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Lamin A/C mutation is independently associated with an increased risk of arterial and venous thromboembolic complications ☆

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ABSTRACT

Background: Lamin A/C (*LMNA*) mutation carriers suffer from a variety of clinical phenotypes, including dilated cardiomyopathy (DCM). Although it has been suggested that carriers are at risk for thromboembolic complications, it is unknown whether this risk is higher than can be expected from the underlying cardiac abnormalities. The purpose of this study was to determine whether a *LMNA* mutation is associated with an increased risk of thromboembolic complications.

Methods: We compared a cohort of 76 *LMNA* mutation carriers with a cohort of 224 idiopathic DCM patients without a *LMNA* mutation, with respect to the prevalence of arterial and venous thromboembolic complications. Furthermore, we carried out a case–control study to explore whether a prothrombotic phenotype was present in *LMNA* mutation carriers without DCM or atrial tachyarrhythmias ($n=14$) and compared this with mutation negative relatives ($n=13$).

Results: The prevalence of thromboembolic complications was higher in the cohort of *LMNA* mutation carriers than in DCM patients (22 vs 11%; $p<0.05$), after respectively mean follow-up of 42 ± 12 and 49 ± 12 years. After adjustment for possible confounders, including atrial tachyarrhythmias and left ventricular ejection fraction, *LMNA* mutation carriership was independently associated with an increased risk of thromboembolic complications (HR 4.8, 95% CI: 2.2–10.6). The results of the case–control study suggested a prothrombotic phenotype in *LMNA* mutation carriers, as reflected by an altered platelet function and increased thrombin generation.

Conclusions: LMNA mutation is independently associated with an increased risk of arterial and venous thromboembolic complications. Laboratory research in *LMNA* mutation carriers without severe cardiac abnormalities suggests a prothrombotic phenotype.

1. Introduction

The *LMNA* gene encodes intermediate filament proteins lamin A and lamin C, which are components of the nuclear lamina [1]. Mutations in *LMNA* are related to more than a dozen different phenotypes, collectively described as laminopathies [2].

The majority of the pathogenic mutations in *LMNA* result in cardiac abnormalities, with or without muscular dystrophy [3,4]. The cardiac phenotype is characterized by conduction disorders, atrial and

Abbreviations: AA, arachidonic acid; AV-block, atrioventricular block; CI, confidence interval; DCM, dilated cardiomyopathy; ICD, implantable cardioverter defibrillator; HR, hazard ratio; *LMNA*, lamin A/C gene; LVEF, left ventricular ejection fraction; MPV, mean platelet volume; SD, standard deviation; TRAP, thrombin receptor activating peptide.

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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ventricular arrhythmias, and dilated cardiomyopathy (DCM) [5]. The ventricular arrhythmias and DCM are often severe, and result in a poor prognosis of individuals carrying a *LMNA* mutation [6,7]. Apart from the cardiac morbidity an increased risk of thromboembolic complications has been suggested in anecdotal reports [8,9]. These reports, with a modest number of *LMNA* mutation carriers, are however inconclusive since it is unknown whether the observed events are higher than expected based on the cardiac abnormality (e.g. atrial fibrillation and/or DCM) per se [10–12].

Therefore, the aim of the present study was to determine whether a *LMNA* mutation is associated with an increased risk of thromboembolic complications, both arterial and venous. We carried out two different studies to investigate this. First, a cohort of *LMNA* mutation carriers was compared with a cohort of idiopathic DCM patients without a *LMNA* mutation, to verify whether a *LMNA* mutation is independently associated with an increased risk of thromboembolic complications. Secondly, we explored whether a prothrombotic phenotype was present in *LMNA* mutation carriers compared with mutation negative relatives.

2. Methods

2.1. Study designs

We carried out two different studies, i.e. a cohort and a case–control study.

2.2. Cohort study

The cohort study was a retrospective observational study, comparing a cohort of *LMNA* mutation carriers with a cohort of idiopathic DCM patients.

2.2.1. Cohort of LMNA mutation carriers

All consecutive individuals (probands and relatives) diagnosed with a pathogenic cardiac disease causing *LMNA* mutation, between January 2000 and December 2010, from two referral centers (the Academic Medical Center Amsterdam and the University Medical Center Groningen, The Netherlands), were eligible for the study. The definition of pathogenic *LMNA* mutation has been described previously [13].

2.2.2. Cohort of DCM patients

All consecutive individuals (probands), diagnosed with idiopathic DCM who were referred in the same period to the outpatient clinics of the clinical genetics departments of the same centers (and underwent *LMNA* screening), who did not carry a *LMNA* mutation were eligible for this cohort.

We only included individuals who were at least 16 years of age and who had been investigated by a cardiologist at least once. Clinical information about the cardiac and muscular phenotype and medical history were collected.

The principal outcome for the analysis was the composite end-point of either an arterial or venous thromboembolic complication.

Arterial thromboembolic complications were defined as ischemic stroke or transient ischemic attack diagnosed by a neurologist or acute peripheral arterial occlusion diagnosed by appropriate imaging.

Venous thromboembolic complications were defined as deep vein thrombosis or pulmonary embolism diagnosed by appropriate imaging.

2.3. Case–control study

All consecutive *LMNA* mutation carriers diagnosed with a pathogenic (cardiac disease causing) *LMNA* mutation and relatives who tested negative for the familial *LMNA* mutation between January 2000 and December 2010 in the Academic Medical Center, Amsterdam, The Netherlands, were eligible for this study. Individuals diagnosed with DCM with left ventricular ejection fraction (LVEF) ≤45% and atrial tachyarrhythmias or receiving vitamin K antagonists were excluded, to rule out their influence on the platelet and hemostatic characteristics. Complete medical history (including medication use), physical examination and blood analysis on platelet and hemostatic characteristics were carried out.

Several exploring platelet and hemostatic characteristics were assessed. Regarding the platelet characteristics, platelet number, mean platelet volume (MPV) and platelet function were measured. Flow cytometry (on a Calibur flow cytometer BD Biosciences) was performed to measure the basal and stimulated platelet activation (P-selectin) and monocyte–platelet complexes. Platelets were stimulated with arachidonic acid (AA, BIO/DATA Corporation, Horsham, PA) or thrombin receptor activating peptide (TRAP, Bachem AG, Bubendorf, Switzerland). Data were analyzed by CellQuest Pro (version 4.02; BD Biosciences).

The hemostatic characteristics were explored by measuring the end products of the coagulation cascade. We therefore, determined fragment 1+2 (marker for in vivo thrombin generation) and ex vivo thrombin generation, after stimulation with tissue factor (initiator of the coagulation cascade). Both the platelet and hemostatic measurements have been described previously [14,15].

The protocol was approved by the institutional review board of the Academic Medical Center, in Amsterdam, The Netherlands. All subjects provided written informed consent.

2.4. Risk factors for thromboembolic complications

2.4.1. Cardiac risk factors for thromboembolic complications

Atrial tachyarrhythmias were defined as paroxysmal (episode of atrial fibrillation for more than 30 s), persistent and permanent atrial fibrillation and atrial flutter. Atrioventricular (AV)-block was defined as a first (PR interval ≥0.20 s), second or third degree block. LVEF was determined by echocardiography, and defined as severely reduced (<35%), moderately reduced (35–55%), or normal (>55%). Cardiac device implantation was defined as both pacemaker and cardioverter defibrillator implantation.

2.4.2. Non-cardiac risk factors for thromboembolic complications

Hypertension was defined as a systolic blood pressure of more than 140 mm Hg or a diastolic blood pressure of more than 90 mm Hg (or both) on at least two occasions or the use of antihypertensive medication. Diabetes mellitus was diagnosed based on the criteria of the American Diabetes Association or the use of antidiabetic drugs [16]. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. Smoking was defined as current (case–control study) or current and former habitual (cohort study) daily use of 10 or more cigarettes. Oral contraceptive use was defined by current or former use of oral contraceptive for more than 1 year, during clinical follow-up by a cardiologist. Individuals were classified as having muscular dystrophy, when it was diagnosed by a neurologist.

2.5. Statistical analysis

The clinical characteristics in both studies were compared using the Student's *t*-test or Mann–Whitney *U* test for continuous variables (depending on whether the variable was supposed to be normally distributed) and the chi-square test in case of categorized variables expressed as proportions.

For the cohort study, we modeled time-to-event, from date of birth until an arterial or venous thromboembolic complication occurred (before the start of antiplatelet or anticoagulant therapy). Individuals were censored when antiplatelet or oral anticoagulant therapy was started (for any reason) or most recent evaluation in individuals without antiplatelet or oral anticoagulant therapy. Multivariate Cox regression analysis was used to assess the association between carrying a *LMNA* mutation and arterial and/or venous thromboembolic complications, independent of confounders for thromboembolic complications. Hazard ratios (HR) and 95% confidence interval (CI) were calculated; robust standard errors were calculated to account for family-clustering in the data [17,18]. Adjustments were made for known thromboembolic risk factors, including gender, cardiac device implantation, atrial tachyarrhythmias, oral contraceptive use, diabetes mellitus, smoking, hypertension, LVEF, AV-block and muscular dystrophy. Missing data were less than 10% per variable and imputed when necessary. Imputations were done randomly based on mean or median proportions of the complete group per variable.

For the case–control study, platelet and hemostatic characteristics were compared between *LMNA* mutation carriers and their mutation negative relatives, using mixed model analyses, with *LMNA* mutation carriers and their relatives as pairs.

The SPSS software version 17.0 (SPSS Inc., Chicago, Illinois) and the R statistical package (version 2.10.1) were used for analyses [19]. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Cohort study

3.1.1. Study population and characteristics

The cohort included 76 *LMNA* mutation carriers from 22 different families (range 1 to 24 individuals per family) and 224 DCM patients without a *LMNA* mutation (Table 1). *LMNA* mutation carriers were significantly younger than DCM patients (45 vs 51 years, $p<0.05$). Furthermore, *LMNA* mutation carriers had more often muscular dystrophy (33 vs 1%; $p<0.05$), atrial tachyarrhythmias (63 vs 21%; $p<0.05$), and conduction disorders (67 vs 14%; $p<0.05$) and a cardiac device was more often implanted (64 vs 51%; $p<0.05$) as compared with DCM patients. In contrast, the prevalence of a LVEF <35% (69 vs 17%; $p<0.05$) and prevalence of hypertension (19 vs 8%; $p<0.05$) were higher in DCM patients compared with *LMNA* mutation carriers. Other thromboembolic risk factors were similar between both groups.