

Pharmacogenetics may explain part of the interindividual variability of dobutamine pharmacodynamics in neonates

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Abstract

Aim: To determine whether the known single nucleotide polymorphisms in adrenoreceptor associated genes affect the hemodynamic response to dobutamine in critically ill neonates. **Methods:** Alleles in the known genetic single nucleotide polymorphisms in $\beta 1$ and $\beta 2$ adrenoreceptor (AR) genes and Gs protein α -subunit gene (GNAS) possibly affecting inotropic effect were identified in patients of neonatal dobutamine pharmacokinetic-pharmacodynamic study. Linear mixed-effect models were used to describe the effect of genetic polymorphisms to heart rate (HR), left ventricular output (LVO) and right ventricular output (RVO) during dobutamine treatment. **Results:** 26 neonates (5 term, 21 preterm) were studied. Dobutamine plasma concentration and exposure time respective HR (adjusted to gestational age) is dependent on $\beta 1$ -AR Arg389Gly polymorphism so that in G/G (Gly) homozygotes and G/C heterozygotes dobutamine increases HR more than in C/C (Arg) homozygotes, with parameter estimate (95% CI) of 38.3 (15.8 – 60.7) bpm per AUC of 100 mg·h, $p=0.0005$. LVO (adjusted to antenatal glucocorticoid administration and illness severity) and RVO (adjusted to gestational age and illness severity) is dependent on GNAS c.393C>T polymorphism so that in T/T homozygotes and C/T heterozygotes but not in C/C homozygotes LVO and RVO increase with dobutamine treatment, 24.5 (6.2 – 42.9) mL kg⁻¹ min⁻¹ per AUC of 100 mg·h, $p=0.0116$ and 33.2 (12.1 – 54.3) mL kg⁻¹ min⁻¹ per AUC of 100 mg·h, $p=0.0025$, respectively. **Conclusion:** In critically ill neonates, $\beta 1$ -AR Arg389Gly and GNAS c.393C>T polymorphisms may play a role in the haemodynamic response to dobutamine during the first hours and days of life.

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The authors confirm that the PI for this paper is Maarja Hallik and that she had direct clinical responsibility for patients.

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Running head: Dobutamine pharmacogenetics in neonates

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What is already known about this subject?

Some genetic variation in human cardiac β -adrenoceptor pathway that might result in different response to inotropic therapy have been described.

In vivo data about the effects of genetic variation to cardiovascular response come from adult studies with small number of healthy volunteers or heart failure patients.

What this study adds?

- The study describes the genetic variability in dobutamine pharmacodynamics in critically ill neonates.
- The large interindividual variability of the cardiovascular effects of dobutamine in early postnatal life may partly be explained by adrenoceptor and Gs protein α -subunit gene sequence variants.
- Results from adult studies cannot be readily extrapolated to neonates.

ABSTRACT

Aim: To determine whether the known single nucleotide polymorphisms in adrenoreceptor associated genes affect the hemodynamic response to dobutamine in critically ill neonates.

Methods: Alleles in the known genetic single nucleotide polymorphisms in $\beta 1$ and $\beta 2$ adrenoceptor (AR) genes and Gs protein α -subunit gene (GNAS) possibly affecting inotropic effect were identified in patients of neonatal dobutamine pharmacokinetic-pharmacodynamic study. Linear mixed-effect models were used to describe the effect of genetic polymorphisms to heart rate (HR), left ventricular output (LVO) and right ventricular output (RVO) during dobutamine treatment.

Results: 26 neonates (5 term, 21 preterm) were studied. Dobutamine plasma concentration and exposure time respective HR (adjusted to gestational age) is dependent on $\beta 1$ -AR Arg389Gly polymorphism so that in G/G (Gly) homozygotes and G/C heterozygotes dobutamine increases HR more than in C/C (Arg) homozygotes, with parameter estimate (95% CI) of 38.3 (15.8 – 60.7) bpm per AUC of 100 mg·h, $p=0.0005$. LVO (adjusted to antenatal glucocorticoid administration and illness severity) and RVO (adjusted to gestational age and illness severity) is dependent on GNAS c.393C>T polymorphism so that in T/T homozygotes and C/T heterozygotes but not in C/C homozygotes LVO and RVO increase with dobutamine treatment, 24.5 (6.2 – 42.9) mL kg⁻¹min⁻¹ per AUC of 100 mg·h, $p=0.0116$ and 33.2 (12.1 – 54.3) mL kg⁻¹ min⁻¹ per AUC of 100 mg·h, $p=0.0025$, respectively.

Conclusion: In critically ill neonates, $\beta 1$ -AR Arg389Gly and GNAS c.393C>T polymorphisms may play a role in the haemodynamic response to dobutamine during the first hours and days of life.

INTRODUCTION

Some variability in drug response can be explained by genetic variability in drug targets. Single nucleotide polymorphisms (SNP) in receptor encoding genes, may result in altered function, substrate binding affinity, expression, and both up- and downregulation of receptors.¹

In the cardiac β -adrenoceptor (AR) pathway β -ARs themselves and Gs protein α -subunit

have been found to date to display some genetic variation in humans that might result in different response to inotropic therapy.²

The β 1-AR gene (*ADRB1* ; RefSeq NM_000684.2) has two commonly variable sites, at amino acid positions 49 and 389. The most studied AR gene polymorphism is the Arg389Gly human β 1-AR gene polymorphism. It is associated with significantly elevated adenylate cyclase (AC)/protein kinase A activities and hence β 1-AR- stimulated cardiac contractility in the Arg389 variant carriers compared with Gly389 carriers.³

Arg389 β 1-AR is more common than Gly389 β 1AR in individuals of European descent.

A second human β 1-AR polymorphism is the p.Ser49Gly variation, associated with increased long-term agonist-promoted receptor downregulation for the Gly49 over the Ser49 variant.^{4,5} The beneficial effect of Gly49 variant on survival of heart-failure patients, is supporting the concept that β 1-AR desensitization is protective in heart failure.⁶

Three nonsynonymous polymorphisms have been shown to exist in the coding region of the β 2-AR gene (*ADRB2*, RefSeq NM_000024.5), at amino acid positions 16, 27, and 164.⁷ Gly16Arg and Gln27Glu variations are in linkage disequilibrium and thus constitute a haplotype, which affects receptor downregulation. And a third polymorphism, Thr164Ile, a rare polymorphism (Ile164, heterozygous frequency ~0.03) that confers impaired receptor G protein coupling and reduced acetylcholine mediated signalling, resulting in severely depressed or absent agonist-promoted cardiac contractility.⁸⁻¹⁰

The β -AR-Gs protein system is essential for the activation of AC in cardiac smooth muscle, which regulates cardiac output and peripheral vascular resistance.^{11,12} Retrospective studies suggest that the c.393C>T polymorphism in the human gene encoding the α -subunit of Gs protein (*GNAS*, RefSeq NM_000516.4) has an effect on response to β -blockade and heart rate after exercise stress test.^{13,14} Data from a prospective study suggest that the c.393C>T polymorphism of GNAS is functionally relevant *in vivo*. The results of a modified dobutamine stress echocardiography protocol show that individuals with homozygous or heterozygous C393 have an increased cardiovascular agonistic response to dobutamine.¹⁵

According to PharmGKB database, β 1-AR gene Arg389Gly and GNAS gene c.393C>T polymorphisms are associated with haemodynamic response to dobutamine *in vivo*.¹⁶ However, there are no data on the effect of the genetic variability of β -ARs or Gs protein α -subunit on haemodynamic response to agonist stimulation in early neonatal life.

Our study aimed to find out whether the known SNPs in the β 1-, β 2-AR and GNAS genes play a role in the variability of cardiovascular response to dobutamine treatment in critically ill neonates.

METHODS

The study was conducted as a sub study of dobutamine pharmacokinetic-pharmacodynamic (PKPD) study, approved by the Ethics Committee of the University of Tartu and registered at the EU Clinical Trials Register under number 2015-004836-36. A prospective 2-centre study was performed from April 2016 to December 2017 in Tallinn Children's Hospital, Tallinn, Estonia and Tartu University Hospital, Tartu, Estonia. Full details of the study can be found elsewhere.¹⁷

Briefly, neonates hospitalised to NICU within the first 72 hours of life and needing inotropic therapy were studied. Written informed consent for genetic analysis was signed by parents or guardians separately from the main dobutamine PKPD study consent before study inclusion.

Whole blood samples of 0.3 ml were collected from indwelling arterial or venous catheter within the study period. The samples were transported immediately to the Department of Clinical Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia. DNA was extracted from blood lymphocytes by Nucleospin Tissue kit (Macherey-Nagel). Sanger sequencing was performed for *ADRB1* gene exon 1, *ADRB2* gene exon 1 and *GNAS* gene exon 5, accordingly including the following variants of interest: β 1-AR p.Gly389Arg, β 1-AR p.Ser49Gly, β 2-AR Gly16Arg, β 2-AR Gln27Glu, *GNAS* c.393C>T. PCR primers used are provided in Supplementary Table 1. Sanger sequencing was performed with same primers as for PCR amplification and products were sequenced bidirectionally using ABI GeneScan 3130XL instrument.

Dobutamine was administered at escalating doses of 5-20 $\mu\text{g kg}^{-1} \text{ min}^{-1}$. Monitoring and recording of heart rate (HR) and mean arterial pressure (MAP) were started before dobutamine infusion and continued throughout the treatment period, left ventricular ejection fraction (LVEF), left and right ventricular cardiac output (LVO, RVO) were measured before and repeatedly during the dobutamine treatment.¹⁷

The effect of SNPs on haemodynamic parameters (HR, MAP, LVEF, LVO and RVO) was analysed with linear mixed-effects models separately for each SNP. The model building process was started with full model including gestational age (GA), Score for Neonatal Acute Physiology Perinatal Extension (SNAPPE-II)¹⁸, age at recruitment, antenatal glucocorticoid administration (only if GA <34 weeks), exposure to dobutamine measured as the area under the time-plasma concentration curve (AUC) calculated according to the published dobutamine population pharmacokinetic model using empirical Bayes estimates of pharmacokinetic parameters in each neonate, and interaction between AUC and SNP as fixed effects and random intercept and random slope of AUC for each neonate.¹⁷ Stepwise backward elimination was used to retain only statistically significant effects into model. Autocorrelation structure of order 1 with time after dose (in minutes) as covariate was used in models for HR and MAP.

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

RESULTS

Overall, 26 of the 28 PKPD study participants were consented to the pharmacogenomic study. The median (range) gestational age was 30.9 (22.7 – 41.0) weeks, birth weight 1668 (465 – 4380) g, age at recruitment 6.3 (2.4 – 27.5) h, SNAPPE-II scores 14.5 (3.0 – 64.0), 5 min APGAR score 7 (1 – 8), baseline LVEF 62.5 (51.0 – 79.0) %, RVO 126 (75 – 306) $\text{mL kg}^{-1} \text{ min}^{-1}$, LVO 128 (71 – 232) $\text{mL kg}^{-1} \text{ min}^{-1}$.

The incidences of investigated alleles were comparable to those of general population of Estonia in all observed SNPs. (Table 1)

We found that β 1-AR Arg389Gly polymorphism influences HR increase during dobutamine treatment and *GNAS* c.393C>T polymorphism affects cardiac output response to dobutamine. The other studied SNPs did not show statistically significant effect on dobutamine induced changes in HR, MAP, cardiac output or LVEF. The linear mixed-effect models, where the effect of SNPs on hemodynamic parameters was statistically significant are presented in Table 2. HR adjusted to GA is dependent on β 1- AR Arg389Gly polymorphism so that in G/G (Gly) homozygotes and G/C heterozygotes dobutamine increases HR more steeply than in C/C (Arg) homozygotes ($p=0.0008$). (Figure 1) LVO adjusted to antenatal glucocorticoid administration and SNAPPE-II is dependent on *GNAS* c.393C>T polymorphism so that in T/T homozygotes and C/T heterozygotes but not in C/C homozygotes LVO increases during dobutamine treatment ($p=0.0095$). (Figure 2) RVO adjusted to SNAPPE-II and GA is dependent on *GNAS* c.393C>T polymorphism so that in T/T homozygotes and C/T heterozygotes but not in C/C homozygotes RVO increases with dobutamine treatment ($p=0.0025$). (Figure 3)

Individual measured haemodynamic parameter values, population (marginal) and individual (conditional) predictions from the final linear mixed effects models plotted against dobutamine AUC are presented in the supplementary figures S1-S3.

DISCUSSION

To the best of our knowledge this is the first study demonstrating the role of polymorphisms in the β -AR pathway related genes in the variability of response to inotropic therapy in critically ill neonates. The main findings of the study were associations between Arg389Gly polymorphism in β 1-AR gene and HR as well as GNAS c.393C>T polymorphism and cardiac output (RVO and LVO).

There is only sparse clinical data about the influence of studied variants and cardiovascular response to inotropes. In our study, β 1-AR Arg389 homozygotes had lower heart rate in response to dobutamine compared to heterozygotes and Gly389 homozygotes, with no effect on RVO or LVO. In contrast, previous studies in adults found that human hearts with Arg389 β 1-AR variant, from both healthy subjects and patients with heart failure, had substantially greater agonist-induced inotropy compared with hearts from Gly389 carriers, in an *ex vivo* experiment.²⁰ Healthy male volunteers homozygous for the Arg389 β 1-AR showed significantly higher increase in fractional shortening upon cumulative doses of dobutamine as compared to subjects carrying one or two copies of the Gly389 allele.²¹ In a study of 10 and 8 male subjects homozygous Arg389 β 1-AR and Gly389 β 1-AR, respectively (to avoid influences of codon 49 polymorphism, all were homozygous Ser49 β 1-AR), dobutamine increased plasma-renin activity, heart rate and contractility and decreased diastolic blood pressure more potently in Arg389 β 1-AR versus Gly389 β 1-AR subjects.²² In healthy individuals, HR and renin responses to dobutamine in incremental doses were more than three-fold greater among β 1-AR Arg389 compared with Gly389 homozygotes.²³ In the same study population, no significant difference was found in haemodynamic response to dobutamine constant infusion between β 1-AR 389 genotypes.²⁴ The difference in the effect on chronotropy in neonates, compared to adults, may be explained by developmental physiology as well as differences in β -AR signalling in the immature heart. As in neonates, especially in the premature and critically ill with cardiac failure, HR is already high before treatment, the lower HR in response to dobutamine treatment in Arg389 β 1-AR subjects may refer to better response to inotropic treatment, with sequence variant specific difference in the change in contractility not captured by our measurement methods.

We also found the effect of GNAS c.393C>T polymorphism on cardiac output during dobutamine treatment, T allele carriers increasing RVO and LVO in response to dobutamine compared to no change or decrease in C/C homozygotes. (Figures S2, S3) The only in vivo prospective study in 18 healthy Chinese adults showed that individuals with homozygous or heterozygous C393 had an increased cardiovascular agonistic response to dobutamine.¹⁵ This controversy remains difficult to explain, as the exact molecular mechanism of the effect of the GNAS c.393C>T polymorphism has not been identified.²⁵ Further studies are urgently needed, as our very first data in the field suggest potential untoward effect of decreasing LVO and RVO with increasing dobutamine exposure in C/C homozygous neonates.

Three of the studied polymorphisms (β 1-AR Ser49Gly and β 2-AR Gly16Arg and Gln27Glu) have been associated with agonist promoted receptor desensitisation and downregulation.^{4,5,8–10} The lack of association between these SNPs and cardiovascular response to dobutamine in our study population may be explained by ontogeny of β -ARs. Results of juvenile animal studies indicate that β -ARs in the neonatal heart do not desensitize in response to agonist stimulation.^{26–29} Furthermore, the maintenance of cardiac β -AR signalling in the face of intense stimulation is likely to play an important role in the physiologic adaptation during perinatal transition.³⁰ One study on preterm foetal sheep demonstrates the downregulation of β -AR with prolonged dobutamine treatment in preterm heart exposed to hypoxia-ischemia.³¹ The lack of the clinical evidence of receptor downregulation in our study may have been influenced by the relatively short observational period.

In addition to the unique situation of transitional circulatory response in neonates several perinatal factors/interventions, like prematurity, glucocorticoid exposure or hypoxia-ischemia may contribute to the effects of sequence variants on dobutamine response.^{31,32} Hence GA, age at recruitment, antenatal glucocorticoid administration status and SNAPPE-II score as a marker for perinatal illness severity were chosen to be included to the multivariable linear models describing the effect of genetic variability to the haemodynamic response to dobutamine.

Developmental changes in the expression of G protein subunits may account for different response to β -adrenergic stimulation in adult and neonatal populations.³³

Enhanced role of β 2-AR and β 3-AR, as compared to β 1-AR, in chronotropic effects of β -adrenergic agonists has been suggested in both immature rat and large mammal heart. Possible underlying mechanism include immature coupling between β 1-AR and Gs in the early postnatal period.³⁴

In our relatively small study group, we could not detect the role of the studied genetic polymorphisms in predisposition to haemodynamic instability and need for inotropic treatment during the transitional period. The allele incidences of all studied SNPs were comparable to the general population. It has been shown that in term neonates β 1-AR Gly49 homozygosity may predispose to transient tachypnoea of the neonate.³⁵ The possible role of AR related genetic variations in perinatal cardiac failure needs to be addressed in larger association studies.

The low number of participants is a major limitation of our study. Assessment of cardiac function by ultrasound is operator dependent with large inter-occasion and -observer variability. To limit this effect all cardiac ultrasound studies were performed by 5 cardiologists in our study and in each patient all examinations were performed by the same specialist throughout the study period. Relatively short haemodynamic data collection period limited the assessment of time-consuming processes like receptor downregulation.

Conclusions. Our results suggest, that the large interindividual variability of the cardiovascular effects of dobutamine in early postnatal life may partly be explained by AR and GNAS sequence variants. Larger pharmacogenomic studies are urgently needed for better targeted dobutamine therapy in neonates. Results from adult studies cannot be readily extrapolated to younger age groups due to developmental differences in beta-adrenergic signalling and the physiology of early transitional circulation.

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The authors have no conflicts of interest to declare.

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Table 1. The incidence of studied SNP alleles in the study population and Estonian population.

Patient ID	β 2-AR 46A>G Gly16Arg	β 2-AR 79C>G Gln27Glu	β 1-AR c.1165G>C p.Gly389Arg	β 1-AR c.145A>G p.Ser49Gly	GNAS c.393C>T p.(Ile131=)
1	A/A	C/C	C/C	A/A	C/C
2	A/A	C/C	C/C	A/A	T/T
3	A/A	C/C	C/C	A/G	T/T
4	A/A	C/C	G/C	A/A	T/T
5	A/A	C/C	G/C	A/A	T/T
6	A/A	C/C	G/C	A/G	C/T
7	A/G	C/C	C/C	A/G	T/T
8	A/G	C/C	G/C	A/G	T/T
9	A/G	C/C	G/G	A/A	C/T
10	A/G	C/G	C/C	A/A	C/T
11	A/G	C/G	C/C	A/A	C/T
12	A/G	C/G	C/C	A/A	C/T
13	A/G	C/G	C/C	A/A	T/T
14	A/G	C/G	C/C	A/G	C/C

Patient ID	$\beta 2$ -AR 46A>G Gly16Arg	$\beta 2$ -AR 79C>G Gln27Glu	$\beta 1$ -AR c.1165G>C p.Gly389Arg	$\beta 1$ -AR c.145A>G p.Ser49Gly	GNAS c.393C>T p.(Ile131=)
15	A/G	C/G	C/C	A/G	C/T
16	A/G	C/G	C/C	A/G	T/T
17	A/G	C/G	G/C	A/A	C/C
18	G/G	C/G	G/C	A/A	C/T
19	G/G	G/G	C/C	A/A	C/C
20	G/G	G/G	C/C	A/A	C/T
21	G/G	G/G	C/C	A/A	C/T
22	G/G	G/G	C/C	A/G	C/C
23	G/G	G/G	C/C	A/G	C/T
24	G/G	G/G	C/C	A/G	C/T
25	G/G	G/G	G/C	A/A	C/T
26	G/G	G/G	G/C	A/A	C/T
Allele incidence in the study population	G – 55.8%	C – 51.9%	C – 80.8%,	A – 80.8%	T – 55.8%
Allele incidence in the Estonian population ¹⁹	G – 63%	C – 58%	C – 76%	A – 84.5%	T – 44.5%

AR-adrenoceptor.

Table 2. Results of model fitting.

HR ~ GA + AUC + AUC: B1AR Arg389Gly (G/G or G/C)

Intercept	Value
GA	191.6
AUC	-1.5
AUC: B1AR Arg389Gly (G/G or G/C vs C/C)	14.3
SD of random intercept	38.3
SD of residual error	15.2
Autocorrelation parameter	12.7
LVO ~ ANATGC:GA34 + SNAPPE-II + AUC + AUC: GNAS c.393C>T (T/T or C/T)	0.94
Intercept	Value
SNAPPE-II	125.3
AUC	1.3
ANATGC:GA34	-4.5
AUC: GNAS c.393C>T (T/T or C/T vs C/C)	-37.6
SD of random intercept	24.5
SD of residual error	30.2
RVO ~ SNAPPE-II + GA + AUC + AUC: GNAS c.393C>T (T/T or C/T)	27.1
(Intercept)	Value
SNAPPE-II	-128.1
	3.1

GA	7.2
AUC	-10.9
AUC: GNAS c.393C>T (T/T or C/T vs C/C)	33.2
SD of random intercept	60.6
SD of residual error	30.2

HR – heart rate; GA – gestational age; AUC - area under the time-plasma concentration curve; SD – standard deviation; LVO – left ventricular output; SNAPPE-II – Score for Neonatal Acute Physiology Perinatal Extension; ANATGC:GA34 – antenatal glucocorticoid administration if born before 34 weeks of gestation; RVO – right ventricular output.

FIGURE LEGENDS

*Φιγυρε 1. Ποπυλατιον (μαργιναλ) πρεδιστιονς οφ ηεαρτ ρατε ιν βεατς περ μινυτε (βπμ) ς αρεα υνδερ τηε δοβυ-
ταμινε τιμε-ςονςεντρατιον ςυρε (ΑΥ^α) βψ β1-αδρενορεςεπτορ (ΑΡ) Αργ389Γλψ πολψμορπησιμ ανδ γεστατιοναλ
αγε ασσορδινγ το τηε φιναλ λινεαρ μιξεδ εφφερςτς μοδελ.*

*Figure 2. Population predictions of left ventricular output vs area under the dobutamine time-concentration
curve (AUC) by GNAS c.393C>T polymorphism and antenatal glucocorticoid administration and SNAPPE-
II score (1st Quantile: 8.50 3rd Quantile:25.75) according to the final linear mixed effects model.*

*Figure 3. Population predictions of right ventricular output vs area under the dobutamine time-concentration
curve (AUC) by GNAS c.393C>T polymorphism, GA and SNAPPE-II score (1st Quantile: 8.50 3rd Quan-
tile:25.75) according to the final linear mixed effects model.*

AUTHORS' CONTRIBUTIONS

MH conceived the study, participated in its design, collected data, participated in data analysis, and drafted the manuscript. HS conducted data analysis and revised the manuscript. TK and ÜM organised and conduc-
ted genetic material analysis, participated in data analysis, and revised the manuscript. M-LI conceived the
study, collected the data, and revised the manuscript. KK supported the work with LC-MS quantification of
dobutamine. TM conceived the study, participated in its design, collected data, and drafted the manuscript.
All authors read and approved the final manuscript.

The data that support the findings of this study are available from the corresponding author upon reasonable
request.



