Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 89–90 (2004) 155–157
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

CYP27B1 polymorphisms variants are associated with type 1 diabetes mellitus in Germans☆

Elizabeth Ramos Lopez, Karoline Regulla, Michael A. Pani, Maren Krause, Klaus-H. Usadel, Klaus Badenhoop*

Department of Internal Medicine I, Division of Endocrinology, University Hospital Frankfurt, Theodor Stern Kai 7, Frankfurt am Main 60596, Germany

Abstract

CYP27B1 (25-hydroxyvitamin D_3-1α-hydroxylase) catalyzes the metabolization of 25-hydroxyvitamin D_3 to $1,25(OH)_2D_3$ the most active natural Vitamin D metabolite. $1,25(OH)_2D_3$ plays a role in the regulation of autoimmunity and cell proliferation and prevents the development of autoimmune diabetes mellitus in animal models besides other autoimmune disorders. One hundred and eighty-seven families with one offspring affected with type1diabetes mellitus were genotyped for the polymorphisms in the promoter region (−1260 C/A) and intron 6 (2338 T/C) of the CYP27B1 gene on chromosome 12 q13.1–13.3 and extended transmission disequilibrium tests (ETDT) were performed.

The haplotype CT (−1260 A/2338 T) was significantly more often transmitted to affected offspring (96 transmitted (T) versus 63 not transmitted (NT), $P = 0.0089$). While the AT (−1260 C/2838 T) was significantly less often transmitted (37 T versus 60 NT, $P = 0.0195$).

This study suggests that CYP27B1 haplotypes may confer susceptibility to type 1 diabetes mellitus in Germans.

Keywords: Diabetes mellitus type 1; Vitamin D; CYP27B1 polymorphisms

1. Introduction

Vitamin D is a secosteroid that acts via the nuclear Vitamin D receptor (VDR). The most active natural Vitamin D metabolite, $1,25(OH)_2D_3$ effectively prevents the development of autoimmune diabetes mellitus [1] and autoimmune thyroiditis [2] in animal models. Also, other autoimmune disorders such as experimentally induced autoimmune encephalitis can be favourably influenced by administering 1α, $25(OH)_2D_3$ [3]. This secosteroid exerts its immunomodulatory actions by inhibiting HLA class II expression on endocrine cells [4], T cell proliferation and secretion of inflammatory cytokines [5] that are thought to act as mediators in autoimmune tissue destruction.

Type 1 diabetes mellitus is a multifactorial disease with a strong genetic component [6]. The main genetic contribution to type 1diabetes mellitus susceptibility lies in the major histocompatibility complex (MHC) on the short arm of chromosome 6 [7]. However, other genes have been associated with susceptibility to type 1diabetes mellitus [8]. Allelic variation in the VDR with regards to this disease was studied in different populations [9]. Furthermore, low Vitamin D levels have been reported to increase the risk for type 1 diabetes mellitus [10] and Vitamin D supplementation to reduce it.

CYP27B1 (25-hydroxyvitamin D_3-1α-hydroxylase) is a mitochondrial P450 enzyme [11], which catalyzes the metabolization of 25-hydroxyvitamin D_3 to $1,25(OH)_2D_3$. It is the key enzyme determining the rate of $1,25(OH)_2D_3$ production. Renal [12] and extrarenal tissues express CYP27B1 suggesting endocrine as well as para- und autocrine functions of this enzyme [13].

Mutations within the CYP27B1 gene, which impair CYP27B1 hydroxylase activity and cause Vitamin D-dependent rickets, have been described [14].

We therefore studied one polymorphism in the promoter region and another in intron 6 of the CYP27B1 hydroxylase gene, enabling a haplotype definition in families.

1.1. Subjects and methods

A total of 187 families (561 subjects) with at least one affected offspring with type 1 diabetes mellitus were recruited

☆ Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July 2003).

* Corresponding author. Tel.: +49-69-6301-5839; fax: +49-69-6301-6405.
E-mail address: badenhoop@em.uni-frankfurt.de (K. Badenhoop).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.03.095

from the endocrine outpatient clinics at the University Hospital Frankfurt am Main (Germany). Type 1 diabetes mellitus was diagnosed according to World Health Organization criteria. The male: female was 1:1.1 and the median age of the affected child at diagnosis was 10.5 years (range 1–37 years). Informed consent was obtained prior to blood sampling.

1.2. Methods

DNA was extracted from whole blood according to standard protocols. All family members were genotyped for the CYP27B1 intron 6 (+2838) C/T polymorphism (Genbank accession no. AF072470) and CYP27B1 promoter (1260) C/A polymorphism (Genbank AB006987) aligned with promoter sequence from Kong et al. [15] using polymerase chain reaction followed, for the first polymorphism, by single strand conformation polymorphism (SSCP) analysis as described previously [16]. The second polymorphism was digested with restriction enzyme Tfi I (New England Bio Labs Beverly, MA) according to manufacturer's instructions for 4 h (RFLP). Digestion products were separated on 2.5% agarose gel. The gel was visualized by SYBR green ultraviolet illumination.

1.3. Statistical analyses

The transmission disequilibrium testing (TDT) [17] was used to detect preferential transmission of the RFLP alleles to affected subjects. The probability of a heterozygous parent transmitting either allele to the affected offspring is equal if there is no linkage between a certain allele and the disease in question. Deviation from the 50:50 transmission pattern suggests an association in the presence of linkage between a gene locus and this disease. In addition, haplotypes were designed and analysed using the extended transmission disequilibrium test (ETDT). The ETDT provides an overall test of transmission distortion for a multiallelic polymorphism either by considering all heterozygous parental genotypes separately (genotype-wise analysis) or by combining information across genotypes to detect effects due to particular alleles (allele-wise analysis sample is approximately X^2 distributed under the null hypothesis that there are no transmission differences between the subsets).

2. Results

No preferential transmission was observed in the polymorphism in the intron 6 (2838 T/C) in type 1 diabetes mellitus (Table 1). The allele C was 94 times transmitted (T) compared with 103 times not transmitted (NT) by heterozygous parents to affected offspring (TDT: $P = 0.524$). Similarly, we could not detect a difference in transmission between fathers and mothers for this polymorphism.

Table 1
Transmitted (T) and not transmitted (NT) CYP27B1 polymorphisms in intron 6 (2838 T/C) polymorphism in 223 families and promoter (−1260 A/C) polymorphism in 222 families with type 1 diabetes mellitus proband

Polymorphism	Families	Allele	T	NT	X^2_{TDT}	P_{TDT}
Intron 6 (2838)	223	C	94	103	0.4112	0.5214
		T	103	94		
Promoter (−1260)	222	A	74	105	5.3687	0.0205
		C	105	74		

Table 2
Combined transmission of CYP27B1 variants (−1260/2838) in 187 families with type 1 diabetes mellitus proband

	CT	CC	AT	AC
T	96	28	37	33
NT	63	28	60	43
X^2_{ETDT}	6.8491	0	5.4536	1.3158
P_{ETDT}	0.0089	1	0.0195	0.2513
ETDT				0.04316

On the other hand, the polymorphism in the promoter region showed a markedly reduced transmission of the allele A (74 transmitted versus 105 not transmitted) to affected offspring (TDT: $P = 0.0205$) (Table 1). The transmission of the allele A showed a tendency ($P = 0.0713$) to be less frequently transmitted by fathers of affected offspring compared with the mothers ($P = 0.2526$) (data not shown).

With these two polymorphisms we designed haplotypes as combination of alleles. The genotype and haplotype frequencies in the investigated population were in Hardy–Weinberg equilibrium.

Our findings showed that the haplotype CT (−1260 A/2838 T) was significantly more often transmitted (96 versus 63, ETDT: $P = 0.0089$) to affected offspring (Table 2).

Furthermore this effect was mainly caused by the paternal transmission of the haplotype CT to affected offspring (transmitted 59 (59.6%) versus not transmitted 40 (40.4%) by fathers $P = 0.052$) (data not shown).

While the haplotype AT (−1260 C/2838 T) was significantly less often transmitted by heterozygous parents to affected offspring 37 times (38.14%) versus 60 times (61.86%) (ETDT: $P = 0.0195$), maternal and paternal transmission frequencies did not differ for the transmission of these haplotypes.

3. Discussion

In this study of the CYP27B1 gene we analysed haplotype variants in Germans families with an offspring affected with type 1 diabetes mellitus. We did not observe an association of the polymorphism C/T in intron 6 with this disease. The polymorphism C/A in the promoter region [15,18] was markedly associated with diabetes mellitus in our population. The reduced transmission rate of allele A in the promoter region suggests a protective role for this

allele. Furthermore it appears, that the transmission of promoter allele A is predominantly reduced from fathers. Thus protection is mainly of paternal origin.

The haplotype CT (−1260 A/2838 T) was more often transmitted to affected offspring. This haplotype might confer susceptibility to type diabetes mellitus in our population. However, the haplotype AT (−1260 C/2338 T) showed a reduced transmission and might therefore be a protective factor in type 1 diabetes mellitus.

Since the single allele (−1260 C) and haplotypes (−1260 A/2838T) are divergent in their susceptibility effects, they may act as markers, indicating gene variation either distant and/or at the functional level.

$1,25(OH)_2D_3$, the most active natural Vitamin D metabolite, can prevent autoimmune diabetes und autoimmune thyroiditis in animal models [2]. Furthermore, it was demonstrated that the $1,25(OH)_2D_3$ has antiproliferative effects, e.g. in prostate and colon rectal cancer [19].

CYP27B1 is the key enzyme for active Vitamin D biosynthesis and plays an important role in the immunomodulatory properties of 1, $25(OH)_2D_3$. It was demonstrated that circulating levels of 1, $25(OH)_2D_3$ are not maintained in the mouse P450C1-gene knockout model [20].

As this polymorphism lies in the promoter region of the key enzyme of the Vitamin D metabolization, it may affect enzyme expression and thus the rate of final hydroxylation of $1,25(OH)_2D_3$. This polymorphism is a new genetic marker for type 1 diabetes mellitus.

Further genomic investigations in larger groups as well as functional studies are underway to confirm our findings.

References

[1] C. Mathieu, M. Waer, J. Laureys, O. Rutgeerts, R. Bouillon, Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D, Diabetologia 37 (1994) 552–558.

[2] C. Fournier, P. Gepner, M. Sadouk, J. Charreire, In vivo beneficial effects of cyclosporin A and 1,25-dihydroxyvitamin D3 on the induction of experimental autoimmune thyroiditis, Clin. Immunol. Immunopathol. 54 (1990) 53–63.

[3] J.M. Lemire, D.C. Archer, 1,25-Dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis, J. Clin. Invest. 87 (1991) 1103–1107.

[4] H.J. Hahn, B. Kuttler, C. Mathieu, R. Bouillon, 1,25-Dihydroxyvitamin D3 reduces MHC antigen expression on pancreatic beta-cells in vitro, Transplant. Proc. 29 (1997) 2156–2157.

[5] M. Thomasset, Vitamin D and the immune system, Pathol. Biol. (Paris) 42 (1994) 163–172.

[6] M.A. Atkinson, N.K. Maclaren, The pathogenesis of insulin-dependent diabetes, N. Engl. J. Med. 331 (1994) 1428–1436.

[7] J. Nerup, P. Platz, O.O. Anderseen, HLA antigens and diabetes mellitus, Lancet (1974) 864–866.

[8] L. Nistico, R. Buzzetti, L.E. Pritchard, B. Van der Auwera, C. Giovannini, E. Bosi, M.T. Larrad, M.S. Rios, C.C. Chow, C.S. Cockram, K. Jakobs, C. Mijovic, S.C. Bain, A.H. Barnett, C.L. Vandewalle, F. Schuit, F.K. Gorus, B.D. Reg, R. Rosi, P. Pozzili, J.A. Todd, The CTLA-4 gene region on chromosome 2q33 is linked to, and associated with type 1 diabetes, Hum. Mol. Genet. 5 (1996) 1075–1080.

[9] M.A. Pani, M. Knapp, H. Donner, J. Braun, M.P. Baur, K.H. Usadel, K. Badenhoop, Vitamin D receptor allele combinations influence genetic susceptibility to type I diabetes in Germans, Diabetes 49 (2000) 504–507.

[10] E. Hypponen, E. Laara, A. Reunanen, M.R. Jarvelin, S.M. Virtanen, Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study, Lancet 358 (2001) 1500–1503.

[11] S.K. Paulson, H.F. Deluca, Subcellular location and properties of rat renal 25-hydroxyvitamin D3-1 alpha-hydroxylase, J. Biol. Chem. 260 (1985) 11488–11492.

[12] D. Zehnder, R. Bland, E.A. Walker, A.R. Bradwell, A.J. Howie, M. Hewison, P.M. Stewart, Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in the human kidney, J. Am. Soc. Nephrol. 10 (2000) 2465–2473.

[13] D. Zehnder, R. Bland, M.C. Williams, R.W. McNinch, A.J. Howie, P.M. Stewart, M. Hewison, Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase, J Clin. Endocrinol. Metab. 86 (2001) 888–894.

[14] W.L. Miller, A.A. Portale, Vitamin D 1alpha-hydroxylase, Trends Endocrinol. Metab. 11 (2000) 315–319.

[15] X.F. Kong, X.H. Zhu, Y.L. Pei, D.M. Jackson, M.F. Holick, Molecular cloning, characterization, and promoter analysis of the human 25-hydroxyvitamin D3-1alpha-hydroxylase gene, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 6988–6993.

[16] S.J. Smith, A.K. Rucka, J.L. Berry, M. Davies, S. Mylchreest, C.R. Paterson, D.A. Heath, M. Tassabehji, A.P. Read, A.P. Mee, E.B. Mawer, Novel mutations in the 1alpha-hydroxylase (P450c1) gene in three families with pseudovitamin D-deficiency rickets resulting in loss of functional enzyme activity in blood-derived macrophages, J. Bone Miner. Res. 14 (1999) 730–739.

[17] P.C. Shan, D. Curtis, An extended transmission/disequilibrium test (TDT) for multi-allele marker loci, Am. J. Hum. Genet. 59 (1995) 323–336.

[18] G.A. Hawkins, S.D. Cramer, S.L. Zheng, S.D. Isaacs, K.E. Wiley, B.L. Chang, E.R. Bleecker, P.C. Walsh, D.A. Meyers, W.B. Isaacs, J. Xu, Sequence variants in the human 25-hydroxyvitamin D3 1-alpha-hydroxylase (CYP27B1) gene are not associated with prostate cancer risk, Prostate 53 (2002) 175–178.

[19] P. Bareis, G. Bises, M.G. Bischof, T. Manhardt, R. Bland, H.S. Cross, M. Peterlik, 25-hydroxy-vitamin D metabolism in human colon cancer cells during tumor progression, Biochem. Biophys. Res. Commun. 285 (2001) 1012–1017.

[20] O. Dardenne, O.J. Prud'homme, A. Arabian, F.H. Glorieux, R. St-Arnaud, Targeted Inactivation of the 25-hydroxyvitamin D(3)-1(alpha)-hydroxylase gene (CYP27B1) creates an animal model of pseudovitamin D-deficiency rickets, Endocrinology 142 (2001) 3135–3141.