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European Journal of Clinical Pharmacology

ISSN 0031-6970

Eur J Clin Pharmacol
DOI 10.1007/s00228-020-02832-0

Springer

European Journal of Clinical Pharmacology
https://doi.org/10.1007/s00228-020-02832-0

PHARMACOGENETICS

Impact of genotype-predicted CYP2D6 metabolism on clinical effects and tolerability of metoprolol in patients after myocardial infarction – a prospective observational study

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Received: 28 August 2019 / Accepted: 8 January 2020

Abstract

Purpose The β-1 adrenergic receptor blocker metoprolol is primarily metabolized by the polymorphic enzyme cytochrome P 450 2D6 (CYP2D6), an enzyme with substantial genetic heterogeneity. Our purpose was to investigate the impact of CYP2D6 metabolism on clinical effects and tolerability of metoprolol in patients after myocardial infarction (MI).

Methods We included 136 patients with MI discharged on treatment with metoprolol with a recommendation to the general practitioner (GP) to increase the metoprolol dose up to 200 mg/day within 2 months if possible. At follow-up, metoprolol dosage after up-titration, metoprolol steady-state trough plasma concentrations, hemodynamic parameters, potential metoprolol-induced adverse drug reactions and number of visits to the GP were measured. *CYP2D6* genotyping including the reduced-function variant alleles *CYP2D6*9*, *CYP2D6*10* and *CYP2D6*41* was performed after end of follow-up.

Results According to the genotype-defined CYP2D6 phenotypes, 30% of the patients were metoprolol extensive metabolizers (EMs), 55% intermediate metabolizers (IMs) and 13% poor metabolizers (PMs; carriers of non-coding and reduced-function variant included). Dose-adjusted metoprolol trough concentrations were significantly higher in IM (2-fold) and PM (6.2-fold) groups vs. the EM group ($p < 0.001$). Only 35% of patients in the PM group achieved the primary end point, i.e. reaching at least 85% of the expected maximum heart rate (HR) during exercise, compared with 78% in the EM group ($p < 0.01$), and maximum observed HR at exercise was significantly lower in the PM group vs. the EM group (129 ± 5 vs. 142 ± 2 bpm, $p < 0.007$). In contrast, metoprolol maintenance dose, blood pressure, exercise capacity, number of visits at the GP and frequency and severity of self-reported potential metoprolol-related adverse drug reactions were not significantly different between the groups.

Conclusion Using a comprehensive *CYP2D6* genotyping panel, the present study demonstrates a > 6-fold increase of dose-adjusted plasma metoprolol trough concentration in CYP2D6 PMs vs. EMs with a parallel lower increase in achieved maximum HR during exercise but without association between genotype and frequency or severity of self-reported adverse drug effects. This may indicate that CYP2D6 PMs potentially could benefit of the increased plasma concentration per dose in a naturalistic setting.

Keywords Metoprolol · CYP2D6 · Genotype · Metabolism · Myocardial infarction

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Published online: 15 January 2020

Introduction

β-1 adrenergic receptor blockers are prescribed to patients with various cardiovascular diseases, including coronary artery disease. The benefit of long-term treatment with oral β-blockers after myocardial infarction (MI) is well established, although most of the supporting data come from trials performed in the pre-reperfusion era [1]. In Norway, the majority of patients discharged after a MI are prescribed a β-blocker, and the most frequently used drug is metoprolol.

Although metoprolol generally exhibits effective reductions in blood pressure (BP) and heart rate (HR), there is substantial individual variability in clinical response. While some patients experience severe bradycardia at standard doses, others obtain insufficient response on cardiovascular parameters. This variability is believed to be mainly related to individual differences in the metabolism of metoprolol via the enzyme cytochrome P450 2D6 (CYP2D6), which is responsible for about 80% of the total clearance of metoprolol [2, 3]. Due to this high preference for CYP2D6, metoprolol has been tested as an exogenous biomarker (probe drug) of CYP2D6 metabolism in vivo [4].

CYP2D6 is highly polymorphic and metabolizes about 25% of all clinically used drugs, including many β-blockers [5, 6]. Among Caucasians, about 5–10% are genetically poor metabolizers (PMs), who totally lack CYP2D6 activity due to homozygous inheritance of non-functional (null) *CYP2D6* alleles, i.e. the variants *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5* and *CYP2D6*6* [6]. The proportion with partial or intermediate CYP2D6 activity (intermediate metabolizers, IMs) is reported to be around 30%, while the remaining are either normal, so-called extensive metabolizers (EMs), or ultrarapid metabolizers (UMs), representing around 55% and 2–5% of Caucasians, respectively [7]. However, a recent study showed that diplotypes comprising the null allele and one reduced-function allele, i.e. the variants *CYP2D6*9*, *CYP2D6*10* or *CYP2D6*41*, exhibited a PM phenotype [8]. Thus, the IM subgroup is very heterogeneous in terms of genotype/phenotype correlations, where carriers of one null allele and one reduced-function allele could be considered as a subgroup where a substantial proportion of Caucasians display a PM phenotype [8].

In pharmacokinetic studies of metoprolol, findings on the relationship between systemic exposure and CYP2D6 metabolism are consistent with a fivefold or larger difference in dose-adjusted concentrations across the various CYP2D6 metabolizer subgroups [2, 9]. Thus, the effective 'dose' (intensity) and β-receptor selectivity of metoprolol treatment are highly dependent on a patient's metabolic capacity via CYP2D6 [2, 9]. Consequently, one may expect that the clinical response of metoprolol will also depend on *CYP2D6* genotype. However, the impact of the *CYP2D6* genotype on cardiovascular effect parameters during treatment with metoprolol is conflicting [10–18]. There is no obvious reason for this observation but the fact that most studies were retrospective and these did not include the reduced-function *CYP2D6* alleles **9*, **10* and **41* are likely relevant issues for the variable study outcomes.

Due to the conflicting findings in previous studies on clinically relevant parameters [10–18], *CYP2D6* genotyping has not yet been implemented as a routine tool for personalized dosing of metoprolol during initiation of therapy, despite the consistent and extensive impact on metoprolol exposure [2, 9]. The aim of the present study was to investigate the impact of CYP2D6 metabolism on clinical effects and tolerability of metoprolol in a prospective design using a comprehensive genotyping panel and accompanying analyses of plasma drug concentrations.

Material and methods

Patient inclusion and follow-up

Diakonhjemmet Hospital is a local urban hospital for approximately 135,000 inhabitants in Oslo, Norway, and the hospital's standard treatment protocol after MI includes metoprolol if no contraindications with a recommendation to the general practitioners to increase the metoprolol dose up to 200 mg/day within 2 months if possible. Patients admitted to Diakonhjemmet Hospital during a period of 30 months with chest pain and increased levels of troponin T suspected to have MI, both with ST elevation (STEMI) and without ST elevation (non (N)-STEMI), with HR > 50 bpm and systolic BP > 100 mmHg on treatment with metoprolol were consecutively asked before discharge to participate in this study. Eligible patients were aged between 18 and 79 years with a life expectancy of more than 6 months, living in Oslo, and understanding either Norwegian or English. Patients already on treatment with metoprolol at a dose of 50 mg/d or more at admission, too frail to perform a bicycle exercise test, or with cognitive impairment were not asked to participate. A total of 150 patients accepted to participate in the study. Ten of the patients did not have signs of coronary artery disease on coronary angiography performed at the nearby university hospital and were therefore not finally discharged on treatment with metoprolol, and four patients either died or were lost to follow-up before *CYP2D6* genotyping and metabolizer classification. These patients were therefore not included. Thus, a total of 136 patients were included in the study.

Patients were discharged on treatment with extended-release metoprolol succinate at a dose chosen at the discretion of the physician responsible for the treatment of the patient, based upon age, body weight, HR and BP. Most patients were discharged on 25–50 mg/day (3% in the EM group and 4% in the IM group were discharged on 12.5 mg/d, whereas 8% in

the EM group, 11% in the IM group and 6% in the PM group were discharged on 100 mg/day). As a standard for Diakonhjemmet Hospital, the discharge report to the general practitioners (GPs) recommended the GPs to gradually increase the metoprolol dose to 200 mg/day within 2 months unless HR fell < 50 bpm, systolic BP fell < 100 mmHg or metoprolol-suspected adverse drug reactions arose. Follow-up consultations at the hospital were performed 3 and 12 months after discharge. The metoprolol dose was up-titrated at the 3-month follow-up visit if the daily dose was below 200 mg with the same exceptions as given in the recommendation to the GPs. Only one patient in the IM group was on treatment with another HR reducing medication than metoprolol (digoxin).

Written informed consent was obtained from all patients. The study protocol was approved by the institutional review board and the Regional Committee for Medical and Health Research Ethics.

CYP2D6 genotyping and metabolizer classification

Analyses of *CYP2D6* variant alleles were performed in whole blood using Taqman-based real-time PCR assays implemented for routine pharmacogenetic analyses at the Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway. The blood was collected at the 3-month follow-up visit, but the analyses of *CYP2D6* variant alleles were performed after the 12-month follow-up visit. The *CYP2D6* pharmacogenetic panel included the non-functional (*null)* alleles *CYP2D6*3* (*rs35742686*), *CYP2D6*4* (*rs3892097*), *CYP2D6*5* (whole gene deletion) and *CYP2D6*6* (*rs5030655*); the reduced-function (*red*) variants *CYP2D6*9* (*rs5030656*), *CYP2D6*10* (*rs1065852*) and *CYP2D6*41* (*rs28371725*); as well as copy number analysis to identify multiplication of functional alleles giving rise to ultrarapid metabolism. The merging of *red*/*null* carriers into the PM subgroup was supported by an initial analysis showing similar metoprolol concentrations in these patients. Absence of the assayed variant alleles was interpreted as *CYP2D6*1* (wild-type).

The patients were divided into the following four CYP2D6 metabolizer subgroups according to *CYP2D6* genotype: (i) PMs, i.e. carriers of *null/null* or *red/null* genotypes; (ii) IMs, i.e. carriers of **1/null* or *red/red* genotypes; (iii) EMs, i.e. carriers of **1/*1* or *red/*1* genotypes; and (iv) UMs, i.e. carriers of three or more functional (**1*) alleles. This classification is not fully compliant with the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for genotype-predicted subgrouping of CYP2D6 phenotypes, but similar to that until very recently used by the Dutch Pharmacogenetics Working Group (DPWG) [19]. However, as our study is based on the previous, until recently used CYP2D6 phenotype nomenclature/classification by DPWG for power calculation and statistical analyses, we have decided to keep the original EM and IM subgroup classifications instead of converting these into the now defined normal metabolizers (NMs).

While the new NM subgroup comprises both carriers of **1/*1*, *red/*1* and **1/null* genotypes, a recent study from our group investigating the *CYP2D6* diplotype-predicted phenotypes in more than 1000 patients showed that the current guidelines are not optimal for activity score definitions of the reduced-function *CYP2D6*9, *10 and *41* variant alleles in Norwegians [8]. While CPIC defines these variants with a generic activity score of 0.5, our calculations showed that the actual enzyme activity score of these reduced-function variant alleles ranged from 0.1 (*41) to 0.3 (*9–10). Thus, we decided to merge carriers of either CYP2D6*9, CYP2D6*10 or CYP2D6*41 with a non-coding allele into the group of CYP2D6 PMs. This is not in line with the new guidelines, where these diplotypes are defined as IMs [19]. However, our merged PM subgroup was supported by an only slightly (~25%), non-significantly higher dose-adjusted steady-state concentration in conventional PMs vs. simultaneous carriers of non-coding and reduced-function variant alleles ($p = 0.1$).

Metoprolol plasma concentration measurements

Blood samples for plasma concentration measurements of metoprolol were drawn at steady-state trough levels before the next metoprolol dose in the morning at the day of clinical follow-up examinations 3 months after hospital discharge. Metoprolol plasma concentrations were analysed at the Department of Clinical Pharmacology, St. Olav's University Hospital, Trondheim, Norway, using an ultrahigh-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method described in detail elsewhere [20]. In brief, 200 μL aliquots of plasma samples and the internal standard metoprolol-d7 (25 μL) were pipetted onto an Ostro 96-well plate (Waters, Milford, MA, USA), using a Tecan Freedom Evo pipetting robot (Tecan, Männedorf, Switzerland). Ice-cold acetonitrile with formic acid (1%) was added for protein precipitation. The eluates were then evaporated to dryness under air, and the samples were reconstituted in 100 μL methanol/water (30:70). A Waters Acquity UPLC I-class FTN system (Waters) equipped with an Acquity UPLC BEH C18 (2.1 × 50 mm, 1.7 μm) column and an Acquity UPLC BEH C18 precolumn (2.1 × 5 mm, 1.7 μm) (Waters) was used for chromatographic separation. Analyses were performed on a Xevo TQ-S tandem quadrupole mass spectrometer (Waters, Manchester, UK) equipped with a Z-spray electrospray interface, using mass transitions of *m/z* 269.3 > 134.0 for metoprolol and *m/z* 275.3 > 191.1 for metoprolol-d7.

The limit of quantification was 5.0 nmol/L, and the method was linear at least up to 1000 nmol/l. Between-day coefficients of variation were assessed at three different concentrations from 8.0 to 750 nmol/L and were < 8% at all concentration levels.

Exercise testing

Three months after discharge, BP was measured with patients resting in supine position before maximum exercise capacity and HR response were examined by a bicycle ergometer exercise test on a XRCISE CYCLE (Cardiowise, Seleon GmbH, Heilbronn, Germany). Expected maximum HR was calculated as 220 minus age. Expected maximum work load was estimated by sex, age, height and body weight. Both maximum expected HR and work load were estimated as expected for healthy individuals without the use of a rate-reducing medication.

The initial workload was 25–50 W depending on age and suspected fitness, and workload was increased stepwise by 25 W every other minute. The reasons for termination of the test were either severe shortness of breath or exhaustion or both.

Adverse drug reaction questionnaire

The potential occurrence of typical metoprolol-induced adverse drug reactions was identified by the use of a questionnaire at the two follow-up visits, with seven questions: Do you have experience of (1) fatigue, (2) impotence (only for men), (3) cold hands and/or feet, (4) reduced exercise capacity, (5) sleep disturbances, (6) nightmares or (7) nausea since inclusion in the study? The patients were asked to grade each potential adverse drug reaction from 0 to 4 indicating no, seldom, occasional, frequent or constant symptoms, respectively.

At discharge from hospital, the patients were asked to prospectively note the number of visits to their GP related to the recent MI, and these numbers were registered at the 3- and 12-month follow-up visits.

End points and statistics

The study was designed to compare PMs with EMs and IMs, and not with UMs, who represent small patient proportion in Scandinavians. The primary end point was achievement of at least 85% of the expected maximum HR under physical stress 3 months after discharge. We used 85% as a cutting point since this is the transition zone between intensive endurance and anaerobic threshold. Secondary end points were observed maximum HR and exercise capacity, achieved percentage of expected maximum HR and exercise capacity, resting BP and HR 3 months after discharge, number of visits to the GP 12 months after discharge and potential metoprolol-induced adverse drug reactions and metoprolol dosage after uptitration 3 and 12 months after discharge. In addition, dose-corrected steady-state trough concentrations of metoprolol (plasma metoprolol concentration/metoprolol dose) at the 3-month follow-up visit were included as a pharmacokinetic end point.

The number of patients to be included in the study was calculated based on the primary end point. Our clinical experience was that about 25% of our patients did not achieve a HR > 85% of expected maximum HR during an exercise test after a MI. We regarded a genotype-related twofold increase in the number of patients that do not achieve this end point as a clinically relevant difference. To achieve this, at least 15 patients had to be included in each phenotypic subgroup. The PM group was expected to be the smallest group and the frequency was expected to be around 10%. Thus, a minimum of 150 patients had to be included ($\beta = 0.20$, $\alpha = 0.05$).

Statistical analysis was performed using the software GraphPad Prism 7.04 (La Jolla, CA, USA). All data are presented as means ± SD or SEM when appropriate. Data from the IM and PM groups were compared with the EM group and analysed by two-tailed, unpaired Student's *t* tests. The groups were tested for equality of variance using a variance ratio test (*F*-test). Variables with unequal variances were analysed with Welch's correction. Categorical variables were analysed by Fischer's exact test. Pearson correlation was used to test the relationship between plasma metoprolol concentration and maximum observed HR at exercise/achieved percentage of expected maximum HR as well as potential metoprolol-induced adverse drug reactions. A p value of < 0.05 was considered statistically significant.

Results

CYP2D6 genotype/phenotype distribution and patient characteristics

The distribution of patients according to genotype-predicted CYP2D6 phenotypes was as follows: UM, $n = 2$ (1.5%); EM, $n = 40$ (30.3%); IM, $n = 73$ (55.3%); and PM, $n = 17$ (12.9%). The two UM patients were excluded from further analyses but interestingly observed with non-detectable steady-state trough levels during treatment with metoprolol doses of 25 and 50 mg/day. In addition, two patients with allele duplication and genotypes comprising non-coding or reduced-function variant alleles were excluded because of unpredictable phenotypes.

Baseline characteristics of the included patients are shown in Table 1. There were no significant differences in baseline characteristics between the three CYP2D6 subgroups.

Table 1 Baseline characteristics of the 130 patients included in the final data analysis

	EM ($n = 40$)	IM ($n = 73$)	PM ($n = 17$)
Age (years), mean ± SD	63 ± 9	62 ± 10	61 ± 11
Males, n (%)	30 (75)	64 (88)	15 (88)
Diabetes mellitus, n (%)	1 (3)	4 (5)	2 (12)
BMI (kg/m^2), mean ± SD	26.5 ± 3.8	26.5 ± 3.7	24.4 ± 3.1
Smoking, n (%)	10 (25)	19 (26)	3 (18)
Atrial fibrillation, n (%)	0	3 (4)	0
STEMI/NSTEMI, n (%)	15/25 (38/62)	32/41 (44/56)	4/13 (24/76)
LVEF ≥ 50%, n (%)	32 (84)	59 (86)	14 (88)
40–49%, n (%)	6 (16)	7 (10)	2 (13)
< 40%, n (%)	0 (0)	3 (4)	0 (0)
NA, n (%)	2 (5)	4 (5)	1 (6)

BMI, body mass index; STEMI, ST-elevation myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; LVEF, left ventricular ejection fraction; NA, data not available

Plasma metoprolol levels and metoprolol maintenance dose

Plasma metoprolol trough concentrations at steady-state were significantly higher in the IM (2.3-fold; $p = 0.008$) and PM (6.8-fold; $p = 0.004$) subgroups compared with the EM subgroup. Furthermore, dose-corrected metoprolol trough concentrations were similarly significantly higher in the IM and PM subgroups compared with the EM subgroup (Fig. 1a). Notably, the metoprolol maintenance dose at 3 and 12 months after discharge and maximum maintenance dose during the 12-month follow-up were not significantly different between EM and the IM and PM groups (Fig. 1b). In the total material, the mean up-titrated daily dose at the 12-month follow-up visit was 68 mg, i.e. only a third of the target daily dose of 200 mg. Only four patients were prescribed the target dose of 200 mg/d (three patients in the EM group and one patient in the IM group), and only 37% of the patients were prescribed a daily dose of 100 mg or more (33% in the EM group, 40% in the IM group and 33% in the PM group).

Hemodynamic parameters

As shown in Fig. 2, only 35% of the patients in the PM group achieved the primary end point, reaching at least 85% of the expected maximum HR, compared with 78% in the EM group ($p < 0.01$). There was no significant difference between the IM group and the EM group. Maximum observed HR at exercise was significantly lower in the PM group than in the EM group, whereas there was no significant difference between the IM group and the EM group. Similarly, achieved percentage of expected maximum HR was significant lower in the PM group compared with the EM group, whereas the percentage was similar in IM group and the EM group. Highly significant but moderate negative correlations were found between plasma metoprolol concentrations and maximum observed HR at exercise ($r = -0.40$; $p < 0.0001$) and achieved percentage of expected maximum HR ($r = -0.41$; $p < 0.0001$). Although numerically lower, there was no statistically significant difference in resting HR in the PM group compared with the EM group both at the 3-month ($p = 0.056$) and 12-month ($p = 0.068$) follow-up visits. Resting HR in the IM group was similar to what was found in the EM group. Furthermore, exercise capacity (Fig. 3a) and BP (Fig. 3b) were not significantly different between the three groups.

Self-reported possible adverse drug reactions

As shown in Fig. 4, the majority of patients in all three groups reported potential metoprolol-related adverse drug reactions both at the 3-month and 12-month follow-up visits. However, few patients (< 15%) reported frequent or constant occurrence of impotence (male patients) and sleep disturbances, whereas even fewer (< 7%) reported frequent or constant occurrence of nightmare and nausea. More patients reported frequent or constant experience of reduced exercise capacity, fatigue and cold hands and/or feet (13–33%) at the 12-month follow-up visit. Despite substantial differences in metoprolol plasma concentrations, there was no increase in the frequency or severity of potential adverse drug reactions in the IM and PM groups vs. the EM group. Furthermore, there was no significant correlation between plasma metoprolol concentrations and the different potential adverse drug reactions.

Visits at the GP

There was no difference in the number of self-reported visits at the GP related to the recent MI 12 months after hospital discharge between the EM group and the IM and PM groups (3.1 ± 0.5 vs. 4.0 ± 0.3 and 3.6 ± 0.6 visits, respectively).

Discussion

The present prospective study confirms the substantial impact of *CYP2D6* genotype on the pharmacokinetics and exposure of metoprolol, but could not find any association between genotype and frequency or severity of self-reported potential adverse drug reactions in patients treated with metoprolol after MI. A significantly lower proportion of CYP2D6 PMs than EMs reached 85% of expected maximum HR during physical exercise. This suggests that metoprolol is generally well tolerated regardless of *CYP2D6* genotype at doses prescribed in the study. However, an important notion is that the prescribed metoprolol doses across the genotype subgroups were only

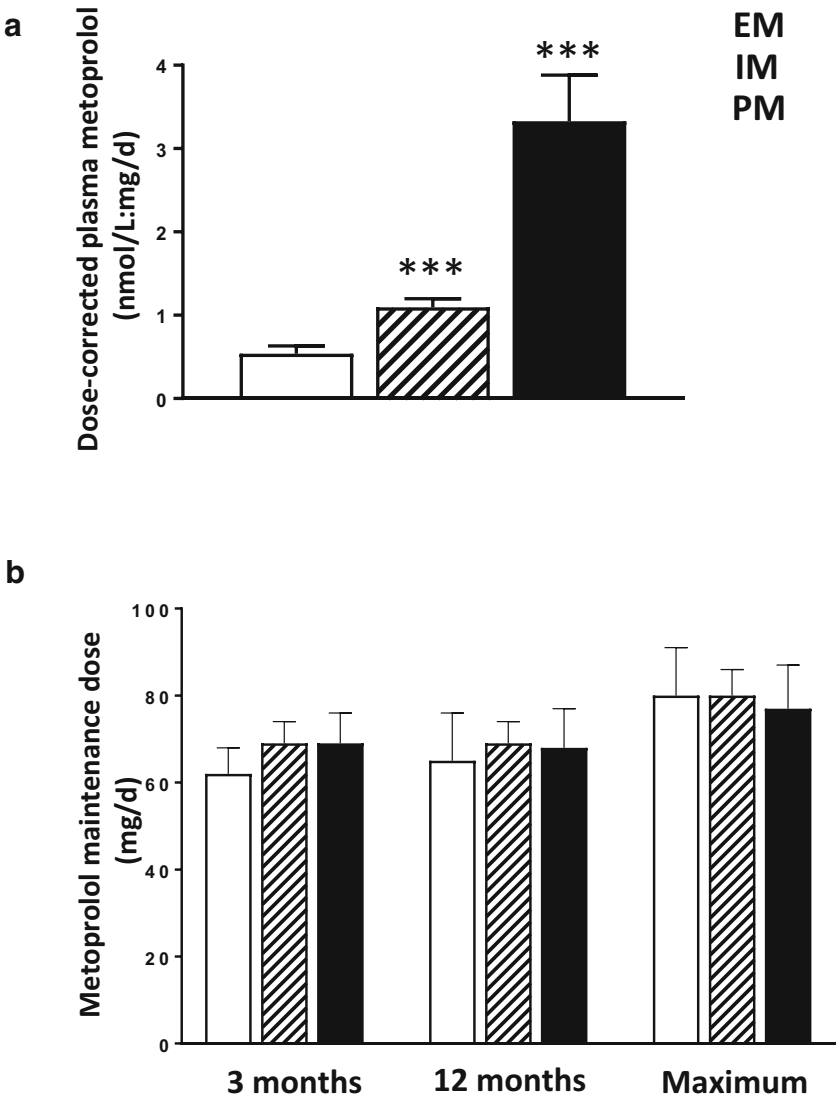

Fig. 1 **a** Dose-corrected plasma trough concentrations of metoprolol. **b** Metoprolol maintenance dose at 3 and 12 months after discharge and maximum maintenance during the 12 months of follow-up. Data are presented as mean ± SEM. ***$p < 0.001$ vs. extensive metabolizers

about one third of the defined target dose after MI. As the GPs apparently were sceptical in performing up-titration of the metoprolol target dose after MI, also in CYP2D6 EMs, the study findings may indicate that CYP2D6 PMs potentially could benefit of the increased plasma concentration per dose in a naturalistic setting. Thus, access to *CYP2D6* genotype may guide the clinicians in prescribing a higher metoprolol dose in EMs and at the same time may keep the dose at lower level in PMs to avoid adverse effects.

Heart rate and exercise capacity

The 6.2-fold higher dose-corrected steady-state trough plasma metoprolol concentration in the PM group than in the EM group is in accordance with previously reported observations [2, 9]. Our data showed a parallel decrease in achieved HR during exercise in the PM group. This finding is probably partly a result of increased inhibition of the sinoatrial node by increasing metoprolol plasma concentrations, supported by a moderate correlation between plasma metoprolol concentrations and achieved maximum HR during exercise. It could also be expected that a reduction of HR during exercise would reduce exercise capacity. However, we found a similar achieved workload during bicycle ergometer exercise as well as experienced a similar exercise capacity by the patient in daily life, independent of the difference in plasma metoprolol concentrations between the three CYP2D6 subgroups. The reason for this somewhat surprising observation may be that the increase in HR during physical activity and exercise in the PM group was enough to perform and feel well. Mean maximum HR during exercise was after all 129 bpm and only 13 bpm less than in the EM and IM groups.

It could be argued that patients with the EM phenotype could be at the upper part of the dose-response curve approaching the upper asymptote and in this way most of the blockade of the β-1 adrenergic receptors is achieved with little

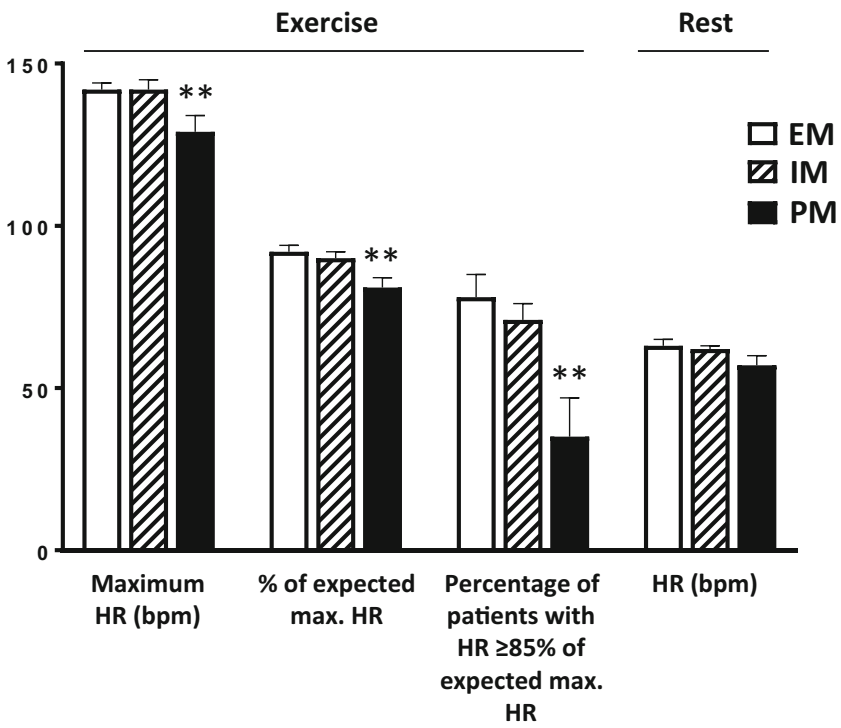

Fig. 2 Heart rate (HR) during exercise and at rest 3 months after discharge. Data are presented as mean ± SEM. $^{**}p < 0.01$ vs. extensive metabolizers

additional β-1 adrenergic receptor blocking effects in PMs. The observed attenuation of increase in HR during strenuous physical activity in PMs indicates at least some additional and clinically important blockade of the β-1 adrenergic receptors. We believe that the attenuated increase in HR may be beneficial in patients with coronary artery disease since avoidance of high HR may protect atherosclerotic lesions from rupturing, the main mechanism leading to an acute coronary syndrome. In light of this hypothesis, the PM phenotype in our population may be beneficial.

Metoprolol-associated adverse drug reactions

Another somewhat surprising observation was that the substantial increase in plasma metoprolol concentration in the PM group was not translated into more frequent or more severe metoprolol-associated adverse drug reactions like fatigue, impotence and cold hands and feet. Moreover, we did not find any correlation between plasma metoprolol concentrations and potential metoprolol-induced adverse drug reactions. Similar observations have been reported previously [15], although others have found opposite results [14]. A plausible explanation is that real metoprolol-induced adverse drug reactions are relative infrequent despite high metoprolol plasma concentrations. In support of this notion, findings from the previous large MERIT-HF trial demonstrated no increase in number of patients that stopped taking metoprolol vs. placebo even though the mean daily dose of metoprolol was 159 mg [21], indicating that metoprolol is well tolerated even at high doses. Keeping this in mind, it is not surprising that we did not find increased number of GP visits in PMs compared with EMs.

Metoprolol target dose

The target dose of metoprolol was 200 mg/day. In the discharge report sent to the patients' GPs, the GPs were instructed to up-titrate the metoprolol dose towards this target dose if not HR was < 50 bpm, systolic BP was < 100 mmHg or metoprolol-induced adverse drug reactions were suspected. The same instructions were given to the study physicians meeting the patients at the 3-month follow-up. Despite this recommendation, the mean up-titrated daily doses at the 12-month follow-up visit were only 68 mg/d regardless of the *CYP2D6* genotype, i.e. 34% of the target dose and only slightly higher than the daily dose observed in a cohort of 18,920 Norwegian patients with MI 12–18 months after hospital discharge (62.3 mg) [22]. The similar prescribed daily doses of metoprolol across the different CYP2D6 subgroups observed in our study were not in accordance with a recent Dutch study by Poulussen et al. reporting a significantly lower prescribed daily dose in CYP2D6 PMs vs. non-PMs, i.e. mean 48 mg/d vs. 84 mg/day [23]. There is no obvious explanation for this discrepancy but may reflect that we included patients after MI who were studied during dose up-titration, while Poulussen et al. included patients on metoprolol maintenance dose in treating various cardiovascular diseases without any common disease-specific dose recommendation.

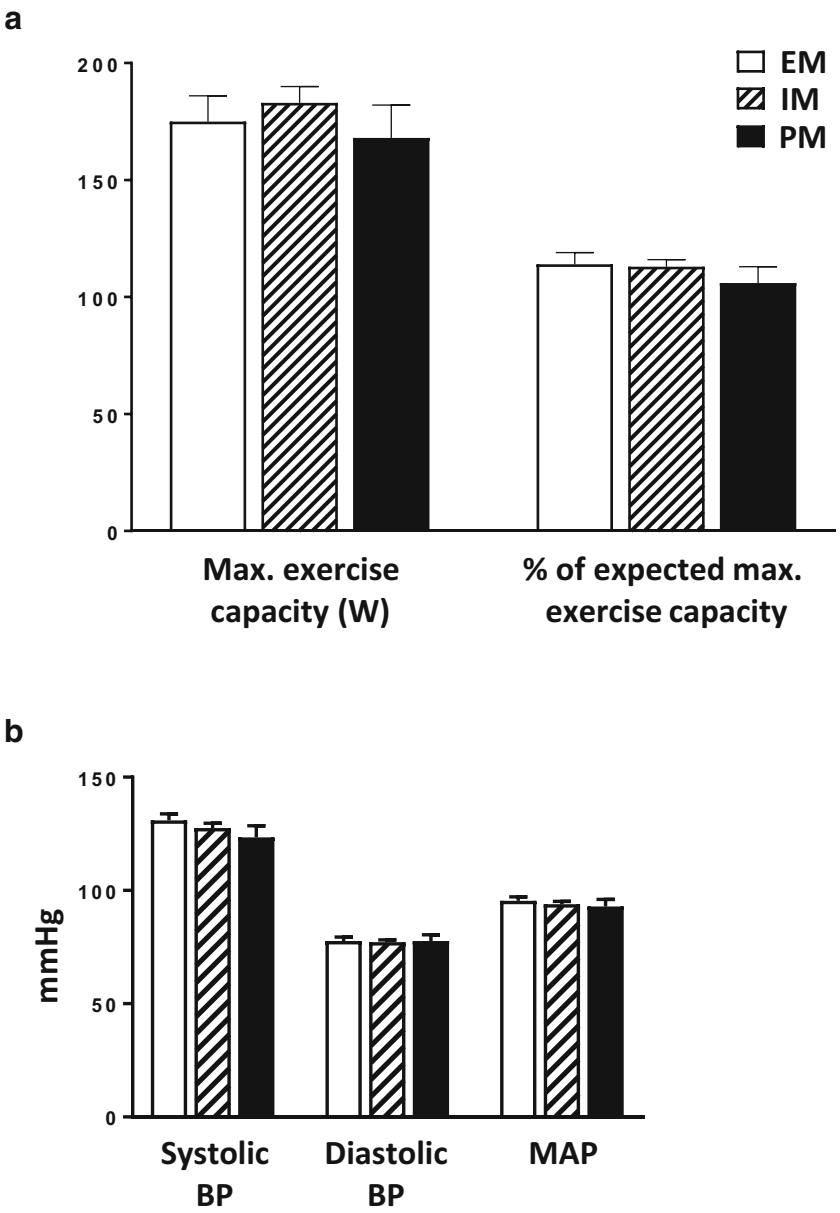

Fig .3 **a** Maximum exercise capacity and percentage of expected maximum exercise capacity as estimated by sex, age, height and body weight at 3 months after discharge. **b** Systolic and diastolic blood pressure (BP) and mean arterial pressure (MAP) at 3 months after discharge. Data are presented as mean ± SEM

Only four of the patients in the present study were actually prescribed the 200 mg per day target dose, whereas only 37% were prescribed a daily dose of 100 mg or more. We suspect that the high frequency of patients reporting possible metoprolol-induced adverse drug reactions at the 3- and 12-month follow-up visits was the main reason for the GPs not to further increase the metoprolol dose. Others have also reported a similarly high frequency of patient-reported poor tolerability of metoprolol preventing dose up-titration [24]. Since we did not find any difference in self-reported potential adverse drug reactions between patients with high (PM group) and much lower (EM group) plasma metoprolol concentration, metoprolol adverse drug reactions either appear at a low daily dose/low plasma concentrations together with a wide therapeutic index of metoprolol or that common complaints are misinterpreted as metoprolol-induced adverse drug reactions. The latter alternative is supported by data from the double-blinded MERIT-HF trial [21], demonstrating similar drug tolerance in patients treated with metoprolol and placebo, and data from another study demonstrating only a small excess in symptomatic fatigue and sexual dysfunction during β-blocker therapy [25].

CYP2D6 genotype panels

Despite the substantial effect on drug exposure, the literature is conflicting regarding the impact of *CYP2D6* genotype on clinical measures of metoprolol treatment [10–18]. There are several possible reasons for this discrepancy, including the fact that frequencies of CYP2D6 variant genotypes and

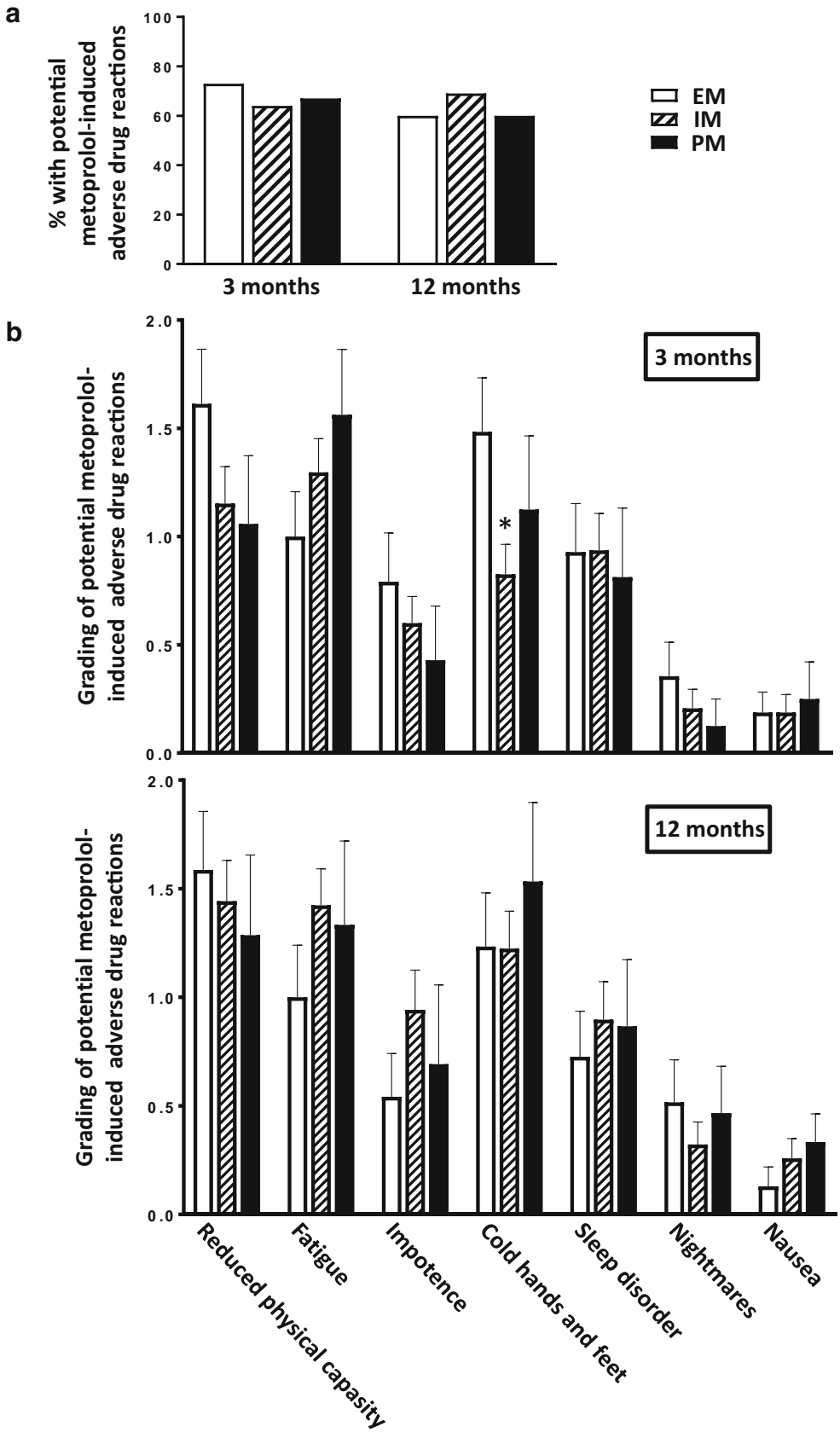

Fig. 4 **a** Percentage of patients reporting potential typical metoprolol-induced adverse drug reactions at 3 and 12 months after discharge. **b** Grading of each potential metoprolol-induced adverse drug reaction from 0 to 4 indicating no, seldom, occasional, frequent or constant symptoms, respectively, at 3 (upper panel) and 12 (lower panel) months after discharge. Data are presented as mean ± SEM. *$p < 0.05$ vs. extensive metabolizers

phenotypes vary significantly between different populations [26, 27]. In addition, an important issue is probably the different CYP2D6 variant allele coverages in the various genotyping panels used in the studies. In the present study, we applied a comprehensive panel including all relevant variant alleles encoding absent (*null*) metabolism, as well as the most frequent reduced-function alleles in Caucasians, i.e. *CYP2D6*9*, **10* and **41*, which in diplotypes with non-coding alleles equalize the PM phenotype when comparing ratios of venlafaxine metabolites as CYP2D6 biomarker [8]. Previous studies on Caucasians have not included *CYP2D6*9*, **10* and **41* in the genotyping panels, implying that patients' CYP2D6 metabolizer phenotypes may have been misclassified as EMs and IMs, instead of IMs and occasionally PMs, respectively. This has probably contributed to conflicting findings on cardiovascular measures between *CYP2D6* genotype subgroups in the previous studies, as an accurate genotype-predicted CYP2D6 metabolizer phenotype is essential for a correct subgroup allocation. It is therefore also important that *CYP2D6* genotyping panels available for practitioners in clinical routine at least include the variant alleles determined in our study.

Strengths and limitations

The main strengths of the present study comprise the prospective and naturalistic study design and the comprehensive *CYP2D6* genotyping panel with inclusion of numerous variant alleles. On the other side, a naturalistic design is associated with methodological limitations as additional factors affecting the study end points are difficult to account for. One of the issues represents possible use of potentially interacting drugs. We did not observe combined use of potent CYP2D6 inhibitors in any of the patients, which probably is the most relevant non-genetic factor affecting CYP2D6 phenotype. However, we have no guarantee that the patients were not using drugs or herbal agents that might have interfered with CYP2D6 phenotype. An additional limitation, which is common in pharmacogenetic studies investigating the impact of variants in single genes, is the number of patients included. The present study was powered to detect a relevant difference in patient proportions achieving 85% of maximum HR during exercise, but more patients would probably have been necessary to detect significant differences in other relevant clinical measures and adverse drug reactions.

In conclusion, the present study including patients treated with metoprolol following MI demonstrates a more than six-fold increase of dose-corrected steady-state trough plasma concentration of metoprolol in CYP2D6 poor vs. extensive metabolizers with a parallel decrease in achieved HR during exercise but without any significant associations between genotype and frequency or severity of self-reported adverse drug reactions. Thus, in this naturalistic patient population, where the prescribed daily doses were lower than intended following MI, CYP2D6 PMs may potentially benefit from the increased plasma concentration of metoprolol without any negative effect on treatment tolerability.

Author contributions Erik Øie and Espen Molden designed the study. All authors contributed to data collection. The first draft of the manuscript was written by Erik Øie and Espen Molden, and the other authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of Diakonhjemmet Hospital and the regional research committee (Regional Committee for Medical and Health Research Ethics South East; reference number 2012/139) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflicts of interest.

Data availability The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

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