IDMC-13

The 13th International Myotonic Dystrophy Consortium Meeting

22-25 June 2022 Osaka, Japan

IDMC-13

The 13th International Myotonic Dystrophy Consortium Meeting

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Welcome to IDMC-13

Masanori P. TAKAHASHI, M.D., Ph.D.
On behalf of the local organizing committee of the IDMC-13

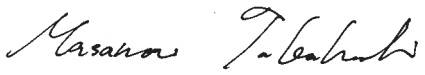
It is a great pleasure to hold IDMC-13 in Osaka, Japan.

Starting in 1997, the International Myotonic Dystrophy Consortium Meeting (IDMC) has been the premier forum fostering interaction and collaboration of clinicians, pharmaceutical companies, and investigators of all types working to understand, treat and ultimately control this most common form of muscular dystrophy.

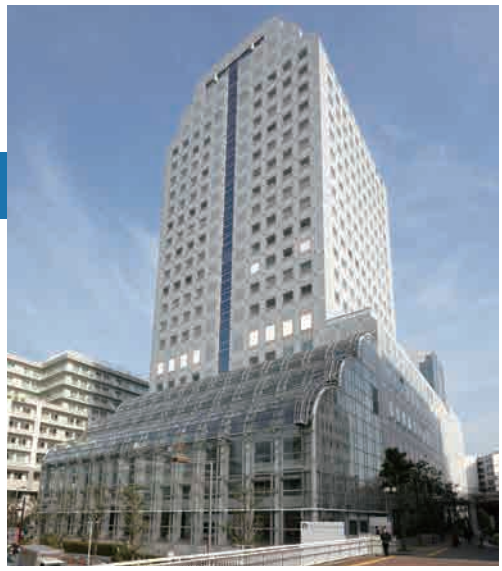
Although the IDMC-13 has been postponed for a year, the strict travel restrictions by the Japanese government continue, and the war in Ukraine makes international travel further difficult.

IDMC-3, the last IDMC in Japan, was in October 2001, one month after 9.11. Many cancelations of participation were expected, but the meeting turned out to be a huge success. Almost everyone showed up at the venue, had lively scientific discussions, and fostered cooperation and trust through social events. It was my first exposure to the IDMC meeting, and I was inspired by the passion of the DM community for basic research, treatment, and medical care.

The IDMC-13 is held in a hybrid format for the first time, and on-demand distribution is also available for one month. There are, unfortunately, no social events, a tradition of IDMC, but we hope the IDMC-13 will be as active and fruitful as previous IDMCs, taking advantage of online participation from anywhere and anytime.



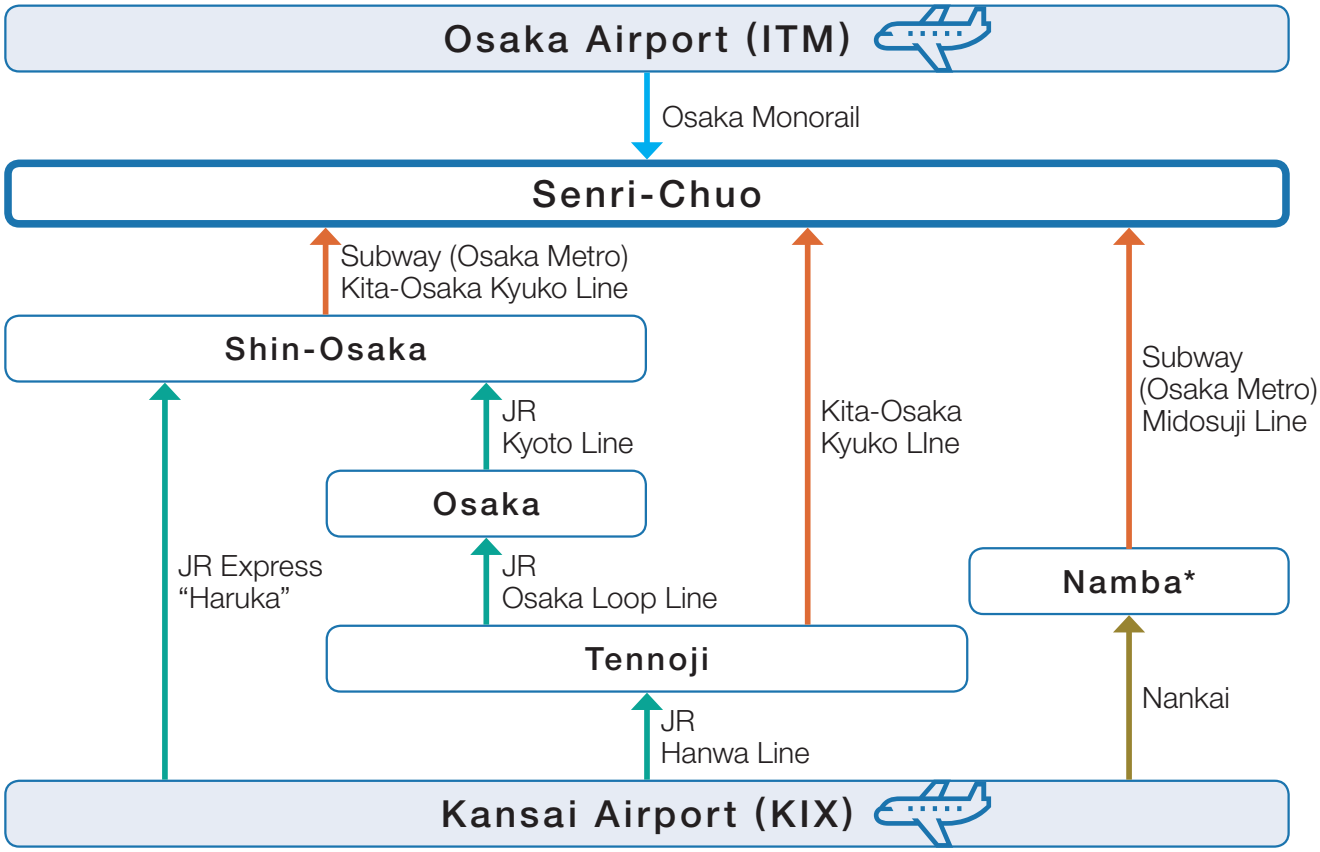
Senri Life Science Center



1-4-2 Shinsenrihigashimachi,
Toyonaka-city, Osaka, Japan
https://www.senrilc.co.jp/access/index2.html
Google Map
https://goo.gl/maps/xSYLG6JjEJgVKiMY9

Transportation

From Osaka Airport (ITM) and Kansai Airport (KIX)



*It takes about 7 minutes from Nankai Namba station to Osaka Metro Namba station.
There are several subway lines in Namba and Teennoji. Please choose Midosuji Line bound for Senri-Chuo.

From JR Shin-Osaka station: Shinkansen (Bullet train), Kyoto Line

Subway (Osaka Metro) Kita-Osaka Kyuko Line: Shin-Osaka → Senri-Chuo

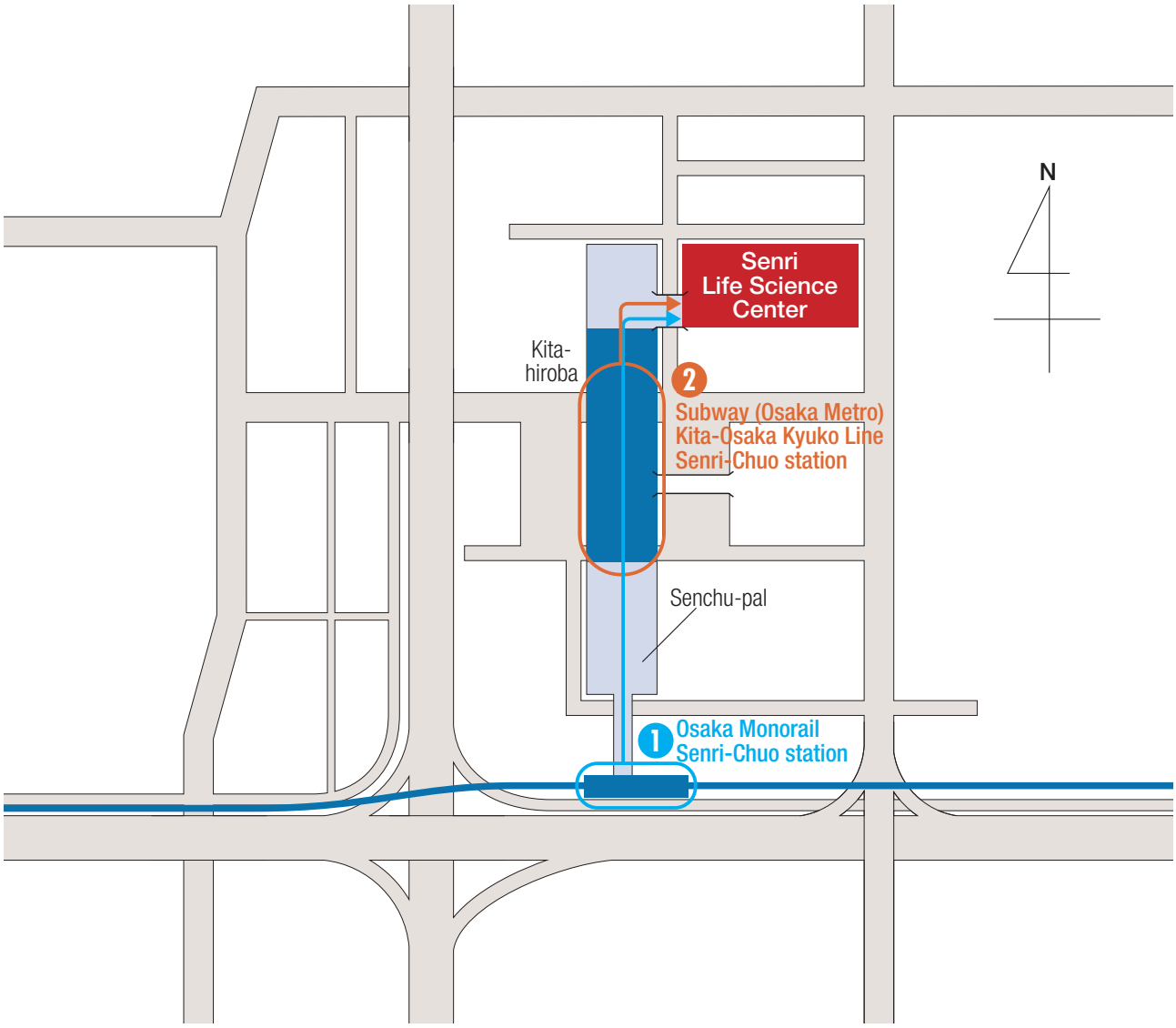
From Senri-chuo station to the venue

❶ From the monorail station

The station gate is located on the deck floor. Go straight north on the deck floor through the shopping mall, and cross the second overpass on the right to reach the venue.

❷ From the subway station

The station gate is located underground. Exit through the North gate (on the side of first train car bound for Senri-Chuo) and go up to the deck floor (2nd floor). The station gate is located underground. Exit through the North gate (on the side of first train car bound for Senri-Chuo) and go up to the deck floor (2nd floor) and cross the overpass on the right (east side) to reach the venue.



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Midori SENOO	Myotonic Dystrophy Patients' Group of Japan (DM-family), Japan
Yuya TSUCHIDA	Myotonic Dystrophy Patients' Group of Japan (DM-family), Japan

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Hirokazu FURUYA, M.D., Ph.D.	Kochi University, Japan
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Masayuki NAKAMORI, M.D., Ph.D.	Osaka University, Japan
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Yuuji AKECHI	Myotonic Dystrophy Patients' Group of Japan (DM-family), Japan
Minako SATOU	Myotonic Dystrophy Patients' Group of Japan (DM-family), Japan

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INTERNATIONAL Myotonic Dystrophy Awareness Day SEPTEMBER 15

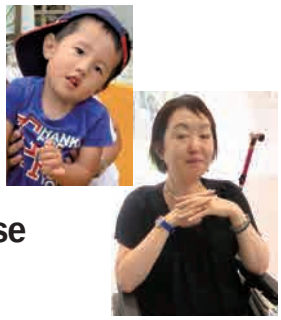
There are things you can do as patients

As patients, we can support DM research

We can share correct DM knowledge

We can make the public aware of DM

Together, we can change the future of this disease



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https://dm-family.net/

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2. 禁忌(次の患者には投与しないこと)
本剤の成分に対し過敏症の既往歴のある患者

4. 効能又は効果

全身型重症筋無力症(ステロイド剤又はステロイド剤以外の免疫抑制剤が十分に奏効しない場合に限る)

6. 用法及び用量

通常、成人にはエフガルチギモド　アルファ(遺伝子組換え)として1回10mg/kgを1週間間隔で4回1時間かけて点滴静注する。これを1サイクルとして、投与を繰り返す。

7. 用法及び用量に関連する注意

7.1　次サイクル投与の必要性は、臨床症状等に基づき、判断すること。[17.1.1、17.1.2参照]
7.2　本剤を投与する場合に、何らかの理由により投与が遅れた際には、あらかじめ定めた投与日から3日以内であればその時点で投与を行い、その後はあらかじめ定めた日に投与すること。あらかじめ定めた投与日から3日を超えていれば投与せず、次のあらかじめ定めた日に投与すること。

8. 重要な基本的注意

8.1　本剤の投与により、血中IgG濃度が低下し、感染症が生じる又は悪化するおそれがある。本剤の治療期間中及び治療終了後は定期的に血液検査を行うなど、患者の状態を十分に観察すること。また、感染症の自他覚症状に注意し、異常が認められた場合には、速やかに医療機関に相談するよう患者に指導すること。[9.1.1、11.1.1、16.8.1参照]
8.2　本剤の投与により、infusion　reactionが発現する可能性があるため、患者の状態を十分に観察し、異常が認められた場合には本剤の投与速度を下げる、又は投与を中止し、適切な処置を行うこと。

9. 特定の背景を有する患者に関する注意

9.1　**合併症・既往歴等のある患者**
9.1.1　**感染症のある患者**
感染症を合併している場合は、感染症の治療を優先すること。感染症が増悪するおそれがある。[8.1、11.1.1参照]
9.1.2　**肝炎ウイルスキャリアの患者**
肝炎ウイルスキャリアの患者に本剤を投与する場合は、肝機能検査値や肝炎ウイルスマーカーのモニタリングを行うなど、B型肝炎ウイルスの再活性化やC型肝炎の悪化の徴候や症状の発現に注意すること。
9.2　**腎機能障害患者**
本剤の血中濃度が上昇するおそれがある。なお、重度(eGFRが30mL/min/1.73m^{2}未満)の腎機能障害を対象とした有効性及び安全性を指標とした臨床試験は実施していない。[16.6.1参照]
9.5　**妊婦**
妊婦又は妊娠している可能性のある女性には治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。IgG抗体は胎盤通過性があることが知られている。本剤の投与を受けた患者からの出生児においては、感染のリスクが高まる可能性があるため、生ワクチン又は弱毒生ワクチンを接種する際には注意が必要である。
9.6　**授乳婦**
治療上の有益性及び母乳栄養の有益性を考慮し、授乳の継続又は中止を検討すること。本剤のヒト乳汁中への移行は不明であるが、ヒトIgGは乳汁中に移行することが知られている。
9.7　**小児等**
小児等を対象とした臨床試験は実施していない。

10. 相互作用

10.2　**併用注意(併用に注意すること)**

薬剤名等	臨床症状・措置方法	機序・危険因子
人免疫グロブリン製剤(ポリエチレングリコール処理人免疫グロブリン等) エクリズマブ(遺伝子組換え)	これらの薬剤の治療効果が減弱する可能性がある。 これらの薬剤による治療を開始する場合、本剤のサイクル投与における最終投与から2週間後以降に投与することが望ましい。	本剤がこれらの薬剤の血中濃度を低下させる可能性がある。
血液浄化療法	本剤の治療効果が減弱する可能性があるため、併用を避けることが望ましい。	本剤による治療中に施行することにより本剤の血中濃度を低下させる可能性がある。
生ワクチン及び弱毒生ワクチン	ワクチンの病原に基づく症状が発現する可能性があるため、本剤による治療中の接種を避けることが望ましい。	生ワクチン又は弱毒生ワクチンによる感染症発現のリスクが増大するおそれがある。
生ワクチン及び弱毒生ワクチン以外のワクチン	ワクチンの効果が減弱する可能性がある。 ワクチンは本剤投与開始の少なくとも4週間前までに接種することが望ましい。 本剤による治療中の場合、本剤のサイクル投与における最終投与から2週間以降にワクチンを投与することが望ましい。	本剤の作用機序により、ワクチンに対する免疫応答が得られない可能性がある。

11. 副作用

次の副作用があらわれることがあるので観察を十分に行い、異常が認められた場合には投与を中止するなど適切な処置を行うこと。

11.1　**重大な副作用**
11.1.1　**感染症 (6.8%)**
帯状疱疹、上咽頭炎、インフルエンザ等の感染症が起こることがある。[8.1、9.1.1参照]

11.2　**その他の副作用**

	5～15%未満	5%未満
神経系障害	頭痛	浮動性めまい
胃腸障害		悪心、嘔吐
傷害、中毒および処置合併症		処置による頭痛
臨床検査		リンパ球数減少、好中球数増加
一般・全身障害および投与部位の状態		疲労
感染症および寄生虫症		帯状疱疹
皮膚および皮下組織障害		発疹

21. 承認条件

21.1　医薬品リスク管理計画を策定の上、適切に実施すること。
21.2　国内での治験症例が極めて限られていることから、製造販売後、一定数の症例に係るデータが集積されるまでの間は、全症例を対象に使用成績調査を実施することにより、本剤の使用患者の背景情報を把握するとともに、本剤の安全性及び有効性に関するデータを早期に収集し、本剤の適正使用に必要な措置を講じること。

24. 文献請求先及び問い合わせ先

アルジェニクスジャパン株式会社
107-0052 東京都港区赤坂二丁目5番8号 ヒューリックJP赤坂ビル
TEL：0800-999-2100

26. 製造販売業者等

26.1　製造販売元
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JP-VJP-22-00289
(2022年6月作成)

患者さんとの絆をつなぐ

重篤な疾患と共に生きる患者さんとそのご家族が、笑顔を取り戻し、人生の喜びを感じていただくことがユーシービージャパンの願いです。
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【効能・効果】 レビー小体型認知症に伴うパーキンソニズム
（レボドパ含有製剤を使用してもパーキンソニズムが残存する場合）

※本効能・効果はOD錠25mgに限られます。

禁忌（次の患者には投与しないこと）
（1）妊婦又は妊娠している可能性のある婦人〔添付文書の「妊婦・産婦・授乳婦等への投与」の項参照〕
（2）本剤の成分に対して過敏症の既往歴のある患者

効能・効果
<トレリーフOD錠25mg>
1.パーキンソン病
（レボドパ含有製剤に他の抗パーキンソン病薬を使用しても十分に効果が得られなかった場合）
2.レビー小体型認知症に伴うパーキンソニズム
（レボドパ含有製剤を使用してもパーキンソニズムが残存する場合）
<トレリーフOD錠50mg>
パーキンソン病
（レボドパ含有製剤に他の抗パーキンソン病薬を使用しても十分に効果が得られなかった場合）

用法・用量
<トレリーフOD錠25mg>
本剤は、レボドパ含有製剤と併用する。
1.パーキンソン病
通常、成人にゾニサミドとして、1日1回25mgを経口投与する。なお、パーキンソン病における症状の日内変動（wearing-off現象）の改善には、1日1回50mgを経口投与する。
2.レビー小体型認知症に伴うパーキンソニズム
通常、成人にゾニサミドとして、1日1回25mgを経口投与する。
<トレリーフOD錠50mg>
パーキンソン病
本剤は、レボドパ含有製剤と併用する。
通常、成人にゾニサミドとして、1日1回25mgを経口投与する。なお、パーキンソン病における症状の日内変動（wearing-off現象）の改善には、1日1回50mgを経口投与する。

<用法・用量に関連する使用上の注意>
1. パーキンソン病に対する本剤の1日50mg投与において、1日25mg投与時を上回るon時の運動機能の改善効果は確認されていない。〔添付文書の「臨床成績」の項参照〕
2.（OD錠）本剤は口腔内で崩壊するが、口腔粘膜からの吸収により効果発現を期待する製剤ではないため、唾液又は水で飲み込むこと。〔「適用上の注意」の項参照〕

使用上の注意（抜粋）
1. 慎重投与（次の患者には慎重に投与すること）
重篤な肝機能障害又はその既往歴のある患者〔血中濃度が上昇するおそれがある。〕
2. 重要な基本的注意　(1)本剤投与中又は投与中止後に**悪性症候群**があらわれることがあるので注意すること。〔「重大な副作用」の項参照〕 (2)連用中は定期的に**肝・腎機能、血液検査**を行うことが望ましい。(3)眠気、注意力・集中力・反射運動能力等の低下が起こることがあるので、本剤投与中の患者には**自動車の運転等危険を伴う機械の操作に従事させない**よう注意すること。(4)**発汗減少**があらわれることがあり、特に夏季に体温の上昇することがあるので、**本剤投与中は体温上昇に留意**し、このような場合には高温環境下をできるだけ避け、適切な処置を行うこと。〔「重大な副作用」の項参照〕 (5)本剤投与中又は投与中止後に、**自殺企図**があらわれることがあるので、患者の状態及び病態の変化を注意深く観察すること。〔添付文書の「その他の副作用」、「その他の注意」の項参照〕
3. 相互作用　本剤は、主として薬物代謝酵素CYP3Aで代謝される。〔添付文書の「薬物動態」の項参照〕
併用注意（併用に注意すること）
抗てんかん剤[フェニトイン、カルバマゼピン、フェノバルビタール、バルプロ酸等]、フェニトイン、三環系抗うつ剤[アミトリプチリン等]、四環系抗うつ剤[マプロチリン等]、レセルピン誘導体[レセルピン等]、フェノチアジン系薬剤[クロルプロマジン等]、ブチロフェノン系薬剤[ハロペリドール等]、スルピリド、メトクロプラミド
4. 副作用
<パーキンソン病の場合>
用量追加承認までの臨床試験842例中393例(46.7%)に臨床検査値異常を含む副作用がみられた。主なものは眠気(8.4%)、食欲不振(6.7%)、ジスキネジア(5.7%)、悪心(4.8%)、幻覚(4.4%)、気力低下(4.2%)等であった。(トレリーフ錠の用量追加承認時)
特定使用成績調査542例中62例(11.4%)に臨床検査値異常を含む副作用がみられた。主なものはめまい・ふらつき(2.4%)、幻覚(1.7%)、ジスキネジア(1.5%)等であった。(トレリーフ錠の再審査終了時)

<レビー小体型認知症に伴うパーキンソニズムの場合>
承認時までの臨床試験435例中120例(27.6%)に臨床検査値異常を含む副作用がみられた。主なものは体重減少(5.3%)、眠気(3.2%)、食欲不振(2.5%)、発疹(1.6%)、幻覚(1.6%)、精神症状の悪化(1.6%)、転倒(1.6%)等であった。(承認時)
(1)**重大な副作用**
1)**悪性症候群**(1%未満) 本剤投与中又は投与中止後に悪性症候群があらわれることがある。観察を十分に行い、発熱、意識障害、無動無言、高度の筋硬直、不随意運動、嚥下困難、頻脈、血圧の変動、発汗、血清CK(CPK)の上昇等があらわれた場合には、冷却、水分補給等の全身管理、及び再投与後に漸減するなど適切な処置を行うこと。なお、本症発症時には、ミオグロビン尿を伴う腎機能の低下がみられることがある。〔「重要な基本的注意」の項参照〕 2)**中毒性表皮壊死融解症(Toxic Epidermal Necrolysis：TEN)、皮膚粘膜眼症候群(Stevens-Johnson症候群)、紅皮症(剥脱性皮膚炎)**(頻度不明) 観察を十分に行い、発熱、紅斑、水疱・びらん、瘙痒感、咽頭痛、眼充血、口内炎等の異常が認められた場合には、投与を中止し、副腎皮質ホルモン剤の投与等の適切な処置を行うこと。3)**過敏症症候群**(頻度不明) 初期症状として発疹、発熱がみられ、さらにリンパ節腫脹、肝機能障害等の臓器障害、白血球増加、好酸球増多、異型リンパ球出現等を伴う遅発性の重篤な過敏症状があらわれることがあるので、観察を十分に行い、このような症状があらわれた場合には、投与を中止し、適切な処置を行うこと。なお、ヒトヘルペスウイルス6(HHV-6)等のウイルスの再活性化を伴うことが多く、発疹、発熱、肝機能障害等の症状が再燃あるいは遷延化することがあるので注意すること。4)**再生不良性貧血、無顆粒球症、赤芽球癆**(頻度不明)、**血小板減少**(1%未満) 観察を十分に行い、異常が認められた場合には、投与を中止し、適切な処置を行うこと。5)**急性腎障害**(頻度不明) 観察を十分に行い、異常が認められた場合には、投与を中止し、適切な処置を行うこと。6)**間質性肺炎**(頻度不明) 発熱、咳嗽、呼吸困難、胸部X線異常、好酸球増多等を伴う間質性肺炎があらわれることがあるので、このような症状があらわれた場合には、投与を中止し、副腎皮質ホルモン剤の投与等の適切な処置を行うこと。7)**肝機能障害、黄疸**(頻度不明) AST(GOT)、ALT(GPT)、γ-GTPの上昇等を伴う重篤な肝機能障害、黄疸があらわれることがあるので、観察を十分に行い、異常が認められた場合には、投与を中止し、適切な処置を行うこと。8)**横紋筋融解症**(1%未満) 観察を十分に行い、筋肉痛、脱力感、CK(CPK)上昇、血中及び尿中ミオグロビン上昇等があらわれた場合には、投与を中止し、適切な処置を行うこと。また、横紋筋融解症による急性腎障害の発症に注意すること。9)**腎・尿路結石**(1%未満) 観察を十分に行い、腎疝痛、排尿痛、血尿、結晶尿、頻尿、残尿感、乏尿等があらわれた場合には、投与を中止するなど適切な処置を行うこと。10)**発汗減少に伴う熱中症**(頻度不明) 発汗減少があらわれ、体温が上昇し、熱中症をきたすことがある。発汗減少、体温上昇、顔面潮紅、意識障害等がみられた場合には、投与を中止し、体冷却等の適切な処置を行うこと。〔「重要な基本的注意」の項参照〕 11)**幻覚**(1%以上)、**妄想**(1%未満)、**錯乱**(1%未満)、**せん妄**(1%未満)**等の精神症状** 観察を十分に行い、このような症状があらわれた場合には、投与を中止するなど適切な処置を行うこと。

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Invited Speakers

Advance in Fukuyama muscular dystrophy & dystroglycanopathy

Tatsushi Toda

Department of Neurology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Tatsushi Toda MD, PhD
2018- President, Japanese Society of Neurology
2017- Member of the Science Council of Japan
2017- Professor, Department of Neurology, University of Tokyo
2009-2017 Professor, Division of Neurology/Molecular Brain Science, Kobe University
2000-2009 Professor, Division of Clinical Genetics, Osaka University
1985 graduated from University of Tokyo

Dr. Tatsushi Toda identified genes for Fukuyama muscular dystrophy (Nat Genet 1993, Nature 1998), muscle-eye-brain disease (Dev Cell 2001), and Parkinson-susceptibility (Nat Genet 2009), and he found antisense therapy for Fukuyama CMD (Nature 2011) and new glycosylation and defect in muscular dystrophy (Cell Rep 2016, Nat Commun 2022).
2008 Asahi Award
2009 Award from Japanese Minister of Education, Culture, Sports, Science and Technology
2017 Japan Academy Prize
2019 Japan Medical Association Award.

Fukuyama muscular dystrophy (FCMD) and muscle-eye-brain (MEB) disease are similar disorders characterized by congenital muscular dystrophy, brain and eye anomalies. Hypoglycosylation of α-dystroglycan (α-DG) are common characteristics of these dystroglycanopathies. We identified the genes for FCMD (fukutin) and MEB (POMGnT1). FCMD is the first human disease found to result from ancestral insertion of a SVA retrotransposon. We show that aberrant mRNA splicing, induced by SVA exon-trapping, underlies the molecular pathogenesis of FCMD. Introduction of antisense oligonucleotides (AONs) targeting the splice acceptor, the predicted exonic splicing enhancer and the intronic splicing enhancer prevented pathogenic exon-trapping by SVA in cells of patients with FCMD and model mice, rescuing normal fukutin mRNA expression and protein production. AON treatment also restored fukutin functions, including O-glycosylation of α-DG and laminin binding by α-DG. We further optimized it to one nucleic acid, NS-035, completed toxicity, safety, and efficacy studies, and initiated investigator-initiated clinical trials with the support of AMED.

We further identified the previously unknown glycan unit ribitol 5-phosphate (Rbo5P), a phosphoric ester of pentose alcohol, as a tandem repeat that functions as a scaffold for the formation of the ligand-binding moiety of α-DG. We determined the enzyme activities of three major α-DGpathy-causing proteins to be involved in the synthesis of tandem Rbo5P. ISPD is cytidine diphosphate ribitol (CDP-Rbo) synthase. Fukutin and fukutin-related protein are Rbo5P transferases that use CDP-Rbo. Consequently, Rbo5P glycosylation is defective in α-DGpathy models. We further demonstrate that prodrug treatments (tetraacetylated CDP-ribitol) can ameliorate muscular dystrophy caused by defects in ISPD.

Exon skipping therapy to Duchenne muscular dystrophy

Shin'ichi Takeda

Honorary Director general of National Institute of Neuroscience
National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

3/77 University of Akita, School of Medicine, M.D.
3/81 Shinshu University, Graduate School, Ph.D.
4/84 Shinshu University School of Medicine, Dept of Medicine, Instructor in Neurology
8/87 Institut Pasteur, Paris, Postdoctoral Researcher in Molecular Biology
9/92 NCNP, NIN, Dept of Neuromuscular Research, Section Chief
10/00 Dept of Molecular Therapy, Director
10/08 NCNP, Translational Medical Center, Director general
4/15 NIN, Director general
4/18 NCNP, Executive Director
4/20- NCNP, NIN, Senior scientific advisor/Honorary director general
Japan Muscle Society (former President)
Associate editor for reviews of J. Neuromuscular Diseases (10/13-)
Associate editor of American J. Pathology (10/14-)
Executive Board Members of CINRG (10/19-)

Duchenne muscular dystrophy (DMD) is the most common childhood genetic disease, affecting one among 5,000 newborn boys, causing progressive muscle weakness, heart and respiratory failure and premature death. This disease is caused by mutations of the DMD gene, and no cure exists for this disease, but promising new molecular therapies are being intensively studied. Exon skipping by antisense oligonucleotides (AONs) is a novel method to restore the reading frame of the mutated DMD gene, and rescue dystrophin expression. We have reported that systemic delivery of AONs targeting exon 6 and 8 of the canine DMD gene to CXMDJ, a dystrophin-deficient canine animal model, efficiently restored functional dystrophin proteins and improved phenotypes of affected dogs (Ann Neurol. 2009;65:667-76). We, then, optimized AON sequences, which allow exon 53 skipping of the human DMD gene, and created the drug, NS-065/NCNP-01, with phosphorodiamidate morpholino oligomer (PMO), together with Nippon Shinyaku Co. Ltd. (NS). We carried an early phase clinical trial and that has been successfully completed without serious adverse events. Following highly effective exon skipping detected by RT-PCR and dystrophin expression (Sci Transl Med. 2018;10(437)), NS-065/NCNP-01, now called as Viltolarsen, has been chosen as fast track for approval process both in Japan and in US, then phase I/II trial in Japan and phase II trial in US were carried by either NS or NS Pharma, Inc. Based on favorable results in these clinical trials in both countries (Ann Clin Transl Neurol. 2020;7:181-190), (JAMA Neurol. 2020;e201264), Viltolarsen has gotten the manufacture and sales approval by PMDA in Japan on March and by FDA in US on August in 2020. Viltolarsen is now available for many DMD patients who have susceptible mutations of the DMD gene for exon 53 skipping.

Conflict of Interest: The speaker has a conflict of interest with Nippon Shinyaku Co. Ltd.

Lesson from other expansion diseases, disease-modifying therapy for SBMA

Gen Sobue
Aichi Medical University, Nagakute, Japan
Nagoya University, Nagoya, Japan,

In 1975, graduated from Nagoya University, School of Medicine.
In 1981, completed a PhD in neurology at Nagoya University Graduate School of Medicine.
From 1981, worked as a lecturer and an associate professor at Aichi Medical University.
In the meanwhile 1982 to 1985, worked as an assistant professor at the University of Pennsylvania, USA.
In 1995, appointed as a professor of neurology at Nagoya University Graduate School of Medicine.
From 2009 to 2012, served as the dean of Nagoya University Graduate School of Medicine.
From 2015, worked as a designated professor of neurology at Nagoya University Graduate School of Medicine and a director of Brain and Mind Research Center at Nagoya University.
From 2019, serve as a president at Aichi Medical University.

Spinal and bulbar muscular atrophy (SBMA) is a lower motor neuron disease caused by an expanded trinucleotide CAG repeat, which encodes the polyglutamine tract, in the androgen receptor (AR) gene. The main symptoms are slowly progressive muscle weakness and atrophy of bulbar, facial and limb muscles. The cardinal histopathological findings of SBMA are an extensive loss of lower motor neurons and intranuclear accumulations of mutant AR protein in the residual motor neurons. SBMA exclusively occurs in adult males, whereas both heterozygous and homozygous females are usually asymptomatic.

To elucidate the pathogenic mechanism and develop the therapeutics, we generated a model mouse with AR-97Q in androgen receptor gene. Androgen deprivation through castration or leuprorelin acetate administration improved the symptoms. Androgen deprivation rescues neuronal dysfunction in animal models of SBMA associated with disappearance of nuclear pathogenic AR accumulation, suggesting that the molecular basis for motor neuron degeneration in this disorder is androgen-dependent nuclear accumulation of the mutant AR. We then performed an investigator-initiated clinical trial of leuprorelin for SBMA. Suppression of disease progression of the swallowing function, serum CK level and incidence of pneumonia by leuprorelin acetate has been demonstrated and leuprorelin was approved by PMDA in Japan. Based on these clinical trials, we also demonstrated that disease-modifying therapy, like the leuprorelin on SBMA, takes long term treatment up to 7 to 8 years long to assess the efficacy on the true endpoint like death or permanent respirator support.

In addition, the ligand-dependent accumulation of the pathogenic AR, an initial step in the neurodegenerative process in SBMA, is followed by several downstream molecular events, such as transcriptional dysregulation and axonal transport disruption. Advances in basic and clinical researches on SBMA are providing the way for clinical application of molecular targeting therapeutics. The clarification of pathophysiology leads to appearance of candidate drugs.

Peter Harper Memorial Lecture

Peter Harper, Myotonic Dystrophy and Me.

J D Brook[1], M T Rogers[2], M A Bowler[3]

[1] School of Life Sciences, University of Nottingham, Queen's Medical Centre, Nottingham, UK.
[2] Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK.
[3] Myotonic Dystrophy Support Group, Gedling, Nottingham, UK.

Peter Harper made outstanding contributions to the study of neurological and neuromuscular disease. In addition to his research, he was a dedicated clinician and exceptional supporter of patients, who provided the basis for modern clinical genetics in the UK and elsewhere. The main conditions with which he is associated are Huntington's Disease and Myotonic Dystrophy (DM). In the case of DM, he literally 'wrote the book' on it.

In this lecture the three presenters will pay tribute to all aspects of Peter's work framed in a chronological sequence of his life, drawing parallels between progress in our understanding of DM and the various contributions Peter made during his lifetime. We will consider his involvement in leading research, writing books of genetic conditions and genetic counselling, patient care and supporting the support groups. We will also consider where research into DM is going.

Overview

Repeat instability

Christopher Pearson
The Hospital for Sick Children

Overview

Repeat-mediated pathomechanisms

Maurice Swanson
Department of Molecular Genetics and Microbiology, Center for NeuroGenetics and the Genetics Institute, University of Florida, College of Medicine, Gainesville, FL, USA

The discoveries of the *DMPK* CTG and *CNBP* CCTG short tandem repeat expansions (STR^{exp}) as the causes of myotonic dystrophy types 1 (DM1) and 2 (DM2) resulted in international research efforts to understand the pathomechanisms associated with disease onset and progression. The resulting biochemical, cell and animal studies led to novel disease models that implicated mutant DMPK and CNBP RNA transcripts, specifically the CUG and CCUG expansions (CUG^{exp}, $CCUG^{exp}$), as major pathogenic factors. While CUG^{exp} and $CCUG^{exp}$ RNAs alter the activities of multiple RNA-binding proteins (RBPs) resulting in the expression of developmentally inappropriate RNA and protein isoforms, they also serve as templates for repeat-associated non-AUG (RAN) translation and the production of RAN proteins. Importantly, these disease models have provided an experimental blueprint for other STR^{exp} disorders. For this overview, these and additional research studies will be briefly reviewed followed by a more detailed discussion of recent experimental findings.

Small Molecules targeting Repeat Sequences causing Neurological Disorders

Kazuhiko Nakatani
SANKEN, The Institute of Scientific and Industrial Research, Osaka University

Dr. Kazuhiko Nakatani graduated from the Department of Chemistry, Osaka City University in 1982. In 1985, he left the university to study at the Department of Chemistry, Columbia University, U.S.A. In 1988, he returned to Japan to complete his doctoral degree at his university and to become a postdoctoral researcher at the Sagami Chemical Research Center. In 1991, he became a research associate at the Faculty of Science, Osaka City University and then in 1993 at the Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University. He was promoted to Assistant Professor at Kyoto University in 1997, and then to Professor at SANKEN, Osaka University in 2005, where he established his own laboratory. He served as the Director of SANKEN from August 2015 to March 2018, and since August 2019, he has been serving as the Executive Vice President for Finance and Facilities at Osaka University.

In DM1, aberrantly expanded CUG repeats trap mRNA metabolism-related proteins such as MBNL1 and induce selective splicing, which is important for the pathogenesis of the disease. In our laboratory, we have been working to develop compounds that exhibit binding activity to G-T and G-U mismatches. Although we have found some effective compounds such as diaminophenanthrene derivatives, their binding selectivity remains an issue because they show affinity for other RNAs such as CCG repeats in addition to CUG repeats. From structure-activity relationship studies using computer simulations and SPR binding data of synthetic molecules, we found a CUG repeat-selective binding molecule JM642 with a diaminoisoquinoline structure. Dr. Masayuki Nakamori, Department of Neurology, Graduate School of Medicine, Osaka University and us studied the effect of JM642 using a DM1 mouse model. We administered JM642 intraperitoneally (20 mg/kg) for 5 days to investigate the effect of releasing splicing factors trapped by toxic RNA to reduce splicing defects leading to pathogenicity. Only the isoform containing exon 22 (+ex22) is expressed from the Atp2a1 gene in control WT mice. On the other hand, the expression level of +ex22 isoform was 15.8% in DM1 model mice, but it was greatly restored to 73.6% in JM642-treated mice. The same reduction in splicing defects was observed in the Clcn1 gene. Apart from this, RNA aggregate formation, which is frequently observed in myoblasts derived from DM1 patients, was greatly reduced from 41% of 255 cells to 6.7% of 286 cells in JM642-treated cells. These results support the mechanism of action in which JM642 binds to CUG repeats and inhibits RNA aggregate formation, resulting in the release of trapped splicing factors, suggesting that the selective and high affinity binding properties of JM642 to CUG repeats are important for the splicing aberration recovery effect. These results with other small molecules targeting HD and SCA31 will be discussed.

Overview

Clinical aspect

Baziel van Engelen
Radboud University Medical Center

Program

Wednesday, 22nd June, 2022

14:00 – 16:00	**Registration**
16:00 – 16:30	**Opening**
16:30 – 18:00	**Keynote Lectures**
18:00 – 19:00	**Peter Harper Memorial Lecture**
19:00 – 20:00	**Organization Updates** **Report on International DM Awareness Day**

Thursday, 23rd June, 2022

8:30 – 9:15	**Keynote Lecture**

Session 1: Repeat-Associated Pathomechanisms

9:15 – 10:15	**1-1 Repeat instability**
10:15 – 11:00	**1-2 RNA-mediated mechanism**
11:00 - 11:15	**Break**
11:15 – 12:15	**1-2 RNA-mediated mechanism**
12:15 – 13:15	**Lunch**
13:15 – 14:00	**1-2 RNA-mediated mechanism**
14:00 – 14:45	**1-3 Cell/organoids and animal models**
14:45- 15:00	**Break**
15:00 – 16:00	**1-4 Tissue specific mechanisms**
16:00 – 16:30	**Mini Lecture**
16:30 – 16:45	**Break**

Session 2: Clinical Aspects

16:45 – 18:00	**2-1 Specific disease features**
18:00 – 18:15	**Break**
18:15 – 20:00	**2-2 Biomarkers, outcome measures, trial design, etc**

Friday, 24th June, 2022

Session 3: Therapeutic Strategies and Targets

8:30 – 10:00	**3 Therapeutic Strategies and Targets**
10:00 – 10:15	**Break**
10:15 – 11:30	**3 Therapeutic Strategies and Targets**
11:30 – 12:00	**Closing**

Wednesday, 22nd June, 2022

14:00	**Registration**	
16:00	**Opening**	
16:30	**Keynote Lecture**	
	Advance in Fukuyama muscular dystrophy & dystroglycanopathy	Tatsushi Toda
17:15	**Keynote Lecture**	
	Exon skipping therapy to Duchenne muscular dystrophy	Shin'ichi Takeda
18:00	**Peter Harper Memorial Lecture**	
	Peter Harper, Myotonic Dystrophy and Me	David Brook Mark Rogers Margaret Bowler
19:00 I 20:00	**Organization Updates** **Report on International DM Awareness Day**	

Thursday, 23rd June, 2022

8:30	**Keynote Lecture**		
	Lesson from other expansion diseases, disease-modifying therapy for SBMA		Gen Sobue
Session 1: Repeat-Associated Pathomechanisms			
	1-1 Repeat instability		
9:15	Overview: Repeat instability		Christopher Pearson
9:45	S1-1-1	Identification of CTG repeat contraction factors in myotonic dystrophy type 1	Laure de Pontual
10:00	S1-1-2	CpG sites surrounding DMPK expansions are heterogeneously methylated in myotonic dystrophy type 1 patients with variant repeats	Jovan Pesovic
	1-2 RNA-mediated mechanism		
10:15	Overview: RNA-mediated mechanisms		Maurice Swanson
11:00	**Break**		
11:15	S1-2-1	RNA subcellular mislocalization in DM1 patient iPSC-derived neurons	Maya L Gosztyla
11:30	S1-2-2	Compensatory mechanism of MBNL paralogs	Larissa Nitschke
11:45	S1-2-3	miR-1 and its target Multiplexin are involved in DM1-associated dilated cardiomyopathy	Anissa Souidi
12:00	S1-2-4	Analysis of DMPK expansion transcript degradation and MBNL-RNA binding kinetics in DM1	Xiaomeng Xing
12:15	**Lunch**		
13:15	S1-2-5	Congenital myotonic dystrophy patients exhibit unique patterns of transcriptomic dysregulation independent of CTG repeat expansion	Melissa Ann Hale
13:30	S1-2-6	Multivalency of MBNL is essential for CUG foci formation in DM1	Chase P Kelley
13:45	S1-2-7	Identification of NIPP1 as a modifier of RNA foci formation	Yoshihiro Kino
	1-3 Cell/organoids and animal models		
14:00	S1-3-1	Expanded CUG repeat RNA induces premature senescence in myotonic dystrophy	Yuhei Hasuike
14:15	S1-3-2	Manipulating expanded *DMPK* expression using CRISPRi/a	Lise Ripken
14:30	S1-3-3	Generation and Characterization of a DM2 BAC Mouse Model	Avery Christian Engelbrecht
14:45	**Break**		
	1-4 Tissue specific mechanisms		
15:00	S1-4-1	MBNL loss of function in the motor unit alters neuromuscular communication	Charles Frison-Roche
15:15	S1-4-2	Choroid Plexus Spliceopathy in DM1	Benjamin Martin Kidd
15:30	S1-4-3	Cell type-specific abnormalities of central nervous system in myotonic dystrophy type 1	Masayuki Nakamori
15:45	S1-4-4	Sense and antisense RAN proteins accumulate in DM1 brain regions with pathological changes	Monica Banez-Coronel
16:00	**Mini Lecture**		
	Small Molecules targeting Repeat Sequences causing Neurological Disorders		Kazuhiko Nakatani
16:30	**Break**		

Thursday, 23rd June, 2022

Session 2: Clinical Aspects

2-1 Specific disease features

16:45	S2-1-1	An International Consensus Document on Evaluation and Management of Arrhythmias in Myotonic Dystrophy	William J. Groh
17:00	S2-1-2	A decade follow-up study of Premanifest DM1: a molecular, muscular and CNS approach	Joana Garmendia
17:15	S2-1-3	An integrative analysis of DNA methylation pattern in Myotonic Dystrophy Type 1 samples reveals a distinct DNA methylation profile between tissues and a dual muscle-associated epigenetic dysregulation	Monica Suelves
17:30	S2-1-4	Independence of Adults with Childhood Phenotype of Myotonic Dystrophy Type 1	Samar Muslemani
17:45	S2-1-5	TREAT-NMD Myotonic Dystrophy Global Registry Network: An International Collaboration in Myotonic Dystrophy Type 2	Stojan Peric
18:00	Break		

2-2 Biomarkers, outcome measures, trial design, etc

18:15	S2-2-1	Longitudinal changes in neuropsychological functioning in Japanese patients with myotonic dystrophy type 1: A 5-year follow-up study	Haruo Fujino
18:30	S2-2-2	Blood based biomarker discovery in DM1	Daniel van As
18:45	S2-2-3	miR-223-3p and miR-24-3p as novel serum-based biomarkers for Myotonic Dystrophy type 1	Leonidas A. Phylactou
19:00	S2-2-4	Myotonic dystrophy type I Tau PET imaging and exploratory study of CSF and plasma biomarkers of neurodegeneration from neurocognitively characterized DM1 patients	Nicolas Sergeant
19:15	S2-2-5	Analysis of circulating myomiRs as potential biomarkers of progression of muscular impairment in myotonic dystrophy type 1 patients	Nemanja Radovanovic
19:30 – 20:00	Overview: Clinical aspect		Baziel van Engelen

Friday, 24th June, 2022

Session 3: Therapeutic Strategies and Targets

8:30	S3-01	Whole transcriptome analysis and functional studies reveal that senescence plays a role in Myotonic Dystrophy type 1	Ander Matheu
8:45	S3-02	Correction of Clcn1 mis-splicing reverses muscle fiber type transition in mice with myotonic dystrophy	Ningyan Hu
9:00	S3-03	The ReCognitION project: Recognition and Validation of Druggable Targets from the Response to Cognitive Behavioural Therapy in Myotonic Dystrophy type 1 patients from Integrated -Omics Networks	Peter-Bram 't Hoen
9:15	S3-04	Identifying potential lead molecules that eliminate toxic nuclear foci in DM1	Anjani Kumari
9:30	S3-05	A CTG repeat-selective screen of a natural product library reveals dietary natural compounds as potential therapeutics for Myotonic Dystrophy.	Subodh K Mishra
9:45	S3-06	Repeat dosing with DYNE-101 is well tolerated and leads to a sustained reduction of *DMPK* RNA expression in key muscles for DM1 pathology in hTfR1/DMSXL mice and NHPs	Stefano Zanotti
10:00	Break		
10:15	S3-07	Elimination of defective muscle stem cells to restore myogenesis in Myotonic Dystrophy type 1.	Nicolas Dumont
10:30	S3-08	Comprehensive transcriptomic characterization of antisense RNA treatments effects on Myotonic dystrophy type 1 cell models	Jorge Patricio Espinosa
10:45	S3-09	EEV-Conjugated Oligonucleotide Results in Nuclear Foci Reduction and Aberrant Splicing Correction in DM1 Cell and Animal Models	Mahasweta Girgenrath
11:00	S3-10	A Phase 1/2 Trial Evaluating the Safety and Pharmacokinetics (PK) of AOC 1001 in Adults with DM1: MARINA Study Design	Nicholas Johnson
11:15	S3-11	Decoy gene therapy to reverse RNA toxicity in DM1	Denis Furling

11:30 – 12:00	Closing

Platform Presentation Abstracts

S1-1-1

Identification of CTG repeat contraction factors in myotonic dystrophy type 1

L de Pontual[1], A Mangin[2], B Yang[3], G Gourdon[1], V Dion[2] and S tomé[1]

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[2]UK Dementia Research Institute at Cardiff University, Cardiff
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Introduction: Myotonic dystrophy type 1 (DM1) is a neuromuscular disease caused by an abnormal CTG repeat expansion in the 3'UTR region of the *DMPK* gene. In patients, the CTG repeat size ranges from 50 to thousands of CTG and usually increases over generations and time in the tissue. Larger expansions are associated with more severe symptoms and a decreasing age of onset. We hypothesize that the development of innovative therapeutic strategies, aimed at decreasing the CTG repeat length, and thus to stop or reverse the progression of the disease, may improve the quality of life of DM1 patients. Although many studies have provided insight into the mechanisms underlying the formation of expansions, how the contractions occur remains elusive. The specific objective of our work is to uncover the bioactive molecules able to induce repeat contractions in trinucleotide repeat (TNR) models and to decipher the mechanisms promoting these contractions using efficient tools.

Methods: We performed a large-scale screen for pharmacologically relevant chemical modulators of instability using the Prestwick Library (>1200 FDA-approved drugs) taking advantage of a chromosomal GFP reporter that can accurately measure both expansions and contractions in the same HEK293 cell population[1]. The effect of selected molecules directly on the dynamics of CTG.CAG repeat instability is studied in HEK293 cells as well as DM1 fibroblasts using targeted long-read sequencing developed by Pacific Biosciences[2].

Results: During the chemical screen, we identified several candidate molecules notably involved in epigenetic regulation pathways, that may change the size of CTG repeats. Some of these molecules induced stabilization or even contractions of CTG repeats in the HEK cell model and in DM1 fibroblasts.

Conclusions: The next step is to better understanding the mechanisms by which these molecules act on CTG repeat instability. The direct perspective of our work is to identify new small molecules and new druggable targets promoting CAG.CTG repeat contractions, thus offering new therapeutic perspectives for DM1 but also for other TNR diseases.

[1]Santillan et al. (2014). *PloS One* 9, e113952.
[2]Mangin et al. (2021). *Int J Mol Sci* 22, 2616.

S1-1-2

CpG sites surrounding *DMPK* expansions are heterogeneously methylated in myotonic dystrophy type 1 patients with variant repeats

J Pešović[1], S Perić[2], N Radovanović[1], L Radenković[1], M Brkušanin[1], G Brajušković[1], V Rakočević Stojanović[2], D Savić-Pavićević[1]

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Introduction: DM1 is phenotypically one of the most variable monogenic diseases. We previously showed that variant repeats, found in about 5% of patients, represent individualspecific modifiers which delay age at onset by stabilizing *DMPK* expansions in somatic cells. Since CTG repeat tract is embedded in large CpG island, it raised the question whether variant expansions and their modifying role are associated with DNA methylation in this region. Our aim was to investigate presence and level of DNA methylation within CpG sites.

Methods: Methylation of CpG sites surrounding CTG repeats was analyzed by targeted bisulfite NGS. We used two-step PCR for library preparation of both upstream and downstream regions spanning 31 and 22 CpG sites, respectively. The study included 15 patients from 9 families with different patterns of CCG variant repeats at the 3′ end of expansions, and 13 control patients with pure *DMPK* expansions.

Results: Our results revealed heterogeneous methylation of CpG sites surrounding variant *DMPK* expansions. Patients from two families with either a large CCG block or a long stretch of CCGCTG hexamers showed high levels of DNA methylation in both regions. Other patients with more scattered variant CCG repasts showed no CpG methylation in upstream region and moderate/low level or absence of CpG methylation in downstream region.

Conclusions: Our results open questions about the role of epigenetic mechanisms in the stabilization of *DMPK* locus and, consequently, about their clinical relevance.

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

S1-2-1

RNA subcellular mislocalization in DM1 patient iPSC-derived neurons

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Introduction: Myotonic dystrophy type 1 (DM1) is a multisystem disorder caused by an expanded CTG triplet repeat in the dystrophia myotonic protein kinase (DMPK) gene. CUG repeat expansions in *DMPK* RNA transcripts form ribonuclear foci that dysregulate RNA-binding proteins (RBPs), including muscleblind-like (MBNL) and CUG repeat binding protein (CELF) family members. In muscles, this causes abnormal splicing, which gives rise to progressive myopathy and myotonia. However, DM1's cognitive symptoms do not appear to originate from missplicing of neuronal transcripts. We hypothesized that DM1's cognitive symptoms originate from RNA mislocalization in neurons.

Methods: We differentiated neurons from congenital DM1 (cDM1) patient-derived iPSCs and neurotypical controls. Then we utilized a version of subcellular fractionation adapted for neuronal cells, followed by RNA sequencing (CeFra-seq), to characterize RNA subcellular localization.

Results: To validate our CeFra-seq protocol, we performed principal component analysis (PCA), which separated our samples by cellular compartment and by genotype. Additionally, the nuclear compartments were enriched for pre-mRNAs, snRNAs, and snoRNAs, consistent with previous reports. When comparing cDM1 to control neurons, we identified transcripts whose expression values differ in the nuclear, cytoplasmic, or insoluble compartments, but do not differ in overall expression, suggesting aberrant subcellular localization. These mislocalized transcripts were highly consistent between neurons differentiated from two different cDM1 patient-derived iPSC lines. Intriguingly, enhanced crosslinking and immunoprecipitation (eCLIP) analysis of CELF2 in cortical organoids revealed that several mislocalized transcripts are 3' UTR binding targets, demonstrating a role for CELF2 in mediating RNA subcellular mislocalization in cDM1 neurons.

Conclusions: Our results highlight neuronal RNA subcellular localization defects as an important layer of misregulation in DM1.

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

S1-2-2

Compensatory mechanism of MBNL paralogs

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Introduction: The effect of pathogenic genetic variations can be compensated by paralogs with redundant functions. An example of such compensation is found with the paralogs of the Muscleblind Like (MBNL) family of RNA binding proteins. Loss of *Mbnl1* results in a significant increase of MBNL2 protein levels in tissues where *Mbnl2* expression is typically low. As such, *Mbnl1*$^{-/-}$ mice develop relatively mild phenotypes, while *Mbnl1*$^{-/-}$*; Mbnl2*$^{+/-}$ mice display severe phenotypes recapitulating Myotonic Dystrophy Type 1 (DM1)[1], a multisystemic disorder in which an expanded CUG RNA repeat sequesters the MBNL paralogs. Still, the mechanism by which MBNL2 is upregulated and the impact on DM1 pathogenesis have yet to be investigated.

Methods: In this study, we used molecular and cellular assays upon *Mbnl1* knockdown and knockout in cell culture and *in vivo* to uncover the mechanism by which loss of *Mbnl1* upregulates MBNL2 protein levels.

Results: We found that loss of *Mbnl1* upregulates MBNL2 via the enhanced inclusion of *Mbnl2* exon 9 that introduces an alternative C-terminus. We show that the C-terminus excluding exon 9 drastically destabilizes MBNL2. Thus, the inclusion of exon 9 upregulates MBNL2 via a switch in C-termini and the loss of protein degradation signal. We further show that the inclusion of *Mbnl2* exon 9 is increased in human DM1 tissues as well as in a DM1 mouse model in which MBNL2 protein levels are upregulated.

Conclusions: This study uncovered the mechanism by which loss of *Mbnl1* upregulates its paralog *Mbnl2* and suggests that the compensatory mechanism is active in DM1. Future work will investigate the importance of the compensatory mechanism in DM1 and explore its utilization for therapeutic purposes.

Funding: Myotonic Dystrophy Foundation

[1]Lee et al., (2013) *EMBO Mol Med.* 5(12):1887-900.

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

S1-2-3

miR-1 and its target Multiplexin are involved in DM1-associated dilated cardiomyopathy

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3 Genevieve Gourdon, the Center of Research in Myology, Paris, France

Introduction: MBNL1, the RNA binding factor involved in DM1, promotes maturation of *miR-1*, a conserved muscle and heart-specific microRNA. Consequently, due to MBNL1 sequestration, the *miR-1* levels are reduced in DM1. On the other hand *miR-1* knockout in mice leads to dilated cardiomyopathy (DCM) phenotype also observed in DM1 patients. However, whether *miR-1* deregulation is involved in DCM observed in DM1 patients has not been investigated.

Methods: We applied FISH approach to analyze *miR-1* expression in the fly hearts. We performed fain and loss of function analyses of *miR-1* and its potential target *Multiplexin* (*Mp*) (*Collagen XV/XVIII*). Heart structure and physiology in different DM1 contexts was assessed using SOHA method. Genetic rescue experiments were used to determine the role of *miR-1* and *Mp* in DM1-associated DCM.

Results: We found that *miR-1* levels are significantly reduced in hearts of DM1 flies and that its down regulation leads to DCM. We identified and validated *Mp* as a new cardiac *miR-1* target, involved in DM1-associated DCM and up regulated in heart samples of our DM1 flies and of DM1 patients with DCM. *Mp* when attenuated in heart tissue ameliorated DCM phenotype in aged DM1 flies.

Conclusions: Mp is essential for proper heart structure and contractility. The increase of Mp is *miR-1*-dependent. Reduced *miR-1* and increased level of its target Mp are both involved in DM1-associated DCM.

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

S1-2-4

Analysis of DMPK expansion transcript degradation and MBNL–RNA binding kinetics in DM1

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Introduction: The pathogenesis of DM1 is associated with nuclear retained mutant expansion DMPK (expDMPK) mRNA. However, little is known about the degradation of expDMPK mRNAs, and which enzymes are responsible. One of our goals is to address this knowledge gap. Due to the presence of abnormally long CUG repeats in the 3' UTR of DMPK and their association with MBNL, mutant DMPK mRNAs fail to be exported to the cytoplasm. Instead, they may undergo degradation via nuclear mRNA decay pathways. Thus, components of the nuclear exosome, decay factors, helicases and various other ribonuclear proteins may be involved in the degradation of mutant DMPK transcripts.

Methods: We seek to determine the key nucleases responsible for the degradation of expansion DMPK RNAs using shRNA expression in DM1 cell lines and their derivatives. Much of this proposal is based on modified DM cell lines produced with MBNL1 and 2 double knock-out. We have adopted a systematic approach to examine the role of UPF1, XRN2 and EXOSC10 in DMPK mRNA degradation and the role of MBNL in protecting mutant DMPK mRNA stability.

Results: We have successfully generated MBNL-deficient DM1 cell lines and shown loss of nuclear foci and dramatically reduced levels of mutant expDMPK transcripts in the resulting cells. This suggests that MBNL proteins play a role in inhibiting the degradation of expDMPK transcripts. Re-introduction of MBNL1 and 2 expression, in MBNL-deficient DM1 cells under the control of a doxycycline-inducible promotor results in a re-appearance of nuclear foci. Following the inhibition of UPF1, the number of foci significantly increased in DM1 cells and the formation of nuclear foci was restored slightly in MBNL-deficient DM1 cells. These results indicate that UPF1 plays a key role in the decay of expDMPK mRNA and the absence of MBNL has a profound effect on this. Thus, sequestered MBNLs may impede the degradation of mutant DMPK mRNA by UPF1 and components of the nuclear exosome.

Conclusions: Our analysis suggests that in the absence of MBNL mutant expDMPK transcripts are rapidly degraded. The MBNL-deficient cells we have generated will allow us to determine which other factors are essential for the degradation of the expansion transcripts. Furthermore, the inducible MBNL expression system established in DM1 cells, will allow us to decode the complex link between patterns of MBNL–RNA binding and MBNL function. For this we will use KIN CLIP (for kinetic CLIP), a form of Chromatin-linked immunoprecipitation (CLIP) that may allow us to elucidate the connection between MBNL-RNA binding and MBNL function in human DM1 cells to provide novel insights for DM pathogenesis.

Acknowledgement: This research work is funded by Myotonic Dystrophy Foundation.

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

S1-2-5

Congenital myotonic dystrophy patients exhibit unique patterns of transcriptomic dysregulation independent of CTG repeat expansion

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Introduction: Despite a shared genetic cause of disease congenital myotonic dystrophy (CDM) patients present with a distinct clinical phenotype and absence of common DM1 symptoms, including myotonia. While global dysregulation of alternative splicing (AS) has been linked to phenotypes, these studies have been performed primarily in adult-onset DM1.

Methods: To characterize spliceopathy of CDM, RNAseq was performed on the largest cohort to date of CDM patients (n=36) ranging in age from 2 weeks to 16 years. 50 biopsies from DM1 and adult/pediatric controls were also sequenced.

Results: Many patterns of AS dysregulation were conserved between CDM and DM1, but CDM patients also displayed significant heterogeneity of AS dysregulation with many clustering with controls. An aggregate splicing dysregulation metric to evaluate changes in global spliceopathy versus age at biopsy revealed a distinct pattern of AS changes during childhood development. While young, infantile patients (<2 years) present with patterns of severe mis-splicing consistent with disease severity, a transition occurs in early childhood (2-8 years) whereby spliceopathy significantly improves, in some cases to levels of unaffected samples. This shift occurs regardless of sex or CTG repeat load. Post 8 years of age, adolescent patients stratified into 2 populations with a full range of global splicing dysregulation. Preliminary gene expression analysis suggests that these alternations in global spliceopathy are connected to *DMPK* expression.

Conclusions: Analysis of these CDM samples illuminates changes in AS dysregulation over the course of disease progression, providing insights into timing of therapeutic intervention and possible new therapeutic strategies.

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

S1-2-6

Multivalency of MBNL is essential for CUG foci formation in DM1

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Introduction: While the effects of MBNL sequestration on RNA processing are well studied, the interactions within nuclear foci that lead to sequestration, i.e., between MBNL and CUG RNAs and among CUG RNAs themselves, remain controversial. *In vitro*, CUG RNAs form liquid-like droplets via base pairing alone; however, in myoblasts, *Mbnl1* knockdown destabilizes CUG RNA foci, arguing that MBNL stabilizes these structures in cells. Here, we explore how MBNL stabilizes CUG RNA foci: We propose a new mechanism in which MBNL uses at least 2 covalently linked zinc fingers (ZnF) to bind multiple RNAs simultaneously, acting as a multivalent bridge that is required for aggregation.

Methods: In MEF cells expressing CUG_{480}, we perform FISH/IF to visualize CUG RNAs in the presence or absence of MBNL variants.

Results: We confirm that knockout of *Mbnl1/2* leads to nuclear export of CUG_{480} RNA and dispersal of CUG RNA clusters in the nucleus. To determine whether RBP multivalency drives these effects, we express MBNL1 variants with one or both ZnF pairs disrupted and study their effects on CUG_{480} localization. We find that MBNL1 with all active ZnFs reestablishes aggregation and nuclear retention, while variants with one active ZnF pair only exert these effects when highly expressed. By deleting the CTD to block dimerization, we find that at least 2 covalently linked ZnF pairs are required to induce aggregation and hinder export.

Conclusions: Multivalent RNA binding by MBNL, mediated both by dimerization and multiple ZnF pairs, stabilizes foci in DM1 and drives nuclear retention. Beyond *DMPK*, other expansion RNAs bind RBPs with multiple RNA-binding domains, implicating RBP multivalency as an essential property that drives RNA toxicity.

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

S1-2-7

Identification of NIPP1 as a modifier of RNA foci formation

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Introduction: RNA foci are characteristic pathological structures found in both DM1 and DM2 and are formed by expanded repeat RNAs that sequester MBNL proteins and cause misregulation of pre-mRNA splicing. Understanding the mechanism of RNA foci formation may lead to novel therapeutic approaches. As reported previously, RNA foci formation is dependent on MBNL proteins[1,2]. However, it is unclear whether other cellular factors are involved in the formation and/or maintenance of RNA foci. Here we tried to identify genes that can affect the formation of CUG repeat RNA foci.

Methods: Using HeLa cells that inducibly express an expanded CUG repeat RNA (Hela-CTG cells), we screened siRNAs targeting >300 genes encoding nucleases or helicases, including putative ones, and evaluated their effects on RNA foci formation by fluorescence in situ hybridization. We also used DM1-derived fibroblasts (DM1500) for the evaluation of selected candidates. Splicing assay of an alternative exon of *MBNL1* was used as a measure of splicing regulation.

Results: We selected one gene, *NIPP1* (*PPP1R8*), known as a regulatory subunit of protein phosphatase 1 and a regulator of pre-mRNA splicing, as a candidate that can repress RNA foci formation of CUG repeat. Its overexpression reduced RNA foci in both HeLa-CTG and DM1500 cells. In addition, the mis-splicing of *MBNL1* was partially restored by the overexpression of NIPP1.

Conclusions: We identified NIPP1 as a novel protein that can repress RNA foci formation, which would pave the way for understanding the molecular pathways of repeat RNA metabolisms.

[1]Dansithong W, Paul S, Comai L, Reddy S (2005) *J Biol Chem* 280:5773-5780.
[2]Kino Y, Washizu C, Kurosawa M, Oma Y, Hattori N, Ishiura S, Nukina N (2015) *Hum Mol Genet* 24:740-756

Session 1-3: Repeat-Associated Pathomechanisms / Cell/organoids and animal models

S1-3-1

Expanded CUG repeat RNA induces premature senescence in myotonic dystrophy

Yuhei Hasuike[1], Hideki Mochizuki[1], Masayuki Nakamori[1]

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Introduction: Myotonic dystrophy type 1 (DM1) is a dominantly inherited disorder due to a toxic gain of function of RNA transcripts containing expanded CUG repeats (CUG^{exp}). Patients with DM1 present with multisystemic symptoms, such as muscle wasting, cognitive impairment, cataract, frontal baldness, and endocrine defects, which resemble accelerated aging. Although the involvement of cellular senescence, a critical component of aging, was suggested in studies of DM1 patient-derived cells, the detailed mechanism of cellular senescence caused by CUG^{exp} RNA remains unelucidated.

Methods: We developed a DM1 cell model that conditionally expressed CUG^{exp} RNA in human primary cells so that we could perform a detailed assessment that eliminated the variability in primary cells from different origins.

Results: Our DM1 model cells demonstrated that CUG^{exp} RNA expression induced cellular senescence by a telomere-independent mechanism. Furthermore, the toxic RNA expression caused mitochondrial dysfunction, excessive reactive oxygen species production, and DNA damage and response, resulting in the increase of cell cycle inhibitors p21 and p16 and secreted mediators insulin-like growth factor binding protein 3 and plasminogen activator inhibitor-1.

Conclusions: This study provides unequivocal evidence of the induction of premature senescence by CUG^{exp} RNA in our DM1 model cells.

Session 1-3: Repeat-Associated Pathomechanisms / Cell/organoids and animal models

S1-3-2

Manipulating expanded *DMPK* expression using CRISPRi/a

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Introduction: A toxic gain-of-function of (CUG)n-expanded *DMPK* transcripts is the underlying cause of DM1. It is still unclear however, which dose of expanded *DMPK* transcripts is actually detrimental to cells. In other words, how much reduction of expanded *DMPK* RNA is required in a patient to obtain a clinical effect?

Methods: We have developed *in vitro* myoblast cell models in which we can tune the expression of expanded *DMPK* RNA using catalytically inactive Cas9 (dCas9) fused to transcription repressors and activators, also known as CRISPR interference and CRISPR activation (CRISPRi/a).

Results: We were successful in upregulating *DMPK* expression up to ten-fold in a transient manner, which was confirmed by RT-qPCR and nuclear foci count using RNA FISH. Missplicing as a result of (CUG)n repeat expression was more severe in these myoblasts. In the opposite direction, we managed to downregulate *DMPK* expression by at least 60% using CRISPRi. Pilot experiments confirm a corresponding rescue of missplicing.

Conclusions: The CRISPRi and CRISPRa systems provide insight in the expanded RNA dose-dependence of pathogenic mechanisms in DM1, which may be translated to *DMPK* expression levels and the corresponding severity of symptoms in certain muscles or organs. CRISPRi may be useful in future therapeutic strategies.

Session 1-3: Repeat-Associated Pathomechanisms / Cell/organoids and animal models

S1-3-3

Generation and Characterization of a DM2 BAC Mouse Model

Avery Engelbrecht[1,2], S. Elaine Ames[1,2], Lisa E.L. Romano[1,2], John Douglas Cleary[1,2], Tao Zu[1,2], Laura P.W. Ranum[1-2]

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Introduction: Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are multisystemic diseases caused by CTG or CCTG repeat expansions located in the *DMPK or CNBP* genes, respectively. RNA gain of function, bidirectional transcription and repeat associated non-ATG (RAN) translation are all found in DM. RAN translation of sense (CCUG) and antisense (CAGG) expansion transcripts produce (LPAC) and (QAGR) RAN proteins. LPAC and QAGR proteins are toxic to cells and found in brain regions with neurodegenerative changes and white matter loss. Understanding the role of RAN proteins in DM2 and developing therapeutic approaches requires animal models that mirror DM2 patient disease features.

Methods: Using a bacterial artificial chromosome (BAC) approach we generated two separate lines of DM2 BAC transgenic mice. We are currently characterizing these mice for RNA foci using HCR FISH, repeat instability by long-range PCR, histopathological and behavioral features.

Results: Both DM2 mouse lines contain the entire *CNBP* gene with substantial flanking sequence and unstable expanded repeats ranging in size from ~750-1300 CCTGs. Southern blot analyses suggest a single insertion site in each line. HCR-FISH detects signal in transgenic mice for CCUG repeats in skeletal muscle and brain. DigiGait abnormalities have been found in the 75 line with ongoing testing on the 236 line.

Conclusions: We have generated a novel DM2 BAC transgenic mouse model that shows initial behavioral and molecular phenotypes. We are continuing to characterize these mice and hope that this model will provide a useful tool for better understanding the molecular mechanisms of DM2 and therapy development.

Funding: Myotonic Dystrophy Foundation

Session 1-4: Repeat-Associated Pathomechanisms / Tissue specific mechanisms

S1-4-1

MBNL loss of function in the motor unit alters neuromuscular communication

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Introduction: DM1 pathophysiology have been studied through skeletal muscle, heart and brain but knowledge about spinal motor neurons (MN) involvement is limited. MN innervate skeletal muscle through neuromuscular junctions (NMJ) and prior reports identified RNA foci colocalizing with MBNL1 in DM1 MN and NMJ[1], which display ultrastructural[2] and possible functional abnormalities.

Methods: To assess and dissect the role of MBNL in neuromuscular communication, we crossed mice invalidated ubiquitously for *Mbnl1* (Mbnl1-KO) with mice deprived of *Mbnl2* specifically in MN to obtain MN-dKO mice.

Results: Young MN-dKO mice show no NMJ or locomotion alterations. However after a couple of months, these mice progressively develop locomotion deficiencies that are associated with NMJ structural and ultrastructural defects, when compared to Mbnl1-KO mice.

Conclusions: MBNL compound loss-of-function in MN significantly affects motor capacities and NMJ structure. Deficiency in NMJ maintenance, rather than development, may be responsible for NMJ abnormalities caused by MBNL loss. Identification of molecular alterations in MN of this mouse model as well as MBNL RNA targets in MN is ongoing. Altogether our work will help for a better knowledge of DM1 physiopathology.

[1]Wheeler TM, Krym MC, Thornton CA (2007) *Neuromuscul Disorders*, *17*(3), 242–247.
[2]Fardeau M, Tomé F (1980) *Ontogenesis and functional mechanisms of peripheral synapses* (pp. 287–298).

Session 1-4: Repeat-Associated Pathomechanisms / Tissue specific mechanisms

S1-4-2

Choroid plexus spliceopathy in DM1

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Introduction: *Dmpk* CTG^{exp} knockin (KI) models for DM1 demonstrated the choroid plexus (ChP) is a highly susceptible glial cell type in the brain[1]. The ChP is important for neurodevelopment, brain homeostasis, circadian rhythms, and sleep via its production and regulation of cerebrospinal fluid (CSF)[2,3]. To clarify how this tissue is affected in DM1, we investigated ChP spliceopathy seen in DM1.

Methods: We performed RNAseq in DM1 ChP and our *Mbnl2* knockout (KO) model from late embryogenesis to adults. We also investigated developmental-specific protein changes.

Results: *Dmpk* CTG^{exp} KIs show a severe ChP spliceopathy that potentially reverts splicing patterns to an earlier developmental state. We show that DM1 ChP shows profound and concordant splicing changes compared to unaffected controls. Next, we determined that ChP mis-splicing is driven by MBNL2 loss and this spliceopathy recapitulates embryonic splicing patterns. Additionally, *Mbnl2* KO, *Dmpk* CTG^{480} KI mice and DM1 show similar mis-splicing events of key ion channels and secreted proteins.

Conclusions: The choroid plexus is affected in DM1, and ChP mis-splicing is a potential factor underlying DM1 CNS symptoms. MBNL2 is the major upregulated RBP during ChP development in mice, and loss of MBNL2 protein leads to robust splicing changes in this tissue that are predicted to affect brain CSF composition.

[1]Nutter et al. (2019) *Genes Dev* 33:1-6.
[2]Lun et al. (2016) *Nat Rev Neurosci* 16(8): 445-457
[3]Myung, et al. (2018) *J Expt Neurol* 12:1-4

Session 1-4: Repeat-Associated Pathomechanisms / Tissue specific mechanisms

S1-4-3

Cell type-specific abnormalities of central nervous system in myotonic dystrophy type 1

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Introduction: Myotonic dystrophy type 1 (DM1) is a multisystem genetic disorder involving the muscle, heart, and central nervous system (CNS). It is caused by toxic RNA transcription from expanded CTG repeats in the 3′-untranslated region (UTR) of *DMPK*, leading to dysregulated splicing of various genes and multisystemic symptoms. Although aberrant splicing of several genes has been identified as the cause of some muscular symptoms, the pathogenesis of CNS symptoms prevalent in patients with DM1 remains unelucidated, possibly due to a limitation in studying a diverse mixture of different cell types, including neuronal cells and glial cells. To elucidate the CNS pathogenesis, we aimed to investigate cell type-specific abnormalities in DM1.

Methods: Previous studies revealed neuronal loss in the cortex, myelin loss in the white matter, and the presence of axonal neuropathy in patients with DM1. Therefore, we conducted molecular analysis on cortical neurons, white matter glial cells, and spinal motor neurons of DM1 patients using a laser-capture microdissection-based approach.

Results: We observed that the CTG repeat instability and CpG methylation status varied among the CNS cell lineages; cortical neurons had more unstable and longer repeats with higher CpG methylation than white matter glial cells, and spinal motor neurons had more stable repeats with lower methylation status. We found gene expression changes in each DM1 CNS lineage. We also identified splicing abnormalities in each CNS cell lineage, such as *DLGAP1* in white matter glial cells and *CAMKK2* in spinal motor neurons. Furthermore, we demonstrated that aberrant splicing of *CAMKK2* is associated with abnormal neurite morphology in DM1 motor neurons.

Conclusions: Our results indicate significant potential of cell type-specific analysis in elucidating DM1 CNS pathogenesis.

Session 1-4: Repeat-Associated Pathomechanisms / Tissue specific mechanisms

S1-4-4

Sense and antisense RAN proteins accumulate in DM1 brain regions with pathological changes

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Introduction: Repeat associated non-AUG (RAN) proteins have been found in 11 repeat expansion diseases, including DM1 and DM2. Toxic LPAC and QAGR RAN proteins accumulate in DM2 autopsy brains, with prominent LPAC RAN aggregates in regions with organized necrosis and macrophage/microglia infiltration, indicating that DM2 RAN proteins are found in brain regions with neurodegenerative changes. In DM1, polyglutamine RAN proteins were previously detected in blood and muscle, but because the antibodies used were not suitable for brain studies, it has been unclear if RAN proteins contribute to CNS pathology in DM1.

Methods: We developed novel antibodies against polyLeucine and polySerine repeat motifs to study sense and antisense RAN translation in DM1. Antibody specificity was validated in transfected cells and in human HD brains previously shown to have polySer and polyLeu accumulation. Immunohistochemistry experiments were conducted on DM1 and control autopsy brains.

Results: Our data show that sense polyLeu and antisense polySer RAN proteins accumulate in DM1 frontal cortex and hippocampus as large cytoplasmic aggregates in neurons or microaggregates in glial cells. White matter regions with intense RAN-positive staining show pathologic features of disease, including activated microglia, astrogliosis and demyelination.

Conclusions: Our results demonstrate that RAN protein aggregates are found in DM1 brain regions with white matter abnormalities and neuroinflammation. These data highlight the need to understand the role of RAN proteins in DM1 and that future therapeutic strategies may need to target both sense and antisense RNAs or RAN proteins to be effective.

Session 2-1: Clinical Aspects / Specific disease features

S2-1-1

An International Consensus Document on Evaluation and Management of Arrhythmias in Myotonic Dystrophy

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Introduction: Cardiac arrhythmias cause morbidity and mortality in patients with myotonic dystrophy (DM). Standardized, evidence-based recommendations on the evaluation and management of arrhythmias could assist clinicians in providing best care.

Methods: A multidisciplinary expert writing committee, organized by the Heart Rhythm Society with members from 12 countries and representing multiple international societies, was tasked with evidence compilation and writing. The document covered arrhythmia care in multiple neuromuscular disorders (NMDs) including a section on DM. Recommendations were formulated based on estimated benefits and risks (class of recommendation [COR]) and level of evidence [LOE]. Edits and reviews were done until a pre-determined consensus (> 67%) was reached.

Results: A total of 16 recommendations were approved for DM with an additional 6 on end-of-life decisions that applied to all NMDs including DM. The recommendations covered both DM1 and DM2. Overall committee consensus was 99%. Recommendations encompassed diagnostic testing, risk stratification, use of pacemakers, treatment for atrial arrhythmias, use of implantable cardioverter-defibrillators, and end-of-life care. Each recommendation included supportive text. COR varied from 1 (strong) to 2b (weak). LOE was primarily non-randomized, observational (B-NR), limited data (C-LD) or expert opinion(C-EO).

Conclusions: An international consensus document on arrhythmia evaluation and management is now available for standardized, evidence-based care of individuals with DM. The use of these recommendations will assist in decreasing adverse arrhythmia outcomes in the DM population.

S2-1-2

A decade follow-up study of Premanifest DM1: a molecular, muscular and CNS approach

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Introduction: DM1 is a heterogeneous neuromuscular disorder characterized by muscular and other body systems' impairment. Nevertheless, patients without muscle signs have previously been described as Premanifest DM1. This is considered a precursory phase, in which subtle signs and/or symptoms (e.g. related to CNS), may emerge and progress gradually. The aim of this project is to characterize and trace Premanifest DM1 with a CNS approach.

Methods: 23 Premanifest DM1, 25 Manifest DM1 and 72 Healthy Controls (HC) were analyzed transversally and longitudinally (over 11.17 years). Clinical, neuropsychological, and neuroradiological (Brain volumes and White Matter Lesions) data of two time-points was analyzed. Transversal analysis, longitudinal intragroup and intergroup analysis, and multiple regression to analyze the muscular progression in Premanifest DM1, were conducted.

Results: Premanifest DM1 was significantly less severe than Manifest DM1, considering molecular, cognitive and brain structure affectation. The latter, was slightly more affected in Premanifest DM1 than HC. 50% variance of muscular progression of Premanifest DM1 patients, was explained by daytime sleepiness and molecular defect.

Conclusions: The results suggest that CNS involvement might appear early in the disease before muscular impairment. Considering the extent of Premanifest DM1 patients that remained muscularly asymptomatic, a "Non-muscular DM1" subtype is suggested, which might differ from the Premanifest phase.

S2-1-3

An integrative analysis of DNA methylation pattern in DM1 samples reveals a distinct DNA methylation profile between tissues and a dual muscle-associated epigenetic dysregulation

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Introduction: We investigated the contribution of epigenetics to the complexity and phenotypic variability of DM1 comparing DNA methylation profiles of the 4 annotated CpG islands in the DMPK locus.

Methods: We analyzed distinct DM1 tissues and derived cells, representing all DM1 patient subtypes by bisulfite sequencing.

Results: In blood, we found no differences in DNA methylation in CpGi 74, 43 and 36, while a CTCF1 DNA hypermethylation gradient was found in the developmental cases. CTCF1 hypermethylation correlated to disease severity and CTG expansion size and 50% of cases showed also hypermethylation in the CTCF2 region. Lymphoblastoid cells preserved the DNA methylation profiles observed in blood from all clinical subtypes. The comparison of DNA methylation levels of distinct DM1 tissues revealed a muscle-specific epigenetic signature with hypermethylation of CTCF1 region accompanied by demethylation of CpGi 43, a region containing an alternative DMPK promoter.

Conclusions: Our results show a distinct DNA methylation profile across DM1 tissues and uncover a dual epigenetic signature in DM1 muscles, involving gain of DNA methylation in the flanking region of CTG expansion accompanied by specific DNA demethylation in the DMPK gene.

S2-1-4

Independence of Adults with Childhood Phenotype of Myotonic Dystrophy Type 1

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Introduction: Among the five phenotypes of myotonic dystrophy type 1 (DM1), little is known about the ability of adults with the childhood phenotype to live independently. Motor and neuropsychological impairments have been shown to influence independence in other phenotypes. This study documents the level of independence in performing activities of daily living (ADL) and explores the impact of executive functions and apathy on ADL performance in adults with the childhood phenotype of DM1.

Methods: ADL performance was assessed with the Independent Living Scale (ILS) and the Activities of Daily Living Profile. The later considered four operations related to executive functions: formulating a goal, planning, carrying out the task and goal attainment. Presence of apathy was assessed with the clinician version of the Apathy Evaluation Scale.

Results: Forty-eight participants (M:24; F:24; 19-57yo) from Saguenay (Canada; n:33) and Nantes (France; n:15) were assessed. According to the ILS total score, half of the participants were categorized as dependent. Only one participant was independent in financial management. Dependence was more frequent in participants with apathy.

Conclusions: Adults with the childhood phenotype exhibit significant difficulties in ADL performance, especially considering their age range. High levels of dependency observed with both outcome measures highlight their need for services to achieve optimal living conditions.

S2-1-5

TREAT-NMD Myotonic Dystrophy Global Registry Network: An International Collaboration in Myotonic Dystrophy Type 2

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[1]On behalf of TREAT-NMD Myotonic Dystrophy Subgroup & TREAT-NMD Global Registry Network

Introduction: Myotonic dystrophy type 2 (DM2) is a rare multi-system disease recognised in the last three decades. The TREAT-NMD Global Registry Network is a global collaboration of registries collecting data on neuromuscular conditions such as DM2.

The aim is to assess the number of DM2 patients included in the network, and analyse socio-demographic and clinical features.

Methods: An email survey was sent to the 22 member DM registries requesting data on number of DM1 and DM2 patients, population catchment, and clinical features.

Results: Of the 13 DM registries that responded, eight enrolled DM2 patients. The total number of DM2 cases was 1,720, with the Czech/Slovakian, German and the USA (MDF) registries enrolling the most patients with 445, 430, and 339, respectively. The highest number of registered cases per 100,000 population was seen in the Czech/Slovakia (4.2) and Serbia (2.0). The DM2:DM1 ratio was highest in the Central European countries. Registry enrolment occurred at a median age of 51 years with 63% being female. Onset of DM2 occurred before the age of 20 in 14% of cases. One fifth of DM2 patients used an assistive device to walk and 4% were non-ambulant. Pacemaker or implantable cardioverter-defibrillator was reported in 4% of DM2 subjects, while 7% used non-invasive ventilation.

Conclusions: TREAT-NMD member registries were able to assemble the largest DM2 cohort to date with an international reach, providing meaningful clinical and demographic data. More DM registries should aim to capture DM2 data to contribute to important collaborations such as this one, which can support future research and clinical trial recruitment.

Session 2-2: Clinical Aspects / Biomarkers, outcome measures, trial design, etc

S2-2-1

Longitudinal changes in neuropsychological functioning in Japanese patients with myotonic dystrophy type 1: A 5-year follow-up study

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Introduction: Myotonic dystrophy type 1 (DM1) is characterized by various symptoms, including the central nervous system. Some studies have reported cognitive declines in patients with DM1, although the available evidence is limited. This study aimed to describe the longitudinal difference in neuropsychological functions in patients with DM1.

Methods: A total of 67 Japanese patients with DM1 were investigated with a neuropsychological battery assessing several domains of cognition, including measures of memory, processing speed, and executive function. The patients received the neuropsychological evaluation approximately five years after the baseline (time 1 and time 2). The procedure of this study was approved by the ethics review boards of the hospitals.

Results: Thirty-eight patients received the second neuropsychological evaluation. Participants of the time 2 evaluation were younger than patients who did not participate at time 2. Patients showed declines in Mini-Mental State Examination, Trail Making Test (TMT), Block Design, and Symbol Digit Modalities Test (SDMT) at time 2 evaluation ($p < 0.05$). Age at time 1 was associated with declines in TMT-A and -B (rho = 0.57, 0.45). The number of CTG repeats was associated with declines in MMSE and SDMT (rho = -0.38, -0.53).

Conclusion: The results suggested the cognitive decline in patients with DM1. These findings need further exploration with the possible effects of age-related changes.

Session 2-2: Clinical Aspects / Biomarkers, outcome measures, trial design, etc

S2-2-2

Blood based biomarker discovery in DM1

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Introduction: The to date largest clinical trial in Myotonic Dystrophy type 1 (DM1), OPTIMISTIC, has demonstrated significant positive effects of cognitive behavioural therapy (CBT) on the capacity for activity and social participation[1]. Through a process of reverse engineering, the ReCognitION study aims to identify druggable biomarkers associated with the clinical improvement observed in the OPTIMISTIC cohort. The lead hypothesis is that biological pathways associated with the therapy response can be induced with repurposed drugs, thereby potentially consolidate or reinforce the positive effects induced by CBT.

Methods: Based on full blood samples collected during OPTIMISTIC, paired mRNA sequencing was done for 27 patients of the intervention group (before and after the CBT intervention) and proteomic profiling (data independent acquisition, DIA) for a total of 451 samples (before and after the intervention, both control and intervention group). Linear mixed effect models were used to identify biomarkers associated with the disease causing CTG-expansion and the mean clinical improvement across all outcome measures.

Results: We identified 608 genes for which their expression was significantly associated with the CTG-repeat expansion, as well as 1176 genes significantly associated with the average clinical response towards the intervention. Remarkably, all 97 genes significantly associated with both returned to more normal levels in patients who improved clinically[2]. Preliminary analysis of the proteomics data revealed that almost 300 peptides and 50 proteins were associated with the CTG-repeat expansion, confirming the disease signature in blood.

Conclusions: DM1 relevant disease signatures can be identified in peripheral blood on multiple biological levels, opening new avenues for drug discovery and therapy efficacy assessment.

[1]Okkersen K et al. Lancet Neurol. 2018;17(8):671–680. [2]van Cruchten RTP et al. MedRxiv. 2022; https://doi.org/10.1101/2022.03.11.22272021
Funding: ReCognitION is funded through the E-Rare Joint Translational Call JTC 2018

Session 2-2: Clinical Aspects / Biomarkers, outcome measures, trial design, etc

S2-2-3

miR-223-3p and miR-24-3p as novel serum-based biomarkers for Myotonic Dystrophy type 1

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Introduction: Limited studies have been performed regarding the development of non-invasive biomarkers for rare diseases like DM1. We previously showed that four miRNAs can serve as biomarkers for DM1 progression. In this study, we aimed to identify novel serum-based biomarkers for DM1.

Methods: Small RNA NGS was performed to profile all small RNA molecules, including miRNAs present in serum samples of DM1 patients and controls. Two miRNAs were selected and validated in a larger panel of patients.

Results: miR-223-3p and miR-24-3p levels were elevated in the serum of DM1 patients. The two miRNAs and the previously identified four miRNAs, miR-1, miR-133a, miR-133b, and miR-206 showed elevated levels in the serum of DM1 mice compared to controls. The levels of miR-223-3p, but not the other five miRNAs, were significantly lower in skeletal muscle and heart of DM1 mice.

Conclusions: Based on our results we suggest two novel miRNAs, miR-24-3p and miR-223-3p, as potential biomarkers for DM1. Moreover, the reduced levels of miR-223-3p in skeletal muscle and heart of DMSXL mice provide significant evidence for its role in the manifestation of the disease[1].

Funding: European Regional Development Fund & Republic of Cyprus; A.G. Leventis Foundation.

[1]Koutsoulidou A., et al. (2021) Mol Ther Methods Clin Dev. 23:169-183

Session 2-2: Clinical Aspects / Biomarkers, outcome measures, trial design, etc

S2-2-4

Myotonic dystrophy type I Tau PET imaging and exploratory study of CSF and plasma biomarkers of neurodegeneration from neurocognitively characterized DM1 patients

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Introduction: Although several therapeutic clinical trials are ongoing in DM1, biomarkers of brain lesional processes and tracers of neurofibrillary tangles made of abnormally modified and aggregated Tau protein isoforms, have not yet been considered as potential theragnostic outcomes. The present study explored a Tau PET tracer, CSF, plasma, and neurocognitive profiles as potential DM1 theragnostic biomarkers.

Methods: Seven genetically confirmed DM1 Canadian patients had a detailed neurocognitive assessment. Biomarker assessments included Tau PET with [18F]-AV1451 tracer, CSF Tau and Ab, blood neurofilaments (NfL), and GFAP using immunoassays.

Results: Three cognitively impaired (CI) DM1 patients presented with an elevated signal of Tau PET tracer bilaterally in the medial temporal lobes. The other DM1 patients had some focal, low diffuse, or no PET signal relative to the reference region. Interestingly, the patient with the greatest Tau PET signal also had the lowest CSF $A\beta_{42}$ and the highest CSF Tau levels. The levels of CSF Tau and phospho-Tau biomarkers were higher in CI DM1 patients but with a mean value lower than that observed in AD. Finally, CSF phospho-Tau was not correlated with Tau PET but did correlate significantly with plasma NfL concentrations.

Conclusions: This pioneering study makes critical gains toward understanding the mechanisms underlying neurocognitive deficits in DM1 and the potential usefulness of Tau PET tracer, CSF Tau, and plasma NfL biomarkers in DM1 as potential theragnostic biomarkers[1].

1. Laforce RJ et al. J Neurol. 2022

This study was possible as a result of a grant from AFM Telethon to Robert Laforce, Jack Puymirat, and Nicolas Sergeant.

Session 2-2: Clinical Aspects / Biomarkers, outcome measures, trial design, etc

S2-2-5

Analysis of circulating myomiRs as potential biomarkers of progression of muscular impairment in myotonic dystrophy type 1 patients

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Introduction: DM1 is a multisystemic disorder, with skeletal muscles being the major affected organ. Progression of muscular impairment is the main parameter of the disease natural history and shows a variation among patients. Currently, there are no non-invasive biomarkers for monitoring DM1 progression. Here, we aimed to analyze level of circulating myomiRs in DM1 patients with a different progression of muscular impairment.

Methods: Sixty-seven DM1 patients with the MIRS assessed more than five years ago were recruited from the Serbian registry of myotonic dystrophies and underwent MIRS assessment and blood sampling. Patients with increased MIRS by at least 1 point during 5 or more years were classified as progressive. MiR-1, miR-206, miR-133a and miR-133b were amplified by real-time PCR using specific TaqMan Assays. MiRNA level was normalized to the external spike-in cel-miR-39-3p and then to the endogenous has-miR-16.

Results: There was a higher level of plasma miR-1 and miR-133a in progressive DM1 patients compared to non-progressive (Wilcoxson test: p=0.039, W=650 and p=0.025, W=636, respectively). The level of miR-206 and miR-133b were not different between examined groups.

Conclusions: MiR-1 and miR-133a showed the potential to be further examined as noninvasive biomarkers for the progression of muscular impairment in DM1.

S3-01

Whole transcriptome analysis and functional studies reveal that senescence plays a role in Myotonic Dystrophy type 1

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Introduction: Myotonic dystrophy type 1 (DM1; MIM #160900) is an autosomal dominant disorder, clinically characterized by progressive muscular weakness and multisystem degeneration. The broad phenotypes observed in DM1 patients resemble the appearance of an accelerated aging process. However, the molecular mechanisms underlying these phenotypes remain largely unknown.

Methods: Human primary fibroblasts from DM1 patients and control donors were used in this study. Functional studies of cell viability, proliferation, DNA damage response and senescence were performed on them in the absence or with senolytics treatment. The gene expression profile in treated cells was determined by RNASeq. Validation of transcriptomic results were performed in human myoblasts samples in vitro and blood samples in vivo. Additionally, we used a *Droshophila* and a mouse model of the disease. The impact of senolytics *in vivo* was evaluated in a *Droshophila* model of the disease in locomotor activity and longevity studies.

Results: Transcriptomic analysis of fibroblasts derived from DM1 patients and healthy individuals revealed a decrease in cell cycle activity, cell division, and DNA damage response in DM1, all of which related to the accumulation of cellular senescence. These Data were corroborated in human myoblasts and blood samples as well as in mouse and *Drosophila* models of the disease. Several studies *in vitro* and *in vivo* confirmed the accelerated increase in senescence and the acquisition of a senescence-associated secretory phenotype in DM1 samples. Functional studies highlighted the impact of BMI1 and downstream $p16^{INK4A}$/RB and ARF/p53/$p21^{CIP}$ pathways in DM1-associated cellular phenotypes. Importantly, treatment with the senolytic compounds, Quercetin, Dasatinib, or Navitoclax, reversed the accelerated aging phenotypes in both DM1 fibroblasts *in vitro* and in *Drosophila in vivo*.

Conclusions: Our results identified the accumulation of senescence as part of DM1 pathophysiology and therefore, demonstrated the efficacy of senolytic compounds in the pre-clinical setting.

S3-02

Correction of Clcn1 mis-splicing reverses muscle fiber type transition in mice with myotonic dystrophy

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Introduction: In patients with DM1 and in the HSALR transgenic and Mbnl1 knockout mouse models of DM1, mis-regulated alternative splicing of muscle chloride channel Clcn1 transcripts causes myotonia, a delayed relaxation of muscles due to repetitive action potentials. In human DM1 muscle, oxidative muscle fibers are upregulated and preferentially atrophic as compared to glycolytic fibers, which can be hypertrophic. The effects of myotonia reversal on myopathy and myosin fiber type transition are unknown.

Methods: We crossed HSALR transgenic mice with Mbnl1 knockout mice to create a double homozygous mouse model of DM1. Tibialis anterior muscles were injected with an antisense morpholino oligo that induces skipping of Clcn1 exon 7a. The contralateral muscle was injected with the 5' - 3' invert of the active drug. We measured Clcn1 splicing by RT-PCR and droplet digital PCR (ddPCR), transgene expression by ddPCR, and myosin fiber type by immunolabeling and ddPCR.

Results: Myotonia was significantly worse in double mutant mice than in either model in isolation, and type 2B glycolytic muscle fibers were nearly absent. In muscles receiving the active treatment, Clcn1 splicing was corrected by 16 days after injection, expression of the muscle regeneration marker embryonic myosin (Myh3) was reduced by more than half, and type 2B glycolytic fibers increased to 40% of the overall total, similar to WT.

Conclusions: Chronic myotonia induces muscle fiber type transition from glycolytic to oxidative in mouse models of DM1. Reversal of Clcn1 mis-splicing is sufficient to rescue muscle fiber type patterns and reduce muscle fiber damage. Targeted correction of Clcn1 splicing is a candidate adjuvant therapeutic approach to improve myopathy in DM1.

Funding: Muscular Dystrophy Association.

S3-03

The ReCognitION project: Recognition and Validation of Druggable Targets from the Response to Cognitive Behavioural Therapy in Myotonic Dystrophy type 1 patients from Integrated -Omics Networks

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Introduction: There are currently no approved therapeutic interventions for DM1. Drug repurposing is an attractive therapeutic strategy for DM1, because patient may get faster access to drugs approved for another clinical indication than to newly developed therapeutic entities. Metformin is an example of a repurposed drug that was shown to impart a clinical benefit in DM1 patients. The ReCognitION project is a follow-up project on the successful OPTIMISTIC clinical trial, which has demonstrated clinical benefit of Cognitive Behaviour Therapy (CBT). ReCognitION's lead hypothesis is that new drug repurposing candidates can be identified from pathways associated with the positive response to CBT and that these drugs can be consolidated or reinforced by conventional drug therapies targeting these pathways.

Methods: In ReCognitION, we have taken a multi-omics approach to identify the molecular signatures associated with the response to CBT. We have generated RNA-seq profiles from blood samples from 27 participants at 0 and 10 months of intervention and we have generated untargeted serum proteomics for all 255 participants at 0 and 10 months. Specific protein targets were validated by Western blotting and their interaction partners were identified through co-immunoprecipitation followed by mass spectrometry.

Results: We have identified several druggable pathways with support from the large scale transcriptomics and proteomics datasets. These include the histone (de)acetylation and retinoid acid signalling pathways. Compounds affecting these pathways are currently being evaluated in induced pluripotent stem cell-derived myogenic and neuronal cell cultures and in the HSA-LR and DMSXL mouse models.

Conclusions: The drug repurposing strategy based on reverse engineering of a positive response to an intervention may constitute a novel paradigm for drug repurposing in rare diseases.

Funding: ReCognitION is funded by the European Union's Horizon 2020 research and innovation porgramme "ERA-NET rare disease research implementing IRDiRC objectives – No 643578" through the E-Rare Joint Translational Call JTC 2018

Session 3: Therapeutic Strategies and Targets

S3-04

Identifying potential lead molecules that eliminate toxic nuclear foci in DM1

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Introduction: Myotonic dystrophy is a dominant multisystemic disorder which currently lacks any available treatment. Myotonic dystrophy type 1 (DM1) is caused by a CTG repeat mutation located in the 3' untranslated region of the *dystrophia myotonica protein kinase* (*DMPK*) gene. The mutant *DMPK* transcripts containing the expanded repeats are retained in the nucleus where they form stable structures detectable as nuclear foci. These nuclear foci sequester RNA-binding proteins such as muscleblind-like splicing regulators (MBNL), thereby interfering with normal RNA metabolism. This results in abnormal regulation of RNA processing, and in particular RNA splicing.

Methods: Medium throughput cell-based assays have been developed in our lab to identify druggable targets for myotonic dystrophy. These assays are based on the identification of nuclear foci using *in situ* hybridization and high-content imaging. Through this screen we have identified small molecules that eliminate the nuclear foci. We are performing further studies to gain insights into the mechanism of action for these molecules that could identify novel therapeutic targets for DM1.

Results: We have screened numerous small molecules that have been suggested to have therapeutic potential for DM therapy including kinase inhibitors, transcriptional inhibitors, macrolides and molecules involved in biomolecular phase separation. Here we summarize our findings from the screening of such small molecules in DM1 and DM2 cell models and report their potency to eliminate nuclear foci and their concentration range.

Conclusions: It is possible that no single drug will provide a suitable treatment for DM and multiple small molecules affecting different molecular targets will be required. Given the multisystemic nature of DM1, using several small molecules against relevant targets in DM1 may provide a suitable approach to alleviate the classic hallmarks of DM1 pathogenesis. Some of the molecules described here may serve as potential lead molecules for developing DM1 therapy. More work will be needed to identify the mechanisms of their action.

S3-05

A CTG repeat-selective screen of a natural product library reveals dietary natural compounds as potential therapeutics for Myotonic Dystrophy.

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Introduction: Myotonic dystrophy (DM) is a multisystemic neuromuscular disease caused by Expansion of CTG and CCTG nucleotide tract in non-coding region of DMPK and CNBP gene respectively. Blocking the transcription of repeat expansion has been proven as a promising therapeutic approach for mitigating toxic RNA associated pathogenesis[1]. The current study identified bioflavonoids as new class of natural compound that exhibited promising therapeutic potential for the treatment of DM.

Methods: We utilized our previously developed DM1 HeLa cell model to screen a natural product library V obtained from NCI[1]. DM Patient derived fibroblast, myotubes and DM1 HSA^{LR} mouse model were used to validate the activity of screening Hits.

Results: The primary screening revealed NP1-NSA1 as a selective modulator of toxic CUG RNA abundance, as it reduced $r(CUG)_{480}$ level by ~45%. Next, NP1-NSA1 notably rescued DM associated mis-splicing events in DM1 and DM2 patient-derived fibroblast cell lines and DM1 patient derived Myotubes. Interestingly, NP1-NSA1 also reduced the transgene expression and spectacularly improved splicing defect in DM1 HSA^{LR} mouse model. NP1-NSA1 also improved the muscle phenotype of DM1-HSA^{LR} mouse without causing any toxicity.

Conclusions: NP1-NSA1 abundantly present in several fruits and vegetable that are part of the daily human diet. This excellent safety profile with little to no adverse effects, positions NP1-NSA1 as a potentially safe lead compound for therapeutic consideration in DM.

1. Reddy, K., et al., *A CTG repeat-selective chemical screen identifies microtubule inhibitors as selective modulators of toxic CUG RNA levels.* Proceedings of the National Academy of Sciences, 2019. **116**(42): p. 20991-21000.

Session 3: Therapeutic Strategies and Targets

S3-06

Repeat dosing with DYNE-101 is well tolerated and leads to a sustained reduction of *DMPK* RNA expression in key muscles for DM1 pathology in hTfR1/DMSXL mice and NHPs

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Introduction: Myotonic dystrophy type 1 (DM1) is a severe neuromuscular disease caused by the expansion of CUG triplets in the 3'-untranslated region of the dystrophia myotonica protein kinase (*DMPK*) RNA. DYNE-101 is being developed for the treatment of DM1 and consists of an antigen-binding fragment (Fab) that binds specifically to transferrin receptor 1 (TfR1), conjugated to a gapmer antisense oligonucleotide (ASO) designed to target the *DMPK* RNA. In hTfR1/DMSXL mice, DYNE-101 reduced the levels of toxic human *DMPK* RNA in nuclei from cardiac and skeletal muscle, consequently improving multiple DM1 splicing defects. The aim of this study was to determine the impact of repeat dosing of DYNE-101 on *DMPK* expression in muscle in hTfR1/DMSXL mice and non-human primates (NHPs).

Methods: hTfR1/DMSXL mice were administered with 4 monthly doses of DYNE-101, or vehicle. Wild-type (WT) NHPs were treated with 2 monthly doses of DYNE-101, or vehicle. A subsequent 13-week GLP toxicology study was conducted in NHPs.

Results: Administration of DYNE-101 led to a robust reduction of mutant human *DMPK* RNA in the heart, diaphragm, gastrocnemius, and tibialis anterior in hTfR1/DMSXL mice. In WT NHPs, administration of DYNE-101 led to substantial suppression of WT *DMPK* expression up to 70% in the heart, diaphragm, gastrocnemius, tibialis anterior, masseter, esophagus, and duodenum. A subsequent 13-week GLP toxicology study demonstrated that repeated administrations of DYNE-101 were well-tolerated in NHPs.

Conclusions: These data support development of DYNE-101 for the clinical treatment of DM1.

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S3-07

Elimination of defective muscle stem cells to restore myogenesis in Myotonic Dystrophy type 1.

Talita C. Conte[1], Gilberto D. Bishop[1], Zakaria Orfi[1] Inès Mokhtari[1,2], Marie-Pier Roussel[2,3], Serge McGraw[1], Elise Duchesne[2,3], and Nicolas A. Dumont[1]

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Introduction: Myotonic Dystrophy type 1 (DM1) induces skeletal muscle wasting and weakness. Muscle stem cells (MuSC), which are responsible for muscle repair are also affected in this disease. In DM1, MuSC show signs of premature senescence (irreversible cell cycle arrest) and reduced myogenesis capacity. The therapeutic potential of therapies targeting senescent MuSC in DM1 remains unexplored.

Methods: We used myogenic cells collected from DM1 patients and age- and sex-matched healthy individuals (n=12 per group; 6 men, 6 women) to characterize cellular senescence and screen for different senolytic drugs that can specifically eliminate senescent cells.

Results: Single cell RNAseq data revealed the presence of a specific subset of DM1 myoblasts expressing a senescent gene signature, characterized by the high expression of inflammatory genes. Immunofluorescence *in vitro* (myoblast culture) and *in situ* (muscle biopsy sections) confirmed an increase in the number of cells expressing the senescent markers p16 (cell cycle inhibitor) or senescence-associated-β-Gal. Drug screening identified a senolytic drug that can specifically eliminate senescent cells and reduce their expression of inflammatory cytokines. Removal of senescent cells re-established myoblast proliferation and differentiation.

Conclusions: This project provides exclusive insights on the pathophysiology of DM1, and it opens the way to a new therapeutic avenue targeting defective MuSC to restore myogenesis and enhance muscle function.

S3-08

Comprehensive transcriptomic characterization of antisense RNA treatments effects on Myotonic dystrophy type 1 cell models

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Introduction: MBNL1 overexpression through antisense oligonucleotide strategies that block repressor miRNAs miR-23b and -218 is a valid approach to treat DM1[1]. Because gene expression alterations at transcription, alternative splicing and polyadenylation levels are major contributors to myotonic dystrophy pathogenesis[2], we used RNA-seq to analyze the effects and recoveries associated with a number of these experimental treatments. The analysis also aimed to correlate these rescues with transcriptomics data coming from patient's muscle biopsies[3].

Methods:
Immortalized MyoD-inducible DM1 and control myotubes were cultured as reported in Cerro-Herrero et al. 2018[1] and 2021[4]. RNA-seq was performed to obtain gene expression data. Expresion changes were analyzed using STAR, RSEM and edgeR, alternative splicing was analyzed using vast-tools, and tappAS characterized differential polyadenylation site usage.

Results:
In the cell model, antagomiR-218 rescues 33.67% of the gene expression alterations to between 10 and 110% of normal levels including several disease-related genes such as CELF1, DMPK and GSK3B[4]. At the meeting, we will present similar data from antagomiR-23b and ongoing analyses that delineate the degree of recovery of the alternative splicing and polyadenylation from antimiR-23b and -218. Furthermore, we will report on potential off-target effects, confirm these candidate therapeutics' mechanism of action, and translate their relevance to the human disease manifestations.

Conclusions: Antisense RNA treatments show significant recovery effects over a wide array of alterations associated with DM1 in cell models. Some of these alterations are also detected on muscle biopsies datasets, which supports the current endeavors of developing a viable treatment against this disease.

S3-09

EEV-Conjugated Oligonucleotide Results in Nuclear Foci Reduction and Aberrant Splicing Correction in DM1 Cell and Animal Models

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Introduction: Myotonic dystrophy type 1 (DM1) is the most common adult-onset muscular dystrophy, manifesting in multisystemic effects including myotonia, muscle weakness and atrophy, cardiac and pulmonary complications, cataracts, endocrine dysfunction, and CNS complications. DM1 is caused by CUG repeat expansions in DMPK mRNA which sequester RNA binding proteins such as MBNL proteins in the nucleus. Aggregations of the toxic mRNA accumulate in the nuclei resulting in MBNL-1 dependent mis-splicing leading to the DM1 phenotype. There are currently no approved therapies for DM1.

Methods: One potential therapeutic approach for DM1 is selective blockade of CUG repeats with an oligonucleotide. To address the inherent challenges of poor delivery of oligonucleotide therapeutics to muscle tissue, we developed a family of proprietary cyclic cell-penetrating peptides (cCPPs), which form the core of our Endosomal Escape Vehicle (EEVTM) technology, that are conjugated to a chemically stable oligonucleotide known as a phosphorodiamidate morpholino oligomer (PMO). Our proprietary EEV technology is designed to facilitate intracellular delivery, endosomal escape, and localization to the nucleus. Here, we developed an EEV-PMO conjugate that specifically binds to and sterically blocks interactions between the CUG repeats and RNA binding proteins.

Results: The EEV-PMO conjugate significantly reduced nuclear foci and corrected splicing defects in a DM1 patient myogenic cell line and in the HSA-LR mouse model of DM1 by sterically blocking the interaction of MBNL proteins and CUG repeat expansion in an allele-selective manner. Furthermore, this EEV-PMO conjugate rescued the myotonia phenotype in vivo.

Conclusions: These results illustrate the significant therapeutic potential of the EEVoligonucleotide approach for DM1, and support development of EEV-conjugated oligonucleotide for DM1 and other neuromuscular diseases.

S3-10

A Phase 1/2 Trial Evaluating the Safety and Pharmacokinetics (PK) of AOC 1001 in Adults with DM1: MARINA Study Design

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Introduction: AOC 1001, an RNA therapeutic designed to target the pathogenic driver of DM1, is a *DMPK* siRNA conjugated to a humanized antibody targeting human transferrin receptor 1. The antibody targets muscles for delivery of siRNA into the cytoplasm and nucleus where it mediates *DMPK* mRNA degradation.

Methods: This phase 1/2 study (NCT05027269) is a two-part, randomized, placebo-controlled, double-blind trial in adults with DM1. In part A, after a single IV dose of AOC 1001, subjects are followed for 6 months to evaluate safety and tolerability. Part B includes 3 cohorts at ascending dose levels. Subjects receive 3 doses of AOC 1001 in the first 3 months, followed by 3 months of post-treatment monitoring. The cohorts will be initiated in a staggered fashion based on safety data reviews of preceding cohorts. The primary endpoint is treatment-emergent adverse events. Secondary endpoints include PK measurements of AOC 1001 and pharmacodynamic measurements, including *DMPK* mRNA knockdown and spliceopathy in muscle biopsies.

The study will enroll 44 symptomatic adults, 18 to 65 years with genetically confirmed DM1 (CTG repeat ≥100). Participants from parts A and B will have the option to participate in an open-label extension study.

Results: Pending trial completion.

Conclusions: Pending trial completion.

S3-11

Decoy gene therapy to reverse RNA toxicity in DM1

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Introduction: Currently, a number of RNA-based therapeutic strategies for DM1 are being developed, including small molecule and antisense oligonucleotide approaches. Here we assessed a gene therapy approach using a modified RNA-binding protein (RBP) with a high affinity for expanded CUG repeats aimed to act as a decoy to displace sequestered endogenous MBNL proteins from RNA foci and reverse RNA toxicity.

Methods: We engineered a truncated MBNL1 protein that keeps the ZnF domains required for the binding to CUG repeats but lacks the C-terminal domain. This MBNL1Δ-decoy has reduced splicing activity but can still compete with MBNL1 for binding to CUGexp. Effect of the MBNL1Δ-decoy was assessed in both human DM1 muscle cells and HSA-LR mouse model.

Results: The binding of the decoy to CUGexp in DM1 muscle cells allows the release sequestered endogenous MBNL1 from nuclear RNA foci, restores MBNL1 activity and corrects the transcriptomic signature of DM1. In addtion, MBNL1Δ-decoy forms less stable ribonucleoprotein complexes than MBNL1 resulting in reduce levels of CUGexp-transcripts. *In vivo*, local or systemic delivery of the AAV-decoy into the skeletal muscle of HSA-LR mice leads to long-lasting correction of splicing defects and improvement of muscle physiology.

Conclusions: This study supports the development of decoy RBPs with high binding affinities for CUGexp as a therapeutic strategy for DM1.

Poster Presentation Abstracts

P-01

Identification of a CCG-enriched expanded allele in DM1 patients using Amplification-free long-read sequencing

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Introduction: Myotonic dystrophy type 1 (DM1) exhibits highly heterogeneous clinical manifestations caused by an unstable CTG repeat expansion reaching up to 4,000 CTG. The clinical variability depends on CTG repeat number, CNG repeat interruptions and somatic mosaicism. Currently, none of these factors are simultaneously and accurately determined due to the limitations of gold standard methods used in clinical and research laboratories. An amplicon method for targeting DM1 locus using Single-Molecule Real-Time sequencing (Pacific Biosciences) was recently developed to accurately analyze expanded alleles[1]. However, amplicon-based sequencing still depends on PCR and the inherent bias towards preferential amplification of smaller repeats can be problematic in DM1. To overcome this limitation, we developed a robust amplification free-targeted (No-Amp) long-read sequencing to specifically characterize the DM1 locus in patients.

Methods: No-Amp long-read sequencing utilizes the CRISPR/Cas9 system to target and isolate the DNA fragment of interest from genomic DNA, in combination with long-read sequencing. This method was used to sequence the DM1 locus in patients with CTG repeat expansion ranging from 130 to > 1000 CTG (CTG repeat size estimated by Southern blot at diagnosis).

Results: We showed that elimination of PCR amplification improves the accuracy of measurement of inherited repeat number and somatic repeat variations, two important key factors in the DM1 severity and age at onset. For the first time, an expansion composed of over 85% CCG repeats was identified in a DM1 family with an atypical clinical profile for whom amplification of the triplet repeat expansion failed by PCR and TP-PCR.

Conclusions: This method allows to simultaneously obtain high resolution information on the number of repeats, a complete and accurate sequence and a measure of somatic mosaicism even for long repeats in the same assay. No-amplification targeted sequencing gives us the opportunity to better understand the dynamics of CTG repeat instability and genotype-phenotype association in DM1.

[1]Mangin et al. (2021). *Int J Mol Sci* 22, 2616.

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

P-02

Transcriptome analysis in a primary human muscle cell differentiation model for myotonic dystrophy type 1

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Introduction: It is well accepted that malfunctioning of two splicing factors, MBNL1 and CELF1, is the main cause for DM1, yet the whole range of symptoms cannot be explained solely by misregulation of these two factors. We thus investigated proliferating and differentiated muscle cells from control and DM1 patients. Additionally, we compared the results to transcriptome data from DM1 muscle biopsies. This revealed that splicing is mis-regulated on a larger scale than anticipated[1].

Methods: We used RNA-sequencing to analyze transcriptomic alterations in DM1 proliferating and differentiated cells and compared the results to publicly available DM1 muscle biopsy data. We further validated selected findings via qPCR and immuno-fluorescence.

Results: We analysed pathways affected in DM1, which revealed that WNT and MAPK signaling, cell adhesion and muscle development are affected, consistent with the observed DM1 phenotype. Further, splicing is mis-regulated on a larger scale than anticipated: a whole set of alternative splicing factors is upregulated, while constitutive splicing is downregulated in a cell differentiation stage dependent manner.

Conclusions: This sheds light on the complexity of DM1 etiology and encourages further exploration of other splicing factors. Moreover, we conclude that it is vital for future therapeutic interventions to target muscle stem cells rather than or in addition to mature muscle.

[1]Todorow V and Hintze S (2021), International Journal of Molecular Sciences.

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

P-03

A genome-scale RNAi knock-down screen identifies modifiers of RNA toxicity in myotonic dystrophy

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Introduction: Transcription of a CTG repeat expansion in the *DMPK* gene produces toxic CUG RNA, causing myotonic dystrophy type 1 (DM1). A major pathogenic consequence of toxic CUG RNA production is the sequestration of MBNL proteins leading to global RNA processing defects.

Methods: Using a previously developed DM1 HeLa cell line that stably expresses r(CUG)480 and r(CUG)0 control[1], we conducted a genome wide siRNA screen of over 16,000 genetic targets to identify novel factors that modify toxic CUG RNA outcomes.

Results: We identified multiple RNA processing factors that when knocked down, reduce CUG RNA levels and promote rescue of MBNL-dependent splicing in the HeLa DM1 cell models and in DM1 patient cells. Quantifying the expression levels of our top hits from DMseq.org patient transcriptomic data, revealed significantly lower RNA levels in several of the hits in DM1 patients compared to unaffected controls, suggesting that differential expression of these hits could modify splicing outcomes for patients. Our work is currently aimed at elucidating the molecular mechanism through which these factors modify RNA toxicity.

Conclusions: Identifying modifiers of toxic CUG RNA and MBNL-dependent splicing in DM1 provides new biological and therapeutic insight and may shed light on a potentially large class of disease modifiers for myotonic dystrophy.

[1]Reddy K, Jenquin JR, McConnell OL, Cleary JD, Richardson JI, Pinto BS, Haerle MC, Delgado E, Planco L, Nakamori M, Wang ET, Berglund JA. Proc Natl Acad Sci U S A. 2019 Oct 15;116(42):20991-21000

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

P-04

Withdrawn

Session 1-2: Repeat-Associated Pathomechanisms / RNA-mediated mechanisms

P-05

Withdrawn

Session 1-3: Repeat-Associated Pathomechanisms / Cell/organoids and animal models

P-06

Characterisation of DM1 cell culture models by In-Cell Western technology

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Introduction: There are multiple cell type models for pre-clinical drug evaluation in DM1, such as patient-derived fibroblasts and myoblasts. Nevertheless, DM1 hallmarks are not homogenous among tissues, which requires the most suitable well-characterized model for each screening. The objective of this work is to evaluate fibroblasts and myoblasts suitability for pre-clinical assays through a novel assessment platform.

Methods: Myoblots and fibroblots are assays for protein quantification in microplates based in the In-Cell Western technology that we have adapted to the study of DM1-relevant proteins in myoblast and fibroblast cultures, respectively. In addition, we have optimized a digital droplet PCR (ddPCR) protocol for the quantification of the mRNA expression of the same proteins.

Results: Optimisation of myoblots and fibroblots allowed us to accurately quantify different proteins in both cell models. We observed differences in protein expression among DM1 and CTRL groups in myoblasts, and variations of these differences between myoblast and fibroblast cultures. Also, different patterns were shown at RNA level. In addition, we treated cultures with well-known small molecules that have been tested in pre-clinical assays for DM1, leading to diverse results when comparing them.

Conclusions: Combination of myoblots/fibroblots and ddPCR analysis of DM1 samples allows a highly reproducible and less laborious characterization of DM1 cultures suitable for evaluation of potentially therapeutic compounds in DM1. Also, we describe different protein expression patterns between fibroblasts and myoblasts cultures, suggesting the need for testing potential treatments in more than one cell model.

Funding: This work was supported by funding from ISCIII (Spain) and the ERDF/FEDER (grant PI18/00114), and the Basque Government (grant 2019111010). A.L-M holds a FPU Fellowship (FPU20/00912) from the Ministry of Universities, Spain. V.A-G acknowledges funding from Ikerbasque (Basque Foundation for Science, Spain.

Session 1-3: Repeat-Associated Pathomechanisms / Cell/organoids and animal models

P-07

RAN Translation in Myotonic Dystrophy Type 1 Primary Cell Cultures

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Introduction: The pathomechanisms in Myotonic Dystrophy type 1 (DM1) are very diverse. Repeat associated non-ATG (RAN) translation was described to contribute to DM1 pathology in 2011, but since then only some reports have explored DM1 antisense presence (DM1-AS), which are the transcripts that originate RAN proteins.

Methods: In order to assess the contribution of RAN translation in DM1, we study the presence of DM1-AS transcripts by RT-PCR and FISH, and the RAN translation via immunoblot and immunofluorescence in distinct DM1 primary cell cultures (myoblast, fibroblast and lymphoblast) isolated from different patients.

Results: DM1-AS transcripts were found in all DM1 cells, with a lower expression in patients compared to controls. Antisense RNA foci were found in the nuclei and cytoplasm of DM1 cells. PolyGln RAN translation was undetectable in all three cell types by immunoblot and immunofluorescence. A 42 kD polyGln containing protein was detected, which was most likely the TATA-box-binding protein. Immunofluorescence revealed a cytoplasmic aggregate, which co-localized with the Golgi apparatus.

Conclusions: DM1-AS transcript levels were lower in patients compared to controls and a small portion of the transcripts included the expanded repeat. Nonetheless, RAN translation was not detectable, with the available current methods, in patient derived DM1 cells.

Session 1-3: Repeat-Associated Pathomechanisms / Cell/organoids and animal models

P-08

MBNL loss of function in visceral smooth muscle as a model of myotonic dystrophy type 1

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Introduction: Recent DM1 patient surveys identified gastrointestinal (GI) disturbances as a prevalent patient complaint that impacts daily life[1–4]. The cause of DM1 GI pathology is unknown and evidence supports a role for visceral smooth muscle dysfunction[5]. The goal of this project is to elucidate the role of muscleblind-like (MBNL) in GI smooth muscle function.

Methods: MBNL loss is induced in mice expressing a smooth muscle specific CreER^{T2} and floxed *Mbnl* alleles (denoted smoCRE;dHOM) and gut motility quantitated using two dye-based methods. The first method measured total gut transit time from gavage of non-absorbable red dye until the first red fecal pellet. The second method used a FITC conjugated, cell-impermeable dextran solution, allowing for the quantification of bolus movement after 25 minutes. RT-PCR was performed on GI smooth muscle to identify DM1-associated splicing changes.

Results: SmoCRE;dHOM mice share homologous misregulated splicing events previously identified in mouse *Mbnl* KO striated muscle and in DM1 tissues. While total gut transit time was highly variable in smoCRE;dHOM mice, the FITC-dextran bolus showed significantly reduced transit in smoCRE;dHOM small bowel compared to floxed-only controls.

Conclusions: The results suggest that smooth muscle specific loss of MBNL affects GI motility. This model will be used to investigate the myogenic basis for DM1 GI pathogenesis.

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[3]Hilbert J, et al. (2017) *Neurology* 89:1348-1354.
[4]Perna A, et al (2020) *Front Neurol* 11:394.
[5]Nowak T, et al (1982) *Gastroenterology* 82:800-810.

Session 1-3: Repeat-Associated Pathomechanisms / Cell/organoids and animal models

P-09

Drug screening using iPSCs derived from myotonic dystrophy type 1 patient

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Introduction: Myotonic dystrophy type 1 (DM1) is caused by CTG trinucleotides repeat expansion within *DMPK* gene[1]. A working hypothesis for the pathogenesis of DM1 is that the toxic RNA gain-of-function by expanded CUG transcripts aggregate in the nucleus, which is called nuclear RNA foci, and alter the regulation of MBNL1 and CELF1 RNA binding proteins, leading to the mis-regulation of the alternative splicing of multiple transcripts[2]. Various types of drug candidates including antisense oligonucleotides have been being developed to target the toxic RNA[2]. Patient-derived induced pluripotent stem cells (iPSCs) are useful for drug development and disease modeling. In this study, we generated iPSCs from DM1-patients and screened drug candidates that reduce nuclear RNA foci using the DM1-patient derived iPSCs.

Methods: iPSCs were generated from peripheral blood mononuclear cells of DM1-patients. RNA foci in DM1-patient iPSCs were detected by fluorescent in situ hybridization (FISH). The effect of antisense oligonucleotides to reduce nuclear RNA foci was examined using DM1-patient iPSCs.

Results: Treatment with antisense oligonucleotides effectively reduced nuclear RNA foci in DM1-patient iPSCs.

Conclusions: We established patient iPSC-based assay which is useful for evaluation of candidate molecules to reverse the cellular phenotypes of myotonic dystrophy.

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[2]Nakamori, M. (2021) Neurology and Clinical Neuroscience

Session 1-3: Repeat-Associated Pathomechanisms / Cell/organoids and animal models

P-10

Withdrawn

Session 1-3: Repeat-Associated Pathomechanisms / Cell/organoids and animal models

P-11

Elucidation of the neuropathological defects in iPSC-derived iNeurons from patients with DM1

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Introduction: DM1 is also characterized by cognitive impairments, but knowledge about the underlying mechanisms that cause the cognitive defects is limited. Therefore, we aim to develop a cell-based model to elucidate the neuropathological defects.

Methods: We successfully generated iPSC from fibroblasts from two patients with DM1 and differentiated these to glutamatergic excitatory neurons (iNeurons). We looked at DM1-specific hallmarks, such as RNA foci and at the electrophysiological activity, measured by multi-electrode array (MEA). In the upcoming period, we aim to characterize DM1 iNeurons in more detail to shed light on underlying processes of disease-related neural defects. We will investigate neuronal morphology (immunocytochemistry and reconstruction with the Neurolucida 360 software), determine the triplet repeat length (Bionano Sapphire), look at DM1-related missplicing and continue establishing an electrophysiological profile of the neurons.

Results: We were successful in differentiating iNeurons from two patients. *In vitro* DM1 neurons seem to be hyperexcitable, which corresponds to earlier findings in a DM1 mouse model.

Conclusions: Initial characterization of DM1 patient-derived neurons revealed electrophysiological aberrations compared to control iNeurons. We aim to dissect the underlying neurobiological mechanisms of these neural network defects and to extend our investigations to other neural cell types such as astrocytes. In parallel, we will use the DM1 neuronal phenotype to test small molecules and oligonucleotides.

Session 1-4: Repeat-Associated Pathomechanisms / Tissue specific mechanisms

P-12

Mechanism of DM1 cardiac pathogenesis

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Introduction: Over half of individuals affected by DM1 have cardiac involvement, such as conduction defects and arrhythmias, which can lead to sudden cardiac death, the second leading cause of death in DM1. RNA containing expanded CUG repeats (CUG_{exp}) transcribed from the mutant allele causes DM1 pathogenesis by disrupting functions of MBNL and CELF1 proteins. While many molecular effects of CUG_{exp} RNA have been identified, most studies have focused on skeletal muscle. The molecular details of how CUG_{exp} RNA induces cardiac involvement are unknown.

Methods: We used our DM1 heart mouse model[1], which expresses cardiomyocyte-specific and tetracycline-inducible RNA containing 960 interrupted CUG repeats (CUG_{960}) to determine the degree to which loss of MBNL and gain of CELF1 activities contribute to DM1 cardiac pathogenesis by testing for phenotypic rescue.

Results: Systemic AAV9 was used for heart-specific overexpression of epitope-tagged MBNL1 and MBNL2, that was confirmed in both left ventricles and atria. AAV9-MBNLs, but not the AAV9-mCherry control, significantly reduced the disrupted alternative splicing events, as well as the QRS and QTc conduction intervals in mice that were prolonged by induced CUG_{960}. Moreover, the AAV9-MBNLs cohort showed trends of rescued heart weight, ventricular wall thickness, and ejection fraction compared to the controls.

Conclusions: The data indicates that MBNLs play a crucial role in DM1 cardiac pathogenesis. Future studies will focus on determining the degree of rescue by MBNL1 and MBNL2 individually, the role of CELF1 in DM1 cardiac pathogenesis, and ranking rescue by comparison to the maximum reversal when the transgene was turned off in mice.

Funding: Myotonic Dystrophy Foundation

[1]Rao et al., (2021) *JCI insight* 6(5):e143465.

Session 1-4: Repeat-Associated Pathomechanisms / Tissue specific mechanisms

P-13

Circadian rhythm disruptions in myotonic dystrophy type I

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Introduction: DM1 patients suffer from sleep dysregulation, including altered sleep architecture and excessive daytime sleepiness. Currently, the mechanism underlying DM1 sleep pathologies is unknown. Disrupted sleep-wake circadian rhythms in DM1 patients and mis-splicing of a core circadian clock gene, *Casein Kinase 1 Delta* (*CSNK1D*), in multiple tissues of DM1 patients and mouse models, suggest that the circadian clock is disrupted in DM1. As the circadian clock regulates sleep-wake rhythms and sleep structure, clock disruption could be an important contributor to DM1 sleep pathologies.

Methods: To examine the effect of the DM1 mutation on the circadian clock, we are studying circadian locomotor activity rhythms in DM1 mouse models and circadian biomarkers in DM1 patients.

Results: Through studies of a *CSNK1D* minigene in a cell culture system, we confirmed that alternative splicing of *CSNK1D* exon 9 is indeed regulated by MBNL with expression of 480 CTG repeats or MBNL promoting exon exclusion and inclusion, respectively. To examine how the circadian clock is affected in DM1, we have performed circadian activity analyses on DM1 mouse models that either lack MBNLs or express expanded CTG repeats and display muscle or CNS features of the disease. Strikingly, all of these models display activity rhythms of reduced amplitude. In addition, *Mbnl2* KO mice, previously shown to have CNS deficits, displayed difficulty in adjusting to a 'jet lag' paradigm, suggesting defects in neuronal communication within the central clock tissue, the suprachiasmatic nucleus. Interestingly, the *DMPK* KI CNS model, which prominently displays repeat foci in the peripheral clock tissue of the choroid plexus, exhibited a decreased circadian period of activity but a normal response to 'jet lag'. To determine whether these observations extend to DM1 patients, we are currently assessing circadian rhythms in patients through analysis of the circadian biomarker, melatonin. These studies involve quantitative analysis of levels of 6-sulfatoxymelatonin, a melatonin metabolite, in patient urine collected at regular intervals over a 48-hour period.

Conclusions: Circadian activity studies in DM1 mouse models indicate that central and peripheral tissue clocks are disrupted in DM1. This combined disruption could contribute to altered sleep-wake rhythms in DM1. In addition, mis-splicing of the clock gene, *CSNK1D*, may be a molecular contributor to these circadian phenotypes; future studies will elucidate

Session 1-4: Repeat-Associated Pathomechanisms / Tissue specific mechanisms

P-14

Myotonic dystrophy RNA toxicity alters morphology, adhesion and migration of mouse and human astrocytes

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Introduction: Brain dysfunction in neurological diseases is frequently mediated by the impairment of neuronal and non-neuronal cells. Although *DMPK* gene expression is higher in cortical astrocytes than in neurons isolated from adult human and mouse brains, the contribution of astroglia to DM1 brain disease has been poorly investigated.

Methods: Transgenic DMSXL mice express expanded human *DMPK* transcripts in multiple cell types of the brain, providing a good model to investigate the impact of RNA toxicity on astroglia.

Results: DMSXL astrocytes exhibit impaired ramification and polarization *in vivo,* as well as defects in adhesion, spreading and migration in culture. In line with these pronounced phenotypes, DMSXL astrocytes express high levels of toxic RNA and accumulate abundant RNA foci, relative to neurons. RNA sequencing revealed MBNL-dependent RNA spliceopathy, which affects primarily transcripts that regulate cell adhesion, cytoskeleton and morphogenesis. To study the impact of defective astrocytes on neurons, we used co-culture cell systems, and found that DMSXL astrocytes impair neuritogenesis.

Conclusions: We demonstrate that DM1 impacts astrocyte cell biology, possibly compromising the support and regulation of synaptic function through defective neuroglia interplay.

Session 1-4: Repeat-Associated Pathomechanisms / Tissue specific mechanisms

P-15

Splicing defects in the grey and white matter of Myotonic Dystrophy Type 1

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Introduction: We found that splicing change between myotonic dystrophy type 1 (DM1) and amyotrophic lateral sclerosis (ALS) was significantly higher in the grey matter (GM) than white matter (WM)[1]. However, imaging analyses have revealed more greater change in WM than in GM[2]. In this study, to resolve the discrepancy between splicing and imaging changes, we comprehensively investigated splicing in GM and WM using RNA sequence (RNA-seq).

Methods: We investigated the autopsied brains samples of three patients with DM1 and three patients with ALS. We performed RNA-seq using RNA that was manually separated based on whether it was from GM and WM on slides of the frontal lobe tissue. We tested the splicing events, that differed only in WM by RNA-seq, using PCR in other samples.

Results: RNA-seq results showed 37, 25, and 3 DM1-affected events only in GM, only in WM, and in both, respectively.

Conclusions: In RNA-seq, we found multiple gene candidates with abnormal splicing in WM. We will need to further examine other samples to show the same abnormal splicing.

1 Nishi M, Kimura T, Igeta M, et. al (2020) PLoS One. 15:e0224912.
2 Minnerop M, Weber B, Schoene-Bake JC, et al. (2011) Brain. 134:3530-46

Session 2-1: Clinical Aspects / Specific disease features

P-16

Current status of reproductive medicine for myotonic dystrophy and views of geneticists in Japan

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Introduction: Anticipation is one of the hallmark of myotonic dystrophy type 1(DM1). There are gender differences in anticipation, with female patients having a higher risk of congenital patient, whereas male patients are less likely. For this reason, male patients have been considered ineligible for prenatal diagnosis (PND) and preimplantation genetic testing (PGT) in Japan. Additionally, since PGT is conducted as a “clinical research” in Japan, approval is required on a case-by-case basis, not only by the ethics committee of the facility but also by the ethics committee of the Japanese Society of Obstetrics and Gynecology. In our previous survey, one-quarter of female patients experienced reproductive treatment[1]. We surveyed the attitudes of geneticists and the current status of reproductive medicine.

Methods: From April to June 2020, we made a questionnaire survey in 1444 certified geneticists. We also surveyed the implementation status of PND and PGT in the 133 facilities admitting the National Liaison Council for Clinical Sections of Medical Genetics from November to December 2020.

Results: 617 geneticists and 77 centers responded to the survey. Two third of geneticists answered that male patient should be eligible for PND. Concerning PGT, 39% of geneticists answered that the restriction should be loosed, and 28% said male patients should be eligible. Some respondents suggested that what is available in other countries should also be available in Japan. In the implementation survey, ten centers had performed PNT/PGT in female patients, while none had performed PND/PGT in male patients, except for one pending PGT application.

Conclusions: The survey revealed that geneticists are permissive to reproductive medicine. The Japanese Society of Obstetrics and Gynaecology held ethics councils on PGT from 2020 to 2021. The ethical regulation of reproductive medicine may change in the future, but the most critical thing is to guarantee the patient's autonomous choice with adequate psychological and social support.

[1]Takahashi MP, Yamamoto R, Kubota T (2020) *Rihshoshinkei* 60:130-136.

P-17

Painful spasm with marked hyper-creatine kinase (CK) level after more than 20 weeks of four pregnant women with myotonic dystrophy type 1 (DM1) and a pregnant woman with paramyotonia congenita (PMC)

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Introduction: **Aggravation of clinical myotonia during pregnancy in myotonic syndromes which composed of DM1, DM2, and non-dystrophic myotonia, at times with increase in painful spasm or myalgia that resolves at the end of pregnancy, has markedly rarely been observed. Because rapidly that phenomenon disappeared after the delivery, the mechanism might be attributable to some increase in the impairment of the muscle membrane electrical activity but remain unclear yet.**

Methods: **In a 30-year-old pregnant woman with DM1 (first patient), painful muscle spasm with marked hyper-CK level (4976 IU/L, normal range; less than 140) was shown after 25 weeks. Surprisingly, immediately after delivery painful spasm was disappeared and serum CK level was markedly reduced (253 IU/L). In addition, in another 3 pregnant women with DM1 and a woman with PMC, the above similar phenomena were shown.**

Results: **In the first patient with DM1, both the pathological findings of the muscle biopsy at the cesarean operation and the image of skeletal muscle MRI at about one week after delivery were inflammatory changes. In addition, after about one month, the inflammatory changes in the MRI findings were improved.**

Conclusions: **The above phenomena were speculated that painful spasm might occur because the skeletal muscle chloride channel was inhibited by the marked elevation of serum progesterone after approximately 22 weeks of the pregnancy. In addition, the elevations of the above hormone might be provoked myositis with hyper-CK with spontaneous pain at effort. Furthermore, the rapidity with which those phenomena disappeared after the delivery might be attributable to the immediate reduction of the above hormone.**

P-18

Sinusitis in myotonic dystrophy: A retrospective study of brain MRI

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Introduction: Although sinusitis is sometimes encountered in myotonic dystrophy patients, there have been only a few case reports. In this study, we investigated the prevalence and clinical characteristics of sinusitis in myotonic dystrophy patients.

Methods: Myotonic dystrophy patients who had undergone brain magnetic resonance imaging (MRI) at Akita National Hospital between January 2014 and December 2021 were enrolled in this study. MRI findings were assessed using the Lund-Mackay (LM) score. Sinusitis on MRI was defined as LM score greater than or equal to 4. For the participants who had sinusitis on MRI, laboratory data including white blood cells (WBC), neutrophils, eosinophils, and C-reactive protein (CRP) were collected from medical records.

Results: Fifty-three patients (31 men and 22 women) aged 19-74 (mean 52) years were assessed in this study. The mean ± standard deviation (SD) of LM score were 2.4 ± 3.2. Fourteen (26%) participants had sinusitis on MRI. These results were higher than those of a previous report[1] of a Japanese middle-aged and elderly population (LM score was 0.88 ± 1.92 and 7.4% participants had MRI abnormality). Laboratory data (mean ± SD) in the sinusitis patients were WBC 7589 ± 1866/μl, neutrophils 64 ± 7%, eosinophils 2.9 ± 2.1%, and CRP 2.8 ± 3.1 mg/dl.

Conclusions: In the present study, sinusitis was a common complication of myotonic dystrophy. The laboratory data suggested that the etiology of sinusitis in myotonic dystrophy was infection in most cases.

[1]S Sugiura, M Yasue, Y Uchida, et al. (2018) *Biomed Res Int.* doi: 10.1155/2018/4096845.

P-19

Withdrawn

Session 2-1: Clinical Aspects / Specific disease features

P-20

Is Myotonic Dystrophy type 1 (DM1) associated with Mild Cognitive Impairment and Dementia?

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Introduction: Abnormal cognitive aging has been associated with DM1. However, knowledge on cognitive performance over time and the extent of decline is limited. The aim of this study was to explore signs of dementia and the preclinical stage of the condition; mild cognitive impairment (MCI) in adult patients with DM1.

Methods: Hundred and twenty-eight patients underwent a cognitive screening with the Montreal Cognitive Assessment (MoCA). Demographic and clinical information were collected.

Results: Signs of MCI and dementia, as measured by scores < 23 on the MoCA, were uncommon. However, when analyzing DM1 phenotypes, 23.8% of patients with mild/late onset DM1, scored below cut off and this group also performed significantly worse than patients with adult onset (p = .001, g = .91). Total score on the MoCA correlated significantly and negatively with age at examination (p < .05). Age, however, only accounted for a minor part of the variance in test scores (R^2 = .04). Disease duration, sex, muscle function, ratings on anxiety and depression, daytime sleepiness, and fatigue were unrelated to MoCA scores.

Conclusions: Signs of MCI and dementia were uncommon in DM1, except from the performance of patients with late onset of the disease, where approximately 1 in 4 scored below cut-off. Therefore, assumptions on abnormal cognitive aging processes, subsequently leading to preclinical and clinical stages of dementia, finds weak support in this sample. However, the performance of patients with late onset DM1 indicates that this subgroup should be explored further, longitudinally and in larger samples.

P-21

Poor visuoconstruction in DM1 through Rey Complex Figure: underlying cognitive processes

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Introduction: DM1 patients perform significantly worse on most cognitive domains compared to healthy controls (HC). Specifically the visuoconstructive task Rey-Osterrieth Complex Figure Test (RCFT) has been associated with consistent impairment. Nevertheless, the classical RCFT correction system might not capture why patients underperform. In this study, the Boston Qualitative Scoring System (BQSS) was used, to depict the cognitive processes underlying poor performance.

Methods: 76 DM1 patients with juvenile, adult, and late-onset, and 68 HC, underwent a comprehensive neuropsychological assessment and RCFT was corrected by both the classical and the BQSS systems. ANCOVA for groups' differences and Spearman's correlation were conducted.

Results: Patients had significantly poorer scores than HC on the RCFT, depicted by both correction systems. With the BQSS, patients performed worse in the global scores of Copy Presence Accuracy and Organization, and specifically in the ability to perceive the holistic view of the picture (configural accuracy), executive functions (planning, perseverations) and drawing ability (neatness). Global scores showed significant correlations with several clinical and cognitive domains.

Conclusions: Both the visuoconstructive and executive dysfunction might explain the poor performance of DM1 patients in the RCFT. Although BQSS offers a process based approach to analyze visuoconstruction, both correction systems are sensible to DM1 cognitive and clinical manifestations.

P-22

Assessment of energy expenditure using doubly labeled water and reported dietary intake in patients with myotonic dystrophy type 1: A preliminary study

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Introduction: The present study aimed to determine the total energy expenditure (TEE) and dietary intake (DI) of patients with myotonic dystrophy type 1 (DM1).

Methods: The participants in the present study were eight patients with DM1 (male: female, 4: 4; mean age, 49±6 years; mean body mass index, 20.8±4.0 kg/m^2). TEE under free-living conditions was measured using the doubly labeled water method. DI was evaluated based on the clinical chart during the measurement period.

Results: The mean energy expenditure, 970±160.9 kcal/day, was superior to the mean DI, 1350.1±252.4 kcal/day, while only one patient lacked DI. There were significant correlations between energy expenditure and fat-free mass (R=0.932, p=0.001), between the amount of body fat and TEE (R=－0.883, p=0.004).

Conclusions: In conclusion, energy expenditure increased with fat-free mass or muscle mass, and TEE decreased with increasing the amount of body fat in patients with DM1. There is the potential for an increase in TEE with the progressive replacement of muscle tissue by fat tissue.

P-23

Differential diagnosis of myotonic dystrophy type 2

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Introduction: Myotonic dystrophy type 2 (DM2) is a rare, multisystemic, autosomal dominant disease with highly variable clinical presentation. DM2 is considered to be highly under-diagnosed. The aim of this study was to determine which symptoms, signs and diagnostic findings in patients referred to neurological outpatient units are the most indicative to arouse suspicion of DM2. We also analyzed differential diagnoses in patients genetically negative for DM2.

Methods: The study included 291 patients with a clinical suspicion of DM2: 69 were genetically confirmed to have DM2 and 222 patients were DM2 negative. Relevant history, neurological, and paraclinical data were obtained from the electronic medical records.

Results: Following parameters appeared as significant predictors of DM2 diagnosis: cataracts (beta=0.410, p<0.001), myotonia on needle EMG (beta=0.298, p<0.001), hand tremor (beta=0.211, p=0.001), positive family history (beta=0.171, p=0.012), and calf hypertrophy (beta=0.120, p=0.043). In the final score, presence of these symptoms was associated with following values: cataracts 3.4, myotonia 2.5, tremor 1.7, family history 1.4, and calf hypertrophy 1.0. Cut-off value of 4.6 points had sensitivity of 81% and specificity of 95% in early diagnosis of DM2. The most common diagnoses were other hereditary myopathies (12% of patients in DM2 negative group) and lumbosacral radiculopathy/plexopathy (5% of patients).

Conclusions: We made an easy-to-administer score for early diagnosis of DM2.

P-24

Canceled

P-25

Withdrawn

P-26

TREAT-NMD Myotonic Dystrophy (DM) Global Registry Network: An Update in 2022

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[1]On behalf of TREAT-NMD Myotonic Dystrophy Subgroup & TREAT-NMD Global Registry Network

Introduction: TREAT-NMD is an international collaboration that aims to accelerate the development of new treatments for neuromuscular diseases. The TREAT-NMD DM Global Registry Network/Subgroup was established in 2017 specifically to develop collaborations amongst member DM registries. Registries collect an agreed minimum dataset with some collecting additional data too. We present here an update of clinical data from the network.

Methods: An email survey was sent to the 22 member DM registries, requesting data on demographics, and respiratory and cardiac measures in DM1 patients.

Results: Responses were received from 13 / 22 registries. Registry enrolments ranged from 13 patients to 1,459, with a total of 6,472 patients. Among nationally recruiting registries, average cases per 100,000 was 2.53.

Non-ambulant patients represented 8.8% of registry patients on average and a further 19.5% required a walking aid. Cardiac conduction defects were reported in 33.8% of patients on average but only 7.9% (range of 3.1% - 16.0%) were fitted with a pacemaker or implantable defibrillator. Daytime sleepiness was present on average in 73.2% of patients, with 13.8% having FVC<50% expected, 13.8% using non-invasive ventilation and 1.3% using invasive ventilation. On average 3.4% of patients used a feeding tube. Patients' data are updated annually for 9 of the 13 registries, and as required for enquiries for the remainder.

Conclusions: This survey reveals the considerable burden of disease related to DM. It also reveals some inter-country differences which is especially useful for discussing best practice. Registry data should be updated regularly, ensuring that it is accurate for such analyses.

P-27

Safety and immunogenicity of mRNA COVID-19 vaccine in patients with muscular dystrophy

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Introduction: Patients with muscular dystrophy (MD) are at an increased risk of coronavirus disease 2019 (COVID-19). Especially, those with myotonic dystrophy type 1 (DM1) are supposed to have high risk because they are often accompanied by major risk factors for severe COVID-19, obesity and diabetes mellites, in addition to cardiopulmonary dysfunction. Vaccination is recommended; however, little is known about its safety and immunogenicity in these patients. Moreover, disease specific predictor like complications or type of MD are unknown.

Methods: We recruited 171 patients with MD including 72 DM1 patients receiving two doses of COVID-19 vaccine. Blood samples were obtained before the first dose and 28–30 days after the second dose from 53 patients, and antibody titers were measured.

Results: Overall, 104 (60.8%) and 115 (67.6%) patients experienced side effects after the first and second doses, respectively. The reactions were generally mild and self-limited. The geometric mean titer of the 53 patients was 239 (95%CI: 159.3-358.7). In multiple linear regression analysis, patients with myotonic dystrophy type 1 (DM1) showed a relatively lower immune response (RR=0.42, 95%CI: 0.21-0.85).

Conclusions: COVID-19 vaccination is safe and immunogenic in patients with MD. Patients with DM1 may have a low immune response.

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Fat accumulation in liver complicated with myotonic dystrophy type 1 is related to insulin resistance rather than muscle mass or CTG repeated elongation.

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Introduction: Recent studies reported dyslipidemia and non-alcoholic fatty liver disease (NAFLD) were not uncommon in myotonic dystrophy type 1 (DM1). The aim of this research was to investigate clinical findings related to NAFLD in DM1.

Methods: Seventy-one patients with DM1 participated. The age of patients was 44 ± 3 years, the number of CTG repeats (CTGn) 825 ± 21 (median ± SE). For fasting blood test, blood sugar (FBS), serum insulin (FIRI), HbA1c, AST, ALT, gamma-GTP, triglyceride (TG), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL) were examined. Liver spleen ratio (LSR) and visceral fat area (Vfat) were measured by CT examination. Body fat percentage (BFP) and skeletal Muscle mass index (SMI) were calculated using dual energy X-ray absorptiometry method. Body mass index (BMI) and homeostasis model assessment insulin resistance (HOMA-R) were also computed. Linear regression analyses and Mann-Whitney U test between groups with or without NAFLD.

Results: Thirty-five patients (49%) were diagnosed as NAFLD based on LSR. LSR significantly correlated to BFP, Vfat, BMI, FBS, FIRI, HbA1c, HOMA-R, TG, AST, and ALT. There was no significant correlation between LSR and SMI, LDL, HDL, CTGn, or age. AST or ALT significantly correlated to FIRI or HOMA-R. Gamma-GTP also correlated to Vfat. There was no significant difference for SMI, CTGn, and age between NAFLD group and non-NAFLD group, even though other parameters showed significant difference between two groups.

Conclusions: NAFLD in DM1 could be related with more glycolipid metabolic impairment represented by insulin resistance rather than muscle volume or CTG repeated elongation.

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Identification of individuals with highly interrupted DM1 alleles by the analysis of co-segregating single nucleotide polymorphisms near the CTG expansion

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Introduction: If the DM1 repeat expansion contains variant repeats, the triplet-primed PCR (TP-PCR) diagnostic test may fail. Often, an interrupted expansion is detectable by small-pool PCR (SP-PCR), or TP-PCR, and Southern blot hybridsation. Sometimes, it still cannot be PCR amplified, but its presence may be inferred by the genotypes of nearby single nucleotide polymorphisms (SNPs).

Methods: SNP alleles near the CTG repeats in a 250-patient DM1 cohort were genotyped using a sequencing panel. Sanger sequencing assays for several SNPs frequently heterozygous in DM1 patients were then developed using samples of known repeat genotype. Blood DNAs were also analysed by SP-PCR, TP-PCR and Southern blot hybridisation.

Results: In the diagnostic TP-PCR test for a patient with atypical DM1 symptoms, only a single, 13-repeat non-disease associated allele was detected. Weak signals for a possible CTG expansion were obtained by SP-PCR and TP-PCR, suggesting a hard-to-amplify interrupted repeat expansion was also present. Three SNP PCR fragments, each expected to be heterozygous in DM1 patients with a 13-repeat allele, were heterozygous, suggesting the patient indeed had a DM1 repeat expansion. SP-PCR using a DNA sample from the patient's sister detected an interrupted expansion, further supporting the existence of an interrupted repeat expansion in the patient[1].

Conclusions: SNP genotypes can be used to infer the presence of highly interrupted mutant DM1 alleles.

[1]Cumming, SA, Oliwa, A, Stevens, G. *et al*., 2021. Neuromuscul. Disord. 31:232-238.

P-30

Clinical Symptoms in an 8-Year Old with Myotonic Dystrophy Type 2

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Introduction: Myotonic dystrophy type 2 (DM2) is a multifactorial disease characterized by progressive muscle weakness, muscle atrophy, myotonia, gastrointestinal dysfunction, and cognitive impairment. Unlike myotonic dystrophy type 1, DM2 is believed to be an almost exclusively adult onset dystrophy.

Methods: Clinical, electrodiagnostic, and genetic evaluations were performed in a large kindred harboring dual diagnoses of DM2 and SCN4A-related non-dystrophic myotonia (NDM).

Results: Segregation of both DM2 and NDM was confirmed in a large family. An 8-year-old male family member presented with a history of speech delay, significant core weakness, ADHD, reflux disease, hypercholesterolemia, and proximal percussion myotonia. Genetic testing was positive for a heterozygous expansion (>75 CCTG repeats) in the CNBP gene, and negative for the familial SCN4A mutation (p.R1463H).

Conclusions: Here we report a genetically confirmed DM2 patient with an early onset of symptoms and negative genetic testing for SCN4A NDM. While the presence of other superimposed disease etiologies cannot be entirely excluded, this patient represents a potential case of childhood onset DM2. Additional genetic and electrodiagnostic testing is pending for this patient and his family.

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Nocturnal transcutaneous carbon dioxide measurement in patients with myotonic dystrophy not receiving respiratory therapy

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Introduction: Respiratory disorders are complicated in patients with myotonic dystrophy. In particular, respiratory distress during sleep causes sudden death. Transcutaneous carbon dioxide monitoring is an available technique. We assessed the respiratory distress during sleep in patients with myotonic dystrophy by this method.

Methods: We enrolled outpatients with myotonic dystrophy not receiving respiratory therapy. They were hospitalized and examined for lung function, daytime arterial blood gas, and saturation of percutaneous oxygen and transcutaneous carbon dioxide pressure during sleep.

Results: Most patients exhibited abnormal transcutaneous carbon dioxide results. Patients who do not decrease saturation of percutaneous oxygen but have increased transcutaneous carbon dioxide existed. In almost all patients, nocturnal transcutaneous carbon dioxide was higher than daytime carbon dioxide of arterial blood gas.

Conclusions: Nocturnal carbon dioxide measurement can readily evaluate detailed respiratory status during sleep. Patients without elevated carbon dioxide at night may be treated with oxygen therapy. Criteria for respiratory therapy indications for transcutaneous carbon dioxide need to be established.

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Cluster Analysis of Phenotypic Characteristics in patients with Myotonic dystrophy type 2

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Introduction: DM2 is a rare, multisystemic disorder affecting all organ systems, mainly heart and skeletal muscles, but the absence of extreme clinical forms makes patient stratification and symptom management challenging. The aim of this study is to explore the potential phenotypic subgroups of DM2 patients using machine learning.

Methods: Our cohort included 124 patients with genetically confirmed DM2 from Serbian registry of myotonic dystrophies. We selected 27 parameters regarding muscle symptoms and other relevant multisystemic features, both clinical and laboratory. We used unsupervised hierarchical clustering, and determined the optimal number of clusters by dendrogram and elbow method. Clustering performance was estimated using standard validity indices.

Results: Cluster analysis showed two major clusters based on phenotypic characteristics, with 77 and 47 patients each. Notably, patients in Cluster B had more severe clinical presentation than patients in much milder cluster A. Clusters didn't differ in age at onset ($p=0.24$), but were different in disease duration ($p=3*10^{-6}$) and age at sampling ($p=2*10^{-4}$), with milder phenotype being observed in patients with shorter disease duration.

Conclusions: Cluster analysis of DM2 patients based only on phenotypic features showed division into two groups with milder and more severe clinical presentation. Since patients had the same age at onset, our results imply that the biggest determinant of the disease progression remains disease duration, even when it is not directly included in clustering analysis.

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Congenital and Childhood Myotonic Dystrophy Type 1 in the UK

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Introduction: Myotonic Dystrophy type 1 (DM1) is the most common adult muscular dystrophy with recent studies suggesting prevalence of 1:2100. DM1 is a progressive, autosomal dominant disorder with no disease-modifying treatment. Manifestations include distal muscle weakness, myotonia, arrythmias and other multisystemic complications. Congenital DM1 is characterised by severe lifethreatening issues at birth. Childhood DM1 shows onset of symptoms between 1 month and 18 years, often presenting with intellectual and learning disabilities. Advances in the understanding of the underlying mechanisms for the molecular pathogenesis of DM1 has enabled development of potential new targeted treatments for congenital and childhood onset DM1. However, information is limited about the prevalence and epidemiology of paediatric patients in the UK.

Methods: CureDM is a UK Charity, focused on raising awareness for congenital and childhood onset DM1 whilst supporting patients and families. An anonymised online questionnaire was sent to DM1 patients in the UK through CureDM and the UK DM patient registry. Responses were received from the patients themselves or by their carers.

Results: 101 congenital and 49 childhood onset DM1 patients/carers completed the questionnaire. The majority of responders documented demographics such as age, sex, closest town, age at onset and diagnosis, CTG repeats, type of inheritance, current symptoms and professionals involved with care. Notable differences in specific symptoms were recorded between congenital and childhood onset DM1, including walking abilities and specialist care received.

Conclusion: Results from this survey can assist in planning, design and recruitment of clinical trials involving patients with congenital and childhood onset DM1 in the UK.

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Myotonic dystrophy type 1 multi-organ involvement: combined or independent phenotypic features?

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Introduction: Extreme phenotypic variability is a hallmark of myotonic dystrophy type 1 (DM1). Both multi-systemic involvement together with large range of age of onset are two key factors underlying disease variability. Somatic mosaicism of the unstable CTGn expansion, which varies between the tissues, has been identified to contribute to organ involvement differences. We investigated whether DM1 organ manifestations are associated with each other or are independent features.

Methods: We assessed tissue/organ involvement by quantifying clinical data from the DM-Scope registry for each affected organs including skeletal muscle, CNS, respiratory, cardiac, digestive, visual and endocrine systems. First, the prevalence of respective diseases manifestations was measured. Then we assessed their correlation with CTG expansion size and each organ feature was compared with other ones to address potential cross-correlation.

Results: DM1 organ clinical findings displayed variable degree of association with each other. The results suggest that some organ manifestations are more closely associated with other tissues. The detailed relations between DM1 features will be presented.

Conclusions: The high variability of clinical features in DM1 makes difficult to form homogenous cohorts for clinical trials and to inform stakeholders. Our results suggest that in DM1 phenotypic features are not randomly distributed. Some organ manifestations correlates with other ones while other disease features seems more independent. The results can contribute to address the complex clinical and genetic characteristics of DM1.

Session 2-1: Clinical Aspects / Specific disease features

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Are research publications aligned with myotonic dystrophy type 1 individuals' expectations?

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Introduction: Myotonic dystrophy type 1 (DM1) is an autosomal-dominant inherited disorder with complex, multi-systemic, and progressively worsening symptoms. Scientific knowledge on DM1 clinical issues and on disease progression has significantly improved over the past years. In parallel, patients' self-reported data - such as patient foundation-driven initiatives using questionnaires - have been helping to address patients' needs and expectations. The aim of our work was to explore the relevance of current DM1 research areas, by comparing research areas of interest with patients' expectations.

Methods: We reviewed the DM1 literature to identify the most frequently studied clinical domains in DM1. Results were compared with DM1 adult individuals' self-reported data collected by a nationwide AFM-Téléthon association survey (n=1013).

Results: Our results showed that researchers mainly focused on skeletal muscle, cardiac- and respiratory defects. Interestingly, some of the most frequent and most embarrassing symptoms for patients such as digestive tract dysfunction, facial involvement, vision, and pain were under-investigated in the literature.

Conclusions: This study shows that scientific publications do not fully overlap with DM1 patients' needs. Researchers mainly focus on disabling and life-threatening features such as muscular-, cardiac- and respiratory defects while some high impacting symptoms on patients' daily life such as pain, digestive tract disorders and facial manifestations are under investigated. Our results may help to guide and improve research in better accordance with DM1 patients' expectations.

Session 2-2: Clinical Aspects / Biomarkers, outcome measures, trial design, etc

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Multicenter study on the impact of non-invasive ventilation in myotonic dystrophy

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Introduction: The purpose of this study was to elucidate the impact of non-invasive ventilation (NIV) on QOL of patients with Type 1 myotonic dystrophy (DM1).

Methods: This is an observational prospective cohort study of patients with DM1 who newly met the clinical indication for NIV. The decision to initiate NIV was based on informed consent at clinical setting. During follow-up period, the cohort was evaluated four times (at 6months, 1 year, 2 years, and 3 years) using myotonic dystrophy health index (MDHI), SF-36, the *respicheck* questionnaire and the Epworth Sleepiness scale (ESS). In this report, first visit data (6-month) is presented.

Results: The study included 26 patients (16 men, 10 women; mean age 48.3±9.7 years) from 10 institutes in Japan. Twenty patients underwent NIV (NIV group) and six declined (non- NIV group) at the enrolment. In NIV group, physical component score (PCS) of SF-36 significantly increased ($p < 0.05$) and mental component score (MCS) and role/social component score (RCS) remained unchanged at 6 months after the initiation of NIV. The greater the improvement in Pco_2, the greater was the rise in PCS. In non-NIV group, all three component scores were unchanged. Both ESS and *respicheck* score tended to improve in the NIV group, and remained unchanged in the non-NIV group.

Conclusions: The results of the present study suggest that NIV might lead to improvement in QOL of patients with DM1 with respiratory insufficiency.

Session 2-2: Clinical Aspects / Biomarkers, outcome measures, trial design, etc

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Technology-assisted rehabilitation for upper limb function in Myotonic Dystrophy type 1

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Introduction: Myotonic Dystrophy type 1 (DM1) is a genetic multisystem disease that causes muscle weakness and myotonia. As a result, upper limb function might be impaired. We know from research on lower limb function in DM1, and other muscular dystrophies, that there are possibilities to improve function also in these deteriorating diseases. The aim of the present study is to study effects of technology-assisted rehabilitation and exercise for upper limb function in DM1.

Methods: A single subject experimental design study including 6-10 participants with DM1. The participants will receive intensive, but personally adapted senso- and robot assisted rehabilitation for arm- and hand function, during stay at an inpatient rehabilitation center for 3 weeks. Tyromotion Amadeo and Armeo Senso, used in this study, have previously been used in rehabilitation research for other neurological conditions. Video games have been used as a motivating factor in the training. The participants will be evaluated weekly using video consultations and patient reported outcome measures, active range of motion and a fine motor skill dexterity test (Nine Hole Peg Test).

Results: Data collection is ongoing. There has been a lot of interest in participating in the study. The use of video consultation in testing is going well, and according to preliminary reports participants seems enjoy the training. Further results will be available at the time of the congress.

Conclusions: Use of rehabilitation technology seems to be feasible in training of upper limb function in people with Myotonic Dystrophy type 1.

Session 2-2: Clinical Aspects /
Biomarkers, outcome measures, trial design, etc

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The current status of medical care for myotonic dystrophy type 1 in Japan: A comprehensive cross-sectional study using the national registry of Japan.

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Introduction: Myotonic dystrophy (DM) is a multisystemic disease with various complications. To clarify the medical care status across multiple organs should be essential to establish the standard of care. Thus, we conducted a cross-sectional analysis using the National Registry data (Remudy) [1].

Methods: Genetically confirmed DM1 patients registered with Remudy were analyzed. Patients younger than 20 y.o. or those with congenital type were excluded.

Results: 809 DM1 patients with a mean age of 44.2 y.o. were enrolled. Ventilators were used in 15.2%. ECG criteria for risk of cardiac events (PR >240 ms, QRS >120 ms) were met in 31.7%, but device implantation was performed in 2.8%. Medication for heart failure was prescribed for 9.6%. Cancer was reported in 3.7%. Patients with impaired glucose tolerance were 21.2%. Among them, 42.9% were treated with oral medication, in which DDP-4 inhibitors were the most common. For myotonia, mexiletine was prescribed in 1.9%, and only 1 % received medication for daytime sleepiness.

Conclusions: This comprehensive cross-sectional analysis revealed the current therapeutic status of DM1 patients in Japan, which should be essential to improve the standard of care.

[1]Sugimoto et al. (2022) *J Neurol Sci.* 432:120080.

Session 2-2: Clinical Aspects / Biomarkers, outcome measures, trial design, etc

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MRI volumetry and the P40 amplitude following posterior tibial nerve stimulation in patients with DM1

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Introduction: In patients with myotonic dystrophy type 1 (DM1), some studies examined MRI volumetry or somatosensory evoked potentials (SEPs). Those reported atrophy in frontal lobes and latency delays of SEP, but there are no reports examining the relationships between SEP and MRI volumetry, as far as the authors are aware.

Methods: Thirteen DM1 patients and 11 normal controls (age and body height matched) participated in SEP recording. The brain MRI was obtained from each patient. The P40 amplitudes following the right tibial nerve stimulation were measured and examined if there are significant correlations against age and the total volume of parcellated regions of interest analyzed with BrainSuites21a. This project was reviewed and approved by the institutional review board at the 1st author's institution and all the participants signed the informed consent. Significance level was set $p < 0.05$.

Results: Significant correlations were obtained between the age and the total volume at right pars opercularis inferior ($r = -0.56$), right pars triangularis posterior ($r = -0.60$), and left pars orbitalis ($r = -0.69$). The z values of the P40 amplitudes were significantly correlated with the total volume at inferior left precentral gyrus ($r = -0.67$), left inferior postcentral gyrus ($r = -0.71$), left thalamus ($r = -0.73$), and left caudate ($r = -0.58$). The total volume of the left precentral inferior gyrus and the left postcentral inferior gyrus correlated ($r = 0.63$).

Conclusions: The negative correlations above suggest that the tibial SEP may reflect impairments of inhibition in sensory (and potentially motor) cortex in DM1.

Session 2-2: Clinical Aspects /
Biomarkers, outcome measures, trial design, etc

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Assessment of intelligence in Myotonic dystrophy type 1: a WAIS-IV short-form proposal

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Introduction: Cognitive deficits and low intellectual functioning have been found in Myotonic Dystrophy type 1 (DM1). Thus, estimation of IQ is relevant as part of a clinical assessment, but this measurement is time-consuming, causes fatigue and limits the possibilities to assess more specific cognitive domains. The aim of this study was to develop a DM1-specific and valid short-form of the most widely used method to measure IQ; the Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV).

Methods: Thirty non-congenital Spanish DM1 patients were assessed with the WAIS-IV. Data were analyzed following two independent strategies: A) multiple linear regression with the aim of maintaining the scale's factorial structure; and B) correlational analyses between scores on all WAIS-IV subtests and Full-Scale IQ (FSIQ). Validity of the resulting short-forms was also analyzed in Swedish and French samples.

Results: From the three short-forms that resulted from the analysis, arguments in favor of the short-form containing Vocabulary, Block Design, Digit Span and Visual Puzzles WAIS-IV subtests, are discussed. This short-form showed a strong and significant correlation with the FSIQ and was considered psychometrically acceptable.

Conclusions: This validated brief IQ estimation, will avoid long assessment procedures in a population characterized by high fatigability, and provide time for further relevant assessment.

Session 2-2: Clinical Aspects / Biomarkers, outcome measures, trial design, etc

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Extracellular RNA splice events in cerebrospinal fluid as candidate biomarkers of myotonic dystrophy

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Introduction: Alternative splicing is mis-regulated in DM1 central nervous system (CNS) tissue. Extracellular RNA (exRNA) in cerebrospinal fluid (CSF) provides a source of CNS-derived splice events that may serve as convenient molecular indicators of DM1 disease activity.

Methods: We examined fresh CSF samples (N = 14) from the MGH CSF biobank. To separate EVs from cells contained in CSF, we used low speed centrifugation followed by filtration of the supernatant. EV size and concentration were measured using microscopy and particle tracking analysis software (NanoSight). After ultracentrifugation (100,000g) to concentrate EVs, we extracted exRNA from the EV pellet, produced cDNA by reverse transcription, and quantified gene expression (transcript copies/µl cDNA) and splice events (% exon inclusion) by droplet digital PCR (ddPCR). Tissue and urine EV samples served as controls.

Results: Mean particle diameter is about 175 nm in CSF vs. 220 nm in urine. In CSF cells, total RNA content is 10 - 100-fold higher and splicing patterns for transcripts *MBNL2*, *CSNK1D*, *MAP3K4*, and *GOLGA4* significantly different as compared to CSF exRNA. Normalized expression of both *DMPK* and *CNBP* was 50 - 60% higher in CSF exRNA than in CSF cells. The quantity of *DMPK* and *CNBP* transcripts in exRNA correlates with the volume of CSF processed.

Conclusions: Quantification of CNS-derived alternative splice products in CSF exRNA is feasible and we expect will show a differential pattern in DM as compared to non-DM. Removal of leukocytes and erythrocytes normally found in CSF will enhance accurate quantification of CNS-derived splice events.

Funding: Elaine and Richard Slye Fund; Myotonic Dystrophy Foundation.

Session 2-2: Clinical Aspects /
Biomarkers, outcome measures, trial design, etc

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Electrical impedance myography predicts muscle function in DM1 patients

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Introduction: Electrical impedance myography (EIM) is a non-invasive, painless technique that uses high-frequency, low-intensity electric current to estimate muscle fiber composition and architecture through the skin. EIM is a potentially valuable tool for monitoring muscle disease status, progression, and response to therapy. The technique requires minimal training and is independent of patient effort. The usefulness of EIM for evaluating disease status and burden in DM1 patients is unknown.

Methods: We measured electrical impedance at 41 frequencies from 1 kHz to 10 MHz in 7 muscle groups of DM1 subjects (N=16) using the portable mScan system (Myolex). From the resistance and reactance, we calculated the phase angle (θ, degrees), the time shift of electric current as it passes through muscle. Subjects also underwent quantitative muscle function testing. A subset of DM1 subjects received EIM at 6 - 8 month intervals. Unaffected (UA) individuals (N=6) served as controls.

Results: Mean phase values at 100 kHz are significantly lower in deltoid, elbow extensor, elbow flexor, gastrocnemius, and tibialis anterior (TA) muscles of DM1 vs. UA subjects. Phase correlates with strength in all muscles tested (r values 0.62 - 0.75; *P* 0.002 - < 0.0001). TA and gastrocnemius phase correlate with 6-minute walk test (r values 0.61 and 0.67; *P* 0.005 and 0.002). First- and second-visit phase values correlate in all muscles tested (r values 0.84 - 0.99).

Conclusions: Phase values predict muscle function in DM1 and are reproducible over time. EIM is a candidate technique to monitor disease status and burden in DM1 muscle tissue.

Funding: Elaine and Richard Slye Fund; U.S. Department of Defense; U.S. National Institutes of Health.

Session 2-2: Clinical Aspects / Biomarkers, outcome measures, trial design, etc

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Muscle-specific miRNAs as potential monitoring biomarkers of muscle wasting progression in DM1

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Introduction: Muscle wasting progression in DM1 is highly variable, exposing a need to develop reliable non-invasive biomarkers for its characterization. We previously showed that the levels of four myomiRs, miR-1, miR-133a, miR-133b and miR-206, correlate with muscle wasting progression in DM1 suggesting that they can serve as biomarkers in clinical practice. Having associated miRNA levels in DM1 patients who are degenerating, the aim of this study was to associate the four myomiRs with muscle wasting during the disease course.

Methods: We analyzed the levels of these myomiRs in the serum of DM1 patients at different time points of the disease course. We used serum samples from DM1 patients participated in 'PhenoDM1' study (Newcastle, UCLH, UK). The participants provided serum samples yearly for three years. DM1 patients were categorized as progressive or non-progressive (stable) at the time of blood collection based on their outcome measures. Total RNA, including miRNA, was extracted from the serum samples followed by Real-Time PCR analysis specific for the four myomiRs.

Results: Our results show that the four myomiR levels remain stable or decrease in stable DM1 patients, whereas, the levels of the four myomiRs follow an increasing trend in progressive DM1 patients. Moreover, we show that the levels of the four myomiRs do not correlate to the age, gender or CTG repeats size.

Conclusions: Based on our results we suggest that the levels of the four myomiRs reflect the progression status of the patients and these molecules could be used as monitoring biomarkers in DM1.

Funding: AFM-Telethon, A.G. Leventis Foundation

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Muscle MRI in Myotonic Dystrophy type 1: a long-term follow-up study

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Introduction: muscle MRI is a useful biomarker of disease severity and activity in DM1. We recently reported data from 134 DM1 patients showing that fat replacement at MRI correlates with clinical impairment, that Muscle MRI can detect muscle involvement also in the milder spectrum of disease (MIRS 1-2) and that STIR positivity and muscle atrophy could represent additional mechanisms for muscle wasting and weakness in DM1[1].
Herein, we report on long-term follow-up (FU) data of muscle MRI in 30 patients affected by DM1.

Methods: we prospectively followed patients of the firstly reported cohort repeating a second muscle MRI study at variable distance from the first one. We analyzed 32 couple of muscle of lower body (LB) and 16 couple of muscle of upper body (UB) by T1 and STIR sequences. T1-, STIR-, and atrophy-scores and their variations between MRIs were considered. Correlations between MRI data and clinical, genetic and other than time-dependent factors were analyzed.

Results: the median FU was 3 years (range 21-53 months). The average T1-score progression was +3.1% in LB (range 0-10.6%) and +0.8% in UB (range 0-4.2%). Patients with higher T1-score variation at FU showed an increase of MIRS rating at FU. 25% of patients did not show any progression in T1-score at FU regardless of disease severity and T1-score at baseline and time lapse between MRIs. Some patients with normal MRI study at baseline (T1 negative/STIR negative) showed STIR positivity in some muscles at FU (3.1-4.7%) and a minimal T1-score progression at FU (+0.3-0.6%) in certain of the STIR positive muscles. Muscle atrophy showed a progression regardless T1-score and STIR positivity or their progression at FU.

Conclusions: Muscle MRI is a sensitive biomarker to assess disease activity and progression in DM1.

[1]Garibaldi et al. EJN 2021

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Gait analysis by IMU sensor in Myotonic Dystrophy type 1

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Introduction: Gait impairment is a common feature of Myotonic Dystrophy type 1 (DM1) leading to a 10x higher risk of falls than in healthy subjects (HS)[1]. Gait analysis has been used to elucidate gait characteristics of DM1 showing an alteration of various spatial-temporal gait parameters[2]. IMU-based gait analysis can be a cost-effective and easy-to-use alternative method[3].
The aim of this study was to compare gait parameters registered through an IMU sensor in patients with DM1 versus HS and to investigate the association between gait parameters and clinical, genetic and instrumental features in patients with DM1.

Methods: 19 DM1 patients and 19 age-, sex- and height-matched HS were enrolled. All subjects performed a 6MWT wearing a lower back-mounted IMU sensor (BTS G-Walk). For all DM1 patients, MIRS score, average number of CTG expansion, disease duration, hand grip test, 9-hole peg test and 6MWT distance were collected.

Results: We found a significant reduction in step length and gait symmetry in DM1 patients, associated to reduction of gait speed and 6MWT distance. Patients with only distal weakness (MIRS 3) showed no difference with HS except for a significant increase of cadence. In patients with proximal involvement (MIRS 4) all the spatial-temporal parameters showed a marked reduction compared with HS and a significant reduction in pelvis tilt. MIRS score showed a moderate-to-strong negative correlation with all gait parameters while hand grip test showed a strong positive correlation with gait symmetry.

Conclusions: Single-IMU gait analysis helps to elucidate gait features in DM1 and could be useful to identify patients with more severe walking impairment and guide treatment and rehabilitation strategies.

[1]Wiles et al. 2006
[2]Galli et al 2012
[3]Bachason et al 2016

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Fatigue in Japanese patients with myotonic dystrophy type 1 (DM1)

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Introduction: Patients with myotonic dystrophy type 1 (DM1) have physical symptoms and psychological difficulties such as depression and fatigue. Several studies have reported that the impact of fatigue was associated with deleterious effects on their quality of life (QoL). However, fatigue in individuals with DM1 has not been sufficiently studied in Japan. Therefore, this study aimed to measure the fatigue of Japanese patients with DM1 and compare the results to those of previous studies in western countries.

Methods: We measured the levels of fatigue and depression of patients with DM1 (n = 59; Men = 35, women = 24) using the Multidimensional Fatigue Inventory-20 (MFI-20) and the Patient Health Questionnaire-9 (PHQ-9) five years ago (Time 1) and recently (Time 2). General healthy participants (n = 692; Male = 328, Female = 364; mean age = 47.0, SD = 13.7 years) also participated in this study and answered these questionnaires by WEB. Patients with DM1 were recruited from 5 national hospitals in Japan. Their mean age was 47.1 (SD = 10.8) years, and the mean of CGT repeat was 1,113.2 (SD = 1,025.2). Most patients were classified as having juvenile or adult forms of DM1.

Results: The mean MFI-20 scores of patients with DM1 and the healthy group were 64.2 (*SD*=12.0) and 55.7 (*SD*=12.8), respectively, in time 1. The mean of PHQ-9 was 8.0 (*SD*=5.5) in patients with DM1 and 4.7 (*SD*=5.2) in the healthy group. Fatigue in patients with DM1 was higher than that of the healthy control (t=4.9, p<.01). Similarly, depression in individuals with DM1 was higher than that of the healthy group (t=4.7, p<.01). Fatigue was associated with depression both in individuals with DM1 and the healthy group (r=.760, p<.001; r=.617, p<.001).

Conclusions: The levels of fatigue in patients with DM1 was higher than that of the general healthy group. This suggests that there is a burden of fatigue on patients with DM1, especially on their QoL.

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TREAT-NMD Myotonic Dystrophy Global Registry Network: Providing Data in Congenital Myotonic Dystrophy to Support FDA Regulatory Decision Making

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[1]On behalf of TREAT-NMD Myotonic Dystrophy Subgroup & TREAT-NMD Global Registry Network.

[2]AMO Pharma Ltd.

Introduction: TREAT-NMD is an international collaboration to accelerate the development of new treatments for neuromuscular diseases. It operates a Global Registry Network, where member registries collect and share agreed disease specific datasets.

In 2019, AMO Pharma contacted TREAT-NMD to request data to support an application to the FDA for a Rare Paediatric Disease (RPD) Designation. They requested details on the prevalence of congenital myotonic dystrophy (cDM1) (≤18 years old) as there was limited scientific literature in this area.

Methods: Registries from 5 countries (USA, Canada, UK, New Zealand & Australia) were selected. The congenital basis of the disorder was based on self-report from caregivers and patients, verified by clinicians.

Results: The registries contained 270 cDM1 patients, with 148 (54.8%) aged ≤18 years. Percentages of patients aged ≤18 years old by country ranged from 42.1% in Australia to 71.4% in New Zealand.

AMO Pharma used the data to successfully support their application for a RPD Designation from the FDA for tideglusib[1]. This will provide an accelerated FDA review in the future.

Conclusions: Registries can be used in all stages of drug development, including providing data to support regulatory applications where data are scarce. cDM1 is an important subset of DM1 with its own research needs and opportunities; DM1 registries need to collect specific data relevant to these patients to support such activities.

Funding: AMO Pharma.

[1]AMO Pharma. (2020) FDA Grants Rare Pediatric Disease Designation to AMO Pharma for AMO-02 for Treatment of Congenital Myotonic Dystrophy. *PR Newswire*. Accessed 07 March 2022.

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Mitochondrial dysfunction in Myotonic Dystrophy type 1 patients

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Introduction: Myotonic dystrophy type 1 (DM1) is an inherited type of muscular dystrophy, for which no viable treatment exists. Resistance exercise intervention has been investigated as a potential way to improve skeletal muscle (SKM) weakness in DM1 patients. The aim of this study is to investigate the oxidative phosphorylation (OXPHOS) in SKM biopsies from a cohort of DM1 patients (n=10 males), who previously undertook a 12-week resistance exercise training.

Methods: Immunofluorescence labelling was used to investigate Laminin, VDAC1 (mitochondrial mass), Ndufb8 (Complex I - CI - subunit), and COX1 (Complex IV - CIV - subunit). Using a linear model between VDAC1 and Ndufb8 or COX1, the 95% predictive interval for the fibres of the combined controls population was used to classify patient fibres as being normal, deficient/lower level than predicted (LLTP) or higher level than predicted (HLTP).

Results: Before exercise, all patients, except P01, present with CI deficiency, and some show additional CIV deficiency. After exercise, seven patients out of 10 display a significant reduction in Ndufb8 LLTP fibres ($p<0.03$), and the majority of patients significantly reduce COX1 LLTP fibres ($p<0.02$). Patients who do not show ameliorations in either CI or CIV deficiency, display a significant increase in HLTP fibers, especially for COX1 ($p<0.001$). Additionally, some patients show increase of mitochondrial mass ($p<0.0001$).

Conclusions: DM1 patients may present with OXPHOS defects in SKM, which are rescued after 12-week resistance exercise training.

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Histomorphological adaptations in myotonic dystrophy type 1: a 3-year follow-up study

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Introduction: Maximal muscle strength (MMS) loss is a strong indicator of physical limitations in myotonic dystrophy type 1 (DM1) and the progressive MMS decline has been documented.[1] The effect of disease progression on histomorphological adaptations of skeletal muscle remains under documented in DM1. This project aims to document histomorphological characteristics of the vastus lateralis over a 3-year period and correlate it with MMS loss of the knee extensors (KE) muscle group.

Methods: Fifteen individuals with adult (n=12) or late (n=3) phenotypes were recruited to complete evaluations in 2016 and 2019. Both evaluations included muscle biopsies of the vastus lateralis and KE MMS assessed by quantified muscle testing. Muscle biopsies were analyzed for fiber typing and minimal ferret diameter (MFD).

Results: In the adult phenotype, fold change KE MMS significantly correlated with MFD fold change of all fibers (ρ=0.678, p=0.015), MFD fold change of type 1 fibers (ρ=0.678, p=0.015), fold change of fiber size variability coefficient (ρ=-0.608, p=0.036) and fold change of type 1 fiber atrophy factor (ρ=-0.685, p=0.014).

Conclusions: Type 1 fiber MFD, namely type 1 fiber atrophy, seems to have a significant influence on MMS of the KE in the adult phenotype. Fiber size variability also has a negative impact on KE MMS.

[1]Roussel MP, Fiset MM, Gauthier L. et al. *J Neurol* 268, 4221–4237 (2021)

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Blood Transcriptome Profiling Links Immunity to Disease Severity in Myotonic Dystrophy Type 1 (DM1)

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Introduction: The blood transcriptome was examined in relation to disease severity in DM1 patients who participated in the Observational Prolonged Trial In DM1 to Improve QoL- Standards (OPTIMISTIC) study[1].

Methods: RNA sequencing was performed on 30 samples selected by MIRS score and CTG-repeat expansion size. Gene enrichment analysis was performed using Ingenuity Pathway Analysis and Reactome. These changes in mRNA expression and associated biological pathways were also compared with the Dystrophia Myotonica Biomarker Discovery Initiative (DMBDI) [+] microarray dataset in blood (with equivalent MIRS/DMPK repeat length).

Results: Changes in gene expression were compared using a number of complementary pathways, gene ontology and upstream regulator analyses. In both datasets we found four significantly enriched pathways (OX40-, NFAT-, T-cell Exhaustion- and SLE-signalling) associated with MIRS severity. Gene ontology analyses suggest a role for mitochondrial protein import, macrophage priming, and Th2 cell expansion. Upstream regulatory analyses implicate the immunity cytokines; IFN gamma, IL2 and IL4.

Conclusions: Symptom severity in DM1 is linked to transcriptomic alterations in innate and adaptive immunity associated with muscle-wasting. Future studies should explore the role of immunity in DM1 in more detail to assess its relevance to DM1.

[1] Okkersen K (2018) Lancet Neurol 17:671-80.
[2] Kurkiewicz A (2020) PLoS ONE 15:1-19.

Funding: FP7/2007–2013 agreement no. 305697 (OPTIMISTIC) and E-RARE agreement no.18-038 (ReCognitION).

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The DM-Scope registry: an innovative framework to promote myotonic dystrophy translational research

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Introduction: Myotonic dystrophy (DM) is the most prevalent form of muscular dystrophy in adulthood and it is a multi-systemic, inherited disorder which affects, besides the skeletal muscle, several other tissues and/or organs, including the respiratory, cardiac, digestive, visual and endocrine systems. The high variability of clinical features in DM makes difficult to determine the proper prognosis for patients and to form homogenous cohorts for clinical trials.

Methods: The French DM-Scope registry was developed in France in 2008 with the financial support of AFM-Téléthon to overcome some of these limitations. The iDM-Scope registry consortium in collaboration with Canadian teams was created in 2016 to address the complex clinical and genetic characteristics of DM and to facilitate translational research. Since 2020, the Registry contributes to setting up a European project for rare neuromuscular diseases, the EURO-NMD Registry Hub.

Results: The DM-Scope registry has a nationwide coverage, composed of 55 neuromuscular centres, encompassing the whole disease clinical and genetic spectrum. This platform gathers 3543 DM patients both children (n = 366) and adults (n = 3177).

Conclusions: In the context of emerging therapies, such integrated platform contributes to the standardization of international DM research and for the design of multicentre clinical trials. With data collected from neuromuscular-expert physicians and/or patients, DM-Scope is a competitive tool, which needs to be further developed/improved as an accelerator to optimize DM patients care and translational research.

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Initial Psychometric Properties of the Congenital Myotonic Dystrophy Type 1 Rating Scale (CDM1-RS)

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Introduction: The CDM1-RS is a novel rating scale for Congenital Myotonic Dystrophy Type 1 (CDM1), a rare, genetic form of muscular dystrophy. It has 11 items, each rated on a severity scale from 0 to 4. The psychometric properties of this scale (e.g. most commonly endorsed items and the association of baseline scores with other outcome measures) have yet to be fully defined.

Methods: The clinician-completed CDM1-RS is the primary outcome measure in an ongoing phase 2/3 clinical trial in CDM1 (NCT03692312). The study is enrolling youth aged 6 to 16 y.o. at 12 sites in the U.S., Canada, Australia and New Zealand. It compares AMO-02/tideglusib versus placebo in a randomized, double-blinded treatment period lasting 5 months. Clinic visits and telehealth (video) evaluations are conducted regularly.

Results: Baseline data from n = 34 enrolees reveals that the most commonly endorsed items are communication difficulties (mean baseline score = 2.4, on the 0 to 4 Likert scale), difficulty thinking (2.3), and problems with hands or arms (2.07). The least endorsed items are signs of pain (0.31), breathing difficulties (0.82), and choking or swallowing issues (0.93). In-person CDM1-RS scores correlate closely with telehealth-administered scores (Pearson R = 0.93, $p < 0.0001$), and with Clinical Global Impression–Severity scores. Also, Clinician CDM1-RS scores correlate with caregiver-completed rating scale scores, and CDM1-RS subscale scores for cognition and ambulation correlate with functional tests (e.g. with the 10-meter Walk/Run test ($p < 0.001$)).

Conclusions: The CDM1-RS is a relatively low-burden rating scale with initial evidence of sound psychometric properties. It can be administered in the clinic or via telehealth. It correlates well with other relevant outcome measures, including those completed by caregivers, as well as with functional/performance-based assessments.

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Sustainable recovery of MBNL activity in autoregulatory feedback loop

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Muscleblind-like (MBNL) are RNA binding proteins essential for developmental regulation of various processes including alternative splicing. The activity of MBNLs is misregulated among others in myotonic dystrophy type 1 (DM1). DM1 is a genetic, neuro-muscular disorder caused by uncontrolled expansion of CTG repeats. Mutant RNAs containing hundreds or thousands repeats efficiently sequester MBNL proteins. As a consequence, global alternative splicing abnormalities are induced. Importantly, the size of expansion differ significantly not only between patients but also different parts of the same muscle as a consequence of somatic expansion. One of potential therapeutic strategy in DM is overexpression of MBNLs. However, the gene therapy tools might induce excessive activity of MBNLs, what in turn might change metabolism of many RNAs. To overcome these limitations, we designed autoregulated MBNL1 overexpression system. The genetic construct contains MBNL1-coding sequence separated by the fragment of ATP2A1 pre-mRNA with MBNL-sensitive alternative exon containing in frame stop codon. Its inclusion leads to arrangement of inactive form of the protein but exclusion give rise in fully active MBNL1. This approach enable the autoregulation of the amount of overexpressed MBNL1 with high dynamic range and its homogenous level in treated cells. We demonstrated beneficial effect of autoregulated construct on alternative splicing pattern in model DM1 cells.

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Generation of novel compounds for Myotonic Dystrophy type 1

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Introduction: Myotonic dystrophy type 1 is an autosomal dominant disorder clinically characterized by progressive muscular weakness and multisystem degeneration that resemble premature aging. It has been previously described that Metformin rescues multiple DM1 phenotypes. Moreover, we have generated and characterized a new family of FKBP12 “reshapers” (known as Ahulkenoids) with promising results against DM1.

Methods: Human primary fibroblasts from DM1 patients and control donors were used in this study. Functional studies of cell viability, proliferation, metabolism were performed on them in the absence or with chimeric compounds.

Results: We have generated a novel family of compounds which contains relevant structural regions of metformin and ahulkenoids. Preliminary results indicate that treatment with this new family of compounds reverses several defects in DM1 derived primary fibroblasts.

Conclusions: We have generated a novel family of compounds which show promising *in vitro* potential in DM1 derived primary fibroblasts.

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Ahulkenoids rescue premature aging phenotypes in Myotonic Dystrophy type 1

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Introduction: Myotonic dystrophy type 1 is an autosomal dominant disorder clinically characterized by progressive muscular weakness and multisystem degeneration that resemble premature aging. In this study, we characterized the impact of a new family of FKBP12 “reshapers” (known as Ahulkenoids).

Methods: Human primary fibroblasts from DM1 patients and control donors were used in this study. Functional studies of cell viability, proliferation, metabolism were performed on them in the absence or with Ahulkenoids treatment. The gene expression profile in treated cells was determined by RNASeq. The impact of Ahulkenoids in vivo was evaluated in a Droshophila model of the disease in locomotor activity and longevity studies.

Results: Treatment with different Ahulkenoids reversed the accumulation of oxidative stress, the impaired cell viability and proliferation as well as mitochondrial activity and metabolism defects in DM1 derived primary fibroblasts. Moreover, RNAseq analysis confirmed the restoration of molecular pathways related to the cell cycle and metabolism. Importantly, treatment with Ahulkenoids significantly improved locomotor activity and extended the lifespan of a Drosophila model of the DM1 disease.

Conclusions: Our results revealed that Ahulkenoids rescue premature aging phenotypes in myotonic dystrophy models and deciphered the benefits of a new family of compounds in the preclinical setting of DM1.

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Musashi-2 overexpression contributes to myotonic dystrophy muscle dysfunction by the repression of miR-7 biogenesis

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Introduction: It has been demonstrated that hyperactivated autophagy contributes to excessive catabolism leading to muscle wasting in DM1. miR-7, downregulated in DM1, regulates autophagy negatively, but the origin of its low levels was unknown.

Methods: To demonstrate the implication of MSI2 in DM1 muscle dysfunction we used gain and loss of function approaches. We inhibited MSI2 by gene-silencing strategies (siRNAs and gapmers) and a small molecule in different cell models. Additionally, we overexpressed MSI2 in skeletal muscles of the HSA[LR] mouse with AAV9.

Results: We found that behind low levels of miR-7 lies the upregulation of MSI2, a protein that binds pri-miR-7 as a repressor. MSI2 was increased in patient-derived myotubes and biopsy samples. Reduction of MSI2 levels or activity boosted miR-7 expression, repressed excessive autophagy, downregulated atrophy-related genes, and enhanced MBNL1 levels. Consistently, AAV-mediated overexpression of MSI2 *in vivo* promoted miR-7 downregulation and modulated atrophy-related genes leading to an enhancement of DM1-like muscle atrophy phenotypes like a reduction in the distribution of fiber sizes, more severe muscle weakness and increase of the percentage of central nuclei.

Conclusions: Taken together, excessive MSI2 levels repress miR-7 biogenesis and contribute to muscle pathology in DM1. Therefore, we propose MSI2 as a new therapeutic target to treat muscle dysfunction in DM1.

[1]Sabater-Arcis, Maria et al. Mol Ther Nucleic Acids. 2021 Aug 19;25:652-667.

P-57

Peptide conjugated antimiRs rescue Myotonic Dystrophy phenotypes in animal and cell models by promoting MBNL1 expression

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Introduction: Myotonic Dystrophy type 1 (DM1) is caused by CTG repeat expansions in the DMPK gene that generate a toxic RNA that sequesters proteins such as MBNL1 and 2 in skeletal muscle, which interferes with a developmental alternative splicing switch. The use of antimiRs against miR-23b and -218 (MBNL translational repressors) has been shown suitable to develop therapies[1], but conventional antimiRs suffer from limited uptake. As a proof-of-concept, we designed antimiRs against both miRs with CPP-PMO chemistry to improve its delivery.

Methods: Toxicity and MBNL1 protein levels were measured in cell and mouse models after treatment with CPP-PMOs, as well as Mbnl1/2 transcript levels, target miRs, muscle strength, myotonia, splicing events, and the arrival of the compounds in different tissues.

Results: In DM1 cells, some CPP-PMOs significantly increased MBNL1 levels at low concentrations. In mouse model, recursive injections of these antimiRs rescued molecular, histopathological and functional phenotypes without significant toxicity alterations. Also, the QDB technique was verified as a good option for the quantification of MBNL1 protein in mouse samples.

Conclusions: CPP-PMOs have a strong therapeutic potential as a treatment for DM1 since they improve many parameters in cell and mouse models. Lastly, the QDB technique can be used to measure MBNL1 protein levels in mouse muscles.

Funding: HR17-00268 TATAMI Project ("La Caixa" Foundation); PROMETEO/2020/081 Project and FDEGENT/2020/01 fellowship (GVA); ERDF funds (Com. Valenciana)

[1] Cerro-Herreros E, et al. (2018) *Nat Commun* 9(1): 2482

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PCSK9 inhibitor mono-treatment and its effect for myotonic dystrophy type I

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Introduction: Hyper low-density lipoprotein (LDL) cholesterolemia is common in patients with myotonic dystrophy type 1 (MyD1). Regarding treatment for MyD, the risk for muscle damage is a criterion that restricts statin use.

Cases/Progress: We retrospectively extracted medical records of four patients with hyper LDL cholesterolemia and MyD1 who had a history of using PCSK9 inhibitors (PCSK9-I). Two men aged 46 (CTGx500) and 52 years (CTGx400) at the start of treatment had a history of acute coronary syndrome (ACS). The remaining patients were two 54-year-old women (CTGx200 and CTGx1400) who did not have ACS. The pretreatment serum creatine kinase (CK) levels were 423, 512, 245, and 32 U/L. Case 1 was treated with atorvastatin (10 mg/day) and PCSK9-I. The other three patients received PCSK9-I alone because of statin intolerance (muscle pain, etc.). In Case 1, LDL-cholesterol (LDL-chol) level decreased from 92 to 19.5 mg/dL after PCSK9-I administration (357 days). Statin therapy was then discontinued and only PCSK9-I was continued. LDL-chol level changed to 48.9 mg/dL (PCSK9-I, 1161 days). In the remaining three patients, the LDL-chol levels decreased from 200, 208, and 198 to 63.1, 97.5, and 49.0 mg/dL (PCSK9-I, 1556, 994, and 1021 days), respectively. No significant adverse events or elevation of serum CK levels were observed in all four patients.

Results: Four patients with hyper LDL cholesterolemia and MyD1 were treated with a PCSK9 inhibitor. Patients with MyD1 were treated with PCSK9 inhibitor alone for an average of 1200 days. The serum LDL-chol level increased without a change in CK levels.

Conclusions: Aggressive treatment is desirable for patients with chronic hyper LDL cholesterolemia, such as patients with MyD. However, hesitation to use statins, which can exacerbate serum CK levels, is quite normal. We suggest that PCSK9 inhibitor monotherapy may be effective in patients with MyD in the long term.

Shakir, M. K. M. et al (2017) *J Clin Lipidol,* 11: 1485-87.

P-59

Efficacy of DPP-4 inhibitors in myotonic dystrophy type 1 with diabetes mellitus: validation by continuous glucose monitoring

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Introduction: Dipeptidyl peptidase 4 inhibitors (DPP4-I) are widely used for the treatment of type 2 diabetes mellitus. However, the efficacy against diabetic patients with myotonic dystrophy type 1 (DM1) has not been verified. The aim of the study was to investigate the effectiveness of DPP4-I in patients with DM1.

Methods: Nine diabetic patients with DM1 participated. The age of patients was 47 ± 8 years (mean ± SD), and HbA1c 7.4 ± 0.5 %. All patients were hospitalized with a fixed amount of meal and exercise, and had the oral administration of 20mg of teneligliptin per day. No patients had any other medicines for diabetes. They were monitored with continuous glucose monitoring (CGM) for 72 hours, and oral glucose tolerance test (OGTT), before and three or more days after administration of teneligliptin.

Results: By administering teneligliptin, Fasting blood sugar (BS) was significantly decreased. BS area under the curve (AUC) during 2 hour OGTT likewise decreased, and serum insulin AUC during OGTT increased. For CGM data analysis, mean BS during CGM, and mean amplitude of glucose excursion value were significantly reduced. Diurnal BS curve by CGM overall shifted downwards. In one case, hypoglycemic episodes were observed after administration. Any other adverse effects were not recognized in all patients.

Conclusions: Our data suggest teneligliptin improves early-phase insulin-secretion capacity, so DPP4-I is a useful therapeutic against DM1 patients with diabetes mellitus.

P-60

Development of psychosocial self-care program for myotonic dystrophy type 1 patients and caregivers

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Introduction: Myotonic dystrophy type 1 (DM1) presents a variety of symptoms that affect patients' quality of life (QoL) and carers' wellbeing. We are developing a psychosocial support program that aims to improve DM1 patients' QoL and caregiver burden through an intervention to increase activities and providing self-care education.

Methods: Results of our study on QoL and caregiver burden, planned outcome measures and the preliminary program content will be described. Opinions on the program of the patients and caregivers, who agreed to collaborate in the development process, and medical staffs will be reported. Implications of these information on revising the program for testing in future pilot study will be discussed.

Results: Our study revealed significantly low physical QoL among the patients, and significant carer's burden in DM1. Preliminary program is designed to improve patients' QoL and carers' burden through increase in activities and psychosocial education to improve stress coping abilities. Reactions to the program were generally positive, but its structure and contents need to be improved for further effectiveness and less burden for participants before execution of the future pilot study.

Conclusions: Our support program for DM1 will be improved for increased feasibility and minimized burden for both participants and staffs. The effectiveness of the finalized program will be tested in a pilot trial.

Grant Support: This work is supported by JSPS Kakenhi 20K03476.

P-61

Strength exercise program improves transcriptome-level changes in myotonic dystrophy type 1

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Introduction: Myotonic dystrophy type 1 (DM1), the most common form of adultonset muscular dystrophy, is characterized by muscle weakness and wasting. While strength-training is clinically beneficial in DM1, the effects at the molecular level are unknown.

Methods: To test if transcriptome-level changes correlate with clinical outcomes after exercise, we performed RNASeq on muscle samples from male DM1 patients before and after a 12-week strength exercise program and on healthy male controls who did not undergo the exercise program.

Results: Differential gene expression (DGE) and alternative splicing (AS) analysis was performed to calculate the percentage of events that shift towards control levels (rescued) and compared to four strength outcomes. For all significant events we calculated dysregulation scores for both AS (average change in percent spliced in) and DGE (average log2-fold change). While rescue of AS was similar among all samples, rescue of DGE was more variable. However, when separated into two groups based on DGE dysregulation score, rescue for the group with a decreased score strongly correlated with clinical strength changes.

Conclusions: We show that transcriptomic changes are likely associated with clinical outcome for DM1 patients who exercise but the response is variable likely due to disease heterogeneity and/or individual responses to exercise. We are investigating what drives these changes to better understand the potential of exercise for DM1.

P-62

MBNL dependent-impaired development connectivity within neuromuscular circuits in Myotonic Dystrophy type 1.

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Introduction: Myotonic dystrophy type I (DM1) is one the most frequent muscular dystrophy in adults. Although DM1 has long been considered mainly as a muscle disorder, growing evidence suggests the involvement in peripheral nerves in the pathogenicity of DM1 raising the question whether motoneurons actively contribute to neuromuscular defects in DM1.

Methods: By using a micropatterned 96-well plate as a co-culture platform, we generated a functional humanized cellular model combining DM1 hiPSC-derived MNs and healthy skeletal muscle cells.

Results: Such approaches led to the identification of pre-synaptic defects which affect development or stability of the neuromuscular junction at an early developmental stage. These neuropathological defects could be reproduced by the loss of RNA-binding MBNL proteins, whose loss of function is associated with muscular defects associated with DM1.

Conclusions: These experiments suggested that the functional defects associated to MNs can be directly attributed to the MBNL family proteins. Altogether, these findings hold several new implications for DM1 pathogenesis.

P-63

IPSC-derived pericytes for the alleviation of muscle symptoms in DM1

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Introduction: Myotonic dystrophy type 1 (DM1) is the most common form of adult muscle disease caused by an expanded CTG repeat in the 3' UTR of *DMPK*. We aim to set up a personalized treatment for DM1 to alleviate the muscular phenotype. Previously, we successfully isolated muscle progenitor cells (pericytes) from quadriceps muscle biopsies of six DM1 patients and two healthy individuals[1]. A main characteristic of pericytes is that they can be delivered into the muscle via systemic injection. However, they have a limited expandability *in vitro* and a limited survival rate for clonal expansion.

Methods: To overcome these limitations, we generated pericyte-derived iPSCs (PC-iPSCs), reaching an unlimited amount of cells and the possibility for clonal selection. We excised the trinucleotide repeat from these cells via CRISPR/Cas9-mediated gene editing or replaced the disease-causing repeat with a healthy repeat via homology directed repair. Corrected PC-iPSCs were differentiated to pericyte-like cells (PiPs) by media changes and cell density limitations.

Results: Our PiPs showed the same markers as pericytes, with no remaining pluripotency (immunocytochemistry and RT-qPCR). However, compared to primary pericytes they were smaller in size and not able to spontaneously form muscle fibers in 2D.

Conclusions: We are currently optimizing the differentiation protocol and will determine the *in vivo* characteristics of PiPs after transplantation in immunodeficient mice.

[1]Ausems CRM *et al*. (2019) *Mol Ther Methods Clin Dev* Sep 12;15:120-132

P-64

Assessing therapeutic potential and mechanism of action of novel small molecules in Myotonic Dystrophy type 1

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Introduction: Myotonic dystrophy type 1 (DM1) is a multisystemic autosomal dominant disease that results in myotonia, cardiac conduction defects, muscle wasting and weakness. DM1 is caused by CTG repeat expansions in the 3' UTR of the dystrophia myotonica protein kinase (DMPK) gene. The CTG repeat expansions produce toxic expansion RNAs that sequester the muscleblind-like (MBNL) family of alternative splicing regulators, leading to the loss of their function. The resulting dysregulation of alternative splicing has been correlated to many DM1 symptoms. As there are currently no effective treatment for DM1, our group has been investigating small molecules, such as diamidines, that can reduce the toxicity effects of the expanded repeats. Unfortunately, many of these compounds are toxic, have many off-target effects, or only demonstrate modest splicing rescue. Therefore we have focused on producing a series of novel small molecules, called modified polycyclic compounds (MPCs), to improve splicing rescue and reduce toxicity.

Methods: We treated DM1 patient-derived fibroblasts and myotubes with MPCs, extracted RNA and analyzed changes in hallmark alternative splicing events and expression of DMPK and MBNL transcripts.

Results: Multiple MPCs (HM19B, 33, and 43) rescued mis-splicing in the nanomolar range with maximum rescue in the 8-16nM (fibroblasts) or 62.5-125nM (myotubes) range. Treatment with MPCs also lead to an upregulation of MBNL2 in both cell types.

Conclusions: Our novel MPCs rescue mis-splicing at low nanomolar levels in cell models, an improvement from previous compounds. While some MPCs upregulate MBNL2 transcript levels, suggesting at a mechanism of action, we are currently further investigating MPCs mechanisms of action.

P-65

Combinatorial drug therapy for Myotonic Dystrophy Type 1

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Introduction: There is no approved therapy for myotonic dystrophy type 1 (DM1), representing a significant unmet medical need for affected individuals. In search for treatments for this multisystemic disorder, our consortium tested combinations of drugs which have individually shown therapeutic potential in muscle and neuronal DM1 cellular models.

Methods: Pairings of the AMPK activator metformin with pan HDAC inhibitors vorinostat and LBH589 were evaluated in immortalized DM1 patient myoblasts and in iPSC-derived neural progenitors and neurons, respectively. The impact of a seven-day treatment was assessed on *DMPK* nuclear aggregates detected by RNA FISH, and on *DMPK* transcripts levels and DM1 associated mRNA splicing defects by PCR.

Results: For DM1 immortalized myoblasts, a low dose of metformin/vorinostat combination resulted in a greater correction of *SERCA1* exon 22 splicing defects as well as a reduction of *DMPK* transcripts levels. The moderating impact of metformin and LBH589 alone or in combination was confirmed in DM1 iPSC-derived neurons where treatments reduced the number of *DMPK* aggregates and partially corrected splicing defects, including *MAPT* exon 10.

Conclusions: Using a combinatorial approach, we have observed a positive impact of low doses of AMPK activator and HDAC inhibitor pairings on DM1 cellular and molecular biomarkers in both myogenic and neural lineages. Although the corrections observed were additive rather than synergistic in nature, these combinations displayed real therapeutic potential and will be next evaluated in mouse models of DM1.

Funding: ReCognitION is funded through the E-Rare Joint Translational Call JTC 2018

P-66

A therapeutic approach targeting muscle stem cells to mitigate myotonic dystrophy type 1

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Introduction: In myotonic dystrophy type 1 (DM1), muscle stem cells (MuSC) exhibit premature senescence and decreased capacity of proliferation and differentiation. DM1 is also associated with an elevated serum inflammatory profile. The therapeutic role of lipid mediators, a new class of molecules playing an active role in resolving inflammation, has never been explored in DM1.

Methods: Muscle tissues and MuSC were obtained from DM1 patients (n=8) and healthy subjects (n=4). Inflammatory gene profile was assessed by single cell sequencing and macrophage quantification was performed on muscle section. The impact of lipid mediators (resolvin [RV] D-1 and -2, maresin [MR] -2) on MuSC function and inflammatory gene expression was assessed by immunofluorescence and RT- qPCR, respectively.

Results: Macrophages and several inflammatory genes (e.g., CXCL-1 and -8, and IL-1a, -1b, -6) were overexpressed in DM1 subjects compared to healthy subjects. RvD2 significantly stimulated proliferation. MR2 and RvD2 significantly increased MuSC differentiation. RvD-2 and MR2 induced a decrease on TNFa or IL1b inflammatory genes.

Conclusions: This project suggests a new therapeutic strategy to target inflammation and MuSC function impairments in DM1.

P-67

Canceled

Session 3: Therapeutic Strategies and Targets

P-68

Advancing antisense therapy against DM1 in a patient-directed manner

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Introduction: No curative therapy is available for myotonic dystrophy type 1 (DM1). Repeat instability and patient variability with respect to repeat length and clinical manifestation result in a heterogeneous DM1 patient population. To develop an effective antisense treatment against DM1, these factors must be addressed.

Methods: We compared the activity of AONs that differ in mechanism of action (blocking- versus RNase H-recruiting) and/or target sequence (repeat or a unique sequence) in DM11 myoblasts. AON efficacy was determined by *DMPK* expression, nuclear foci and disease-associated missplicing, along with RNA sequencing to investigate on- and off-target effects. We also addressed the impact of DM1 heterogeneity on activity using primary cultures originating from DM1 patients with varying repeat lengths.

Results: Although the repeat blocking AON and both gapmers led to *DMPK* knock-down and were equally potent in correcting aberrant splicing, the repeat blocking AON was more effective in MBNL1 protein displacement and had the fewest off-target effects. AON efficacy in our primary DM1 cell panel was less straight-forward, as these cultures exhibited a much milder disease phenotype regardless of repeat length, limiting the corrective potential of the tested AONs.

Conclusions: The efficacy and safety profile of the AONs suggest that the repeat blocking-type AON is the most suitable candidate for further development. The complexities observed for primary DM1 cells highlight the importance of testing drug candidates against a broad panel of cell lines to capture patient heterogeneity.

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Target-agnostic drug discovery approach using informative high-content imaging for identification of a myogenic modulator in DM1 context

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Introduction: Myotonic Dystrophy Type 1 (DM1) is the most common form of muscular dystrophy in adult. Despite major efforts made to generate therapeutic strategies designed to target the molecular substrates associated to DM1, there is currently no cure for this disease. Development of an effective therapeutic approach for DM1 is confronted with a major challenge due to its complex physiobiology although the causal mutation has been well characterized. This example illustrates the needs to implement innovative strategies for the discovery of new medicines relying on more predictive translational models capable to capture the biological complexity of the disease.

Methods: Our objective was to develop unbiased screening approaches using stem cell-based disease models that promise to more realistically recapitulate the complex biology of the disease. We combined the use of disease-specific human stem cells differentiated into relevant cell types with informative high-content imaging screening based on multiparametric approaches and artificial intelligence to identify new therapeutic.

Results: We have successfully developed image analysis tools to screen over 7000 compounds in two doses. Hits will be validated through their ability to reduce DM1 molecular hall marks and functional assays will be performed on in vitro neuromuscular junction system derived from DM1 pluripotent stem cell.

Conclusions: This work will result in the identification and optimization of new myogenic inductors compounds capable to normalize the myogenic defects associated with DM1.

P-70

STARFiSH: Study of Testosterone and rHGH in FSHD: A Proof-of-Concept Study

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Introduction: Large scale clinical trials and preclinical data have found that testosterone combined with recombinant human growth hormone (rHGH) (combination therapy) is well tolerated and effective in synergistically improving respiratory function, lean body mass, protein synthesis, strength, and aerobic endurance in healthy adult human populations. This approach has never been studied in a population with muscular dystrophy.

Methods: We conducted a proof-of-concept clinical study of the safety and tolerability of daily rHGH combined with biweekly testosterone injections in ambulatory adult men with either genetically confirmed fascioscapulohumeral muscular dystrophy (FSHD) or clinical symptoms of FSHD with a first degree relative with genetically confirmed FSHD. All participants were serially and closely monitored during a 24-week period of combination therapy followed by a 12-week washout period. We collected safety and pharmacokinetics data and recorded changes in body composition, functional status, and disease-burden.

Results: Twenty participants were enrolled, with 19 completing all study visits. No participants experienced a serious adverse event. One participant discontinued testosterone use while continuing rHGH due to an asymptomatic increase in his hematocrit. Data regarding changes in body composition, functional performance, and disease burden are currently being analyzed and will be presented at the upcoming conference.

Conclusions: Combination therapy is safe and well tolerated in a select population of patients with FSHD. As a non-disease specific therapeutic approach, combination therapy has promise and potential to be further tested as a treatment modality for both men and women with different types of muscular dystrophy.

P-71

Exercise enhances the beneficial effect of AICAR in DM1 mouse muscles in a sex-specific manner

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Introduction: Myotonic Dystrophy type 1 (DM1) is caused by a CUG expansion in DMPK mRNAs. In addition to causing RNA toxicity by misregulating RNA-binding proteins and alternative splicing, the mutant mRNAs alter multiple signaling pathways in DM1 cells. In a previous study[1], we found that AMPK signaling, a key regulator of muscle metabolism and plasticity, is markedly repressed in a DM1 mouse model and patient-derived DM1 myoblasts. Furthermore, we showed that AICAR and exercise, known activators of AMPK, improve the DM1 muscle phenotype and RNA toxicity. Here, we hypothesized that greater improvements could be achieved by combining exercise with AMPK-drug based activation.

Methods: DM1 mice (HSA^{LR}) were treated with AICAR, exercised with swimming, or treated with a combination of AICAR and exercise for 4 weeks.

Results: Our data show that swimming exercise enhances the beneficial effect of AICAR in mitigating RNA toxicity by further decreasing mutant mRNA aggregation and MBNL1 sequestration in myonuclei. Accordingly, a greater rescue of key aberrant alternative splicing events was observed in the combinatorial group. In addition, our results show that combining AICAR and exercise further improves histological features and promotes muscle fiber hypertrophy in DM1 skeletal muscle. Importantly, these improvements were more pronounced in female DM1 mice, demonstrating a sex-specific effect.

Conclusions: These new findings further demonstrate the therapeutic benefit of activating AMPK and the potential of combining AMPK-activating strategies for improving DM1 muscle. Our data also uncover an important sex-specific effect of AMPK activation in DM1 mice.

[1]Ravel-Chapuis et al. *Hum Mol Genet,* 2018

P-72

Switch-off the trouble: *DMPK* promoter targeting by CRISPRi as an original specific therapy in DM1

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Introduction: Myotonic dystrophy type 1 (DM1) originates from an amplification of CTG microsatellites in the *DMPK* gene. The pathology is primarily explained by a toxic gain of function where the expanded-CUG *DMPK* transcripts induce mainly the loss of function of the MBNL proteins, triggering a wide spliceopathy. Several therapies have been tested to neutralize the toxic *DMPK* transcripts or their consequences. Here, we investigated a new therapeutic strategy consisting of the silencing of the *DMPK* promoter by a CRISPRi system in patient-derived myotubes.

Methods: Our DMPK repressing strategy by CRISPRi was tested in immortalized myoblasts from a DM1 patient or a healthy donor. Stable cell lines expressing a deactivated Cas9 conjugated to an inhibitory KRAB domain in addition to their own sgRNAs were produced by lentiviral transduction. The efficacy and specificity of our therapeutic strategy were then assessed in differentiated myotubes.

Results: Our *DMPK* promoter inhibition strategy is highly efficient to reduce toxic *DMPK* transcript quantities up to 80%. This level of inhibition allows to correct the DM1 hallmark defects by reducing the presence of foci, improving the spliceopathy and normalizing an electrophysiological parameter in DM1 myotubes. Furthermore, this approach displays unprecedented high specificity as evidenced by a complete lack of off-target effects on the transcriptome from unaffected myotubes.

Conclusions: We conclude that *DMPK* promoter inhibition is a promising strategy to be developed for DM1 treatment.

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Fatigue and Sleepiness in the OPTIMISTIC Trial

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Introduction: Fatigue and sleepiness/sleep disorders have high prevalence and impact in myotonic dystrophy 1 (DM1). The OPTIMISTIC trial treated severely fatigued DM1 participants with cognitive behavioural therapy (CBT) +/- graded exercise therapy (GET) reducing the DM1-Activ-c score, indicating clinical improvement. Here we investigate changes in the fatigue and sleepiness measures further.

Methods: We randomized 255 DM1 participants across four sites to standard care or CBT for 10 months, aiming to increase activity and social participation. Two sites offered additional GET to CBT. Fatigue and daytime sleepiness scale (FDSS) and Checklist Individual Strength subscale Fatigue (CIS Fatigue) metrics were taken at baseline and 5, 10 and 16 months and compared across the treatment groups (standard care, CBT alone, CBT+GET) using Mann Whitney U tests. The moderation of the data by age, gender and FDSS components were also investigated.

Results: There was a significant reduction in FDSS score in the CBT+GET group (n=33) compared to standard care (n=125) at 5, 10, and 16 months (P<.001). In contrast, CBT alone (n=96) showed a significant improvement over standard care at 5 months only (P<.05). This was replicated in CIS fatigue scores and across both genders, age groups and FDSS components.

Conclusions: The combination of CBT and GET is effective in reducing fatigue and daytime sleepiness in participants with DM1.

P-74

Altered behavioral responses show GABA sensitivity in Muscleblind-like (Mbnl2) deficient mice: Implications for CNS symptoms in myotonic dystrophy

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Introduction: Evidence indicates that loss of Muscleblind-like protein 2 (MBNL2) function in brain is a major driver of central nervous symptoms in Myotonic Dystrophy Type 1 (DM1), which may be partly attributed to RNA mis-splicing events. Similar splicing and behavioral deficits to DM1 are found in *Mbnl2* deficient mice. Increased hypersomnia, fatigue, and surgical complications associated with general anesthesia suggest possible sensitivity to GABAergic inhibition. We hypothesize that MBNL2 deficient mice exhibit behavioral sensitivity to anesthesia, benzodiazepines, and $GABA_A$-R modulation. We further examine if MBNL2 loss affects total $GABA_A$-R mRNA subunit levels and splicing of *Gabrg2* known to regulate GABA sensitivity and associated behaviors.

Methods: We examine behaviors indicative of emergence and recovery from general anesthesia in *Mbnl2* KO mice from the anesthetic, sevoflurane, the benzodiazepines, diazepam, and zolpidem, and effects of $GABA_A$-R antagonist, flumazenil on baseline sleep activity.

Results: We report that *Mbnl2* KO mice exhibit delayed recovery following sevoflurane, delayed emergence and recovery from zolpidem, and enhanced sleep time that is modulated by the flumazenil differentially in *Mbnl2* KO compared to WT mice. A significantly higher proportion of MBNL2 KO mice also loss their righting reflex (LORR) from a standard diazepam dose. While no other $GABA_A$-R subunit mRNA levels are altered in *Mbnl2* KO mice, we validate via rt-qPCR that *Gabgr2S* mRNA levels are significantly elevated, whereas *Gabgr2L* are significantly reduced.

Conclusions: Findings propose that loss of MBNL2 function in DM1 affects GABAergic sensitivity with implications for neurotransmission in Myotonic Dystrophy.

Funding: NIH NINDS R01NS112291, Myotonic Dystrophy Foundation Pre-doctoral grant

Session 3: Therapeutic Strategies and Targets

P-75

Hybrid Assistive Limb treatment for Patients with Myotonic dystrophy

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Introduction: Hybrid Assistive Limb treatment is effective for walking training of the unstable ambulant patients with neuromuscular diseases[1].In our hospital, adaptive patients with neuromuscular diseases have undertaken Hybrid Assistive Limb walking training 2 or 3 times per week, with total 9 times for one treatment program. Some of the patients repeatedly undertook the treatment program with interval several months. As regular work, we measured 2-minute walking distance before and after each one treatment program to evaluate the efficacy of Hybrid Assistive Limb treatment.

Methods: As subject, myotonic dystrophy patients were recruited. We analyzed 2-minute walking distance of the first treatment program. We also confirmed the number of one treatment program in each patient.

Results: Eighteen patients were included. There were 9 male and 9 female. Their age ranged from 28.8 to 61.1years with mean 48.0 years. Ten patients used night non-invasive ventilation. Mean 2-minute walking distance before one treatment program was 82.4(SD 34.1) m, and that after treatment was 101.4 (36.9) (paired t-test, $p < 0.05$). Half of the patients undertook one program, the others more than once. The highest number of program was 8 times. The patients who repeatedly undertook the program have kept the 2-minute walking distance.

Conclusions: Hybrid Assistive Limb treatment improved the 2-minute walking distance of patients. The short time effectiveness of this treatment is apparent. Long term effectiveness should be evaluated in this slowly progressive disease.

[1]Nakajima et al. Orphanet J Rare Dis (2021) 16:304

P-76

Aurintricarboxylic Acid Decreases RNA Toxicity in a C. elegans Model of Repeat Expansions

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Introduction: Pathologic expansions of DNA nucleotide tandem repeats may generate toxic RNA that triggers disease phenotypes. RNA toxicity is the hallmark of multiple expansion repeat disorders, including myotonic dystrophy type 1 (DM1). To date, there are no available disease-modifying therapies for DM1.

Methods Our aim was to use drug repositioning to ameliorate the phenotype of affected individuals in a nematode model of DM1. As the RNA interference pathway plays a key role in mediating RNA toxicity, we investigated the effect of aurintricarboxylic acid.

Results: We demonstrated that by perturbing the RNA interference machinery using aurintricarboxylic acid, we could annihilate the RNA toxicity and ameliorate the phenotype.

Conclusions: As our approach targets a universal disease mechanism, it is potentially relevant for more expansion repeat disorders.

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(Early bird registrations only)

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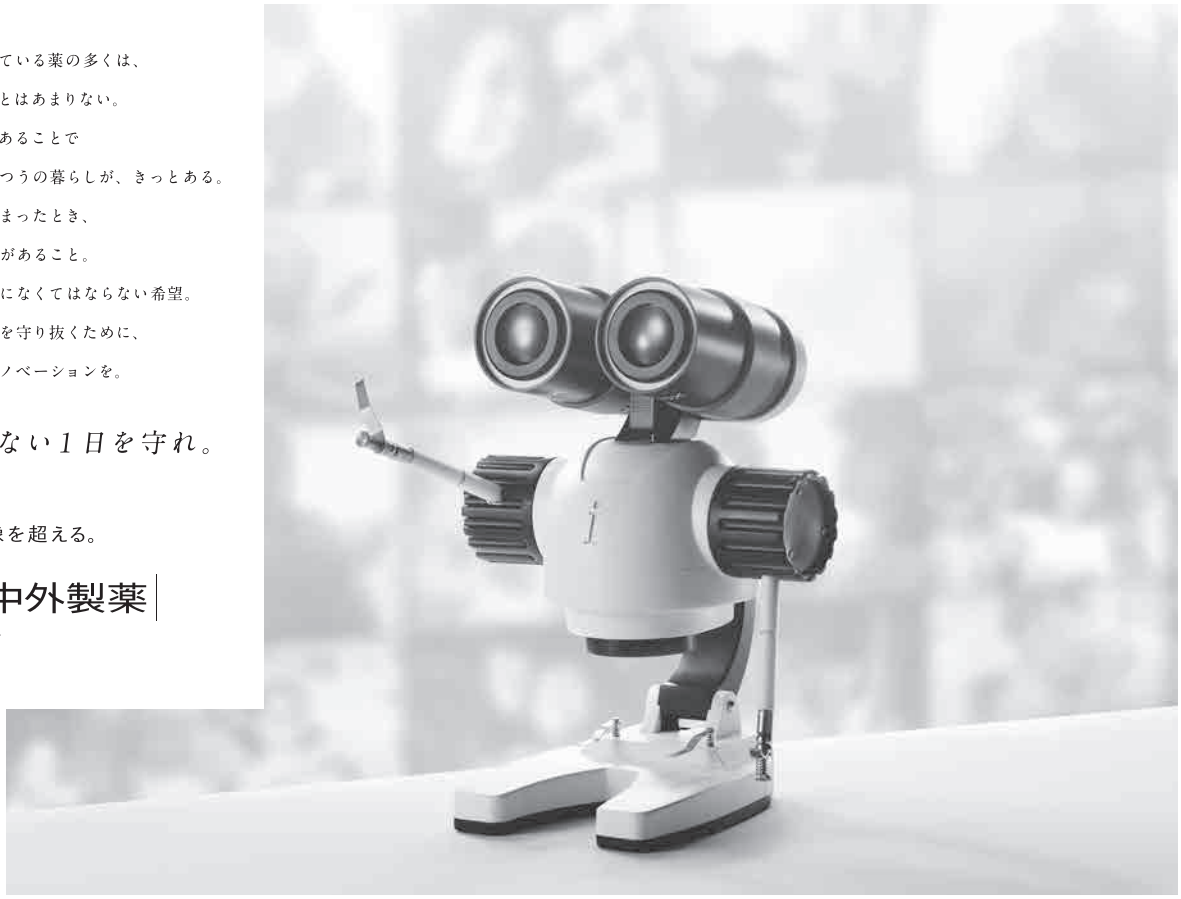
私たちがつくっている薬の多くは、
ふだん接することはあまりない。
けれどその薬があることで
守られているふつうの暮らしが、きっとある。
病気になってしまったとき、
ちゃんと治療法があること。
それはこの世界になくてはならない希望。
当たり前の日々を守り抜くために、
今日も新たなイノベーションを。

なんでもない1日を守れ。

創造で、想像を超える。

中外製薬
ロシュ グループ



Chugai Innovation Lab

健康未来、創ります
日本新薬

新しい生きるを、創る。

独自技術で難病に挑み、
ひとりの「生きる」に希望をとどける。
ユニークな機能性食品で、
みんなの「生きる」を健やかにする。
新しい時代の、新しい生きるを、
わたしたちは、創っていく。

IDMC-13

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