Medizinische Fakultät
der
Universität Duisburg-Essen

aus der Klinik für Anästhesiologie und Intensivmedizin

# Association of guanine nucleotide alpha subunit (GNAS) gene haplotypes with diastolic dysfunction in patients undergoing coronary artery bypass graft operation

I n a u g u r a l - D i s s e r t a t i o n
zur
zur Erlangung des Doktorgrades der Medizin
durch die Medizinische Fakultät
der Universität Duisburg-Essen

Vorgelegt von

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2019

**DOI:** 10.17185/duepublico/70680
**URN:** urn:nbn:de:hbz:464-20191119-092238-3



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Tag der mündlichen Prüfung: 2. Oktober 2019

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# 1. Introduction

## 1.1. Signal transduction in chronic heart failure

Patients with cardiovascular diseases, as for example chronic heart failure (CHD), often present with increased sympathetic tone, i.e. postsynaptic and intravascular catecholamine concentrations are increased. A chronic stimulation of the adrenergic system leads to a desensitization and downregulation of β-receptors. This desensitization originates from a phosphorylation of the receptors through G protein coupled receptor kinases (GRK) and the Protein Kinases A and C which are activated with heart failure. The receptor is thereby uncoupled from the Adenylate Cyclase stimulator (Gαs) (Port & Bristow, 2001).

The downregulation mechanisms include the decrease of the receptor number, which is probably adjusted through GRKs which lead to endocytosis particularly of the β1 receptors (Leineweber & Heusch, 2009), and the internalization of β-receptors resulting in a loss of surface receptors after long term agonist stimulation (Mahan, Koachman, & Insel, 1985). At the same time, the high catecholamine concentration leads to an increase of Adenylate Cyclase inhibitor (Gαi). This leads in turn to downregulation of β1 receptors and a rearrangement of the signal transduction towards the β2 receptors. This signal transduction to β2 seems to produce a weaker contractility than β1 does, added to that β2 are uncoupled increasingly from the Gαs (Bers, 2002).

It is noteworthy that β2 couples to Gαi, which entails another restriction from chronotropy and inotropy (Brodde, Bruck, & Leineweber, 2006). As a result, an important mechanism for short-term increase of contractility and chronotropy gets lost.

## 1.2. The role of coronary revascularization in chronic heart failure

Coronary artery disease (CAD) is a common pathological background in patients with heart failure and it is associated with increased mortality. Coronary arterial bypass grafting (revascularization or CABG) is a therapeutic option and may be associated with preservation of cardiac function and improved outcomes in patients with CAD (Hwang, Melenovsky, & Borlaug, 2014).

Patients assigned to CABG had lower rates of death from any cause or hospitalization for cardiovascular causes ( Velazquez, E. J., Lee, K. L., Deja, M. A., Jain, A., Sopko, G., Marchenko, A., 2011).

## 1.3. The role of beta blockers in heart failure

ß-blockers were not a therapeutic option in the management of heart failure for a long time until it was noticed that in patients with decompensated heart failure, reduced heart rate improved symptoms (Swedberg, 1998). While the mode of action was unknown, Bristow et al. showed that the adenylate cyclase response to β adrenoceptor blockade was attenuated in patients with heart failure (Bristow, Port, Hershberger, Gilbert, & Feldman, 1989). As a result, ß-blockers took a role in the management and later became a standard treatment (Swedberg, 1998). The pharmacological basis for the beneficial effects of β-blockers in heart failure seems to be reduction in catecholamine-driven changes like increase of the heart rate, myocardial contractility and relaxation, reducing left ventricular afterload. The ß-blockers improve the outcome after myocardial infarction and they became (along with the diuretics) the main treatment of the heart failure (Baker, Hill, & Summers, 2011).

## 1.4. Description of the GNAS gene

The guanine nucleotide alpha subunit (GNAS) complex locus is an important gene located in the human long chain of the chromosome 20, and its products are responsible for many endocrine, bone and tumor diseases (Turan & Bastepe, 2015). It is composed of 13 exons and 12 introns (Kozasa, Itoh, Tsukamoto, & Kaziro, 1988). The GNAS gene encodes the α subunit of the G transmembrane protein, namely the stimulating subunit of the G receptor (Gαs), which in turn, when stimulated, induces a cascade of reactions through the adenylate cyclase on the cell membrane (Weinstein, Xie, Zhang, & Chen, 2007). Adenylate cyclase stimulates the cation channels on the heart muscle cell membrane to cause a calcium influx and hence depolarization of the myocardium (Wainger, DeGennaro, Santoro, Siegelbaum, & Tibbs, 2001). The response activates the protein kinase A (PKA) and in turn the L-type calcium channels, phospholamban and Troponin I leading to increase inotropy and lusitropy (Wettschureck & Offermanns, 2005).

The cardiac function is highly regulated by the sympathetic nervous system via Gαs. In cardiac myocytes, Gαs couples to β receptors. Here, the predominating receptor type is the β1 receptor while β2 receptors comprise of only 30% of the total β receptors ( Lowes, B. D., Gilbert, E. M., Abraham, W. T., Minobe, W. A., Larrabee, P., Ferguson, D., 2002). If nor-epinephrine or epinephrine as neurotransmitters of the sympathetic nervous system bind to β receptors, the described cascade runs off with formation of cyclic adenosine monophosphate (cAMP), which in turn activates Phosphokinase A, followed by phosphorylation of other proteins including Phospholamban, L type Calcium channels or Ryanodine receptors (Bers, 2002). This leads to an increase of inotropy and chronotropy. Contrarily to β1 receptors, β2 receptors couple to Gαi and can cause therefore a lowering in cAMP followed by a

potential reduction of contractility ( Kuschel, M., Zhou, Y. Y., Cheng, H. P., Zhang, S. J., Chen, Y., Lakatta, E. G., & Xiao, R. P., 1999), (Madamanchi, 2007).

## 1.5. Single nucleotide polymorphisms

A single nucleotide polymorphism (SNP) points to a position in the genome at which two different bases can alternatively be found. When the frequency of two alternative bases amounts more than 1 percent in the population, it distinguishes it from the point mutations whose frequency accounts for less than 1 percent. Moreover, SNP's distinguish themselves by their stability and that they almost do not change even after several generations (Brookes, 1999).

SNPs can be differentiated regarding the location within the genome:

- Intronic SNP's (iSNP) are located in introns and may influence splicing of a gene.
- Coding SNP's (cSNP) located in the exon which can change the structure of the protein.
- Regulatory SNP's (rSNP) in the promotor location can adjust the genetic expression.
- Silent SNP's (sSNP) have no functional influence. Statistically, a SNP occurs every 300-1000 base pairs supposing approx. 3-10 million SNP's in the human genome (Schork, Fallin, & Lanchbury, 2000).

Haplotypes refer to SNPs which are inherited together on a single chromosome and are not prime of variation by chance. In other words haplotype refer to a specific combination of single nucleotide polymorphisms (SNPs). Haplotypes are characterized by haplotype tagging SNP's (htSNPs or tag SNPs). While these are inherited together with other SNPs genotyping a smaller number of tag SNP's is therefore sufficient to determine a haplotype.

## 1.6. Haplotype structure of GNAS

Recently, our group sequenced the complete GNAS1 promotor and Intron 1 and 11 SNP's defining 3 frequent Haplotypes were identified resulting from 2 htSNPs: G(-1211) A (or the surrogate marker C2273T being in 100% linkage disequilibrium with G(-1211)) and C2291T (Fig. 1)( Frey, U. H., Adamzik, M., Kottenberg-Assenmacher, E., Jakob, H., Manthey, I., Broecker-Preuss, M., 2009). We could show that the haplotype 3 is associated with a significantly higher promotor activity compared to haplotype 1 and 2. As a result patients became divided in homozygotes (2 copies of the Haplotype 3 alleles), heterozygote (1 copy) and negatively (no copy) for the Haplotype 3 (Fig. 1). In vitro experiments in cultured human cells showed a raised promotor activity in the Haplotype 3 carriers.

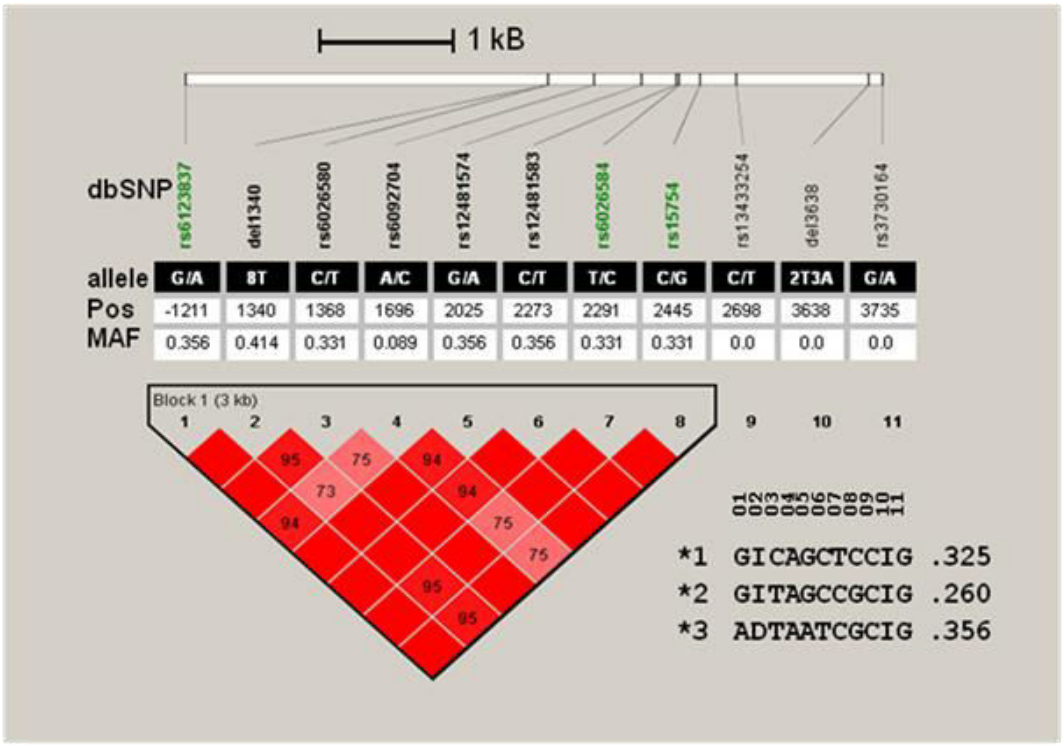
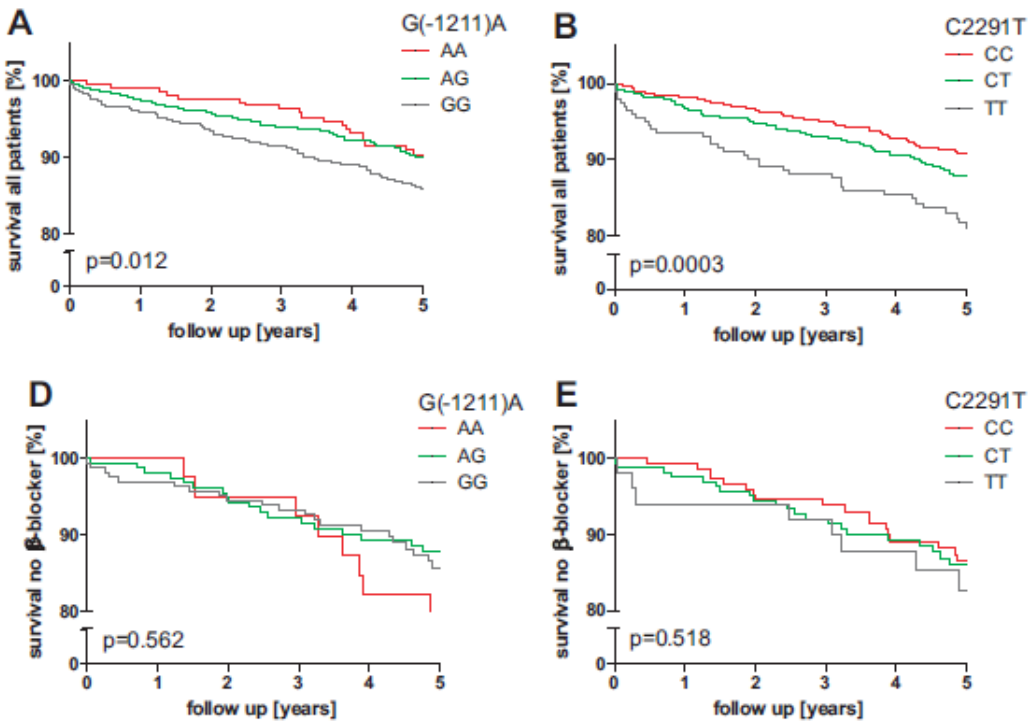



Fig.1: The identification numbers of the SNP's (dbSNP), the SNP allele the position of the SNP's relatively to the translation starting point (PoS) and the Minor allele frequency (MAF). Three most frequent Haplotypes are illustrated on the right with her respective frequencies. Besides, the haplotypes were formed with SNP's of which MAF amounted to >0.1. The coupling imbalances were provided with the software Haploview. The red squares indicate a coupling imbalance of 100%, the others are given with the respective figures. (Frey et al., 2009)

In a pilot study we investigated whether differences on GNAS diplotype classification leads to a different mortality after coronary artery bypass operations. 185 patients scheduled for CABG under β blockade were enclosed. The analyses showed that the cardiac mortality was genotype dependent. For G (-1211) A: AA: 0%; AG: 2.6%, and GG: 10.5%. For T2291C the cardiac specific one year mortality was: CC: 2.3%; CT: 6.3%, and TT: 20.0%. The results were confirmed in an international cooperation project on more than 1600 patients. Here, the analysis of the diplotypes proved a better risk classification than the analysis of single genes. the cardiac specific one year mortality was: diplotype *3/*3: 0%; *3/*2: 2.4 % *3/*1: 2.9 % *2/*2: 4.5%, *2/*1: 9.1% and *1/*1: 20.0%. (Frey et al., 2014) (Fig. 2)



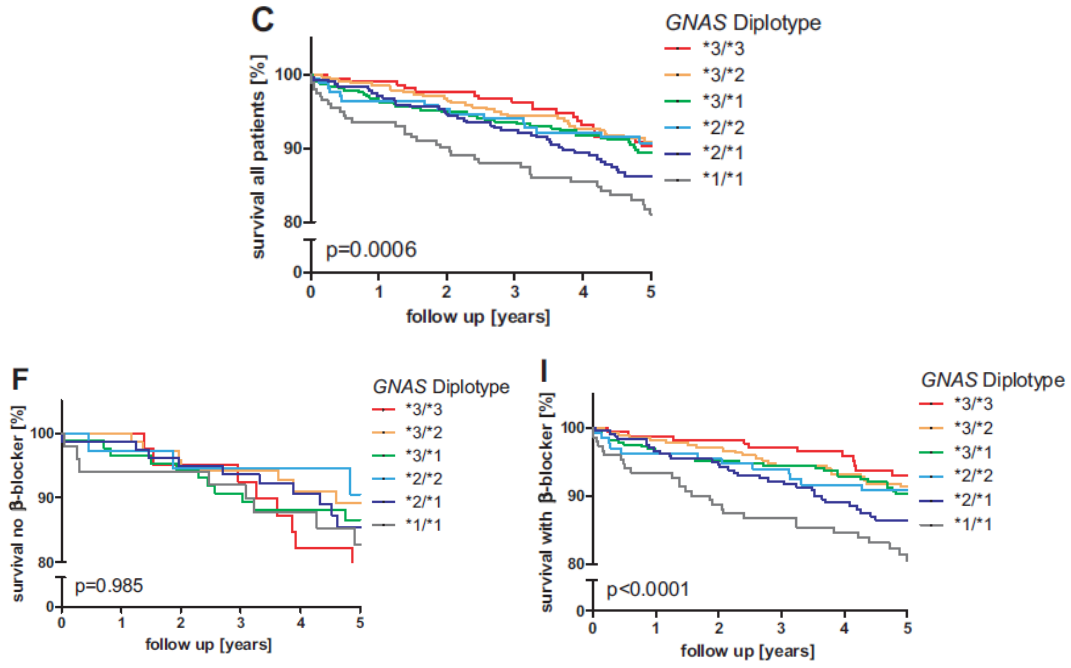



Fig.2: GNAS diplotypes and survival (Frey 2014)

**A**= G(-1211)A -related 5 years mortality of all patients, **B**= C2291T-related 5 years mortality, **C**= Diplotype related 5 years mortality of all patients, **F**= Diplotype related 5 years mortality of patients off ß-blockers, **I**= Diplotype related 5 years mortality of patients on ß-blockers, **D**= G(-1211)A -related 5 years mortality of ß-blocked patients, **E**= C2291T-related 5 years mortality of ß-blocked patients

Regarding the risk factors, except of the gender, there were no significant differences between the different genotypes. Interestingly, ejection fraction as a marker of systolic function showed no differences. When long term survival was investigated there were clear differences between the different genotypes. Nevertheless, the effect on the survival was only detected in patients who were under beta blocker therapy (Frey, U. H., Muehlschlegel, J. D., Ochterbeck, C., Fox, A. A., Shernan, S. K., Collard, C. D., 2014).

## 1.7. Impact of GNAS gene on the clinical setting

During the CABG operation the cardiopulmonary bypass leads to inflammatory response and to a desensitization of myocardial β receptors ( Booth, J. V., Landolfo, K. P., Chesnut, L. C., Bennett-Guerrero, E., Gerhardt, M. A., Atwell, D. N., 1998).

While there is molecular evidence showing an improved heart function with increased Gas expression and an improved long term survival after CABG systolic function was not affected.

## 1.8. The diastolic function

The cardiac cycle composes of two phases, the systole and the diastole. Both of them are active processes requiring energy. Every phase is prone to dysfunction. Therefore we differentiate between systolic and diastolic heart dysfunction.

The diastole begins with the calcium re-uptake in the sarcoplasmic reticulum via Na/Ca ATPase channel, after an intracellular hypocalcemia ensues, troponin detaches from the myofilaments and relaxation occurs (Bers, 2008).

Diastolic dysfunction of the heart is a silent sign which proceeds to the revealed, global or systolic heart failure (Schmidt & Pieske, 2012). The concern about its health and economic burden was raised at the early 90's with many publications, as it has about 8% mortality per year which accounts for about the half of the systolic failure mortality and the costs of therapy of both are nearly equal (Wettschureck & Offermanns, 2005), (Grossman 1991).

Nevertheless, this special type of heart failure carries an independent adverse risk for the outcome in patients undergoing non cardiac operations (Fayad, Ansari, Yang, Ruddy, & Wells, 2016).

Diastolic dysfunction is an abnormality in the relaxation of the left ventricle. This leads to an interference of the filling of the left ventricle in the diastole. A diastolic malfunction is independent of the left ventricular systolic function. Only 11-15% of the patients with asymptomatic diastolic dysfunction develop within 5 years of evident symptoms of a cardiac insufficiency (systolic heart failure).

It is well known that under a given end diastolic ventricular blood volume a higher end diastolic pressure ensues, the causes are ventricular hypertrophy and wall stiffness. A secondary increase in the left atrial pressure with atrial dilatation and increased pulmonary vein pressure ensues (Aurigemma & Gaasch, 2004).

The diagnosis of this kind of heart failure is made if the patient suffers from symptoms of heart failure but his ejection fraction is normal, according to the American guidelines the absence of valve disorders is another parameter for the diagnosis, and according to the latest European guidelines there should be an evidence of left ventricular relaxation abnormality. The echocardiography plays a pivotal role in the detection of patients with diastolic insufficiency, partly because the physical investigation, electrocardigarm and X-ray of the Thorax do not deliver specific information about the differentiation between diastolic and systolic heart failure.

A normal or nearly normal left ventricular ejection fraction (40 percent to 50 percent) is necessary for the diagnosis. In addition, echocardiography can facilitate the exclusion of mitral-or aortic regurgitation or constrictive pericarditis, which are also related with the signs and symptoms of the cardiac insufficiency with normal ejection fraction (Fig. 3).

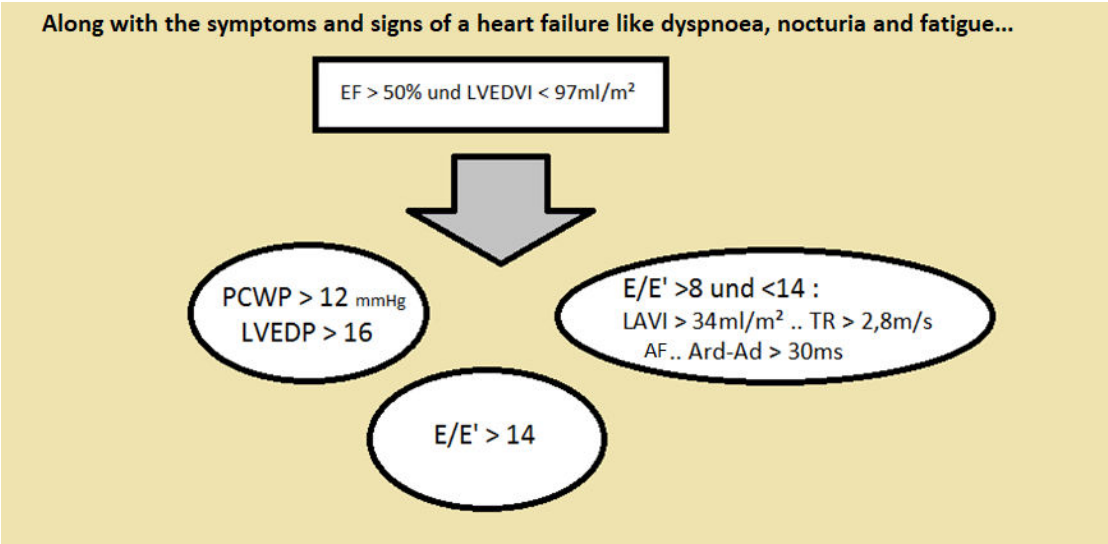



Fig. 3: Diagnosis of diastolic dysfunction. EF= ejection fraction, LVEDI= left ventricular end-diastolic volume index, PCWP= pulmonary capillary wedge pressure, LAVI= left atrial volume index, TR= tricuspid regurgitation, Ard= A Doppler wave of pulmonary vein, Ad= A Doppler wave of mitral inflow

According to the recommendations for the evaluation of left ventricular diastolic function by echocardiography of 2009 the recommended variables for identifying diastolic dysfunction and their abnormal cut off values (as illustrated in Fig.4) are: lateral e´ <10 cm/sec, average E/e´ ratio > 14, left atrial volume index > 34 mL/$m^2$, (Nagueh et al., 2009).

An update of the recommendations in 2016 stated that four parameters are required for the diagnosis of a diastolic dysfunction, the lateral e´ <10 cm/sec, the average E/e´ ratio > 14, the left atrial volume index > 34 mL/m² and the new parameter was a tricuspid valve regurgitation velocity of more than 2,8 m/sec (Nagueh et al., 2016).

The Doppler echocardiography, which measures the speed of the atrioventricular blood flow through an opening (e.g. valve) is the main diagnostic tool for the detection of the diastolic dysfunction. In normal sinus rhythm, the diastolic flow from

the left atrium to the left ventricle through the mitral valve has two components, the E wave which reflects the early passive diastolic filling, and the A wave, in the late diastole which reflects the atrial contraction (Aurigemma & Gaasch, 2004).

An important physiological and anatomical landmark of the diastolic dysfunction is the left atrial dilatation with an increase of both end diastolic volume and end diastolic pressure. For a given ejection fraction the pressure-volume curve of the left atrium is shifted to the left indicating a wall stiffness and reduced compliance. More end diastolic pressure is produced for the same blood volume and clinically symptoms of heart failure like dyspnea appear.

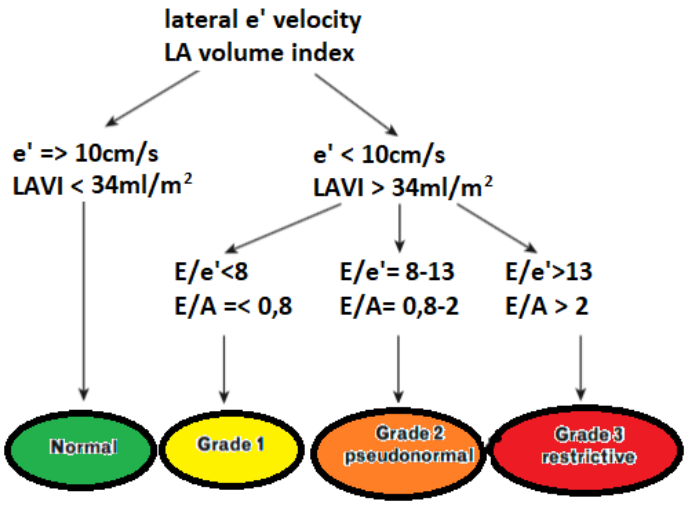



Fig. 4: Grading of diastolic dysfunction

E/A= mitral inflow Doppler, e`= tissue Doppler of the lateral mitral annulus,

LAVI= Left atrial volume index

With the echocardiographic measurements, the diastolic malfunction can be divided into four grades, those relationship with the diastolic dysfunction is explained in figure 5:

Grade 1: Abnormal relaxation pattern

Grade 2: Pseudo-normal diastole

Grade 3: Restrictive diastolic dysfunction (reversible)

Grade 4: Restrictive irreversible dysfunction

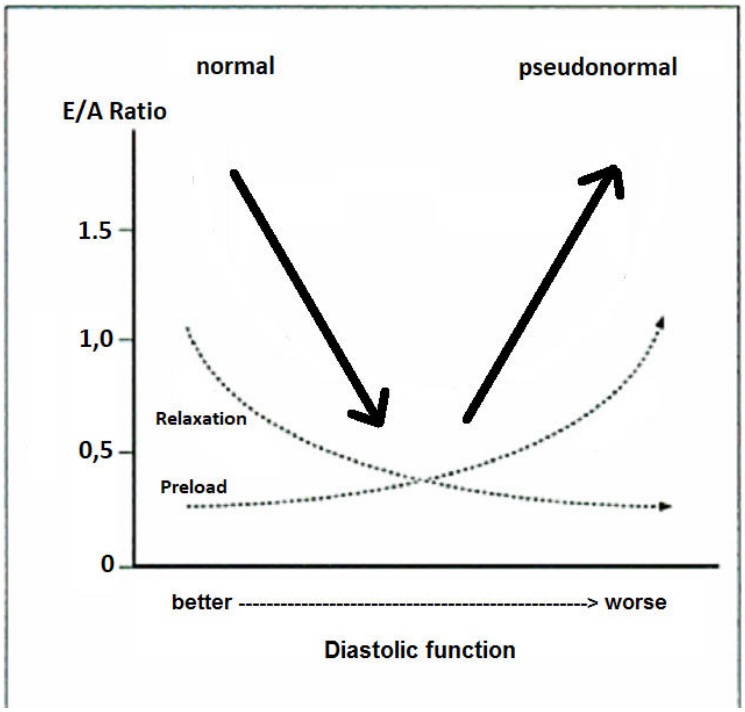



Fig. 5: Relationship between E/A ratio and diastolic dysfunction.

The worse the diastolic function the more the end-diastolic volume

These structural changes of the atrium have deleterious consequences as evidence supports the increased incidence of atrial fibrillation in patients with diastolic dysfunction (Henry et al., 1976). In those patients where it is not possible to measure the E/A ratio due to absence of atrial contraction there are some other parameters to augment the diagnosis and grading of the diastolic dysfunction, such as the early ventricular filling deceleration time and the systolic pulmonary artery pressure (Ahn, Kim, & Kim, 2015).

## 1.9. Diastolic dysfunction and CABG

In a study from 2014, diastolic dysfunction (DD) was present in about 80% of the preoperative examinations. In the postoperative course, 44% of the patients had either new or worsened DD, and 20% of them presented with either unchanged or improved DD. (Ashes et al., 2014). There is yet not a clear association between DD grade and indication to a CABG.

## 1.10. Prognosis of diastolic dysfunction

Patients who had a worsening of the systolic or the diastolic function within two years had a substantial risk of death. Those with worsened left ventricular filling patterns had worse prognosis than those who had either no change or improvement in left ventricular filling (21% vs. 12% 24 months' mortality) (Aljaroudi et al., 2012).

There is a direct correlation between the diastolic dysfunction grade and adverse cardiac outcome (Swaminathan et al., 2011). The long term outcome was different depending on the diastolic function.

Interestingly high grade diastolic dysfunction is associated to increased post-surgical atrial fibrillation appearance, mainly on the 2nd postoperative day (Ashes et al., 2014).

As mentioned above, perioperative risk of patients with diastolic dysfunction is higher in all kinds of surgical interventions and in cardiac surgical patients, diastolic dysfunction predicts a difficult weaning from the cardiopulmonary bypass as well as post-extubation pulmonary edema in non-cardiac operations (Bernard et al., 2001), (Cho et al., 2014).

## 1.11. Aim of the Study

Cardiac overexpression of the ß-adrenoceptor-coupled G-protein subunit Gαs in mice enhances inotropic responses to sympathetic stimulation. Our group could translate these finding into humans demonstrating that functional single nucleotide polymorphisms (SNPs) in the human Gαs (GNAS) gene modulate its expression and function (Frey et al., 2009). However, no association of SNPs in regulatory regions of GNAS with the systolic function of the heart were detected, which let us to suppose that there is another GNAS related factor affecting the outcome after CABG operations.

While cardiac function is not only described by systolic function, but also by diastolic function, we tested the hypothesis that the GNAS Haplotypes affect the diastolic function having therefore an influence on the outcome.

The hypothesis was therefore that the GNAS haplotype *3 is associated with better diastolic function as detected in intraoperative echocardiography.

# 2. Materials and Methods

## 2.1. Materials

### 2.1.1. Transesophageal echocardiography (TEE) Equipment

The following TEE equipment was used for examining the patients (fig. 6). The GE Healthcare Vivid S5 high-performance cardiovascular ultrasound system with a mobile designed console (GE, Solingen, Germany) assists in measuring the most commonly used parameter to describe the left ventricular function including the diastolic dysfunction. A Software feature called “Smart Depth” in 2D and Color mode is used by the Vivid S5 to automatically change frequency, focus position and transmit pattern as the user changes depth. It is possible to send and store the examinations clips or pictures to the Echo-PAC© Clinical Workstation Software (GE Corporation, Solingen, Germany) to be analyzed offline.


Fig. 6: GE TEE high-performance cardiovascular ultrasound system with a mobile console unit

## 2.2. Methods

### 2.2.1. Study protocol

After approval of the ethic committee of the Duisburg-Essen University (No. 15-6633-BO), 522 Patients were screened for this prospective observational trial during 01.04.2016 and 31.08.2017. This study was registered in the German clinical trial register under the number DRKS00010666. The consort diagram is shown below (Fig. 7).

# CONSORT Flow Diagram

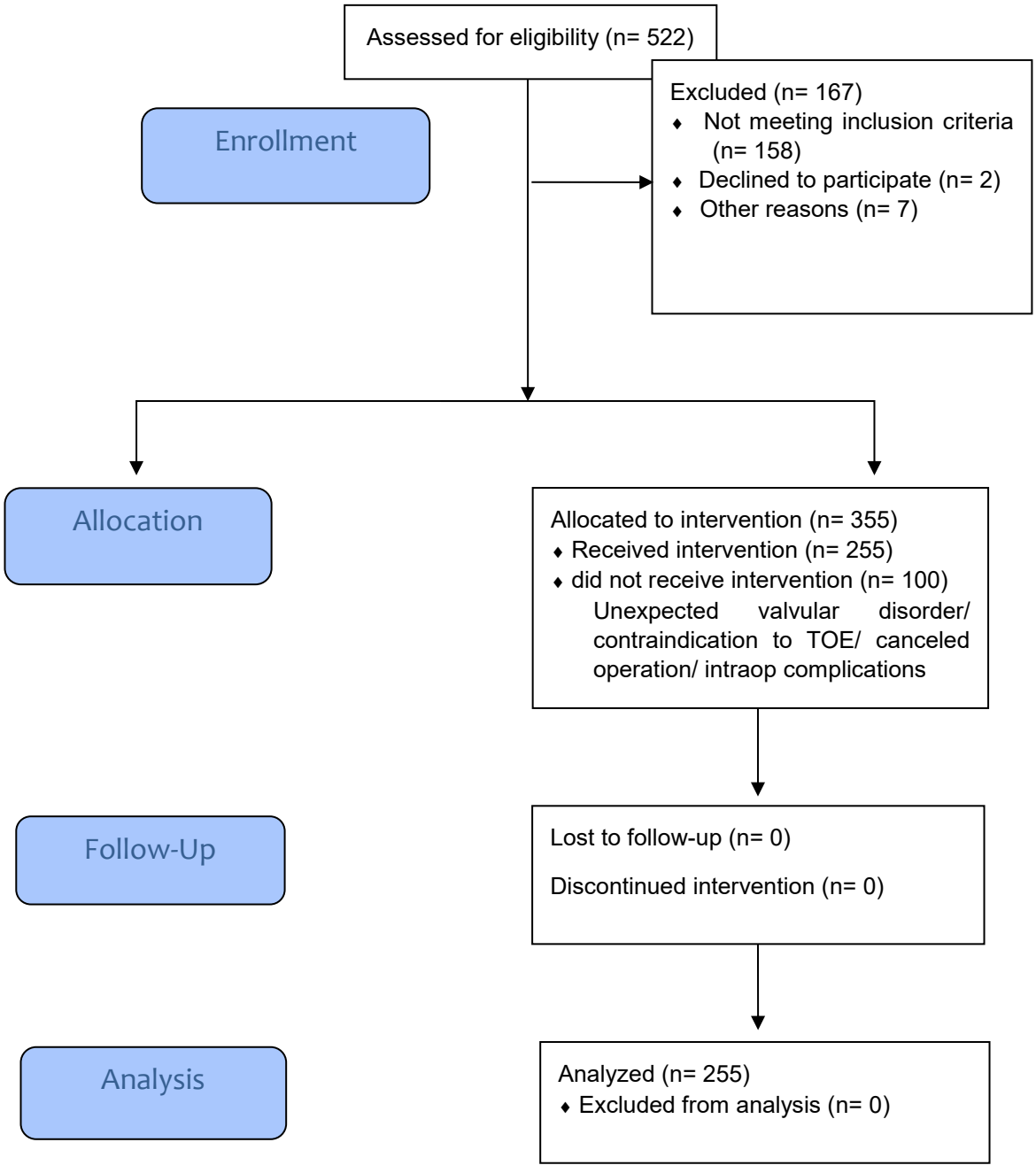



Fig.7: Consort diagram showing the Patients' enrolment

### 2.2.2. Inclusion and exclusion criteria:

#### Inclusion criteria:

Coronary heart disease

On-pump CABG operation

Preoperative chronic beta blockade

Age between 45 and 85 years

Antegrade Bretschneider cardioplegia

Mild Hypothermia (32C°)

Preoperative standard medication (Statins, beta blocker, Acetyl salicylic acid)

Standard anesthesia (Isoflurane, Sufentanil)

Intraoperative standard heparinization protocol (heparin injection with activated clotting time (ACT) > 400 seconds, reverse with Protamine)

Postoperative standard protocol (500 mg Acetylsalicylic acid after 2 hours, Low dose Heparin after 4h).

#### Exclusion criteria:

Acute myocardial infarction

Combined structural valve abnormality

Emergency operation

Pre- and intraoperative application of phosphodiesterase inhibitors

Reoperation (Redo)

Chronic Obstructive Pulmonary Disease GOLD III and IV

Renal failure with creatinine > 1.5 mg/dl

Dual antiplatelet therapy (Clopidogrel + Acetyl salicylic acid)

Repeated heart lung machine

Repeated Aortic cross clamping

Intraoperative complications ("Low-flow" bypass, failed bypass reconstruction, valve injury, Aortic dissection, pumping failure, implantation of heart assist devices, like intra-aortic balloon pump (IABP) installation), Aortic cross clamp time >150 min

Moderate / deep Hypothermia (<32C°)

### 2.2.3. The Genetic part

Anesthesia was induced with Etomidate (0,2 mg/kg), Sufentanil (1 µg/kg) and the muscle relaxant Rocuronium (0,6 mg/kg) and maintained with Isoflurane 0,8%.

After anesthesia induction 2 ml Ethylenediaminetetraacetic acid (EDTA) blood for the Genotyping was taken from the arterial line. The assigned anesthesiologist was responsible for withdrawing the blood sample after induction.

I perfomrmed nearly 20% of the whole number of the following procedure in the laboratory of our department.

#### 2.2.3.1. Deoxyribonucleic acid (DNA) extraction

With the *peqGOLD* Blood *DNA Mini Kit plus*, 200µl EDTA blood in sterile 1.5 ml centrifuge tubes added to 25 µl Protein K and 200µl DNA lysis BL buffer mixed in a vortex vibrator for 10 sec and then incubated for 10 minutes in 60°C. The lysate was

added to 350 µl binding solution BL in a *Perfect bind DNA Column*. These columns were put in a collection tube before centrifuging them. After the centrifugation the collecting tube was changed and 400 µl *DNA wash buffer BL1* was added and then centrifuged.

In a new collection tube, the lysate was washed with 600 µl *DNA wash buffer BL 2* which was mixed with ethanol 1:9, and after its centrifuge this last step was repeated in new collecting tubes of 2 ml. For a last time the last step was repeated without adding any buffering solution.

In a new 1.5 ml low bind centrifuge tube, we add 200 µl of warm elusion buffer to our Perfect bind DNA column and let it 2 minutes in room temperature to centrifuge it finally for 1 minute and get the Eluate.

DNA samples were then quantified spectrophotometrically using an Eppendorf Biophotometer (Eppendorf, Hamburg) using the default absorbance algorithm, then stored until use. Genotyping was performed using standard methods

### 2.2.3.2. Polymerase chain reaction (PCR)

PCR reaction for sequencing with a definite primer in this study was done as follows:

***1- GNAS polymorphism GNAS G(-1211)A:***

GNAS C2273T SNP was used as surrogate marker for G(-1211)A (Frey et al. 2008)

PCR synthesis Primer:

GS_int1_SNP_Se 5' AGGCAGAATTATGCTGTTGGGA 3'

GS_int1_SNP_AS 5' AGATCCGTGCCTCAGTTTCCAC 3'

***2- GNAS polymorphism GNAS 2291:***

PCR synthesis Primer:

GS_int1_SNP_Se 5' AGGCAGAATTATGCTGTTGGGA 3'

GS_int1_SNP_AS 5' AGATCCGTGCCTCAGTTTCCAC 3'

Amplification was performed in a 25 ml reaction volume and consisting of 10 µl nuclease free water, 12.5 µl Tag green master mix, 0,5 µl forward primer, 0,5 µl reverse primer, 0,5 µl DMSO, 1 µl template. PCR was performed using a PCR machine (Biorad, Munich, Germany). The Thermal cycle was programmed for 90 seconds at 95°C for initial denaturation, followed by 35 cycles of 30 sec at 95°C for denaturation, 30 sec at 55 °C as annealing, 90 sec at 72 °C for extension, and final extension at 72 °C for 5 min.

### 2.2.3.3. Genotyping of the GNAS SNPs

#### 2.2.3.3.1. Allelic discrimination (TaqMan®)

An allelic discrimination assay is a multiplexed (more than one primer/probe pair per reaction) end-point (data is collected at the end of the PCR process) assay that detects variants of a single nucleic acid sequence. The presence of two primer/probe pairs in each reaction allows genotyping of the two possible variants at the single-nucleic polymorphism (SNP) site in a target template sequence.

For each sample in an allelic discrimination assay, a unique pair of fluorescent dye detectors is used. One fluorescent dye detector is a perfect match to the allele 1 and the other fluorescent dye detector is a perfect match to the allele 2 (Fig. 8).

An allelic discrimination run using an Allelic Discrimination (AD) plate document. The SDS software analyses the data, then you assign allele calls (automatically or manually).

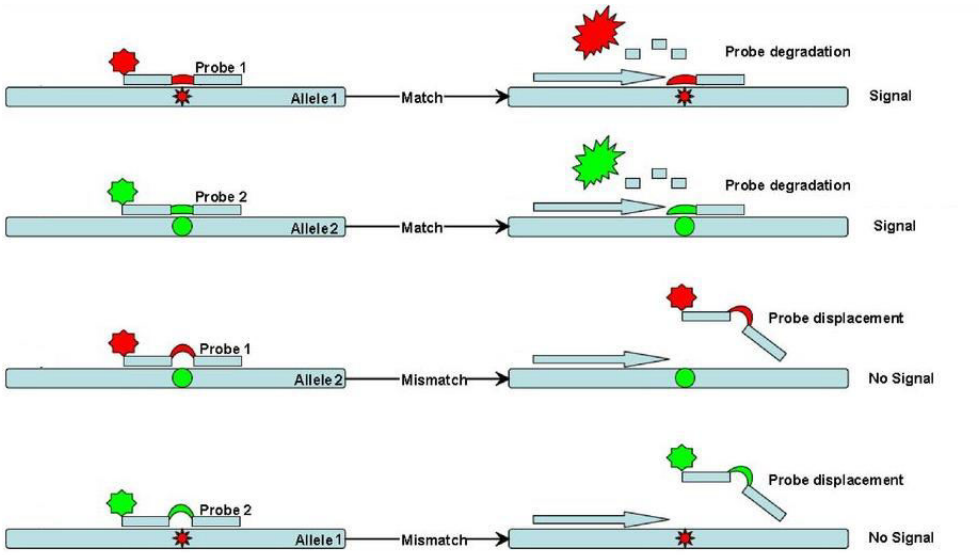



Fig. 8: TaqMan Allelic detection

Polymorphisms were genotyped using allelic discrimination assays and genotyping was successful in 99% of cases. Allelic discrimination assays use the fluorogenic 5′ nuclease chemistry, also known as TaqMan® probe-based chemistry (Applied Biosystems, Darmstadt, Germany, to enable detection of a specific PCR product as it accumulates during PCR cycles. A typical reaction mix contained a universal TaqMan® PCR Master-mix from Applied Biosystems, one primer and probe concentration, deionized water and template in varying amounts. Thermocycling programs and data retrieval varied depending on the cycling unit used.

The premixed SNP genotyping assays (assays-on-demand, which contain primers and probes mixed) were used. As shown in figures 9 and 10, a clear differentiation between the alleles were achieved.

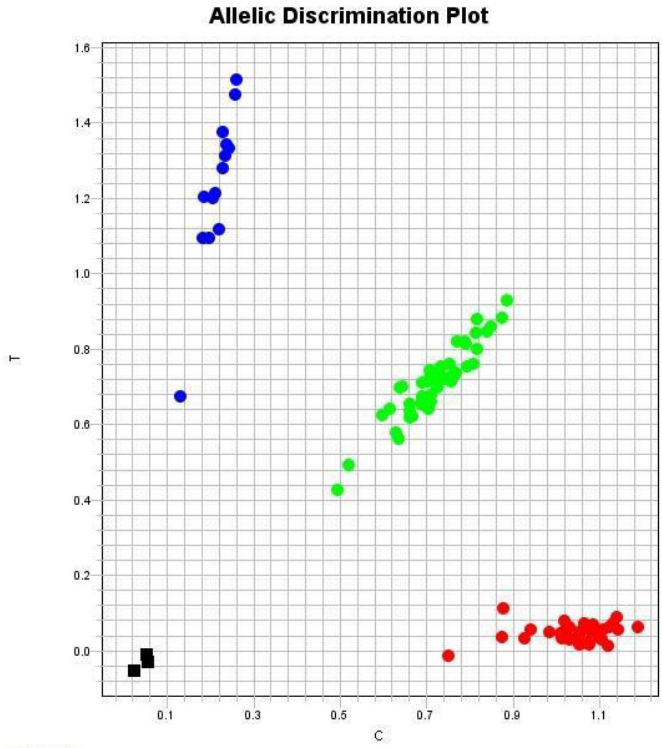



Fig 9: Genotyping of the GNAS C2273T SNP (Homozygous TT blue/ Homozygous CC red/ heterozygous green/ test calibration black)

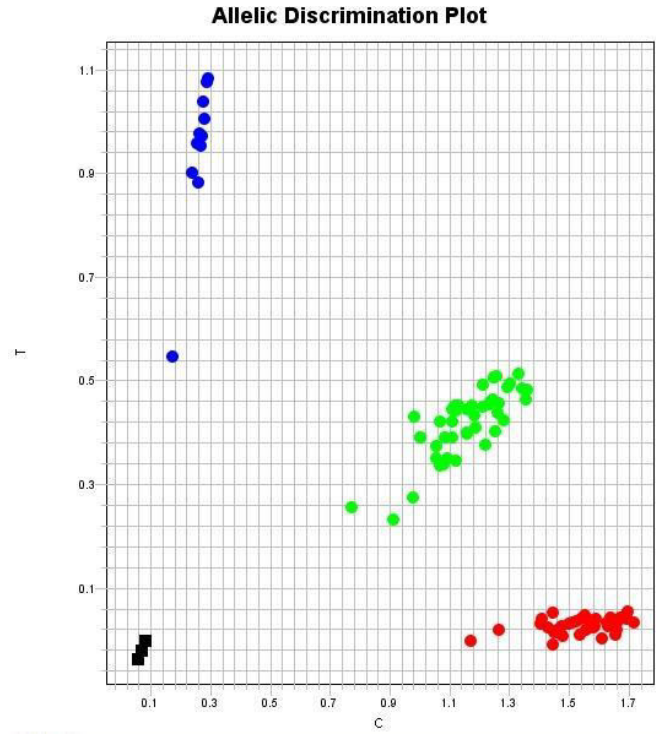



Fig 10: Genotyping of the GNAS C2291T SNP (Homozygous TT blue/ Homozygous CC red/ heterozygous green/ Test-calibration black)

### 2.2.4. The Echocardiographic part (Transesophageal approach)

The diastolic dysfunction was defined in our study as follows:

Lateral e´ <10 cm/sec

Average E/e´ ratio > 14

Left atrial volume index > 34 mL/$m^2$

E/A ratio 0,8 – 2,0 (The pseudonormal diastolic dysfunction was differentiated through other parameters from the normal variant).

With the TEE, the diastolic function of the patients were measured and determined according to the ACE and EACVI guidelines of 2009 (Nagueh et al., 2009).

As recommended from the guidelines, we considered a patient as having a diastolic dysfunction when presenting with more than two echocardiographic criteria.

I performed approximately 80% of the examinations to avoid interobserver bias as much as posssible.

If tissue Doppler reveals an E/E' which does not prove definitively diastolic dysfunction (15 > E/E' > 8), other investigations are required. These were Doppler of the Mitral valve for E/A ratio, Doppler of the Pulmonary veins, Left ventricular systolic and diastolic dimensions, volume index of the left atrium, or atrial fibrillation (Paulus et al., 2007).

Before every operation the patients underwent a careful history regarding their past medical and surgical issues, clinical examination were done as well as ECG, Echocardiography and lab variables. Investigations and the purpose of the study were explained and a written consent was taken from the patients preoperatively.

### 2.2.5. Clinical variables

#### Questionnaires, preoperative:

Demographic data

Metabolic equivalents

Current medication

Past operations

Age

Height

Weight

Smoking status

Implantable cardioverter defibrillator or pace maker

Family history of coronary artery disease

Percutaneous coronary arteriography in the past

Myocardial infarction

New York heart association (NYHA) classification

Diabetes

Hypertension

Renal function

Peripheral arterial disease

Cerebrovascular event

Lung function

## Medication:

Acetyl salicylic acid

GP IIb/IIIa Receptor blockers

Cumarines

ß-Blockers

ADP-Receptor blockers

Agiotensine Converting enzyme inhibitors

Angiotensine 1 Blockers

Calcium Antagonists

Lipid inhibitors

Diuretics

Nitrates

Operations' date

Operations' duration

Extracorporeal circulation time

Cross clamping duration

Number of Grafts

Blood or coagulation factors transfusion

## 2.2.6. Hemodynamic diagnostics

**Transesophageal Echocardiography (TEE) (after induction of the anaesthesia in the operation theatre)**

1- Cardiac Output (continuity equation), Ejection Fraction (biplane Simpson). The ejection fraction was used to rule out a systolic dysfunction.

2- E wave / A wave / deceleration time (DT) / e´:

As shown in the figure 11, the 'e' wave or the early diastolic phase inflow starts after the T wave of the electrocardiogram (ECG). In a sinus rhythm the late diastolic wave or 'a' wave follows the P wave of the ECG.

The e` illustrated in the figure 12, is the signal of the tissue Doppler on the mitral annulus. It is the tissue movement equivalent to the early ventricular inflow wave.

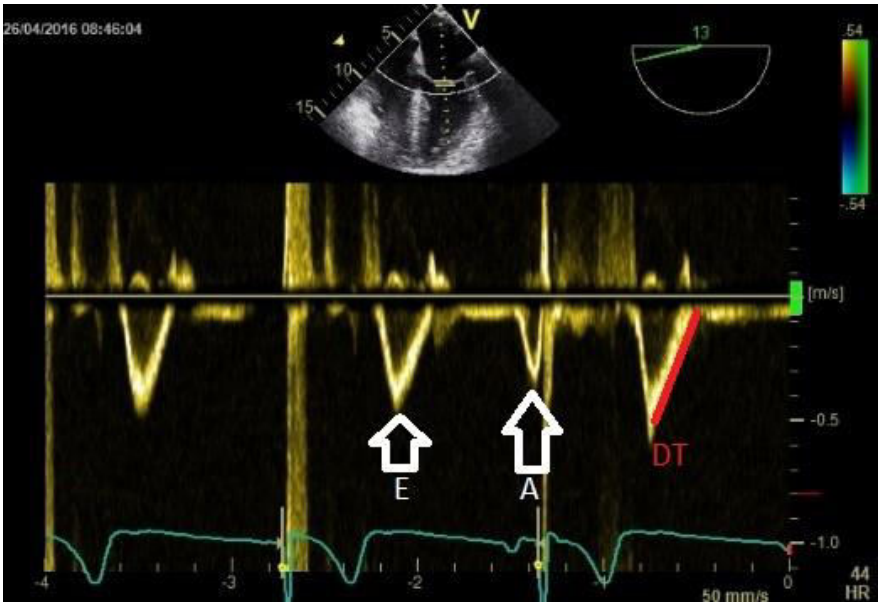



Fig. 11: E wave begins after the T wave on ECG and A wave after the P wave

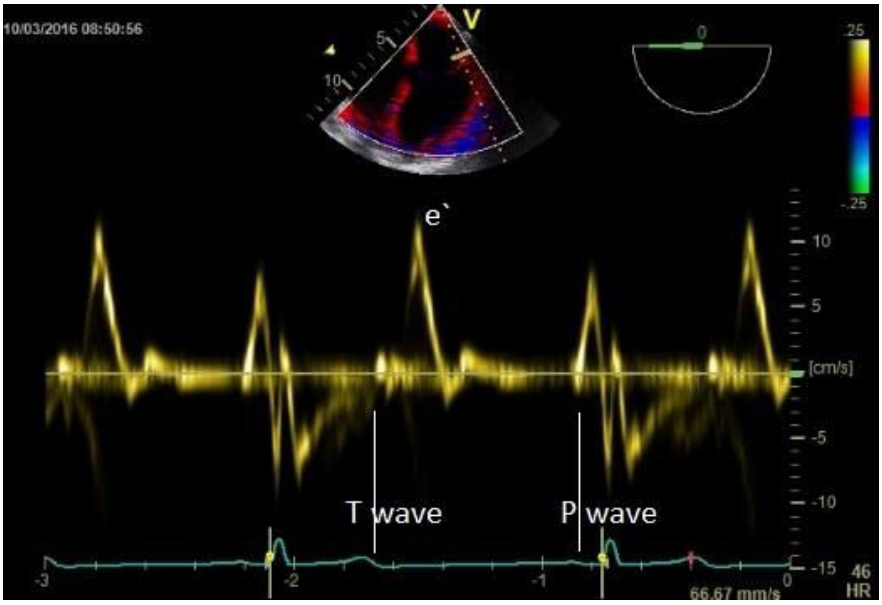



Fig. 12: Tissue Doppler of the lateral mitral annulus

3- S wave (pulmonary) / D wave / Ar dur / A-Ar dur (atrial reversal duration):

It is possible to measure the flow of the pulmonary vein, as it is possible to detect a flow reversal (to the opposite direction) with the PW mode as show in the figure 13.

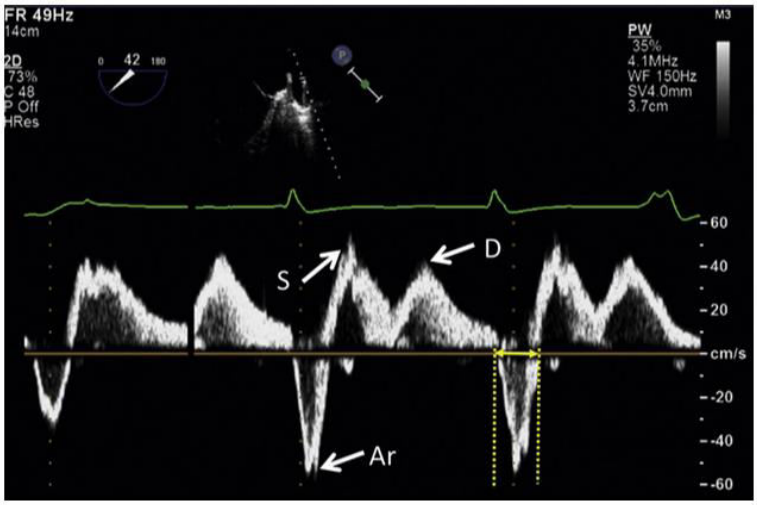



Fig.13: The different waves of a pulmonary vein Doppler

4- left atrial volume index, to measure it we need two pictures of the atrium with 90° rotation and excluding the left atrial appendage and pulmonary veins. (Fig. 14):

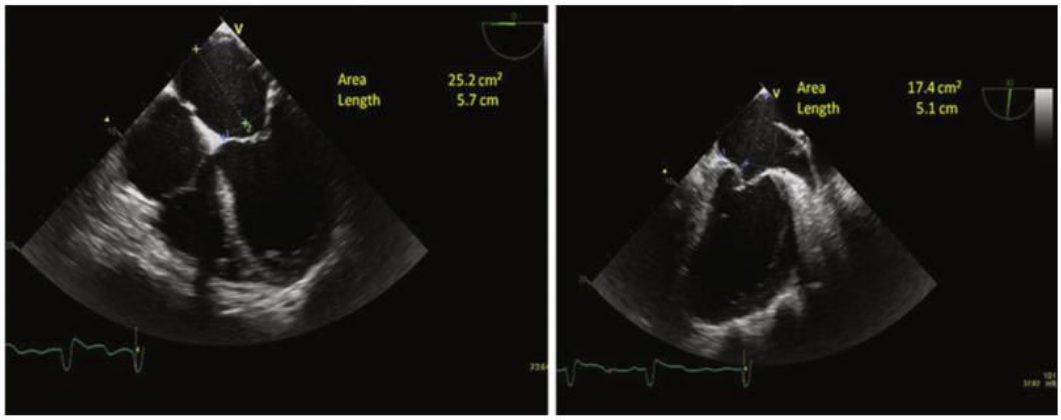



Fig. 14: An example for LA Volume measurement with TEE as biplane method in 4 CV and 2 CV.

## 2.3. Statistics

Continuous variables were reported using the t-Test in case of normally distributed variables and the Mann-Whitney in case of not normally distributed variables. Between-group comparisons were made by using a non-parametric test when not normally distributed. Categorical variables were reported as percentages. Between-group comparisons were made by using a $\chi^2$ test or a Fisher's exact test if any expected count was less than five. For categorical variables with more than two possible values, exact P-values have been estimated. A two-sided P-value <0.05 was considered statistically significant. All statistical tests were done using SPSS version 24 (IBM 2016, USA).

The Hardy–Weinberg equilibrium states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences. These influences include genetic drift, gene flow and meiotic drive. In population genetics studies, the Hardy-Weinberg equation can be used to measure whether the observed genotype frequencies in a population differ from the frequencies predicted by the equation 'p + q = 1'. (Graffelman, Sanchez, Cook, & Moreno, 2013)

In a diploid organism with alleles *A* and a at a given locus, there are three possible genotypes: *AA*, *Aa*, and *aa*. If we use p to represent the frequency of *A* and *q* to represent the frequency of *a*, we can write the genotype frequencies as (*p*) (*p*) or $p^2$ for *AA*, (*q*) (*q*) or $q^2$ for *aa*, and 2(*p*)(*q*) for *Aa*. The equation for genotype frequencies is

*p2+ 2pq + q2 = 1. (Rodriguez, Gaunt, & Day, 2009)*

Our results were tested with the Hardy-Weinberg Equilibrium test and no deviation from the norm was detected.

The multiple logistic regression can be used when there is one nominal variable and two or more measurement variables to detect how the measurement variables affect the nominal variable statistically, or to predict probabilities of the dependent nominal variable, or for suggestions about which independent variables have a major effect on the dependent variable. Choosing variables can be made using an objective method, or by careful examination of the data to subjectively choose the best variables from the observers' point of view.

# 3. Results

## 3.1. Demographic statistics

255 patients aged from 45 to 85 years, which were diagnosed with coronary artery disease and requiring CABG surgery in the cardiosurgical centre of the university hospital of Essen-Germany were finally analysed. Demographic data are presented in table 1.

**Table 1, Demographics:**

| Variable | All (n=255) | No diastolic dysfunction (n=70) | Diastolic dysfunction (n=185) | p-value |
|---|---|---|---|---|
| Age, median (IQ- range) | 68 (15) | 62 (13) | 70 (15) | <0.001[a] |
| Male, n (%) | 211 (83) | 59 (84) | 152 (82) | 0.853[d] |
| BMI, median (IQ-range) | 28 (5) | 28.5 (5) | 28 (5) | 0.662 [a] |
| Non Smokers, n (%) | 99 (39) | 23 (33) | 76 (40) | 0.344[d] |
| Non COPD, n (%) | 196 (77) | 59 (84) | 137 (74) | 0.217[d] |
| Ejection fraction, m (IQ-range) | 56 (10) | 57 (9) | 55 (10) | 0.020[a] |
| NYHA, n (%) 0<br>1<br>2<br>3<br>4 | 13 (5.1)<br>34 (13.3)<br>108 (42.4)<br>92 (36.1)<br>8 (3.1) | 2 (3)<br>9 (13)<br>28 (40)<br>30 (43)<br>1 (1) | 11 (6)<br>25 (13.5)<br>80 (43)<br>62 (33.5)<br>7 (4) | 0.522[c] |
| Myocardial infarction, n (%) | 87 (34.1) | 17 (24.3) | 70 (37.8) | 0.054[d] |
| IDDM, n (%) | 30 (11.8) | 3 (4.3) | 27 (14.6) | 0.001[c] |

| | | | | |
|---|---|---|---|---|
| Glucose, m (IQ-r) | 113 (52) | 103.5 (38) | 116.5 (59) | <0.001[c] |
| Antidiabetic treatment, n (%) | 47 (18.7) | 5 (7.2) | 42 (23) | 0.014[c] |
| ß Blockers, n (%) | 194 (76.1) | 47 (67) | 147 (80) | 0.048[d] |
| Atrial Fib POP, n (%) | 80 (31.5) | 19 (27.1) | 61 (33.2) | 0.450[d] |
| EF Biplane(%) intraop, m (IQ-r) | 55 (14) | 55 (11) | 54 (15) | 0.200[a] |
| Mitral valve E/A ratio, m (IQ-r) | 1.25 (0.59) | 1.35 (0.67) | 1.2 (0.55) | 0.010[a] |
| E/E´ ratio, m (IQ-r) | 8.6 (5) | 5.2 (1.4) | 7.8 (3.3) | <0.001[a] |
| E´ lateral in m/s, m (IQ-r) | 0.08 (0.04) | 0.1 (0.01) | 0.06 (0.03) | <0.001[a] |
| LA Vol. Index ml/$m^2$, m (IQ-r) | 25 (16) | 25.4 (13.7) | 24.6 (15.8) | 0.545[a] |

[a] Mann-Whitney U-test, [b] Wilcoxon-Test, [c] $\chi^2$ Pearson, [d] Fisher´s exact test

As shown on table 1, patients on ß blockers in our sample were significantly associated with diastolic dysfunction. Also patients either with diabetes mellitus or hyperglycemia where more prone to diastolic dysfunction. The results show also that previous heart infarction carries a risk for the disorder.

We could not detect any relevance of the atrial fibrillation and the left atrial volume index. Although these parameters are useful tools for the diagnosis of diastolic dysfunction, they are not pathognomic.

## 3.2. Genetic distribution

### 3.2.1. Genotype frequencies and diastolic dysfunction:

The table 2 shows the frequencies and percentage of the GNAS C2291T haplotypes according to their occurrence in diastolic dysfunction. A slight tendency of the genotype towards the dysfunction group could be identified.

**Table 2, GNAS C2291T genotype distribution:**

| | DD n=185 | % in DD | No DD n=70 | % in non DD |
|---|---|---|---|---|
| CC (%) | 89 (75) | 48% | 29 (25) | 41.5% |
| CT (%) | 77 (70) | 41.5% | 33 (30) | 47% |
| TT (%) | 19 (70) | 10.5% | 8 (30) | 11.5% |
| Sum | 185 | 100% | 70 | 100% |

In table 3 (cross table) the detailed distribution of the genotypes according to the diastolic function of the patients taken from.

**Table 3, Cross table of the GNAS C2291T and the diastolic dysfunction:**

| GNAS C2291T | | | | | | |
|---|---|---|---|---|---|---|
| | | | CC | CT | TT | |
| Diastolic dysfunction | no | Number | 29 | 33 | 8 | 70 |
| | | % in n DD | 41.40% | 47.10% | 11.40% | 100.00% |
| | | % C2291T | **24.60%** | **30.00%** | **29.60%** | **27.50%** |
| | | % Sum | 11.40% | 12.90% | 3.10% | 27.50% |
| | yes | Number | 89 | 77 | 19 | 185 |
| | | % in DD | 48.10% | 41.60% | 10.30% | 100.00% |
| | | % C2291T | **75.40%** | **70.00%** | **70.40%** | **72.50%** |
| | | % Sum | 34.90% | 30.20% | 7.50% | 72.50% |
| Sum | | Number | 118 | 110 | 27 | 255 |
| | | % in DD | 46.30% | 43.10% | 10.60% | 100.00% |
| | | % C2291T | 100.00% | 100.00% | 100.00% | 100.00% |
| | | % Sum | 46.30% | 43.10% | 10.60% | 100.00% |

The table 4 shows the frequencies of the GNAS C2273T genotypes according to their occurrence in diastolic dysfunction. No significant associations could be detected (p=n.s.)

**Table 4, GNAS C2273T haplotype distribution:**

| genotype | DD n=185 | % in DD | No DD n=70 | % in non DD |
|---|---|---|---|---|
| CC (%) | 71 (74) | 38.5% | 25 (26) | 35.5% |
| CT (%) | 86 (71) | 46.5% | 35 (29) | 50 % |
| TT (%) | 28 (74) | 15 % | 10 (26) | 14.5 % |
| Sum | 185 | 100% | 70 | 100% |

In table 5 (cross table) the detailed distribution of the genotypes according to the diastolic function of the patients is shown.

**Table 5, Cross table of the gene GNAS C2273T and diastolic dysfunction:**

| GNAS C2273T | | | | | | |
|---|---|---|---|---|---|---|
| | | | CC | CT | TT | Sum |
| Diastolic dysfunction | no | Number | 25 | 35 | 10 | 70 |
| | | % in n DD | 35.70% | 50.00% | 14.30% | 100.00% |
| | | % C2273T | **26.00%** | **28.90%** | **26.30%** | **27.50%** |
| | | % Sum | 9.80% | 13.70% | 3.90% | 27.50% |
| | yes | Number | 71 | 86 | 28 | 185 |
| | | % in DD | 38.40% | 46.50% | 15.10% | 100.00% |
| | | % C2273T | **74.00%** | **71.10%** | **73.70%** | **72.50%** |
| | | % Sum | 27.80% | 33.70% | 11.00% | 72.50% |
| Sum | | Number | 96 | 121 | 38 | 255 |
| | | % in DD | 37.60% | 47.50% | 14.90% | 100.00% |
| | | % C2273T | 100.00% | 100.00% | 100.00% | 100.00% |
| | | % Sum | 37.60% | 47.50% | 14.90% | 100.00% |

The results were tested with the Hardy-Weinberg Equilibrium test and no deviation from the norm was detected ($p = 0.6$).

### 3.2.2. Diplotype related diastolic dysfunction

Table 6 shows the respective diplotype distribution according to DD. Again, no significant difference between the 6 diplotypes regarding to the diastolic dysfunction. (p value = 0.9).

**Table 6: Diplotype frequencies in our patients**

| Haplotype | DD n=185 | % in DD | No DD n=70 | % in non DD |
|---|---|---|---|---|
| 3*/3* (%) | 28 (74) | 15% | 10 (26) | 14% |
| 3*/2* (%) | 41 (75) | 22% | 14 (25) | 20% |
| 3*/1* (%) | 45 (68) | 24% | 21 (32) | 30% |
| 2*/2* (%) | 20 (80) | 11% | 5 (20) | 7% |
| 2*/1* (%) | 32 (73) | 17% | 12 (27) | 17% |
| 1*/1* (%) | 19 (70) | 11% | 8 (30) | 12% |
| Sum | 185 | 100% | 70 | 100% |

We next tested whether GNAS genotypes and diplotypes are related to diastolic dysfunction in a binary logistic multivariable model including demographic variables that showed significant associations in table 1. As shown on table 7, 8, and 9, neither the T2291C, nor the C2273T and the diplotype were independently associated with diastolic dysfunction while age, diabetes, beta-blocker therapy, preoperative systolic ejection fraction were independently associated with the incidence of DD.

**Table 7: GNAS SNP 2291 genotypes in relation to diastolic dysfunction as well as its depending factors.**

| demographic variables | | | 95% Confidence interval | |
|---|---|---|---|---|
| | p-value | Hazard ratio | Lower value | Higher value |
| Age | 0,000 | 1,068 | 1,034 | 1,104 |
| Diabetes (t. I and II) | 0,003 | 1,832 | 1,235 | 2,720 |
| Bblocker | 0,042 | 2,037 | 1,025 | 4,046 |
| Preoperative EF | 0,012 | 0,952 | 0,916 | 0,989 |
| Myocardial infarction | 0,078 | 1,832 | 0,935 | 3,592 |
| GNAS 2291 | 0,474 | 0,848 | 0,539 | 1,333 |

**Table 8: GNAS SNP 2273 genotypes in relation diastolic dysfunction as well as its depending factors**

| demographic variables | | | 95% Confidence interval for HR | |
|---|---|---|---|---|
| | Significance | Hazard ratio | Lower value | Higher value |
| Age | 0,000 | 1,068 | 1,034 | 1,103 |
| Diabetes (t. I and II) | 0,002 | 1,867 | 1,225 | 2,776 |
| ß blocker | 0,049 | 1,983 | 1,003 | 3,919 |
| Preoperative EF | 0,012 | 0,952 | 0,917 | 0,989 |
| Myocardial infarction | 0,085 | 1,805 | 0,921 | 3,535 |
| GNAS 2273 | 0,743 | 0,929 | 0,600 | 1,440 |
| Constants | 0,326 | 0,213 | | |

**Table 9: GNAS diplotypes and demographic variables in relation to diastolic dysfunction**

| demographic variables | | | 95% Confidence interval | |
|---|---|---|---|---|
| | Significance | Hazard ratio | Lower value | Higher value |
| Age | 0,000 | 1,068 | 1,034 | 1,104 |
| Diabetes (t. I and II) | 0,002 | 1,854 | 1,247 | 2,756 |
| ß-blocker | 0,049 | 1,987 | 1,004 | 3,931 |
| Preoperative EF | 0,012 | 0,952 | 0,916 | 0,989 |
| Myocardial infarction | 0,084 | 1,808 | 0,923 | 3,542 |
| GNAS diplotypes | 0,989 | 1,001 | 0,828 | 1,212 |
| Constants | 0,266 | 0,183 | | |

# 4. Discussion:

Diastole is the interval of the cardiac cycle between the closure of the aortic valve and the closure of the mitral valve. This interval consists of four phases: isovolumic relaxation, early diastolic filling (e wave in PW-Doppler), diastasis, and late diastolic filling (a wave in PW-Doppler). There are numerous factors that influence diastolic function, including ventricular relaxation, compliance, atrial compliance and contractility, and mitral valve function. External factors are the effects of pericardial pressure, intrathoracic pressure, right ventricular function, and left ventricular (LV) systolic function. Echocardiography allows the physician to see and measure multiple indices of diastolic function noninvasively as statistically shown by Geenstein et al. in 2018. (Greenstein & Mayo).

Postoperative mortality after coronary artery bypass grafting operation is associated with a genetic predisposition. We recently showed that specific diplotypes were associated with reduced one-year mortality (Frey et al., 2014). The reason for this observation is still unknown. The systolic function of the patients´ left ventricle was not related to the outcome.

We supposed that the diastolic function of the heart plays a role in the outcome as increased morbidity has been documented in patients with isolated diastolic dysfunction (Tschope et al., 2005). Even older studies that examine relatively young patients with documented normal coronary anatomy suggest increased morbidity in the setting of diastolic dysfunction. One small study with an average age of 55 years and isolated LV diastolic dysfunction, defined as an elevated LV end diastolic pressure at cardiac catheterization, showed that 45% of patients developed symptoms of congestive heart failure within 5 years, and 25% required hospitalization for these

symptoms.(Brogan, Hillis, Flores, & Lange, 1992).

The mortality rate in patients with diastolic heart failure ranges from 5% to 8% annually (O'Connor, Gattis, Shaw, Cuffe, & Califf, 2000). Also there is a direct correlation between the diastolic dysfunction grade and adverse cardiac outcome, for example it is associated to increased post-surgical atrial fibrillation appearance. (Swaminathan et al., 2011) (Ashes et al., 2014).

We examined 255 patients undergoing CABG. The withdrawn blood was used to identify the two important SNPs of the GNAS gene and the patients received an intraoperative evaluation of the diastolic heart function. Intra-operative diagnosis and strategies to manage patients with left ventricular diastolic dysfunction are not well clarified, and diastolic dysfunction could be considered as a "Trojan horse"! (Apostolakis, Baikoussis, Parissis, Siminelakis, & Papadopoulos, 2009)

The results show a correlation between age, β blocker therapy, previous myocardial infarction and glucose intolerance. (p value = 0.04 or less)

Von Bibra et al has mentioned the correlation between age, overweight and Insulin resistance from one side and the diastolic dysfunction from the other side. (von Bibra & Paulus, 2016)

The ß blocker therapy and previous myocardial infarction are not described in the literature as direct correlates to the diastolic dysfunction, but as mentioned before the consequences of the disease in terms of stiffness and increased filling pressure as well as the according therapy. Because it is impossible in these patients to know which condition came first, the diastolic dysfunction or the cardiac insult, we assume here a who came first or ‘hen-egg like’ correlation.

Our results showed that none of the GNAS polymorphisms play a role in the diastolic

dysfunction. This leads us to think about other possible mechanisms causing this disease. It is well known that some demographic criteria are correlated to it, so it is possible to be a non-genetic origin or linked with other disorders. For example Nair et al identified circulating micro RNAs (miRNA) which showed distinct patterns of expression in patients with diastolic dysfunction (Nair, Kumar, Gongora, & Gupta, 2013). In the future miRNAs can be used as diagnostic tool alone or in combination with other biomarkers like (B-type natriuretic peptide) BNP (Yan et al., 2017).

Another work evaluate the therapeutic applicability of miRNAs in diastolic dysfunction (Schulte, Westermann, Blankenberg, & Zeller, 2015).

In another aspect, the gender was not significantly correlated with the diastolic dysfunction, though there were a slight tendency towards the female gender. The patients had a similar ejection fraction (55 +/- 5) tend to be smoker and no difference in the NYHA score was observed.

This slight tendency towards the female gender was seen but not explicit mentioned in a work of Kuznetsova et al. The diastolic dysfunction is in general an age and gender dependent syndrome which can be observed in individuals with previous heart attacks or in diabetics.(Kuznetsova et al., 2009; von Bibra & Paulus, 2016)

A structural or functional valvular disease should be ruled out as this may influence the atrial function and cause a secondary diastolic dysfunction. We could confirm these factors in our study without any correlation to a genetic predisposition, perhaps because there is cellular and not a molecular cause. The new definition of diastolic dysfunction is not based on the E/A ratio anymore, but the E/e` and the newly accepted tricuspid regurgitation speed, which means that the atrial contraction is not a must to measure the grade of diastolic dysfunction any more. This allows the

examiner to make a more objective view of the disease with more different variables, as one-third to one-half of patients with diastolic dysfunction have normal left atrial size ( Lewis, G. A., Schelbert, E. B., Williams, S. G., Cunnington, C., Ahmed, F., McDonagh, T. A., & Miller, C. A., 2017).

In a study diastolic heart failure has also been associated with other comorbid conditions, in addition to age, the median ejection fraction in these patients was 58%. Overall, 55% of patients were female, which supports our results, 65% had ischemic heart disease (60% of these had peripheral artery disease), 28% had diabetes. (Franklin & Aurigemma, 2005). In our study 38% were diabetics, from them 86% had diastolic dysfunction, with the type I diabetes mellitus (IDDM) showing a 90% association with DD, while in the non-diabetics the fraction of the pathological findings was 65%.

Although there is a small tendency to the GNAS C2291T SNP with diastolic dysfunction, it doesn't show a statistical significance (p value = 0.4).

In the 2273 SNP there were no obvious difference between the genotypes.

Our results were tested with the Hardy-Weinberg Equilibrium test and no deviation from the norm was detected (p value = 0.6).

Also the diplotype analysis didn´t show any significant difference between the 6 diplotypes regarding to the diastolic dysfunction. (p value = 0.9).

It can be assumed that the myocardial ischemia which leads to the treatment with a beta blocker or its symptoms is the cause for the myocardial stiffening and the progress of the disease. Myocardial structure and intra-myocardial signalling were shown to be altered in diastolic dysfunction. Paulus et al. proposed that a systemic pro-inflammatory state induced by comorbidities as the cause of myocardial structural

and functional alterations (Paulus & Tschope, 2013). Their paradigm presumes the following sequence of disorders, comorbidities such as obesity, diabetes mellitus, chronic obstructive pulmonary disease, and salt-sensitive hypertension induce a systemic pro-inflammatory state which in turn causes coronary microvascular endothelial inflammation followed with reduced nitric oxide bioavailability, cyclic guanosine monophosphate content, and protein kinase G (PKG) activity in adjacent cardiomyocytes. These factors favour hypertrophy development and increases resting tension because of hypophosphorylation of titin; and both stiff cardiomyocytes and interstitial fibrosis contribute to high diastolic left ventricular stiffness and dysfunction. Myocardial remodelling in diastolic heart failure differs from heart failure with reduced ejection fraction, in which remodelling is driven by loss of cardiomyocytes (Paulus & Tschope, 2013). This new paradigm for the development of heart failure with preserved ejection fraction has identified a systemic pro-inflammatory state induced by comorbidities as the origin of microvascular endothelial cell inflammation and subsequent concentric cardiac remodelling and dysfunction (Tschöpe & Van Linthout, 2014).

Interestingly, in patients with diastolic dysfunction have recently been found to improve by the use of the neprilysin inhibitor (LCZ696), which inhibits the breakdown of natriuretic peptides. Neprilysin activity is induced in obese patients. The finding that IL-1ß induces neprilysin activity may explain the increased neprilysin and BNP paradox in obese patients with diastolic dysfunction, which is associated with increased inflammation. BNP can be used to screen diabetic patients for the presence of left ventricular dysfunction (Epshteyn et al., 2003).

As the left ventricular hypertrophy and left atrial dilation are considered hallmarks of diastolic dysfunction, and are included in its definition and in the last

echocardiographic guidelines (Nagueh et al., 2016) and in the inclusion criteria of current randomized controlled trials, it should be noted that there is considerable morphological heterogenecity. Approximately one half of patients with diastolic dysfunction do not have left ventricular hypertrophy and remodelling is more common in patients with hypertension, although approximately one half of them and those with normal left ventricular mass have hypertension. Diastolic dysfunction also forms part of the guideline definition of heart failure with preserved ejection fraction. Using invasive conductance pressure-volume assessments, Westermann et al. (Westermann et al., 2008) found that mean left ventricular relaxation time constant, end-diastolic pressure, diastolic stiffness, and stiffness constant were higher in patients with ejection fraction more than 50% at rest, and mean end-diastolic volume, stroke volume, and cardiac output were reduced during tachycardic atrial pacing compared with mean values in age, sex, and comorbidity-matched control subjects, although there was a strong trend toward a higher prevalence of hypertension in the normal ejection fraction group. ( Lewis, G. A., Schelbert, E. B., Williams, S. G., Cunnington, C., Ahmed, F., McDonagh, T. A., & Miller, C. A., 2017)

To summarise it is to assume that the SNPs of the gene GNAS does not influence the diastolic dysfunction and the link between the different outcomes between the GNAS haplotypes stays unknown. One can assume that the predisposition to the disease is multifactorial and there is (until now) not a single known factor which can tell us a definite prognosis.

## 5. Summary:

**Thesis:** SNPs (single nucleotide polymorphisms) in regulatory regions of GNAS gene impact upon cardiac performance in vivo showed no association with the systolic function of the heart, because the cardiac function is not only described by the systolic function, but also by the diastolic function, the question is whether the GNAS Haplotypes affect the diastolic function having therefore an influence on the outcome.

Methods: We examined 255 patients undergoing coronary aortic bypass grafting. Two millilitre blood was withdrawn to identify the two important SNPs of the GNAS gene and the patients receive an intraoperative evaluation of the diastolic heart function.

Gene extraction was performed, followed with its genotyping using the polymerase chain reaction technique. The Haplotypes were matched to the echocardiographic results of the transoesophageal echo which were performed in the cardiosurgical operations theatre.

For a two group comparison we used the $\chi^2$ test or the Fisher's exact test if any expected count was less than five. For categorical variables with more than two possible values, exact P-values have been estimated. A two-sided P-value $<0.05$ was considered statistically significant. All statistical tests were done using SPSS version 24 (IBM 2016, USA). The results show a correlation between diastolic dysfunction and age, ß blocker therapy, previous myocardial infarction and glucose intolerance.

But unfortunately the SNPs of the gene GNAS does not seem influence the diastolic dysfunction and the link between the different outcomes between the GNAS haplotypes stays unknown.

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## 7. Table of figures:

## 8. Tables' list:

# 9. Abbreviations' list:

| | |
|---|---|
| ACT | Activated clotting time |
| Gai | G Protein Adenylate Cyclase inhibitor |
| Gas | G Protein Adenylate Cyclase stimulator |
| CHD | Chronic heart failure |
| cSNP | Coding SNP |
| CABG | Coronary arterial bypass grafting |
| CAD | Coronary artery disease |
| cAMP | Cyclic adenosine monophosphate |
| DNA | Deoxyribonucleic acid |
| DD | Diastolic dysfunction |
| ECG | Electrocardiogram |
| EDTA | Ethylene-diamine-tetra-acetic acid |
| GRK | G protein coupled receptor kinases |
| GOLD | Global Initiative for Chronic Obstructive Lung Disease |
| GNAS | Guanine nucleotide alpha subunit |
| htSNP | Haplotype tagging SNP |
| IABP | Intra-aortic balloon pump |
| iSNP | Intronic SNP |
| LV | Left ventricle |
| miRNA | Micro Ribonucleic acid |
| NYHA | New York heart association |
| PCR | Polymerase chain reaction |
| PKA | Protein kinase A |
| PKG | Protein kinase G |
| rSNP | Regulatory SNP |
| sSNP | Silent SNP |
| SNP | Single nucleotide polymorphism |
| TEE | Transoesophageal echocardiography |
| IDDM | Type I diabetes mellitus |

# 10. Patients´ agreement consent

Patientennummer

_____

**Einwilligungserklärung zur wissenschaftlichen Untersuchung**

Ich bin damit einverstanden, dass die bei einer Operation gewonnenen Blutproben (insgesamt 2 ml) für wissenschaftliche Zwecke verwendet werden. Ich bin im Rahmen folgender Studie einverstanden:

## Untersuchung genetischer Einflüsse auf die diastolische Herzfunktion

Mir ist bewusst, dass die Teilnahme an der Studie vollkommen freiwillig ist und mein vorheriges Einverständnis erfordert. Ich bin außerdem darüber informiert worden, dass diese Proben nicht kommerziell verwertet werden.

Ich bin damit einverstanden,

- dass die medizinischen Daten aus der Untersuchung meiner Blutproben aufgezeichnet werden.
- dass die Blutproben für weitere Tests im Rahmen der beschriebenen wissenschaftlichen Untersuchungen aufbewahrt werden.
- dass die medizinischen Daten aus der Untersuchung meiner Blutproben in pseudonymisierter Form (d.h. ohne Name des Patienten) zur wissenschaftlichen Auswertung innerhalb der Klinik weitergegeben werden können.
- dass die medizinischen Daten aus der Untersuchung meiner Blutproben als personenbezogene Daten von autorisierten Mitarbeitern der Klinik (Priv.-Doz. Dr. Ulrich Frey) eingesehen werden können, um die ordnungsgemäße Durchführung der Studie zu überprüfen.

Ich erkläre mich hiermit mit der Untersuchung meiner Blutproben und der Datenerhebung der klinischen Daten einverstanden.

Ich habe eine Kopie der Einwilligungserklärung und der Patienteninformation erhalten.

**<u>Information und Einwilligungserklärung zum Datenschutz</u>**
**Im Rahmen des Forschungsprojektes werden persönliche Daten und medizinische Befunde über Sie erhoben. Die Weitergabe, Speicherung und Auswertung dieser projektbezogenen Daten erfolgt nach gesetzlichen Bestimmungen und setzt vor Teilnahme an dem Projekt folgende freiwillige Erklärung voraus:**

**Ich erkläre mich einverstanden, dass im Rahmen der Studie erhobene Daten auf Fragebögen und / oder elektronischen Datenträgern aufgezeichnet und ohne Namensnennung (pseudonymisiert) ausgewertet werden dürfen. Außerdem bin ich damit einverstanden, dass die Studiendaten in anonymisierter Form für wissenschaftliche Darstellungen und Veröffentlichungen verwendet werden dürfen.**

Vom Patienten persönlich auszufüllen:

Datum _____

Unterschrift _____

Name in Druckbuchstaben_____

Vom aufklärenden Arzt auszufüllen, der das Informationsgespräch geführt hat:

Datum _____

Unterschrift _____

Name in Druckbuchstaben_____

## 11. Acknowledgement:

Firstly, I would like to express my sincere gratitude to my advisor Prof. Dr. U. Frey for the continuous support of my doctor thesis and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my work.

Last but not the least, I would like to thank my family, my parents and my wife and my three daughters for supporting me spiritually throughout writing this thesis and my life in general.

Der Lebenslauf ist in der Onlineversion aus datenschutzrechtlichen Gründen nicht enthalten