Universidad Zaragoza
1542

Trabajo Fin de Máster

Tratamientos actuales para el Síndrome de Duplicación de *MECP2*

Current treatments for *MECP2* Duplication Syndrome

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Máster en Biotecnología Cuantitativa

FACULTAD DE CIENCIAS

2020

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I Acknowledgements

En primer lugar, me gustaría agradecer la atención, el esfuerzo y la dedicación que me han proporcionado mis tutores Adrián Velázquez, Olga Abián y David Ortega. A este último le tengo que agradecer, además, todo el valiosísimo tiempo que ha dedicado a enseñarme las diferentes técnicas de expresión, purificación y cribado de proteínas, además de sus consejos y su imprescindible apoyo durante la elaboración de este TFM. A pesar de la compleja y desafortunada situación que ha truncado el desarrollo del máster, y por tanto del trabajo desarrollado en el laboratorio, estoy convencido de que los conocimientos que he adquirido durante este mes en las instalaciones del BiFi serán puestos en práctica muy pronto. También les reservo un cariñoso saludo a Ana, Pablo, Cote, Nunilo, Laura, Sonia, Bea, Violeta y a todas las demás personas que me han acompañado, aconsejado y arropado durante mi –breve pero intensa– estancia. En tan solo un mes me habéis hecho sentir parte de vuestra familia.

Gracias al BiFi y a la Universidad de Zaragoza por darme la oportunidad de formarme como investigador y por ofrecerme los recursos académicos y científicos con los que cuenta. También tengo que agradecer a todo el profesorado del Máster en Biotecnología Cuantitativa su dedicación y paciencia. También a mis compañeras de clase, Ijeoma, Arantxa y Jéssica. Ha sido un placer compartir aula y aprender no solo *con* vosotras, sino también *de* vosotras.

A mis amigos y colegas de profesión que dejé en Valencia, ojalá pronto nos volvamos a ver, y no solo en el ámbito profesional. Y, por supuesto, un eterno gracias a mi familia, todo lo que soy se lo debo a ellos.

II Abstract and keywords

Abstract

Methyl-CpG-binding protein 2 (MeCP2) is a small, intrinsically disordered protein which has a role in gene activation and repression, and chromatin compaction. When *MECP2* gene, located in the X chromosome, suffers a duplication, MeCP2 expression increases, leading to the outbreak of seizures, hypotonia, autistic features and respiratory infections. This disorder is known as *MECP2* Duplication Syndrome (M2DS).

The aim of this Master's thesis is to gather all the existing drug trials and auxiliar treatments for M2DS.

The search returned 10 cases of drug trials (6 performed on humans, 1 on human iPSCs and 3 on M2DS mouse models). Anticonvulsants are the most frequently prescribed drugs on young patients, although antisense oligonucleotides (ASOs) and CRISPR-Cas9 gene editing are being developed in preclinic studies. The main auxiliar therapies found for M2DS were occupational, physical and speech therapy.

Valproic acid (VPA) is the most used anticonvulsant for the treatment of seizures in children with M2DS, but other drugs have also proven to be effective, such as lamotrigine (LTG) and topiramate (TPM). The screening of small compounds able to destabilize the MeCP2-DNA union could also be a fruitful source of long-term drugs against M2DS. Auxiliar therapies will continue to be imperative until a full phenotype reversal can be achieved in human patients via gene editing.

Keywords Clinical trials | Non-clinical studies | Drug discovery | *MECP2* Duplication Syndrome

1 Introduction

1.1 MeCP2

Methyl-CpG-binding protein 2 (MeCP2) is a small molecular weight protein encoded by the *MECP2* gene (1) which is located in the human chromosome Xq28 (2,3). It has two isoforms, E1 MeCP2 and E2 MeCP2, which differ in a few N-terminal amino acids (4,5) and in their expression level during brain development and between tissues (6,7). Although they perform similar functions, E1 mutations usually provoke changes in the chromatin regulation, while E2 mutations affect microtubules formation and ribosomal expression (8). Both isoforms have a molecular weight around 53 kDa, many phosphorylation sites and a high isoelectric point (9).

1.1.1 Structure and function

MeCP2 is an intrinsically disordered protein (10,11), which means that its structure does not fold into a stable structure, being this feature typical of molecular recognition factors (12). It was firstly described as able to bind to methylated CpG islands and it also binds to chromosomes at sites that contain methylated DNA (13), but later it was proved its ability to also bind to non-methylated and hydroxymethylated regions (14), recruiting acetone deacetylases (2,15) and different transcription factors (16). It has been demonstrated that this protein has important function in complex metabolic pathways, altering chromatin's structure and playing a toggle switch role for many genes (15).

This protein is organized into five domains (**Figure 1**) N-terminal domain, NTD (or HMGD1); methyl binding domain, MBD; intervening domain, ID (also known as HMGD1); transcriptional repressor domain, TRD; and C-terminal domain, CTD. Thanks to this multi-domain structure and its folding plasticity, MeCP2 can bind to other proteins or *cis*-elements (12).

Figure 1. Domains of methyl-CpG-binding protein 2. HMGD1, high mobility group protein-like domain 1; HMGD2, high mobility group protein-like domain 2; MBD, methylated DNA binding domain; TRD, transcriptional repression domain; CTDα, C- terminal domain alpha; CTD_{β}, C-terminal domain beta. Figure adapted from (17).

To the date, only three of its five domains have been corroborated to bind to DNA: MBD (18), TRD (16) and ID (19).

Molecular Dynamics models have been useful to predict MeCP2's disordered folding, but the X-ray structure determination has not been done for all the five domains since a whole protein crystallization process has not been achieved yet (20). Only MBD's structure has been solved (**Figure 2**) (11). MBD can discriminate between methylated and non-methylated DNA, and displays a strong selectivity for binding symmetrically methylated duplex DNA in vitro (21).

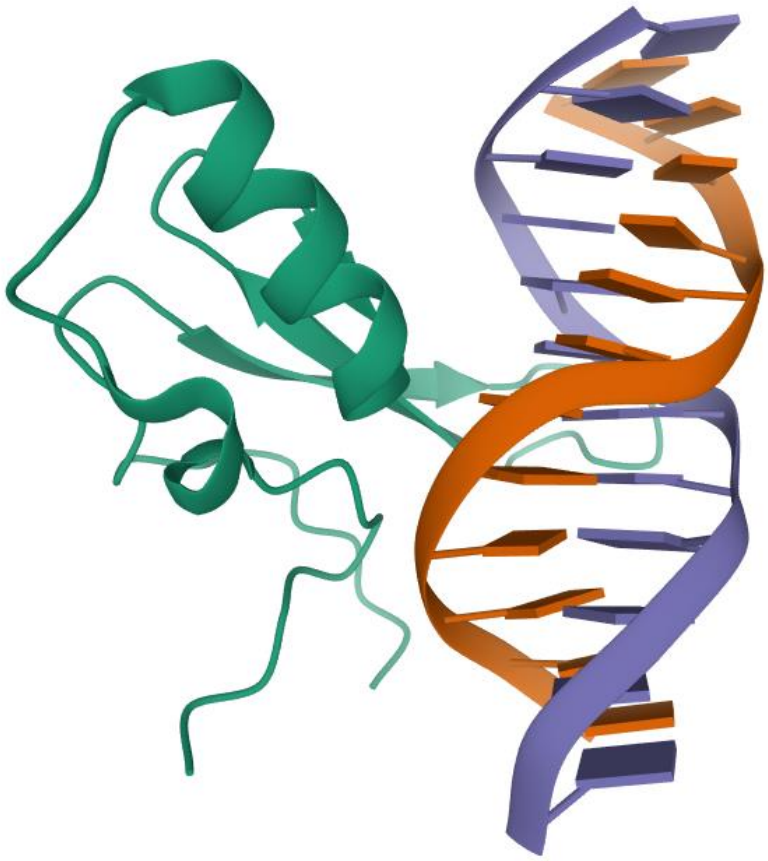

Figure 2. Methyl Binding Domain (MBD) domain of the Methyl-CpG-binding protein 2 (green) attached to a double strand of DNA (orange/purple). 3D visualization done with RCSB Protein Data Bank 3D View online tool (www.rcsb.org/3D-view). Protein data taken from (22).

Regarding the rest of the domains, NTD and ID are also known as High Mobility Group-Like Domains (HMGD1 and HMGD2 respectively), and they are intrinsically disordered

(16). It has been suggested that NTD plays a role in regulating MBD binding, whilst ID stabilizes MeCP2-chromatin interaction, enabling downstream repressor recruitment or secondary interactions required for the structural modulation of chromatin (19).

The ubiquity and the high frequency of its binding sites along the mammalian genome led to MeCP2 being classified as a global transcriptional repressor (13). Its ability to bind to methylated DNA made researchers think that this protein acted as a methylated DNA binding transcriptional repressor (1).

Most of the studies related to MeCP2's function have tried to elucidate its exact mechanism in gene repressing (17). The presence of a non-methylated binding domain, TRD, was confirmed after removing the MBD domain in recombinant MeCP2, that showed repression activity in vitro (13).

A study on *MECP2* overexpression carried out in mice showed that either lacked or overexpressed MeCP2 in hypothalamus activated ~85% of the genes (23). In this same study, six of the affected genes were selected, and MeCP2 showed binding affinity to their promoter, confirming MeCP2's ability to recruit activation or repression factors depending on the binding conformation and the environment (24).

1.1.2 MeP2's role on epigenetics

The term "epigenetics" refers to phenotypic, heritable changes on chromatin without affecting the DNA sequence. These modifications make genes more or less accessible to recognition and transcription factors, so that different stages of gene regulation can be achieved through these reversible changes (25). MeCP2 is a link between two different epigenetic mechanisms: DNA methylation and histone deacetylation (26).

Methylation reactions on DNA are performed by DNA methyltransferases (DNMT), either creating a methylation pattern or maintaining it along the cell cycle. Nonetheless, methylation patterns are not steady. They can suffer modifications, such as the conversion of 5-methylcytosine to 5-hydroxymethylcytosine (27). As previously said, MeCP2 is able to recognize patterns of methylated DNA and to bind to them, acting as an "epigenetic reader" for the recruited factors (17,28).

On the other hand, the acetylation/deacetylation mechanism of histones is carried out by histone-acetyl transferases (HATs) and histone-deacetylases (HDACs), respectively. MeCP2 can recruit adaptor proteins for HDACs, modifying the chromatin structure (6).

Finally, it has been proved that MeCP2 can play the role of a histone H1 (29), compacting nucleosomes in an unspecific way and locally regulating gene expression (15,30).

1.2 MeCP2-linked disorders

MeCP2 has an important role in neuronal chromatin organization, transcriptional regulation, histone H1-binding competition and DNA methylation-binding dependence (31). To optimally maintain all these functions, MeCP2's expression levels must remain inside a narrow range of concentrations (32,33). Protein misfolding, mutations or changes in the expression levels can alter the amount of functional protein in the cell, resulting in the outbreak of neuromuscular disorders (24,34). Rett Syndrome and *MECP2* Duplication Syndrome are the most common disorders related to changes in the expression of MeCP2 (31).

1.2.1 Rett Syndrome

Rett Syndrome (RTT) is a rare genetic neurodevelopmental disease caused by loss-of-function mutations or deletions in the *MECP2* gene (10). It affects mostly females, because males only have one X chromosome, and a defective *MECP2* allele of their only copy would cause premature death, although some point mutations on this gene can make survival possible for male patients (35).

1.2.2 *MECP2* Duplication Syndrome

MECP2 Duplication Syndrome (M2DS) is a neuromuscular disorder caused by a duplication of the *MECP2* gene locus in Xq28. This second copy of *MECP2* increases the expression of MeCP2 to anomalous levels, leading to the apparition of neuromuscular symptoms (34).

Although RTT and M2DS show opposite effects regarding MeCP2's expression, phenotypic and clinical effects are surprisingly similar in both disorders (36). Gene expression levels have also been measured in mouse models for both syndromes, resulting in opposite expression patterns and molecular effects (37).

Intellectual disability cases related to *MECP2* were mostly focused on RTT, until Lubs *et al.* described in 1999 a X-linked mental retardation with progressive, severe central nervous system deterioration disorder which affected young males, and whose causal gene was located on Xq28 (38). A relationship between MeCP2 overexpression and mental retardation in males was stablished in 2005 (39).

Since then, several molecular, genetic and phenotypic studies on different human populations have been carried out regarding this disorder (36,40–56), contributing to a better understanding of the disease.

1.2.2.1 *MECP2* peripheral genes implications

Xq28 duplications do not only affect *MECP2K* locus. There are evidences claiming that duplication size is related to clinical severity due to peripheral genes and genetic elements present in the duplicated region (57).

The relevance of adjacent genetic elements close to *MECP2*'s locus (**Figure 3**) lies in the fact that they are prone to remain within the duplicated region (47,58–60).

Figure 3. Location of *MECP2* (dark red arrow) and some of its peripheral genes close to the minimal duplicated region according to (59). Figure taken from (3).

It has been suggested that the length of the duplicated region, and thus the duplicated genes, adding more symptoms to the clinical picture and aggravating the disorder (39,56,59).

Interleukin-1 receptor-associated kinase 1 (*IRAK1*) is one of the most frequently duplicated genes along with *MECP2*, and some authors suggest that its overexpression is the main cause of recurrent respiratory infections (47,54,55). Host cell factor C1 (*HCFC1*) gene and solute carrier family 6, member 8 (*SLC6A8*) gene have been related to mental retardation, although their role in this syndrome is still unknown (47,61).

L1 cell adhesion molecule (*L1CAM*) gene is less often affected by duplications (53,54), but Yi *et al.* reported the presence of this gene in the duplicated region of 10 out of 16 of their patients (47). Overexpression of this gene is usually related to the lack of nerves in a region of the intestine, which is characteristic of Hirschsprung disease (44). In this same study, duplication of the filamin A, alpha (*FLNA*) gene, whose overexpression cause intestine obstruction, was also detected in 9 out of 16 patients.

1.2.2.2 Diagnosis

Nowadays, diagnostic laboratories offer clinical testing for X-linked mental retardation disorders, and *MECP2* duplications can be easily detected using Quantitative Real-Time PCR, Multiplex Ligation-dependent Probe Amplification (MLPA), or Comparative Genomic Hybridization (CGH) (58,62). Recently, Chromosomal Microarray Analysis has been suggested as a genetic broad-spectrum tool for the diagnosis of children with intellectual disability (63) .

Molecular genetic analysis in patients with developmental disability and prenatal diagnosis in the case of female carriers is strongly recommended to enable early diagnosis of M2DS and to follow the fetus's development (47). In the case of female patients, a X chromosome inactivation analysis (XCI) is often done, since knowing the X chromosome inactivation ratio skewness is crucial for the patient's diagnosis (60,64).

The neurological deterioration degree of a patient, diagnosis of type and frequency of phenotypic features should be carried out via electroencephalogram (65).

De visu diagnosis based on facial features has been described for RTT in order to enable early detection of the disorder (66). Lately, new diagnosis tools based on deep machine learning and face analysis are being developed (67), such as Face2Gene (FDNA).

1.2.2.3 Epidemiology

Although no population-based studies have been carried out to assess the incidence of this syndrome, *MECP2* duplications are found in 1.6 and 2.2% of unexplained X-linked mental retardation cases in males, according to two different and independent studies (49,56).

Females are often asymptomatic because of the skewed X-inactivation that preferentially inactivates the rearranged chromosome (36,60,68). However, many of them show psychopathic features such as depression or chronic anxiety, and in some cases their phenotype is similar to those of male patients (46,47,58,69). There are reported cases of female patients with mental retardation but no noticeable dysmorphic features (70,71).

No large population studies have been carried out for the estimation of the life expectancy in males with M2DS, but several studies on small groups of patients have evidenced that there is a high risk of early death due to hypotonia and respiratory infections, mainly (38,48,49,69).

1.2.2.4 Phenotype

To date, M2DS's etiology has been deeply researched, and the main phenotypic features related to this syndrome include dysmorphic features, delayed psychomotor development resulting in absent to very limited speech, neurological symptoms, such as mental retardation, abnormal gait, epilepsy and spasticity, and deficient gastroesophageal and gastrointestinal mobility (58,60,65,69).

A) Dysmorphic features

There are facial features that most of the patients share, such as brachycephaly, macrotia, midface hypoplasia, depressed nasal bridge, and/or slightly upturned nares (58,65). To make an objective study on face dysmorphia, these facial features have been already compilated for a M2DS patient (**Figure 4**) using Face2Gene (67).

Figure 4. Consensus face of a *MECP2* Duplication Syndrome patient obtained from Face2Gene (FDNA). Figure taken from (72).

B) Psychomotor development

Motor hypotonia is the most evident impairment in newborns, and it can lead to feeding difficulties, excessive drooling, gastro-esophageal reflux, and failure to thrive (65). This condition usually requires the use of a nasogastric tube to feed the baby (46,58).

Due to this poor psychomotor condition, most affected children suffer a delay in their developmental milestones, such as crawling or sitting. In the few cases when unsupported ambulation is achieved, patients tend to make an excessive extension of the lumbar region while walking, and they eventually develop lumbar hyperlordosis (65). As many other neurodegenerative disorders, patients lose the ability to walk through the years due to the progressive loss of muscular tone (31,38,51).

Epilepsy episodes are commonly observed in young males during their second or third decade of life, although their frequency, duration and magnitude can vary a lot between patients (52,73,74). It has been observed that the onset of epilepsy and the emergence of neurologic regression occur simultaneously (75).

MeCP2 also has a role in the development of GABAergic circuits in the brain. To achieve an optimal regulation of this process, MeCP2 levels in the thalamus must remain at a narrow range of concentrations (32,33). Otherwise, as in the case of RTT and M2DS, an anomalous MeCP2 concentration alters GABA (γ-aminobutyric acid) levels, which are the main cause of MeCP2-related epilepsy and various types of seizures, such as myoclonic seizures, stereotypies and involuntary facial movements (69,76,77).

It is also noticeable that most of the patients suffer speech lose (65). There are reported cases in which patients started speaking during their second year of life, and they progressively lost their speech ability until they were unable to say any word at age 4 (58,59) and 6 (55).

C) Neurological development

Severe central nervous system deterioration has been identified as a main feature of M2DS since the first cases of X-linked mental retardation in males were studied (38). Actually, the duplication of the *MECP2* region is one of the most common causes of severe mental retardation and progressive neurological symptoms in males (39).

It has been demonstrated that one or more functional variants of *MECP2* existing at significant frequencies in a population increase the risk of autism/autism spectrum

disorders (78). Deficits in communication, lack of a stable social interaction, repetitive behaviors and restricted areas of interest are some of the autistic features commonly described in patients who suffer mutations in the *MECP2* region (58).

Some studies on the incidence of autism spectrum disorders in young patients suggest that the rate of successful RTT and M2DS diagnoses is significantly higher when the clinical diagnostic tools include detection tests for mutations on the *MECP2* locus (79–81).

Nonetheless, unlike RTT (82), a broad research on autism with M2DS patients has not been done so far, but successive studies regarding young male populations with diagnosed M2DS usually describe autistic features that they show. A main patient's behavioral symptoms also includes ataxia (10,56), gait abnormality (53,56,83) and sporadic eye movements (46,60,69). Although they have been more deeply studied in RTT patients (10,82), stereotypic hand movements have been also observed not only in human patients (83), but also in transgenic animals with a *MECP2* duplication (34,84–87).

Mental retardation is another characteristic related to X-linked mutations (88). The Intelligence Quotient (IQ) is a common index used to diagnose mental retardation. Although this quotient can be affected by environmental factors such as fetal alcohol syndrome, perinatal hypoxia, or infectious diseases during pregnancy (89), there is a relationship between low IQ scores and high frequency of X-linked mutations in a population (90).

D) Recurrent infections

Since the first study with children who had a *MECP2* duplication, where 100% of the described patients tested positive to respiratory infections (38), most of the newer studies about X-linked duplication in young males conclude that this affection is the main cause of early death in patients with M2DS (50,51,55,91).

IRAK1 gene is the most likely to be the cause of recurrent respiratory infections (47,54,55), although Bijlsma *et al.* described a young male patient who presented this symptom despite not having an extra copy of this gene (36).

E) Gastroesophageal and gastrointestinal mobility

Ramocki *et al.* reported 25 out of 33 (76%) of their M2DS patients suffering from intestinal pseudo-obstruction (58). This condition can be fatal if not treated, and is commonly matched with severe constipation and/or gastro-esophageal reflux (46).

When the duplicated region includes them, overexpression of L1 cell adhesion molecule (*L1CAM*) gene and filamin A, alpha (*FLNA*) gene could aggravate the intestinal obstruction (44,47). In fact, a direct relationship between *FLNA* and intestinal obstruction has been already seen in patients with a large duplicated region (41).

2 Objectives

This review aims to summarize and illustrate the state-of-the-art on drug and palliative treatment studies involving M2DS, and also to describe the auxiliar therapies that come along with the drug treatments.

3 Methodology

A comprehensive research was performed by searching in PUBMED, MEDLINE and Google Scholar and clinical trial registries and pharmaceutical companies using the keywords “mecp2 duplication syndrome treatment and/or drug or compound or molecule”.

Papers which did not describe symptoms, drug usage and/or outcome of the trial, or those where the patient’s improvement could be a result of a surgical procedure or non-drug therapies, were excluded.

Then, another search was done using the keywords “mecp2 therapy and/or occupational or physical or speech”.

4 Results

4.1 Drug-based clinical trials

After the search, clinical and preclinical drug studies in *MECP2* Duplication Syndrome have been compiled at **Table 1**.

Table 1. Clinical and preclinical drug studies in *MECP2* Duplication Syndrome

Assay type	Title	Described symptoms	Management	Outcome (reference)
In vivo (mouse)	Reversal of social recognition deficit in adult mice with *MECP2* duplication via normalization of MeCP2 in the medial prefrontal cortex	Social recognition deficit, aversive-like behaviors, heightened anxiety-like behaviors, fear generalization	MeCP2 levels restoration via CRISPR-Cas9	Social recognition deficit was reversed when medial prefrontal cortex (mPFC) MeCP2 levels were restored (92)
In vivo (human)	Valproic acid as a monotherapy in drug-resistant methyl-CpG-binding protein 2 gene (*MECP2*) duplication-related epilepsy	Epilepsy, hypotonia, dysmorphic features	LEV, LTG, OXC, LCM, TPM, CLB, VPA	VPA reduced the frequency of epilepsy episodes (93)
In vitro, patient's fibroblasts (human)	Altered neuronal network and rescue in a human *MECP2* duplication model	Epilepsy, pneumonia, developmental delay, hypotonia, dysmorphic features	Chemical library	Histone deacetylase inhibitor NCH-51 decreased MeCP2 levels of fibroblasts from *MECP2* Duplication Syndrome patients (94)
In vivo (human)	Improved seizure control by lacosamide in a patient with *MECP2* duplication syndrome	Epilepsy, hypotonia, developmental delay	LCM, LEV	Lacosamide reduced the frequency of epilepsy episodes (95)
In vivo (mouse)	Reversal of phenotypes in *MECP2* duplication mice using genetic rescue or antisense oligonucleotides	Social recognition deficit, hypoactivity, anxiety-like behaviors, motor abnormalities	Antisense oligonucleotides (ASOs)	ASO treatment corrected MeCP2 mRNA levels (96)
In vivo (mouse)	$GABA_A$ a receptor antagonism ameliorates behavioral and synaptic impairments associated with MeCP2 overexpression	Anxiety-like behaviors, motor coordination deficits, impaired novel object recognition, extinction learning	$GABA_A$ antagonist picrotoxin (PTX)	Chronic treatment of PTX ameliorated specific behavioral phenotypes, including motor coordination, episodic memory impairments, and synaptic plasticity deficits (97)
In vivo (human)	Treatment of epilepsy in 6 patients with MECP2 duplication syndrome	Epilepsy, focal tonic seizures, atypical absences, myoclonic seizures, head dropping, swallowing difficulties	TPM, VGB, ZNS, AZA, RUF, LEV, CLB, STM, CZP, LTG, ESM, VPA	One of the patients (1/6) became seizure-free after rufinamide and clonazepam (98)
In vivo (human)	Electroclinical pattern on *MECP2* duplication syndrome: eight new reported cases and review of literature	Epilepsy, myoclonic seizures, head dropping, head nodding	VPA, ETS, LEV, LTG, TPM	Two patients (2/6) became seizure-free after VPA (99)
In vivo (human)	Four unrelated patients with Lubs X-linked mental retardation syndrome and different Xq28 duplications	Epilepsy, myoclonic seizures	Anticonvulsants (unknown)	One patient (1/2) became seizure-free (43)
In vivo (human)	Submicroscopic duplication in Xq28 causes increased expression of the MECP2 gene in a boy with severe mental retardation and features of Rett syndrome	Epilepsy, hypotonia	TPM, CLB, LTG	All patients were refractory to the treatment (40)

AZA: azathioprine, CLB: clobazam, CZP: clonazepan, ESM: ethosuximide, LCM: lacosamide LEV: levetiracetam, LTG: lamotrigine, OXC: oxcarbazepine, RUF: rufinamide, STM: sulthiame, TPM: topiramate, VGB: vigabatrin, VPA: valproic acid, ZNS: zonisamide,

4.1.1 Anticonvulsants

Seizures are one of the most frequent symptoms in M2DS, and one of the most important constraints that limit the patient's daily activities (65), so the administration of anticonvulsants is mandatory in most of the cases (58).

Anticonvulsants are the most frequently used drugs against seizures in patients with MeCP2-related disorders. The compounds listed below had an effect on the patients in the compiled studies.

4.1.1.1 GABAergic compounds

Anomalous MeCP2 levels alter nervous system's GABAnergic circuits, which leads to the outcome of epileptic episodes in young patients (32,33). Although GABA has an inhibitory role in a mature brain, it acts as a excitation factor during the central nervous system development (100). Treatments with GABAergic drugs look for either the restauration of normal GABA levels, or the normalization of the GABA receptors ($GABA_A$ and/or $GABA_B$) response via inhibition or activation (32,33,97,101).

Valproic acid (sodium valproate, VPA) is a short-chain fatty acid, and is the most used antiepileptic drug today in the treatment of seizures (101). Although its exact mechanism of action is still unknown, it has been proved that VPA increments GABA levels in mice and humans (93,101,102). This compound is widely used to reduce the frequency and the intensity of the seizures related to RTT and M2DS (103).

In general terms, a simultaneous administration of VPA and other anticonvulsants has been proved to be effective against several kinds of seizures in young male patients with a *MECP2* duplication (93).

Clobazam (CLB) has demonstrated clinical benefit, also against MeCP2-related seizures (40), and has been administered safely in more than 50 European studies (104).

Clonazepam (CZP) is administered as an adjunctive treatment for partial and generalized seizures (105). It has been proved efficient against M2DS epilepsy episodes (98).

Picrotoxin (PTX), also known as cocculin, is a non-competitive $GABA_A$ antagonist (106) that was successfully used to reverse the wild-type phenotype in mice with a *MECP2* duplication (97). This compound is extremely toxic to humans, and there are evidences of PTX acting not only as an antagonist, but also as a $GABA_A$ blocker (107,108).

Topiramate (TPM) is a monosaccharide which increases GABA levels in the central nervous system (109,110). It has been successfully used in females with RTT (103,111) and in male patients with M2DS (40,93,98).

Vigabatrin (VGB) reduces the catalytic activity of the GABA transaminase enzyme, allowing GABA to maintain a higher concentration (112). Although it has been proved efficient in RTT cases (110,113,114), its efficacy against M2DS's seizures will need more preclinical and clinical studies (98).

4.1.1.2 Na^+ channel blockers

Seizures derived from MeCP2 overexpression can also be palliated by a controlled reduction of the motor neurons' excitation threshold (115). Voltage-gated sodium channels in neurons suffer a refractory period in which they are impermeable to sodium ions, so nerve impulses are not possible during this period. The time of recovery of these channels depends on cell type, and on the chemical environment (116). There are several compounds that can inhibit, partially or totally, the activity of sodium channels, leading to stabilization of neuronal membranes (115).

Lacosamide (LCM) is a sodium channel blocker, and it is the only anticonvulsant able to stabilize the refractory period in affected neurons without affecting the brain activity (117). It has been successfully tried in M2DS patients (95).

Lamotrigine (LTG) has been used in RTT, preventing not only convulsion episodes, but also hand stereotypies and autistic behaviors in young females (118,119). It has been administered in at least four documented occasions to M2DS patients, and it showed effectivity when used together with other anticonvulsants, such as VPA (40,93,98,99).

Oxcarbazepine (OXC) is commonly prescribed to patients with bipolar disorders, but it can also decrease the frequency of seizures related to epilepsy (120). Despite its success regarding other disorders, the effectiveness of OXC against M2DS seizures have not been proved yet (98), so further studies will be needed.

Rufinamide (RUF) is an anticonvulsant used for treating the seizures related to Lennox-Gastaut Syndrome, another disorder which causes early-onset epilepsy, usually administered together with other antiepileptic compounds (121,122).

Zonisamide (ZNS) is a sulfonamide derivative which not only prolongates sodium channel inactivation time, but also blocks T-type calcium channels (123). This compound

is well tolerated by children, and effective against early onset seizures (124). Despite its good results against infant epilepsy, only few studies on ZNS have been carried out in the case of MeCP2-related disorders (98,99,125).

4.1.2 Gene therapy

4.1.2.1 Antisense oligonucleotides

Antisense oligonucleotides are nucleotide chains from 8 to 50 nucleotides in length that bind, partially or totally, to messenger RNA through Watson-Crick base pairing, modulating the function of said mRNA (126). Recently, a promising field has emerged regarding the treatment of single-gene disorders with ASOs (127).

Although ASOs are one of the most specific treatments for chronic diseases, oligonucleotides have to overcome several chemical and physical barriers, such as the lipidic barrier, the ubiquitous presence of nucleases and other defense mechanisms against foreign polynucleotides and, on a larger scale, the blood–brain barrier (128,129).

Concerning these obstacles, a promising field regarding ASOs' delivery into targeted tissues is currently flourishing, and several methods for carrying the oligonucleotides inside cells at a sufficient quantity are being developed for neuromuscular disorders (130–132).

ASOs against *MEPC2* have been successfully used in model mice, achieving the recovery of the wild-type phenotype (96). There are two patents of ASOs for modulation of expressed MeCP2 levels via *MECP2* knock-down in mice (133,134), but there are no references on their use in human patients.

4.1.2.2 CRISPR-Cas9

CRISPR (clustered regularly interspaced short palindromic repeats) is a series of DNA sequences, derived from bacteriophages, that some species of bacteria and archaea archive in their chromosome for subsequent recognition, as a primitive immune system. Cas9 is the enzyme responsible of recognizing and cutting the foreign DNA sequences that correspond to those present in CRISPR.

CRISPR-Cas9 is a promising gene editing technology that uses Cas9 as a complementary oligonucleotide carrier that involves specific DNA sequence recognition and nuclease activity in the same enzyme. This allows the Cas9/oligonucleotide complex to search for

the target gene and cut it away from the chromosome, letting the cell DNA repair mechanisms to ligate the strands again (135). In addition, Cas9's nuclease domain can be removed, creating a reader protein with high specificity in which other peptidic functional subunits, such as a fluorescent protein, which marks the DNA sequence and allows its detection via fluorescence microscopy (136), or a nickase, which cuts only one of the DNA strands instead of both, and gives less off-target effects than a nuclease domain (137), can be attached to the recognition enzyme for broadening the spectrum of gene seeking/gene editing possibilities.

Nonetheless, this system involves risks that include point mutations, reading frame shift, random recombination, and unspecific gene targeting (138). This is the main reason why CRISPR-Cas9 has not been widely tried in human patients yet.

MECP2 gene has already been successfully targeted and modified in human induced pluripotent stem cells (iPSCs) via CRISPR-Cas9 (139). In the case of RTT model iPSCs, point mutations on *MECP2* can be repaired using an alternative Cas9 method known as homology-directed repair (HDR), which uses the attached oligonucleotide as a wild-type template for this gene and allows the recovery of the functional MeCP2 protein (140).

Regarding M2DS, Yu *et al.* (92) achieved a complete reversal of the autistic behavior in mice by injecting Cas9 intraperitoneally, and using an adeno-associated virus (AAV) as a vector for the Cas9/oligonucleotide complex.

4.2 Auxiliar therapies

Aside from the drug-based treatments described above, there are other therapies focusing on supporting and improving the patient's development. These therapies are complementary to the chemical treatment of the symptoms, but they cannot replace a drug treatment unless the doctor in charge specifically stablishes it (58).

4.2.1 Occupational therapy

Occupational therapy can be defined as the design of the occupational framework by the occupational therapist in collaboration with the recipient of services (the patient) to advance therapeutic evaluation or achieve a therapeutic goal (141,142).

Regarding MeCP2-related disorders, this kind of therapy improves the patient's motor abilities via mechanical stimuli (143) and their intellectual skills through reading and playing with toys (144).

Another important benefit is the consolidation of a close, coordinated circle of people, usually the therapist and the child's family, that will track the patient's behavior in order to collect useful data about their communication skills, autistic features evolution, in addition to supervising their seizure frequency, type and intensity, as a supporting tool for drug treatment (145,146).

4.2.2 Physical therapy

Physiotherapy is crucial for the improvement of motor learning in young patients with neuromuscular disorders (147). In M2DS patients, hypotonia and swallowing difficulties are the main symptoms to be treated by physical therapy (58).

After an exhaustive diagnosis of each case, the physiotherapist evaluates those practices that will be more suitable for performing an adequate treatment (148), and once these practices have been proved to maintain or increase the patient's muscular tone, they can be performed by parents or legal guardians after a brief course (149).

Besides that, swallowing difficulties sometimes involve the use of a feeding tube if the symptoms are severe enough (58).

4.2.3 Speech therapy

Speech therapy is related to evaluation, diagnosis, and treatment of communication disorders, not only concerning to speech, but also non-verbal languages such as music or signs (150).

Autism is the main cause of speech absence in M2DS patients (65,151). The probability for a person with autism of acquiring speech after the age of five is extremely low, although there are reported cases of late acquisition (152), and there are no evidences of M2DS patients acquiring speech after a speech therapy treatment. Nonetheless, clinical music therapy has been successfully tried with RTT patients (153).

5 Discussion

There is a remarkable difference between the quantity of drug studies on RTT compared to the ones on M2DS so far. Gomathi, Padmapriya & Balachandar (103) registered a total of 104 studies (65 on animal/cell line, 5 on iPSC and 34 on clinical trials) related to drug trials for treating RTT's symptoms. In comparison, only 6 studies on human patients, 1 with human iPSC cells, and 3 with mice were found for M2DS drug trials.

This difference in the number of early drug trials between both syndromes may lie in the time gap between the discovery of RTT by Andreas Rett in 1966 (154) and the first reported cases of M2DS in 1999 (38), a fact that has led researchers to mistakenly describe M2DS cases as RTT cases, or as the catch-all term X-linked disorders.

Due to the phenotypic similarities between these two syndromes (58), some the of the compounds that were successful when treating RTT symptoms are likely to work with M2DS too. It is probable that most of the drug trials on M2DS patients remain undocumented.

Although almost all the clinical trials compiled after this study aim to reduce the occurrence of seizures, the broader range of drugs tried against RTT include also epigenetic modifiers, hormone therapy, metabolic boosters, neurotrophic factor boosters, antidepressants, and mitochondrial effectors (103).

Some of them, such as the metabolic boosters L-Carnitine and lovastatin, could also be administered to M2DS patients and therefore combat the "floppy child" phenotype and decrease hypotonia. Neither of the syndromes causes a deficiency of L-Carnitine (38,52,103), but the administration of this amino acid could help the patients to increase their muscle tone and to improve their physical skills, communication and sleep (155).

The same applies for antidepressants and serotonergic drugs. There are studies on RTT which demonstrate the efficacy of fluoxetine and buspirone, two serotonin-reuptake inhibitors, of reducing the frequency of hyperventilation episodes in a young female (156). These drugs could also help M2DS patients to cope with respiratory issues.

In a general way, RTT patients' response to anticonvulsants is usually better (103) than in M2DS (93,98). Nonetheless, the rate of success of lamotrigine to reduce seizures frequency in M2DS (40,93,98,99) is comparable to that of the reported RTT cases (103,118,157), and the same occurs with the GABAergic drug topiramate (40,93,98,111).

However, one of the difficulties when stablishing an effective treatment against seizures is the high variability of the response of the M2DS patients to the different anticonvulsants (93). Valproic acid has turned out to be the most promising anticonvulsant for the reduction of the epilepsy episodes in M2DS young patients (93,98,158).

To date, all the drug trials on M2DS with human patients aim for fighting the main symptoms of the disorder, but none of them prevents the patient from requiring medication and auxiliar therapies for the rest of their lives, as the available drugs do not address the root of the disease.

Both RTT and M2DS have been proved to be reversible if MeCP2 wild-type levels are restored (92,159), but they will continue to be chronic diseases until a gene editing treatment is available for human patients.

Although antisense oligonucleotides (ASOs) and CRISPR-Cas9 work very well in mice and single cells, some clinic studies on carriers and vectors are needed in order to arrive to the final step of drug development with these two promising therapies.

Furthermore, gene editing treatments with CRISPR-Cas9 would theoretically reverse M2DS phenotype in human patients, as it would carry out a deletion of the extra genes involved in the disease.

Another less developed strategy, but also very promising in genetic disorders, is the screening of small molecules, known as "molecular chaperones", that help the protein to fold properly or to strengthen the protein-ligand binding. This has already been done to stabilize the MeCP2-DNA interaction (9,160), aiming for the address the lack of functional MeCP2 in RTT. A reverse approach could also be applied to M2DS, by finding a compound capable of increasing the MeCP2-DNA binding energy, thus destabilizing it and counteracting the abnormally high levels of expressed protein.

6 Conclusions and future directions

There are far more studies on treatments for Rett Syndrome than *MECP2* Duplication Syndrome, probably because M2DS patients have traditionally been mistaken for Rett Syndrom cases until the first cases of M2Ds were described.

Among palliative treatments, anticonvulsant drugs are the most frequently prescribed ones, and they usually succeed against epilepsy in young patients. Valproic acid is the most used anticonvulsant for the treatment of the seizures derived from M2DS. Nonetheless, physiotherapy, occupational therapy and speech therapy play an important role in the maintenance of the patient wellness and the achievement of independency, and they are still imperative in M2DS cases, because there are still no compounds able to address the root of the disease.

Antisense oligonucleotides (ASOs) and CRISPR-Cas9 are two promising gene therapies against chromosomal duplications. Additionally, small molecules screenings have proven to be effective to normalize MeCP2's function in RTT, and the same approach could be performed for M2DS.

The original intention of this Master's thesis was to carry out a small molecules screening with a chemical library to find compounds which bound to free, recombinant MeCP2, favoring the destabilization of the MeCP2/DNA complex. In order to contribute to the discovery of new drugs against *MECP2* Duplication Syndrome, it would be interesting to perform this study in the future.

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