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POLYMORPHISM OF CYP2D6, CYP2C19, CYP2C9 AND CYP2C8 IN THE FAROESE POPULATION

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Informative title: Phenotypes and genotypes for CYP2D6 and CYP2C19 and genotypes for CYP2C9 and CYP2C8 in the Faroese population

Abstract Objective: The purpose of the study was to study the distribution of poor and extensive metabolizers of CYP2C19 and CYP2D6 and to genotype for *CYP2C8* and *CYP2C9* among 312 randomly selected Faroese.

Methods and Results: The participants were phenotyped for CYP2D6 with the use of sparteine. The distribution of the sparteine metabolic ratio (sparteine/didehydrosparteines) was bimodal, and 14.5% (n=44) (95% CI 10.7-18.9%) of the subjects were phenotyped as poor metabolizers. The frequency of poor metabolizers was higher (P=0.0002; χ^2 test) among the Faroese than in other European populations (7.4%). Genotype analysis for the *CYP2D6**3, *4, *6 and *9 alleles were performed using real-time PCR (TaqMan®), and we found 14.6% (n=45) (95% CI 10.8-19.0%) with deficient *CYP2D6* genes (*3/*4, *4/*4, *4/*6, *6/*6) in the Faroese population.

The subjects were phenotyped for CYP2C19 with the use of mephenytoin and 10 subjects, i.e. 3.2% (95% CI 1.6-5.9%) were phenotyped as poor metabolizers. Genotype analysis for the *CYP2C19**2 and *3 alleles was performed by PCR analysis and 2.9% (n=9) (95% CI 1.3-5.4%) of the Faroese were found to have a deficient *CYP2C19* gene all explained by the *CYP2C19**2/*2 genotype. The allele frequencies of the *CYP2C9**2 and *CYP2C9**3 alleles were 8.8% (95% CI 6.7–11.4%) and 5.3% (95% CI 3.7–7.4%), respectively, while the *CYP2C8**3 allele frequency was 6.9% (95% CI 5.0-9.2%). Real-time PCR (TaqMan®) was used for both *CYP2C9* and *CYP2C8* genotype analyses.

Conclusion: The frequency of CYP2D6 poor metabolizers is two fold higher among the Faroese population compared to other Caucasians, while the frequencies of Faroese subjects with decreased CYP2C19, CYP2C8 and CYP2C9 enzyme activity are the same as seen in other Caucasian populations. A possible consequence might be a higher incidence of side effects among Faroese patients taking pharmaceuticals that are CYP2D6 substrates.

Keywords: CYP2D6, CYP2C19, CYP2C8, CYP2C9, Faroe Islands, polymorphism.

Introduction

The polymorphisms of CYP2D6 and CYP2C19 are relatively well known, and more than 80 mutations of the *CYP2D6* gene and about 15 mutations of the *CYP2C19* gene have been described [1]. More than 75 drugs are metabolized by CYP2D6, including antidepressants, selective serotonin reuptake inhibitors and opioids [2]. CYP2C19 metabolizes more than 25 drugs including proton pump inhibitors and some antidepressants [3]. Populations in different environments and with different genetic backgrounds show interethnic differences in drug metabolism [4]. Geographical differences in the metabolism of sparteine (CYP2D6) and also S-mephenytoin (CYP2C19) are well known. The CYP2D6 poor metabolizers represent 5-10% of Caucasians [5] and 0-2% of African and Orientals [6],[7]. The frequency of CYP2C19 poor metabolizers varies from approximately 3% in Caucasians and Africans to 13-23% of Orientals [4]. Genetic polymorphisms have also been identified for CYP2C8 that oxidizes drugs such as taxol, cerivastatin, as well as endobiotics such as retinoic acid and arachidonic acid [8]. The most frequent allele associated with defective metabolism is the *CYP2C8*3* allele, which occurs with a frequency of 13% in Caucasians and 2% in Afro-Americans [9]. The polymorphic CYP2C9 enzyme is responsible for the metabolism of several commonly used drugs, including S-warfarin, phenytoin and the nonsteroidal anti-inflammatory drugs [10]. The *CYP2C9*2* allele has been found in 11-13% of Caucasians, 1-4% of Africans, while it is not present in Orientals [11]. A SNP linkage disequilibrium between the *CYP2C8* and *CYP2C9* genes has been reported. According to Yasar *et al.* 2002 [12], 96% of the subjects with *CYP2C8*3* alleles also carried *CYP2C9*2* alleles, while 85% of the subjects genotyped as *CYP2C9*2* also were genotyped as *CYP2C8*3*.

The Faroe Islands include 18 islands situated northwest of Scotland and halfway between Iceland and Norway.

The Faroese population derives mainly from Vikings of predominantly Norse extraction, who settled in the Faroe Islands in the 9th century [13],[14]. However, an influx of men and women from the British Isles, either as independent colonisers, or brought there by Vikings, may have contributed significantly to the current gene pool of the Faroese population [15]. It has been suggested that the Faroes were populated by Irish monks or hermits as early as 650 A.D. and they may have left before the Norse settlement [16].

Genetic studies of the Faroese population are limited, but increased incidence of diseases associated with specific gene mutations are known, presumably due to founder effects [17], isolation, inbreeding or simply genetic drift. Examples of diseases are glycogen storage disease type IIIA (GSD IIIA) [18], hereditary hemochromatosis HFE [19], cystic fibrosis CF [20], primary ciliary dyskinesia [17] and classical non-AIDS-related Kaposi's sarcoma [21].

According to the findings cited above it is likely that the Faroese population is of Nordic origin, but in some aspects genetically different from other Caucasian populations. Hence, we were interested in studying the activity of the important drug metabolising enzymes CYP2D6 and CYP2C19, as well as the occurrence of variant alleles within the *CYP2D6*, *CYP2C19*, *CYP2C9* and *CYP2C8* genes, among Faroese subjects.

Methods

Subjects

From the Faroese national register we received the names of 900 randomly selected Faroese individuals between 18 and 60 years, which was the inclusion criteria. Approximately one third was excluded based on the following criteria: concomitant medication, pregnancy or breastfeeding and allergy to medicine. One third refused to participate of various reasons. Hence three hundred and twelve healthy Faroese (144 females and 168 males) were recruited. The mean age of the participants was 39 years. All subjects consented to participate on the basis of verbal and written information in Faroese. The study was approved by the Faroese Ethics Committee.

Out of the 312 volunteers, eight individuals were excluded from the sparteine test: Two women were excluded from the phenotyping part because of uncertain pregnancy status, but blood samples were taken for genotype analysis. Further, the analysis of the urine from four persons showed that they had not ingested the sparteine capsule, even though they had collected urine. For two subjects the urine volume was not recorded and consequently these two persons were therefore excluded. In total, 304 persons were included in the CYP2D6 phenotype analysis.

The two possibly pregnant women, were also excluded from the mephenytoin test. Thereby, 310 persons were included in the CYP2C19 phenotype analysis.

For the *CYP2D6*, *CYP2C19*, *CYP2C8* and *CYP2C9* genotype analysis, one person was excluded because it was not possible to get a blood sample from him. He was however included in the

phenotype analysis. Two samples could not be genotyped for *CYP2D6* either due to homozygosity for the *CYP2D6*5* allele or poor quality of DNA, and were excluded from the genotype analyses. Consequently, 311 samples were genotyped for *CYP2C19*, *CYP2C8* and *CYP2C9*, and 309 for *CYP2D6*.

Phenotyping

After emptying the bladder the participants took an oral dose of 100 mg racemic mephenytoin (Mesantoin! , Sandoz Pharmaceuticals Corporation, East Hanover, NJ) and 100 mg sparteinesulphate-pentahydrate (The Central Pharmacy, Odense University Hospital, Denmark). They also received 200 mg of caffeine at the same time due to participation in another study on CYP1A2. It has been demonstrated, that the simultaneous administration of low dose of these drugs does not influence the outcome of their respective metabolic indices [22],[23]. Urine was collected from 0 h to 12 h after dosing. The total volume was recorded, and aliquots of 2x10 ml were stored at -80° C until analysis. Sparteine, 2,3- and 5,6- didehydrosparteine in urine (% of dose) was assayed by gas chromatography [24]. The metabolic ratio (MR) was calculated as:

$$\text{MR} = \frac{\% \text{ of dose sparteine}}{(\% \text{ of dose 2,3-didehydrosparteine} + \% \text{ of dose 5,6-didehydrosparteine})}$$

in the 12 h urine.

Individuals with a sparteine MR greater than 14 were classified as poor metabolizers (PMs). Historically the antimode between poor and extensive metabolizers is 20, but this is an arbitrary value and scientifically not better founded than for instance 14. Inspection of the data from the genotype analysis in this particular study encourages us to set an antimode of 14 instead of 20. In this manner the phenotype results were analysed accordingly with respect to genotype accordance. Three subject with MR ratios below 20 (14.3, 14.5 and 18.9) had a *CYP2D6* deficient genotype.

The ratio between *S*-and *R*-mephenytoin in the urine was determined using gas chromatography according to Wedlund et al. [25] as modified by Sanz et al. [26].

All urine samples with *S/R* ratio >0.5 were reanalysed after acid treatment according to Tybring & Bertilsson [27]. In extensive metabolizers the *S/R* ratio increases to several times the original value,

whereas it remains the same in true poor metabolizers. Subjects having S/R ratios >1, which did not increase significantly after acid treatment, were thus classified as poor metabolizers.

Genotyping

A 10 ml blood sample was drawn in EDTA containing Vacutainer tubes (BD, Franklin Lakes, NJ), and the blood was immediately transferred to a DNA glass (Terumo Europe, Haasrode, Belgium) and kept frozen at -80° C until extraction of DNA for PCR analysis. DNA was isolated by PUREGENE^{T M} genomic DNA purification kit by Gentra Systems, Minnesota 55441, USA. Genotype analysis for the *CYP2D6**3, *4, *6 and *9 alleles were performed by PCR before using the Taqman® technology. The realtime analysis was performed at the ABI PRISM 7700 Sequence Detection System equipped with the allelic discrimination module (software version 1.7; Applied Biosystems). Genotype analysis for the *CYP2C19**2 and *3 alleles was performed as described previously [28],[29]. Primers and probes for *CYP2D6* and *CYP2C19* were purchased from Applied Biosystems (Foster City, CA).

The samples were also genotyped for the *CYP2C9**2 and *3 alleles as well as the *CYP2C8**3 allele. The method for *CYP2C8**3 and *CYP2C9**3 have previously been described [12]. For the *CYP2C9**2 allele new primers and probes, specific for the *CYP2C9* gene, were designed (table 3 and 5), using Primer Express 1.0 from Applied Biosystems, Invitrogen (Paisley, Scotland, UK).

Primers and probes used in this study are summarized in Table 1-3.

Results

Among 304 Faroese subject phenotyped with sparteine, 44 had an MR ratio above 14 and were assigned as poor metabolizers. Thus, the incidence of *CYP2D6* poor metabolizers was 14.5% (95% CI 10.7-18.9%). The frequency of poor metabolizers was 2 fold higher (P=0.0002; χ^2 test) among the Faroese compared with other European populations (7.4%) [5].

The MR ratio in the Faroese ranged from 0.10 to 230 and the frequency distribution in extensive and poor metabolizers of sparteine is shown in Figure 2.

By genotype analysis the frequency of the *CYP2D6**3, *4, *6 and *9 alleles was determined for 309 individuals and a high frequency of the *CYP2D6**4 allele (33.4%) (95% CI 29.7–37.3%) was

observed. The frequency of *CYP2D6**1, *3, *6 and *9 was 62.0% (95% CI 57.6-65.4%), 0.2% (95% CI 0.004–0.9%), 1.9% (95% CI 1.0–3.3%) and 0.8% (95% CI 0.3–1.9%) respectively. The frequency of Faroese subjects carrying *CYP2D6* deficient genes was found to be 14.6% (n=45) (95% CI 10.8-19.0%).

Out of the 312 persons recruited, 304 persons were phenotyped for *CYP2D6* and 44 of these were classified as poor metabolizers. 309 persons were genotyped for *CYP2D6* and 45 carried a deficient gene.

Among 310 Faroese subjects phenotyped with mephenytoin, 18 individuals had an S/R ratio ≥ 0.5 , and they were all clearly classified as poor or extensive metabolizers after acid treatment and reanalysis of the samples, and 10 were assigned as poor metabolizers having a S/R ratio ≥ 1 . The prevalence of poor metabolizers of *S*-mephenytoin in the population studied was therefore 3.2% (95% CI 1.6-5.9%). Statistically, this frequency was not significantly different from other Europeans ($P=0.80$; χ^2 test) [5] The S/R ratio distribution in extensive and poor metabolizers of *S*-mephenytoin is presented in Figure 3.

Nine out of the ten poor metabolizers of *CYP2C19*, identified through the mephenytoin test, were by the genotype analysis found to be homozygous for the *CYP2C19**2 mutation. The last one was genotyped as *CYP2C19**1/*2. This person had a S/R ratio of 1.18 before and 1.26 after acid treatment and were therefore by the phenotype analysis classified as poor metabolizer. The *CYP2C19**3 mutation was not found in the Faroese population, and the frequency of subjects with *CYP2C19* deficient genes was found to be 2.9% (95% CI 1.3-5.4%).

The allele frequencies of *CYP2C9**1, *2 and *3 and *CYP2C8**1, *3 were 86.7% (95% CI 83.7–89.2%), 8.8% (95% CI 6.7–11.4%), 5.3% (95% CI 3.7–7.4%), 93.0 (95% CI 90.8–95.0%) and 6.9% (95% CI 5.0–9.2%) respectively. This is in agreement with previous reported allele frequencies in other Caucasians [30].

95% (95% CI 84.2-99.4%) of the subjects with *CYP2C8**3 alleles also carried *CYP2C9**2 alleles, while 75% (95% CI 61.0-85.3%) of the subjects having the *CYP2C9**2 variant also carried *CYP2C8**3 alleles.

The results of the *CYP2D6*, *CYP2C19*, *CYP2C9* and *CYP2C8* genotyping in the Faroese population are summarized in Table 4-7.

Discussion

This study is the first to estimate the distribution of CYP2D6 and CYP2C19 poor and extensive metabolizers and the distribution of *CYP2C8* and *CYP2C9* genotypes in the Faroese population. The observed frequency of CYP2D6 poor metabolizers is about 14% in Faroese subjects, which is about twice the frequency reported in other Caucasians populations. The reason for the high frequency of CYP2D6 poor metabolizers among Faroese is unknown but might be due to the geographical isolation of the Faroe Islands, giving rise to a genetically homogeneous population.

The frequency of CYP2D6 poor metabolizers found by the phenotype analysis (14.5%) is slightly different from the frequency of subjects with *CYP2D6* deficient genotypes found by the genotype analysis (14.6%). There are different reasons for this finding but they can all be explained.

One of the two women, who did not ingest the sparteine capsule because of possible pregnancy, was genotyped as *CYP2D6**4/*4. Two of the four subjects, where the urine analysis showed that they had not ingested the sparteine capsule, also had a *CYP2D6* deficient genotype. Further 304 subjects were included in the CYP2D6 phenotype analysis while 309 were genotyped for CYP2D6. However we consider the result demonstrates a concordance between the frequencies found by the phenotype and genotype analysis.

The CYP2D6 polymorphism has been studied as a risk factor for Parkinson's disease (PD), and a higher frequency of CYP2D6 poor metabolizers among PD patients has been reported [31],[32]. The number of PD patients is relatively higher on the Faroe Islands compared to other countries [33],[34],[35] and the frequency of CYP2D6 poor metabolizers is twice as high as in other Caucasians. Therefore there may be an association between these two facts.

Tricyclic antidepressants (TCA) are metabolized by CYP2D6, which also catalyses the metabolism of a number of other antidepressants [36],[37]. Poor metabolizer pheno- and genotype may result in very high plasma drug concentration and lead to side effects [38]. Hence, the high frequency of CYP2D6 poor metabolizers among Faroese presumably result in a respectively larger part of the population suffering from adverse effects when taking CYP2D6 substrates, especially TCA's than seen in other populations.

As a consequence of the high frequency of CYP2D6 poor metabolizers, the response rate to analgesic treatment with tramadol is probably decreased in several Faroese patients. Experimental pain studies have showed reduced efficacy of tramadol in poor metabolizers [39],[40].

In conclusion the frequency of CYP2D6 poor metabolizers is twofold higher in the Faroese population compared to other Caucasians, while the frequencies of Faroese subjects with decreased CYP2C19, CYP2C8 and CYP2C9 enzyme activity are the same as seen in other Caucasian populations. Further studies of e.g. the association between PD and CYP2D6 poor metabolizers among Faroese are warranted. Attention to possible side effects of drug treatments in Faroese CYP2D6 poor metabolizers is needed.

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Table 1. Sequence of oligonucleotide *CYP2D6* primers and probes used in this study

Table 2. Sequence of oligonucleotide *CYP2C19* primers used in this study

Table 3. Sequence of oligonucleotide *CYP2C9*2* primers and probes used in this study

Table 4: Allele frequencies (A) and genotype frequencies (B) of *CYP2D6* with 95% confidence intervals in the Faroese population.

Table 5: Allele frequencies (A) and genotype frequencies (B) of *CYP2C19* with 95% confidence intervals in the Faroese population.

Table 6: Allele frequencies (A) and genotype frequencies (B) of *CYP2C9* with 95% confidence intervals in the Faroese population.

Table 7: Allele frequencies (A) and genotype frequencies (B) of *CYP2C8* with 95% confidence intervals in the Faroese population.

Table 1.Sequence of oligonucleotide *CYP2D6* primers used in this study

Primers	Sequence 5'→3'
CYP1	att tcc cag gaa tcc
CYP4	ccg gcc ctg aca ctc ctt ct
<i>CYP2D6</i> *3 sense	tcc tga ccc agc tgg atg a
<i>CYP2D6</i> *3 antisense	ctt ctc cat ctc tgc cag gaa
<i>CYP2D6</i> *4 sense	cga ccc ctt acc cgc atc
<i>CYP2D6</i> *4 antisense	ctc acg get ttg tcc aag aga
<i>CYP2D6</i> *6 sense	ctt ggg cct ggg caa ga
<i>CYP2D6</i> *6 antisense	agg gca gca caa agg ca
<i>CYP2D6</i> *9 sense	gag acc tga ctg agg cct tcc t
<i>CYP2D6</i> *9 antisense	tca ttc ctc ctg gga cgc t

Sequence of oligonucleotide *CYP2D6* probes used in this study

Probes	Sequence 5'→3'
<i>CYP2D6</i> *3 wtMGB	FAM- tca tcc tgt gct cag tt –MGB
<i>CYP2D6</i> *3 mutMGB	VIC- tca tcc gtg ctc agt t –MGB
<i>CYP2D6</i> *4 wt	FAM- acc ccc agg acg ccc ctt t –tamra
<i>CYP2D6</i> *4 mut	JOE- acc ccc aag acg ccc ctt tc –tamra
<i>CYP2D6</i> *6 wtMGB	FAM- cgg tca ccc act gc –MGB
<i>CYP2D6</i> *6 mutMGB	VIC- cgg tca ccc ctg c –MGB
<i>CYP2D6</i> *9 wtMGB	FAM- tet cac ctt ctc cat ct –MGB
<i>CYP2D6</i> *9 mutMGB	VIC- tet cac ctc cat ctc –MGB

Table 2.Sequence of oligonucleotide *CYP2C19* primers used in this study

Primers	Sequence 5'→3'
MEP1	aat tac aac cag agc ttg gc
MEP2	tat cac ttt cca taa aag caa g
MEP3	tat tat tat ctg tta act aat atg a
MEP4	act tca ggg ctt ggt caa ta

Table 3.

Sequence of specific oligonucleotide primers and MGB-TaqMan[®] probes used for the discrimination of the *CYP2C9*2* alleles.

Primers	Sequence 5'→3'
Forward primer (<i>CYP2C9*2</i>)	caa tgg aaa gaa atg gaa gga gat
Reverse primer (<i>CYP2C9*2</i>)	aag ata gta gtc cag taa ggt cag tga tat g
Probe (for discrimination of <i>CYP2C9*1</i>)	ttg aac acG gtc ctc
Probe (for discrimination of <i>CYP2C9*2</i>)	ttg aac acAa gtc ctc a

The discrimination between *CYP2C9*1* and *CYP2C9*3* has been described elsewhere (Yasar et al., Biochem Biophys Res Commun. 2002 Nov 22;299(1):25-8).

Table 4: Allele frequencies (A) and genotype frequencies (B) of *CYP2D6* with 95% confidence intervals in the Faroese population.

A		
Variant allele	Frequency	95 % confidence interval
<i>CYP2D6</i> *1	0.616	0.576 - 0.654
<i>CYP2D6</i> *3	0.002	0.00004 – 0.009
<i>CYP2D6</i> *4	0.334	0.297 – 0.373
<i>CYP2D6</i> *6	0.019	0.010 – 0.033
<i>CYP2D6</i> *9	0.008	0.003 – 0.019

B		
Genotype	Frequency	95 % confidence interval
<i>CYP2D6</i> *1/*1 (EM)	0.418	0.363 – 0.475
<i>CYP2D6</i> *1/*4 (EM)	0.395	0.341 – 0.452
<i>CYP2D6</i> *1/*6 (EM)	0.019	0.007 – 0.042
<i>CYP2D6</i> *1/*9 (EM)	0.009	0.002 – 0.028
<i>CYP2D6</i> *4/*9 (EM)	0.006	0.0008 – 0.023
<i>CYP2D6</i> *3/*4 (PM)	0.032	0.00008 – 0.018
<i>CYP2D6</i> *4/*4 (PM)	0.125	0.090 – 0.167
<i>CYP2D6</i> *4/*6 (PM)	0.013	0.004 – 0.032
<i>CYP2D6</i> *6/*6 (PM)	0.032	0.0008 – 0.018

Table 5: Allele frequencies (A) and genotype frequencies (B) of *CYP2C19* with 95% confidence intervals in the Faroese population.

A		
Variant allele	Frequency	95 % confidence interval
<i>CYP2C19*1</i>	0.818	0.786 – 0.848
<i>CYP2C19*2</i>	0.188	0.158 – 0.221
<i>CYP2C19*3</i>	0	

B		
Genotype	Frequency	95 % confidence interval
<i>CYP2C19*1/*1</i> (EM)	0.662	0.607 – 0.715
<i>CYP2C19*1/*2</i> (EM)	0.312	0.261 – 0.367
<i>CYP2C19*2/*2</i> (PM)	0.032	0.016 – 0.058

Table 6: Allele frequencies (A) and genotype frequencies (B) of *CYP2C9* with 95% confidence intervals in the Faroese population.

A		
Variant allele	Frequency	95 % confidence interval
<i>CYP2C9*1</i>	0.867	0.837 – 0.892
<i>CYP2C9*2</i>	0.088	0.067 – 0.114
<i>CYP2C9*3</i>	0.053	0.037 – 0.074

B		
Genotype	Frequency	95 % confidence interval
<i>CYP2C9*1/*1</i>	0.733	0.680 – 0.781
<i>CYP2C9*1/*2</i>	0.177	0.136 – 0.224
<i>CYP2C9*1/*3</i>	0.106	0.074 – 0.146
<i>CYP2C9*2/*3</i>	0.016	0.005 – 0.037

Table 7: Allele frequencies (A) and genotype frequencies (B) of *CYP2C8* with 95% confidence intervals in the Faroese population.

A		
Variant allele	Frequency	95 % confidence interval
<i>CYP2C8*1</i>	0.930	0.908 – 0.950
<i>CYP2C8*3</i>	0.069	0.050 – 0.092

B		
Genotype	Frequency	95 % confidence interval
<i>CYP2C8*1/*1</i>	0.862	0.818 – 0.898
<i>CYP2C8*1/*3</i>	0.138	

Legend to figures

Figure 1. The position of the Faroe Islands

Figure 2. The distribution of sparteine metabolic ratios (MR) in 304 Faroese

Figure 3. The distribution of S- and R-mephenytoin ratios (S/R) in 310 Faroese



Figure 1. The position of the Faroe Islands

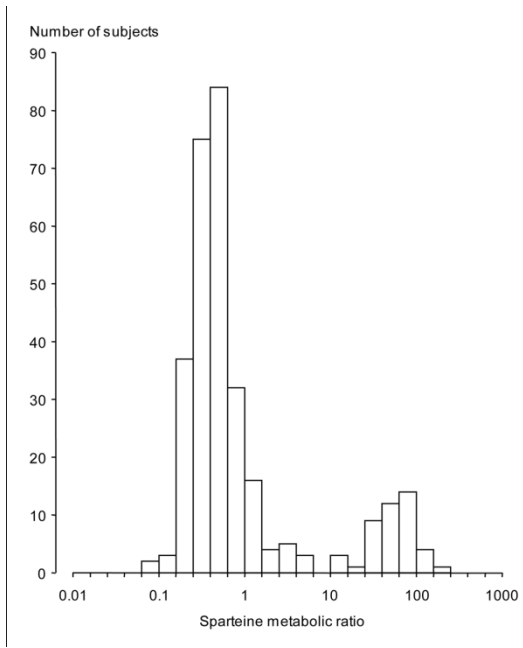


Figure 2. The distribution of sparteine metabolic ratios (MR) in 304 Faroese

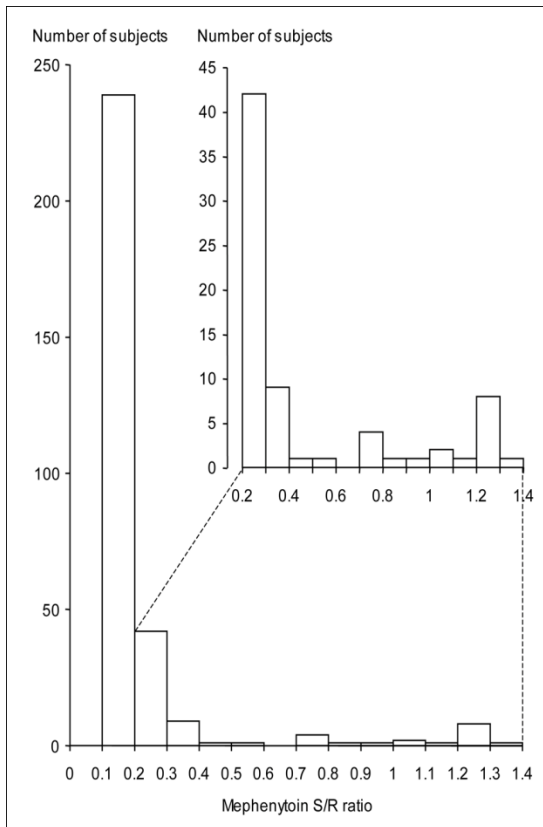


Figure 3. The distribution of S- and R-mephenytoin ratios (S/R) in 310 Faroese