

Vitamin D receptor (VDR) gene and type 2 diabetes mellitus

Protocol for a HuGE association review

Date edited: 4th January 2006

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Background

Diabetes mellitus is a chronic condition characterised by increased blood concentrations of glucose. It is caused by deficiencies in pancreatic insulin production which may be inherited or environmentally acquired. There are three ways of diagnosing diabetes:

- symptoms (e.g. polyuria, polydipsia, weight loss, blurred vision) plus random glucose ≥ 11.1 mmol/l
- fasting glucose plasma glucose ≥ 7 mmol/l
- 2-hr post load plasma glucose (75g) ≥ 11.1 mmol/l.

There is also a metabolic state between diabetes and normal glucose homeostasis. This is known as impaired glucose regulation and defined as either abnormal fasting glucose (impaired fasting glucose (IFG)) or abnormal post-load glucose levels (impaired glucose tolerance (IGT)). Blood concentrations of glucose in all three states are summarised in Table 1 below.

Table 1 Blood glucose levels in diabetes, IGT and IFG (from (7))			
	Glucose concentration, mmol l ⁻¹ (mg dl ⁻¹)		
	Whole blood		
	Venous	Capillary	Plasma
Diabetes Mellitus:			
Fasting <i>or</i>	≥ 6.1 (≥ 110)	≥ 6.1 (≥ 110)	≥ 7.0 (≥ 126)
2-h post glucose load	≥ 10.0 (≥ 180)	≥ 11.1 (≥ 200)	≥ 11.1 (≥ 200)
Impaired Glucose Tolerance (IGT):			
Fasting (if measured) <i>and</i> 2-h post glucose load	< 6.1 (< 110) and ≥ 6.7 (≥ 120)	< 6.1 (< 110) and ≥ 7.8 (≥ 140)	< 7.0 (< 126) and ≥ 7.8 (≥ 140)
Impaired Fasting Glycaemia (IFG):			
Fasting	≥ 5.6 (≥ 100) and < 6.1 (< 110)	≥ 5.6 (≥ 100) and < 6.1 (< 110)	≥ 6.1 (≥ 110) and < 7.0 (< 126)
and (if measured) 2-h post glucose load	< 6.7 (< 120)	< 7.8 (< 140)	< 7.8 (< 140)

Type 1 diabetes (also known as insulin dependent) is primarily due to auto-immune mediated destruction of pancreatic β -cell islets. 90% of diabetes cases are type 2 diabetes (T2DM) which is characterised by insulin resistance and/or abnormal insulin secretion. Whilst people with type 1 diabetes are dependent on exogenous insulin to prevent ketoacidosis people with T2DM may require exogenous insulin but may be able to control blood glucose levels by diet alone or oral hypoglycaemic agents. The epidemiologic transition (1) with its shift from communicable diseases to non-communicable diseases has brought the public health importance of diabetes, especially type 2, into the fore in recent years. The increasing burden of T2DM has been referred to as an epidemic, (2). This review will only consider T2DM.

Risks, prevalence and consequences of T2DM

The global burden of disease study estimates deaths due to diabetes to be nearly 1 million for 2002, 55% of which are women and 95% in those over 44 years (3). The World Health Organisation (WHO) estimates there will be nearly 300 million affected individuals world wide by 2025 (4). The

prevalence is around 1-2% in the general population with increased prevalence in some ethnic minorities (South Asian, African, Afro-Caribbean and Chinese) as well as in less affluent populations, those who are physically inactive and in those who have central obesity or a high body mass index. Some populations such as Pima Indians, Australian Aboriginal communities, Pacific and Indian Ocean islanders have a far greater prevalence (up to 40%, (2)). Impaired glucose regulation is also a risk factor for developing diabetes. In the UK T2DM accounts for 85% of diabetes (5). The presence of diabetes is associated with a range of microvascular and macrovascular complications (myocardial infarction, stroke, amputation or death from peripheral vascular disease, heart failure, angina, retinopathy, neuropathy) (6). Diabetes also carries an increased risk of cataracts, infections, soft tissue conditions, skin conditions and mental disorders (7). These risks may occur before symptoms of diabetes so that detection of T2DM can help prevent the development of these complications.

The genetic basis of diabetes

There are markers of type 1 diabetes which are detectable even if a subject is normoglycaemic (e.g. presence of islet cell antibodies). One gene locus, the human histocompatibility antigen locus, accounts for approximately 50% of the risk of type 1 diabetes (8). This allows early detection and modification of malleable risk factors.

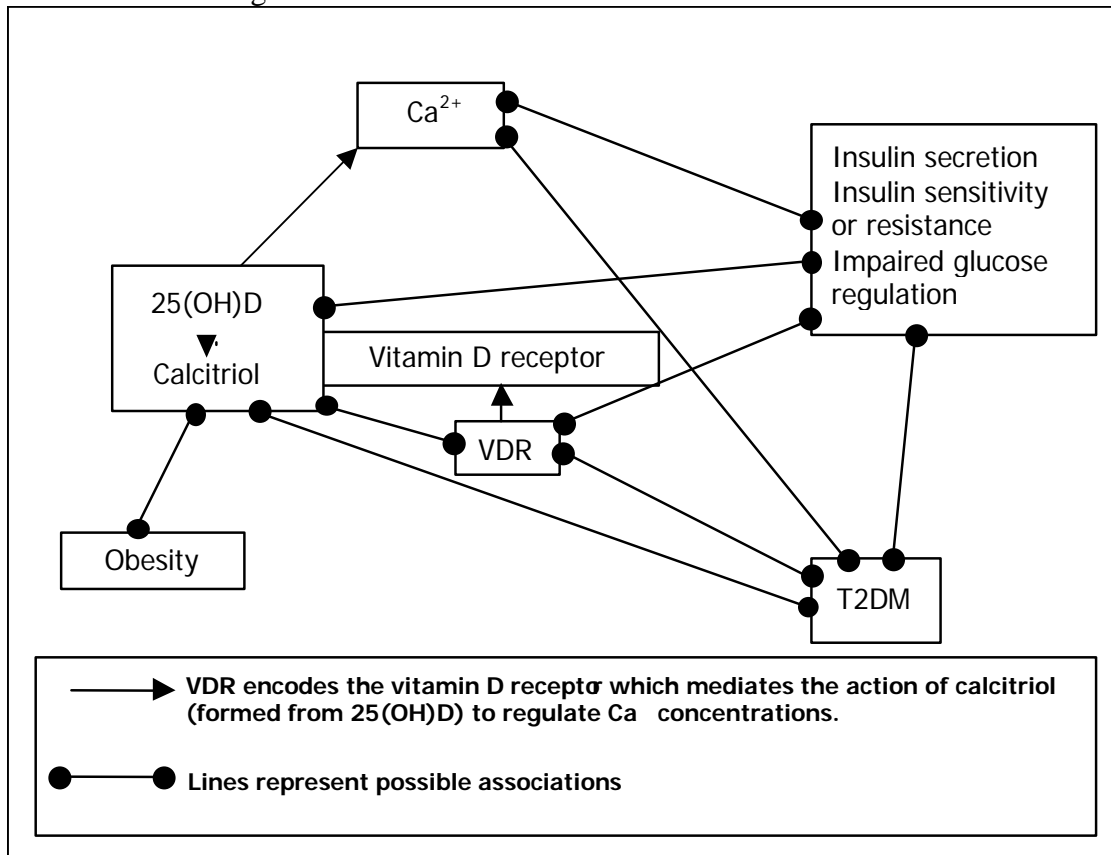
As the aetiology of T2DM is less clear this is not the case with this type of diabetes. However, there is increasing evidence that genetic susceptibility plays a major role in the pathogenesis of T2DM (9). Risk varies widely across populations, and concordance among identical twins is high but the high population prevalence suggests common, low penetrance genes. There is evidence of diabetic gene defects for several genes; glucokinase, hepatocyte nuclear factors, insulin promoter factors (all of which lead to maturity onset diabetes of the young rather than the more common typical T2DM), possibly the insulin and insulin receptor genes and mitochondrial genes. Most of the progress in identifying candidate genes for T2DM has been with these rare, autosomal dominant forms of T2DM. The examination of a large number of genes based on their role in the pathway of insulin action or secretion has led to the identification of several candidate genes in typical T2DM. These include certain polymorphisms in the insulin receptor genes and activators, the sulphonyl receptor gene, adiponectin and protein phosphatase 1. Genome scans have also identified other likely chromosomal areas. Candidate genes fall into broad categories of those involved in pancreatic β -cell function, genes influencing insulin action and glucose metabolism in the main target tissues of muscle, liver and fat and other genes (e.g. to do with lipid metabolism or energy homeostasis) (10).

The genetic basis of T2DM involves multiple genes that have modest effects on diabetes susceptibility that interact with each other and may be related to environmental conditions. The large and increasing burden of diabetes and the potential to modify risk through adequate treatment and lifestyle changes makes the identification of methods for early detection an important public health challenge. Identification of genetic polymorphisms which affect diabetes risk and accurate quantification of those risks will aid the development of more complex models to study, predict and treat this condition.

Vitamin D receptor gene (VDR)

The vitamin D receptor mediates the majority of the effects of vitamin D (as the active form $1\alpha,25$ -dihydroxyvitamin D (calcitriol)) on gene expression via formation of a heterodimer with the retinoid X receptor which binds to promoter regions of many target genes. There are 6 known polymorphisms in the VDR locus (Figure 1) with a range of possible effects (Table 2) (11).

Figure 2. Possible associations of vitamin D and T2DM



Given the complexity of the possible pathways, this review will consider associations between VDR and T2DM, calcitriol or 25(OH)D, [Ca²⁺] and insulin secretion, sensitivity, resistance or impaired glucose regulation. Dependent upon results it may be possible to use the principal of Mendelian deconfounding (16) to investigate these associations further.

Objectives

To quantify the magnitude of association between any identified variants of the VDR gene and type 2 diabetes mellitus and various intermediate outcomes including plasma glucose, insulin sensitivity and vitamin D.

We aim to supplement the published literature using new data from case-control studies held by the MRC Epidemiology Unit (University of Cambridge).

Methods

Criteria for considering studies

Polymorphisms

Study selection will be limited to those addressing any identified VDR polymorphism.

Clinical outcomes

Studies which report outcomes as:

- Clinical diagnosis of T2DM (with clinical criteria defined)

- Diagnosis of glucose intolerance/impaired glucose tolerance or impaired fasting glucose (using WHO criteria, 1991 (7)).
- Fasting or post-glucose challenge plasma glucose levels
- Measurements of insulin resistance or sensitivity (eg homeostasis model assessment (HOMA), insulin sensitivity index (S_i))
- Levels of vitamin D (calcitriol) or metabolites
- Serum calcium ion levels

Types of studies

Primary observation studies investigating associations between a VDR polymorphism and T2DM. These may be case-control studies with population-based control groups, population-based cohort studies or population-based cross-sectional studies.

Search methods

Electronic searches will be performed using PubMed, EMBASE, Biosis, ISI Science Citation Index, HuGENet and LocusLink (now EntrezGene) Further papers will be sought from reference lists. To produce a manageable number of references it will be appropriate to search only for papers mentioning the VDR gene. Search strategies will be:

PubMed:

vdr[All Fields] OR ("calcitriol receptors"[Text Word] OR "receptors, calcitriol"[MeSH Terms] OR vitamin d receptor[Text Word]) OR ("calcitriol receptors"[Text Word] OR "receptors, calcitriol"[MeSH Terms] OR calcitriol receptor[Text Word]) OR (("vitamin d"[MeSH Terms] OR vitamin d[Text Word]) AND (("hormones"[TIAB] NOT Medline[SB]) OR "hormones"[MeSH Terms] OR "hormones"[Pharmacological Action] OR hormone[Text Word]) AND receptor[All Fields]) OR (("vitamin d"[MeSH Terms] OR vitamin d[Text Word]) AND nuclear[All Fields] AND receptor[All Fields]) OR (("ergocalciferols"[MeSH Terms] OR ergocalciferol[Text Word]) AND receptor[All Fields]) OR (("ergosterol"[MeSH Terms] OR ergosterol[Text Word]) AND receptor[All Fields])

Biosis:

"vdr" or "vitamin d receptor" or "calcitriol receptor" or "vitamin d hormone receptor" or "vitamin d nuclear receptor" or "ergocalciferol receptor" or "ergosterol receptor"

ISI:

TS=vdr or TS=vitamin d receptor or TS=calcitriol receptor or TS=vitamin d hormone receptor or TS=vitamin d nuclear receptor or TS=ergocalciferol receptor or TS=ergosterol receptor

Embase:

CALCITRIOL ADJ RECEPTOR
 CALCITRIOL-RECEPTOR#.DE.
 VITAMIN ADJ D ADJ HORMONE ADJ RECEPTOR
 VITAMIN ADJ D ADJ NUCLEAR ADJ RECEPTOR
 ERGOSTEROL ADJ RECEPTOR
 ERGOCALCIFEROL ADJ RECEPTOR

From the original search, title, keywords and abstract (where available) will be scanned by one reviewer. If the article does not match the inclusion criteria above it will be excluded although if exclusion is not clear full text will be obtained. Two reviewers will then independently assess eligibility with a third opinion sought to resolve any disagreements not resolved by discussion. Further information will be sought from authors where appropriate.

Quality assessment

Little *et al* (20) propose criteria for assessing the methodological quality of gene-disease association studies. Quality assessment for this review will be based on this. The most important criteria to be evaluated are subject selection, validity of genotyping, and analysis for population stratification, gene-gene and gene-environment interactions. A descriptive, qualitative assessment of quality will be made with reviewers noting the following indicators:

- Study population selection method
- Molecular technique used and whether laboratory workers were blinded to disease status
- Whether conformity to the Hardy-Weinberg equilibrium was assessed (and if so whether deviance was detected)
- Whether linkage disequilibrium was noted between genotypes studied
- Any individual studies assessed as being at high risk of bias will be excluded from meta-analyses as a sensitivity analysis.

Data extraction

A data extraction form will be designed for the review and data will be independently extracted by two reviewers. Differences between results will be resolved by a third reviewer if agreement cannot be reached by discussion.

Data extracted will include:

- Allele, genotype and haplotype frequencies for cases and controls separately or for the study population with data on intermediate phenotypes
- Conformity to Hardy-Weinberg equilibrium and linkage disequilibrium data
- Associations reported as odds ratios, relative risks or risk differences with significance levels
- Participants (number, age, sex, ethnicity, locality, selection criteria)
- Study design
- Outcome measures as numbers of cases and controls for each genotype or means/medians and standard deviations, standard errors or confidence intervals
- DNA extraction and genotyping methodology
- Primary data on VDR polymorphisms and T2DM or biomarkers will also be collected from collaborators. This will be data from the Ely study (1071 participants selected from UK primary care practice lists and tested for diabetic status after 4.5 and 20 years) and the Norfolk EPIC (from a subgroup of the nearly 24,000 participants of the population-based study).

Data synthesis

Study heterogeneity will be assessed visually with forest plots. The presence of heterogeneity can be determined using a χ^2 test. However, since one might expect heterogeneity in most cases it is more useful to determine the impact of the heterogeneity on the meta-analysis than to just test for its presence. This will be done using the I^2 statistic (19):

$$I^2 = 100\% \times (Q - df)/Q$$

where Q is the χ^2 statistic from a test for heterogeneity and df represents the degrees of freedom of the test.

Meta-analysis will be employed where appropriate to increase power to detect associations (18). Meta-analyses will be conducted separately where possible for each polymorphism and association with T2DM as an outcome. Separate analyses will be conducted for each polymorphism and each

biomarker (fasting glucose, glucose intolerance, insulin sensitivity, insulin secretion, calcitriol or Ca^{2+} levels) if possible.

Data concerning T2DM will be collected as 3×2 tables and analysed according to a suitable assumption concerning mode of inheritance (dominant, recessive or co-dominant). Where this is not known a model free approach will be used, for example by performing two pair-wise comparisons. For dominant or recessive models, odds ratios will be calculated from collapsed 2×2 tables and for co-dominant models logistic regression will be used. Meta-analysis will be undertaken on the log odds ratio scale.

Biomarker data will be analysed by comparing mean levels, providing that sample sizes are sufficiently large or the distributions are not highly skewed. If some studies have small sample sizes and distributions are highly skewed then log-transformed values (equivalently, geometric means) will be sought.

Studies will be weighted by precision, such that studies with smaller standard errors have a greater weight. Meta-analyses may be performed using a fixed-effect and random-effects assumption. Results from random-effects analyses will be reported unless there is a marked discrepancy between the two. This corresponds to a relationship between effect size and precision. Funnel plots will be used to investigate further this potential source of bias, which may be due to publication bias or to artificial or real effects in small study sizes.

Potential conflict of interest

None known

References

- (1) Omram A. The Epidemiologic Transition: A Theory of the Epidemiology of Population Change. *Bull World Health Organ* 2001; 79(2):161-170.
- (2) Zimmet P, Alberti K, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; 414:782-787.
- (3) WHO. World Health Report 2004 - changing history. 2004.
- (4) King H, Aubert R, Herman W. Global burden of diabetes, 1995-2025: prevalence, numerical estimates and projections. *Diabetes Care* 1998; 21:1414-1431.
- (5) Department of Health. National Service Framework for Diabetes. 1999.
- (6) Intensive blood-glucose control with sulphonylurea or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; 352(9131):837-853.
- (7) WHO. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. 1999. Geneva, Department of Noncommunicable Disease Surveillance.
- (8) Cox N, Wapelhorst B, Morrison V, Johnson L, Pinchuk L, Spielman R et al. Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. *Am J Hum Genet* 2001; 69:820-830.
- (9) Elbein. Perspective: The search for genes for type 2 diabetes in the post-genome era. *Endocrinology* 2002; 143(6):2012-2018.
- (10) Barroso I, Luan J, Liddelberg R, Harding A-H, Franks P, Jakes R et al. Candidate gene association study in type 2 diabetes indicates a role for genes involved in beta cell function as well as insulin action. *PLoS Biology* 2004; 1(1):041-055.
- (11) Uitterlinden AG, Fang Y, van Meurs JBJ, Pols HAP, van Leeuwen JPTM. Genetics and biology of vitamin D receptor polymorphisms. *Gene* 2004; 338(2):143-156.
- (12) Hirschhorn J, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. *Genetics in Medicine* 2002; 4(2):45-61.
- (13) Lohmueller K, Pearce C, Pike M, Lander E, Hirschhorn J. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nature Genetics* 2003; 33:177-182.
- (14) Salanti G, Sanderson S, Higgins J. Obstacles and opportunities in meta-analysis of genetic association studies. *Genetics in Medicine*. In press.

- (15) Stroup D, Berlin J, Morton S, Olkin I, Williamson D, Rennie D et al. Meta-analysis of observational studies in epidemiology. *JAMA* 2000; 283(15):2008-2012.
- (16) Tobin MD, Minelli C, Burton PR, Thompson JR, Commentary: Development of Mendelian randomization: from hypothesis test to 'Mendelian deconfounding'. *Int. J. Epidemiol.*, February 1, 2004; 33(1): 26 - 29.
- (17) Centre for Reviews and Dissemination. Undertaking systematic reviews of research on effectiveness. 2nd Edn. 4. 2001. University of York.
- (18) Alderson P, Green S, Higgins JPT, editors. *Cochrane Reviewers' Handbook 4.2.2* [updated December 2003]. In: *The Cochrane Library, Issue 1, 2004*. Chichester, UK: John Wiley & Sons, Ltd.
- (19) Higgins J, Thompson S, Deeks J, Altman D. Measuring inconsistency in meta-analysis. *BMJ* 2004; 327:557-327.
- (20) Little J, Bradley L, Bray M, Clyne M, Dorman J, Ellsworth D et al. Reporting, appraising and integrating data on genotype prevalence and gene-disease associations. *Am J Epidemiol* 2002; 156(4): 300-310.
- (21) Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF et al. Improving the quality of reports of meta-analyses of randomised controlled trials: the Quorum Statement. *The Lancet* (1999) 354: 1896-900.