Identification of biomarkers of hypertrophic cardiomyopathy development and progression in Dutch MYBPC3 founder mutation carriers

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The objective of this study is to identify biomarkers predictive for the development of HCM in asymptomatic MYBPC3 mutation carriers and to identify prognostic biomarkers in mutation carriers in whom the disease has already been revealed. In order…

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Myocardial disorders
Study type	Observational invasive

Summary

ID

NL-OMON47925

Source
ToetsingOnline

Brief title
BIO FOr CARe

Condition

- Myocardial disorders
- Cardiac and vascular disorders congenital

Synonym
hypertrophic cardiomyopathy / thick heart muscle

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Utrecht
Source(s) of monetary or material Support: Nederlandse hartstichting

Intervention

Keyword: Biomarker, Founder mutation, Hypertrophic cardiomypathy, MYBPC3

Outcome measures

Primary outcome

Main study determinants/potential predictors

- Age (at carrier diagnosis)

- Gender

- cMyBP-C protein content in exosomes isolated from plasma

- creatine/guanidine acetic acid ratio in plasma

- delta creatine/guanidine acetic acid ratio in plasma prior/after exercise test

- acylcarnitine profile in plasma

- delta acylcarnitine profile in plasma prior/after exercise test

Dependent of the study question of the prospective cohort the endpoints are:

1) development of severe HCM

2) progression of HCM to severe HCM

For the case-control study at baseline: a severe HCM phenotype.

A severe cardiac phenotype will be defined as one or more of the following:

septal thickness of >= 20 mm, cardiac arrest due to ventricular arrhythmia, LVEF

< 40% or indication for myectomy or cardiac transplant.

Secondary outcome

not applicable

Study description

Background summary

HCM is characterized by an, usually asymmetric, hypertrophied, non-dilated left ventricle (especially septal wall thickness of >= 15 mm) in absence of other disorders that may induce cardiac hypertrophy, like hypertension and aortic valve stenosis7. The inheritance pattern of HCM is autosomal dominant, and most mutations have been identified in genes encoding sarcomeric proteins8. HCM can be an innocent condition and not cause any (sub)clinical symptoms throughout life in some patients. It is estimated that approximately one quarter of mutation carriers remain asymptomatic up to their seventies5. At the other end of the spectrum, however, HCM can lead to severe and even lethal cardiac events4,5,9-11. For instance, HCM can be diagnosed as a result from cardiac arrest in adolescence in a proband. Subsequently, an asymptomatic parent or even grandparent, who may not even show signs of HCM on ECG or cardiac ultrasound, can turn out to be a carrier of the causal mutation that was detected in the severely affected proband. As yet, there is no solid explanation for how an identical mutation can lead to extreme phenotypes at both ends of the disease spectrum. In the Netherlands, DNA-diagnostics for HCM is offered since 1996, and in about halve of the index cases a pathogenic mutation is identified3. The majority of these mutations (approximately 70%) is located in the MYBPC3 gene, encoding the cardiac myosin-binding protein C (cMyBP-C)3. There are three Dutch MYBPC3 founder mutations accounting for more than half of the identified MYBPC3 mutations, which are termed c.2373dupG, c.2827C>T p.Arg943X and c.2864_2865delCT3. It estimated that in the Netherlands more than 6000 MYBPC3 founder mutation carriers are present (32.000 x 20%). Studying these carriers with different cardiac phenotypes offers an unique cohort with a similar genetic background in which disease penetrance and factors involved can be assessed. The reduced penetrance and variable expression of HCM is a very challenging aspect in the counseling of (asymptomatic) mutation carriers and determination of cardiac surveillance schemes and medical treatment. Initially, learning about carriership which predisposes to a potential lethal condition can cause a lot of distress and insecurity about one*s future perspectives. Annual to biannual cardiologic examinations are now recommended for asymptomatic mutation carriers, but approximately 25% of these *patients* will undergo these tests throughout life without developing any clinical signs or symptoms of the disease5. Moreover,

once the onset of HCM has been documented, it remains impossible to predict the course of disease. Therefore, there is an urgent need to develop prediction models which that enables quantification of the risk of asymptomatic mutation carries on disease development. In addition, biomarkers that may predict the progression of disease in mutation carriers in whom the onset of disease has been documented are required to improve personal medical treatment.

Age and gender have been shown to be major disease determinants in MYBPC3 mutation carriers6. A potential source of biomarkers with additional predictive value for HCM development and progression are exosomes. Extracellular vesicles, including exosomes are nanovesicles secreted into the extracellular environment, like blood and saliva, upon internal vesicle fusion with the plasma membrane12. Extracellular vesicles contain cytosolic components and are expected to serve as excellent sources for biomarkers of different forms of disease as they reflect a *liquid biopsy* of pathological tissues. Indeed, exosomal markers are already used in clinics for diagnosis and prognosis of several tumors12. My collaborators have recently observed the presence of sarcomeric proteins in extracellular vesicles isolated from blood of patients with atherosclerotic disease (unpublished data). The founder mutations in MYBPC3 all occur >50 nucleotides away from the last exon-intron boundary within the mRNA, targeting them for nonsense medicated RNA decay (NMD)13. Reduced mRNA of mutated MYBPC3 alleles has been observed in (end-stage) cardiac tissues14,15. Efficiency of the NMD machinery differs between individuals and upregulation of the wild type allele, or variation in degradation of toxic cMyBP-C peptides by the ubiquitin-proteosome system has also been proposed to explain the variability of HCM in mutation carriers16. Furthermore, recent murine studies have shown that stress induces a reduction in cMyBP-C content in mice with a heterozygous MYBPC3 truncating mutation, leading to an exacerbation of cardiac dysfunction17. This supports the haploinsufficiency hypothesis, which implies that a reduction in amount of cMyBP-C levels in MYBPC3 mutation carriers induces cardiac deterioration13. Therefore, the regulation of total cMyBP-C content could very well be partly responsible for the variable expression and reduced disease penetrance in mutation carriers. cMyBP-C levels in exosomes isolated from blood from MYBPC3 mutation carriers may reflect disease
onset and/or severity.

Another potential source of biomarkers for HCM development and progression are energy metabolites. Energy depletion has been proposed to be a major pathophysiological mechanism driving HCM18. In vitro and in vivo studies have shown that HCM causing mutations induce a higher Ca2+ sensitivity of the contractile unit18. Subsequently, inefficient cross-bridging leads to more ATP usage, thereby compromising the energy level of the cardiomyocyte18. Indeed, using magnetic resonance spectroscopy, a reduction of phospho-creatine to ATP ratio, which reflects energy status, has been observed in the myocardium of HCM mutation carriers, even prior to left ventricle hypertrophy occurred19. Furthermore, ATPase activity during force

development within the cardiomyocyte was shown to be higher in MYBPC3 mutation carriers20. Perhexiline treatment, which is proposed to shift myocardial metabolism from fatty acid to glucose utilization, was shown to improve energy capacity in symptomatic HCM patients21. In keeping with this, metabolic analyses of heterozygous MYBPC3 knock-in mice were indicative of an altered fatty acid transport into mitochondria upon perhexiline treatment, suggestive of a reduction of fatty acid beta oxidation22. This all suggests that a shift in plasma energy metabolites may represent an early stage of HCM development which may be exemplified during successive stages of the disease. However, no study of metabolic energy biomarkers in blood of HCM patients has been reported to date.

Study objective

The objective of this study is to identify biomarkers predictive for the development of HCM in asymptomatic MYBPC3 mutation carriers and to identify prognostic biomarkers in mutation carriers in whom the disease has already been revealed. In order to establish the predictive value of these potential biomarkers, the generation of a nation-wide prospective cohort is required. This prospective cohort will include plasma samples of MYBPC3 founder mutation carriers with and without a phenotype at baseline, and plasma will be additionally collected at different time points and, thereby, at different stages of the disease. Plasma will be taken during (bi)annual control visits of all participating mutation carriers in the cohort, preferably prior and after exercise tests. Rationale behind this is that exercise may increase the level of a plasma biomarkers (specifically energy metabolites) because of the stress exposed to the cardiomyocyte during extreme exercise. We aim to include 1000 MYBPC3 founder mutation carriers in the prospective cohort. Follow-up time will be for indefinite duration (until cardiac transplant, death or withdrawal for other (personal) reasons).

We will perform a case-control study at the baseline of the creation of our prospective cohort to identify potential biomarkers. We will analyze potential biomarkers in 100 included HCM patients with a severe phenotype at baseline versus 100 included mutation carriers with no cardiac phenotype at baseline (as determined by ECG and cardiac ultrasound). The prediction models for HCM development and progression, using the potential biomarkers identified in the case control setting, will subsequently be developed and validated using samples of the prospective cohort.

For future studies, this prospective cohort will be an excellent source to study (epi)genetic and environmental factors involved in HCM development and progression as well as for large-scale hypothesis-free metabolic and proteomic approaches.

Study design

1. Inclusion of MYBPC3 founder mutation carriers

For the prospective cohort, we aim to included 1000 mutation carriers (with and without documented HCM). The major exclusion criterium will be cardiac transplant, as the disease heart is then no longer present to deposit biomarkers in blood.
For our case-control study at baseline, we will select 100 MYBPC3 founder mutation carriers with no cardiac phenotype (as determined by recent ECG and cardiac ultrasound) and 100 MYBPC3 founder mutation carriers with a severe cardiac phenotype. A severe phenotype will be defined as one or more of the following: septal thickness of >= 20 mm, cardiac arrest due to ventricular arrhythmia, LVEF < 40% or indication for myectomy or cardiac transplant. Since the pitfall of the case control setting for predictive research is that a difference in biomarker may be a consequence of the cardiac disease rather than a cause, we aim to select patients that have just been documented with a severe cardiac phenotype. Furthermore, since advanced age is such a major determinant of HCM development and progression, the controls will be in a similar age group.
Mutation carriers will be selected from our own outpatient clinic and from the outpatient clinics from our collaborators from the AMC (dr. I.Christiaans, dr. P. van Tintelen, Prof. Dr. A.A. Wilde), Erasmus MC (dr. M. Michels, dr. R.A. Oldenburg), UMCG (Prof. dr. R.A. de Boer, dr. Y.M. Hoedemakers) and UMCN (dr. E. Cramer, dr. C. Marcelis).

2. Extracellular vesicle analysis of MYBPC3 founder mutation carriers
The laboratory of my collaborators Prof. Dr. G. Pasterkamp and Dr. H. den Ruijter in the UMCU is pioneering in biomarker research of exosomes in cardiovascular disease. Therefore, this part of the research proposal will be conducted in their laboratory. Extracellular vesicles of MYBPC3 founder mutation carriers will be isolated from blood using the Xtractt protocol.

For the case control study at baseline, cMyBP-C levels in extracellular vesicles will be determined in all 200 mutation carriers using an electrochemiluminescence immunoassay. This sensitive method of cMyBP-C protein detection was recently developed by my collaborator dr. D.W. Kuster (VUMC)24.

3. Metabolic analysis of MYBPC3 founder mutation carriers
The metabolic laboratory within my department, led by my collaborators Dr. J.J. Jans and Prof. Dr.N.M. Verhoeven-Duif has a longstanding expertise in metabolic investigations both in diagnostic and research settings.
For the case control study at baseline, we will focus specifically on plasma metabolites in the total 200 MYBPC3 mutation carriers that reflect energy regulation and consumption (creatine/guanidine acetic acid and acylcarnitine profiles). Levels of these plasma metabolites will be compared between carriers with (n=100) an without (n=100) a cardiac phenotype. Dedicated tandem mass spectrometry methods have been developed in the metabolic laboratory to quantitatively assess these metabolites.

4. Development of a prediction model
In order to identify the potential biomarkers for HCM development and/or progression, metabolites and exosomal proteins will be analyzed in a case control setting at the baseline of our prospective cohort of MYBPC3 founder mutation carriers. Differences between the groups will be evaluated using the student T-test (continuous variables) or chi square analysis (dichotomous variables).
We will subsequently apply multivariable logistic regression analysis to relate the potential predictors (age at determining carriership, gender, biomarker values) to the presence or absence of HCM and its progression in our prospective cohort.

5. Ancillary studies
In order to study the effect of environmental risk factors, a questionnaire has been generated together with collaborators from the UMCG (Prof. Dr. R.A. de Boer en dr. Y. Hoedemaekers). The questionnaire was based on the study of James et al. in patients with arithmogenic cardiomyopathy (ref), The questionnaire will be sent to the participants that have agreed to receive a questionnaire in the consent form.
If we are able to obtain the financial means, in future studies we will perform hypothesis-free approaches to identify potential novel predictive biomarkers for HCM development in MYBPC3 mutation carriers, using large scale metabolomics and proteomics studies.
In addition, we intend to study genetic modifiers of HCM development and progression in MYBPC3 mutation carriers using geonome-wide-association studies and NGS-based techniques (funding already obtained from CVON: DOSIS, by Dr. F Asselbergs and co-workers). Therefore, during the first blood withdrawal, 10 ml extra blood will be sampled for storage of leucocytes (from which DNA can be isolated). Blood spots that will allow future additional metabolic analyses, will be obtained from blood already derived from plasma sampling.

To study the full spectrum of HCM, we will also include HCM patients with other mutations in MYBPC3 or mutations in other genes (mainly, but not exclusively, MYH7, MYL2,TPM1, TNNC1, TNNT2, TNNI3) and asymptomatic relatives that are carriers of these mutations. These patients and asymptomatic mutation carriers will receive a slightly altered patient information letter and informed consent.

Items for ancillary research will be addressed in the current patient information letter and informed consent (IC). Separate amendments to the current study will be submitted when applicable.

A user-committee will be established which will consist of all principal investigators of each participating center. This committee will determine for what purposes the biosamples for the BIO FOr CARe study can be used.

Incidental findings found in the described future studies will be reported to the TC bio of the UMC Utrecht. If the TC bio decides that the incidental

finding is clinically relevant and needs to be reported to the patient, appropriate action will be undertaken (consultation with the general practitioner or treating specialist, in order to get the information in the appropriate manner to the patient). In the IC, the patient needs to declare that he is aware that he can be informed about incidental findings.

Study burden and risks

Burden and risks are minimal (once or biannual blood withdrawal). If we are able to predict the disease course in MYBPC3 founder mutation carriers, this will benefit those carriers in the future. Prevention and pharmaceutical therapies may be designed based upon the acquired results. This will probably not benefit the participating mutation carriers directly, but may benefit their progeny.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)

Elderly (65 years and older)

Inclusion criteria

MYBPC3 founder mutation carrier and 18 years or older

Exclusion criteria

Cardiac transplant

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	12-01-2017
Enrollment:	1000
Type:	Actual

Ethics review

Approved WMO

Date:	24-03-2016
Application type:	First submission
Review commission:	METC NedMec

Approved WMO

Date:	05-07-2016
Application type:	Amendment
Review commission:	METC NedMec
Approved WMO Date:	07-12-2016
Application type:	Amendment
Review commission:	METC NedMec
Approved WMO Date:	27-07-2017
Application type:	Amendment
Review commission:	METC NedMec
Approved WMO Date:	21-09-2017
Application type:	Amendment
Review commission:	METC NedMec
Approved WMO Date:	07-11-2017
Application type:	Amendment
Review commission:	METC NedMec
Approved WMO Date:	04-01-2019
Application type:	Amendment
Review commission:	METC NedMec
Approved WMO Date:	31-07-2019
Application type:	Amendment
Review commission:	METC NedMec
Approved WMO Date:	21-11-2019
Application type:	Amendment
Review commission:	METC NedMec

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL55889.041.15