Genetic aspects of osteoporosis

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Osteoporosis

Skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture

Bone Strength = Bone Mineral Density (BMD) and Bone Quality

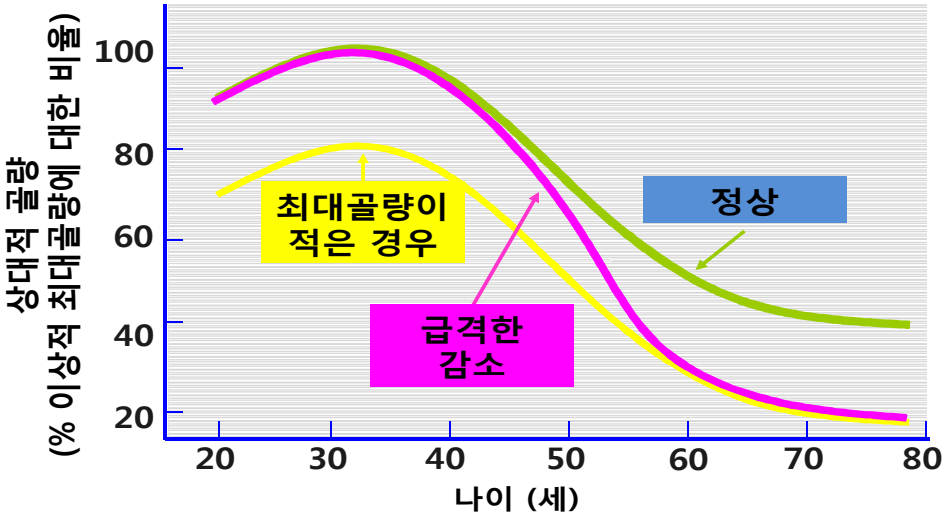
70%

Architecture, Turnover Rate
Damage Accumulation
Degree of Mineralization
Properties of the Collagen/mineral Matrix

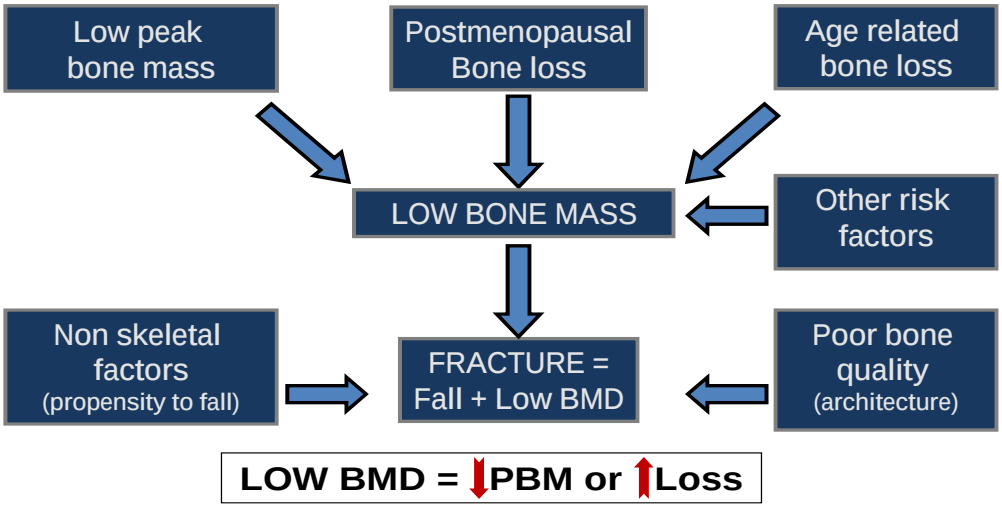
- 50-85% of the variance in peak bone mass is genetically determined

Smith, 1973, J Clin Invest

연령별 골밀도 변화

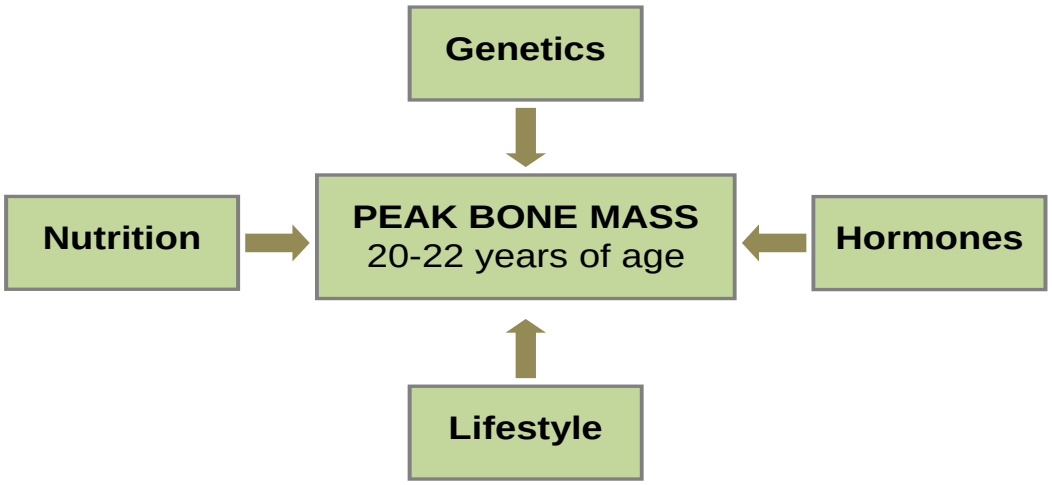


Pathogenesis of osteoporotic fracture



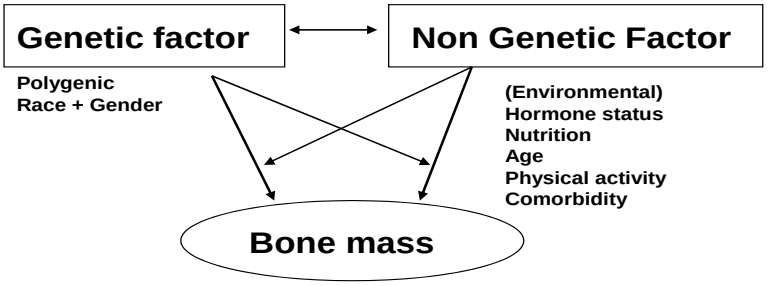
Melton LJ & Riggs BL. Osteoporosis: Etiology, Diagnosis and Management
Raven Press, 1988, pp155-179

Determinants of Peak Bone Mass



Osteoporosis

- Polygenic, multifactorial disease
- Population variance is determined by the interaction of multiple genes with environment



Heritability

By twin studies: Monozygotic vs dizygotic twins

- Assumption that they similarly share common environmental factor
- bone mass
  - spine, hip: 70-85%
  - wrist: 50-60%
- bone size and structure
- bone loss?
- bone turnover
- fracture: 25~35%
- muscle strength, falls
- BMI, age at menarche, age at menopause
- quantitative ultrasound properties of bone
- hip axis length, femoral neck geometry
- serum levels of PTH and 1-25(OH)2VitD

Bone mass, size, structure

- Bone mass; up to 85% genetic contribution to bone mass at any age
- Bone structure at both hip and spine

Phenotype	Sibling pairs (n)	H^2
Lumbar spine BMD	425	0.89
Femoral neck BMD	425	0.77
Pelvic axis length	309	0.83
Femoral neck axis length	309	0.81
Femoral head width	309	0.75
Femoral calcar width	309	0.68
Femoral medulla width	302	0.63
Femoral neck width	309	0.61
Femoral shaft width	302	0.58
Lumbar vertebral middle height	206	0.83
Lumbar vertebral posterior height	206	0.66
Lumbar vertebral anterior height	206	0.68
Lumbar vertebral upper width	206	0.72
Lumbar vertebral lower width	206	0.61

Heritability

Fracture

- Hx of hip Fx in their mother doubled the risk of hip fracture
- Increased risk remained after adjusting for BMD, indicating that factors other than bone mass are involved

Falls

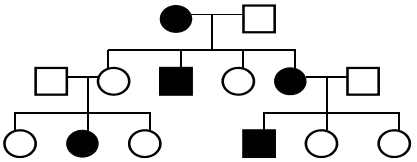
- Highly complex phenotype with multiple environmental risk factors
- Although falls also have a heritable component, the susceptibility genes for fracture resulting from falls are unlikely to relate to the genes underlying bone strength

Quantitative Trait Loci (QTL)

- Chromosomal regions which harbor genes that regulate quantitative phenotype such as bone mass and skeletal geometry

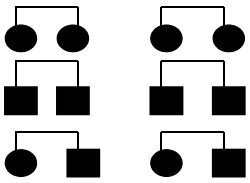
Genetic approaches to complex disease like osteoporosis

Linkage analysis

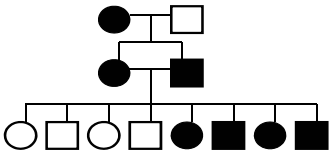


Allele-sharing methods

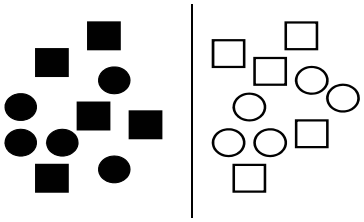
Sib-pair analysis



Experimental crosses in animals

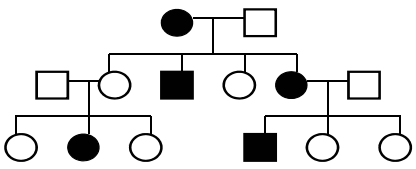


Association studies



Zajickova K, 2003, Endocrine Regulation

Linkage analysis



Linkage analysis

- Searching genome, testing polymorphic markers evenly spaced (5-10cM) on all chromosome
- Allowing susceptibility genes to be identified that are not candidates based on the current understanding of the pathophysiology of osteoporosis
- LOD score: the ratio of the odds that the candidate locus is linked to the trait under study as opposed to being unlinked
  - > +3.0: significant evidence of linkage
  - > +1.9: evidence suggestive of linkage
  - < -2.0: exclude linkage

Linkage of BMD using a genome screen in pairs of sisters

- BMD

Chromosome	Phenotype	Marker	LOD score
1q21-23	Lumbar spine	D1S484	3.11
22q12-13	Lumbar spine	D22S423	2.13

- Bone structure

Chromosome	Phenotype	Marker	LOD score
5q11-12	Femoral neck axis length	D5S647	4.3
4q11-12	Femoral neck axis length	D4S428	3.9
4q12-13	Femoral shaft width	D4S392	3.5
17q21-23	Femoral head width	D17S791	3.6
3q22-24	Pelvic axis length	D3S1569	3.1

Peacock et al., 2002, Endoc Rev

Genome wide linkage analysis

- May identify new regions for study: top down approach
- Very large (30-50cM), containing between 20-70 megabase of DNA with several hundred of genes
- Next step is to "fine map" these regions
- Increasing the marker density to a 2.5-5cM efficiently extracted additional information
- After identifying the candidate genes in a fine mapped region, the next task is to identify polymorphism in these genes

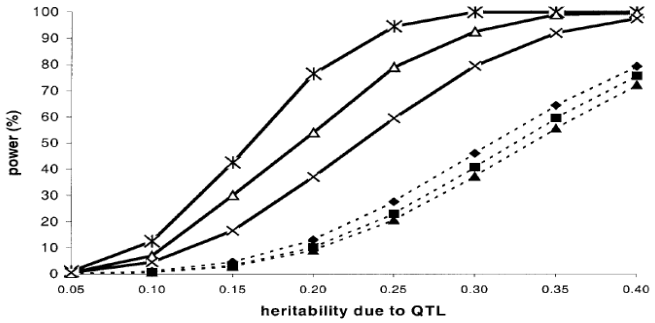
Contradictory results from linkage analysis

- Complexity of genetic inheritance of osteoporosis
- Genes that regulate BMD differ in different populations
- Genes that predispose to osteoporosis have modest effects, which are difficult to detect by conventional linkage analysis

Statistical power of current linkage analysis for osteoporosis

- More than 8000 randomly ascertained sibling pairs are needed in a whole-genome scan to detect a major locus with heritability as large as 30%

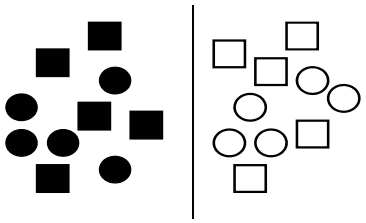
- Comparison of the statistical power of independent sib pairs and pedigrees



N=630 pedigree

N=595 sib pairs

Association study



Candidate gene association study

- Population-based study or case-control study
- Two groups matched so that they differ only in their disease status
- Positive results: likely to be causative for disease, but may be the result of linkage disequilibrium with the candidate gene or possibly with another gene in close proximity

Candidate genes - BMD

Candidate gene	Protein	Chromosome
AHSG	α_2HS-glycoprotein	3q27
VDR	VDR	12q12–q14
ESR1	ER 1 (α)	6q25.1
ESR2	ER 2 (β)	14q23
COL1A1	Collagen, type 1, α 1	17q21.3–q22.1
COL1A2	Collagen, type 1, α 2	7q22.1
IL6	IL-6	7p21
TGFB1	TGFβ	19q13.2
CALCR	Calcitonin receptor	7q21.3
IGFI	IGF-1	12q22–q23
BGLAP	Bone γ-carboxyglutamide protein (osteocalcin)	1q25–q31
MTHFR	Methylenetetrahydrofolate reductase	1p36.3
IL1RN	IL-1β receptor antagonist	2q14.2
TNFRGF5	TNF receptor superfamily/1β	1p36.3–p36.2
CASR	Calcium-sensing receptor	3q21–q24
CYP19	Aromatase (cytochrome P450)	15q21.1
P57, KIP2	Cyclin-dependent kinase inhibitor 1c	11p15.5
HLA DRB1	Major histocompatibility complex, class 11, DR β1	6p21.3
APOE	Apolipoprotein E	19q13.2

- None have been replicated over all populations

Possible mechanisms how genetic polymorphisms may influence a phenotype

- Effect on mRNA stability
- Effect on the rate of gene transcription (amount of mRNA)
- Modification of resulting amino acids sequence
- Functional variation by the polymorphism might be detectable only in specific tissue, such as bone or intestine
- Linkage disequilibrium with another trait-causing mutation in nearby locus

Zajickova K, 2003, Endocrine Regulation

Candidate gene association study in Korean women I

Calcitropic hormones and receptors		
Vitamin D receptor	*Bsm*I, *Apa*I, *Taq*I	Park JH et al., 1998*
	*Fok*I	Lee JY et al., 1999*; Chio YM et al., 2000*
		Kim JG et al., 2001*; Chung DJ et al., 2001
	*Bsm*I	Kwon DJ et al., 2003*; Lim SK et al., 1995*
		Kwon IS et al., 2001
	*Bsm*I, *Taq*I	Kim JG et al., 2002*
	*Apa*I	Kim JG et al., 2003
	poly(A) repeat	Kim JG et al., 2003*
Estrogen receptor	*Pvu*II, *Xba*I	Yoon HK et al., 1997; Han KO et al., 1997*
		Kim JG et al., 2001*
	*Xba*I	Kwon IS et al., 2001*
	*Pvu*II	Kwon DJ et al., 2003; Nam HS et al., 2005*
Calcitonin	(CA) repeat	Kim JG et al., 2003*
Calcitonin receptor	*Alu*I	Kim JG et al., 2005
Parathyroid hormone	*BstB*I	Kim JG et al., 2004
Calcium-sensing receptor	CA repeat	Kim JG et al., 2004

Candidate gene association study in Korean women II

Local regulators		
Transforming growth factor-1	T869C	Kim JG et al., 2001; Kwon DJ et al., 2003
Interleukin-6	CA repeat	Kim JG et al., 2001
	VNTR repeat	Lee HK et al., 1999
Insulin-like growth factor I	CA repeat	Kim JG et al., 2002*
Interleukin-1 receptor antagonist	86-bp repeat	Kim JG et al., 2002*; Han KO et al., 2002*
Osteoprotegrin	A163G, T245G	Oh KW et al., 2005*
	A163G, G1181C	Lee SW et al., 2004*; Kwon DJ et al., 2003*
Interleukin-10	A592C	Park BL et al., 2004*
Bone matrix components		
Collagen type I α1	Sp 1	Han KO et al., 1999*
Miscellaneous		
Peroxisome proliferator-activated receptor-γ	C161T in exon 6	Rhee EJ et al., 2005*

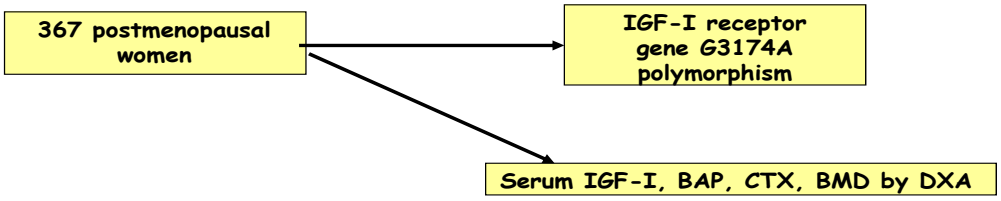
IGF-I receptor gene SNP and bone

Lee DO et al., 2007, J Bone Miner Metab

IGF-I receptor gene G3174A polymorphism

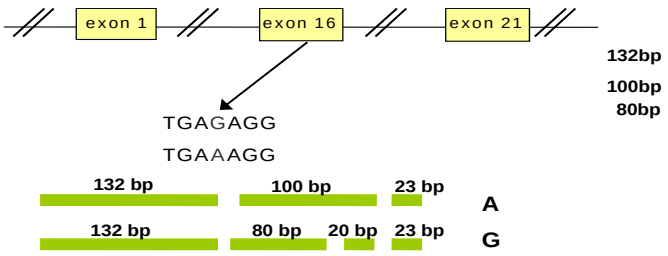
- IGF-I produces its biological effects through IGF-I receptor.
- G(3174)A Polymorphism in exon 16 of IGF-I receptor gene
- To evaluate the relationship among IGF-I receptor gene G3174A polymorphism, serum IGF-I levels and BMD.

Study design

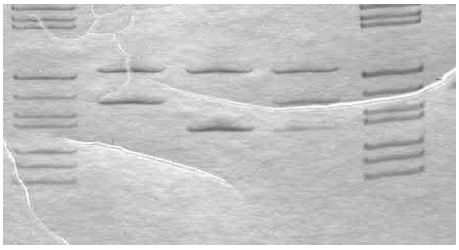


- Inclusion criteria
  - No spontaneous menses for at least one year
  - Serum FSH ≥ 50mIU/ml
- Exclusion criteria
  - Previous bilateral oophorectomy
  - Current hepatic, renal, parathyroid, thyroid disease, DM
  - Medication known to affect bone metabolism

IGF-I receptor gene G3174A polymorphism



IGF-I receptor gene *MnlI* restriction site and RFLP pattern of alleles



Genotyping of the G/A polymorphism of the IGF-I receptor gene by PAGE-silver stain
Lane M : DNA markers

Demographic data, serum IGF-I, bone turnover markers according to IGF-I receptor polymorphism

	IGF-I receptor genotypes		
	AA (n=40)	GA (n=162)	GG (n=165)
Age (yrs)	57.0 ± 1.1	55.2 ± 0.5[a]	57.6 ± 0.5[a]
Menopause duration (yrs)	8.3 ± 1.1	7.8 ± 0.6	8.4 ± 0.5
Body mass index (kg/m^2)	24.3 ± 0.5	24.2 ± 0.2	24.4 ± 0.2
Serum OST (ng/ml)*	14.3 ± 1.4	14.3 ± 0.5	16.6 ± 2.2
Serum BAP (U/L)*	17.3 ± 1.1	18.6 ±0.6	17.9 ±0.7
Serum Crosslaps (pM/L)*	1929.2 ± 470.4	1700.1 ± 158.2	2119.9 ± 444.7

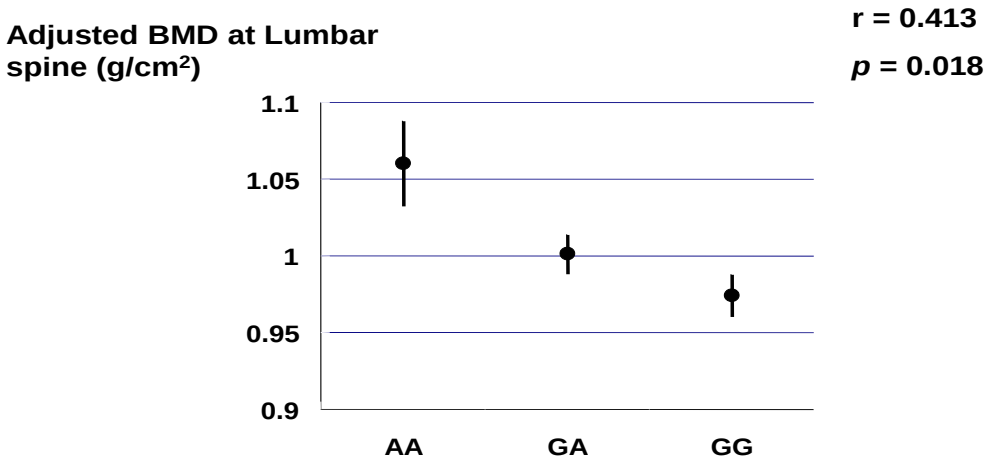
Data are expressed as mean ± SE. OST; osteocalcin, BAP; bone alkaline phosphatase
a; p<0.001 by Tukey's test,
*; Values, adjusted for age, menopause duration and BMI , **; ANCOVA

BMD according to IGF-I receptor genotypes

Bone mineral density*	IGF-I receptor genotypes			*p* value**
	AA (n=40)	GA (n=162)	GG (n=165)	
Lumbar spine (g/cm^2)	1.060 ± 0.028^{a,b}	1.001 ± 0.013^{a}	0.974 ± 0.014^{b}	0.01
Femoral neck (g/cm^2)	0.848 ± 0.023	0.826 ± 0.010	0.803 ± 0.010	0.09
Ward's triangle (g/cm^2)	0.670 ± 0.026	0.664 ± 0.012	0.631 ± 0.011	0.07
Trochanter (g/cm^2)	0.726 ± 0.018	0.728 ± 0.009	0.708 ± 0.009	0.29

Data are expressed as mean ± SE.
a; p<0.01, b; p<0.005 by Tukey's test
*; Values, adjusted for age, menopause duration and BMI, **: ANCOVA

Correlation between adjusted BMD and three genotypes at IGF-I receptor



Conclusion

- The IGF-I receptor gene G3174A SNP are one of genetic factors which **may associated with** BMD in postmenopausal Korean women.

Major problems in association study

- Choice of candidate gene
- Analysis of each candidate in isolation of the others is difficult to interpret statistically
- Simple polymorphisms in introns with doubtful biological effect
- Studies in multiple populations are required
- No chance of finding genes outside those hypothesized
- Spurious associations: racial admixture
- Complex characterization of the boundaries of linkage disequilibrium is essential for the accurate interpretation

Contradictory results from association studies

- Interaction of environmental factors: same allele may function differently in different environment
- Linkage Disequilibrium (LD)
- Limited sample size
- Difference in genotype distribution among different ethnic group
- Interaction with other gene

Vitamin D receptor (VDR)

- Bsm*I*, Apa*I*, Taq*I* : btw exon 8, 9
- Association with BMD, bone turnover markers, Ca absorption, peak bone mass, age-related bone loss
- Dependent on environmental factors (Ca and Vit D intake)
- Various results with haplotype
- Polymorphism in exon 2
  - creates an alternative translational start site, resulting in the production of two isoforms of the VDR protein, which differ in length by three amino acids
  - uncertain significance in function and association

Vitamin D receptor (VDR)

Cdx polymorphism

- Decreased transcriptional activity
- Regulating BMD through intestinal VDR content
- Influenced by estrogen status
- Associated in Japanese women but not in Korean women

Type I Collagen

COLIA1 Sp1 polymorphism

- Affecting binding site for the transcription factor Sp1 in the first intron of COLIA1
- Positive association with bone mass, osteoporotic fracture, osteoporosis, postmenopausal bone loss, femoral neck geometry, response to etidronate
- Clear allele dose-effect at both lumbar spine and femoral neck BMD, and fracture prevalence
- Common in Caucasian, but rare in Asians and Africans

Estrogen Receptor (ER) gene

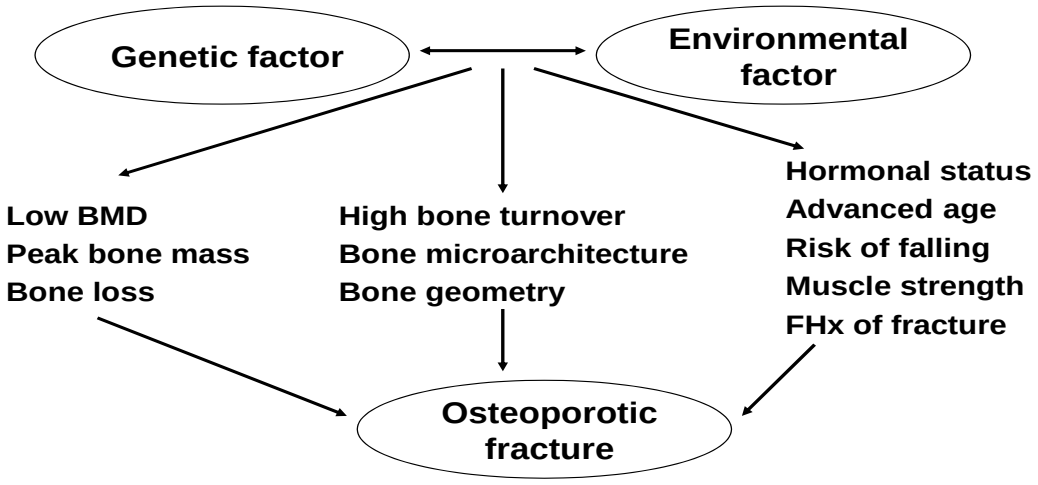
- TA repeat polymorphism in promotor region
- Pvu*II*, Xba*I* polymorphism in first intron
- Strong LD with one another
- Molecular mechanism by which they influence bone mass is unclear

- Greater bone mass, greater bone turnover and bone loss with XX, pp genotype: tends to decrease with age and disappear eventually through the late postmenopausal period

TGFß-1 gene

Polymorphism in exon 1

- Changing protein coding from leucine to proline
- Association with BMD, circulating TGFß-1 level
- Mechanism by which the polymorphism of the gene influence TGFß is unclear



Zajickova K, 2003, Endocrine Regulation

Osteoporosis is highly heritable disease.... And so what?

Osteoporosis is mediated by a large number of genetic variants of modest effect size (<5%)

Clinical Implication

- Genetic markers for fragility fracture – Targeted therapy
- Development of new drug
- Distinguish Tx responders from non-responders
- Identifying Pt at risk of developing unwanted side effects
- Gene therapy?