Androgen and Vitamin D Receptor Gene Polymorphisms: the Long and Short of Prostate Cancer Risk

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In this issue of the Journal, in a study of candidate genes and prostate cancer susceptibility, Ingles et al. (1) report on the association of polymorphisms in the genes encoding the androgen receptor (AR) and the vitamin D receptor (VDR) with the risk of prostate cancer. The polymorphisms, discussed in detail below, both result from variations in length of nucleotide repeats; the AR polymorphism is due to an expansion of CAG codons, whereas the VDR polymorphism is due to an increased number of adenosines in a poly-A tract. The short AR alleles (with <20 CAG repeats) were associated with an adjusted odds ratio of 2.1-fold greater risk of prostate cancer, and the long VDR alleles (with poly-A of ≥18) were associated with a 4.6-fold greater risk. In both cases, the at-risk genotypes were more strongly associated with advanced disease than with localized disease limited to the prostate. These polymorphisms are among the strongest genetic risk factors found yet for prostate cancer and are therefore of great potential significance. It should be emphasized, however, that the variant alleles under study are distributed among the normal population and that a polymorphism does not necessarily cause the cancer but is merely associated with increased risk of having the cancer.

Given the importance of androgens for prostate growth, one might expect that a modification in the AR could cause a genetic effect on prostate cancer risk; the VDR, however, is another story, and the relationship of vitamin D to the prostate and prostate cancer has been recognized only recently (2-6). However, the AR and VDR have many similarities. Both are members of the steroid–thyroid–retinoid receptor superfamily of hormone-dependent nuclear transcription factors. The AR gene has been localized to the proximal long arm of the X chromosome and the VDR to chromosome 12q14. The hormonal ligands for both receptors circulate as prehormones, testosterone and 25-hydroxyvitamin D, respectively. In both cases, the hormonal activity can be converted to the highly active form by specific enzymes, 5α-reductase to produce 5α-dihydrotestosterone (DHT) and 1α-hydroxylase to produce 1α,25-dihydroxyvitamin D$_3$ (calcitriol), respectively. In both cases, the nuclear receptor is essential for hormone action, and inactivating mutations in the receptors can cause hormone-resistant syndromes, androgen insensitivity syndrome, and hereditary vitamin D-resistant rickets. Both hormones act via their cognate receptors to regulate cell proliferation; DHT stimulates and calcitriol inhibits prostate cell growth. Also, both hormones stimulate secretion of prostate-specific antigen (5). The study by Ingles et al. (1) reports on a new similarity that now links the polymorphisms in both receptors to the risk of prostate cancer.

The AR gene is very polymorphic, having a highly variable trinucleotide microsatellite of CAG repeats in exon 1 that results in the incorporation of different numbers of glutamine residues in the N-terminal region of the AR protein. The numbers of CAG repeats, and thus polyglutamines, vary greatly in the population, with normal numbers ranging between nine and 30. An expansion of the microsatellite to 40-62 CAG repeats is associated with Kennedy’s disease, an X-linked spinal and bulbar muscular atrophy frequently associated with mild androgen insensitivity. The longer AR variants have decreased transactivation activity and recently were also shown to have decreased binding affinity for androgens (7). Even within the normal range of CAG repeats, transactivation activity in vitro is inversely related to the number of glutamine residues in the polyglutamine tract (8). This functional difference might be the factor associated with increased prostate cancer risk, since reduced androgen responsiveness of the long AR variants might be protective to the prostate, whereas the relative increased responsiveness of the short variants might predispose the prostate to chronic androgen overstimulation and a higher risk of developing cancer.

Vitamin D is best known for its role in maintaining calcium homeostasis and regulating bone metabolism. In recent years, however, it has become clear that the vitamin D endocrine system regulates cell proliferation, cell differentiation, and the immune system (9). Because of the relative novelty of vitamin D effects on the prostate, this editorial will focus on the VDR polymorphisms. The findings demonstrating that the VDR is expressed in the prostate and that calcitriol and its analogues inhibit prostate cell proliferation (4-6,10-12) prompted Ingles et al. also to examine a VDR polymorphism for association with prostate cancer risk. They chose to study a polymorphism in the 3′-untranslated region (3′ UTR) of the VDR, where the length of a poly-A tract varies. This polymorphism was originally identified by Morrison et al. (13) in studies examining the association between VDR alleles and bone mineral density as predictors of the risk of developing osteoporosis. Ingles et al. designated a stretch of 17 poly-A’s as the short (S) allele and 18 poly-A’s as the long (L) allele. The presence of the L allele, whether in the heterozygous (LS) or homozygous (LL) state, is associated with a fourfold to fivefold increase in prostate cancer risk. This L/S polymorphism is related to other common linked polymorphisms in intron 8 and exon 9 that were described by Morrison et al. (13). These other polymorphic sites are usually detected by polymerase chain reactions followed by restriction fragment length polymorphism analyses. The alleles are resolved by di-
gestion with the enzymes *Bsm* I (where the absence of the en-
zyme site is denoted the B allele, while the presence of the site
is denoted the b allele) and *Taq* I (similarly denoted T or t).
Importantly, none of these polymorphisms is associated with a
change in the VDR amino acid sequence, since the poly-A tract
is in the 3’UTR, the B site is intronic, and, although the T site is
in the 9th exon, both variants code for the same amino acid
(isoleucine).

These VDR polymorphisms have been studied extensively as
risk factors for osteoporosis, and a brief review of these data
provides additional insight into the significance of these findings
for prostate cancer. The cause of osteoporosis is multifactorial
and, like prostate cancer, involves both genetic and environmen-
tal risk factors. Most studies have used bone mineral density,
determined by bone densitometry, as a surrogate marker for
osteoporosis. In initial studies, Morrison et al. (13) claimed that
subjects homozygous for BB exhibited an approximately 12% 
decrease in bone mineral density compared with individuals ho-
mozygous for bb, with heterozygous individuals (Bb) having
intermediate bone mineral density (13). Numerous subsequent
studies attempting to confirm this association yielded conflicting
results. Eventually, enough additional data accumulated to in-
dicate that there was probably an association between VDR poly-
morphisms and bone mineral density, but that the effect on bone
mineral density was much smaller than originally proposed (14).
Multiple analyses were required, with adjustments to account for
confounding variables that also caused low bone mineral den-
sity, such as body size, estrogen status, and age. Since the preva-
ience of the polymorphisms differed substantially in different
ethnic groups, it was clearly important to control for these dif-
f erences as well.

In a recent study published after the study by Ingles et al. was
submitted, Taylor et al. (15) examined the association of the *Taq*
I polymorphism in the VDR gene with prostate cancer risk. They
found that men homozygous for the t allele had one-third less
risk of developing prostate cancer requiring prostatectomy than
men who were heterozygous or homozygous for the T allele.
The studies by Ingles et al. and Taylor et al. are in agreement,
based on linkage disequilibrium between the T allele and the L
allele, that these VDR polymorphisms are associated with in-
creased risk of prostate cancer. The study by Taylor et al. found
a threefold increase in risk associated with the T allele, and the
study by Ingles et al. found a 4.6-fold increase in risk associated
with the L allele. The studies also agree on the absence of a gene
dosage effect, with the increased risk of heterozygotes (LS or LT)
approaching the high risk of the homozygotes (LL or TT).

A major difficulty in accepting the hypothesis that VDR poly-
morphisms cause low bone mineral density as well as altered
risk of prostate cancer is that, unlike the AR polymorphism, the
VDR polymorphisms do not alter the amino acid sequence of the
VDR protein. It is possible that they could alter the level of
messenger RNA (mRNA) expression or stability and thus alter
the abundance of the VDR. It is also possible that the VDR
polymorphisms are linked to a nearby gene and merely mark the
relevant locus. Morrison et al. (13) suggested that the B/t/S
genotype might have increased mRNA expression compared with
the b/T/L genotype. In a preliminary study, Gross et al. (16)
could not find any detectable differences in VDR characteris-
tics in intact fibroblasts grown from skin biopsy specimens from
individuals with BB and bb genotypes. In addition, their study
included measurements of ligand binding, mRNA levels, VDR
protein abundance, and transactivation activity in response to
graded concentrations of calcitriol. The potential mechanism of
these genotype effects on either osteoporosis or prostate cancer
risk remains unknown.

The recent recognition that these VDR polymorphisms are
associated with an increased incidence of other diseases lends
credence to the hypothesis that these genotypes may be impor-
tant predictors of osteoporosis or prostate cancer risk. Uitterlin-
den et al. (17) and Keen et al. (18) have reported a relationship
between VDR alleles and the risk of osteophyte formation and
osteoarthritis. In contrast to a decreased risk of osteoporosis, the
TT genotype was associated with over a threefold increased risk
of osteoarthritis. These findings may help to explain the known
inverse relationship between osteoarthritis and osteoporosis,
both of which are under strong genetic control. Carling et al. (19)
have shown a difference in the prevalence of the bb genotype in
individuals with primary hyperparathyroidism, with the bb allele
present in 60% of patients and in only 33.3% of controls.

A new polymorphism in the VDR recently has become the
subject of intense study. The hVDR gene, cloned by Baker et al.
(20), has two potential ATG translational initiation sites that are
separated by three codons. A polymorphism, located in the first
ATG (21), presumably determines the site of translation initia-
tion; hence, the VDR variants at this polymorphic site would
differ in length by three amino acids. Gross et al. (22) and
Miymoto et al. (23) have shown that the elongated VDR is
associated with reduced bone mineral density and therefore with
an increased risk of osteoporosis. However, it has not yet been
proven whether this start codon polymorphism results in a
change in VDR function. It will be of interest to determine
whether this VDR polymorphism, which has no known relation-
ship to the poly-A-linked polymorphisms, is also associated with
altered risk for prostate cancer.

The relationship between vitamin D and prostate cancer is a
relatively new idea, bolstered by epidemiologic (2,3) and labo-
atory (4-6) experiments. One therapeutic trial (24) has at-
ttempted to treat prostate cancer patients with calcitriol, but it
failed to show any benefit in patients with advanced cancer.
Vitamin D may play a role in other cancers (including cancers of
the breast and colon, leukemia, and melanoma) as well as in
immunosuppression and psoriasis (9). The use of systemically
administered calcitriol to treat these diseases, however, is lim-
ited by the induction of hypercalcemia and hypercalciuria when
high doses of calcitriol are used. However, on the horizon is the
potential development of new vitamin D analogues with in-
creased antiproliferative activity but decreased tendency to
cause hypercalcemia (10).

In conclusion, to highlight the potential difficulties of genetic
association studies, it may be helpful to note that the claims
about VDR polymorphisms and osteoporosis risk, initially in-
flated, are still controversial. The experience with the study of
VDR polymorphisms and osteoporosis risk indicated that there
are many possible confounders, such as ethnicity and various
environmental factors, that may confuse these genetic associa-
tion findings. Also, the statistical power to detect relationships
must be adequate. In the study by Ingles et al. (I) in this issue of
the Journal, the data are somewhat limited, the number of cases
is small, and the controls have been obtained as part of a separate study. Ingles et al. are appropriately prudent in interpreting their data with caution and suggesting the need for additional studies. However, when they wrote their report, they were unaware of the independent results obtained by Taylor et al. (15), who corroborated the association using a different but linked VDR polymorphism. Although the risk found by Ingles et al. (fourfold to fivefold) is greater than that found by Taylor et al. (threefold), these parallel and confirmatory results greatly strengthen the findings of an association between VDR polymorphisms and prostate cancer risk. Several aspects of these studies still dictate caution, however. I am puzzled by the lack of increased risk in individuals harboring both VDR and AR at-risk alleles. Furthermore, the lack of a gene dosage effect with no increased risk seen with two at-risk VDR alleles is curious. With additional studies on osteoporosis, the direction of the risk seems to have held up, even though the magnitude of the effect was substantially decreased from original predictions. An increased risk of prostate cancer of 4.6-fold appears to be very large for a VDR polymorphism, and this level of increased risk may decrease with further study. However, the implications of these data are great, and it is hoped that additional studies with larger samples, in different ethnic and age groups, and with other VDR polymorphisms will confirm and extend these exciting and provocative findings.

References