The role of vitamin D receptor gene polymorphisms in bone biology

André G. Uitterlinden *, Yue Fang, Arjan P. Bergink, Joyce B.J. van Meurs, Hans P.T.M. van Leeuwen, Huibert A.P. Pols

Genomic Laboratory Room Ee575, Department of Internal Medicine, Erasmus University Medical School, Dr. Molewaterplein 50, PO Box 1738, GD-3015 Rotterdam, The Netherlands

Abstract

The role of vitamin D and its receptor (VDR) in skeletal metabolism is well known but the vitamin D endocrine system seems to play an important role in other metabolic pathways as well, such as those involved in osteoarthritis, the immune response and cancer. One approach to understand the vitamin D endocrine system is to study the influence of variations in the DNA sequence of important proteins of this system. For example, deleterious mutations in the VDR gene cause 1,25-dihydroxyvitamin D-resistant rickets, a rare monogenetic disease. More subtle sequence variations (polymorphisms) in the VDR gene occur much more frequently but their effects are poorly understood. Their influence on the vitamin D endocrine system is currently under scrutiny in relation to a number of so-called complex diseases and traits such as osteoporosis. The interpretation of polymorphic variations in the VDR gene is severely hindered by the fact that several of the polymorphisms used have unknown effects. However, current data indicate that dozens of additional polymorphic variations exist in the VDR gene that could each have different types of consequences. Therefore, efforts are focussed on finding novel sequence variations and to study their interaction in molecular- and cell-biological experiments as well as in genomic epidemiological studies. The ultimate goal of this approach is to identify the combinations of functional sequence variants that modulate the vitamin D endocrine system and confer risk of disease.
© 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Genetic; Steroid: receptor; Haplotype; Osteoporosis

1. Introduction

The steroid hormone vitamin D, its receptor (VDR) and the metabolizing enzymes involved in the formation of the biologically active form of the hormone, together are major players in the vitamin D endocrine system. This system plays an important role in skeletal metabolism but also seems to play an important role in other metabolic pathways, such as those involved in osteoarthritis, the immune response and cancer (Haussler et al., 1998).

One approach to understand the vitamin D endocrine system is to study the influence of variations in the DNA sequence of important proteins of this system. For example, deleterious mutations in the VDR gene cause 1,25-diydroxyvitamin D-resistant rickets, a rare monogenetic disease. More subtle sequence variations (poly-

morphisms) in the VDR gene occur much more frequently but their effects are poorly understood. Their influence on the vitamin D endocrine system is currently under scrutiny in relation to a number of so-called complex diseases and traits such as osteoporosis. This so-called candidate gene approach in the genetic dissection of the complex trait osteoporosis is currently gaining increased importance over genome search approaches using linkage analysis (Lander and Schork, 1994; Risch and Merikangas, 1996).

The interpretation of polymorphic variations in the VDR gene is severely hindered by the fact that many of the polymorphisms currently used are anonymous. The analysis of the genomic organization has shown that the VDR gene is quite large (at least 80 kb; see Fig. 1) and has an extensive promotor region capable of generating multiple tissue-specific transcripts (Miyamoto et al., 1997; Crofts et al., 1998). In view of the genome-wide observed frequency of single nucleotide polymorphisms (Nickerson et al., 1998; Rieder et al., 1999; Cambien et al., 1999; Cargill et al., 1999; Wang et al., 1998), one can

* Corresponding author. Tel.: +31-10-408-7573; fax: +31-10-408-9461

E-mail address: uitterlinden@endov.fgg.eur.nl (A.G. Uitterlinden).
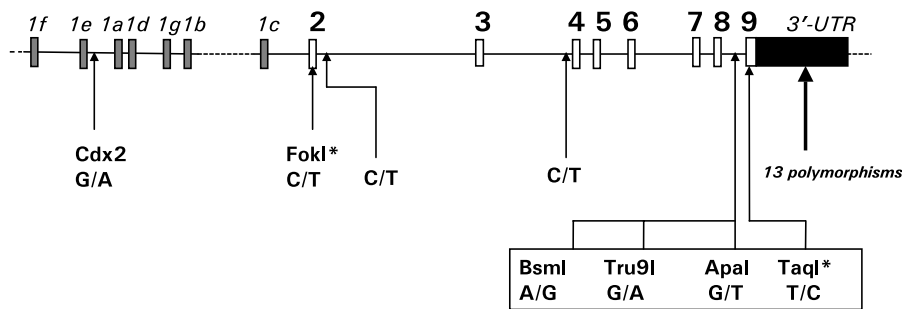
VDR GENE POLYMORPHISMS

Fig. 1. Position of polymorphisms in the vitamin D receptor gene. * indicates that these polymorphisms are in the coding sequence. For the *Fok*I-RFLP, see text. The *Taq*I-RFLP is a silent (synonomous) base change not changing the amino acid encoded by this codon.

expect over 100 polymorphisms present in the VDR region alone, including in areas that are functionally relevant such as the promoter region. Studies on different polymorphisms in the VDR gene are briefly discussed below.

1.1. Association studies using the BsmI-, ApaI-, and TaqI-RFLPs

The candidate gene that actually initiated the 'molecular genetics of osteoporosis' is the vitamin D receptor (VDR) gene. Three adjacent RFLPs for *Bsm*I, *Apa*I and *Taq*I, respectively, in intron 8/exon 9 at the 3′-end of the gene, are most frequently studied. Morrison et al. (1992, 1994) reported that the *Bsm*I-RFLP in the last intron of the VDR gene was related to serum osteocalcin concentration and subsequently to BMD in a twin study and in postmenopausal women. Although the initial observations on the twin study have been withdrawn (Morrison et al., 1997), in the following years dozens of papers were published analysing the same RFLP in relation to BMD. Some of these confirmed the observation, while others could not find an association or found another allele associated. In the largest study published so far and which analysed 1782 Dutch elderly men and women, no effect of single RFLPs on BMD was observed while a small effect was detected employing haplotypes constructed of the three adjacent 3′-RFLPs (Uitterlinden et al., 1996). In line with this, a meta-analysis of 29 studies (excluding the Dutch cohort) on the relationship of VDR genotype with BMD, Cooper and Umbach (1996) concluded that VDR genotype is associated with BMD in elderly subjects but with only 1–2% difference between extreme genotypes. In addition, Gong et al. (1999) analysed 75 articles and abstracts on VDR genotype and BMD, and concluded that BMD is associated with VDR genotype, especially in females before the menopause, and that genetic heterogeneity and non-genetic factors play a role in finding the associations. This notion is supported by other studies that found evidence to suggest an influence

on peak BMD in younger subjects (aged 7–29 years) (Sainz et al., 1997; Rubin et al., 1999), found a relation of VDR genotype with bone loss (Keen et al., 1995; Gough et al., 1998), and observed influence of dietary calcium intake on the strength of the associations with bone density (Krall et al., 1995; Kiel et al., 1997). Interestingly, a study of American Caucasian women (Feskanich et al., 1998), of Danish Caucasian women (Langdahl et al., 2000), and of Dutch Caucasian women (Uitterlinden et al., 2001) suggested VDR genotype to be associated with increased fracture risk. This effect was mostly independent of (the small) genotype-related differences in BMD (Uitterlinden et al., 2001). However, because other groups have not seen a relationship between fracture risk and VDR genotype (Houston et al., 1996; Berg et al., 1996), this relationship remains uncertain and needs further scrutiny.

Of particular interest in this respect is the fact that not always the same risk allele is being found associated with bone parameters (but also with other endpoints; see below) preventing the straightforward interpretation of these associations. While the initial studies by Morrison et al. (1992, 1994, 1997) suggested the "B" allele of the *Bsm*I-RFLP site to be the risk allele associated with low BMD, other studies either could confirm this, did not find any effect, or found the (opposite) "b" allele to be the risk allele associated with low BMD. Such conflicting findings—which are not exclusive for the field of genetic association analysis of osteoporosis—could have several reasons. The most likely explanations are that given the small effect, e.g., on BMD, very often the statistical power is much too low and no conclusions on the presence or absence of an effect can in fact be drawn. More important, however, is the fact that the 3′-*Bsm*–*Apa*–*Taq*-RFLPs are not functional, as far as we know. The *Bsm*I- and *Apa*I-RFLPs are located in intron 8 and not affecting any splicing site and/or transcription factor binding site. The *Taq*I-RFLP is a "synonomous" polymorphisms meaning that it is present in the coding sequence (i.e., exon 9) but that it is not changing the amino acid sequence of the encoded protein.

When polymorphisms are (supposedly) non-functional, they are still useful in association studies because they can be used as markers. When association is found with a marker allele, the association is then believed to be caused by a truly functional allele which is linked to the marker allele and which is located elsewhere but usually nearby in the same gene. Such linkage between marker allele and functional allele depends on the extent of linkage disequilibrium across that area of the chromosome. Consequently, differences in LD between the marker allele and the truly functional allele can lead to altered associations. Finally, interactions between different genes and/or environmental factors play a role in the action of this important steroid hormone receptor transcription factor. For example, dietary Ca-intake is known to differ substantially between countries while circulating serum vitamin D levels, which are determined by several metabolizing enzymes, also differ between populations. Consequently, such gene–gene and gene–environment interactions can of course differ between different populations. In addition, pleiotropic effects of this gene can play a role in influencing an association, but these will be discussed below.

1.2. Other VDR polymorphisms

The alleles of the BsmI, ApaI, and TaqI polymorphisms in intron 8 and exon 9 are closely linked and haplotypes can be constructed over this 2.2 kb region (Morrison et al., 1992; Uitterlinden et al., 1996). The LD of these RFLPs extends into the 3′-untranslated region (UTR) which is a 3.2 kb sequence immediately adjacent to exon 9 (Morrison et al., 1992; Ingles et al., 1997; Durrin et al., 1999). More than 10 different sequence variations in the 3′-UTR have been described including a poly(A) repeat polymorphism. Analysis of the LD over this 5.5 kb region at the 3′-UTR of the VDR gene in different ethnic population groups indicated that the LD differed among populations (Ingles et al., 1997). A single RFLP, such as the BsmI-RFLP which is the most frequently used in association studies of the VDR gene, is therefore not a good marker for the LD with other sequence variations and, thus, the use of the BsmI-RFLP might contribute to heterogeneity among association studies.

This notion is strongly supported by the recent comprehensive sequence analyses of other genes such as the LPL gene (Nickerson et al., 1998; Rieder et al., 1999) and the description of haplotype structures in the genes (Claiborne Stephens et al., 2001; Daly et al., 2001). These studies showed that there are islands of LD across a gene in which blocks of dozens of SNPs are linked together and form haplotype alleles. A practical advantage is that only a few SNPs have to be genotyped to identify the haplotype allele. A disadvantage is that when association is observed with such a haplotype allele, functional studies have to be performed to identify the "causative" allele.

In our previous studies (Uitterlinden et al., 1996), we have identified at least three major haplotype alleles to exist across the 3′-UTR region. Functional studies are currently ongoing to identify what the causative polymorphisms in this region are. This will be imperative to understand the associations found using VDR polymorphisms derived from this region of the gene. However, when we evaluate a particular candidate gene of interest, such as the VDR gene, it is important to take into account all polymorphisms which could influence expression and activity of the mRNA and protein. This is illustrated in Fig. 2 for a hypothetical gene carrying functional SNPs in the promoter, coding, and 3′-UTR region. Because the promoter and 3′-UTR both influence mRNA levels (production and stability, respectively), their interaction determines net availability of mRNA for translation into protein. In Fig. 2, it is shown that only particular combinations of risk alleles on a haplotype across the complete gene result in high levels of mRNA encoding a defective protein. Thus, although subjects might have identical genotypes for a number of SNPs across a gene, different bio-response effects might be observed because the individuals differ with respect to the haplotype.

More than 25 different polymorphisms are currently known to be present at the VDR locus (see Fig. 1), so far mostly near the 3′-end of the gene. However, also towards the 5′-end of the gene in and near the promotor region other sequence variations have been reported. For example, a substitution (T to C) at exon 2 eliminates the first ATG translation inition site and allows a second one 9 bp downstream to be used. Thus, two variant forms of the VDR protein can be translated which differ by three amino acids resulting in proteins of 427 (M1) and 424 (M4) amino acids. The existence of these two different forms of the VDR protein has been demonstrated while the shorter form was found to give greater transcriptional activation (Arai et al., 1997; Jurutka et al., 2000). The sequence change can be detected as a FokI-RFLP and the "f" allele (corresponding to M1, the longer protein) has been found associated with low BMD in several study populations (Arai et al., 1997; Gross et al., 1996; Gennari et al., 1999) but this finding is not consistent (Eccleshall et al., 1998; Ferrari et al., 1998). The RFLP seems not to be in linkage disequilibrium with the 3′-polymorphisms. It is therefore unlikely to explain the association results of the BsmI, ApaI, and TaqI polymorphisms and, in view of the considerable distance between the two sites (± 40 kb) and the different nature of the polymorphisms, should be treated as a different marker. This also holds true for the recently described G to A sequence variation in the Cdx-2 binding element just upstream of exon 1A (Yamamoto et al., 1999; Arai et al., 1999). Arai and
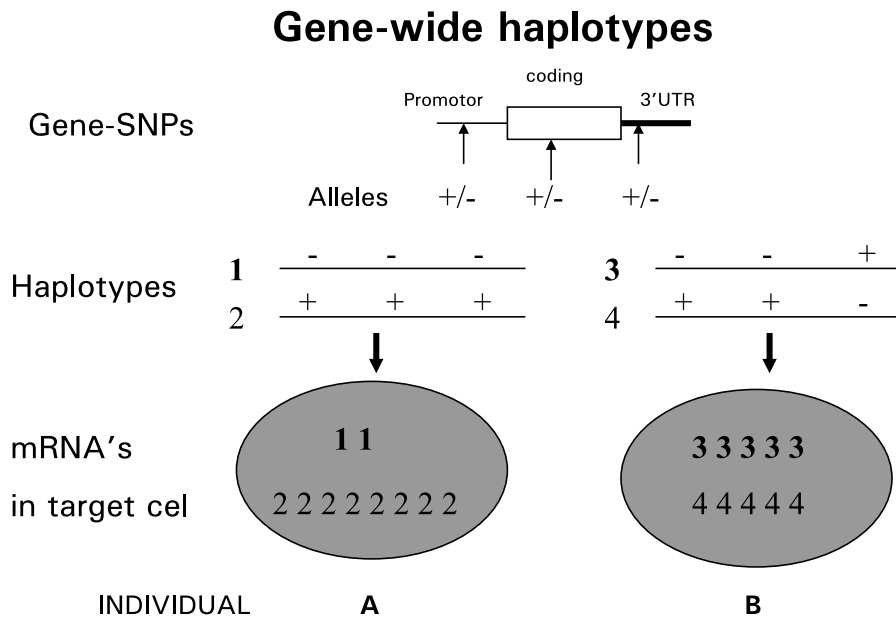
Gene-wide haplotypes

Fig. 2. The importance of gene-wide haplotypes. Three adjacent SNPs in different parts of the gene are shown for two individuals with identical genotypes (they are both heterozygous for all three SNPs) but with different haplotypes (1, 2 for subject A and 3, 4 for subject B). + indicates ab allele of the polymorphisms with a positive effect (e.g., high production of mRNA for promoter SNPs and, e.g., high affinity of the encoded protein for a ligand) while − indicates a negative effect (e.g., low production of mRNA and low affinity of the protein for the ligand). The promoter area regulates production of mRNA while the 3′-UTR is involved in stability/degradation of mRNA and their interaction regulates the net availability of the mRNA for translation into the protein. The result of the particular haplotype combinations is that individual A (− − −/+ + +) has less of the risk protein than individual B (− − +/+ + −) in the target cell. This could not have been predicted by analysing single SNPs and/or only looking at genotypes of individual SNPs but is only evident upon analysis of the haplotypes.

colleagues reported the G allele to have a decreased transactivation capacity and to be associated with 10% decreased lumbar spine BMD in 123 Japanese women.

1.3. Pleiotropic effects

The vitamin D endocrine system has been shown to be involved in a number of endocrine pathways related to calcium metabolism, immune-modulation, regulation of cell growth and differentiation (of keratinocytes, osteoblasts, cancer cells, T-cells), etc. (Haussler et al., 1998). Thus, for a pleiotropic "master" gene such as the VDR, one can expect to find associations of this gene with multiple traits and disease phenotypes. Indeed, the VDR gene has been found associated with a number of different phenotypes of which, especially, the associations with osteoarthritis, hyperparathyroidism, cancer, and infection-susceptibility, so far are supported by several independent and large studies reporting similar associations. However, also here different alleles are sometimes reported to be the risk allele and, thus, the same considerations as described above should be taken into account. In addition, the potential confounding effects which arise from this pleiotropy can influence the associations observed. For example, VDR gene variants can influence calcium metabolism through differential absorption in the intestine and, at the same time, influence bone turnover, while also the occurrence of

osteophytosis (as a part of osteoarthritis) can be influenced, together resulting in a net effect on BMD measured at a certain site, at a certain age and in a subject with a certain diet.

1.4. Functional studies

The interpretation of the VDR association studies is severely hindered by the fact that most of the polymorphisms used are anonymous, i.e., have an unknown functional effect. The likely explanation for any observed association is then to assume the presence of a truly functional sequence variation elsewhere in the gene which is—to a certain extent—in linkage with an allele of the anonymous polymorphism used. As can be understood from the complex organization of the VDR gene (see Fig. 1), the identification of these functional polymorphisms in the VDR gene is a challenging enterprise. While these results are still eagerly awaited, several investigators have nevertheless analysed multiple bio-response parameters using the anonymous polymorphisms, including the *Fok* I, *Bsm* I, and *Bsm* − *Apa* − *Taq* haplotypes, and a polyA tract in the 3′-UTR. These studies include in vitro cell-and molecular-biological studies, and in vivo measurements of biochemical markers and response to treatments with vitamin D, calcium, and even HRT or bisphosphonates. In Fig. 3, the different levels of biological organization

VDR BIORESPONSE PARAMETERS
Potential Effects of Functional DNA Polymorphisms

ORGANIZATIONAL LEVEL   PARAMETERS WHICH VDR POLYMORPHISMS MIGHT INFLUENCE

-mRNA   **-level, stability, splicing/isoforms,translation**

-Protein   **-level, stability, isoforms (M1, M4, A, B, etc.),**

protein-protein interaction (TFIIB, RXR)

-transcriptional activity (binding to VDREs)

-Cells   **-Cell growth inhibition**

-Humans   **-Serum parameters: osteocalcin**

-Intervention with vitamin D$_3$

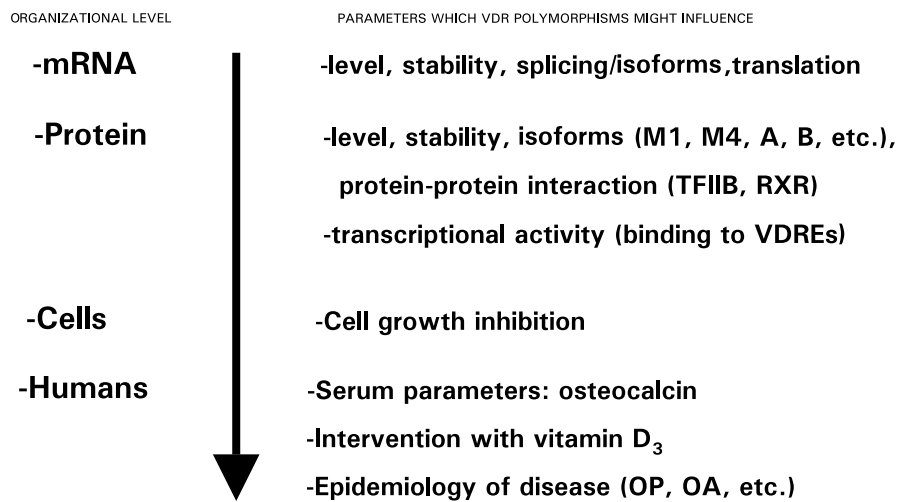**-Epidemiology of disease (OP, OA, etc.)**

Fig. 3. Functional effects of polymorphisms in the VDR gene can occur at different organizational levels of biological organization. M1, M4, A and B identify different protein isoforms (see text and Sunn et al., 2001). TFIIB is a transcription factor with which VDR can interact through the N-terminal domain, RXR is the retinoic acid X receptor with which the VDR forms heterodimers. VDREs are vitamin D receptor binding elements found in promoter areas of some genes influenced by vitamin D (OP = osteoporosis, OA = osteoarthritis).

are indicated at which functional effects of VDR alleles could be observed and measured. Ideally, an allele of a functional polymorphism will show consistent effects at each of these levels of organization.

In view of what has been discussed above, it is not very surprising that these "functional" studies have not shown one allele being consistently associated with all the different parameters. Major caveats of these studies are (a) the use of the anonymous rather than functional polymorphisms to group subjects and cells by genotype and (b) the use of different types of bioresponses and different cell types and cell culture conditions in which the vitamin D response might not be evident under the conditions of the experiment. Therefore, the identification of a functional polymorphism and the use of different well-defined cell types will help in clarifying the molecular mechanisms underlying the associations observed.

Part of the initial efforts to identify functional sequence variations have been focussed on the 3′-regulatory region because this is close to the anonymous markers used so far in associations studies (see Fig. 1). While the *Bsm*I, *Apa*I and *Taq*I-RFLPs are located near the 3′-end of the gene, the LD extends into the 3′-regulatory region containing the UTR. Morrison et al. (1994) already showed the 3′-UTR to contain sequence variations that were suggested to explain the observed associations and provided evidence of differential luciferase activity for the two UTRs that are linked the two most frequent haplotypes "baT" (haplotype 1 according to Uitterlinden et al. (1996)) and "BAt" (haplotype 2).

Durrin et al. (1999) have shown certain parts of the UTR, the so-called destabilizing elements, to be involved in determining the stability of the VDR-mRNA. Yet, when eight individuals, selected by their genotype of the poly(A)-stretch in the 3′-UTR, were sequenced no polymorphisms were found in the destabilizing elements of the 3′-UTR. Furthermore, the UTRs linked to the two most common variants (the "baT" and "Bat" haplotype) were not found to differ with respect to mRNA stability (Durrin et al., 1999). However, only few individuals were sequenced and so variations could have still been missed while also heterologous constructs (human VDR-UTR sequences coupled to a rabbit -globulin gene) and cell types (mouse NIH3T3 cells) were used to test for functionality. Especially, since it is known that 3′-UTRs display cell-type-specific effects on mRNA stability this could be important in demonstrating functionality of sequence variations in the UTR.

Recently, Whitfield et al. (2001) demonstrated functional significance of the translation initiation codon polymorphism (detected as *Fok*I-RFLP) and the poly(A) stretch in the 3′-UTR. In a series of 20 fibroblast cell lines of different VDR genotype, the relative transcription efficiency was measured of the endogenous VDR-protein which was differing by the genotype at the *Fok*I-RFLP (F and f alleles) and the poly(A) stretch with long (L) and short (S) alleles which is acting as a transcription factor for a 1,25-dihydroxyvitamin D$_3$-responsive reporter gene. This study provided evidence for the so-called high (of the "FL" genotype) and low (of the "fS" genotype) VDR activity.

The study also illustrated the importance of analysing multiple polymorphisms in a single gene in relation to each other, as is illustrated in Fig. 2.

2. Conclusions

It is likely that still more polymorphisms, including functional ones, will be discovered in the complex promoter region of the VDR gene and larger population studies will be necessary to document the LD over the region and to evaluate the associations with relevant endpoints such as BMD and fracture risk. In particular, studies should be undertaken in which the VDR gene is systematically scanned for sequence variations such as has been done for other candidate genes (Claiborne Stephens et al., 2001; Cooper and Umbach, 1996). Haplotype analyses should be used to identify groups of SNPs linked together and, thus, simplify the association analyses and understand the associations observed. Until clearly functional polymorphisms are identified in the VDR gene, it is not very useful to perform meta-analyses to evaluate the consistency of associations and estimate effect size of a polymorphism. Because of difference in LD and the resulting haplotypes, allelic heterogeneity is expected a priori while for truly functional polymorphisms, the same risk allele is expected to display similar effects and associations.

Taken together, it is clear that multiple polymorphic variations exist in the VDR gene which could each have different types of consequences (as is illustrated in Fig. 2). Thus, 5′-promotor variations can affect mRNA expression patterns and levels while 3′-UTR sequence variations can affect the mRNA stability and/or protein translation efficiency. In combination, these genotypic differences are likely to affect the VDR protein levels and/or function, depending on the cell type, developmental stage and activation status.

In summary, one can conclude that VDR gene variants seem to influence a number of biological endpoints, including those related to osteoporosis. Yet, the associations have different magnitudes with BMD probably being one of the weaker effects. In different study populations, different alleles of the anonymous RFLPs can be found associated with the same endpoint. This probably reflects that linkage disequilibrium, between the anonymous marker alleles and the causative alleles in (or very near) the VDR gene, is likely to be different between populations. This notion is supported by the recent observation of islands of linkage disequilibrium in genes which were scrutinized for polymorphisms (Nickerson et al., 1998; Rieder et al., 1999). Finding functional sequence variants that matter, establishing the phase of alleles across the entire VDR gene, and defining haplotype patterns is therefore required to put the associations observed with VDR gene polymorphisms in biological perspective.

References

Arai, H., Miyamoto, K.-I., Taketani, Y., Yamamoto, H., Iemori, Y., Morita, K., Tonai, T., Nishisho, T., Mori, S., Takeda, E., 1997. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J. Bone Miner. Res. 12, 915–921.

Arai, H., Miyamoto, K., Yoshida, M., Kubota, M., Yamamoto, H., Taketani, Y., Yoshida, S., Ikeda, M., Takeda, E., 1999. The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. J. Bone Miner. Res. (Abstract. T084) 14, S191.

Berg, J.P., Falch, J.A., Haug, E., 1996. Fracture rate, pre- and postmenopausal bone loss are not associated with vitamin D receptor genotype in a high-endemic area of osteoporosis. Eur. J. Endocrinol. 135, 96–100.

Cambien, F., Poirier, O., Nicaud, V., Herrman, S., Mallet, C., Ricard, S., Behague, I., Hallet, V., Blanc, H., Loukaci, V., Thillet, J., Evans, A., Ruidavets, J.-B., Arveiler, D., Luc, G., Tiret, L., 1999. Sequence diversity in 36 candidate genes for cardiovascular disorders. Am. J. Hum. Genet. 65, 183–191.

Cargill, M., Altshuler, D., Ireland, J., Sklar, P., Ardlie, K., Patil, N., Lane, C.R., Lim, E.P., Kalyanaraman, N., Nemesh, J., Ziaugra, L., Friedland, L., Rolfe, A., Warrington, J., Lipshutz, R., Daley, G.Q., Lander, E.S., 1999. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nature Genet. 22, 231–238.

Claiborne Stephens, J., Schneider, J.A., Tanguay, D.A., Choi, J., Acharya, T., Stanley, S.E., Jiang, R., 2001. Haplotype variation and linkage disequilibrium in 313 human genes. Science 293, 489–493.

Cooper, G.S., Umbach, D.M., 1996. Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. J. Bone Miner. Res. 11, 1841–1849.

Crofts, L.A., Hancock, M.S., Morrison, N.A., Eisman, J.A., 1998. Multiple promotors direct the tissue-specific expression of novel N-terminal variant human vitamin D receptor gene transcripts. Proc. Natl. Acad. Sci. USA 95, 10529–10534.

Daly, M.J., Rioux, J.D., Schafner, S.F., Hudson, T.J., Lander, E.S., 2001. High-resolution haplotype structure in the human genome. Nat. Genet. 29, 229–232.

Durrin, L.K., Haile, R.W., Ingles, S.A., Coetzee, G.A., 1999. Vitamin D receptor 3′-untranslated region polymorphisms: lack of effect on mRNA stability. Biochim. Biophys. Acta 1453, 311–320.

Eccleshall, T.R., Garnero, P., Gross, C., Delmas, P.D., Feldman, D., 1998. Lack of correlation between start codon polymorphism of the vitamin D receptor gene and bone mineral density in premeno-pausal French women: the OFELY study. J. Bone Miner. Res. 13, 31–35.

Ferrari, S., Rizzoli, R., Manen, D., Slosman, D., Bonjour, J.-P., 1998. Vitamin D receptor start codon polymorphisms (*Fok*I) and bone mineral density: interaction with age, dietary calcium, and 3′-end region polymorphisms. J. Bone Miner. Res. 13, 925–930.

Feskanich, D., Hunter, D.J., Willett, W.C., Hankinson, S.E., Hollis, B.W., Hough, H.L., Kelsey, K.T., Colditz, G.A., 1998. Vitamin D receptor genotype and the risk of bone fractures in women. Epidemiology 9, 535–539.

Gennari, L., Becherini, L., Mansani, R., Masi, L., Falchetti, A., Morelli, A., Colli, E., Gonelli, S., Cepollaro, C., Brandi, M.L., 1999. *Fok*I polymorphism at translation initiation site of the vitamin D receptor gene predicts bone mineral density and

vertebral fractures in postmenopausal Italian women. J. Bone Miner. Res. 14, 1379–1386.

Gong, G., Stern, H.S., Cheng, S.-C., Fong, N., Mordeson, J., Deng, H.-W., Recker, R.R., 1999. The association of bone mineral density with vitamin D receptor gene polymorphisms. Osteop. Int. 9, 55–64.

Gough, A., Sambrook, P., Devlin, J., Lilley, J., Huisoon, A., Betteridge, J., Franklyn, J., Nguyen, T., Morrison, N., Eisman, J., Emery, P., 1998. Effect of vitamin D receptor gene alleles on bone loss in early rheumatoid arthritis. J. Rheumatol. 25, 864–868.

Gross, C., Eccleshall, T.R., Malloy, P.J., Villa, M.L., Marcus, R., Feldman, D., 1996. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican–American women. J. Bone Miner. Res. 11, 1850–1855.

Haussler, M.R., Whitfield, G.K., Haussler, C.A., Hsieh, J.-C., Thompson, P.D., Selznick, S.H., Encinas Dominguez, C., Jurutka, P.W., 1998. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J. Bone Miner. Res. 13, 325–349.

Houston, L.A., Grant, S.F.A., Reid, D.M., Ralston, S.H., 1996. Vitamin D receptor polymorphism, bone mineral density, and osteoporotic vertebral fracture: studies in a UK population. Bone 18, 249–252.

Ingles, S.A., Haile, R.W., Henderson, B.E., Kolonel, L.N., Nakaichi, G., Shi, C.-Y., Yu, M.C., Ross, R.K., Coetzee, G.A., 1997. Strength of linkage disequilibrium between two vitamin D receptor markers in five ethnic groups: implications for association studies. Canc. Epi. Biom. Prev. 6, 93–98.

Jurutka, P.W., Remus, L.S., Whitfield, G.K., Thompson, P.D., Hsieh, J.C., Zitzer, H., Tavakkoli, P., Galligan, M.A., Dang, H.T.L., Haussler, C.A., Haussler, M.R., 2000. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol. Endocrinol. 14, 401–420.

Keen, R.W., Major, P.J., Lanchburry, J.S., Spector, T.D., 1995. Vitamin D receptor gene polymorphism and bone loss. Lancet 345, 990.

Kiel, D.P., Myers, R.H., Cupples, L.A., Kong, X.F., Zhu, X.H., Ordovas, J., Schaeffer, E.J., Felson, D.T., Rush, D., Wilson, P.W., Eisman, J.A., Holick, M.F., 1997. The *Bsm*I vitamin D receptor restriction fragment length polymorphism (bb) influences the effect of calcium intake on bone mineral density. J. Bone Miner. Res. 12, 1049–1057.

Krall, E.A., Parry, P., Lichter, J.B., Dawson-Hughes, B., 1995. Vitamin D receptor alleles and rates of bone loss: influences of years since menopause and calcium intake. J. Bone Miner. Res. 10, 978–984.

Lander, E.S., Schork, N.J., 1994. Genetic dissection of complex traits. Science 265, 2037–2048.

Langdahl, B.L., Gravholt, C.H., Brixen, K., Eriksen, E.F., 2000. Polymorphisms in the vitamin D receptor gene and bone mass, bone turnover and osteoporotic fractures. Eur. J. Clin. Invest. 30, 608–617.

Miyamoto, K.-I., Kesterson, R.A., Yamamoto, H., Taketani, Y., Nishiwaki, E., Tatsumi, S., Inoue, Y., Morita, K., Takeda, E., Pike, J.W., 1997. Structural organization of the human vitamin D receptor chromosomal gene and its promotor. Mol. Endocrinol. 11, 1165–1179.

Morrison, N.A., Yeoman, R., Kelly, P.J., Eisman, J.A., 1992. Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphisms and circulating osteocalcin. Proc. Natl. Acad. Sci. USA 89, 6665–6669.

Morrison, N.A., Qi, J.C., Tokita, A., Kelly, P.J., Crofts, L., Nguyen, T.V., Sambrook, P.N., Eisman, J.A., 1994. Prediction of bone density from vitamin D receptor alleles. Nature 367, 284–287.

Morrison, N.A., Qi, J.C., Tokita, A., Kelly, P.J., Crofts, L., Nguyen, T.V., Sambrook, P.N., Eisman, J.A., 1997. Prediction of bone density from vitamin D receptor alleles (correction). Nature 387, 106.

Nickerson, D.A., Taylor, S.L., Weiss, K.M., Clark, A.G., Hutchinson, R.G., Stengard, J., Salomaa, V., Vartiainen, E., Boerwinkle, E., Sing, C.F., 1998. DNA sequence diversity in a 9.7-kb region of the human lipoprotein lipase gene. Nature Genet. 19, 233–240.

Rieder, M.J., Taylor, S.L., Clark, A.G., Nickerson, D.A., 1999. Sequence variation in the human angiotensin converting enzyme. Nature Genet. 22, 59–62.

Risch, N., Merikangas, K., 1996. The future of genetic studies of complex human diseases. Science 273, 1516–1517.

Rubin, L.A., Hawker, G.A., Peltekova, V.D., Fielding, L.J., Ridout, R., Cole, D.E.C., 1999. Determinants of peak bone mass in a young female Canadian cohort. J. Bone Miner. Res. 14, 633–643.

Sainz, J., van Tornhout, J.M., Loro, L., Sayre, J., Roe, T.F., Gilsanz, V., 1997. Vitamin D receptor gene polymorphisms and bone density in prepubertal American girls of Mexican descent. New Engl. J. Med. 337, 77–82.

Sunn, K.L., Cock, T.-A., Crofts, L.A., Eisman, J.A., Gardiner, E.M., 2001. Novel N-Terminal variant of human VDR. Mol. Endocrinol. 15, 1599–1609.

Uitterlinden, A.G., Pols, H.A.P., Burger, H., Huang, Q., van Daele, P.L.A., van Duijn, C.M., Hofman, A., Birkenhäger, J.C., van Leeuwen, J.P.T.M., 1996. A large-scale population-based study of the association of vitamin D receptor gene polymorphisms with bone mineral density. J. Bone Miner. Res. 11, 1242–1248.

Uitterlinden, A.G., Weel, A.E.A.M., Burger, H., Fang, Y., van Duijn, C.M., Hofman, A., van Leeuwen, J.P.T.M., Pols, H.A.P., 2001. Interaction between the vitamin D receptor gene and collagen type 1α1 gene in susceptibility for fracture. J. Bone Miner. Res. 16, 379–385.

Wang, D.G., Fan, J.-B., Siao, C.-J., Berno, A., Young, P., Sapolsky, R., Ghandour, G., Perkins, N., Winchester, E., Spencer, J., Kruglyak, L., Stein, L., Hsie, L., Topaloglou, T., Hubbell, E., Robinson, E., Mittmann, M., Morris, M.S., Shen, N., Kilburn, D., Rioux, J., Nusbaum, C., Rozen, S., Hudson, T.J., Lipshutz, R., Chee, M., Lander, E.S., 1998. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science 280, 1077–1082.

Whitfield, G.K., Remus, L.S., Jurutka, P.W., Zitzer, H., Oza, A.K., Dang, H.T.L., Haussler, C.A., Galligan, M.A., Thatcher, M.L., Dominguez, C.E., Haussler, M.R., 2001. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol. Cell. Endocrinol. 177, 145–159.

Yamamoto, H., Miyamoto, K.-I., Bailing, L., Taketani, Y., Kitano, M., Inoue, Y., Morita, K., Pike, J.W., Takeda, E., 1999. The caudal-related homeodomain protein Cdx-2 regulates vitamin D receptor gene expression in the small intestine. J. Bone Miner. Res. 14, 240–247.