OTHERS

Original article

Genotypic groups as risk factors for cardiac magnetic resonance abnormalities and complications in thalassemia major: a large, multicentre study

Laura Pistoia[1], Antonella Meloni[1], Paolo Ricchi[2], Aldo Filosa[2], Roberto Lisi[3], Aurelio Maggio[4], Rosamaria Rosso[5], Giuseppe Messina[6], Nicola Dello Iacono[7], Liana Cuccia[8], Saveria Campisi[9], Massimiliano Missere[10], Massimo Midiri[11], Antonino Vallone[12], Stefania Renne[13], Nicolò Schicchi[14], Riccardo Righi[15], Maurizio Mangione[1], Vincenzo Positano[1], Alessia Pepe[1]

[1]*"G. Monasterio Foundation", National Research Council – Region of Tuscany, Pisa;*
[2]*"A. Cardarelli" Hospital, Naples;*
[3]*"Garibaldi Hospital Centre, Catania;*
[4]*"V. Cervello" Hospital, Palermo;*
[5]*"Vittorio Emanuele" University Hospital, Catania;*
[6]*"Bianchi-Melacrino-Morelli" Hospital, Reggio Calabria;* [7]*"Casa Sollievo della Sofferenza" Research Hospital, San Giovanni Rotondo (FG);*
[8]*"Benfratelli-Di Cristina" Civilian Hospital, Palermo;*
[9]*"Umberto I" Hospital, Siracusa;*
[10]*"Giovanni Paolo II" Research Hospital", Campobasso;*
[11]*"Paolo Giaccone" Polyclinic, Palermo;*
[12]*"Garibaldi" Hospital Nesima, Catania;*
[13]*"Giovanni Paolo II" Hospital, Lamezia Terme;*
[14]*"Umberto I-Lancisi-Salesi" Combined University Hospital, Ancona;*
[15]*"Delta" Hospital, Lagosanto (FE); Italy*

Background - Background. The causes and effects of genotypic heterogeneity in beta-thalassemia major (β-TM) have not been fully investigated. The aim of this multicentre study was to determine whether different genotype groups could predict the development of cardiovascular magnetic resonance abnormalities and cardiac complications.

Materials and methods - We considered 708 β-TM patients (373 females, age 30.05±9.47 years) consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) network. Data were collected from birth to the first cardiac magnetic resonance scan. Myocardial iron overload was assessed using a T2* technique. Biventricular function was quantified by cine images. Macroscopic myocardial fibrosis was evaluated by a late gadolinium enhancement technique.

Results - Three groups of patients were identified: β^+ homozygotes (n=158), β^+/β° heterozygotes (n=298) and β° homozygotes (n=252). Compared to β^+ homozygotes, the other two groups showed a significantly higher risk of myocardial iron overload and left ventricular dysfunction. We recorded 90 (13.0%) cardiac events: 46 episodes of heart failures, 38 arrhythmias (33 supraventricular, 3 ventricular and 2 hypokinetic) and 6 cases of pulmonary hypertensions. β° homozygotes showed a significantly higher risk than β^+ homozygotes of arrhythmias and cardiac complications considered globally.

Discussion - Different genotype groups predicted the development of myocardial iron overload, left ventricular dysfunction, arrhythmias and cardiac complications in β-TM patients. These data support the importance of genotype knowledge in the management of β-TM patients.

Keywords: *beta-thalassemia, genotype, magnetic resonance imaging, prognosis.*

INTRODUCTION

Heart disease is the primary cause of mortality and morbidity in β-thalassemia major (β-TM) patients, despite the survival of these patients having improved in the last decades[1]. Cardiac iron measured by T2-star ($T2^*$) cardiovascular magnetic resonance (MR) is one of the strongest predictors of cardiac complications[2,3]. $T2^*$ cardiac MR is a non-invasive

Arrived: 23 January 2020
Revision accepted: 28 April 2020
Correspondence: Alessia Pepe
e-mail: alessia.pepe@ftgm.it

technique able to quantify cardiac iron loading with a high reproducibility[4]. This technique allows constant monitoring of cardiac iron deposition, enabling chelation therapy to be tailored[5]. Different patterns of cardiac iron overload have been identified among β-TM patients and have been related to different clinical outcomes[6]. Moreover, cardiac MR is the gold standard method in cardiology for quantifying biventricular volumes and function with excellent reproducibility[7].

The variability observed in clinical manifestations as regards cardiac iron overload patterns, different degrees of heart dysfunction, and cardiac complications, could be partially explained by the wide heterogeneity observed at the molecular level. More than 200 disease-causing mutations affecting the beta globin gene (*HBB*) have been identified and a spectrum of different genotypes has been found among β-TM patients[8,9]. β-TM is characterised by the absence (β^0) or reduced output (β^+) of the β chains of haemoglobin. The clinical severity of the disease depends on the degree of imbalance between the α and non-α globin chains in red cell precursors, which causes abnormal maturation of the red blood cells and their destruction in the bone marrow (ineffective erythropoiesis). The main determinant of the degree of α/non-α chain imbalance is the type of gene mutation[10]. Nevertheless, the correlation between genotype and phenotype is extremely complex, because other genetic and environmental factors interact with the different allelic variants[11]. The wide spread of molecular techniques has led to increasing interest in studying the β globin genes, improving the diagnosis of and clinical approach to patients with β-TM.

The aim of this multicentre study was to assess, through a retrospective analysis, whether different genotype groups could predict the development of cardiac MR abnormalities and cardiac complications.

MATERIALS AND METHODS

Patients

We studied seven hundred and eight β-TM patients (335 males/373 females, mean age 30.05±9.5 years), consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) project. MIOT is an Italian network constituted by ten MR imaging sites and 66 thalassemia centres, in which cardiac MR examinations are performed using homogeneous, standardised and validated procedures[4,12]. All the MIOT centres share a web-connected, centralised database, into which clinical and instrumental data are collected from birth to the latest MR examinations[12]. All patients were regularly transfused to maintain a pre-transfusion haemoglobin concentration above 9-10 g/dL. MR imaging was performed within the week preceding the regular, scheduled blood transfusion. Compliance with chelation therapy was determined by the investigators at each thalassaemia centre and, based on the correlation between the time history of drug administration and the prescribed regimen, was defined as excellent (>80%), good (60-80%) or insufficient (<60%). The study complied with the Declaration of Helsinki. Informed consent to inclusion in the study was obtained from all patients. The institutional ethics committees approved this study.

Sample collection and analysis and genotype characterisation

Blood samples from all 708 β-TM patients were collected into ethylenediaminetetra-acetic acid (EDTA) for DNA extraction. Genomic DNA was extracted from peripheral blood leucocytes using the salting-out method[13]. All coding and non-coding regions of the β globin gene were amplified by polymerase chain reaction (PCR) in different fragments ranging from 200 bp to 13.4 kb and partially overlapped. The PCR conditions were different, depending on the protocol used by the laboratory of the thalassemia centre in which the patient was treated.

β thalassemia mutations were identified by reverse hybridisation assay (β-globin strip assay, Nuclear Laser, Vienna Lab, Austria).

Magnetic resonance image acquisition and analysis

MR imaging was performed with 1.5 T scanners (GE, Siemens and Philips), using an eight-element cardiac phased-array receiver surface coil with breath-holding at end-expiration and electrocardiographic gating.

The T2* technique was used to assess iron overload. A multislice approach was used for the heart. Three parallel short-axis views (basal, medium, and apical) of the left ventricle (LV) were obtained at nine echo times[14,15]. For the liver, a mid-transverse slice was obtained at nine echo times using a T2* gradient-echo multiecho sequence[16]. T2* images were analysed using custom-written, previously validated software **(HIPPOMIOT®, Company?, Town?, State?)**[17]. The software provided the T2* value for all the 16

segments of the LV, according to the standard American Heart Association (AHA)/American College of Cardiology (ACC) model[18]. The global heart T2* value was obtained by averaging all segmental values. For the liver, the T2* values were calculated in a circular region of interest[19] and were converted into liver iron concentration using Wood's calibration curve[20,21].

For the quantification of biventricular functional parameters, steady-state free precession cines were acquired in sequential 8 mm short-axis slices. Images were analysed using MASS® software (Medis, Leiden, the Netherlands). Left and right atrial areas were measured from the four-chamber view in the ventricular end-systolic phase. The inter-centre variability for the quantification of cardiac function had been previously reported[22].

To detect the presence of macroscopic myocardial fibrosis, short-axis images with late gadolinium enhancement were acquired 10-18 min after intravenous administration of gadobutrol (Gadovist®; Bayer Schering Pharma; Berlin, Germany) at the standard dose of 0.2 mmoL/kg. Vertical, horizontal, and oblique long-axis views were also acquired. Late gadolinium enhancement images were not acquired from patients with a glomerular filtration rate <30 mL/min/1.73 m^2 or from patients who refused this investigation. Late gadolinium enhancement was considered present when visualised in two different views[23,24].

Timing and diagnostic criteria

Patients' data were recorded from birth to the first cardiac MR imaging scan. Only the first cardiac complication was taken into account for each patient.

For all cardiac complications, the study end date was the date of the onset of the complication. If no cardiac complication occurred, the date of MR imaging was considered the end of the follow-up. As regards MR outcomes (heart and liver iron, ventricular dysfunction and macroscopic fibrosis), the date of the MR scan was considered the end of the follow-up.

Heart failure was diagnosed by clinicians based on symptoms, signs and instrumental findings according to AHA/ACC guidelines[25]. Pulmonary hypertension was diagnosed if the trans-tricuspid velocity jet was greater than 3,2 m/s[26]. Arrhythmias were diagnosed if documented by electrocardiography or 24-h Holter electrocardiography if requiring specific medications. Arrhythmias were classified according to the AHA/ACC guidelines[27].

The term "cardiac complications" comprised heat failure, arrhythmias and pulmonary hypertension.

A T2* measurement of 20 ms was taken as a "conservative" normal value[17,28].

LV and right ventricular (RV) dysfunction were diagnosed, respectively, in the presence of LV and/or RV ejection fraction <1 standard deviations (SD) from the mean values normalised to age and gender. LV and RV dilation were diagnosed, respectively, in the presence of LV and/or RV end systolic volume (ESV) >2 SD from the mean values normalised to age and gender[29]. Left and right atrial dilation were diagnosed if left and right atrial areas were greater than 15 cm^2/m^2 [30].

A MR liver iron concentration ≥3 mg/g/dry weight was considered indicative of significant iron load[31].

STATISTICAL ANALYSIS

All data were analysed using SPSS version 13.0 (IBM SPSS Statistics, Chicago, IL, USA). Continuous variables are described as means ± SD and categorical variables are expressed as frequencies and percentages.

The Cox proportional-hazard model was used to test the association between the considered prognostic variable (genotype) and the outcomes. All the continuous prognostic variables were made discrete by grouping patients into subcategories. The results are presented as hazard ratios (HR) with 95% confidence intervals (95% CI). Kaplan-Meier curves were generated by relating the development of an outcome over time to each significant prognosticator. The log-rank test was used to compare different strata in the Kaplan-Meier analysis. We did not apply a correction model for the variables significantly different among the groups because they were not associated to dependent variable. A two-tailed probability value of 0.05 was considered statistically significant.

RESULTS

Patients' characteristics

The demographic and clinical characteristics of the patients in the three genotypic groups are summarised in **Table I**. The mean duration of the observation period was 29.55±9.41 years. The time interval between the cardiac complication and first cardiac MR was 4.08±5.62 years.

Table I - *Patients' characteristics*
Demographic and clinical characteristics in the three groups. Continuous variables are presented as mean ± standard deviation

Parameters/variables	β⁺/β⁺ (n=158)	β°/β⁺ (n=298)	β°/β° (n=252)	P
Sex (M/F)	81/77	143/155	111/141	0.346
Hb pre-transfusion in the last 12 months before CMR (g/dL)	9.71±1.00	9.64±0.58	9.61±0.63	0.498
Ferritin levels in the last 12 months before CMR (ng/L)	1,535.66±1,239.79	1,566.14±1,469.43	1,491.42±1,362.36	0.859
Age of starting regular transfusion (years)	2.62±4.87	2.14±3.20	2.58±4.55	0.297
Units transfused in the last 12 months before CMR	34.65±11.15	37.92±12.63	41.48±12.73	<0.001
Age at occurrence of first cardiac complication (years)	29.36±12.57	29.39±7.76	31.36±8.23	0.578
Chelation starting age (years)	5.00±4.38	5.16±5.66	4.37±3.62	0.196
Chelation therapy at CMR, None, n (%)	0/154 (0.0)	1/284 (0.4)	1/233 (0.4)	0.733
Chelation therapy at CMR, DFO, N, (%)	55/154 (35.7)	93/284 (32.6)	80/233 (34.2)	0.803
Chelation therapy at CMR, DFP, N, (%)	38/154 (24.7)	49/284 (17.2)	42/233 (17.9)	0.138
Chelation therapy at CMR, DFX, N, (%)	38/154 (24.7)	74/284 (26.0)	49/233 (20.9)	0.398
Chelation therapy at CMR, Combined DFO+DFP, n (%)	17/154 (11.0)	51/284 (17.9)	47/233 (20.1)	0.061
Chelation therapy at CMR, Sequential DFO/DFP, n (%)	5/154 (4.0)	15/284 (5.9)	15/233 (6.5)	0.389
Chelation therapy at CMR, Sequential DFO/DFX, N, (%)	1/154 (0.6)	1/284 (0.4)	0/233 (0.0)	0.504
Chelation therapy at CMR, Sequential DFP/DFX, n (%)	0/154 (0.0)	1/284 (0.4)	0/233 (0.0)	0.506
Excellent/good compliance to chelation therapy (%)	92	89.2	93.2	0.264

M: male; F: female; CMR: cardiac magnetic resonance; DFO: desferrioxamine; DFP: deferiprone; DFX: deferasirox.

We recorded 35 different genotypes among the β-TM patients. The commonest genotypes were CD39 homozygosity (*HBB*: c.118C>T/*HBB*: c.118C>T) (23.3%) and CD39/IVS-1,110 heterozygosity (*HBB*: c.118C>T/*HBB*: c.93-21G>A) (16,1%). Each allele belonging to a genotype was classified according to the corresponding phenotypic expression (β⁺ or β°) and three groups of patients were identified: β⁺ homozygotes (n=158), β⁺/β° compound heterozygotes (n=298), and β° homozygotes (n=252).

Due to a lack of collaboration, cardiac function was not assessed in 73 patients: 20 (12.7%) in the β⁺/β⁺ group, 29 (9.7%) in the β⁺/β° group, and 24 (9.5%) in the β°/β° group, p=0.544). For technical reasons, bi-atrial areas were known for only 531 patients. Mean end-systolic volumes indexed of the LV and RV at the study end date were 87.03±17.84 and 82.73±17.72 ml/m², respectively. Mean left and right atrial areas were 13.18±2.72 and 12.21±2.41 cm²/m², respectively. One hundred and twenty-nine (20.3 %) patients refused the administration of the contrast medium: 35 (25.4%) in the β+/β+ group, 58 (21.6%) in the β⁺/β° group, and 36 (15.8%) in the β°/β° group; p=0.07).

Influence of genotype on cardiac outcomes

Table II shows the effect of genotype group on the development of different cardiac MR abnormalities. The homozygous β° and heterozygous β⁺/β° groups showed a significantly higher risk of myocardial iron overload and LV dysfunction, compared to the homozygotes β+ group. No prospective association was detected between genotype group and macroscopic myocardial fibrosis and bi-ventricular and bi-atrial dilation.

We recorded 90 (13.0 %) cardiac events: 46 cases of heart failure, 38 arrhythmias (33 supraventricular, 3 ventricular and 2 hypokinetic) and six cases of pulmonary hypertension. **Table III** shows the influence of genotype on cardiac complications. Patients in all genotype groups had a similar risk of developing heart failure and pulmonary hypertension. The homozygous β° group showed a significantly higher risk of arrhythmias than the homozygous β⁺ group and a higher risk, at the limit of significance, than the compound heterozygotes. Globally, β° homozygotes showed a significantly higher risk than β⁺ homozygotes of cardiac complications. **Figure 1** illustrates the Kaplan-Meier curves for cardiac complications, showing that genotype was a significant prognosticator. The log-rank test revealed a significant difference in both curves (genotype group predicting arrhythmias: p=0.001; genotype group predicting cardiac complications: p=0.021).

DISCUSSION

This multicentre study aimed to evaluate different genotypic groups as a risk factor for the development of cardiac MR abnormalities and cardiac complications. Given the increasing availability of molecular analysis techniques, there is a growing interest in the identification of genetic factors that could predict patients' phenotypic characteristics[32,33].

To our knowledge, there are a few studies that compare β° and β⁺ thalassemia. A previous study by Sagar *et al*.[33] conducted on regularly transfused β thalassemia patients showed that iron-induced toxicity, as indicated by DNA damage, was greater in β° homozygotes than in the heterozygotes or β⁺ homozygotes. They attributed the higher level of DNA damage of β° homozygotes to the higher α/non-α globin chain imbalance. A recent cross-sectional study conducted on β-TM patients compared cardiac iron overload, detected by T2* MR imaging, in different genotypic groups[34]. The study showed significantly lower global and segmental heart iron burden in β+ homozygotes than in compound β⁺/β° heterozygotes and β° homozygotes and a concordant better left systolic heart function. Moreover, patients in the homozygous β° group showed significantly greater need for transfusions, when compared with the milder groups. Accordingly, in the present study we confirmed that the homozygous β° and heterozygous β⁺/β° groups showed a higher risk of myocardial iron overload and left ventricular dysfunction, compared to the milder genotype group. This finding suggests that patients in the three groups could have different degrees of anaemia during their life, requiring different transfusion volumes that expose them to different risks of myocardial iron overload and dysfunction, despite similar ages of starting transfusions.

Homozygous β° genotype group emerged as a risk factor for the development of cardiac arrhythmias (particularly supraventricular) and cardiac complications considered globally. It is known that atrial arrhythmias may result from past damage caused by high cardiac output or cardiac iron load[35]. The higher arrhythmic risk observed in our study could, therefore, be due to the more severe anaemia and subsequent high cardiac output state of β°/β° TM patients since birth when compared to the milder forms[36,37]. In any case, in our cohort of well-transfused patients at the time of their first MR imaging scan in adult age, the stigmata of hyperkinetic circulation were attenuated and this could explain why the three genotypic groups showed similar risks of atrial and ventricular dilation at the end of the follow up by MR imaging. Moreover, differences in the risk of arrhythmias could be explained by the different risks of myocardial iron accumulation in the three groups. Historical histological cardiac data showed that supraventricular arrhythmias were correlated with the extent of iron deposits in atrial myocardium[38]. Unfortunately, at the time of the study, it was not possible to have a non-invasive atrial tissue characterisation in terms of cardiac iron and fibrosis due to technical constraints. However, in the now widespread cardiac MR era cardiac iron has been demonstrated to be a significant prognosticator for arrhythmias (most of which are supraventricular)[3].

Table II - *Risk of CMR abnormalities*
Cox proportional hazards model for the effect of genotype group on different CMR abnormalities

	N (%) with positive outcome	Cox Regression	
		HR (95% CI)	P
Global heart T2*<20 ms:			
$\beta^+\beta^+$ Genotype group	24 (15.2)	Reference	
$\beta^+\beta^0$ Genotype group	78 (26.2)	1.8 (1.14-2.85)	0.012
$\beta^0\beta^0$ Genotype group	83 (32.9)	1.92 (1.26-3.02)	0.005
Left ventricular dysfunction:			
$\beta^+\beta^+$ Genotype group	15/138 (10.9)	Reference	
$\beta^+\beta^0$ Genotype group	55/269 (20.4)	1.82 (1.03-3.23)	0.040
$\beta^0\beta^0$ Genotype group	53/228 (23.2)	1.80 (1.02-3.20)	0.044
Right ventricular dysfunction:			
$\beta^+\beta^+$ Genotype group	17/138 (12.3)	Reference	
$\beta^+\beta^0$ Genotype group	51/269 (19.0)	1.50 (0.86-2.60)	0.150
$\beta^0\beta^0$ Genotype group	42/228 (18.4)	1.25 (0.71-2.20)	0.432
Left ventricular dilation:			
$\beta^+\beta^+$ Genotype group	4/138 (2.9)	Reference	
$\beta^+\beta^0$ Genotype group	15/269 (5.6)	1.91 (0.63-5.76)	0.250
$\beta^0\beta^0$ Genotype group	10/228 (4.4)	1.29 (0.40-4.11)	0.671
Right ventricular dilation:			
$\beta^+\beta^+$ Genotype group	7/138 (5.1)	Reference	
$\beta^+\beta^0$ Genotype group	15/269 (5.6)	1.13 (0.46-2.78)	0.787
$\beta^0\beta^0$ Genotype group	7/228 (3.1)	0.52 (0.18-1.49)	0.224
Left atrial dilation:			
$\beta^+\beta^+$ Genotype group	23/106 (21.7)	Reference	
$\beta^+\beta^0$ Genotype group	40/225 (17.8)	0.70 (0.42-1.18)	0.181
$\beta^0\beta^0$ Genotype group	46/202 (22.8)	0.82 (0.50-1.36)	0.445
Right atrial dilation:			
$\beta^+\beta^+$ Genotype group	8/106 (7.5)	Reference	
$\beta^+\beta^0$ Genotype group	24/225 (10.7)	1.28 (0.57-2.85)	0.553
$\beta^0\beta^0$ Genotype group	21/202 (10.4)	1.12 (0.49-2.52)	0.794
Myocardial fibrosis:			
$\beta^+\beta^+$ Genotype group	15/103 (14.6)	Reference	
$\beta^+\beta^0$ Genotype group	40/211 (19.0)	1.10 (0.60-1.99)	0.762
$\beta^0\beta^0$ Genotype group	39/192 (20.3)	1.09 (0.60-1.98)	0.772

Table III - *Risk of cardiac complications*
Cox proportional hazards model for the effect of genotype group on different cardiac complications

	N (%) with positive outcome	Cox Regression	
		HR (95% CI)	P
Heart failure:			
β⁺β⁺ Genotype group	10 (6.3)	Reference	
β⁺β⁰ Genotype group	17 (5.7)	1.32 (0.59-2.94)	0.497
β⁰β⁰ Genotype group	19 (7.5)	0.97 (0.44-2.13)	0.941
Arrhythmias:			
β⁺β⁺ Genotype group	1 (0.6)	Reference	
β⁺β⁰ Genotype group	13 (4.4)	7.45 (0.97-57.00)	0.053
β⁰β⁰ Genotype group	24 (9.5)	15.05 (2.04-111.29)	0.008
Pulmonary hypertension:			
β⁺β⁺ Genotype group	1 (0.6)	Reference	
β⁺β⁰ Genotype group	3 (1.0)	2.03 (0.21-19.52)	0.541
β⁰β⁰ Genotype group	2 (0.8)	1.31 (0.12-14.48)	0.825
Cardiac complications:			
β⁺β⁺ Genotype group	12 (7.6)	Reference	
β⁺β⁰ Genotype group	33 (11.1)	1.57 (0.81-3.04)	0.182
β⁰β⁰ Genotype group	45 (17.9)	2.30 (1.22-4.35)	0.010

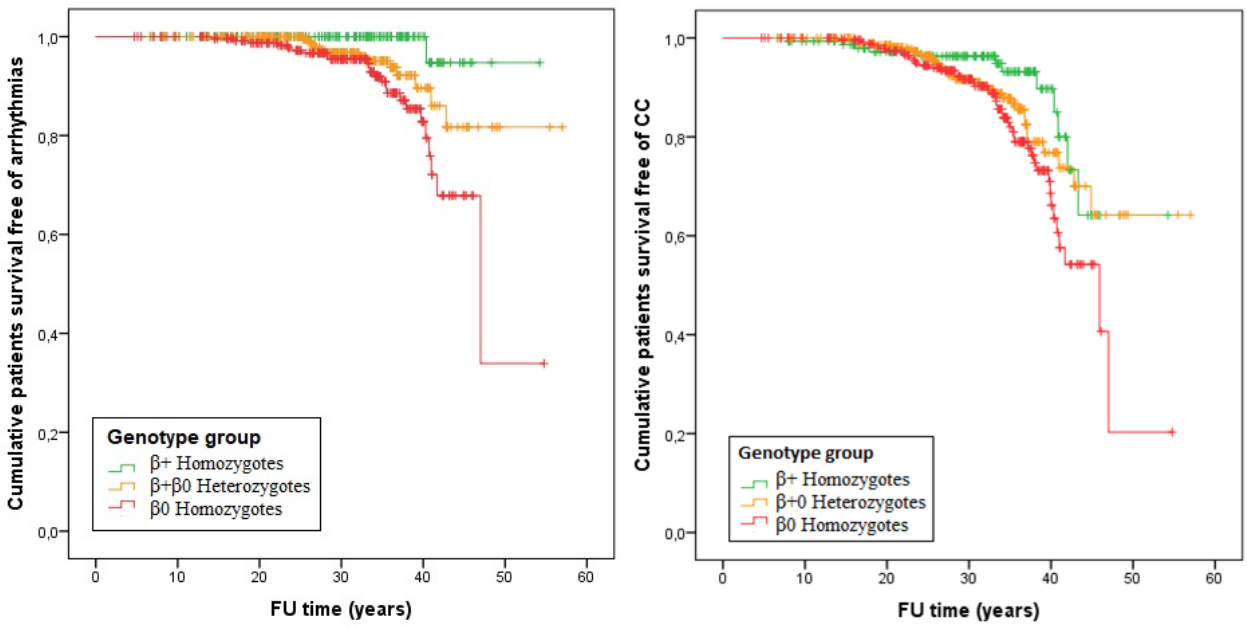

Figure 1 - Kaplan-Meier curves showing the impact of genotype group on the development of arrhythmias and cardiac complications (CC)

Although cardiac MR T2* studies have demonstrated that cardiac iron is correlated with heart failure[2,3], we did not find differences in the risk of heart failure between the three genotypic groups, probably due to the more accurate monitoring of iron burden and tailoring of consequent chelation therapy in preventing heart failure in the last two decades[2,39]. We could speculate that atria maintain a stronger memory respect to the ventriculi regarding damage related to the higher risk of myocardial iron overload and left ventricular dysfunction in the homozygous β°group Furthermore, iron overload cardiomyopathy is slowly progressive and clinical symptoms occur late in the disease process[40] and the pathophysiology of thalassaemic cardiomyopathy is complex, multifactorial and not only related to a direct effect of myocardial iron infiltration[22,41].

LIMITATIONS

A limitation of this study is that data about modifier genes, which can either ameliorate the disease phenotype or increase its severity, are not available. The second limitation is that, although patients in the three genotype groups showed no significant differences in pre-transfusion haemoglobin and ferritin concentrations, type of chelator used and therapy compliance in the last 12 months before the MR imaging, they could have experienced a different exposure to iron toxicity during their life. Moreover, with regards to iron quantification, there are no MR imaging data available before the first MR reported in the study.

CONCLUSIONS

In conclusion, a β° homozygous genotype could be a major determinant in the development of myocardial iron overload, left ventricular dysfunction, arrhythmias and cardiac complications in patients with β-TM. Our findings highlight that the heterogeneity of the molecular background of β-TM could be responsible for the clinical variability. Despite the complex relationship between genotype and phenotype, the knowledge of genotypic group in relation to the seriousness of the α/non-α globin chain imbalance could help in the clinical management of β-TM patients.

ACKNOWLEDGEMENTS

We would like to thank all the colleagues involved in the MIOT project (https: //miot.ftgm.it/). We thank Claudia Santarlasci for her skilful secretarial work and all patients for their cooperation.

FUNDING AND RESOURCES

The MIOT project receives non-profit support from industrial sponsorships (Chiesi Farmaceutici S.p.A., ApoPharma Inc. and Bayer S.p.A).

AUTHORSHIP CONTRIBUTIONS

LP and AP conceived the study and wrote the paper. AM contributed to the interpretation of the results and data analysis. PR, AF, RL, AM, RR, GM, NDL, LC, SC, MAS MIS, MAS MID, AV, SR, NS and RR collected the data. AP, VP, MAU and MAG were responsible for data collection. All authors contributed to the critical revision and final approval of the version to be published.

The Authors declare no conflicts of interest.

REFERENCES

1. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 2004; **89**: 1187-93.
2. Pepe A, Meloni A, Rossi G, et al. Prediction of cardiac complications for thalassemia major in the widespread cardiac magnetic resonance era: a prospective multicentre study by a multi-parametric approach. Eur Heart J Cardiovasc Imaging 2018; **19**: 299-309.
3. Kirk P, Roughton M, Porter JB, et al. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. Circulation 2009; **120**: 1961-8.
4. Ramazzotti A, Pepe A, Positano V, et al. Multicenter validation of the magnetic resonance T2* technique for segmental and global quantification of myocardial iron. J Magn Reson Imaging 2009; **30**: 62-8.
5. Pepe A, Meloni A, Capra M, et al. Deferasirox, deferiprone and desferrioxamine treatment in thalassemia major patients: cardiac iron and function comparison determined by quantitative magnetic resonance imaging. Haematologica 2011; **96**: 41-7.
6. Meloni A, Restaino G, Borsellino Z, et al. Different patterns of myocardial iron distribution by whole-heart T2* magnetic resonance as risk markers for heart complications in thalassemia major. Int J Cardiol 2014; **177**: 1012-9.
7. Aquaro GD, Camastra G, Monti L, et al. Reference values of cardiac volumes, dimensions, and new functional parameters by MR: a multicenter, multivendor study. J Magn Reson Imaging 2017; **45**: 1055-67.
8. Refaldi C, Cerino M, Bignamini D, Volpato E, Cassinerio E, Cappellini MD. Molecular basis of β-thalassaemias in Italy. Blood Transfus 2005; **3**: 136-43.
9. Rigoli L, Meo A, Miceli MR, et al. Molecular analysis of beta-thalassaemia patients in a high incidence area of southern Italy. Clin Lab Haematol 2001; **23**: 373-8.
10. Cao A, Galanello R. Beta-thalassemia. Genet Med 2010; **12**: 61-76.
11. Thein SL. Genetic modifiers of beta-thalassemia. Haematologica 2005; **90**: 649-60.
12. Meloni A, Ramazzotti A, Positano V, et al. Evaluation of a web-based network for reproducible T2* MRI assessment of iron overload in thalassemia. Int J Med Inform 2009; **78**: 503-12.
13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; **16**: 1215.

14. Pepe A, Positano V, Santarelli MF, et al. Multislice multiecho T2* cardiovascular magnetic resonance for detection of the heterogeneous distribution of myocardial iron overload. J Magn Reson Imaging 2006; **23**: 662-8.
15. Meloni A, Positano V, Pepe A, et al. Preferential patterns of myocardial iron overload by multislice multiecho T*2 CMR in thalassemia major patients. Magn Reson Med 2010; **64**: 211-9.
16. Positano V, Salani B, Pepe A, et al. Improved T2* assessment in liver iron overload by magnetic resonance imaging. Magn Reson Imaging 2009; **27**: 188-97.
17. Positano V, Pepe A, Santarelli MF, et al. Standardized T2* map of normal human heart in vivo to correct T2* segmental artefacts. NMR Biomed 2007; **20**: 578-90.
18. Cerqueira MD, Weissman NJ, Dilsizian V, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Circulation 2002; **105**: 539-42.
19. Meloni A, Luciani A, Positano V, et al. Single region of interest versus multislice T2* MRI approach for the quantification of hepatic iron overload. J Magn Reson Imaging 2011; **33**: 348-55.
20. Wood JC, Enriquez C, Ghugre N, et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. Blood 2005; **106**: 1460-5.
21. Meloni A, Rienhoff HY, Jr., Jones A, Pepe A, Lombardi M, Wood JC. The use of appropriate calibration curves corrects for systematic differences in liver R2* values measured using different software packages. Br J Haematol 2013; **161**: 888-91.
22. Marsella M, Borgna-Pignatti C, Meloni A, et al. Cardiac iron and cardiac disease in males and females with transfusion-dependent thalassemia major: a T2* magnetic resonance imaging study. Haematologica 2011; **96**: 515-20.
23. Pepe A, Positano V, Capra M, et al. Myocardial scarring by delayed enhancement cardiovascular magnetic resonance in thalassaemia major. Heart 2009; **95**: 1688-93.
24. Meloni A, Favilli B, Positano V, et al. Safety of cardiovascular magnetic resonance gadolinium chelates contrast agents in patients with hemoglobinopathies. Haematologica 2009; **94**: 1625-7.
25. Jessup M, Abraham WT, Casey DE, et al. 2009 focused update: ACCF/AHA guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. Circulation 2009; **119**: 1977-2016.
26. Cogliandro T, Derchi G, Mancuso L, et al. Guideline recommendations for heart complications in thalassemia major. J Cardiovasc Med (Hagerstown) 2008; **9**: 515-25.
27. Buxton AE, Calkins H, Callans DJ, et al. ACC/AHA/HRS 2006 key data elements and definitions for electrophysiological studies and procedures: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Data Standards (ACC/AHA/HRS Writing Committee to Develop Data Standards on Electrophysiology). J Am Coll Cardiol 2006; **48**: 2360-96.
28. Anderson LJ, Holden S, Davis B, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 2001; **22**: 2171-9.
29. Meloni A, Righi R, Missere M, et al. Biventricular reference values by body surface area, age, and gender in a large cohort of well-treated thalassemia major patients without heart damage using a multiparametric CMR approach. J Magn Reson Imaging 2020; In press.
30. Maceira AM, Cosin-Sales J, Roughton M, et al. Reference left atrial dimensions and volumes by steady state free precession cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2010; **12**: 65.
31. Angelucci E, Brittenham GM, McLaren CE, et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med 2000; **343**: 327-31.
32. Ricchi P, Ammirabile M, Spasiano A, et al. Extramedullary haematopoiesis correlates with genotype and absence of cardiac iron overload in polytransfused adults with thalassaemia. Blood Transfus 2014; **12** (Suppl 1): s124-30.
33. Sagar CS, Kumar R, Sharma DC, Kishor P. DNA damage: beta zero versus beta plus thalassemia. Ann Hum Biol 2015; **42**: 585-8.
34. Pistoia L, Meloni A, Salvadori S, et al. Cardiac involvement by CMR in different genotypic groups of thalassemia major patients. Blood Cells Mol Dis 2019; **77**: 1-7.
35. Aessopos A, Berdoukas V. Cardiac function and iron chelation in thalassemia major and intermedia: a review of the underlying pathophysiology and approach to chelation management. Mediterr J Hematol Infect Dis 2009; **1**: e2009002.
36. Mozos I. Mechanisms linking red blood cell disorders and cardiovascular diseases. BioMed Res Int 2015; **2015**: 682054.
37. Russo V, Rago A, Papa AA, Nigro G. Electrocardiographic presentation, cardiac arrhythmias, and their management in beta-thalassemia major patients. Annals of noninvasive electrocardiology: the official journal of the International Society for Holter and Noninvasive Electrocardiology. Inc 2016; **21**: 335-42.
38. Buja LM, Roberts WC. Iron in the heart. Etiology and clinical significance. Am J Med 1971; **51**: 209-21.
39. Pepe A, Meloni A, Pistoia L, et al. MRI multicentre prospective survey in thalassaemia major patients treated with deferasirox versus deferiprone and desferrioxamine. Br J Haematol 2018; **183**: 783-95.
40. Pennell DJ, Udelson JE, Arai AE, et al. Cardiovascular function and treatment in beta-thalassemia major: a consensus statement from the American Heart Association. Circulation 2013; **128**: 281-308.
41. Kremastinos DT, Farmakis D. Iron overload cardiomyopathy in clinical practice. Circulation 2011; **124**: 2253-63.