ていた(図 2)。一方、同時に行ったビタミンD3では、これまでの結果の通り、細胞の活性と培地中の活性の両方が有意に上昇していており(図2)、γ-GTPの尿中への遊離に関して、ビタミンD3とおそらくPTHの作用のメカニズムは異なったものであることが示唆された。

PTH 受容体を発現している近位尿細管細胞はあまり知られておらず、文献上報告があり入手可能なオポッサム由来の OK 細胞についてγ-GTP 活性その他を検討してみた。しかし、γ-GTP を高発現していないことが判明し、近位尿細管のモデルとして本研究の目的には適していないと考えられた。

D. 考 察

活性型ビタミン D3 によるγ-GTP の誘導は、いわゆる一過性のものではなく、長期曝露による細胞の性質の変化などに伴う誘導現象ではないかと考えられた。ビタミンD3の場合は、細胞におけるγ-GTP レベルが上昇したために、培地中への分泌・遊離も増えると示唆された。細胞膜からのγ-GTP の遊離、すなわち膜貫通ドメインの切断が亢進するとは考えにくい。

ところがビタミンD3とは対照的に、PTHの作用を模倣する目的で用いたforskolinの効果は、細胞内のγ-GTP 酵素活性には変化がなく、いわゆる遺伝子発現の増加や誘導を起こさないと考えられた。そのため、細胞膜にアンカーしているγ-GTP を培地中へ遊離させるステップが亢進していると示唆される。

どのようにして分泌が増加するのかは今のところ不明であるが、いわゆるシグナルアンカードメインを切断するようなプロセッシングプロテアーゼが存在し、その発現の増加や活性化がおこるのではないかと推測している。このような現象を手がかりとして全身の骨代謝と関連した内分泌的な変化と尿中γ-GTP 活性の増加を結びつける分子や機序を明らかにできればと考えている。

E. 結 論

活性型ビタミン D3 によって近位尿細管上皮細胞におけるγ-GTP 活性を上昇させること、PTH が培地中（生体系では尿中にあたる）への分泌を亢進させるらしいことが培養細胞を用いた結果から推察され、生体内ではこれらの組み合わせを通して尿中γ-GTP 活性が変動する可能性が考えられた。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況

1. 特許取得
なし

2. 実用新案登録
なし

3. その他
なし

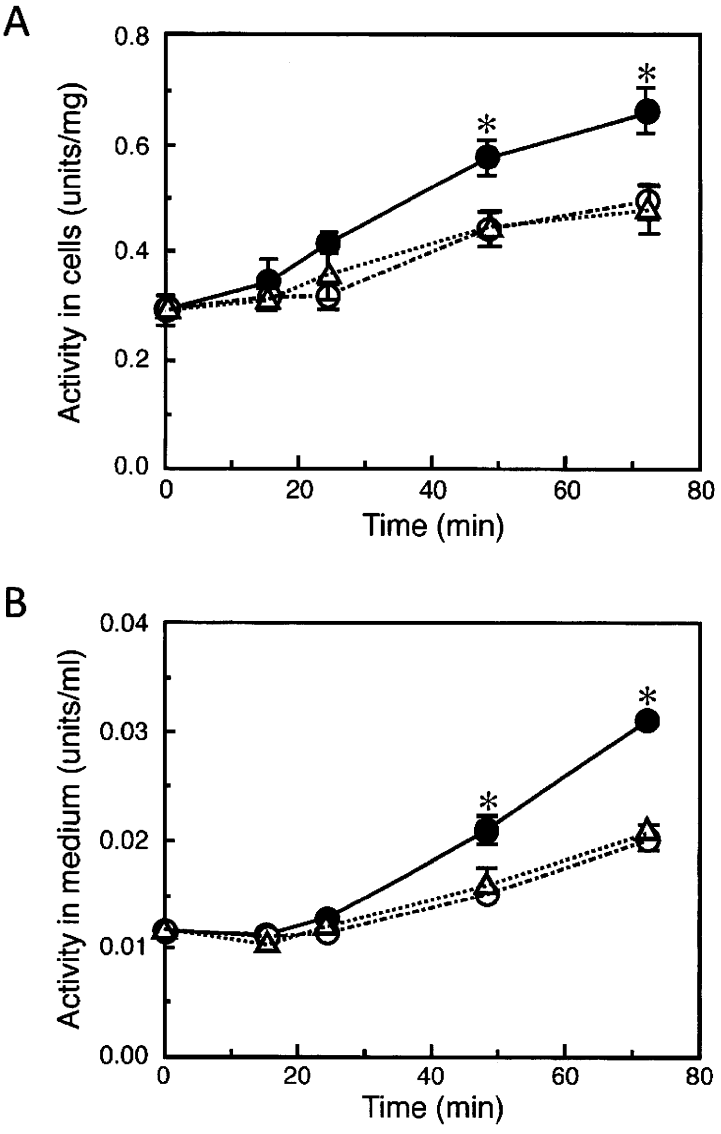

図1. 腎近位尿細管上皮細胞(LLC-PK1)における活性型ビタミンD3刺激後のγ-GTP活性値の変化.
A: 細胞内活性値, B: 培養上清中活性値

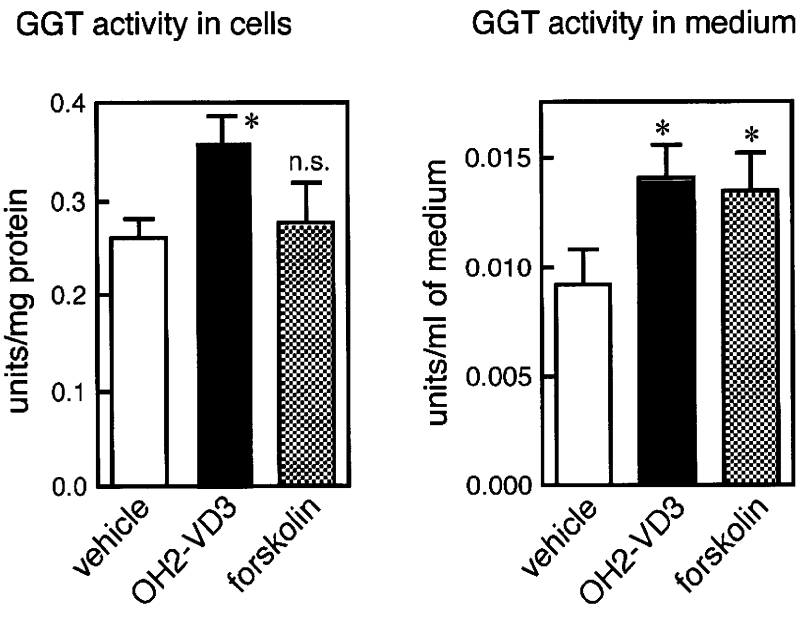

図2. Forskolin存在下における腎近位尿細管上皮細胞(LLC-PK1)からのγ-GTP(GGT)活性の上昇. A: 細胞内活性値, B: 培養上清中活性値

厚生労働科学研究費補助金（長寿科学総合研究事業）
分担研究報告書

4．尿γ-GTP 測定による骨粗鬆症ｽｸﾘｰﾆﾝｸﾞの費用対効果

濃沼 信夫（東北大学大学院医学系研究科・教授）

尿中γ-GTP 測定による骨粗鬆症スクリーニングの費用対効果について検討した。骨密度測定によるスクリーニングでは、効率よく有病者を発見しうるが、そのコストが高い課題がある。一方、γ-GTP 検査では廉価であるが、擬陽性者が少なくない課題がある。今回のモデル検診では、γ-GTP 検査は、擬陽性者の検診コストを加えても、骨密度測定の 4 割ほど安く実施できた。また、検診成績をみると、γ-GTP 検査では骨密度測定より約 2 倍の有病者を発見できた。すなわち、モデル検診の結果からは、γ-GTP 検査は従来法の半分のコストで、2 倍の有病者を発見したことになる。γ-GTP 検査による骨検診は、既存の方法よりも費用対効果の高いことが示唆される。

キーワード：骨検診、骨吸収マーカー、閉経後骨粗鬆症

A．目的

骨粗鬆症による骨折を減らすには骨粗鬆症の早期の医療介入が有効であるが、介入機会となる住民健診等による骨粗鬆症検診（骨検診）の受検率はきわめて低い。受検率が低い原因として、有料検診であることや検診対象が 5 歳刻みという不便な設定になっていることが挙げられる。

尿中γ-GTP は、骨吸収亢進と連動することが示されており、骨吸収マーカーとして利用できる。また、この測定コストは廉価である。本研究では、この方法を骨粗鬆症のスクリーニングに用いた場合の費用対効果について検討する。

B．研究方法

昨年度(2009 年度)に愛知県内で実施したモデル検診で蓄積したデータをもとに、効果を骨粗鬆症または骨量減少症の早期発見とし、費用はスクリーニングおよび二次検診に要する費用として費用対効果を試算した。

C．研究結果

骨検診の結果と費用は表１～２のとおりである。検診法ごとの受検者数が異なるため、人数補正を行ってそれぞれの費用対効果について検討した。

骨密度測定による検診効果と既存の骨吸収マーカー(NTX)による検診効果を費用面で比較すると、一次検診コストは検査料が若干安い分だけ骨吸収マーカーによる検診の方がやや経済的であった。しかし、骨吸収マーカーでは「要精密検査」に区分される人数が多く、その分二次検診の受検者数も増えるため、検診全体の合計額はややコスト高となった。それでも、骨吸収マーカーによる検診の方がより多くの有病者を発見しており、有病者ひとりを発見するのに要したコストは骨密度測定の半分であった。

一方、尿γ-GTP 法による検診では、一次検診コストはさらに低コストとなり、骨密度測定の約 10 分の 1 であった。二次検診受検者数は、既存マーカー同様、「要精密検査」の該当者を多くスクリーニングするため、従来法の 2 倍ほどの人数が受検したが、一次検診コス

トが廉価であったため、総額でも骨密度法の半額であった。また、最終的に発見した有病者数は骨密度法の2倍となり、有病者ひとりを発見するのに要したコストは骨密度法の1/4であった。

D. 考 察

尿γ-GTP 測定にかかる料金は濃度補正のためのクレアチニン測定を加えても210円である。今回実施した骨密度測定の1回あたりの料金は2100円であり、尿γ-GTP 測定はその1/10である。

検査法としての特異度、正診率を高くすることでさらに費用対効果を向上させることができると考えられる。

検体の採取方法、保存方法の他、対象年齢、カットオフ値や経時的な変化をとらえることで診断能力を向上させることができないか、などを検討することは今後の課題である。

E. 結 論

2009 年度に実施した骨検診のデータからは、尿γ-GTP 検査法は、骨密度測定による検診よりも費用対効果の高いことが示唆される。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

	一次検診				二次検診*/診断			二次検診の	治療開始
実施地域	検査法	受検者数	要精検	%	受検者数**	骨量減少	骨粗鬆症	有病率(%)	人数
大府	橈骨BMD	522	68	13.0	17(25%)	7	8	88	7
	NTX	732	206	28.1	50(24%)	20	16	68	19
	γ-GTP***	732	341	46.6	52(15%)	20	18	73	20
東浦	踵骨BMD	510	65	12.7	17(26%)	9	2	64.7	2
	NTX	522	186	35.6	39(21%)	20	3	59	8
	γ-GTP	522	199	38.1	35(18%)	16	2	51.4	2

* 二次検診は医療機関における自己負担での受診
** ( )内の数字は要精検に区分された全員に対する割合
*** γ-GTPのカットオフ値は45.2IU/g・Cre.

	一次検診の	受検者数		発見された有病者数*	費用(千円)			BMD検査との差額	有病者一人を発見する費用
実施地域	検査法	一次	二次	(骨量減少または骨粗鬆症)	一次	二次**	合計		
大府	橈骨BMD	522	17	15 (7)	1,096	271	1,367		91.1
	NTX	732	50	36 (19)	923	798	1,721	(+)354	47.8
	γ-GTP	732	52	38 (20)	145	829	944	(−)423	24.8
東浦	踵骨BMD	510	17	11 (2)	1,071	271	1,342		122.0
	NTX	522	39	23 (8)	658	622	1,280	(−) 62	55.7
	γ-GTP	522	35	18 (2)	104	558	663	(−)679	36.8

* ( )内の数字は治療を開始した人数
** 二次検診費用は医療機関での骨粗鬆症の標準的検査と診察に掛かる金額を15,950円として計算

厚生労働科学研究費補助金（長寿科学総合研究事業）
分担研究報告書

6．医療経済から見た骨粗鬆症

田中　清（京都女子大学・教授）

骨粗鬆症は予防医学的疾患であり、生活習慣病的に理解される。患者数が莫大であり、DXA 法などの専用測定機器を用いて、見いだされた対象者に対して薬物療法を行うという、いわば high risk approach とは別に、スクリーニングとしてふさわしい検査をまず行い、生活習慣改善などの非薬物療法による介入を考えるという、population approach の考え方もまた、非常に重要である。今年度は、その後半部分について検討を行った。老人ホーム入居者に対して、ビタミンＤによる介入を行うシミュレーションを行い、モデル化分析を行ったところ、5 年後の寝たきり者の割合は減少し、要する費用は、ビタミンＤ介入群において低いという結果であった。一次予防としての骨粗鬆症対策が、本研究の目的であり、次年度骨粗鬆症健診のあり方について、特に尿中γ-GTP 測定の意義について、検討を加える予定である。

キーワード：医療経済，骨粗鬆症，生活習慣病

A. 目的

骨粗鬆症は、骨折のリスクが増加した状態であり、決して骨折した状態ではない。骨折していなくても、将来の骨折を防ぐために治療する、すなわち現在無症状であっても、将来の有害な事象を防止するために治療するというのは、生活習慣病の考え方であり、骨粗鬆症は生活習慣病的にとらえることができる。

しかし骨粗鬆症には、他の生活習慣病とは異なる大きな特徴がある。まず診断には、脊椎Ｘ線撮影による骨折の判定や、専用の機器を用いての骨密度測定が必要であり、この点血圧測定によって診断される高血圧や、血糖値や血清脂質測定によって診断される脂質異常症とは大きく事情が異なる。骨粗鬆症は患者数が莫大だが、適切に診断・治療を受けている患者の割合が極めて低いと言われており、この点もその一因であろう。

また治療に関しては、近年ビスフォスフォネート・SERM・PTH などの、骨折抑制のしっかりしたエビデンスを持つ薬剤が次々に臨床的に用いられ、さらに新薬の開発も進んでいる。しかし骨折抑制のエビデンスがあれば、それだけで充分であるとは言えない。

骨折発生を半減させる薬剤があったとして、骨折のリスクが極めて高い集団を対象に治療介入を行えば、極めて費用対効果に優れるが、予防できる骨折の絶対数は、社会全体としてごく一部に過ぎないという論文が発表されている（Sanders KM et al. Bone 38:694-700, 2006)。すなわち社会全体としてみた場合、骨折の大多数は、中～低リスクの集団から発生するので、その対策を講じる必要があるということである。しかし、このような集団に対して広く予防的薬物療法を行うことは、費用対効果が悪いだけではなく、副

作用の面からも、現実的ではない。

すなわち骨粗鬆症の診断・治療を考えた場合、ある程度骨折リスクの高い集団を対象とするのであれば、現在骨密度測定の標準法である DXA 法にて診断し、ビスフォスフォネート・SERM・PTH などにより治療すればよい訳だが、骨粗鬆症を生活習慣病としてとらえ、その一次予防を考えた場合には、異なったアプローチが必要となる。

疾患の予防には、高リスク者を対象に、集中的に介入を行う high risk approach と、社会全体のリスクを低い方に平行移動させる population approach がある。上記の DXA 法による診断・新薬による治療は、high risk approach の考え方だが、population approach としての骨粗鬆症については、診断・治療とも未だ確立した戦略がなく、今後の大きな課題であろう。

そこで本研究では、以下の二点を検討することとした。まず診断に関しては、すなわち骨粗鬆症の一次予防としてのスクリーニング検査のあり方、その医療経済評価を検討するとともに、それによって見いだされた対象者に対する、生活習慣改善を中心とした、非薬物療法の介入に関する医療経済評価を行うこととした。今年度はまずその後半部分に関して報告する。

B. 方法

高齢者に対する、ビタミンDによる介入による骨折予防効果の費用対効果を検討した。Base Case は、80 歳女性・椎体既存骨折 1 つあり・老人ホーム入所中とした。シミュレーションとして、カルシチュウ 1 日 2 錠服用 (Ca610mg、D3 400IU)を服用するものとし、これにより大腿骨近位部骨折が 25%減少するものとした (Maturitas 2003)。またこの年齢の年間死亡率は、簡易生命表から 0.06 とし、大腿骨近位部骨折の発生率 は 0.0415 (萩野)、大腿骨近位部骨折後寝たきりになる割合を 18.8% (太田)、骨折後 1 年以内の死亡率を 20%とした。また大腿骨近位部骨折後 1 年以内に要する費用を 148 万円 (林)、寝たきりとなった場合の費用 を 284 万円 (林) とした。

結果は TreeAge 2009 を用いて、マルコフモデルを作成して分析した。最初 healthy の状態からスタートし、一定の割合で大腿骨近位部骨折・死亡に移行するものとし、骨折後は一定の割合で死亡・寝たきり・大腿骨近位部骨折後状態に移行するものとした。

C. 結果

図に示すように、ビタミン D 介入により、寝たきり者の割合は低下した。5 年間に要する費用は、ビタミン D 介入群 94,714 円、非介入群 108,786 円であった。

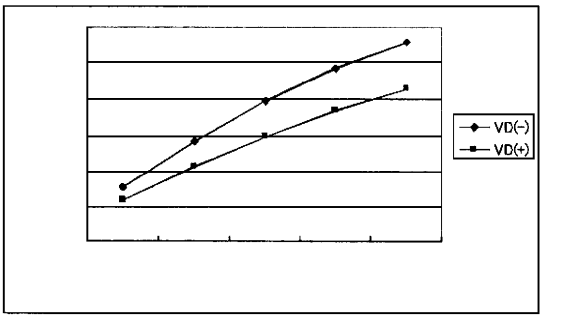

図 1. ビタミン D 介入・非介入によるシミュレーション 5 年後の寝たきり者の割合

D. 考察

老人ホーム入居者に対して、ビタミン D 介入を行うことは、費用対効果に優れるという結果が得られた。本結果は、引用するデータ

を変更して結論がどのように変わるかという感度分析も行っておらず、予備的なものであるが、従来わが国においては、このような分析は行われてこなかった。

また本検討の結果は、おそらく大幅な過小評価である。すなわちビタミンDによる、それ以外の骨折に対する予防効果は算定していないし、また骨折後には、「寝たきり」以外の要介護の状態に移行する可能性も高いが、その分も算定していない。

「目的」の項にも述べたように、一次予防としての骨粗鬆症対策が、本研究の目的であり、次年度骨粗鬆症健診のあり方について、特に尿中γ-GTP 測定の意義について、検討を加える予定である。

E. 結論

骨粗鬆症は生活習慣病的な疾患として理解されるべき疾患であり、その診断・治療のいずれに関しても、primary prevention としての理解も必要である。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

3. 研究成果の刊行に関する一覧表

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4.　本研究の基盤となった研究論文

ELSEVIER

Bone 39 (2006) 1276–1282

BONE

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Urinary γ-glutamyltransferase (GGT) as a potential marker of bone resorption

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Abstract

We recently identified γ-glutamyltransferase (GGT) as a novel bone-resorbing factor. The present study was undertaken to determine whether GGT is a marker of bone resorption in two genetic models of hyper- and hypo-function of osteoclasts, as well as in postmenopausal women with accelerated bone resorption, using type I collagen N-telopeptide (NTX) and deoxypyridinoline (DPD) as established biochemical markers. Urinary excretion of GGT, corrected for creatinine, was found to be increased in osteoprotegerin (OPG)-deficient osteoporotic mice as well as in patients with postmenopausal osteoporosis (67–83 years of age); in both cases the urinary level decreased after treatment of patients or mice with alendronate, a selective inhibitor of bone resorption, concomitantly with a reduction in DPD and NTX. Conversely, in osteopetrotic *op/op* mice, urinary GGT increased in parallel with DPD after induction of osteoclasts with M-CSF injection. Constant infusion of parathyroid hormone (PTH) also increased urinary GGT along with DPD. In a survey of 551 postmenopausal women (50–89 years of age) at their regular health checkup, urinary GGT excretion exhibited a high correlation with DPD ($\rho=0.49$, $p<0.0001$). The calculated sensitivity and specificity for diagnosing elevated bone resorption, as determined by a DPD value higher than 7.6 nM/mM Cr, were 61% and 92%, respectively, when a cut-off value of 40 IU/g Cr was assigned for urinary GGT. Since GGT activity can be measured inexpensively in large numbers in a very short time, the measurement of urinary level may provide a convenient and useful method for mass screening to identify those with increased bone turnover and hence at increased risk for bone fracture.
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Keywords: Osteoclast; Bone resorption; GGT

Introduction

Osteoporotic fractures are a major cause of morbidity and mortality in the aging population [1]. The annual incidence of hip fractures has been increasing and exceeded one hundred thousand cases in the most recent 2002 survey in Japan. The diagnosis of osteoporosis is made on the basis of bone mineral density (BMD) measurement, as in other countries [9]; however, due to the limited availability of devices for dual X-ray absorptiometry (DXA), the number of those who actually receive medical treatment is estimated to be only 20–30% of the more than 10 million patients with osteoporosis in our country. Thus, it is of utmost importance to develop a noninvasive, simple and inexpensive method to estimate bone fragility and the associated increased risk of fractures.

Although low BMD is the most reliable surrogate for the assessment of fracture risk, other traits, referred to collectively as "bone quality", entailing size, architecture, turnover, damage accumulation and mineralization, contribute to bone strength as well [1,21]. Among these, biochemical markers of bone turnover have been shown to predict the risk of fractures independently of BMD [5,6]. Bone undergoes continuous remodeling, in which bone resorption always precedes formation. Elevated

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osteoclastic bone resorption plays a central role in the pathogenesis of osteoporosis, leading to fragility and fracture [24], with anti-resorptive drugs, represented by bisphosphonates, currently regarded as the first choice of treatment [4]. Recent studies using mouse genetics have identified regulators of osteoclast differentiation and function, among which receptor activator of NF-κB ligand (RANKL) has attracted considerable attention [2]. A humanized monoclonal antibody against RANKL, which has recently been shown to increase BMD by inhibiting bone resorption, is emerging as a new treatment option [14]. The discovery of the extracellular signals that control osteoclastogenesis is much anticipated and expected to provide an attractive target for the development of new diagnostic and therapeutic strategies.

In a search for new bone-resorbing cytokines using a *Xenopus* oocyte expression cloning technique, we have recently identified γ-glutamyltransferase (GGT or γ-GTP) as an osteoclastogenic factor, and demonstrated that recombinant human GGT as well as purified GGT from rat kidney stimulates bone resorption [16]. Further, during the course of our study examining the involvement of GGT in bone and joint diseases characterized by accelerated bone resorption, we unexpectedly found that GGT activity in urine, but not in serum, correlates with bone resorption. The present study was undertaken to determine whether GGT is a potential marker of bone resorption, using genetic mouse models as well as human subjects.

Materials and methods

Reagents

Alendronate sodium hydrate was purchased from Teijin Pharma Ltd. (Osaka, Japan). Human PTH (1–34) and M-CSF were kindly provided by Asahi Kasei Pharma (Tokyo, Japan) and Morinaga Milk Industry (Tokyo, Japan), respectively.

Animal experiments

Osteoprotegerin (OPG)-deficient male mice and BALB/cA mice were purchased from Clea Japan Inc. (Tokyo, Japan), and acclimated under standard laboratory conditions at 24±2°C and 50–60% humidity. The mice were allowed free access to tap water and commercial standard rodent chow (CE-2) containing 1.20% calcium, 1.08% phosphate and 240 IU/100 g vitamin D_3 (Clea Japan Inc., Japan). At the age of 9 weeks, OPG homozygous and heterozygous knockout mice (as control) were treated s.c. with vehicle (saline) or 1 mg/kg BW alendronate 5 times a week for 2 weeks, and urine was collected during the final 24 h. Blood samples were centrifuged to obtain the serum.

Eight-week-old female BALB/cA mice were infused with PTH at a rate of 4.3 pmol/h for 4 days. In brief, human PTH (1–34) was resolved in 2% L-cysteine solution, and loaded into Alzet osmotic minipumps. After equilibrated in saline at 37°C overnight, the pumps were implanted in a subcutaneous space on the back. Urine was collected during the final 24 h for biochemical analysis.

op/+ heterozygous mice were obtained from Jackson Laboratory (Bar Harbor, ME), and fed CE-2 powder chow (Clea Japan Inc., Japan). At the age of 5 weeks, *op/op* homozygous mice were treated i.p. with 5 μg M-CSF twice daily for 3 days, and urine and serum samples were collected before and after M-CSF treatment. Tibiae were removed for micro-CT scanning and tartrate-resistant acid phosphatase (TRAP) staining.

The animal experiments were carried out in accordance with the institutional ethical guidelines for animal care, and the experimental protocols were approved by the animal care committee of NCGG.

Subjects

Blood and spot urine samples were collected at 10:00–12:00 am from 10 patients with postmenopausal osteoporosis (67–83 years of age; average, 76.7), who visited the National Center for Geriatrics and Gerontology Hospital from April 2003 through August 2004, before and after alendronate treatment for measurement of blood GGT as well as urinary GGT and NTX. The diagnosis of osteoporosis was made based on the criteria recommended by the Japanese Society for Bone and Mineral Research [18], i.e., at least one non-traumatic vertebral fracture and a BMD lower than 80% of the young adult mean (YAM) or BMD lower than 70% of YAM without fracture.

Urine samples were also collected from 551 volunteer postmenopausal women (50–89 years of age; average, 66) at their regular health checkup for the measurement of GGT and deoxypyridinoline (DPD). The human studies were approved by the institutional review board, and written informed consent was obtained from all individuals.

Biochemical analysis

GGT activity and creatinine concentrations in the serum and urine were determined by using an autoanalyzer (model AU5232, Olympus) on the day following sample collection after storage at room temperature, since we found that GGT activity in the urine was stable for up to 1 week at room temperature or at 4°C but was lost after freezing at −20°C and subsequent thaw. Intra- and inter-assay variations for GGT were 0.58–1.77% and 0.29–1.78%, respectively. NTX and free DPD concentrations in the urine were measured using Osteomark [7] and Osteolinks-DPD (Sumitomo Seiyaku Biomedical Co., Ltd., Osaka, Japan) assay kits [20], respectively, and the values were corrected for creatinine. Intra- and inter-assay variations were 1.8–4.5% and 4.7–10.8% for NTX, and 1.4–7.4% and 4.2–6.4% for DPD, respectively. Leucine aminopeptidase, alkaline phosphatase, acid phosphatase and *N*-acetyl-β-D-glucosaminidase (NAG) in the urine were determined by using autoanalyzers (model AU5200 and AU600, Olympus).

Urinary GGT, creatinine and free DPD concentrations for the 551 volunteer women were measured by using "γ-GTP C-TestWako" (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and "Determina-L CRE" (Kyowa Medex Co., Ltd., Tokyo, Japan) assay kits, respectively.

For fractionation of GGT activity, urine was collected from 6 healthy volunteers (3 females and 3 males; aged 29–35 years). After centrifugation at 17,000×*g* for 15 min, the supernatant was further centrifuged at 200,000×*g* for 3 h. GGT activity in the pellet and supernatant fractions after each centrifugation was measured using γ-GTP C-TestWako (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Bone analysis

For bone analysis, right tibiae were dissected and stored in 70% ethanol for micro-computed tomography scanning. Left tibiae were fixed in 4% paraformaldehyde, and TRAP staining was performed by a standard technique [17].

Micro-computed tomography scanning was performed on proximal tibias by using a μCT-40 (SCANCO Medical AZ, Bassersdorf, Switzerland) with a resolution of 12 μm, as described previously [8].

Statistical analysis

Data are expressed as the mean±SD. Changes in GGT and DPD or NTX excretion before and after alendronate treatment were analyzed by unpaired or paired Student's *t* test. The relation between GGT and DPD was assessed by Spearman rank order correlation analysis. $P<0.05$ was considered statistically significant.

Results

In order to determine if GGT is involved in bone diseases associated with accelerated bone resorption, we first assessed

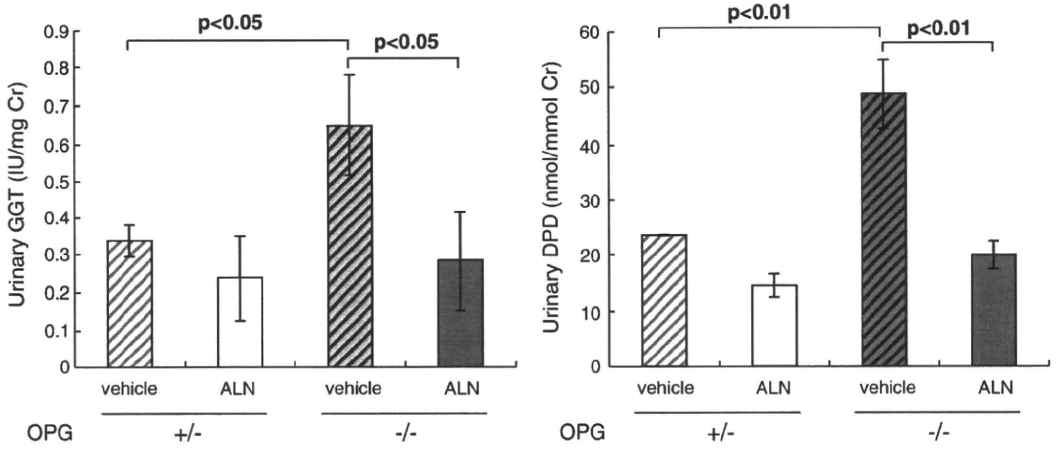

Fig. 1. Reduction in urinary GGT excretion after treatment of OPG-deficient mice with alendronate. Nine-week-old osteoprotegerin (OPG)-deficient male mice were treated s.c. with vehicle (saline) or 1 mg/kg BW alendronate (ALN) 5 times a week for 2 weeks, and urinary excretion of GGT (left) and DPD (right) was then determined. OPG heterozygous knockout mice served as the control. $n=3$ for vehicle groups and $n=7$ for treatment groups.

blood and urinary levels of GGT in a genetic model of osteoporosis, i.e., osteoprotegerin (OPG)-deficient mice [2]. OPG is a decoy receptor of RANKL, an essential cytokine for the formation of osteoclasts, and mice lacking OPG exhibit osteoporosis due to unopposed RANKL signaling and accelerated bone resorption [2]. Serum GGT activity in these mice was very low (less than 4 IU/l), compared with that in humans (normal range being 10–63 IU/l), and did not differ between OPG homozygous and heterozygous knockout mice or after treatment with alendronate, a selective inhibitor of bone resorption (data not shown). In contrast, as shown in Fig. 1, urinary excretion of GGT as well as of DPD was significantly increased in OPG homozygous knockout mice, compared with the levels of the control heterozygous mice. Treatment of OPG-deficient mice with alendronate resulted in a significant reduction in both urinary GGT and DPD excretion to the control levels found in the heterozygous mice. These findings suggest that urinary excretion of GGT, not serum levels, reflects the activity of bone resorption in the body.

Urinary excretion of leucine aminopeptidase (0.048±0.023 in WT vs. 0.065±0.021 U/mg Cr in OPG KO) and alkaline phosphatase (0.032±0.054 in WT vs. 0.021±0.015 IU/mg Cr in

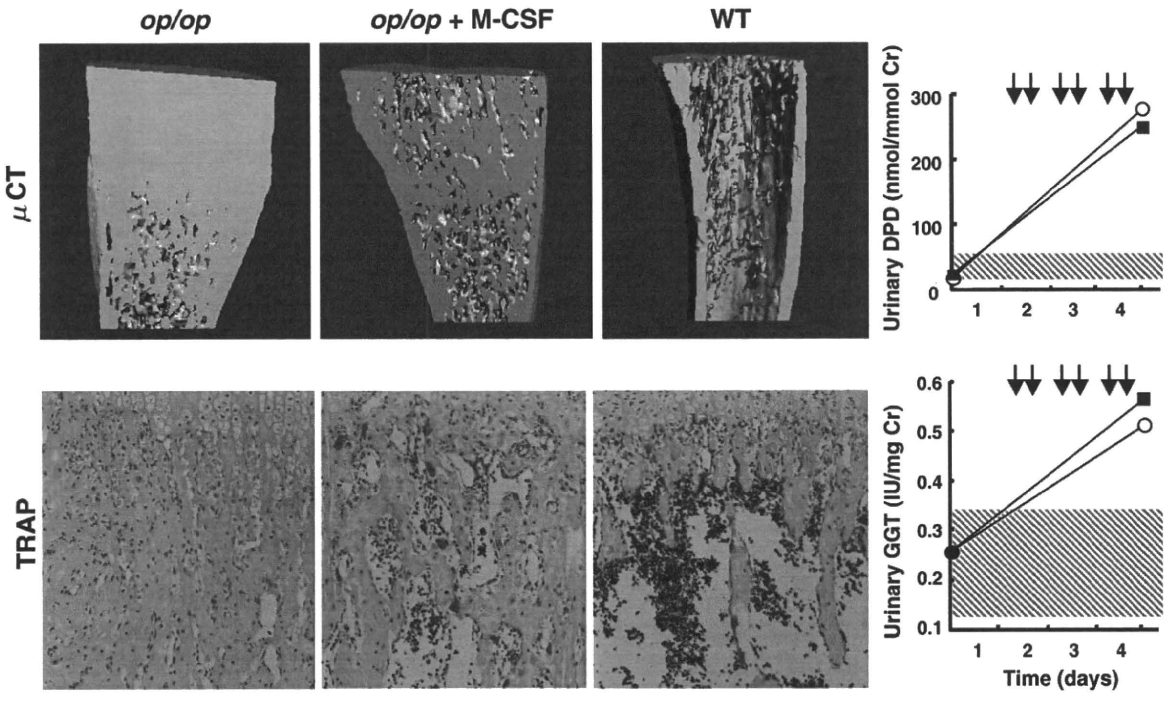

Fig. 2. Increase in urinary GGT excretion after osteoclast induction in *op/op* mice. Six-week-old osteopetrotic *op/op* female mice ($n=2$ each) were treated i.p. with 5 μg M-CSF twice daily for 3 days, and urinary excretion of DPD and GGT was then determined before and after injections. Age- and sex-matched wild-type mice served as the control as shown as the shaded area (mean±SD, $n=10$). Arrows indicate M-CSF injections. Representative micro-CT images and photomicrographs of TRAP staining of the proximal tibia are shown.

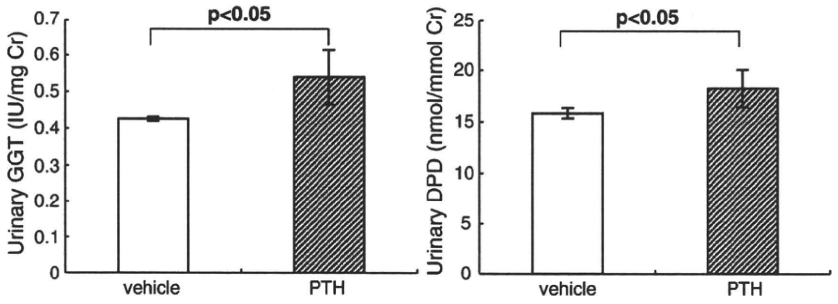

Fig. 3. Increase in urinary GGT excretion after constant PTH infusion. Eight-week-old female BALB/cA mice ($n=3$ each) were subjected to constant infusion of PTH (1–34) at a rate of 4.3 pmol/h for 4 days through Alzet osmotic minipumps, and urinary excretion of DPD and GGT was determined during the final 24 h. Age- and sex-matched mice with constant infusion of vehicle (2% L-cysteine) served as the control. Osteoclast surface and eroded surface per bone surface were markedly increased in the tibial metaphyses and lumbar vertebrae of PTH-infused mice.

OPG KO), enzymes located at the brush border membrane of renal tubules, did not differ significantly between wild-type and OPG knockout mice. Of lysosomal enzymes, urinary excretion of acid phosphatase did not differ (0.0030±0.0011 in WT vs. 0.0026±0.0015 IU/mg Cr in OPG KO), while that of *N*-acetyl-β-D-glucosaminidase (NAG) was significantly increased in OPG KO mice (0.066±0.028 vs. 0.193±0.074 IU/mg Cr, $p<0.01$). Thus, certain enzymes of proximal renal tubular cells including GGT may be excreted during elevated bone resorption.

We also employed a gain-of-function approach with another genetic model, osteopetrotic *op/op* mice, to examine whether urinary GGT excretion increases following the induction of osteoclasts with M-CSF injection. Fig. 2 shows representative micro-CT images (upper panel) and bone sections stained with TRAP activity (lower panel) at the proximal tibia. *op/op* mice at 6 weeks old exhibited typical osteopetrosis with very few osteoclasts, although osteoclasts appeared spontaneously with aging [17]. Administration of M-CSF twice daily for 3 days caused marked increases in bone marrow cavity and TRAP-positive osteoclasts (Fig. 2). Urinary DPD and GGT excretion both increased after M-CSF treatment (Fig. 2, right panel).

Continuous excess of PTH and PTH-related protein is associated with elevated bone resorption, as seen in patients with primary hyperparathyroidism and hypercalcemia of malignancy, respectively. As a model mimicking these conditions, we infused PTH (1–34) to mice constantly through osmotic minipumps. Histological examination on sections of tibial metaphyses and lumbar vertebrae revealed that osteoclast number and eroded surface per bone surface markedly increased following PTH infusion (data not shown). As shown in Fig. 3, constant infusion of PTH also increased urinary excretion of GGT significantly along with DPD. Collectively, our loss- and gain-of-function experiments using genetic and pharmacological models with excessive and deficient osteoclastic bone resorption, respectively, indicate that urinary GGT changes in parallel with DPD and reflects bone resorption activity in the body.

Based on these data, we analyzed urinary excretion of GGT in osteoporotic patients with elevated bone resorption. Urine samples were collected from 10 patients with postmenopausal osteoporosis (67–83 years of age; average, 76.7), before and after alendronate treatment. As shown in Fig. 4, urinary excretion of GGT decreased significantly along with NTX and DPD following treatment with alendronate. Serum GGT concentrations in these patients were within normal limits (10–63 IU/l) and did not change following treatment (data not shown).

In order to gain some insight into the form in which GGT exists in human urine, urine collected from healthy volunteers was fractionated by centrifugation, and the GGT activity in each fraction was determined. As shown in Table 1, when urine was centrifuged at 17,000×*g* (17 K) to remove cells and cell debris,

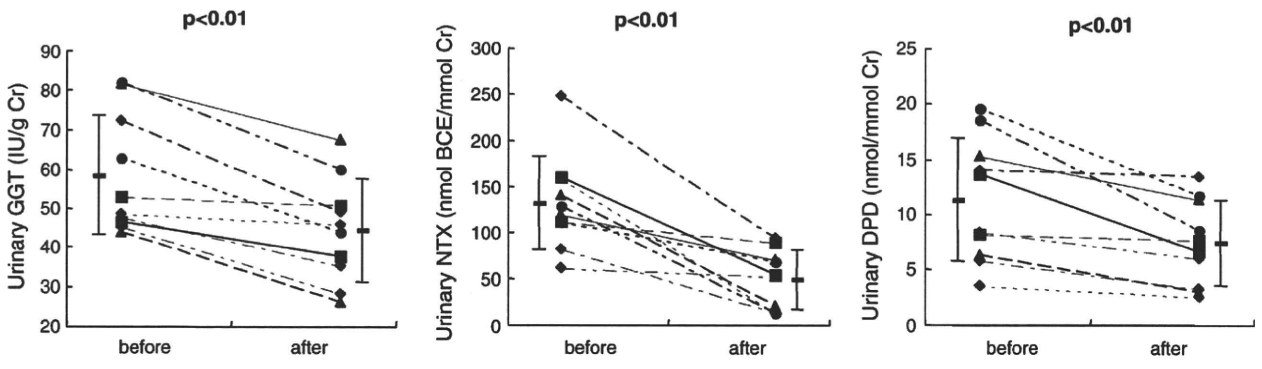

Fig. 4. Reduction in urinary GGT excretion after treatment of postmenopausal osteoporosis with alendronate. In 10 patients with postmenopausal osteoporosis (mean age: 76.7 years old), urinary excretion of GGT (left) decreased concomitantly with a reduction in urinary NTX (middle) and DPD (right) after treatment with alendronate (for 7 months on average). Individual data are shown with the mean±SD.

Table 1
Fractionation of urinary GGT activity

	Male				Female			
Urinary GGT	1	2	3	Mean	1	2	3	Mean
(IU/L)	32.7	37.0	63.6	44.4	45.3	30.8	14.6	30.2
17 K								
S	29.3	34.6	55.7	39.9	43.4	29.9	13.9	29.1
P	3.5	4.6	6.5	4.9	4.3	3.3	1.8	3.1
200 K								
S	6.5	6.3	9.5	7.4	5.0	6.3	3.5	4.9
P	23.7	26.9	47.0	32.5	36.5	28.9	13.3	26.2
% of P	72.5	72.7	73.9	73.2	80.6	93.8	91.1	86.8

Urine was collected from healthy volunteers (3 males and 3 females), and GGT activity in the whole urine was determined (top). After centrifugation at 17,000×*g* (17 K) and 200,000×*g* (200 K), GGT activity was determined in both supernatant (S) and pellet (P) fractions.

more than 90% of the total GGT activity in the urine was recovered in the supernatant fraction. When the 17 K supernatant was further subjected to centrifugation at 200,000×*g* (200 K), 73.2 to 86.8% of the total GGT activity was found in the pellet fraction, suggesting that GGT in human urine does not exist as a soluble form but rather is mostly associated with certain microstructures that sediment at 200 K.

Finally, to determine if urinary GGT can be used for screening individuals with elevated bone resorption in the general population, we assessed the urinary excretion of GGT and DPD in 551 volunteer postmenopausal women (50–89 years of age; average, 66) at their regular health checkup. As shown in Fig. 5A, there was a high correlation between urinary excretion of GGT and DPD in this population ($p<0.0001$). Of these 551 individuals, 113 had increased bone resorption, as judged from DPD values higher than 7.6 nM/mM Cr (17.0±15.0), the cut-off value for diagnosing elevated bone resorption recommended by the Japanese Society for Bone and Mineral Research. These individuals exhibited significantly elevated urinary excretion of GGT (85.7±95.0 IU/g Cr), compared with those that had normal DPD values (GGT: 22.0±12.0 IU/g Cr, DPD: 3.8±1.9 nM/mM Cr; Fig. 5B). When a cut-off value of 40 IU/g Cr was assigned for urinary GGT, the calculated sensitivity and specificity for discriminating those with elevated bone resorption, as determined by a DPD value higher than 7.6 nM/mM Cr, were 61% and 92%, respectively, and 75% and 79% for a GGT cut-off value of 30 IU/g Cr.

Discussion

GGT is an ectopeptidase that catalyzes the transfer of a γ-glutamyl moiety to an acceptor and plays a critical role in glutathione degradation and cysteine metabolism [11,23]. Mice deficient in GGT exhibit growth retardation, cataracts and severe osteoporosis, and die early at 10–18 weeks of age [12]. Osteopenia of GGT-deficient mice is due mainly to impaired bone formation, which is reversible by supplementation with *N*-acetylcysteine, suggesting that GGT plays an important physiological role in regulating bone formation through cysteine metabolism [10]. We have identified GGT as a bone-resorbing factor in the expression cloning of an osteoclastogenic activity contained in murine T lymphoma, which caused marked osteolysis in mice, and demonstrated that recombinant GGT at 100 IU/l, a level often seen in patients with excess alcohol intake or fatty liver, is indeed capable of stimulating osteoclastogenesis in bone marrow cultures [16]. Furthermore, the generation of transgenic mice overproducing GGT has revealed that excess GGT causes osteopenia due to accelerated bone resorption (Hiramatsu et al. manuscript submitted). Taken together, it is suggested that GGT levels should be maintained within a set physiological range and both deficiency and excess can lead to osteoporosis, but by distinct mechanisms, i.e., through suppressed bone formation and elevated bone resorption, respectively. Interestingly, a mutated GGT molecule devoid of enzyme activity is fully active in promoting osteoclast formation (Hiramatsu et al. manuscript submitted), suggesting that the osteoclastogenic function of GGT is dissociated from its enzyme activity, does not involve glutathione or cysteine metabolism, and may represent a novel mode of action as a cytokine.

In the present study, we demonstrate that the urinary excretion of GGT changes in parallel with established biochemical markers of bone resorption, NTX and DPD, and therefore reflects bone resorption activity both in animal models and human subjects. Whereas serum GGT activity derives mainly from the liver, GGT is most abundantly expressed in the proximal tubule of the kidney, where this ectoenzyme is located

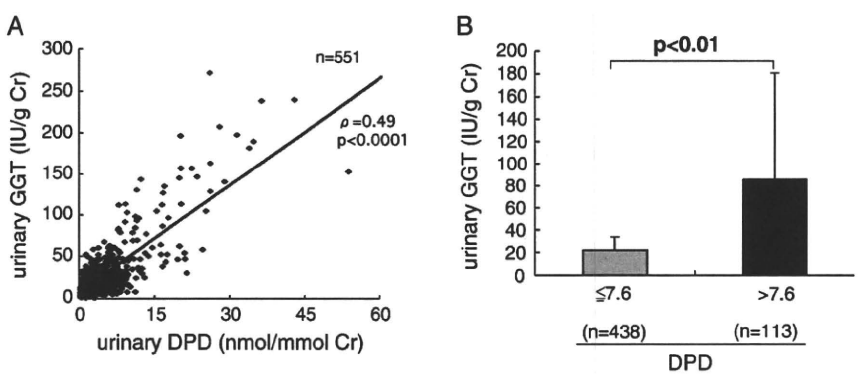

Fig. 5. Correlation between urinary GGT and DPD excretion in postmenopausal women. (A) In 551 postmenopausal women (50–89 years old; mean age, 66), urinary excretion of GGT showed a highly positive correlation with urinary DPD ($p<0.0001$). (B) Of these individuals, 113 showed elevated bone resorption (DPD >7.6 nmol/mmol Cr); and their urinary GGT excretion was increased significantly compared with that of the individuals with lower DPD values ($p<0.01$).

on the apical membrane [23]. Although the exact mechanism underlying GGT excretion in high bone turnover states remains to be determined, increased GGT activity in the urine of humans as well as experimental animals with accelerated bone resorption, without appreciable increase in serum concentrations, prompts us to hypothesize that the GGT anchored to the plasma membrane of renal tubular cells and exposed to the tubular lumen is prone to being shed into the urine in response to some signaling cue from bone turnover. Communication between bone and kidney is known for collagen cross-links; the conversion of peptide bound to free DPD in the kidney has been reported to become more efficient as bone turnover decreases [15].

By fractionation we found that most of the GGT activity in human urine was recovered in the pellet fraction after centrifugation at 200,000×*g*, suggesting that GGT is not excreted in a soluble form but rather in association with certain microstructures. A recent proteomic analysis of exosomes [membrane vesicles that originate as internal vesicles of multivesicular bodies (MVBs)] in the urine identified protein components of MVBs, among which GGT was included [19]. Taken together with our observations, it is tempting to speculate that increased GGT activity in a high bone turnover state is associated with exosomes and the shedding of exosomal GGT from the proximal renal tubules is stimulated in response to some cue from elevated bone resorption [25]. This may provide an explanation for the unexpected observation that unlike serum GGT activity, which is stable after freezing at −20°C and thawing, most of the urinary GGT activity is lost after freezing at −20°C. Alternatively, the possibility that GGT is produced in bone sites undergoing elevated resorption and is excreted in the urine after filtration through the glomerulus cannot be completely ruled out, although it seems unlikely that GGT, with a relatively high molecular weight, is filtered through the glomerulus under physiological conditions. Further studies are required to identify the molecular form(s) of GGT in the urine, and to clarify the specific mechanism(s) by which its excretion is enhanced in diseases with elevated osteoclastic activity.

Osteoporosis is pandemic in industrialized countries, and early diagnosis with timely measures is crucial for mitigating further bone loss and preventing bone fracture [1,13]. The widely used measurement of BMD alone is not sufficient for assessing fracture risk and can miss most of the postmenopausal women who experience fracture [22]. In addition to BMD, several other factors are known to impact the quality of bone, including bone geometry and microstructure, microdamage, and bone turnover, but only biochemical markers of bone turnover are available for use in clinical practice [5]. Measurement of these biochemical markers, however, is time consuming and costly, and a simple and inexpensive method for mass screening is urgently required. Since GGT activity can be measured inexpensively in-house for large numbers of patients in a very short time with little variability, the measurement of the GGT urinary level may provide a highly convenient and useful method for screening individuals who have increased bone turnover and therefore an increased risk for bone fracture. It is to be noted that since urinary GGT activity can be increased in renal dysfunction due to drug intoxication, diabetes and hypertensive nephropathy [3,26,27], the results should be interpreted with caution.

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