The *CYP3A4*18* Genotype in the Cytochrome P450 3A4 Gene, a Rapid Metabolizer of Sex Steroids, Is Associated With Low Bone Mineral Density

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Osteoporosis is influenced by genetic factors. The interindividual variability in the activity of CYP3A, the metabolic enzyme of sex hormones, may result from genetic polymorphisms. In a study of 2,178 women of ages 40–79 years, the presence of the *CYP3A4*18* variant was found to be significantly associated with low bone mass. *In vitro* functional analyses indicate that *CYP3A4*18* is a gain-of-function mutation in sex steroid metabolism, resulting in rapid oxidation of estrogens and testosterone; *in vivo* pharmacokinetics using midazolam (MDZ) verify the altered activity of the CYP3A4*18, showing lower metabolic turnover in the mutant than in the wild type. Molecular modeling reveals the structural changes in the substrate recognition sites of CYP3A4*18 that can cause changes in enzymatic activity and that potentially account for the difference between the catalytic activities of estrogen and MDZ, depending on the genotype. The results indicate that a genetic variation in the *CYP3A4* gene—as a gain-of-function mutation in the metabolism of certain CYP3A substrates, including sex steroids—may predispose individuals to osteoporosis.

Osteoporosis is a multifactorial disease with a strong genetic component. Genetic factors in uence bone mass, bone size, bone quality, and bone turnover, and they may modulate the risk of osteoporosis.[1] Many candidate genes have thus far been suggested, but none has yet been supported strongly and consistently by subsequent studies.

e members of the cytochrome P450 3A (CYP3A) subfamily are the major enzymes in the nicotinamide adenine dinucleotide phosphate-oxidase–dependent oxidative metabolism of various endogenous and exogenous compounds, including sex hormones. A wide interindividual variability in the expression and catalytic activity of CYP3A has been reported in the general population.[2] e interindividual variation, exceeding 30-fold in some populations, may in uence the circulating levels of endogenous sex steroids and thereby mediate the risk of certain estrogen-associated diseases such as osteoporosis.[3–5]

is variation is, at least partly, caused by multiple environmental factors, including induction by drugs, chemicals, and endogenous compounds, but genetic factors are also among the most plausible mechanisms.

The CYP3A activity of the adult human liver is the sum activity of at least two CYP3A family members: CYP3A4 and CYP3A5. To date, approximately 40 allelic variants in the *CYP3A4* gene have been reported as showing marked ethnic di erences in allele frequencies.[6,7] CYP3A5, the second-most important CYP3A protein in the liver, has characteristic polymorphic expression caused by genetic variation; certain genetic variations, such as *CYP3A5*3* and *CYP3A5*6*, give rise to an aberrantly spliced mRNA with a premature stop codon, which produces a nonfunctioning protein.[8,9]

We therefore hypothesized that genetic variations of CYP3A proteins, the important metabolizing enzymes of estrogen, might be among the major determinants in the development of osteoporosis. To identify the candidate genetic variations in the *CYP3A4* gene, we sequenced the entire coding region and performed detailed structural and functional studies, including

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