

Vitamin D Receptor Gene Polymorphisms Influence Insulin Secretion in Bangladeshi Asians

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The etiology of type 2 diabetes is likely to involve defects of both insulin secretion and insulin signaling. In experimental animals, vitamin D is necessary for normal insulin release and maintenance of glucose tolerance (1). The β -cell possesses specific receptors for the activated hormone 1,25-dihydroxy vitamin D₃ [1,25-(OH)₂D₃] and vitamin D-dependent calcium-binding proteins (1,2). Insulin secretion is impaired by vitamin D deficiency and restored by 1,25-(OH)₂D₃ administration (3). We have found in Bangladeshi Asians living in East London, U.K., that vitamin D status (serum 25-OH vitamin D concentration) was reduced in those at risk for type 2 diabetes compared with subjects not at risk (4). A positive correlation was found between 30-min specific insulin concentrations, and negative correlations of glycemia were found with vitamin D status during oral glucose tolerance testing (OGTT). Short-term vitamin D replenishment in a subset of subjects increased insulin secretion but did not alter established glucose intolerance.

We have recently described an association between vitamin D receptor (VDR) polymorphisms and type 1 diabetes in South Indian Asians (5). Vitamin D has important immunomodulatory properties (6), and depletion, or relative vitamin D resistance, could play a part in the etiology of both type 1 and 2 diabetes through effects on insulin secretion. The purpose of the present study, designed as an arm of a long-term study of vitamin D repletion in the Bangladeshi-Asian population, was to determine whether insulin secretion was associated with VDR polymorphisms.

The experimental protocol was approved by the district ethical committee. Of the healthy Bangladeshi Asians categorized at risk for type 2 diabetes, 171 were recruited as previously described (4). Subjects with previously diagnosed diabetes were excluded from the study. Spot glucose levels >6.0

mmol/l less than 2 h postcibum or >4.6 mmol/l at times greater than 2 h postcibum, on two separate occasions, were regarded as defining patients as at risk for type 2 diabetes (4). The subjects thus defined all underwent a standard glucose tolerance test. Clinical characteristics of the subjects studied were 40.4% men, mean age 45.9 years \pm 10.3 (range 30–65), mean BMI 26.6 \pm 3.8 (16.6–35.9), and mean 25-OH vitamin D concentration 17.6 \pm 8.5 ng/ml (3.5–56.9; 43% vitamin D deficient defined by <15 ng/ml). Within the study population, 75% had normal glucose tolerance, 16.5% had impaired glucose tolerance, and 8.5% had newly diagnosed type 2 diabetes (defined by the World Health Organization criteria, 1985).

DNA from all subjects were genotyped for three restriction fragment length polymorphism (RFLP) sites (*BsmI* and *Apal* in successive introns between exons 7 and 9 of the VDR gene, and *TagI* within the 9th exon) (7), as described previously (5). Genotypes were designated by a lowercase letter for the presence of a restriction site (i.e., "a" for a *Apal* site and an uppercase letter for absence (i.e., "A") (5). Samples were analyzed at least twice in the event of ambiguous typing, and all results were assessed blindly by an independent investigator.

Uncorrected *P* values suggest an association between the insulin secretion index and all three RFLPs (Table 1); after correction for six factors (the number of genotypes [\times 3] and the two principal comparisons [insulin secretion or resistance], that is, \times 6), only the *Apal* association reaches significance (corrected *P* value [*P*_c] = 0.006). The insulin secretion index was used as a measure of insulin secretion and calculated from the 30-min glucose and insulin values during an OGTT using the formula (30-min insulin-basal insulin)/(30-min glucose-basal glucose) (8). Specific human insulin was measured by enzyme immunoassay (Medgenix, Florius, Belgium; coefficient of variation [CV] <7.2%). A gene dosage effect was found for estimated insulin secretion; the lowest with the aa genotype (68.5 [95% CI 48.9–95.8]), highest with AA (146.8 [112.8–191.0]), and aA intermediate (97.2 [76.6–116.2]). When these calculations were repeated to exclude those subjects with diabetes, the association between *Apal* genotype and insulin secretion remained significant (*P* = 0.003; *P*_c = 0.018). Linear regression analysis confirmed independent contributions from both changes in insulin (30-min insulin-basal insulin; *P* < 0.0001) and changes in glucose (30-min glucose-basal glucose; *P* = 0.04) contributing to the association between the insulin secretion index and *Apal* genotype. An analysis of variance (ANOVA) with 25-OH vitamin D as a covariate demonstrated no interaction with vitamin D (*P* = 0.426).

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ANOVA, analysis of variance; CV, coefficient of variation; OGTT, oral glucose tolerance test; 1,25-(OH)₂D₃, 1,25-dihydroxy vitamin D₃; *P*_c, corrected *P* value; RFLP, restriction fragment length polymorphism; VDR, vitamin D receptor.

TABLE 1
Insulin secretion by vitamin D receptor gene

Genotypes	n	Clinical data				Statistical analysis of insulin secretion index		
		Age (years)	% men	BMI	Vitamin D ng/ml	Insulin secretion index	*P value	Corrected P value
<i>Apal</i>	164							
AA	52	44 (42–47)	31	26 (25–27)	15 (13–17)	146.8 (112.8–191.0)	0.001	0.006
Aa	86	47 (45–49)	47	27 (26–28)	15 (14–17)	92.7 (76.6–116.2)		
aa	26	46 (42–50)	35	26 (24–27)	17 (13–22)	68.5 (48.9–95.8)		
<i>Bsml</i>	164						0.039	0.23
BB	37	46 (43–49)	33	26 (25–27)	17 (14–19)	120.5 (89.0–163.1)		
Bb	75	47 (45–49)	43	27 (26–28)	18 (16–20)	117.3 (94.5–145.6)		
bb	52	45 (42–48)	42	27 (26–28)	18 (15–20)	80.7 (64.6–100.7)		
<i>TaqI</i>	64						0.01	0.06
TT	76	45 (43–47)	40	27 (26–28)	18 (16–20)	87.5 (72.2–106.0)		
Tt	70	48 (45–50)	42	26 (25–27)	18 (16–20)	112.0 (90.9–138.1)		
tt	18	43 (39–48)	37	26 (25–28)	15 (13–18)	173.3 (102.0–294.4)		

Data are mean (range), %, or P value. *P values were calculated by ANOVA using SPSS for Windows (release 7). Because the insulin secretion index was not normally distributed, the data were log-transformed before analysis.

No differences with genotype were found for 32,33 split proinsulin (an estimate of insulin resistance assayed by an immunoradiometric assay [CV <7.5%]) (7) in all subjects (*Apal* $P = 0.45$, *Bsml* $P = 0.34$, and *TaqI*; $P = 0.41$) or similarly when the type 2 diabetic subjects were excluded. There were no differences in mean 25-hydroxy-vitamin D concentrations measured by radioimmunoassay (Medgenix, Florius, Belgium; lower limit of detection 2.1 ng/l, CV <3.8), age, sex ratio, or BMI according to *Apal*, *Bsml*, or *TaqI* genotypes.

We have demonstrated an association between vitamin D receptor gene polymorphisms and insulin secretion in Bangladeshi Asians, categorized as at risk for type 2 diabetes, which is independent of vitamin D status. Together with previous data on South Indian patients with type 1 diabetes (5), this provides evidence for the VDR as a novel candidate gene contributing to a component of susceptibility to both types of diabetes; however, a direct effect with type 2 diabetes needs to be studied by further association and/or linkage studies with type 2 diabetes itself. A possible explanation for different RFLP associations (*Bsml* with IDDM in South Asians and *Apal* RFLP with insulin secretion in Bangladeshi Asians) is genetic heterogeneity between these populations, suggesting that the RFLPs per se are not directly responsible for disease. Furthermore, associations might reflect separate disease mechanisms operating for type 1 diabetes susceptibility and for insulin secretion.

The VDR gene is located on chromosome 12q12–12q14 (9). A locus on 12q24 (NIDDM2) has recently been found to be linked to insulin secretion in type 2 diabetes in a region containing the maturity onset diabetes of the young (MODY3) locus (10,11); MODY3 encodes hepatic nuclear factor-1 α (12). The distance between 12q12–12q14 (VDR location) and 12q24 (NIDDM2/MODY3) precludes our observations being explained by associations with either NIDDM2 or MODY3. In a genome scan for traits associated with type 2 diabetes in the Pima Indians, 12q12–12q14 was not identified as a susceptibility region, although the region containing vitamin D binding protein (chromosome 4q12) was found to be linked to fasting insulin (13).

The VDR polymorphism associations with type 1 diabetes and with insulin secretion are probably best explained by either a direct association with a VDR locus variant in linkage disequilibrium with these RFLPs or with a closely linked locus. It is unlikely that a diabetes-associated variant will be found in the VDR-coding region since sequencing of "osteoporosis-associated" haplotypes has not identified a coding region variant (7). Furthermore, *Apal*, *Bsml*, and *TaqI* restriction sites are localized to introns and are unlikely to cause disease. DNA sequencing studies are now indicated in subjects possessing these diabetes-related VDR genotypes and haplotypes.

Although vitamin D plays a role in insulin secretion, the exact mechanism is unclear and may involve both genomic and nongenomic pathways (1). The effects of 1,25-(OH) $_2$ D $_3$ on insulin secretion are partly dependent on calcium status (14,15). There also is evidence that 1,25-(OH) $_2$ D $_3$ directly influences insulin secretion in the β -cell through a rise in intracellular-free calcium concentration via the nonselective calcium channel, rather than the calcium-dependent inositol 1,4,5-triphosphate receptor-mediated pathway (16). Furthermore, 1,25-(OH) $_2$ D $_3$ has been shown to inhibit β -cell growth by upregulation of vitamin D receptors, suggesting an alternative mechanism whereby VDR variants might alter insulin responses through early β -cell differentiation (17).

These results, allied to our recent studies on VDR associations in type 1 diabetes, require further investigation in other population groups possessing differing levels of risk for vitamin D depletion. To explain the functional role of the VDR in both types of diabetes, it will be necessary to determine the effects of these of VDR polymorphisms on β -cell function.

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Author Queries (please see Q in margin and underlined text)

Q1: <<AU: Correct that this sentence cites reference 1?>

Q2: <<AU:Correct that 'those' refers to patients? Sentence correct as edited?>

Additional Query: Please define the subscript "c" in P_c (used on page 1).