Calcium and Vitamin D Intake Interactions with Genetic Variants on Bone Phenotype

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Published online: 12 June 2012
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Abstract The notion that environmental factors interact with genetic variants to affect phenotypes associated with complex diseases has arisen since the early days of genetic research. Among the environmental factors, nutrition holds a strong and permanent position, as it is a factor present throughout the life span. Calcium and vitamin D are the most important nutrients with regard to the development and health of the skeleton and have been associated with a variety of bone metabolic diseases (eg, osteoporosis). Multiple interactions between these two nutrients and genetic variants have been identified in the genetic research on bone phenotypes. A summary of these interactions is presented in this review. Furthermore, some ideas for the improvement of the studies in this field are also discussed within the current framework of the genetic research into bone phenotypes.

Keywords Gene–diet interactions · Bone mineral density · Bone mass · Peak bone mass · Bone loss · Calcium · Vitamin D · Intake · Genetic variants · Phenotype · Fractures · Metabolic disease · Fragility fractures

Introduction

The development of most common chronic human diseases is believed to be associated with genetic and environmental

factors as well as the interactions between them. The study of gene–environment interactions has been present since the early days of genetic research [1].

Due to technological development, high-throughput genotyping technologies such as genome-wide association studies (GWAS) and next-generation sequencing allow the assessment of a vast number of genetic variants in relation to different phenotypes [2, 3]. Although a noteworthy number of variants in many genes have been identified using these technologies, a significant amount of heritability of the assessed traits remains unexplained [4, 5•]. Among the reasons for this observation is the existence of gene–environment interactions [6].

The assessments of these interactions were traditionally investigated using candidate gene approaches [1]. The use of the new “hypothesis-free” approaches was expected to increase the current knowledge in this research field as well. However, as the number of assessed variants increases, the assessment of interactions becomes more difficult for many reasons. To ensure increased statistical power in GWAS, different large populations are pooled. Common issues are the lack of availability of common environmental data for all subjects or their assessment with different tools, which make the pooling of data very difficult. Furthermore, the exposure to some factors (eg, nutrition) can be significantly different between different populations, which is another problem that needs to be addressed. Finally, a “hypothesis-free” approach in which both environmental factors and the genetic variants are assessed (>1 million nowadays) in a gene–environment-wide association methodology is almost statistically impossible, and even if the environmental factors are preselected, the sample size for the interactions assessment should be at least four times larger than the sample used for detection of genetic main effects only [2, 5•]. Therefore, most available data on gene–environment interactions still come from candidate gene approach studies.

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Among the different environmental factors known to interact with genetic variants, nutrition is considered very important, as humans depend on food for survival and nutrition is modifiable throughout life [7]. The field of gene–environment interactions is an extremely active research field. However, the variety of factors that take part of the nutritional exposure as well as the diversity of methods available to assess nutritional intake and habits pose problems in these types of studies. In particular, comparisons between different studies and populations should be made with caution [8].

Metabolic bone diseases and especially osteoporosis have an increasing prevalence and represent a major public health problem [9]. These diseases have a multifactorial etiology and a significant and polygenic heritable component [10]. Calcium and vitamin D are the most essential nutrients for bone health. Therefore, they are the nutritional factors most commonly assessed for possible interactions with genes in the study of bone phenotypes.

In the present review, calcium and vitamin D interactions with genetic variants for bone phenotypes are described. Among these phenotypes, bone mineral density (BMD), bone loss, peak bone mass, and fragility fractures have been selected, as they represent the most common traits for bone diseases. As there are no available data from GWAS for the reasons that were previously mentioned, data coming from candidate gene studies are presented.

Calcium

Actions

Calcium is the most abundant inorganic component of bones. The mineralization of bone mass is the most significant action of calcium in bones. It is also an essential nutrient for important physiologic processes, including nerve impulse transmission, muscle contraction, regulation of blood clotting and pressure, enzyme regulation, membrane permeability, and cellular metabolism [11–13]. Milk and dairy products are the basic dietary sources for calcium, along with certain fish (salmon and small fish consumed with bones [eg, sardines]), seafood (clams and oysters), vegetables (turnip and mustard greens, broccoli, cauliflower, and kale), legumes and legume products (tofu), and dried fruits. Nowadays, a variety of enriched foods (fruit juices and bread) are also available [12].

Calcium–Gene Interactions and Bone Mineral Density

BMD is the most commonly assessed bone phenotype. The current diagnosis for osteoporosis is based on BMD values [14]. The majority of calcium–gene interactions for BMD

refer to single nucleotide polymorphisms (SNPs) of the *VDR* gene (vitamin D receptor), which encodes a nuclear receptor of the steroid hormone receptor family. Vitamin D's actions are mediated mostly by its binding with this receptor [15].

In premenopausal Caucasian women, femoral neck BMD was significantly higher in carriers of the minor allele B of the BsmI SNP only in higher calcium intake (>1,036 mg/d), while for the bb genotype, there was no difference in BMD based on calcium intake [16]. In contrast, the bb genotype of the same SNP was associated with higher trochanter BMD compared with the BB genotype only with calcium intake greater than 800 mg/d, while this effect was reversed with intake of less than 500 mg/d in a large population of older adults [17]. However, significant differences exist between these two studies that could explain the differences in the reported results. The mean calcium intake was such that the possible long-term exposure to different levels of calcium could explain the disparity between results. Also, age differences were important, as in premenopausal women, BMD is usually stable and close to peak bone mass, while in older adults, BMD is decreased.

Furthermore, other polymorphisms of the *VDR* have been implicated in interactions with calcium. Among the most recent reports for the Cdx-2 variant, Stathopoulou et al. [18•] demonstrated that Caucasian postmenopausal women with the minor allele A had significantly lower lumbar spine BMD only with lower calcium intake (<680 mg/d). Furthermore, the B allele of the BsmI SNP and the t allele of the TaqI SNP were associated with osteoporosis in the same group. On the contrary, among those with higher calcium intake, none of the SNPs was associated with osteoporosis or BMD [18•]. Also, Fang et al. [19] demonstrated that in a large sample of adults, the A allele of Cdx-2 polymorphism of *VDR* was associated with greater BMD values only in those with low calcium intake (<600 mg/d). However, this finding did not reach statistical significance because as the population had increased calcium intake, only a small number of individuals were categorized in this group; therefore, statistical power was decreased.

Apart from *VDR*, other genes have also been shown to interact with calcium intake for BMD. Concerning the low-density lipoprotein receptor–related protein 5 (*LRP5*) gene, the rs4988321 SNP was associated with a calcium intake interaction in postmenopausal women. The A allele demonstrated significantly lower lumbar spine BMD only in those with lower calcium intake (<680 mg/d), whereas in those with higher intake, there were no differences in BMD between genotypes [20•].

The interleukin-6 (*IL6*) gene has also been implicated in interactions with calcium intake for BMD. The *IL6* -174 G/C SNP was associated with lower hip Ward's area BMD in GG individuals in the group with lower calcium intake (<941 mg/d) [21].

Calcium–Gene Interactions and Bone Density Changes

There have been some studies in which calcium supplementation has been examined. In these studies, the rate of bone density changes has been assessed, as calcium supplementation is usually tested in older adults with increased risk of osteoporosis. Actually, the first report for calcium–*VDR* interaction concerned a sample of Caucasian postmenopausal women in a supplementation study of 500 mg/d [22]. This study demonstrated that among those with increased calcium intake in the intervention group, all genotypes for BsmI polymorphism had decreased rates of bone loss, while in the placebo group, the BB genotype was associated with increased bone loss compared with carriers of the b allele [22]. Therefore, BB genotype could have the better clinical response in calcium supplementation. In another study of calcium supplementation (800 mg/d), Ferrari et al. [23] demonstrated that in older adult Caucasians, only the Bb genotype of the BsmI SNP was associated with differences in BMD changes compared with the other genotypes. However, the sample size of this study was small. Furthermore, in another study, the rate of bone loss in the hip of postmenopausal Caucasian women in 6 years was higher for the carriers of the minor allele t of the TaqI SNP of *VDR* only in those with lower levels of calcium intake (100–456 mg/d) compared with individuals with the common allele T. Also, no difference was found in BMD changes between genotypes with higher calcium intake. In this study, a significant interaction with the collagen 1a1 (*COL1A1*) gene was observed. Carriers of s genotype of the 1 Sp1 (MscI) polymorphism had higher rates of bone loss in the low calcium intake group, while this effect was reversed with higher intakes (705–2,237 mg/d) [24].

A recent study investigated the effect of calcium and vitamin D supplementation in Caucasian postmenopausal women with low bone density and the possible interaction with *VDR* and estrogen receptor 1 gene (*ESR1*). Significant differences were observed between responders and nonresponders concerning the frequency of four polymorphisms (BsmI and Fok I of *VDR* and T/C of codon 10 and C/G of codon 325 of *ESR1*) [25•].

Calcium–Gene Interactions and Peak Bone Mass

Similarly, a 1-year intervention study of foods enriched with calcium in prepubertal girls concerning peak bone mass showed that carriers of the B allele of the BsmI polymorphism of *VDR* were associated with lower BMD at baseline; however, they responded more favorably in the intervention. Also, the BMD of bb subjects was not modified during the intervention [26].

Another SNP of the *VDR* that has been shown to interact with calcium intake is the FokI variant. A trend of calcium

intake–FokI SNP was demonstrated in a study in girls and premenopausal women, while the FF genotype girls had better responses to calcium supplementation with regard to BMD increase [27].

Concerning *IL6* and peak bone mass, in a sample of premenarche Chinese, the G allele of the -634 C/G SNP was associated with lower total body bone mineral content compared with CC individuals only in the group with lower calcium intake (<460 mg/d), but not in those with higher intake [28]. However, the study sample was relatively small.

Calcium–Gene Interactions and Fractures

Fragility fractures are the clinical manifestation of osteoporosis and therefore the most important bone phenotype. The majority of medications for osteoporosis aim to prevent fractures [29, 30]. Although there is a considerable heritable component (25 %–48 %) [31], fractures represent one complicated and multifactorial phenotype. Their identification should be based on clinical data, which is difficult, especially for fractures in the spine, which are often misdiagnosed. Therefore, as genetic studies usually require large populations with complete phenotypic data, few studies are available regarding the genetic background of fractures. Among them, those that have assessed gene–environment interaction are even more scarce. We were able to find only one study that identified a significant interaction. Specifically, Fang et al. [32], in a study of older adult Caucasians, showed that homozygous individuals for haplotype 1 of the D site of the albumin promoter binding protein (*DBP*) gene had a 47 % increased risk of clinical fractures compared only with non-carriers with low calcium intake (<1,090 mg/d).

Vitamin D

Actions

Vitamin D is considered more of a steroid hormone than a vitamin (an essential nutrient). It is basically produced photochemically by the skin and metabolized into active molecules through a well-regulated endocrine system [33, 34]. The maintenance and regulation of calcium homeostasis is the primary biological action of vitamin D; however, a wide range of actions has been described during the past few decades that includes effects on cellular differentiation, immune system, blood pressure regulation, insulin production, and the nervous system [34, 35]. Vitamin D deficiency causes rickets in children and osteomalacia in adults and is associated with decreased bone mass and fractures as well as with type 2 diabetes, cardiovascular disease, specific cancers, and autoimmune diseases [36, 37].

Nutritional sources of vitamin D are restricted. Oily fish, cod liver oil, and liver are among the most significant sources, while smaller concentrations are found in butter and dairy products. However, nowadays, a significant amount of vitamin D dietary intake comes from supplemented foods (juices, bread, dairy products) [36, 38]. Although nutritional intake is important for vitamin D status, it is not usually assessed in genetic studies of bone diseases. Also, as the quantification of dietary intake is difficult due to the limited number of validated assessment methods, most studies that investigate the effect of vitamin D intake include the administration of supplements. Therefore, few data are available on vitamin D–gene interactions for bone phenotypes.

Vitamin D–Gene Interactions and Bone Phenotypes

In a study of vitamin D supplementation for at least 2 years in a small sample of older women, bb genotype of BsmI polymorphism of *VDR* responded less favorably than B allele to treatment. In particular, the difference in change of BMD was significantly higher in B allele carriers between the intervention and placebo groups [39].

The transforming growth factor- β (*TGF- β*) gene was associated with responsiveness to vitamin D supplementation in a study on postmenopausal Japanese. Individuals with the CC genotype for the T29-C polymorphism in exon 1 of *TGF- β* had a significant increase of BMD after 1 year of treatment compared with controls with the same genotype. In contrast, T allele carriers had a similar BMD decrease to that of controls [40]. Also, in the previously mentioned study by Elnenaei et al. [25•], BsmI and Fok I of *VDR* and T/C of codon 10 and C/G of codon 325 of *ESR1* were associated with response to vitamin D and calcium supplementation for 3 months.

Nonsignificant Interactions

Most genetic studies that investigate bone phenotypes do not assess gene–nutrition interactions. However, some studies did not identify interactions between calcium intake and SNPs, although these were assessed. These reports refer to the *VDR* gene, as it is the most commonly studied. In particular, Macdonald et al. [41] used a large sample of Caucasian postmenopausal women to identify possible effects of *VDR* polymorphisms and gene–calcium interactions. Associations of the G allele of Cdx-2 and t allele of TaqI polymorphism with decreased femoral neck BMD in the low calcium intake group (mean intake, 768/mg/d) were identified, which, however, were not statistically significant when adjustment for body weight was performed. Furthermore, in the same calcium intake group, the B allele had decreased spine BMD and greater bone loss compared with

the b allele of BsmI SNP; this result also lost its significance following adjustment for osteoarthritis [41]. Furthermore, in a Chinese population, FokI polymorphism of the *VDR* gene was tested for possible associations with BMD and vertebral fractures. Although a marginal effect of the SNP was revealed only in an older adult female sample, no significant interactions were found [42].

The discrepancy between studies may be due to the different levels of calcium intake being examined in each population, as the levels of typical intakes in each population can modify the genetic effect [17].

Conclusions

The impact of nutrition in bone health is well-described, and calcium and vitamin D play a pivotal role, as higher intake of both nutrients is associated with more favorable values of bone traits [43]. Based on the results of most studies, it seems that the gene–calcium and vitamin D interactions can have an important role, especially in those with low intakes. The majority of studies indicate that differences between genotypes are observed in individuals with calcium intakes lower than 600 mg/d, while among those with higher intakes, there are no significant differences in bone phenotypes. This could also be the reason why in populations with generally increased calcium intake, no genetic effect can be identified, as it is probably masked by the calcium effect. Furthermore, the complex effect of calcium intake on genotypes may depend on the level of calcium intake being examined. For example, in the study by Ferrari et al. [23], the Bb genotype of BsmI polymorphism of the *VDR* gene was affected by calcium intake, while in the study by Krall et al. [22], the BB genotype's effect was modified by calcium intake. In the first study, calcium intake was 1,226 to 1,235 mg/d, whereas in the second, it was 274 to 530 mg/d. Therefore, in each population, these effects should be assessed based on the level of exposure to the environmental factor. Finally, in most cases, the risk allele is the one that better responds to supplementation treatments. These observations lead to the conclusion that adequate calcium and vitamin D intake (at least at the recommended levels [37, 44]) can reduce the genetic risk of certain SNPs and improve bone health regardless of the effect of certain genes. However, more studies and experimental validations are needed to establish these effects. Although the study of genetics of chronic diseases makes significant progress, the assessment of gene–nutrition interactions is limited. Large populations are needed to fully explore these interactions. Large consortia can usually yield a satisfactory sample size. Nevertheless, careful and homogeneous collection of nutritional data is crucial. Furthermore, especially in the case of calcium and vitamin D, the stratification of the sample should be well-

designed to ensure the assessment of interactions in a wide range of intakes that reflect the different levels of intake within specific populations. In this way, it may be possible to identify true gene–calcium and vitamin D interactions and to better understand the factors that underlie bone phenotypes, as well as to possibly produce individualized markers and strategies for disease prevention and treatment.

Disclosure No potential conflicts of interest relevant to this article were reported.

References

- Papers of particular interest, published recently, have been highlighted as:
- Of importance,
 - Of major importance
- Hunter DJ. Gene-environment interactions in human diseases. *Nat Rev Genet*. 2005;6:287–98.
 - Manolio TA. Genomewide association studies and assessment of the risk of disease. *N Engl J Med*. 2010;363:166–76.
 - Zeggini E. Next-generation association studies for complex traits. *Nat Genet*. 2011;43:287–8.
 - Ndiaye NC, Azimi Nehzad M, El Shamieh S, et al. Cardiovascular diseases and genome-wide association studies. *Clin Chim Acta*. 2011;412:1697–701.
 - Thomas D. Gene–environment-wide association studies: emerging approaches. *Nat Rev Genet*. 2010;11:259–72. *This is an excellent review on methodologic issues concerning gene–environment interactions, especially in GWAS.*
 - Zuk O, Hechter E, Sunyaev SR, Lander ES. The mystery of missing heritability: Genetic interactions create phantom heritability. *Proc Natl Acad Sci U S A*. 2012;109:1193–8.
 - Mutch DM, Wahli W, Williamson G. Nutrigenomics and nutrigenetics: the emerging faces of nutrition. *FASEB J*. 2005;19:1602–16.
 - Jenab M, Slimani N, Bictash M, et al. Biomarkers in nutritional epidemiology: applications, needs and new horizons. *Hum Genet*. 2009;125:507–25.
 - Bonura F. Prevention, screening, and management of osteoporosis: an overview of the current strategies. *Postgrad Med*. 2009;121:5–17.
 - Karasik D, Kiel DP. Evidence for pleiotropic factors in genetics of the musculoskeletal system. *Bone*. 2010;46:1226–37.
 - Chung M, Balk EM, Brendel M, et al. Vitamin D and calcium: a systematic review of health outcomes. *Evid Rep Technol Assess (Full Rep)* 2009:1–420.
 - Gropper SS SJ, Groff JC. Advanced nutrition and human metabolism. Edited by Gropper SS SJ, Groff JC. Wadsworth: Cengage Learning; 2008.
 - Dennehy C, Tsourounis C. A review of select vitamins and minerals used by postmenopausal women. *Maturitas*. 2010;66:370–80.
 - World Health Organization: Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. *World Health Organ Tech Rep Ser* 1994, 843:1–129.
 - Norman AW. Minireview: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology*. 2006;147:5542–8.
 - Salamone LM, Glynn NW, Black DM, et al. Determinants of premenopausal bone mineral density: the interplay of genetic and lifestyle factors. *J Bone Miner Res*. 1996;11:1557–65.
 - Kiel DP, Myers RH, Cupples LA, et al. The BsmI vitamin D receptor restriction fragment length polymorphism (bb) influences the effect of calcium intake on bone mineral density. *J Bone Miner Res*. 1997;12:1049–57.
 - Stathopoulou MG, Dedoussis GV, Trovas G, et al. The role of vitamin D receptor gene polymorphisms in the bone mineral density of Greek postmenopausal women with low calcium intake. *J Nutr Biochem*. 2011;22:752–7. *This recent publication assessed gene–calcium interactions of VDR gene polymorphisms.*
 - Fang Y, van Meurs JB, Bergink AP, et al. Cdx-2 polymorphism in the promoter region of the human vitamin D receptor gene determines susceptibility to fracture in the elderly. *J Bone Miner Res*. 2003;18:1632–41.
 - Stathopoulou MG, Dedoussis GV, Trovas G, et al. Low-density lipoprotein receptor-related protein 5 polymorphisms are associated with bone mineral density in Greek postmenopausal women: an interaction with calcium intake. *J Am Diet Assoc*. 2010;110:1078–83. *This recent research identified for the first time an SNP–calcium interaction of a relatively new osteoporosis candidate gene, LRP5.*
 - Ferrari SL, Karasik D, Liu J, et al. Interactions of interleukin-6 promoter polymorphisms with dietary and lifestyle factors and their association with bone mass in men and women from the Framingham Osteoporosis Study. *J Bone Miner Res*. 2004;19:552–9.
 - Krall EA, Parry P, Lichter JB, Dawson-Hughes. Vitamin D receptor alleles and rates of bone loss: influences of years since menopause and calcium intake. *J Bone Miner Res*. 1995;10:978–84.
 - Ferrari S, Rizzoli R, Chevalley T, et al. Vitamin-D-receptor-gene polymorphisms and change in lumbar-spine bone mineral density. *Lancet*. 1995;345:423–4.
 - Brown MA, Haughton MA, Grant SF, et al. Genetic control of bone density and turnover: role of the collagen 1 α 1, estrogen receptor, and vitamin D receptor genes. *J Bone Miner Res*. 2001;16:758–64.
 - Elnenaei MO, Chandra R, Mangion T, Moniz C. Genomic and metabolomic patterns segregate with responses to calcium and vitamin D supplementation. *Br J Nutr*. 2011;105:71–9. *This recent publication searched for gene–calcium and vitamin D interactions and differences in response to supplementation. The methodology used for the assessment of response to treatment based on genotype is very interesting.*
 - Ferrari SL, Rizzoli R, Slosman DO, Bonjour JP. Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphisms? *J Bone Miner Res*. 1998;13:363–70.
 - Ferrari S, Rizzoli R, Manen D, et al. Vitamin D receptor gene start codon polymorphisms (FokI) and bone mineral density: interaction with age, dietary calcium, and 3'-end region polymorphisms. *J Bone Miner Res*. 1998;13:925–30.
 - Li X, He GP, Zhang B, et al. Interactions of interleukin-6 gene polymorphisms with calcium intake and physical activity on bone mass in pre-menarche Chinese girls. *Osteoporos Int*. 2008;19:1629–37.
 - Johnell O, Kanis J. Epidemiology of osteoporotic fractures. *Osteoporos Int*. 2005;16 Suppl 2:S3–7.
 - Kanis JA, Oden A, McCloskey EV, et al. A systematic review of hip fracture incidence and probability of fracture worldwide. *Osteoporos Int* 2012, In press.
 - Ferrari S. Human genetics of osteoporosis. *Best Pract Res Clin Endocrinol Metab*. 2008;22:723–35.

32. Fang Y, van Meurs JB, Arp P, et al. Vitamin D binding protein genotype and osteoporosis. *Calcif Tissue Int*. 2009;85:85–93.
33. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol*. 2005;289:F8–F28.
34. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr*. 2008;88:491S–9S.
35. Lin R, White JH. The pleiotropic actions of vitamin D. *Bioessays*. 2004;26:21–8.
36. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr*. 2008;87:1080S–6S.
37. Dawson-Hughes B, Mithal A, Bonjour JP, et al. IOF position statement: vitamin D recommendations for older adults. *Osteoporos Int*. 2010;21:1151–4.
38. Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab*. 2011;96:53–8.
39. Graafmans WC, Lips P, Ooms ME, et al. The effect of vitamin D supplementation on the bone mineral density of the femoral neck is associated with vitamin D receptor genotype. *J Bone Miner Res*. 1997;12:1241–5.
40. Yamada Y, Harada A, Hosoi T, et al. Association of transforming growth factor beta1 genotype with therapeutic response to active vitamin D for postmenopausal osteoporosis. *J Bone Miner Res*. 2000;15:415–20.
41. Macdonald HM, McGuigan FE, Stewart A, et al. Large-scale population-based study shows no evidence of association between common polymorphism of the VDR gene and BMD in British women. *J Bone Miner Res*. 2006;21:151–62.
42. Lau EM, Lam V, Li M, et al. Vitamin D receptor start codon polymorphism (Fok I) and bone mineral density in Chinese men and women. *Osteoporos Int*. 2002;13:218–21.
43. National Osteoporosis Foundation: Clinician's Guide to Prevention and Treatment of Osteoporosis. Edited by National Osteoporosis Foundation. Washington, DC: National Osteoporosis Foundation; 2010.
44. NIH State-of-the-Science Conference Statement on Multivitamin/Mineral Supplements and Chronic Disease Prevention. NIH Consens State Sci Statements 2006, 23:1-30.