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CYP2D6 metabolism in frail elderly compared to non-frail elderly

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ABSTRACT

Background: Frailty is a clinical phenotype that is associated with adverse health outcomes. Since frail patients may be more prone for adverse drugs events and about 15–20% of commonly prescribed drugs are metabolized by CYP2D6, we hypothesized that CYP2D6 metabolism is altered in frail patients compared to healthy subjects.

Methods: The ^{13}C-dextromethorphan breath test (^{13}C-DM-BT) was used to determine CYP2D6 phenotype using ^{13}C-dextromethorphan (^{13}C-DM) as a probe. Eleven frail and twenty-two non-frail (according to the Fried criteria) subjects aged 70–85 years were phenotyped for CYP2D6.

Results: Length, weight and BMI were significantly correlated to CYP2D6 phenotype whereas frailty score and individual parameters of frailty, Karnofsky score, and ADL score were not significantly correlated to the CYP2D6 phenotype. There was no difference in CYP2D6 phenotype between frail (mean AUC_{DOB2h} 319 ± SD 169‰min) and non-frail subjects (mean AUC_{DOB2h} 298 ± SD 159‰min, p=0.728), even when corrected for BMI.

Conclusion: Frail and non-frail subjects did not differ in CYP2D6 phenotype. Our study does not suggest a role for CYP2D6 in explaining why frail subjects are more sensitive to adverse drug reactions.

INTRODUCTION

Frailty is a clinical phenotype that is associated with adverse health outcomes [1, 2] and is characterized by an excessive reduction of lean body mass, sarcopenia, chronic under-nourishment, reduced function, and poor endurance. Frailty might influence the pharmacokinetics and pharmacodynamics of drugs [3]. Studies investigating the effect of frailty on the pharmacokinetics of drugs in older people are scarce. In the limited studies published, the, interpretation of the results is complicated by inconsistent assessment of frailty. From a pathological point of view, there is an association between inflammation and frailty [4]. Frailty was associated with higher inflammatory markers such as IL-6 and lower esterase activity [4, 8]. Inflammation has the potential to downregulate drug metabolism and transporter pathways [4, 9] reducing the systemic clearance of some drugs. Indeed, sulfation of metoclopramide [7] and glucuronidation of acetaminophen [6] were significantly decreased in frail elderly compared to fit elderly. In contrast, very old (>80 years) as well as frail people were able to maintain the ability to metabolize CYP3A4 substrates in a phenotyping study using the erythromycin breath test [5]. CYP2D6 has been involved in the metabolism of approximately 15–20% of all drugs such as betablockers, antipsychotics (like haloperidol), most selective serotonin reuptake inhibitors (SSRIs), and antitumour agents such as tamoxifen and gefitinib and is therefore a candidate to explain the increased risk of drug side effects in frail elderly. Studies on ageing and CYP2D6 phenotype are conflicting: there is no evidence that slow debrisoquine (test probe for CYP2D6) metabolizers are more common at older age [10]. Clinically however, older people are at greater risk of adverse reactions when CYP2D6 deficient using propranolol as a probe drug. There was more than a 50% reduction in clearance of total propranolol in older patients deficient in the CYP2D6 pathway compared with young subjects [11]. Therefore, there is a need to understand the factors which determine heterogeneity of metabolism in elderly, which might be partly attributed to frailty, in order to individualize pharmacotherapy. To date, no phenotyping studies have been reported on CYP2D6 phenotype and frailty. The goal of this investigation was to study CYP2D6 phenotype in frail and non-frail elderly, to test the hypothesis if frailty is associated with a decreased CYP2D6 mediated metabolism. The ^{13}C-dextromethorphan breath test (DM-BT) was used to determine CYP2D6 phenotype with limited breath sampling using ^{13}C-dextromethorphan (^{13}C-DM) as a phenotype probe [13].

MATERIALS AND METHODS

Population

To assess the impact of frailty on CYP2D6 metabolism, and to exclude age as a possible confounder, it was chosen to select frail and non-frail subjects in the age of 70 to 85 years, who were recruited from the outpatient clinic of the Leiden University Medical Center. The subjects were referred by general practitioners from the Leiden region, or were recruited by advertisements. The protocol was approved by the institutional review boards of the Leiden University Medical Center, the Netherlands. All subjects provided written informed consent. Since no data exist regarding the phenotype parameter Delta-over baseline $(DOB)_{0\text{-}2h}$ and DOB_{50min} is considered to sufficiently correlate with $DOB_{0\text{-}2h}$, the power calculation was based on existing DOB_{50} values. To achieve 80% power at a 0.05 significance level in order to detect a hypothesized difference in CYP2D6 phenotype parameter DOB_{50} of 0.8, based on results with ^{13}C-DM-BT from our previous study [8], between frail and non-frail elderly, the calculated sample size was 42 (21 frail, 21 non-frail patients). Blood samples were collected to determine *CYP2D6* genotype by the Amplichip array (Amplichip, Roche). *CYP2D6* genotype was translated into an ultrarapid (=UM e.g. XN *1), extensive (=EM, e.g. *1/*1), heterozygous extensive (=hetEM, e.g. *1/*4), intermediate (=IM, e.g. *41/*41) or poor metabolizer (=PM, *4/*4) predicted phenotype. CYP2D6 activity score was determined for each patient according to the method introduced by Gaedigk [14].

Baseline evaluation

Patients were interviewed to assess physical function, demographics, self-estimated health, weight loss, energy expenditure, frequency of falls, medications used, and a diagnosis of M. Parkinson, cognitive impairment, cardiovascular events (myocardial infarction, stroke and peripheral vascular disease), cancer, diabetes, renal disease, and hearing and visual impairment. Comorbidity was defined as the presence of three or more of these conditions. Physical function was ascertained by Karnofsky score and questionnaires regarding activities of daily living (ADL). Subjects' length and weight were recorded. Blood samples were collected under fasting conditions, to analyze alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase, lactate dehydrogenase, total bilirubin, creatinine, dibucain number (pseudocholinesterase), (free) testosterone and sex hormone-binding globulin (SHBG) by the Clinical Chemical Laboratory of the Leiden University Medical Center. Cognitive function was assessed with the Mini-Mental State Examination [15]. Physical function included maximal grip strength (kilograms) in the dominant

hand (3 measures averaged), using a Jamar® hand-held dynamometer (Sammons Preston Rolyan, Bolingbrook, Illinois, USA), assessment of time to walk 4 meters at usual walking speed and assessment of time to stand up from a sitting position for five subsequent times.

Fried frailty score

Subjects were tested for frailty using the Fried [1] criteria. A patient was identified as a frail subject when at least 3 of Fried criteria were met, while a subject was considered non-frail when none of the criteria were met in order to discriminate sufficiently between the subgroups. A phenotype of frailty was identified by the presence of three or more of the following determinants of frailty:

1. Unintentional weight loss, of ≥5 kilograms in the prior year or, at follow-up, of ≥5% of body weight in prior year.
2. Handgrip strength <30 kg (men) or <18 kg (women).
3. Normal walking speed of less than 0.76 m/s (=more than 6 seconds for 4 m).
4. Poor energy expenditure in the last three months reflected in more than 4 hours sitting a day, less than one stroll/month, and no cycling or jogging.
5. The presence of self-reported exhaustion, identified by a positive answer to two questions from the CES-D scale [16]:
   - Does it take you struggling to get going?
   - Does everything you do take effort?

The number of frailty characteristics present was identified in each subject. Subjects were identified as non-frail by the absence of all Fried criteria.

In- and exclusion criteria

Men and women aged 70–85 years with a body-mass index between 20 and 30 kg/m^2 were included in the study. Subjects were excluded from the study when the investigator identified the following: acute illness, metastasized cancer, inability or unwillingness to fast overnight prior to the study session, inability or unwillingness to abstain from drinking alcohol for 24 h prior to the DM-BT, a diagnosis of severe asthma or severe chronic obstructive pulmonary disease (COPD), the existence of metabolic or gastrointestinal disorders which influence absorption and/or gastric emptying, a demonstrated adverse reaction to previous dextromethorphan exposure, impaired hepatic function as defined by ≥ grade 3 AST, alkaline phosphatase or total bilirubin or a history

of liver cirrhosis, stage IV renal insufficiency defined by a MDRD of <30 mL/min, use of medication known to slow gastric emptying or gastrointestinal motility within 24 hours of the breath test, use of medication inhibiting CYP2D6 and/or mono-amine oxidase (MAO) inhibitors in the last two weeks, and use of dextrometorphan cough syrup/tablets within 24 hours of the breath test.

DM-BT

DM-BT was used to determine CYP2D6 phenotype. Participants were asked to fast for at least 12h and abstain from alcohol intake for 24 h prior to the administration of the DM-BT. During the breath test, patients were observed and potential side effects were recorded. A baseline breath sample was collected into 1.3 L breath bag prior to first administration of 2 Alka-Seltzer Gold (A.S.G.)® tablets and 50 mg=24 ml of a 2.07 mg/mL DM-^{13}C formulation (Cambridge Isotope Laboratories Inc.®, Andover, Massachusetts, USA)

Breath samples were collected into 1.3 L breath bag at 30, 40, 50, 60, 90 and 120 minutes postdose. Following the collection of the breath samples, the breath was analyzed for $^{13}CO_2/^{12}CO_2$ ratio using a POCone® (Otsuka Pharmaceutical Ltd. Tokyo, Japan) spectrophotometer. A DOB value was calculated for each timepoint, reflecting CYP2D6 activity [13]. Using the trapezoidal method, the area under the curve (AUC) of DOB versus time (min) was calculated for each subject.

Data analysis

Data are presented as mean with SD or range for summary statistics. Statistical analyses of data were done with IBM SPSS Statistics, version 22. Comparisons of demographic data were done by use of the unpaired Student's t test. Analyses of DM-BT data were done by use of ANOVA or regression analyses as indicated.

RESULTS

Study participants

All patients were Caucasians. Due to difficulties in recruiting frail patients, eventually 35 (13 frail, 22 nonfrail) patients were invited for the study. Two frail patients were excluded to participate in the study because they were not eligible according to the pre-specified Fried criteria. Eventually, thirty-three patients (22 non-frail and 11 frail) were enrolled in this study. Of the 22 non-frail

patients 12 were male and 10 were female and of the 11 frail 6 were male and 5 were female. The patients' mean (± SD) age was 77.3 years (± 4.3) and the mean (± SD) weight was 76.1 kg (SD ± 12.7). Age, weight and BMI did not differ between frail and non-frail patients (Table 9.1).

The predicted CYP2D6 phenotype (following the genotyping result) were not equally distributed: in the frail group there 0/11 subjects had a PM predicted phenotype, whereas in the non-frail group PM phenotype occurred in 3/22 subjects. IM predicted phenotype occurred in 6/11 frail versus 7/22 non frail subjects respectively whereas EM predicted phenotype was almost equally distributed among the two groups (frail 3/11 and non-frail 8/22). However, the mean gene activity score did not differ between the two groups (1.33 (SD 0.50) versus 1.28 (SD 0.752)).

In frail subjects, compared to non-frail subjects, the total number of comorbidities was higher compared to non-frail subjects. More than 50% of the frail subjects had three or more comorbidities, compared to 0% in the non-frail subjects. Consistent with the presence of frailty, frailty score (3.36 vs 0, $p<0.001$) and Karnofsky score were lower in frail subjects compared to non-frail subjects (77.3% vs 96.8%, $p<0.001$). None of the frail subjects had a 100% Karnofsky score. No difference in daily activities and cognitive function (by means of a Mini Mental State Examination) were observed between the two groups.

Handgrip strength was slightly lower in frail men compared to non-frail men but the difference was not statistical significant (31.3 vs. 35.0 kg, $p=0.317$). For women however, the difference in handgrip strength was more obvious between the two groups (18.2 kg in frail vs. 28.2 kg in non-frail patients, $p=0.01$). In frail patients, a significant lower walking speed was observed compared to non-frail subjects (7.9 sec. for 4 m compared to 4.6 sec. $p=0.017$), more or less consistent with the pre-specified cut-off value of 6 sec. in the Fried criteria for frail subjects. The chair stand test was performed at a lower speed in frail compared to non-frail subjects (15.9 sec. vs. 13.2 sec), but the difference was not significant ($p=0.119$).

Liver and renal function did not significantly differ between the 2 groups. No difference in the dubicain number was observed between the two groups.

In male patients, no difference in total and free serum testosterone levels was observed between frail and non-frail men. Serum DHEAS levels were lower in frail subjects (1.56 µmol/l) compared to non-frail (2.36 µmol/L) subjects, but the result was not significant ($p=0.15$). Smoking was not reported in any subject. Use of low amounts of alcohol beverages was reported in two patients in the non-frail subgroup.

Table 9.1 Demographic and frailty characteristics of the study population

	Frail	Non-frail
Number (N)	11	22
Men	6	12
Women	5	10
Age (years), mean (min–max)	74.5 (70–79)	78.6 (70–85)
BMI (kg/m^2), mean (min–max)	26.1 (19.1–32.5)	25.4 (19.9–30.6)
Predicted phenotype	9	18
PM	0	3
IM	6	7
EM	3	8
Gene activity score	1.33 (SD 0.50)	1.28 (SD 0.752)
Comorbidities (N)		
0	2	18
1	1	2
2	2	2
3	2	0
4	3	0
5	1	0
Karnofsky score[§], mean (range)	77.3 (60–90)	96.8 (90–100)
Frailty score[¶], mean (range)	3.36 (3-4)	0
ADL*, mean (range)	0.8 (0–3)	0.1 (0–1)
MMSE score[‡], mean (range)	28.8 (27–30)	29 (27–30)
Max. grip strength (kg) [£], mean (range)		
Men	31.3 (26.0–42.0)	35.0 (22.1–51.0)
Women	18.2 (8.0–28.0)	28.2 (20.0–39.0)
4m walking time (sec.)[χ], mean (range)	7.9 (4.7–25.8)	4.6 (2.8–5.8)
Chair-stand test (sec.)[φ], mean (range)	15.9 (10.8–29.3)	13.2 (8.3–20)
Laboratory investigations		
ALAT (U/L)[1], mean (range)	15.4 (7.4–27.5)	10.1 (4.0–18.4)
ASAT (U/L)[2], mean (range)	32.1 (14.9–56)	25.2 (14.0–50.4)
LDH (U/L)[3], mean (range)	220 (129–407)	206 (139–349)
Bilirubin (µmol/L)[4], mean (range)	12.2 (4.5–28)	10.7 (6.3–16.5)
Creatinin (µmol/L)[5], mean (range)	103 (84–130)	106 (61–164)
Dibucaine[6], mean (range)	76.8 (73.1–78.1)	77.0 (75.6–78.7)
Testosterone (nmol/L)[7], mean (range)	19.3 (16.5-22.8)	15.6 (5.6–30.0)
Free testosterone (pmol/L)[8], mean (range)	240 (163–415)	235 (114–364)
DHEAS (µmol/L)[9], mean (range)	1.56 (0.42–3.47)	2.36 (0–5.43)

Abbreviations: BMI, Body Mass Index; MMSE, Mini Mental State examination.

¶ Fried Frailty score (ref): The total sum for the presence of each item: unintentional weight loss, of ≥5 kilograms in the prior year or, at follow-up, of ≥5% of body weight in prior year; handgrip strength <30 kg (men) or <18 kg (women); normal walking speed of less than 0.76 m/s (=more than 6 seconds for 4 m); poor energy expenditure in the last three months reflected in more than 4 hours sitting a day, less than one stroll/month, and no cycling or jogging; the presence of self-reported exhaustion, identified by a positive answer to two questions from the CES-D scale (Weissman, Sholomskas

DM-BT results

No difference in CYP2D6 activity was observed between frail and non-frail subjects (AUC_{DOB2h} 319 ± 169 ‰min in frail versus 298 ± 159 ‰min, p=0.728, Figure 9.1). Also, no difference in CYP2D6 activity was observed between frail men and non-frail men and between frail women and non-frail women respectively. Recruitment of subjects was limited to patients aged 70–85 years in order to assess the influence on frailty without the interference of age as a possible confounder. Indeed, it was shown that in the chosen small age frame, no correlation between age and CYP2D6 phenotype by DM-BT was detected (r^2=0.008 p=0.631 for age versus AUC_{DOB2h}). CYP2D6 activity by DM-BT was significantly higher in women than in men (AUC_{DOB2h} 362 ± 162 ‰min vs. 252 ± 142 ‰min, p=0.036, Table 9.2) for the whole group. In the non-frail group, there was a non-significant (p=0.059) trend towards a higher CYP2D6 phenotype in women compared to men.

Parameters which may be associated with drug metabolism were separately correlated to the Fried frailty score and the CYP2D6 phenotype (Table 9.3): Weight, length and BMI were negatively correlated to the phenotype (r=-0.518 (p=0.002), -0.472 (p=0.006) and -0.350 (p=0.046) respectively). With the knowledge that most patients had normal liver- and renal function, liver tests and creatinine values were not correlated to CYP2D6 phenotype. With the use of a fixed DM dose of 50 mg, and because the mean weight in men (82 ± SD 9 kg) was significantly higher

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et al., 1977): - Does it take you struggling to get going?- Does everything you do take effort?

*‡ Activities of daily living: One point for each inability to independent performance of the following 9 functions: bathing, shopping, cooking, eating, dressing, using toilet, transferring, sexual performance, and controlling continence, as reported by all participants.

‡ The mini–mental state examination (MMSE) or Folstein test is a brief 30-point questionnaire test that is used to screen for cognitive impairment. It is commonly used in medicine to screen for dementia. It is also used to estimate the severity of cognitive impairment and to follow the course of cognitive changes in an individual over time, thus making it an effective way to document an individual's response to treatment. Any score greater than or equal to 27 points (out of 30) indicates a normal cognition. Below this, scores can indicate severe (≤9 points), moderate (10–18 points) or mild (19–24 points) cognitive impairment.

£ Maximal grip strength (kilograms) in the dominant hand (3 measures averaged), using a Jamar® hand-held dynamometer.

x Time in seconds to walk 4 meters at normal speed.

φ Time in seconds for a subject to stand up for 5 times from a chair with the arms in a crossed position in front if the chest.

[1] Serum alanine aminotransferase, reference: Men: 0–45 U/L; women: 0–34 U/L.

[2] Serum aspartate aminotransferase, reference: Men: 0–35 U/L; women: 0–31 U/L.

[3] Serum lactate dehydrogenase, reference: 0–248 U/L.

[4] Serum bilirubin, reference: 3.1–6.4 µmol/L.

[5] Serum creatinin, reference: men: 64–104 µmol/L; women: 49–90 µmol/L.

[6] Serum dibucaine number get 70–100.

[7] Serum testosterone (men only): reference: 8–50 nmol/L.

[8] Serum free testosterone (men only): reference: 120–750 pmol/L.

[9] Serum Dehydroepiandrostenedione-sulfate (DHEAS): reference: men 2–15 µmol/L; w

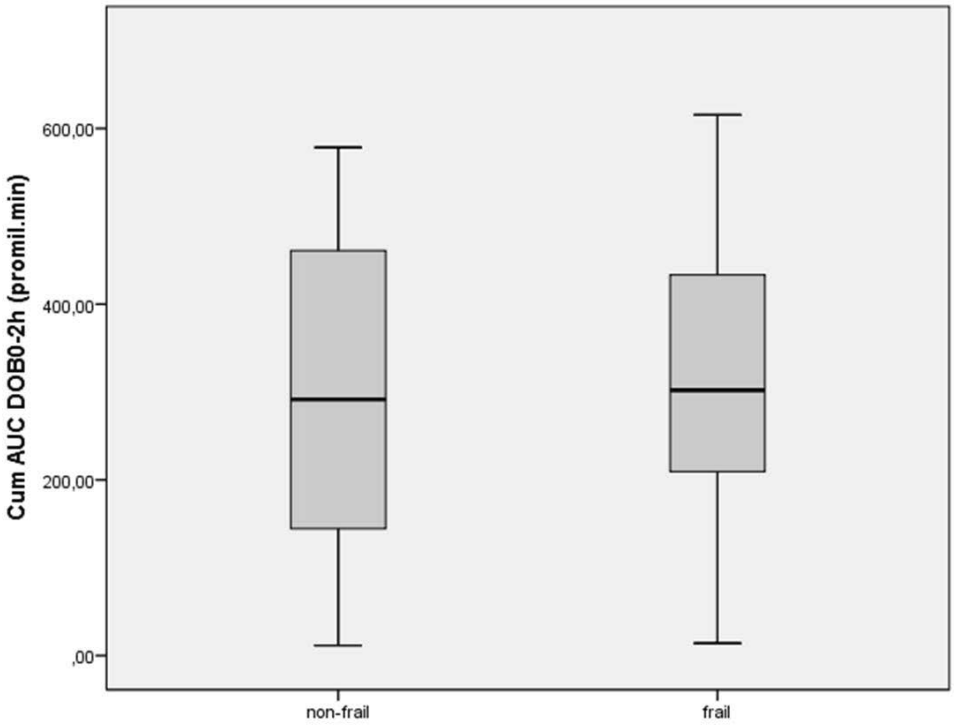

Figure 9.1 Boxplot of CYP2D6 phenotype measured by dectrometorphan breath test between frail and non-frail subjects.
Abbrevations: Cum, cumulative; AUC, Area under the curve; DOB, Delta-Over-Baseline; h, hour; min., minute; promil., promille.

Table 9.2 CYP2D6 phenotype by frailty subgroup

	^{13}C-DM-BT (AUC_{DOB2h} (‰min)) (Mean ± SD)	No.	ANOVA
Sex			
Men	252 ± 142	15	p=0.036 (men vs. women)
Women	362 ± 162	18	
Frailty			
Frail	319 ± 169	11	p=0.728 (frailty vs. non-frailty)
Men	276 ± 168	6	p=0.62 (frail men vs. non-frail men)
Women	370 ± 174	5	p=0.982 (frail women vs. non-frail women)
Non-frail	298 ± 159	22	
Men	240 ± 134	12	p=0.059 (non-frail men vs. non-frail women)
Women	367 ± 165	10	

than in women (69 ± SD 13 kg), we observed a higher CYP2D6 phenotype in women compared to men (Table 9.2).

Regarding separate parameters of frailty, Karnofsky score, HADS score ADL score were and maximum grip strength and the time needed to walk 4 meters were not correlated to CYP2D6 phenotype. The chair-stand test was positively correlated to frailty score but negatively correlated to CYP2D6 phenotype (0.467 (p=0.006) and -0.372 (p=0.039) respectively). Dubicain number, (free) testosterone and dehydroepiandrostenedione-sulfate (DHEAS) levels in men were neither correlated to frailty nor to CYP2D6 phenotype. No correlation between frailty score, whether or not corrected for body mass index, and CYP2D6 phenotype was identified.

Table 9.3 Correlation of specific parameters with frailty score and CYP2D6 phenotype

Parameter	Pearson correlation with frailty score (r)	Pearson correlation with AUC_{DOB2h} (r)
Gender	0.07 (p=0.714)	0.366 (p=0.036)*
Weight (kg)	0.176 (p=0.326)	-0.518 (p=0.002)*
Length (m)	0.207 (p=0.247)	-0.472 (p=0.006)*
BMI (kg/m^2)	0.10 (p=0.585)	-0.350 (p=0.046)*
Karnofsky score	0.86 (p<0.001)*	0.152 (p=0.397)
MMSE score	-0.143 (p=0.426)	0.288 (p=0.104)
HADS score	0.510 (p=0.013)*	0.185 (p=0.400)
ADL score	0.603 (p<0.001)*	-0.135 (p=0.461)
Max. grip strength (kg)	-0.379 (p=0.02)*	-0.028 (p=0.873)
4m walking time (sec)	0.467 (p=0.006)*	-0.024 (p=0.896)
Chair-stand test (sec)	0.467 (p=0.006)*	-0.372 (p=0.039)*
Laboratory investigations		
Dibucain	-0.021 (p=0.909)	-0.010 (p=0.959)
Testosterone	0.045 (p=0.868)	-0.024 (p=0.931)
Free testosterone	0.030 (p=0.913)	-0.43 (p=0.874)
DHEAS	-0.195 (p=0.293)	0.002 (p=0.994)
LDH	0.123 (p=0.504)	0.052 (p=0.776)
Bilirubin	0.138 (p=0.450)	-0.219 (p=0.229)
ASAT	0.248 (p=0.171)	-0.032 (p=0.862)
ALAT	0.366 (p=0.039)	0.089 (p=0.630)
Creatinine	-0.024 (p=0.895)	-0.180 (p=0.324)
Frailty score		-0.032 (p=0.861)
Corrected for BMI		0.030 (p=0.987)

*Correlation is significant at the 0.05 level (two tailed).

DISCUSSION

This is the first study investigating CYP2D6 phenotype in frail versus non-frail subjects by means of a non-invasive DM-BT phenotyping test. We hypothesized that CYP2D6 metabolism was decreased in frail elderly in line with the increased incidence of adverse drug reactions in frail subjects compared to non-frail subjects [17]. Our findings however do not suggest altered CYP2D6 metabolism in frail elderly compared to healthy elderly in the age group of 70–85 years. Consistent with a frail phenotype, functional scores (ADL, Karnofsky) were lower in the frail subgroup. Despite the fact that no difference was observed in the time to stand up from a chair and in biochemical parameters associated with frailty such as dubicain and testosterone, between frail and non-frail subjects, the high combined frailty score in the frail elderly group was compatible with a frail population. In light of the absence of any Fried criteria in the non-frail group, we therefore considered that the frail and non-frail study participants were distinctive enough to test our hypothesis. Weight, length and BMI were negatively and significantly correlated to CYP2D6 metabolism, but even when frailty score was corrected for BMI, no difference in CYP2D6 phenotype was observed between the 2 groups.

The finding of absence of altered CYP2D6 activity in frail subjects is consistent with the study of Schwartz et al. [5]. In this study, erythromycin breath tests (ERBTs) to phenotype CYP3A, were performed in 60 individuals aged 65 to 101 years, 27 of whom were classified as frail. Frail subjects did not display altered CYP3A metabolism compared to non-frail subjects. Phenoconversion, in which the phenotype is altered by concomitant use of CYP450 inhibitors or inducers might alter phenotype results. In contrast to the study by Schwartz [5] however, concomitant medication such as CYP2D6 inhibitors was excluded in our study and could therefore not have biased our results. There are some limitations in this study: First, due to difficulties in recruiting frail patients, eventually 33 (11 frail, 22-nonfrail) patients were invited for the study, which number is less than originally calculated (21 versus 21). Therefore, the study might display too less power to show any difference between the two groups. However, no trend in decreased phenotype was observed in the frailty group and it is not considered very likely that an increased number of patients would have yielded different results.

Second, we applied the Fried criteria to assess frailty in our subjects. Although this phenotype model has been validated and generally accepted, potentially important factors such as cognitive impairment, a highly prevalent condition associated with functional decline and disability, has not been included in the Fried criteria. Although other models do in fact include such parameters,

they have not been validated in clinical practice [18]. At last, the patient population was not racially diverse, and we cannot address potential racial differences in drug clearance.

We observed however, a difference in CYP2D6 phenotype between men and women in our study. Female subjects had a more active CYP2D6 phenotype compared to male subjects. In both frail and non-frail subjects, a trend towards higher CYP2D6 metabolism was suggested for women, but results were not significant. Men had a significantly higher weight than women however, and using a fixed DM dose of 50 mg instead of a weight based-dose, CYP2D6 phenotype was increased in men accordingly compared to women. When corrected for weight, there was no difference in CYP2D6 phenotype between the groups.

Frailty, as currently defined, can be considered representative of a generalized decline in function that can be predictive of the need for assistance or interventions to avoid dependency. The criteria do not include measures of medical disease severity or organ function but suggest a clinical syndrome.

Our findings do not suggest that the clinical syndrome of frailty *as such* is an important determinant of CYP2D6 mediated drug metabolism. Since we did observe a large heterogeneity in CYP2D6 metabolism in women using tamoxifen in the adjuvant setting for breast cancer [9], predicting factors other than frailty might be more important. However we cannot exclude that CYP2D6 induction or inhibition by several drugs could be altered with the presence of frailty.

In conclusion, we did not observe alterations regarding CYP2D6 phenotype in frail subjects compared to non-frail subjects and therefore we do not advise to modify dosing of CYP2D6 mediated drugs in frail-elderly in respect to the observed unchanged drug metabolism.

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