

Subclinical myocardial dysfunction in Rett syndrome

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Aims

Rett syndrome (RTT) is a rare neurodevelopmental disorder frequently linked to methyl-CpG-binding protein 2 (*MeCP2*) gene mutations. RTT is associated with a 300-fold increased risk of sudden cardiac death. Rhythm abnormalities and cardiac dysautonomia do not to fully account for cardiac mortality. Conversely, heart function in RTT has not been explored to date. Recent data indicate a previously unrecognized role of *MeCP2* in cardiomyocytes development. Besides, increased oxidative stress markers (OS) have been found in RTT. We hypothesized that (i) RTT patients present a subclinical biventricular dysfunction and (ii) the myocardial dysfunction correlate with OS.

Methods and results

We evaluated typical ($n = 72$) and atypical ($n = 20$) RTT female and healthy controls ($n = 92$). Main outcome measurements were (i) echocardiographic biventricular systo-diastolic parameters; (ii) correlation between echocardiographic measures and OS levels, i.e. plasma and intra-erythrocyte non-protein-bound iron (NPBI) and plasma F2-Isoprostanes (F2-IsoPs).

A significant reduction in longitudinal biventricular function (tricuspid annular plane systolic excursion, mitral annular plane systolic excursion, S' of lateral and septal mitral annulus, S' of tricuspidal annulus) was evidenced in RTT patients vs. controls. No significant changes in the LV ejection fraction were found. Peak-early filling parameters (E , E' of lateral mitral annulus, E' of tricuspidal annulus) and right ventricular systolic pressure were reduced. A-wave, E/A, and E/E' were normal. OS markers were increased, but only F2-IsoPs correlated to LV systolic dysfunction.

Conclusion

These data indicate a previously unrecognized subclinical systo-diastolic biventricular myocardial dysfunction in typical and atypical RTT patients. A reduced preload is evidenced. The biventricular dysfunction is partially related to OS damage.

Keywords

Myocardial function • Rett syndrome • Echocardiography • Oxidative stress • *MeCP2* • Perfusion index

Introduction

Rett syndrome (RTT) is a severe neurological disorder with autistic features, mainly caused by *de novo* loss-of-function mutations in the methyl-CpG-binding protein 2 (*MeCP2*) gene encoding the

transcriptional regulator *MeCP2*, which is abundantly expressed in the brain. The protein acts as both transcriptional repressor and activator regulating a large set of target genes.¹

Although RTT is a rare disease (1:10.000 live births), it represents the second most common cause for mental retardation in

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females and the most common cause of mental retardation caused by a gene mutation (missense, frameshift, non-sense).¹ While a single monogenic mutation in the *MeCP2* gene is known to cause RTT in up to 95% of cases,^{2,3} mutations in other two genes are involved in RTT: cyclin-dependent kinase-like 5 (*CDKL5*)⁴ and fork-head box protein G1 (*FOXP1*).⁵

Typical RTT shows an apparently normal developmental period followed by neurological regression at ~6–18 months of age exhibiting a diagnostic tetrad, i.e. 'main criteria': (i) partial or complete loss of acquired purposeful hand skills, (ii) partial or complete loss of acquired spoken language, (iii) gait dyspraxia, and (iv) stereotypic hand movements,⁶ and its natural history progresses in four clinical stages (I–IV).⁷

Atypical RTT (~26% of all cases) is diagnosed on the basis of a period of regression followed by recovery or stabilization with the presence of at least 2 of the 4 main criteria plus 5 out of 11 supportive criteria.⁷ To date, the early seizure variant (linked to *CDKL5* mutations), the preserved speech variant (linked to *MeCP2* mutations), and the congenital variant (recently linked to *FOXP1* mutations) are the only recognized clinical variants according to a revised diagnostic criteria and nomenclature consensus.⁶

No effective treatment to prevent or arrest the neurological regression exists.

MeCP2 protein expression has also been reported in different tissues outside the central nervous system. However, non-neurological phenotypes in RTT patients have less well been studied, such as pulmonary⁸ and cardiovascular disease.

Patients affected by RTT present a 300-fold increased risk of sudden cardiac death due to fatal arrhythmias, such as torsades

de pointes/ventricular fibrillation. A sympato-vagal imbalance and electrocardiographic findings of QT interval prolongation^{9,10} have been demonstrated in RTT,¹¹ but they do not fully account for the observed increased cardiac mortality risk. Nevertheless, very little is known on myocardial function in RTT.¹² Recently, *MeCP2* gene has been implicated in cardiomyocyte development in a murine model. In fact, DNA methylation plays a key role in neonatal cardiomyocyte differentiation.¹³ On the other hand, increased oxidative stress (OS) markers have been reported in typical RTT^{14,15} and experimental settings have demonstrated a detrimental effect of systemic OS on myocardial function.

We, therefore, evaluated the left and right ventricular (LV and RV, respectively) longitudinal systolic and diastolic function in RTT by means of echocardiography and investigated on a possible relationship between myocardial performance and OS markers levels.

Methods

Patients

Ninety-two patients with RTT (72 typical RTT and 20 atypical RTT) (Table 1) and 92 healthy age- and gender-matched control subjects were enrolled. Baseline clinical data were recorded. Body surface area (BSA) was expressed as square metres and calculated by the Mosteller formula¹⁶ which is considered the most valid algorithm for BSA calculation.¹⁷ Blood samplings in the control group were carried out during routine health checks, sports, or blood donations. Amino-terminal pro B-type natriuretic peptide (NT-proBNP), as a biomarker of ventricular volume expansion and pressure overload,

Table 1 Baseline features of Rett syndrome patients

Item	RTT categories			
	Typical, n = 72	Atypical		
		ESV, n = 8	PSV, n = 9	CV, n = 3
Age (years)	11.8 ± 4.9	9.3 ± 3.3	18.1 ± 8.7	11.7 ± 8.0
Mutated gene	<i>MeCP2</i>	<i>CDKL5</i>	<i>MeCP2</i>	<i>FOXP1</i>
Loss of acquired purposeful hand skills	72 (100%)	5 (62%)	1 (11%)	2 (67%)
Loss of acquired spoken language	72 (100%)	7 (87%)	0	3 (100%)
Gait dyspraxia	72 (100%)	8 (100%)	9 (100%)	1 (33%)
Stereotypic hand movements	72 (100%)	3 (37%)	8 (89%)	2 (67%)
Breathing disturbances when awake	44 (61%)	3 (37%)	2 (22%)	1 (33%)
Bruxism when awake	50 (69%)	1 (12%)	1 (11%)	0
Impaired sleep pattern	40 (55%)	4 (50%)	1 (11%)	0
Abnormal muscle tone	72 (100%)	8 (100%)	8 (100%)	3 (100%)
Peripheral vasomotor disturbances	65 (90%)	4 (50%)	2 (22%)	2 (67%)
Scoliosis/kyphosis	47 (65%)	1 (12%)	8 (89%) ^a	1 (33%)
Growth retardation	72 (100%)	8 (100%)	5 (55%)	2 (67%)
Small cold hands and feet	72 (100%)	8 (100%)	8 (100%)	2 (67%)
Inappropriate laughing/screaming spells	28 (39%)	0	0	0
Diminished response to pain	72 (100%)	8 (100%)	7 (78%)	2 (67%)
Intense eye communication	65 (90%)	8 (100%)	8 (100%)	2 (67%)

ESV, early seizure variant; PSV, preserved speech variant; CV, congenital variant.

^aMajority of kyphosis. In bold: main criteria according to Neul et al.⁶

was measured in serum samples by a commercially available immune assay technique; normal values in females were <150 pg/mL. All samplings were carried out at the Child Neuropsychiatry Unit, University Hospital A.O.U.S., Siena, Italy. The study was approved by the institutional review board, University General Hospital of Siena. Informed consent was obtained from the legal caregivers prior to enrolment into the study.

Pulse oximetry

Continuous and non-invasive measurements of oxyhaemoglobin saturation (SpO₂), perfusion index (PI), pleth variability index (PVI), carboxy-haemoglobin (CO-Hb), and meta-hemoglobin (Met-Hb) were carried out using pulse oximetry motion artefact-free technology.

PI is an objective indicator of skin perfusion and is calculated as: $PI = AC/DC \times 100\%$, where AC is the variable amount of light absorbed by the pulsating arterial inflow and DC is the constant amount of light from the signal of the pulse oximeter absorbed by the skin, other tissues, and non-pulsatile blood.¹⁸ PVI is a measure of the dynamic changes in the PI that occur during the respiratory cycle and is calculated as: $PVI = PI \text{ maximum} - PI \text{ minimum} / PI \text{ maximum} \times 100\%$.¹⁹

Each patient and control subject was equipped with an adequate pulse oximeter probe attached at the index of the left hand and wrapped to prevent outside light from interfering with the signal. The pulse oximeter was connected to a Masimo Radical 7 monitor (Masimo SET; Masimo Corp.) and plethysmographic waveforms were recorded on a personal computer using PhysiLog software (PhysioLog version 1.0.1.1; Protolink, Richardson, TX, USA) and analysed by an observer who was blinded to clinical, biochemical, and respiratory function data. Randomly measured, 1 h-long data were obtained in triplicate and averaged data were used for data analysis.

Echocardiography

The study was performed using commercially available echocardiography equipment (Phillips IE 33 Vision 2009; qLAB 7.0 software; 5 and 8 MHz transducers). The heart rate was recorded and two-dimensional right and left chambers quantification (areas and volumes) performed. Right ventricular systolic pressure (RVSP) was estimated using continuous wave Doppler on the tricuspid valve regurgitation jet (TRJ) and applying the simplified Bernoulli equation ($dP = 4v^2$; $RVSP = dP \times TRJ + \text{estimated right atrial pressure}$). The left ventricle ejection fraction (LVEF) was measured by Simpson's method in B-mode, as a surrogate of global LV systolic function. Mitral flow velocities were recorded using pulsed wave (PW) Doppler on the mitral valve, including peak early (E) and peak late (A) inflow velocities and E/A ratio.^{20,21} Mitral annular plane systolic excursion (MAPSE) and tricuspid annular plane systolic excursion (TAPSE), using M-mode, were determined in order to evaluate LV and RV longitudinal systolic function.²² PW tissue Doppler imaging (TDI) of the lateral (_{lat}) and septal (_{sep}) mitral annulus and of tricuspid annulus (_{tr}) was obtained from four-chamber apical views and analysed for systolic (S'_{lat} , S'_{sep} , S'_{tr}) and early (E'_{lat} , E'_{tr}) diastolic peak velocities. The E'/E'_{lat} ratios were determined as surrogate of LV filling pressures.^{23,24}

In order to reduce the operator-dependent bias, all measurements were performed by a single operator who was blinded for the clinical and laboratory data of RTT patients. Intra-observer variability was assessed in 15 patients by repeating the measurements on two different admissions; variability was calculated as the mean percent error and ranged between 3.56 and 8.29%.

Blood sampling

Blood was collected in heparinized tubes and all manipulations were carried out within 2 h after sample collection. Blood samples were centrifuged at 2400 g for 15 min at 4°C. The platelet poor plasma was saved and the buffy coat was removed by aspiration. The red blood cells were washed twice with physiological solution, resuspended in Ringer's solution, as previously reported²⁵ for the determination of intra-erythrocyte non-protein-bound iron (IE-NPBI). Plasma was used for the determination of NPBI (P-NPBI), and free F₂-Isoprostanes (F₂-IsoPs). For F₂-IsoPs measurements, butylated hydroxytoluene (BHT) (90 μM) was added to plasma, as an antioxidant, and the sample was stored under nitrogen at -70°C until analysis.

Intra-erythrocyte and plasma non-protein-bound iron

Intra-erythrocyte and plasma NPBI were determined as a desferrioxamine-iron complex by high performance liquid chromatography, as previously reported.²⁵

Plasma F₂-Isoprostanes

Plasma F₂-IsoPs were measured by gas chromatography/negative ion chemical ionization tandem mass spectrometry (GC/NICI-MS/MS) analysis. The measured ion was the product ion at m/z 299 from 15-F_{2t}-IsoP, also referred as 8-iso PGF_{2α} or 8-epi PGF_{2α}, the most represented F₂-isoprostane isomer.^{25,26}

Statistical analysis

All variables were tested for normal distribution (D'Agostino-Pearson test) and data were presented as mean value with 95% confidence intervals (95% CI) for normally distributed variables or medians with 95% CI for non-normally distributed data, respectively. Differences between groups were evaluated using independent sample t-test (continuous normally distributed data), Mann-Whitney rank sum test (continuous non-normally distributed data), χ^2 test (categorical variables with minimum number of cases per cell ≥ 5) or Fisher's exact test (categorical variables with minimum number of cases per cell < 5), one-way analysis of variance for multiple comparisons (ANOVA), Student-Newman-Keuls *post hoc* test, or Kruskal-Wallis test. Associations between variables were tested by univariate regression analysis, while unadjusted odds ratios were determined by univariate logistic regression. Two-tailed *P*-values of < 0.05 were considered significant. Correction for multiple comparison was made in order to control for the increase in type I error occurring when statistical tests are used repeatedly (i.e. the multiple testing problem) so that in the case of multiple *t*-tests or Mann-Whitney rank sum test the standard *P*-value 'alpha' level (0.05) was recalculated as α/n , where *n* is the total number of comparisons (Bonferroni's correction). The MedCalc version 9.5.2.0 statistical software package (MedCalc Software, Mariakerke, Belgium) was used.

Results

Clinical status

RTT patients showed a reduced BSA in comparison with that of the control subjects. Significantly a higher heart rate, reduced NT-proBNP serum levels and PI values, together with increased PVI values were observed in the RTT patients (total cohort) vs. controls (Table 2). No congenital cardiac defects were detectable

Table 2 Baseline clinical evaluations in Rett syndrome patients and healthy control subjects (data are means \pm SD)

Variables	RTT		Controls (c), (n = 90)	ANOVA, P-value	Post hoc analysis
	Typical (a), (n = 72)	Atypical (b), (n = 20)			
Height (cm)	130.9 \pm 18.5	126.5 \pm 19.2	138.3 \pm 24.5	0.030	c > a \approx b
Body weight (kg)	33.9 \pm 17.42	37.6 \pm 25.8	38.6 \pm 18.6	0.131	NA
Head circumference (cm)	48.2 \pm 4.8	50.1 \pm 2.1	52.5 \pm 2.3	<0.001	c > a \approx b
BSA (m ²)	1.09 \pm 0.34	1.18 \pm 0.45	1.19 \pm 0.39	0.084	NA
Systolic BP (mmHg)	101.9 \pm 11.2	100.8 \pm 8.5	102.7 \pm 8.0	0.662	NA
Diastolic BP (mmHg)	60.3 \pm 5.3	59.3 \pm 6.2	60.4 \pm 6.0	0.803	NA
Heart rate (bpm)	84.7 \pm 7.4	84.9 \pm 8.8	75.7 \pm 7.2	<0.001	c < a \approx b
PI (%)	0.97 \pm 0.38	1.17 \pm 0.57	4.38 \pm 0.86	<0.001	c > a \approx b
PVI (%)	42.0 \pm 10.4	39.3 \pm 9.0	22.9 \pm 4.9	<0.001	c < a \approx b
NT-proBNP (pg/mL)	15.5 \pm 9.1	20.4 \pm 10.6	70.1 \pm 56.1	<0.001	c > a \approx b

BP, blood pressure; BSA, body surface area (as calculated by the Mosteller's formula;¹⁶ NT-proBNP, N-terminal pro-hormone of the brain natriuretic peptide; PI, perfusion index (derived from pulse oximetry); PVI, pleth variability index (derived from pulse oximetry); NA, not applicable.

with the exception of a single mitral valve prolapse in one case with atypical RTT (1.1%).

Echocardiography

Two-dimensional volumes

Right ventricular end-diastolic areas were significantly reduced in RTT (total cohort) vs. the control group while right atrium areas were reduced in typical RTT vs. controls and in typical vs. atypical RTT. LV volumes and left atrium areas were not significantly reduced in RTT vs. the control group (Table 3).

Ventricular function

Examining LV systolic variables, a mild but significant reduction in MAPSE ($P < 0.001$), S'_{lat} ($P < 0.001$), and S'_{sep} ($P = 0.001$) was found in RTT patients (total cohort) vs. controls, while no variations were observed between typical and atypical RTT (Table 3). The LVEF was preserved in RTT and controls. In regard to RV systolic function, TAPSE ($P < 0.001$) and S'_{tr} ($P < 0.001$) were significantly reduced in RTT vs. controls, with no difference between typical and atypical RTT.

Diastolic LV function analysis revealed a reduction in mitral inflow E-wave ($P = 0.003$) and E'_{lat} ($P = 0.002$) in RTT vs. controls, while no differences were found in A-wave and E/A ratio. E/E'_{lat} ratio, an indicator of LV filling pressures, was similar in RTT and control subjects (Table 3). E'_{tr} , an indicator of RV diastolic function, was reduced in typical RTT patients vs. controls ($P = 0.029$), while no correlation was found in atypical RTT vs. control and in typical vs. atypical RTT. RVSP ($P < 0.001$) was reduced in RTT patients vs. control.

Oxidative stress markers

All the analysed markers of OS (P-NPBI, IE-NPBI and F₂-IsoPs) were increased in RTT patients compared with healthy controls (Table 4).

However, a correlation between OS and compromised heart function was detectable only for F₂-IsoPs and three LV systolic function parameter (S'_{lat} : CC -0.3696 , $P = 0.008$; S'_{sep} : CC -0.2920 ,

$P = 0.046$; MAPSE: CC -0.3304 , $P = 0.016$), whereas no significant relationship between the three markers and LV diastolic or RV systo-diastolic echocardiographic parameters was found.

Discussion

To the best of our knowledge, our findings demonstrate for the first time the presence of subclinical myocardial dysfunction in patients with RTT, adding a new perspective to what is currently known on non-neurological phenotypes in RTT.

Previous studies have highlighted an increased risk of sudden cardiac death in RTT girls.^{11,12} Prolongation of corrected QT interval in electrocardiogram and cardiac dysautonomia partially account for the increased cardiac mortality risk.^{9,10} In particular, the reduction in a physiological heart rate variability in 24 h ECG ambulatory monitoring has been associated with an increase in adrenergic tone and suggested to be involved in the pathogenesis of lethal ventricular arrhythmias.¹⁰ On the other hand, myocardial function has not been explored in RTT.

In our study, we found a mild to moderate decrease in systolic and diastolic LV and RV longitudinal function in both typical and atypical RTT while the LVEF was preserved. Biventricular dysfunction was subclinical as patients do not appear to present clinical signs evocative for heart failure, NT-proBNP levels are low or normal and the LVEF is preserved.

Previous studies have demonstrated that single LVEF measurement may be normal despite existing mild or moderate systolic dysfunction.²⁷ Analysis of LV longitudinal function by M-mode and TDI has been shown to detect impairments of LV systolic performance more accurately.^{22,24} In this regard, according to the four stage classification, the dysfunction observed in RTT somewhat resembles that of stage B of heart failure, where structural myocardial abnormalities without clinical symptoms or signs of heart failure are developed.²⁷

With regard to diastolic function, the first phase of diastole (i.e. peak early filling) evaluated with PW Doppler (E-wave) and TDI

Table 3 Echocardiographic evaluation in Rett syndrome patients and healthy control subjects (data are means \pm SD)

Variables	RTT		Controls (c), (n = 90)	ANOVA, P-value	Post hoc analysis
	Typical (a), (n = 72)	Atypical (b), (n = 20)			
RA (cm ² /m ²) ^a	7.0 \pm 2.3	8.4 \pm 2.5	8.6 \pm 2.1	<0.001	c \approx b > a
RV (end-diastolic area, cm ² /m ²) ^a	19.9 \pm 3.8	18.8 \pm 3.2	21.2 \pm 2.2	0.002	c > a \approx b
LA (cm ² /m ²) ^a	10.0 \pm 1.3	9.7 \pm 1.2	10.1 \pm 0.7	0.383	NA
LV (end-diastolic volume, mL/m ²) ^a	43.2 \pm 8.1	42.1 \pm 8.3	43.4 \pm 4.1	0.733	NA
S' _{lat} (cm/s)	8.77 \pm 1.79	8.97 \pm 1.57	10.51 \pm 3.03	<0.001	c > a \approx b
S' _{sep} (cm/s)	7.65 \pm 1.66	7.35 \pm 1.41	8.30 \pm 1.11	0.001	c > a \approx b
MAPSE (mm)	13.32 \pm 2.48	13.60 \pm 3.88	15.84 \pm 2.80	<0.001	c > a \approx b
LVEF (%)	62.1 \pm 4.15	61.53 \pm 4.47	62.89 \pm 3.76	0.3730	NA
E (cm/s)	91.7 \pm 16.89	89.37 \pm 12.23	99.43 \pm 11.87	0.003	c > a \approx b
A (cm/s)	51.05 \pm 11.36	52.94 \pm 13.84	54.38 \pm 15.69	0.3730	NA
E/A	1.89 \pm 0.57	1.76 \pm 0.48	1.95 \pm 0.52	0.398	NA
E' _{lat} (cm/s)	15.98 \pm 3.72	16.52 \pm 3.02	18.57 \pm 4.81	0.002	c \approx b > a
E/E'	6.13 \pm 2.16	5.64 \pm 1.18	5.86 \pm 2.31	0.626	NA
S' _{tr} (cm/s)	11.60 \pm 3.03	10.72 \pm 1.71	13.35 \pm 1.87	<0.001	c > a \approx b
TAPSE (mm)	18.54 \pm 3.52	18.70 \pm 3.46	21.74 \pm 4.00	<0.001	c > a \approx b
E' _{tr} (cm/s)	15.19 \pm 6.0	13.67 \pm 2.23	17.25 \pm 3.75	0.029	c \approx b > a
RVSP (mmHg)	19.09 \pm 4.95	18.56 \pm 4.50	23.71 \pm 2.80	<0.001	c > a \approx b

RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle; S'_{lat}, peak systolic velocity of lateral mitral annulus; S'_{sep}, peak systolic velocity of septal mitral annulus; MAPSE, mitral annular plane systolic excursion; LV EF, left ventricle ejection fraction; E, peak early diastolic mitral flow; A, peak late diastolic mitral flow; E'_{lat}, peak early diastolic velocity of lateral mitral annulus; S'_{tr}, peak systolic velocity of tricuspid annulus; TAPSE, tricuspid annular plane systolic excursion; E'_{tr}, peak early diastolic velocity of tricuspid annulus; RVSP, right ventricle systolic pressure; NA, not applicable.

^aRA, RV, LA and LV were normalized to body surface area.

Table 4 Oxidative stress markers in Rett syndrome and healthy controls subjects

Oxidative stress markers	RTT clinical categories				Healthy controls (e), n = 92	ANOVA, P-value	Post hoc analysis
	Typical (a), n = 72	Atypical	ESV (b), n = 8	PSV (c), n = 9			
P-NPBI (nmol/mL)	1.0 \pm 0.5	1.0 \pm 0.1	0.5 \pm 0.2	0.5 \pm 0.08	0.25 \pm 0.2	<0.0001	a \approx b > c \approx d > e
IE-NPBI (nmol/mL)	1.4 \pm 0.8	1.3 \pm 0.45	0.85 \pm 0.32	0.9 \pm 0.18	0.8 \pm 0.4	<0.0001	a \approx b > c \approx d \approx e
F ₂ -IsoPs (pg/mL)	60 \pm 22	59 \pm 10	33 \pm 15	42 \pm 4	24 \pm 5	<0.0001	a \approx b > c \approx d > e

P-NPBI, plasma non-protein-bound iron; IE-NPBI, intra-erythrocyte non-protein-bound; F₂-IsoPs, plasma-free F₂-isoprostanes; ESV, early seizure variant; PSV, preserved speech variant; CV, congenital variant.

velocities (E'_{lat}, E'_{tr}) were significantly decreased in RTT. Diastolic relaxation is an active process, as E-wave is determined by preload and E' is affected by dysfunction in ventricle relaxation. On the other hand, late diastolic phase is mainly affected by ventricular compliance and atrium systolic function;^{21,28} this phase was not altered in RTT patients. E/E' ratio has been shown to correlate with pulmonary capillary wedge pressure (PCWP): E/E' >15 is associated with PCWP >12 mmHg and define an increase in LV end-diastolic pressure.^{23,24} E/E' was superimposable in RTT and controls. Moreover, RVPS is reduced in RTT. Our findings indicate the presence of a chronic reduced preload associated with a longitudinal diastolic dysfunction in RTT.

Besides, baseline clinical assessment of RTT patients evidences increased heart rate and PVI and reduced NT-proBNP and PI (Table 2). PI is an objective indicator of skin perfusion, while PVI measures the dynamic changes in PI during respiratory cycle; then, PI directly and PVI indirectly correlate with peripheral vasoconstriction and intravascular volume status.^{18,19} NT-proBNP is secreted from the cardiac ventricle and is increased in response to ventricular volume expansion and pressure overload;²⁹⁻³¹ it is used as a biomarker in patients with heart failure to evaluate the state of compensation and the degree of ventricular dysfunction and to assess prognosis.³² Therefore, the overall evaluation of clinical and echocardiographic variables indicates the existence

of a previously unrecognized chronically reduced preload in RTT patients.

From a pathophysiological point of view, the reasons for which RTT patients show the observed signs of subclinical myocardial dysfunction remain to be clarified.

A factor that might contribute to myocardial dysfunction in RTT patients is OS.¹⁵ Systemic OS is characterized by an excess of circulating peroxidation products, known to impair processes such as neonatal cardiomyocytes development. F₂-IsoPs are formed by free radical-catalyzed peroxidation of phospholipid-bound arachidonic acid, a cyclooxygenase-independent pathway. F₂-IsoPs are initially formed on phospholipids *in situ*,¹⁴ and subsequently released into the circulation. Owing to their relatively low reactivity, they can be easily measured in biological samples. To date, F₂-IsoPs are considered specific and reliable OS markers *in vivo*.^{25,33} We observed an inverse relationship between F₂-IsoPs levels and LV systolic function variables. As a consequence, an excess of circulating reactive oxygen species may contribute to the LV systolic dysfunction observed in RTT, both typical and atypical. However, OS does not appear to be the major cause of the myocardial dysfunction described here, because not all OS markers correlated with the severity of LV dysfunction and even F₂-IsoPs levels did not correlate well with the degree of global RV or diastolic LV dysfunction.

An unrecognized role of the *MeCP2* gene mutation may be involved in the observed myocardial dysfunction. Although there is no universal consensus regarding the functions of the *MeCP2* gene, which is mutated in the great majority of RTT patients (90–95%),² three major points regarding *MeCP2* protein seem to be well-established: (i) *MeCP2* is a methylated-DNA-binding protein, sharing a common methyl-CpG-binding domain motif; (ii) *MeCP2* is very abundantly expressed in the brain; (iii) through the association with co-repressors, such as Sin3a,³⁴ *MeCP2* protein represses transcription. This prevailing view of *MeCP2* as being a methyl-CpG-binding repressor has been challenged by a recent publication reporting that *MeCP2* is also able to activate transcription in specific brain regions.¹

The mechanisms by which *MeCP2* may affect the cardiac phenotype are still to be explored. Recently, a role for *MeCP2* in heart development and cardiomyocyte maturation has been described. In particular, emerging evidence indicates that *MeCP2* is a developmental regulator involved in the cardiac differentiation, in which co-operative repression of transcription is mediated through DNA methylation and chromatin condensation in the myocardium.^{13,35} Thus, it is possible that an intrinsic structural abnormality may be present in the myocardium of patients with a dysfunctional *MeCP2* protein.

Furthermore, recent studies have demonstrated mitochondrial abnormalities in a mouse model of RTT.³⁶ Likewise, patients with mitochondrial disorders may harbour mutation in the *MeCP2* gene and present symptoms similar to that of RTT patients (hypotonia, small stature, developmental delay).^{37,38} It is, therefore, conceivable that the reduced longitudinal myocardial function in RTT may be referred, at least in part, to a coexisting mitochondrial abnormality.

These data lead to considerations that: (i) preload is chronically reduced in RTT and (ii) subclinical myocardial dysfunction is a

structural intrinsic abnormality of the RTT myocardium which can be partially attributed to enhanced OS.

At this point of knowledge, it cannot be ruled out the possibility that the highlighted myocardial dysfunction could also be related to multiple factors including altered loading, mild hypoxia, and/or autonomic dysfunction or be the consequence of non-cardiac factors, such as breathing disorders and motor dysfunction, with their effects on cardiovascular system. In addition, we have previously reported that chronic alveolar hypoxia is evident in patients with typical RTT, that is at least partly due to the respiratory bronchiolitis and interstitial lung disease detectable at the high resolution CT scans of lungs in about half of typical RTT patients.⁸ Hence, a possible role of chronic hypoxia in subclinical RTT myocardial dysfunction cannot be ruled out at this stage.

There are limitations to our study. Clinical evaluation is naturally hampered by the fact that >90% of RTT patients have no verbal skills and 50% cannot walk; accordingly, the evaluation of NYHA functional class and exercise tolerance is hampered in this particular group of patients with severe developmental regression. Likewise, the echocardiographic evaluation is limited by the lack of patient co-operation in this particular population. However, in spite of these possible technical limitations, intra-observer variability was found to be quite low.

In conclusion, to the best of our knowledge, this is the first echocardiographic evidence for subclinical biventricular dysfunction with preserved LVEF in RTT girls. The findings may resemble those of stage B of heart failure. OS may only partially explain this dysfunction. A chronically reduced preload is present in RTT patients. Our data add a previously unrecognized risk factor to the natural history of RTT, while future investigations are needed to clarify how myocardial dysfunction may affect the clinical course of the RTT natural history and whether additional cardiac risk factors or abnormalities may coexist.

Note

Still unpublished data on the basic mechanisms of Rett syndrome in an experimental mouse model (*MeCP2* null *y/–*) indicate the presence of a mitochondrial dysfunction in the hearts of symptomatic Rett mice, with a complex pattern of respiratory chain defects (we heartily thank Prof. John Christodoulou for the personal communication, as well as his collaborators Dr. Sarah Williamson, Dr. Simranpreet Kaur and Dr. Wendy Gold). Thus, it is conceivable that the subclinical myocardial dysfunction that we observed in Rett girls could indeed be linked to a previously unrecognized mitochondrial abnormality in the cardiomyocytes.

Ethics approval

This study was conducted with the approval of the Institutional Review Board and informed consents were obtained from either the parents for underage subjects or from the subject in case of adult subjects (responsible physician for enrolment: J.H.).

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