The serotonin transporter-linked polymorphic region (5-HTTLPR) of the serotonin transporter gene (SLC6A4) S allele is linked to pathogenesis of depression and slower response to selective serotonin reuptake inhibitors (SSRIs); depression and SSRIs are independently associated with bone loss. We aimed to determine whether 5-HTTLPR was associated with bone loss. This cross-sectional study included psychiatric patients with both 5-HTTLPR analysis and bone mineral density (BMD) assessment (hip and spine Z-scores if age < 50 years and T-scores if ≥ 50 years). BMD association with 5-HTTLPR was evaluated under models with additive allele effects and dominant S allele effects using linear regression models. Patients were stratified by age (< 50 and ≥ 50 years) and sex. Of 3016 patients with 5-HTTLPR genotyping, 239 had BMD assessments. Among the younger patients, the S allele was associated with lower Z-scores at the hip (P = 0.002, dominant S allele effects; P = 0.004, additive allele effects) and spine (P = 0.0006, dominant S allele effects; P = 0.01, additive allele effects). In sex-stratified analyses, the association of the S allele with lower BMD in the younger patients was also significant in the subset of women (P ≤ 0.003 for both hip and spine BMD under the additive allele effect model). In the small group of men younger than 50 years, the S allele was marginally associated with higher spine BMD (P = 0.05). BMD T-scores were not associated with 5-HTTLPR genotypes in patients 50 years or older. The 5-HTTLPR variants may modify serotonin effects on bone with sex-specific effects.
with fluoxetine had impairment of bone mineral accrual. These phenotypes resulted from a reduction in bone formation without an increase in bone resorption and were not influenced by effects on skeletal mechanosensitivity or serum biochemistries.

A more recent study in mice showed that fluoxetine acts on bone remodeling through two distinct mechanisms. Ortuno et al. showed that fluoxetine has peripheral antiresorptive properties, directly impairing osteoclast differentiation and function through a serotonin reuptake-independent mechanism that is dependent on intracellular calcium levels and the transcription factor Nfatc1. The study showed that short-term, 3-week treatment with fluoxetine resulted in a local antiresorptive response that increased bone mass, but there was a net loss of bone with longer term, 6-week use of fluoxetine, which was mediated by a centrally triggered increase in sympathetic activity. The brain serotonin-dependent rise in sympathetic output increased bone resorption sufficiently to counteract its local antiresorptive effect, resulting in a net effect of impaired bone formation and bone loss. Further, neutralization of the central nervous system-mediated mode of action via co-treatment with the β-blocker propranolol prevented fluoxetine-induced bone loss in the treated mice while leaving the peripheral effect intact. Collectively, these findings led the authors to conclude that their result provided evidence for a dual mode of action of SSRIs on bone remodeling and suggested a therapeutic strategy to block the deleterious effect on bone homeostasis from their chronic use. It remains unclear, however, whether these findings apply to humans treated with SSRI medications.

Thus, although several studies have addressed antidepressants and bone loss, few have investigated 5-HTTLPR genotypes. Our study explored the relationship between the 5-HTTLPR genotype and bone loss. We hypothesized that the 5-HTTLPR S allele variant is associated with bone loss in psychiatric patients.

**MATERIALS AND METHODS**

We conducted a cross-sectional study of adult psychiatric patients seen during a 10-year period (1 January 2003, to 21 February 2013). Patients were identified through electronic search of our institutional clinical databases, and they were selected if they had completed both 5-HTTLPR genotyping analyses and BMD assessment. Patients who gave research authorization were included in this study, which was approved by the Mayo Clinic Institutional Review Board. There were no other exclusion criteria.

Collected information included demographics, 5-HTTLPR genotype, and hip and spine BMD. Data were also included if they were available for potential confounders, such as hysterectomy, history of smoking, and selected medications. Medications of interest included estrogen replacement, bisphosphonates, raloxifene, teriparatide, SSRI antidepressants, SNRI antidepressants, tricyclic antidepressants, monoamine oxidase inhibitor antidepressants, other antidepressants and anticonvulsants. Use of the medication in the 5 years preceding the BMD assessment was recorded without the dosage or duration of use.

Pharmacogenetic testing, including 5-HTTLPR, became clinically available in our institution in 2003. Genotyping was performed by Mayo Medical Laboratories or AssureRx Health (Mason, OH, USA). The genotypes identified consisted of homozygous long/long (LL), heterozygous long/short (LS), and homozygous short/short (SS). At that time, neither laboratory genotyped for the triallelic LA/LG/S variation, in which the L allele with the G mutation at rs25531 is thought to result in gene expression similar to that produced by the short allele.16

The Mayo Medical Laboratories procedure extracted DNA from EDTA-preserved whole blood with an EZ1 DSP kit (Qiagen, Valencia, CA, USA). The L and S forms of 5-HTTLPR were determined with PCR amplification similar to the method described by Wendland et al. PCR fragment sizes were determined with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). A length of 530 base pairs was considered a long promoter and a length of 486 base pairs was considered a short promoter polymorphism. The rare promoter polymorphisms that were longer or shorter were resolved with this method and given a customized reference. The AssureRx Health procedure was similar, except that DNA was extracted from a buccal swab.

Areal BMD measurements (in grams per square centimeter) at the lumbar spine and total hip were made by dual-energy x-ray absorptiometry (DXA) according to established clinical protocols. By using X-rays of different energy levels, DXA allows for the subtraction of soft tissue absorption from total absorption, thereby permitting determination of bone absorption specifically. DXA lumbar spine scans were evaluated according to International Society for Clinical Densitometry (ISCD) criteria. Thus, vertebras with deformities were deleted, and the mean value for L1-L4 BMD was recalculated from the remaining vertebras. Consistent with ISCD recommendations, DXA results for women and men 50 years or older were reported as T-scores (equal to the number of s.d. above or below the reference mean for a Healthy 20-year-old of the same sex as the patient). For women and men younger than 50 years, DXA results were reported as Z-scores (equal to the number of s.d. above or below the mean for a healthy age- and sex-matched reference mean). The most recent BMD was used for patients with multiple BMD measurements during the study period.

The association of BMD with genotype was evaluated with linear regression models and assumptions of additive allele effects (coding the genotype in terms of the number of S alleles) and dominant S allele effects (LL vs LS or SS genotype) as predictors of hip and spine T- and Z-scores. Analyses were performed in strata defined by age (<50 or ≥50 years); T-scores were used for patients 50 years or older, and Z-scores for patients younger than 50 years. Analyses were adjusted for age in models for patients 50 years or older and were repeated in sex-specific strata, with the understanding that analyses of men were underpowered because of the small sample of men with available BMD data. Potential confounders, including use of estrogen, antidepressants or anticonvulsants within 5 years before the BMD measurement; history of hysterectomy; and smoking history were evaluated using t-tests. P values were interpreted in the context of the multiple testing that was performed. While analyses of the younger and older groups are independent, analyses of hip and spine within an age group, and those under additive versus dominant models, are not independent. We therefore used an approximate 0.01 significance threshold for determining significance for the analyses of combined male/female samples. We used the same threshold for the secondary sex-stratified analyses, recognizing that these analyses are considered exploratory and should be interpreted with greater caution given the small sample sizes, particularly for males. All statistical analyses were conducted with SAS version 9.4 software (SAS Institute, Cary, NC, USA).

**RESULTS**

Of 3016 psychiatric patients with 5-HTTLPR genotyping over a 10-year period, 239 had at least 1 BMD measurement, including 217 with hip measurements and 190 with spine measurements. Of the 239 patients, 198 (82.8%) were female, 231 (97%) were white, and the mean (s.d.) age was 52.0 (14.0) years (Table 1). The overall 5-HTTLPR genotype frequencies were LL (n = 82; 34%); LS (n = 121; 51%); and SS (n = 36; 15%).

The ISCD defines the normal range for BMD Z-scores in adults younger than 50 years to be above −2.0. Thus, the observed mean BMD Z-scores of −0.66 for the total hip and −0.97 for the lumbar spine are both within the normal range for adults younger than 50 years. The mean hip and lumbar spine Z-scores for premenopausal women were also within the normal range for women younger than 50 years.

Of the possible confounders analyzed, only SSRI use (P = 0.01) and antipsychotic use (P = 0.03) within 5 years before BMD measurement were associated with BMI in patients younger than 50 years, with lower hip BMD Z-scores in SSRI or antipsychotic users as compared to patients who did not use SSRIs or antipsychotics. Final models in patients younger than 50 years were adjusted for only SSRI use because antipsychotic use was not a significant predictor (all P > 0.10) in the multivariable models. Estrogen use and the use of other psychotropic medications (for example, anticonvulsants and non-SSRI antidepressants) were not significant confounders.

In patients 50 years or older, the 5-HTTLPR genotype was not associated with hip or spine BMD T-scores in analyses assuming
with lower hip Z-scores ($P=0.002$ for additive and $P=0.003$ for dominant analyses) and spine Z-score ($P=0.003$ assuming additive allele effects and $P=0.0006$ for a dominant effect) in women younger than 50 years. However, in the small sample of men younger than 50 years, the S allele was marginally associated with a higher spine Z-score ($P=0.05$ under an additive model).

**DISCUSSION**

To our knowledge, this is the largest study to investigate the relationship between 5-HTTLPR and bone loss. Our findings suggest that the 5-HTTLPR S allele is associated with lower BMD in adults younger than 50 years. In contrast, no evidence was found for an association between the S allele and BMD in adults 50 years or older. This association between the 5-HTTLPR genotype and BMD in adults younger than 50 years was driven by the larger sample of women; in particular, sex-stratified analysis showed that premenopausal women younger than 50 years with the S allele had lower BMD compared to women younger than 50 years without the S allele. Surprisingly, an opposite association of the S allele with higher spine BMD was observed in men younger than 50 years, a finding that warrants further investigation because it was based on a small sample.

The murine study by Warden et al., showed that mice with homozymous mutations in the gene encoding the serotonin transporter had reduced bone mass, altered bone microarchitecture, and inferior mechanical properties, suggesting that inhibition of the serotonin transporter in adult humans may lead to bone loss by a similar mechanism. For the younger adults in this cohort treated with SSRIs, the observation that growing wild-type mice treated with fluoxetine had impairment of bone mineral accrual may also be relevant. The mouse phenotypes were attributed to a reduction in bone formation without an increase in bone resorption and were not influenced by effects on skeletal mechanosensitivity or serum biochemistries. These observations may suggest that the 5-HTTLPR S allele is linked to lower BMD in adults or premenopausal women younger than 50 years but not in adults 50 years or older because of effects on the growing skeleton. Given that gonadal sex steroid deficiency or age-related factors are likely to be more common and perhaps correspondingly more important as causative factors for bone loss in adults 50 years or older, it is conceivable that a modest effect associated with the S allele in older adults was not detectable in this small cohort. The biphasic mechanism causing bone loss observed in the mouse study by Ortuño et al., may be directly relevant to this study cohort. The antiresorptive effect seen on osteoclasts early in therapy in the mice would likely not be observable in this human study population in which participants received longer term treatment with SSRIs. The antiresorptive effect in the mice was shown to be independent of serotonin reuptake and to act instead through osteoclast intracellular calcium and the transcription factor Nfatc1. The longer term treatment effect in the mice leading to increased central nervous system sympathetic outflow and bone loss may also be operative in humans.

It is not clear whether the 5-HTTLPR S allele might also be associated with lower BMD because of decreased platelet serotonin levels. Expression of the S allele in circulating platelets would be expected to alter platelet 5-HT function and lead to increased circulating or tissue fluid 5-HTT levels. In turn, the increased 5-HTT levels might potentially interact with the serotonin receptor on osteoblasts, resulting in inhibition of Wnt signaling, reduced osteoblast activity, decreased bone formation, and lower BMD. This effect was not seen in the mouse study by Ortuño et al.,

The medical literature on 5-HTTLPR, BMD, depression and antidepressant use is conflicting, which speaks to the complexity of these relationships. In a study of male youths treated with

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**Table 1. Demographic and clinical characteristics by age group**

| Characteristics | Age < 50 years | Age ≥ 50 years |
|-----------------|---------------|---------------|
|                 | (n = 79)a     | (n = 138)a    |
| Age, years      | 40 (16-49)    | 59 (50-90)    |
| Hip T- or Z-score | −0.5 (−3.1 to 1.9) | −1.0 (−3.9 to 2.7) |
| Spine T- or Z-score | −0.6 (−3.2 to 2.9) | −0.9 (−4.4 to 1.9) |
| Female          | 66 (84)       | 111 (80)      |
| White           | 75 (95)       | 135 (98)      |
| Antidepressantb |                |               |
| SSRI            | 61 (77)       | 98 (71)       |
| SNRI            | 29 (37)       | 68 (49)       |
| MAOId           | 0             | 0             |
| TCA            | 19 (24)       | 38 (28)       |
| Other          | 29 (37)       | 61 (44)       |
| Anticonvulsantb | 33 (42)       | 56 (41)       |
| Antipsychoticb  | 30 (38)       | 41 (30)       |
| Past smoker     | 14 (18)       | 34 (25)       |
| Hysterectomy    | 6 (8)         | 7 (5)         |
| Hormone replacementb | 16 (20) | 46 (33) |

Abbreviations: MAOI, monoamine oxidase inhibitor; SNRI, serotonin-norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant. Continuous data are summarized as median (range); categorical data as number of patients (percentage of sample). bMedication use refers to any prescribed treatment in the previous 5 years.

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**Table 2. Association of BMD with 5-HTTLPR genotype using additive and dominant S allele effects**

| BMD test | Additive S allele effects | Dominant S allele effects |
|----------|---------------------------|---------------------------|
|          | No. | Sample | Estimate | P-value | Estimate | P-value |
| Age ≥ 50 years |     |        |          |         |          |         |
| Hip T-score | 138 | All    | 0.055    | 0.71    | 0.222    | 0.27    |
|            | 111 | Female | 0.127    | 0.47    | 0.283    | 0.22    |
|            | 27  | Male   | −0.116   | 0.63    | 0.077    | 0.82    |
| Spine T-score | 117 | All    | 0.044    | 0.82    | 0.308    | 0.21    |
|            | 96  | Female | 0.032    | 0.89    | 0.291    | 0.30    |
|            | 21  | Male   | 0.098    | 0.74    | 0.404    | 0.33    |
| Age < 50 years |     |        |          |         |          |         |
| Hip Z-score | 79  | All    | −0.378   | 0.004   | −0.614   | 0.002   |
|            | 66  | Female | −0.484   | 0.002   | −0.700   | 0.003   |
|            | 13  | Male   | 0.151    | 0.41    | 0.141    | 0.68    |
| Spine Z-score | 73  | All    | −0.442   | 0.01    | −0.935   | 0.0006  |
|            | 61  | Female | −0.633   | 0.003   | −1.047   | 0.0006  |
|            | 12  | Male   | 0.588    | 0.05    | 0.186    | 0.78    |

Abbreviations: BMD, bone mineral density; 5-HTTLPR, serotonin transporter-linked polymorphic region. aAdjusted for age. bAdjusted for selective serotonin reuptake inhibitor use.
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CONCLUSIONS
This study suggests that 5-HTTLPR S allele is associated with lower BMD at the hip and spine, which was most evident in women younger than 50 years. Our results suggest that 5-HTTLPR variants may modify serotonin effects on bone in a sex-specific interaction. Future studies are needed to explore the utility of 5-HTT genotypes in psychiatric patients who may be vulnerable to increased bone loss.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGMENTS
This study was made possible by the Mayo Clinic Center for Translational Science Activities through grant UL1 TR000135 from the National Center for Advancing Translational Sciences, a component of the National Institutes of Health. This study also received support from the Mayo Clinic Department of Psychiatry & Psychology Small Grant Award.

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