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Author/s:

Schönewolf-Greulich, B; Bisgaard, AM; Dunø, M; Jespersgaard, C; Rokkjær, M; Hansen, LK; Tsoutsou, E; Sofokleous, C; Topcu, M; Kaur, S; Van Bergen, NJ; Brøndum-Nielsen, K; Larsen, MJ; Sørensen, KP; Christodoulou, J; Fagerberg, CR; Tümer, Z

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Mosaic *MECP2* variants in males with classical Rett syndrome features, including stereotypical hand movements

Bitten Schönewolf-Greulich^{1,2}, Anne-Marie Bisgaard¹, Morten Dunø³, Cathrine Jespersgaard², Mette Rokkjær⁴, Lars Kjærsgaard Hansen⁵, Eirini Tsoutsou⁶, Christalena Sofokleous⁶, Meral Topcu⁷, Simran Kaur⁸, Nicole J Van Bergen⁸, Karen Brøndum-Nielsen³, Martin J. Larsen⁹, Kristina P. Sørensen⁹, John Christodoulou⁸, Christina R. Fagerberg⁹, Zeynep Tümer^{2,10}

1. Center for Rett Syndrome, Department of Paediatrics and Adolescent Medicine, Rigshospitalet, Denmark
2. Kennedy Center, Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Glostrup, Denmark
3. Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
4. Department of Pediatrics, Kolding Hospital, Kolding, Denmark
5. Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark
6. Medical Genetics Department, National and Kapodistrian University of Athens, "Aghia Sophia" Children's Hospital, Athens, Greece
7. Hacettepe Medical School, Ankara, Turkey
8. Murdoch Children's Research Institute and Dept of Paediatrics, University of Melbourne, Melbourne, Australia
9. Department of Clinical Genetics, Odense University Hospital, Odense, Denmark
10. Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

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The authors declare no conflict of interests.

Correspondence to:

Bitten Schönewolf-Greulich
Center for Rett Syndrome
Kennedy Center
Rigshospitalet
Gl. Landevej 7
2600 Glostrup
Copenhagen, Denmark
Tel.: (+45) 43260150

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ABSTRACT

Rett syndrome is rarely suspected in males because of the X-linked dominant inheritance. In the literature only six male patients have been reported with *MECP2* mosaicism. NGS methods have enabled better detection of somatic mosaicism compared to conventional Sanger sequencing, however mosaics can still be difficult to detect. We present clinical and molecular findings in two males mosaic for a pathogenic *MECP2* variant. Both have been reexamined using deep sequencing of DNA isolated from four different cell tissues (blood, muscle, fibroblasts and oral mucosa). Deep sequencing of the different tissues revealed that the variants were present in all tissues. In one patient the molecular diagnosis could only be established by reexamination after a normal whole exome sequencing, and the other case is an example of reverse genetic diagnostics. Rett syndrome should be considered in males with neurodevelopmental delay and stereotypical hand movements. Subsequent to clinical diagnosis males should be investigated with NGS based technologies of *MECP2* with high read depth and a low threshold for variant calls. If the initial analysis on full blood derived DNA fails to confirm the suspicion, we recommend repeating the analysis on another tissue, preferentially fibroblasts to increase the diagnostic yield.

INTRODUCTION

Rett syndrome (RTT) (OMIM# 312750) is a neurodevelopmental syndrome first described in 1966 by Andreas Rett, who investigated a group of girls with regression of cognitive and motor function especially of speech and hand use and with development of hand stereotypies¹. The latest international diagnostic criteria published by Neul et al. 2010² are being used to establish the clinical diagnosis of RTT. The primary causative gene is the X-linked gene *MECP2* (methyl-CPG binding protein 2)³. Around 3900 females with *MECP2* variants have been reported in the international RettBASE⁴. In males, however, variants are not so common and only about 60 cases have been reported. The phenotype of males with single nucleotide variants is often more severe with neonatal encephalopathy as the main symptom in contrast to the classical RTT phenotype described in the majority of females⁵. The RTT phenotype observed in the few published male cases is occasionally explained by the presence of mosaicism⁶⁻¹¹ or a coincidental 47,XXY karyotype detected with Sanger sequencing of *MECP2* or chromosome analysis, respectively, using DNA derived from blood^{12,13}. The six published cases of males with *MECP2* mosaicism have been diagnosed with non-syndromic neurodevelopmental delay, Rett-like, atypical RTT or classical RTT⁶⁻¹¹. None of these patients were evaluated according to the 2010 criteria². Recently, the unaffected father of a RTT patient hemizygous for c.925C>T (p.Arg309Trp) in *MECP2* was found to be mosaic for this variant¹⁴. This variant is, however, also described as resulting in a less severe phenotype than RTT and was also detected in an unaffected mother in non-mosaic state¹⁵.

Today, next generation sequencing (NGS) methods, such as genome sequencing, exome sequencing or targeted gene panels are commonly used as a first-tier approach to screen for genomic variants causing neurodevelopmental syndromes, including RTT. In the present study, two males with neurodevelopmental syndromes and mosaic for an *MECP2* variant are described. Only one of them was suspected of having RTT initially without finding a variant in the known RTT genes. The six previously published cases were also reevaluated according to the 2010 guidelines² either by using the information published or by personal communication with the authors and the symptoms were compared with the two present cases.

“insert Figure 1 about here”

MATERIALS AND METHODS

Subjects

In this study two male patients are included. Patient A is an 8-year-old male (Figure 1- A1, A2), patient B is a 10-year-old male (figure 1 – B1, B2). The phenotypic features of both patients are listed in table 1 (elaborated subject description in supplementary file A).

Genetic study

The two clinical cases were examined as part of a project investigating patients with Rett-like syndromes: “*Rett-like childhood syndromes – identification of disease causing genes and mechanisms, a clinical and molecular study*”. The project was approved by the Danish regional ethics committee and the regional Danish data protection agency and the families have consented to the publication of photos and clinical information.

Apart from the thorough clinical description of the present cases, we also contacted the authors of the previously described males with mosaic *MECP2* variants in order to update and expand their previously published clinical presentations. Further information was gathered for two of the cases^{8,9} and features of the remaining cases are extracted from the publications^{6,7,10,11}.

DNA was extracted from blood, buccal swab, skin and muscle biopsies using standard methods. To investigate the overall presence of the VAF in different tissues, all DNA samples from the two patients were sequenced with the same method with high coverage.

PCR amplification of *MECP2* exon 4 was performed and the resulting products were fragmented. NGS was carried out on the IonProton (ThermoFisher, Waltham, USA) using standard procedures and settings (TorrentSuite v5.4). All samples were sequenced in a single run. The sequencing depth varied from 19.428-39.344 reads.

Post hoc Sanger sequencing was carried out for the same samples in order to see whether the variant was detectable by this method.

RESULTS

Table 1 summarizes the clinical features of the present cases together with the features of the previously published male patients with mosaic *MECP2* variants⁶⁻¹¹. The published cases were diagnosed with non-syndromic neurodevelopmental delay, Rett-like, atypical RTT or classical RTT. Re-evaluation of the published cases according to the Neul 2010 criteria² suggest that all the patients have classical RTT, regardless of what the initial diagnosis was.

DNA extracted from blood, buccal swab, fibroblasts and muscle biopsies from both patients were sequenced. The resulting VAF values are shown in table 1. Patient A displayed a VAF of 8.4-15.8%, with the highest VAF found in muscle and the lowest in blood (table 1). In patient B the VAF varied from 33-45%, with the highest VAF found in fibroblasts and the lowest in oral mucosa.

Post hoc Sanger sequencing showed that the variant in patient A was difficult to assess compared to patient B (supplementary file B).

Table 1. Clinical and molecular data of male cases with mosaicism

	Present cases		Published cases with new information		Previously published cases without new information			
	Case A	Case B	Psoni <i>et al</i> ⁹	Topcu <i>et al</i> ⁸	Pieras <i>et al</i> ¹⁰	Kleefstra <i>et al</i> ¹¹	Clayton-Smith <i>et al</i> ⁶	Armstrong <i>et al</i> ^{7*}
Gender	male	male	male	male	male	male	male	male
Age at evaluation (years)	8	9	14	12	4	11	6	14
RTT diagnosis considered at age	4 years	Never considered due to male gender and facial expression	6 years	2 years	3 years	Considered but disregarded due to male gender	6 years	uk
Head circumference	50 cm (<10 th centile)	50,5 cm (<10 th centile)	54,5 cm (50 th centile)	47 cm (25 th centile)	50 cm (50 th centile)	51,5 cm (10 th centile)	<25 th centile	uk
Nucleotide change (NM_004992.3)	c.1308dupT	c.808C>T	c.316C>T	c.808C>T	c.360T>G	c.473C>T	c.241_242del	c.398G>A
Predicted effect on protein sequence	p.(Gln437Serfs*50)	p.(Arg270*)	p.(Arg106Trp)	p.(Arg270*)	p.(Tyr120*)	p.(Thr158Met)	p.(Gly81Glnfs*9)	p.(Arg133His)
Detected in:								
Leukocytes	9%	36%	mosaic	36%	mosaic	mosaic	mosaic	mosaic
Muscle tissue	16%	40%	nt	nt	nt	nt	nt	nt
Oral mucosa	12%	33%	nt	nt	nt	nt	nt	mosaic
Fibroblasts	15%	45%	mosaic	nt	mosaic	nt	nt	nt
Hair roots	nt	Nt	nt	41%	nt	nt	nt	nt
Genetic test	NGS	NGS	Sanger Sequencing	Restriction digestion	Sanger Sequencing	Sanger Sequencing	Sanger Sequencing	Sanger Sequencing
Required diagnostic Criteria for RTT								
Age of onset for a period of regression followed by recovery or stabilization	18 mo	8-10 mo	18 mo	11 mo	no regression	12 mo	2 years	uk
Exclusion criteria for RTT	0/3	0/3	0/3	0/3	1/3	0/3	0/3	uk
Brain injury	no	No	no	no	no	no	no	uk
Neurometabolic disease, or severe infection	no	No	no	no	no	no	no	uk
Grossly abnormal development < 6 mo	no	No	no	no	yes	no	no	uk
Main criteria for RTT	4/4	4/4	4/4	4/4	4/4	4/4	4/4	uk
Hand skills	poor	poor	lost	lost	none	lost	poor	uk
Language	lost	none	poor	none	none	none	yes	uk

Gait	ataxic	poor	No	none	ataxic	lost	ataxic	uk
Stereotypic hand movements	yes	Yes	yes	yes	yes	yes	yes	uk
Supportive criteria for RTT	9/11	8/11	1/11	7/11	5/11	5/11	7/11	uk
Breathing disturbance	yes	Yes	uk	no	no	uk	yes	uk
Bruxism	yes	Yes	uk	yes	yes	yes	yes	uk
Impaired sleep pattern	yes	Yes	uk	uk	yes	yes	no	uk
Abnormal muscle tone	yes	Yes	Yes	yes	yes	yes	yes	uk
Peripheral vasomotor disturbances	yes	No	No	yes	uk	uk	yes	uk
Scoliosis/kyphosis	no	Yes	No	yes	uk	uk	yes	uk
Growth retardation	yes	Yes	No	yes	uk	uk	yes	uk
Small cold hands and feet	yes	Yes	No	yes	uk	yes	yes	uk
Inappropriate laugh/screaming spells	yes	No	No	yes	yes	yes	uk	uk
Diminished response to pain	yes	Yes	uk	uk	uk	uk	uk	uk
Intense eye communication	no	No	No	uk	yes	uk	uk	uk
RTT diagnosis (based on the Neul criteria 2010²)	Classical RTT	Classical RTT	Classical RTT	Classical RTT	Classical RTT	Classical RTT	Classical RTT	Classical RTT, according to publication

nt,not tested; uk, unknown.*This case was published as having classical RTT without a detailed clinical description but as a patient with classical RTT and 8/9 of the symptoms according to the 2001 criteria².

DISCUSSION

We present two males with a mosaic *MECP2* variant. The RTT diagnosis was not even considered for patient B prior to exome sequencing at the age of 9 years, whilst for patient A the suspected clinical RTT diagnosis could not be confirmed by Sanger or initial exome sequencing. Among the more than 100 patients with a constitutional *MECP2* variant registered at the Danish National Center for Rett syndrome¹⁶ between 2001-2016, there is only one male patient¹⁷. Our finding of two male patients in a relatively short period suggests that RTT in males may be underdiagnosed. Males with a pathogenic constitutional *MECP2* variant are rare probably because of a severe phenotypic outcome, but in a mosaic state the phenotypic effect has been suggested to resemble that of females with RTT⁵. When reviewing the clinical data of the eight males with a mosaic *MECP2* variant, including the present cases it is clear that they all present with a classical RTT phenotype: a regression period with loss of skills and thus poor hand function, an abnormal gait and poor speech development. Finally, they all have a very recognizable feature of RTT, namely stereotypic hand movements (Figure 1). However, their diagnosis of RTT was delayed in most of the cases (2-6 years) presumably because of the male gender¹¹ or not even considered (Patient B) (Table 1); whereas the corresponding mean age of the diagnosis in females with RTT in Denmark is 2.5 years¹⁸.

Aside from the importance of raising clinicians' awareness of males with RTT, the present cases also illustrate the pitfalls in the diagnosis of mosaicism. Sanger sequencing is not the method of choice for detection of mosaicism. NGS based methods necessitate high sequencing depth (optimal >100x coverage) to reliably detect low-frequent mosaic variants. Diagnostic exome sequencing analyses are often carried out at lower read depth (approximately 30x)¹⁹ and some regions are captured poorly; therefore a targeted gene panel with high coverage of the gene of interest (e.g. *MECP2*) and high read depth may be a preferred approach for the molecular genetic diagnosis of mosaic males who have typical or atypical RTT. The decision to select the most relevant approach can be reached through collaboration between clinicians and molecular geneticists to enhance the diagnostic rate of RTT in male patients.

The mosaic variants probably arise very early in embryogenesis and therefore most likely are present in all types of tissues. However there can be asymmetry in the selective pressure among cell-lines²⁰ resulting in different VAF's in different tissues. Standard genetic analyses are performed in DNA from blood samples and although mosaicism will probably be present in all cell types and not restricted to a single tissue, it is recommended to also test another tissue whenever mosaicism is suspected²⁰.

In conclusion, hand stereotypies are a clinical hallmark of RTT, and together with a regression period with loss of hand skills and speech, these unique symptoms can aid the clinician to consider RTT and apply the Neul clinical criteria in both males and females. This applies both to the clinical assessment before genetic testing and in cases where a *MECP2* variant is found without a prior suspicion of RTT.

Furthermore, the interpretation of NGS data should have a low threshold for variant calls in case-specific highly relevant genes, high coverage in critical gene regions, and examination of various tissues is relevant to confirm a low-frequency degree of mosaicism.

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PATIENT CONSENT

Parental/guardian consent was obtained.

ETHICAL APPROVAL

The project was approved by the Danish regional ethics committee and the regional Danish data protection agency.

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LEGENDS FOR TABLES AND FIGURES

Figure 1

A1: Patient A 1 years old using his hands

A2: Patient A 8 years old with hand stereotypies

B1: Patient B 4 years old still able to sit independently

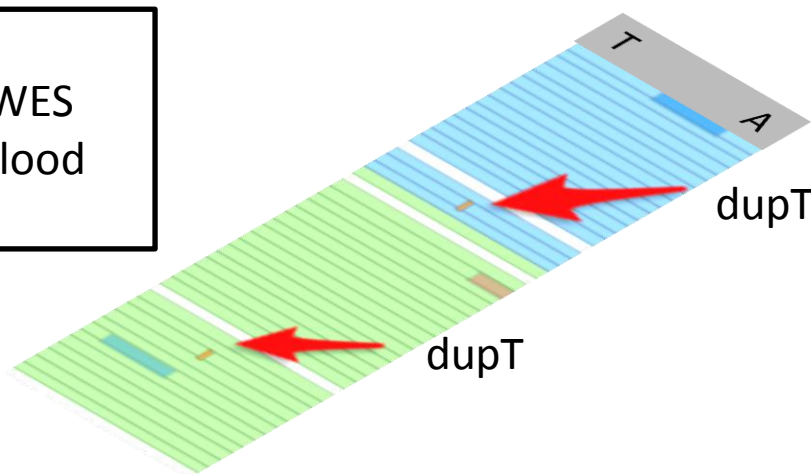
B2: Patient B 9 years old he sits with support and has almost constant hand stereotypies



Figure 1.tif

Mosaicism in Rett syndrome males

WES
blood



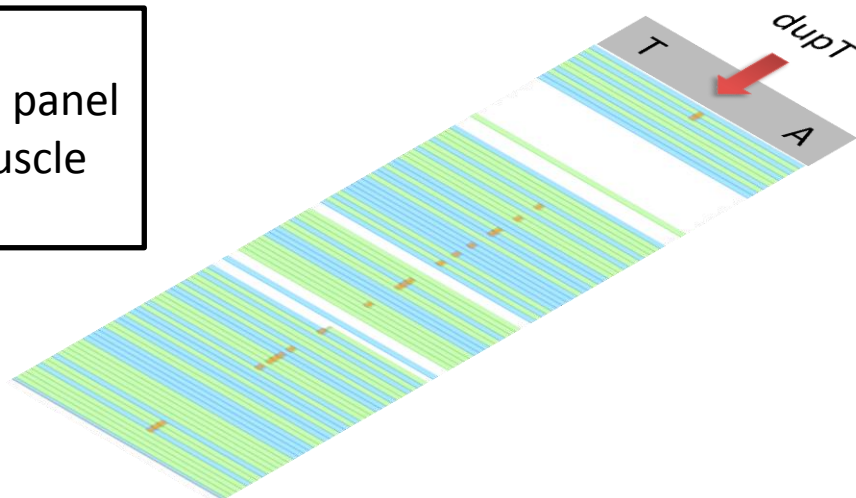
ChrX between 153,295,970 and 153,295,971

Insertions

Base(s)	Count	% of Total	Mean Quality
T	2	4.2	31.5
Non-Insertions	46	95.8	?
Total	48	100	?

MECP2
c.1308dupT

NGS panel
muscle



ChrX between 153,295,970 and 153,295,971

Insertions

Base(s)	Count	% of Total	Mean Quality
T	31	23.8	32.4
Non-Insertions	99	76.2	?
Total	130	100	?

MECP2
c.1308dupT

Even very low-grade mosaicism in *MECP2* can cause Rett syndrome in males. The c.1308dupT variant of a male patient was present in 4.2% in blood and 23.8% in muscle tissue (encircled). Analysis of *MECP2* in males should be carried out with very high read depth and variant call threshold should be low. A negative finding blood should be repeated using other tissues.