	2 (3.3%)
Less than monthly	13 (21.3%)
No seizures currently	28 (45.9%)
Taking Seizure Medications	**N=63**
Yes	38 (61.3%)
Medicine: Keppra (20), valproic acid (9), clobazam (6), topiramate (5), and oxcarbazepine (4), CBD oil (4), cannabidiol (2), diazepam (2), ethosuximide (2), ketogenic diet (2), lamotrigine (2), baclofen (1), brivaracetam (1), clonazepam (1), midazolam (1), phenobarbital (1), prednisone (1), vigabatrin (1), zonisamide (1)	
Previous Imaging	
MRI	69/72 (95.8%)
Abnormal MRI	40/68 (58.8%)
Specific MEF2C alteration type	**N=73**
Variant (point mutation or INDEL)	29 (39.7%)
Deletion involving the *MEF2C* gene	40 (54.8%)
Uncertain	4 (5.5%)

Table S2: Developmental Milestones by Age Group

		Roll over		**Sit Up**		**Crawl**		**Reach for Objects**	
Age Group	N=73	Yes	No	Yes	No	Yes	No	Yes	No
Infant (9 months to <24 months)	9	7	2	4	5	2	7	5	4
Preschool (>2 years to <6 years)	35	34	1	27	8	23	12	29	6
Child (>6 years to <13 years)	11	9	2	10	1	9	2	9	2
Adolescent (>13 years to <19 years)	10	9	1	10	0	6	4	9	1
Adult (>19 years to <45 years)	8	7	1	8	0	5	3	8	0

		Transfer Items from Hand to Hand		**Uses Pincer Grasp**		**Finger Feeds Self**		**Feeds Self Using Utensils**	
Age Group	N=73	Yes	No	Yes	No	Yes	No	Yes	No
Infant (9 months to <24 months)	9	4	5	2	7	2	7	1	8
Preschool (2 years to <6 years)	35	26	9	8	27	13	22	5	30
Child (6 years to <13 years)	11	7	4	4	7	7	4	2	9
Adolescent (13 years to <19 years)	10	8	2	2	8	6	4	3	7
Adult (19 years to <45 years)	8	8	0	1	7	5	3	4	4

Table S3: Proportions of patients >2 years of age able to use words to communicate (either "a small number of words or signs for minimal communication", "series of single words of 2-word combinations used meaningfully, or "phrase/sentences of 3 words or more") by alteration type, gender, and age group.

Variable	Total Group (N)	Uses Words	Does Not Use Words	Association Test p-value
1- Alteration Type	62	-	-	0.1194†
Deletion	36	2 (5.6%)	34 (94.4%)	-
Variant	26	5 (19.2%)	21 (80.8%)	-
2- Gender	64	-	-	0.0033†*
Male	34	0 (0.0%)	34 (100.0%)	-
Female	30	7 (23.3%)	23 (76.7%)	-
3- Age Group	64	-	-	0.0416§*
Preschool (2 years to <6 years)	35	1 (2.9%)	34 (97.1%)	-
Child (6 years to <13 years)	11	2 (18.2%)	9 (81.8%)	-
Adolescent (13 years to <19 years)	10	2 (20.0%)	8 (80.0%)	-
Adult (19 years to <45 years)	8	2 (25.0%)	6 (75.0%)	-

* Significant at $p<0.05$
† Fisher's Exact Test
§ Cochran-Armitage Trend Test

Table S4: Proportions of patients >18 months of age able to walk by alteration type, gender, and age group.

Variable	Total Group (N)	Able to Walk	Unable to Walk	Association Test p-value
1- Alteration Type	65	-	-	0.2083†
Deletion	38	17 (44.8%)	21 (55.3%)	-
Variant	27	17 (63.0%)	10 (37.0%)	-
2- Gender	67	-	-	0.0867†
Male	36	15 (41.7%)	21 (58.3%)	-
Female	31	20 (64.5%)	11 (35.5%)	-
3- Age Group	67	-	-	0.0483§*
Infant (>18 months to <24 months)	3	1 (33.3%)	2 (66.7%)	-
Preschool (2 years to <6 years)	35	14 (40.0%)	21 (60.0%)	-
Child (6 years to <13 years)	11	8 (72.7%)	3 (27.3%)	-
Adolescent (13 years to <19 years)	10	6 (60.0%)	4 (40.0%)	-
Adult (19 years to <45 years)	8	6 (75.0%)	2(25.0%)	-

* Significant at $p<0.05$
† Chi-Square Test
§ Cochran-Armitage Trend Test

Table S5: Proportions of respondents reporting hyperactivity and anxiety by alteration type, gender, and age group.

Does your child have hyperactivity?				
Variable	**Total Group (N)**	**Yes**	**No**	**Association Test p-value**
1- Alteration Type†	68	-	-	0.0807
Deletion	39	12 (30.8%)	27 (69.2%)	-
Variant	29	15 (51.7%)	14 (48.3%)	-
2- Gender†	72	-	-	0.9515
Male	37	14 (37.8%)	23 (62.2%)	-
Female	35	13 (37.1%)	22 (62.9%)	-
3- Age Group§	72	-	-	0.5971
Infant (9 months to <24 months)	8	1 (12.5%)	7 (87.5%)	-
Preschool (2 years to <6 years)	35	14 (40.0%)	21 (60.0%)	-
Child (6 years to <13 years)	11	7 (63.6%)	4 (36.4%)	-
Adolescent (13 years to <19 years)	10	2 (20.0%)	8 (80.0%)	-
Adult (19 years to <45 years)	8	3 (37.5%)	5 (62.5%)	-
Does your child have anxiety?				
Variable	**Total Group (N)**	**Yes**	**No**	**Association Test p-value**
1- Alteration Type†	66	-	-	0.6400
Deletion	37	6 (16.2%)	31 (83.8%)	-
Variant	29	6 (20.7%)	23 (79.3%)	-
2- Gender†	70	-	-	0.3936
Male	37	5 (13.5%)	32 (86.5%)	-
Female	33	7 (21.2%)	26 (78.8%)	-
3- Age Group§	70	-	-	0.6655
Infant (9 months to <24 months)	7	0 (0.0%)	7 (100.0%)	-
Preschool (2 years to <6 years)	34	6 (17.7%)	28 (82.3%)	-
Child (6 years to <13 years)	11	2 (18.2%)	9 (81.8%)	-
Adolescent (13 years to <19 years)	10	2 (20.0%)	8 (80.0%)	-
Adult (19 years to <45 years)	8	2 (25.0%)	6 (75.0%)	-

† Chi-Square Test
§ Cochran-Armitage Trend Test

Table S6: Proportions of respondents reporting seizures by alteration type, gender, and age group.

Does your child have seizures?				
Variable	**Total Group (N)**	**Yes**	**No**	**Association Test p-value**
1- Alteration Type†	69	-	-	0.3928
Deletion	40	37 (92.5%)	3 (7.5%)	-
Variant	29	25 (86.2%)	4 (13.8%)	-
2- Gender†	73	-	-	0.4114
Male	38	34 (89.5%)	4 (10.5%)	-
Female	35	29 (82.9%)	6 (17.1%)	-
3- Age Group§	73	-	-	0.8165
Infant (9 months to <24 months)	9	7 (77.8%)	2 (22.2%)	-
Preschool (2 years to <6 years)	35	30 (85.7%)	5 (14.3%)	-
Child (6 years to <13 years)	11	11 (100.0%)	0 (0.0%)	-
Adolescent (13 years to <19 years)	10	9 (90.0%)	1 (10.0%)	-
Adult (19 years to <45 years)	8	6 (75.0%)	2 (25.0%)	-

† Chi-Square Test
§ Cochran-Armitage Trend Test

Table S7: Proportions of respondents reporting an abnormal MRI by alteration type, gender, and age group.

Abnormal MRI?				
Variable	**Total Group (N)**	**Yes**	**No**	**Association Test p-value**
1- Alteration Type†	64	-	-	0.5951
Deletion	37	23 (62.2%)	14 (37.8%)	-
Variant	27	15 (55.6%)	12 (44.4%)	-
2- Gender†	68	-	-	0.5411
Male	37	23 (62.2%)	14 (37.8%)	-
Female	31	17 (54.8%)	14 (45.2%)	-
3- Age Group§	68	-	-	0.0669
Infant (9 months to <24 months)	8	4 (50.0%)	4 (50.0%)	-
Preschool (2 years to <6 years)	34	17 (50.0%)	17 (50.0%)	-
Child (6 years to <13 years)	10	7 (70.0%)	3 (30.0%)	-
Adolescent (13 years to <19 years)	9	7 (77.8%)	2 (22.2%)	-
Adult (19 years to <45 years)	7	5 (71.4%)	2 (28.6%)	-

† Chi-Square Test
§ Cochran-Armitage Trend Test

Appendix F

IRB Documents for *MEF2C*-Related Disorders Natural History Survey

MEF2C-Related Disorders Natural History Survey Project Protocol

A. Background and Significance

MEF2C-related disorders are neurodevelopmental disorders caused by pathogenic variants in the *MEF2C* gene or by microdeletions or duplications of the 5q14.3 region containing part or all of the *MEF2C* gene. These disorders display some similarities to Rett syndrome and other neurodevelopmental disorders. It is characterized by intellectual disability, lack of verbal language, motor delay, abnormal movements, autistic behaviors, and often epilepsy (Paciorkowski *et al*., 2014). The available literature regarding *MEF2C*-related disorders is limited with only approximately 90 variants being described to date. A larger scale study would be beneficial to gather additional data and improve the clinical description.

The goal of this research is to gather information about *MEF2C*-related disorders by collection of developmental and medical history by use of a survey designed for parents of children with this condition. Researchers at the Greenwood Genetic Center, Clemson University, and the Medical University of South Carolina will analyze and report the data collected by the surveys to raise awareness and increase knowledge regarding *MEF2C*-related disorders. This information could assist clinicians in better recognizing and diagnosing patients, and could better prepare researchers for clinical trials or drug development.

B. Design and Methods

(1) Study Design

This study will involve researchers at Greenwood Genetic Center, Clemson University, and the Medical University of South Carolina obtaining consent from patient families to gather clinical information via an online survey through REDCap. The patients will be chosen based on a previous diagnosis with a *MEF2C* variant or deletion or duplication involving the *MEF2C* gene. Responses to the survey will be analyzed to gain a better understanding of *MEF2C*-related disorders.

(2) Patient Selection and Inclusion/Exclusion Criteria

Patient families will be made aware of the survey via email, social media, and verbal communications. Any patient with a previously reported *MEF2C* alteration (variant, deletion, duplication) will qualify to participate. The

family/parent/guardian filling out the survey will have the option to submit identifying contact information via email for any future studies or opportunities, but this is optional and not required for taking the survey. The survey responses will remain completely anonymous.

(3) Data Collection Methods

The surveys will be completed by the patient's parent, guardian, or caregiver online via REDCap. These surveys will be electronically returned to the Greenwood Genetic Center. Only the researchers and reviewing faculty at the Greenwood Genetic Center, Clemson University, and Medical University of South Carolina will have access to the survey responses, including any identifying protected health information if the patient families consent to provide this information.

C. Adverse Event Criteria and Reporting Procedures

This study is considered minimal risk. As with any study involving collection of data, there is the possibility that unauthorized individuals may gain access thereby breaching the confidentiality of the data. Every precaution will be taken to secure the participants' personal information to ensure confidentiality. The investigators do not foresee any adverse events, but any adverse event will be reported to the IRB immediately.

D. Data Management Methods

The patient survey will be collected and securely saved within the HIPAA compliant web-based application REDCap (Harris et al., 2009). The patient survey response data will be extracted from REDCap and stored on a password protected computer at the Greenwood Genetic Center to which only the researchers have access. Greenwood Genetic Center is fully compliant with HIPAA regulations. Survey responses may be uploaded to Box online, which allows data sharing between Greenwood Genetic Center, Clemson University, and Medical University of South Carolina. Only the researchers involved in this project at these institutions will have access to Box. The Box platform and associated products have been compliant with HIPAA, HITECH, and the final HIPAA Omnibus rule since November 2012 (Box – Secure File Sharing). Only survey answers will be added to Box, and no patient identifiers will be added to Box. Any paper copies will be stored in a locked file cabinet to which only the research team has access.

E. Data Analysis Plan

Descriptive analyses will be calculated from the data obtained from the survey. This data will be evaluated to gain a better understanding and knowledge base of *MEF2C*-related disorders.

F. References

Box - Secure File Sharing, Storage, and Collaboration. (n.d.). Retrieved from https://www.box.com/

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SELF REGIONAL HEALTHCARE

INSTITUTIONAL REVIEW BOARD

Request for Waiver of the Requirement to Obtain Signed Consent from Subjects

(not applicable to FDA regulated studies)

☐ The only record linking the subject and the research would be the consent document and the principal risk would be the harm resulting from breach of confidentiality. (Note: Each subject must be asked whether they want documentation.)
Explain why:

OR

☐ The research presents no more than minimal risk* and involves no more procedures for which written consent is normally required.
Explain why: The research is in the form of an online survey in which there will be no link to personal identifiers and the survey responses. Subjects will have the option to provide their contact information to be contacted for future studies, but this information will not be linked to their survey responses.

**minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.*

If documentation is waived, will the subjects be provided with a written statement regarding research? ☐ YES. Attach copy of written statement that will be provided. ☐ NO. Explain below why a written statement is not necessary or appropriate:
Subjects will see written information prior to starting the survey (like a consent form, however they won't be signing anything). Since the survey is online and subjects can take the survey in various locations, they will not be provided with a physical copy.

Patient Informed Consent
MEF2C-Related Disorders Natural History Survey

Principal Investigator
Steven A. Skinner, MD
Greenwood Genetic Center
106 Gregor Mendel Circle
Greenwood, SC 29646
864-941-8164
sas@ggc.org

Study Coordinator
Jessica A. Cooley Coleman, MB(ASCP)CM
Greenwood Genetic Center
106 Gregor Mendel Circle
Greenwood, SC 29646
864-941-8188
jcooley@ggc.org

Purpose of Study
You are being asked to take part in a research study. Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. Please read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information.

The purpose of this study is to gather information to better characterize the symptoms of *MEF2C*-related disorders.

Study Procedures
Patients who carry a *MEF2C* variant or deletion or duplication involving the *MEF2C* gene are eligible to participate in this survey study. We hope to enroll approximately 50 individuals in this survey, but there will not be a limit to how many individuals can participate.

Parents/Guardians agree:

1. to complete a survey in which their child's medical information will be collected and stored in the web-based application REDCap.
2. to have the survey responses uploaded to Box, an online application that will allow data sharing between researchers at Greenwood Genetic Center, Clemson University, and Medical University of South Carolina.

It is estimated that it will take 20-30 minutes to complete the survey.

Risks
This study presents minimal risks. Any time health information is collected, there is a risk that unauthorized individuals may gain access thereby breaching the confidentiality of the data. However, the data is stored in a secure location which should not be accessible to people outside

of the research team, and precautions including password protections will be taken to secure personal information.

You may decline to answer any or all questions and you may terminate your involvement at any time if you choose.

Benefits
The benefits of this study include developing a large information databank which will help physicians better diagnose and better understand this genetic condition in future patients.

Confidentiality
You will be asked for contact information; however, you may opt out of giving this information. Regardless if you give information or opt out, the data collected in the survey will remain anonymous. If you provide contact information, there may be future approved studies for which you would be contacted for additional information. Every effort will be made by the researcher to preserve your confidentiality, including the following:

- Assigning code names/numbers for participants that will be used on all research notes and documents
- Keeping notes, interview transcriptions, and any other identifying participant information in a locked file cabinet or locked computer document in the personal possession of the researcher.

Participant data will be kept confidential except in cases where the researcher is legally obligated to report specific incidents. These incidents include, but may not be limited to, incidents of abuse and suicide risk.

Compensation
You will not be paid for participation in this study.

Authorization to Use or Disclose Protected Health Information

By proceeding with this survey, you are authorizing the Greenwood Genetic Center to use and disclose (share) your protected health information for this research. You must authorize this use and sharing of your information to be in the study. The protected health information used for this research will include information collected about you and your child during the survey.

Greenwood Genetic Center is required by law to protect your health information. This is detailed in the Greenwood Genetic Center Notice of Privacy Practices, which is available at www.ggc.org and can be provided upon request. The researchers in this study agree to use your protected health information only as directed by you and as required by state and federal law. Several people and organizations may access your protected health information. They will need this information to conduct the research or to assure the quality or safety of the research. These groups include:

- members of the research team and other authorized staff at Greenwood Genetic Center, Clemson University, and Medical University of South Carolina,
- the Institutional Review Board (IRB) of Self Regional Healthcare,

- and the Office for Human Research Protections (OHRP) or possibly other federal or state government agencies.

You may change your mind and withdraw your permission to use and share your protected health information at any time. To take back your permission, you must email Jessica Cooley Coleman [jcooley@ggc.org] or Dr. Steven Skinner [sas@ggc.org].

The results of this study may be shown at scientific meetings or published in scientific journals to inform other doctors and health professionals. As the data is anonymous, your identity will not be included in any publication or presentation.

Consent for Use of Information for Future Research

As part of the study, we will collect information. If you provide your contact information, we may wish to contact you for a future study about *MEF2C*-related disorders. Information that can identify you may be kept permanently in a laboratory, repository, or computer database at the Greenwood Genetic Center. Only members of the research team and other authorized staff at the Greenwood Genetic Center, Clemson University, and Medical University of South Carolina will be able to see information that can identify you.

Contact Information

If you have questions at any time about this study, or you experience adverse effects as the result of participating in this study, you may contact the researcher whose contact information is provided on the first page. If you have questions regarding your rights as a research participant, or if problems arise which you do not feel you can discuss with the Primary Investigator, please contact the Institutional Review Board at (864) 725-4252 or (864) 725-4851.

Voluntary Participation

Your participation in this study is voluntary. It is up to you to decide whether or not to take part in this study. If you decide to take part in this study, you will be asked to give your consent in order to proceed with the survey. After you consent to the survey, you are still free to withdraw at any time and without giving a reason. Withdrawing from this study will not affect the relationship you have, if any, with the researcher.

Consent

If you would like to participate, you are consenting that you have read and understand the provided information and have had the opportunity to ask questions. You understand that your participation is voluntary and that you are free to withdraw at any time, without giving a reason and without cost. If you consent, please check the box below and then proceed with the survey online. Your answers will not be submitted until you have completed and submitted the survey.

Do you consent to taking this survey?

- o Yes
- o No

Advertising Script
MEF2C-Related Disorders Natural History Survey

My name is Jessica Cooley Coleman and I am a doctoral student in the Healthcare Genetics PhD program at Clemson University. For my research, I have decided to study *MEF2C.* My fellow researchers at Clemson and I have collaborated with researchers at the Greenwood Genetic Center and Medical University of South Carolina (MUSC) to create a survey so that we can better characterize the symptoms of *MEF2C*-related disorders, sometimes referred to as *MEF2C* haploinsufficiency syndrome. Currently, there is limited information in the literature about individuals who carry an alteration in the *MEF2C* gene. We are hoping to collect information from families by use of this survey and increase knowledge regarding *MEF2C*-related disorders. We hope this information will help medical providers better diagnose and understand this condition in the future. Also, this survey will help direct future research efforts. I hope you will consider taking our survey. I will be happy to answer any survey-related questions you may have via email at jcooley@ggc.org.

Additionally, if you would like to provide your contact information in the case of future studies or opportunities, please send an email containing your first and last name and preferred email address to MEF2C@ggc.org. Please note that this email address is only for providing contact information for the possibility of future contact and therefore will not be monitored for questions. This contact information will not be linked to your survey responses and providing your contact information is optional and not required for taking the survey. Thank you for your time and consideration.

To proceed with the survey, please visit:
https://redcap.healthsciencessc.org/surveys/?s=M3NRP9MXMM

-Jessica Cooley Coleman

MEF2C-Related Disorders Natural History Survey

Survey instrument may be available upon request.

Appendix G

Clemson University – Medical University of South Carolina
MEF2C RNAseq Visiting Researcher Proposal

Background

MEF2C is a transcription factor in the MEF2 (myocyte enhancer factor 2) family, expressed in the nervous, muscular, and immune system. In the brain, *MEF2C* orchestrates the expression of numerous genes critical for neurotypical brain development and function. *MEF2C* is particularly known to play a role in neurogenesis, synaptic formation, and remodeling (Assali et al., 2019). *MEF2C* is expressed in different brain cell types, including excitatory and inhibitory neurons, as well as microglia, which regulate synapse formation and elimination.

Pathogenic variants in the *MEF2C* gene or microdeletions of the 5q14.3 region containing part or all of the *MEF2C* gene cause *MEF2C* Haploinsufficiency Syndrome (MCHS) in humans. MCHS is characterized by intellectual disability, lack of verbal language, motor delay, abnormal movements, autistic behaviors, and often epilepsy (Paciorkowski et al., 2013). These symptoms are thought to be caused by haploinsufficiency of *MEF2C* particularly in the neurons. *Mef2c* global heterozygous mice and microglia-restricted conditional *Mef2c* heterozygous mice (*Mef2c* cHet^{Cx3cr1}) display social deficits and repetitive behaviors, reminiscent of autism-like behaviors (Harrington, Bridges et al., 2020). In addition, the loss of one copy of *Mef2c* in GABAergic neurons (*Mef2c* cHet^{VGat}) induces deficits in social preference and working memory (unpublished data), both prefrontal cortex (PFC)-dependent behaviors. These different mutant mice can therefore serve as animal models for the human syndrome, MCHS, to study the role of *Mef2c* in autism-like behaviors, brain function, and gene expression.

Project Plan

Previous RNAseq studies in global *Mef2c* heterozygous mice showed dysregulation of hundreds of genes in the cortex as well as an upregulation of microglial

genes (Harrington, Bridges et al., 2020). The authors hypothesized that microglia have a delayed maturation in the *Mef2c* heterozygous mice as certain genes are enriched in the postnatal day 35 mice that should no longer be active under normal microglia development.

Given previous findings, the next step is to isolate cortical microglia nuclei and perform single nuclei RNAseq to assess gene expression differences between global *Mef2c* heterozygous mouse microglia and wildtype microglia.

Since *Mef2c* seems to play an important role in GABAergic cells, another interesting direction is to assess gene expression differences between *Mef2c* cHet^{Vgat} mice and wildtype mice, in the specific GABAergic subtype neurons of the PFC, using single-nuclei RNA-seq.

Jessica Cooley Coleman, doctoral candidate in the Healthcare Genetics PhD program at Clemson University, will perform the role of visiting researcher at MUSC from June to December 2021, with research and data analysis extending to May 2022 if necessary. The project consists of three potential phases.

1) Isolate the nucleus of the specific cell type of interest (i.e., microglia or *Vgat-Cre; Mef2c*$^{fl/+}$ neurons).
   - Nuclei will be sent to a core laboratory for RNAseq library preparation and sequencing.

2) Learn and perform bioinformatic analysis of data generated by the RNAseq runs to determine which genes are dysregulated due to the hypofunction of *Mef2c*.

3) Validate significant up/down-regulated genes from the RNAseq results by performing qPCR, or RNAscope, to quantify the level of gene expression.

References

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Appendix H

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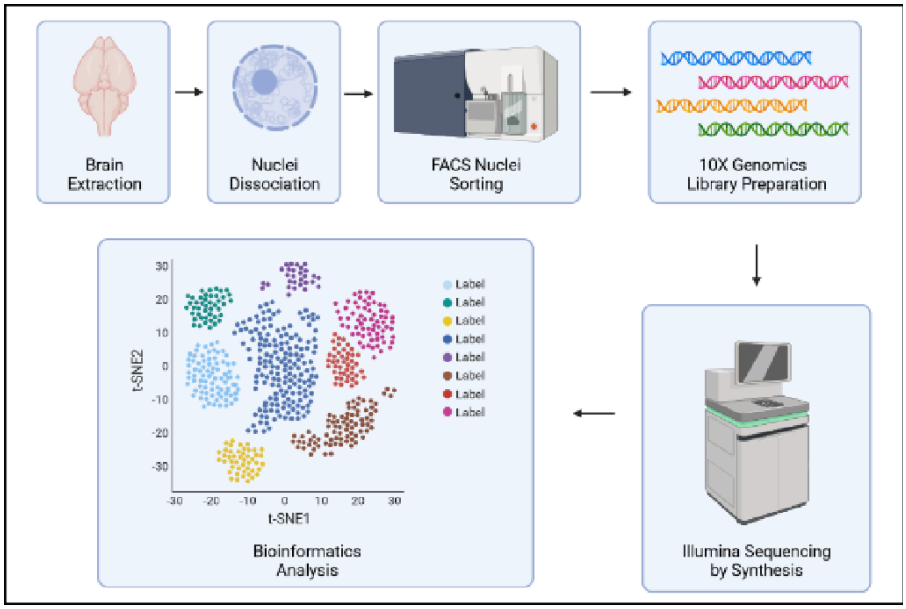

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