Effects of UGT1A1*28 polymorphism on raloxifene pharmacokinetics and pharmacodynamics

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

- Raloxifene exhibits large and unexplained interindividual pharmacokinetic variability (coefficient of variation 30–50%).
- There is some evidence that UDP-glucuronosyltransferase 1A1 (UGT1A1) may play a key role in metabolic clearance of raloxifene.
- UGT1A1 has a common genetic polymorphism, UGT1A1*28, that could lead to slower elimination of raloxifene and contribute to the high pharmacokinetic variability.

WHAT THIS STUDY ADDS

- Subjects with UGT1A1*28/*28 genotype exhibited a twofold higher raloxifene exposure compared with the hetero- and homozygotes for the wild-type allele. This indicates that raloxifene pharmacokinetics may be significantly affected by the UGT1A1*28 polymorphism.
- It was also demonstrated that the *28 homozygotes gained a significantly greater increase in hip bone mineral density after 12 months' raloxifene treatment.

AIMS

Raloxifene concentrations were reported to correlate approximately with serum bilirubin levels. Bilirubin is a typical UGT1A1 substrate. Based on these facts, we postulated a hypothesis that UGT1A1 is the key enzyme for metabolic clearance of raloxifene and that the common UGT1A1*28 polymorphism significantly contributes to the large pharmacokinetic variability of raloxifene.

METHODS

Serum samples from postmenopausal osteoporotic patients treated with raloxifene were assayed for the concentrations of raloxifene and its glucuronides by liquid chromatography–mass spectrometry–mass spectrometry. The same samples were also genotyped for the presence of UGT1A1*28 polymorphism by the single-strand conformation polymorphism method. The pharmacodynamic effect was evaluated by measuring the change in bone mineral density (BMD) in femoral neck, hip and lumbar spine after 12 months' raloxifene therapy.

RESULTS

Patients homozygous for the *28 allele showed significantly, twofold higher raloxifene glucuronide concentrations compared with the hetero- and homozygotes for the wild-type allele: $558 \pm 115 \text{ nmol l}^{-1}$ compared with $295 \pm 43 \text{ nmol l}^{-1}$, respectively ($P = 0.012$). This indicates a higher raloxifene exposure in the *28/*28 group. Consequently, a significantly greater increase in hip BMD was observed in subjects homozygous for the *28 allele compared with the group carrying at least one copy of the wild-type allele: $4.4 \pm 2.4\%$ compared with $0.3 \pm 1.4\%$ ($P = 0.035$).

CONCLUSIONS

In this study it is shown that a relatively common UGT1A1*28 polymorphism may considerably influence raloxifene pharmacokinetics and pharmacodynamics. Underlying mechanisms and clinical implications of our findings are also discussed.

Introduction

Raloxifene is a selective oestrogen modulator used for the prevention and treatment of osteoporosis in postmenopausal women. Recently, it has also been approved for reducing the risk of invasive breast cancer in postmenopausal women with osteoporosis and in postmenopausal women at high risk of invasive breast cancer [1]. Raloxifene exhibits a large (30–50%) variability in pharmacokinetic parameters [2], the source of which is still unknown. Raloxifene is mainly eliminated after conjugation to glucuronide metabolites. The glucuronides are secreted into the intestinal lumen and cleaved by bacterial microflora, and the resulting free raloxifene can be reabsorbed and metabolized again, forming an enterohepatic cycle [2]. The major isoforms responsible for raloxifene conjugation are thought to be UGT1A1, UGT1A8 and UGT1A10 [3]. The main role in the presystemic metabolic clearance of raloxifene has been attributed to UGT1A10 [3, 4], which is abundantly expressed in the small intestine but is absent in the liver [5]. On the other hand, subjects with liver disease were observed to have 2.5-fold higher raloxifene concentrations compared with normal subjects [2]. Moreover, raloxifene concentrations have been found to correlate approximately with serum bilirubin levels [2]. The most important enzyme for bilirubin conjugation is UGT1A1. These are the key facts that lead us to hypothesize that UGT1A1 is the major UGT isoform responsible for metabolic clearance of raloxifene. A major promoter polymorphism in UGT1A1 gene, the elongated (TA)_n repeat, A(TA)₇TAA (UGT1A1*28) instead of A(TA)₆TAA (UGT1A1*1), leads to lower expression of the enzyme and, ultimately, to the Gilbert's syndrome when present in a homozygous state [6]. The occurrence of Gilbert's syndrome, characterized by elevated levels of unconjugated bilirubin, is quite common among Whites and even more common among Blacks (10–13% and 36% of the population, respectively) [6]. This polymorphism has already been linked to some significant drug-related adverse events [7], for example with the anticancer drug irinotecan [8] and the anti-human immunodeficiency virus drug indinavir [9]. The same polymorphism could also represent one of the main causes for the high variability observed in raloxifene pharmacokinetics and consequently its pharmacodynamics. In order to test this hypothesis, we measured the concentrations of raloxifene and its two conjugates, raloxifene-6-glucuronide (M1) and raloxifene-4'-glucuronide (M2) in serum samples obtained from 57 postmenopausal osteoporotic women on raloxifene therapy. The pharmacodynamic response in the enrolled subjects was evaluated by measuring the change in bone mineral density (BMD) after 12 months' treatment. The same subjects were also genotyped for UGT1A1*28 polymorphism.

Materials and methods

Chemicals

Unless stated otherwise, all materials were of the highest obtainable purity grade and purchased from Sigma-Aldrich (Steinheim, Germany). Raloxifene metabolites M1 and M2 were synthesized as authentic standards by incubating raloxifene with human recombinant UGT1A1 enzymes expressed in Supersomes™ from BD Gentest (Woburn, MA, USA). The detailed method is described elsewhere [10].

Serum samples

A total of 57 postmenopausal female patients treated for osteoporosis were enrolled in the study. Written informed consent was obtained from each individual and the study protocol was approved by the Slovenian National Medical Ethics Committee. The patients were selected according to the following inclusion criteria: ≥5 years of menopause, aged <70 years, presence of osteoporosis, defined as low BMD (T score <−2.5 SD) or radiographically apparent vertebral, femoral or radius fracture. Patients were excluded if they had substantial postmenopausal symptoms or abnormal uterine bleeding, endometrial carcinoma, a history of or suspected breast carcinoma at any time, or a history of non-skin cancer in the previous 5 years, had taken an oestrogen, androgen, calcitonin, bisphosphonate or lipolytic therapy within the previous 6 months, had been receiving fluoride therapy during the previous 3 years, undergone systemic glucocorticoid therapy for >1 month within the past year, had been taking antiseizure drugs, had a history of thromboembolic disorders (except in association with an injury), had a history of thyroid disorder, primary hyperparathyroidism or malabsorption, had serum creatinine levels >170 μmol l^{−1} or abnormal hepatic function, had been smoking or had consumed more than four alcoholic drinks per day. The patients were treated for 12 months with 60 mg raloxifene per day and were followed in the University Medical Centre (Maribor, Slovenia). Blood samples were collected 4–6 h after administration of the last dose (steady state was assumed). After centrifugation, serum samples were stored at −85 °C until analysis.

Determination of raloxifene and metabolites in serum

The method used for determination of M1, M2 and raloxifene had been developed and validated previously in our laboratory and is described in full detail elsewhere [10]. Briefly, 25 μl of internal standard solution (haloperidol, 100 μg l^{−1}) was added to 500 μl of a serum sample, which was then subjected to an automated solid-phase extraction procedure on Strata-X cartridges (Phenomenex, Torrance, CA, USA). The extracts were dried under a flow of pure nitrogen, reconstituted in mobile phase (0.1% formic acid in water and 10% acetonitrile) and then analysed by a triple-quadrupole liquid chromatography–mass

spectrometry–mass spectrometry in positive electrospray ionization mode with multiple reaction monitoring. Chromatographic separation was achieved using a gradient elution (from 10 to 100% acetonitrile, the rest was water with 0.1% formic acid) on a Luna C18(2) 50 × 2.0 mm column (Phenomenex) at a flow rate of 500 µl min⁻¹. The following mass transitions were used for quantification: m/z 650→474 for M1 and M2, m/z 474→112 for raloxifene and m/z 376→165 for internal standard. The limits of detection were 12, 17 and 12 pmol l⁻¹ for M1, M2 and Ral, respectively. No significant matrix interferences and good accuracy as well as precision were achieved [10].

UGT1A1*28 genotype determination

The genotyping was carried out according to the method developed in our laboratory [11]. In brief, genomic DNA was isolated from leucocytes of peripheral blood by the Miller salting-out procedure. Polymerase chain reaction (PCR) was carried out using primers (5'-TGA AAT TCC AGC CAG TTC AA-3' and 5'-AGA GGT TCG CCC TCT CCT AC-3'). The PCR reaction mixture (25 µl) contained genomic DNA (100 ng), 1× PCR buffer, 0.2 mM each of the four deoxyribonucleotides, 2.0 mM MgCl₂, 0.42 µM each of the two oligonucleotide primers and 0.6 units of AmpliTaq Gold™ polymerase (Applied Biosystems, Foster City, CA, USA). Cycling conditions consisted of an initial denaturation step at 95 °C for 12 min, 35 cycles of denaturation at 94 °C for 30 s, annealing for 30 s at 56.5 °C and primer extension at 72 °C for 30 s. This was followed by final extension at 72 °C for 7 min. Aliquots of PCR products were electrophoresed on 2% agarose gel to check their quality and quantity.

In single-strand conformation polymorphism analysis (SSCP), following the PCR amplification, the PCR products (2 µl) were mixed with 5 µl formamide loading buffer [95% formamide, 0.05% xylene cyanol, 0.05% bromphenol blue, 20 mM NaOH and 20 mM ethylenediamine tetraacetic acid (EDTA)]. The mixture was heated at 95–97 °C for 3 min and then immediately transferred to ice. The total volume was loaded on 8% (37 : 1) polyacrylamide gel. Electrophoresis was run in a Protean II electrophoresis unit (BioRad, Laboratories, Inc., Hercules, CA, USA) in 0.5× TBE buffer (50 mM

Tris-borate, pH 8.3 and 0.5 mM EDTA) at a constant power of 20 W at 9 °C for 3 h. After electrophoresis, SSCP patterns were visualized by silver staining. The genotype was determined according to the position of the sample bands relative to the standards indicated the presence of a homozygous wild-type (UGT1A1*1/*1), a heterozygous (UGT1A1*28/*1 or UGT1A1*1/*28) or a homozygous polymorphic (UGT1A1*28/*28) genotype.

Bone mineral density measurements

Bone mineral densities of total hip (HIP), femoral neck (FN) and lumbar spine (L1–4) were measured before and after 12 months' treatment with 60 mg raloxifene hydrochloride daily by dual energy X-ray absorptiometry with a Hologic QDR-2000+ densitometer. Quality control was assured daily according to applicable standard procedures and protocol provided by manufacturer's instructions.

Statistical methods

The Kolmogorov–Smirnov goodness-of-fit test was used to determine the normality of data distribution and Levene's test was used to test the homogeneity of variances prior to the ANOVA. A square root transformation was applied to the concentrations of M1 in order to obtain a normal data distribution. The Bonferroni post hoc test was used to compare the means from three UGT1A1 genotype groups (*1/*1; *1/*28; *28/*28). To test the influence of genotype on change in BMD after treatment, the nonparametric Mann–Whitney test was applied. The significance criterion (α) was set at $P < 0.05$. All data analyses were performed by SPSS version 15.0.0 (SPSS Inc., Chicago, IL, USA).

Results

Patients homozygous for polymorphic allele *28 had significantly elevated levels of both metabolites compared with patients with at least one copy of the wild-type allele: 558 ± 115 nmol l⁻¹ and 295 ± 43 nmol l⁻¹, respectively ($P = 0.012$) (Tables 1 and 2, Figure 1, lower diagram). Looking at the three genotype groups and both metabo-

Table 1

The concentrations of raloxifene species (M1, M2, Ral) and the measured changes in bone mineral density (BMD) after 12 months' treatment in the observed UGT1A1 genotype groups

UGT1A1 genotype	Genotype frequency (%)	c(M1) (nmol l ⁻¹)	c(M2) (nmol l ⁻¹)	c(Ral) (nmol l ⁻¹)	Δ BMD HIP (%)	Δ BMD FN (%)	Δ BMD L1–L4 (%)
*1/*1	35	48 ± 11 (25, 73)	203 ± 47 (47, 103)	3.8 ± 0.8 (2.0, 5.5)	-0.6 ± 1.0 (-2.6, 1.4)	2.5 ± 1.0 (0.3, 4.6)	2.2 ± 0.8 (0.4, 4.0)
*1/*28	46	58 ± 12 (33, 83)	274 ± 54 (54, 161)	2.9 ± 0.5 (1.9, 4.0)	1.1 ± 2.4 (-3.8, 6.0)	0.9 ± 1.3 (-1.7, 3.4)	1.9 ± 1.0 (-0.3, 4.0)
*28/*28	19	102 ± 24 (48, 155)	457 ± 94 (243, 670)	4.3 ± 1.1 (1.8, 6.9)	4.4 ± 2.4 (-0.9, 9.6)	1.8 ± 1.1 (-0.7, 4.2)	2.4 ± 1.3 (-0.6, 5.3)

The values of ΔBMD are presented as arithmetic means ± standard errors and the numbers in parentheses represent 95% confidence intervals.

Table 2

The influence of the homozygous UGT1A1*28 genotype on raloxifene species concentrations

Raloxifene species	Genotype	n	c [nmol l ⁻¹]	P	Stat. significance
M1	*1/*1, *1/*28	37	54 ± 8 (37, 71)	0.025	S
	*28/*28	10	102 ± 24 (48, 155)		
M2	*1/*1, *1/*28	37	241 ± 36 (167, 314)	0.021	S
	*28/*28	10	457 ± 94 (243, 670)		
M1+M2	*1/*1, *1/*28	37	295 ± 43 (208, 381)	0.012	S
	*28/*28	10	558 ± 115 (298, 819)		
RAL	*1/*1, *1/*28	37	3.3 ± 0.5 (2.4, 4.3)	0.340	NS
	*28/*28	10	4.3 ± 1.1 (1.8, 6.9)		

The values of concentrations are arithmetic means ± standard error, the numbers in parentheses represent 95% confidence intervals.

lites separately, the concentrations of M1 in the *28/*28 and *1/*1 groups were visibly different as well: 102 ± 24 nmol l⁻¹ and 48 ± 11 nmol l⁻¹, respectively (Figure 1). However, because of the pronounced data variability the difference was only just below the statistical significance threshold: *P* = 0.068. More importantly, the difference in concentrations of the major raloxifene metabolite, M2, was quite larger and statistically significant between the same two genotype groups: 203 ± 47 nmol l⁻¹ compared with 457 ± 94 nmol l⁻¹, respectively (*P* = 0.032). Although visibly present, the same pattern could not be statistically proven for unconjugated raloxifene concentrations, because of the excessive variability (coefficients of variation of up to 84%) and the insufficient sample size (*n* = 47) (Tables 1 and 2, Figure 1).

Furthermore, a significant increase in HIP BMD after 1 year's raloxifene treatment was observed in subjects homozygous for the *28 allele compared with the group carrying one or two copies of the wild-type allele: 4.1 ± 2.4% compared with 0.3 ± 1.4%, respectively (*P* = 0.036) (Table 3, Figure 2, lower diagram). On the other hand, the changes in BMD of L1–L4 and FN were not

Figure 1

Box plot presentations of raloxifene species concentration distribution among three UGT1A1 genotype groups. Labels c(m1), c(m2), c(Ral) and c(m1 + m2) stand for serum concentrations of M1, M2, unconjugated raloxifene and summed concentrations of both glucuronides M1 and M2, respectively. The outliers are marked with circles (○) and the extremes with asterisks (*)

Table 3

The changes after 12 months' treatment in measured bone mineral density (BMD) of HIP, FN and L1–L4 in the patient group homozygous for *28 allele compared with the group with at least one copy of the wild-type allele

Site	Genotype	n	Δ BMD [%]	P	Stat. significance
HIP	*1/*1, *1/*28	45	0.3 ± 1.4 (−2.4, 3.1)	0.036	S
	*28/*28	11	4.4 ± 2.4 (−0.9, 9.6)		
FN	*1/*1, *1/*28	45	1.6 ± 0.8 (−0.1, 3.2)	0.94	NS
	*28/*28	11	1.8 ± 1.1 (−0.7, 4.2)		
L1–L4	*1/*1, *1/*28	45	2.0 ± 0.7 (0.6, 3.4)	0.59	NS
	*28/*28	11	2.4 ± 1.3 (−0.6, 5.3)		

The differences were tested for significance by the Mann–Whitney test. The values ΔBMD are arithmetic means ± standard error, the numbers in parentheses represent 95% confidence intervals.

significantly different in any of the observed genotype groups (*1/*1, *28/*1 and *28/*28) (Table 3, Figure 2).

Discussion

Our results clearly show the significant influence of UGT1A1 genotype on serum concentrations of raloxifene conjugates. The anticipated effects of UGT1A1*28 polymorphism and the resulting lower UGT1A1 activity would be an increase in serum levels of parent raloxifene and a decrease in serum levels of its glucuronides. In contrast, in the *28/*28 group, significantly higher levels of glucuronides were observed. The levels of unconjugated raloxifene were visibly elevated as well, but due to the small sample size and the pronounced data variability, statistical confirmation of this observation could not be made. The contradictory increase of metabolite levels in the *28/*28 group with a lower UGT1A1 activity may be explained by the metabolic enzyme–transporter interplay. Many authors have described the functional connection

Figure 2

Box plot diagrams showing distribution of % changes in bone mineral density in FN, L1–L4 and HIP after 1 year's therapy with raloxifene in three UGT1A1 genotype groups (*1/*1, *1/*28, *28/*28). In the lower diagram, the genotype groups containing wild-type allele (genotypes *1/*1 and *1/*28) are grouped together in order to facilitate demonstration of the influence of the *28 allele. The outliers are marked with circles (○) and the extremes with asterisks (*)

between metabolic enzymes and transporters [12–14]. Inhibition of one process leads to the inhibition of a linked process of metabolic conversion and excretion of the formed metabolites [12, 14]. Therefore, in the *28/*28 group, decreased conjugating activity in the liver may cause a lower formation rate and lower excretion rate of raloxifene conjugates as well. The principle route of raloxifene elimination is through the liver and bile to faeces [15]. When this route is obstructed it leads to an accumulation of raloxifene in the body [2]. Intriguingly, at the same time increased levels of raloxifene conjugates were observed. The conjugates are formed not only by the liver (with the major contribution of UGT1A1), but also in the intestinal wall [3] and perhaps in the kidney also [16]. The intestinal wall has been recognized as the principal site of raloxifene presystemic metabolic clearance [3, 4], capable of producing high amounts of glucuronides [4]. Therefore, the rate limiting step of raloxifene excretion may not be the formation of conjugates in the whole body, but rather the formation and excretion of metabolites by the liver. This is why the liver function has such a strong influence on raloxifene clearance (cirrhosis and cholestasis included) [2].

The same UGT1A1*28 effect could not be demonstrated for unconjugated raloxifene levels. Although only unconjugated raloxifene can bind with high affinity to the oestrogen receptors to evoke a pharmacodynamic response [17], the lack of statistical significance for unconjugated raloxifene levels does not decrease the impact of our findings. In fact, unconjugated raloxifene represents <1% of total serum raloxifene [15] (Table 1). Even this small fraction of unconjugated raloxifene found in systemic circulation does not originate directly from the parent compound absorbed from the gut, but is mostly formed by the cleavage of the circulating glucuronides [2]. In other words, the circulating glucuronides are the main source of active raloxifene. Furthermore, the terminal parts of the unconjugated raloxifene plasma level curve after a single oral dose were found to be parallel to raloxifene glucuronide levels [15]. This observation indicates an equilibrium and interconversion between raloxifene species [2, 15]. Hence, raloxifene metabolites can be thought of as transport forms and a depot of active raloxifene. Consequently, given the extremely low and highly variable raloxifene concentrations, the serum levels of raloxifene metabolites can be used as a measure of raloxifene exposure. Accordingly, subjects homozygous for the *28 allele with significantly higher raloxifene conjugate levels have been effectively exposed to a higher raloxifene load.

A similar UGT1A1 expression effect has also recently been found for ezetimibe [18]. Like raloxifene, ezetimibe is metabolized by UGT1A1 and, when taken together with the UGT1A1 inducer rifampin, a significant decrease in plasma levels of ezetimibe and its glucuronide was found [18]. The state with induced UGT1A1 activity and the result-

ing lower ezetimibe and its glucuronide plasma concentrations was analogous to our group of patients carrying two wild-type alleles (*1/*1) and the resulting higher UGT1A1 activity leading to a faster elimination of raloxifene and its lower exposure compared with the *28/*28 group.

Furthermore, the same influence of UGT1A1 genotype has also been observed in bilirubin concentration distribution [11, 19]. Bilirubin is a typical UGT1A1 substrate. Subjects homozygous for *28 allele showed markedly increased serum bilirubin levels [11]. Moreover, the serum bilirubin levels were approximately 2.5-fold higher in the *28/*28 group compared with the wild-type homozygotes [11], very similar to our finding of a 2.2-fold increase of raloxifene exposure in the *28/*28 group. In addition, a recently published *in vitro* study has shown that liver microsomes from subjects homozygous for the variant *28 allele demonstrated an approximately threefold lower UGT1A1 activity compared with microsomes originating from the wild-type homozygotes [20]. All these facts taken together with a previously reported (but until now unexplained) correlation between raloxifene and bilirubin serum levels strongly support our hypothesis concerning the major role of UGT1A1 in raloxifene systemic metabolic clearance and excretion.

Therefore, our study explains at least one significant factor that clearly contributes to the large pharmacokinetic variability of raloxifene. Other factors with potential influence on the disposition of raloxifene could be certain concomitant medications, herbal medicines and vegetable food intake, which can significantly affect UGT expression [21, 22]. Also, variations in the liver blood flow, as well as liver function and kidney function, may strongly affect glucuronidation metabolic clearance [16, 23]. Furthermore, enteric passage, bacterial flora, gall bladder emptying, conjugate transporter activity and other factors affecting the enterohepatic circulation could also strongly influence raloxifene clearance [24].

The displayed genetic effect on raloxifene exposure was also shown to be reflected in the observed pharmacodynamic response. The *28/*28 group showed a significantly greater increase in HIP BMD compared with the group with at least one copy of the wild-type allele. This observation can be explained by the higher raloxifene exposure in the *28/*28 group and may also lead to a greater reduction in bone fracture risk [25]. In our study there were no drug-related adverse events. Even in a 36-month, large-scale, multicentred raloxifene clinical trial, there was no significant difference in the risk of venous thromboembolic adverse events between the groups taking 120 mg and 60 mg raloxifene daily [2, 25, 26]. However, our sample was too small and the observation period too short to warrant any definitive conclusions about the UGT1A1 genotype influence on the risks of adverse events.

Moreover, findings from the multiple outcomes of raloxifene study show that compared with placebo, women

taking 120 mg of raloxifene daily had a 33% lower risk of developing mild cognitive impairment and a suggestion of a lower risk of Alzheimer's disease and of any cognitive impairment [27]. However, the 60-mg dose, which is the only dose registered, does not seem to offer any significant protection against cognitive function deterioration compared with that of placebo [27].

Furthermore, it has been discovered that the *28/*28 female population eliminates β -oestradiol significantly more slowly than the rest and thus have a greater risk of developing breast or ovarian cancer [28, 29]. Considering the recently discovered raloxifene breast cancer preventive effect [30], one can speculate that in these cases there may exist a significant and beneficial genotype–raloxifene–pharmacokinetics interaction. Subjects with the *28/*28 genotype and a greater risk of breast cancer would benefit the most from raloxifene treatment due to higher raloxifene exposure. The validity of this hypothesis should be evaluated in a larger cohort of patients over a longer observation period. In addition, the observed UGT1A1*28/*28 genotype effect on raloxifene pharmacodynamics could also be partly caused by the life-long greater β -oestradiol levels in these subjects. The significant difference of $4.1 \pm 3.8\%$ found in hip BMD between *28/*28 and wild-type allele containing groups was much greater than the reported difference in BMD at femoral neck between the 60 and 120 mg raloxifene doses (0.3%, $P = 0.05$), whereas it was even insignificant at other skeletal sites [2, 25]. This could be interpreted as evidence for an additional underlying (and synergistic) mechanism of raloxifene-mediated BMD increase in the *28/*28 group apart from the twofold greater raloxifene exposure. A placebo or control group would be useful to determine whether the observed difference in response was due to raloxifene pharmacokinetics difference or not.

In conclusion, our study has shown that the relatively common UGT1A1*28 polymorphism, when present in a homozygous state, may significantly affect raloxifene pharmacokinetics and may double the raloxifene exposure. Furthermore, the homozygous state may also cause a significantly greater raloxifene-induced increase in hip BMD. Our findings call for future research in this field, especially in the light of the recently discovered protective effect of raloxifene against breast cancer.

Competing interests

None declared.

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