Association between (AC)_n dinucleotide repeat polymorphism at the 5'-end of the aldose reductase gene and diabetic nephropathy: a meta-analysis

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Abstract

Association between the (AC)_n dinucleotide repeat polymorphism at the 5'-end of the aldose reductase gene and the occurrence of diabetic nephropathy was conducted. We examined eight studies consisting of ten Caucasian type 1 diabetes mellitus case-control comparisons and eight studies consisting of nine type 2 diabetes mellitus case-control comparisons, which were based on our inclusion criterion and available in the literature. The meta-analysis demonstrated a large heterogeneity among the studies on the type 1 diabetic subjects and a significant association was observed between the (AC)_n dinucleotide repeat polymorphism at the 5'-end of the aldose reductase gene and diabetic nephropathy. The Z-2 allele appeared to be a genetic risk factor for susceptibility to diabetic nephropathy with a random effects odds ratio (OR) of 1.40 (95% confidence interval, CI (1.07, 1.84)). The Z+2 allele showed a protective effect on diabetic nephropathy with a random effects OR of 0.77 (95% CI (0.65, 0.91)). The meta-analysis, however, showed no association between the genetic polymorphism and diabetic nephropathy in type 2 diabetic subjects. Neither the risk Z-2 allele nor the protective Z+2 allele in type 1 diabetic subjects appeared to have an effect on nephropathy in type 2 diabetic subjects, while their fixed effects OR was 1.09 (95% CI (0.96, 1.22)) and 0.88 (95% CI (0.67, 1.15)) respectively. The current meta-analysis demonstrated a correlation between the (AC)_n dinucleotide repeat polymorphism and the occurrence of diabetic nephropathy in Caucasian type 1 diabetic subjects in contrast to type 2 diabetic subject population in which such an association could not be demonstrated.

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Introduction

Diabetic nephropathy is a severe long-term complication of both type 1 and 2 diabetes mellitus, which is considered as one of the leading causes of end-stage renal disease requiring either dialysis or renal transplantation. Although the precise process that causes diabetic nephropathy remains unknown, some studies suggested that environmental risk factors, including the duration of disease, quality of glycaemic control, hypertension and hyperlipidaemia, contribute to the pathogenesis of the microvascular damage, which is associated with diabetic nephropathy. On the other hand, accumulated evidence supported the role of genetic factors in the susceptibility to diabetic nephropathy in family-based, case-control and epidemiological studies (Chowdhury *et al.* 1999).

An increased flux of glucose through the polyol pathway, in which aldose reductase (ALR2) is the first and rate-limiting enzyme, is one of the main mechanisms implicated in the progression of diabetic

microvascular complications (Srivastava *et al.* 2005). The ALR2 gene localized on chromosome 7q35 extends over 18 kilobases and contains ten exons that code for a 1384 bp mRNA. An (AC)_n dinucleotide repeat polymorphism in the promoter region and some single nucleotide polymorphisms (SNPs) have been identified in the ALR2 gene (Demaine 2003). After Ko *et al.* (1995) initially reported that the (AC)_n repeat polymorphism was associated with early onset of retinopathy in Chinese patients with type 2 diabetes, the association between the ALR2 gene polymorphisms and chronic microvascular complications in patients either with type 1 or type 2 diabetes had been examined in several populations; however, some studies reported inconsistent observations (Heesom *et al.* 1997, Dyer *et al.* 1999, Kao *et al.* 1999, Maeda *et al.* 1999).

The most common allele has been designated as Z, which consists of (AC)₂₄ repeats, whilst the Z-2 allele and conversely the Z+2 allele contain (AC)₂₃ and (AC)₂₅ repeats respectively. Because of the low frequencies of the variant ALR2 microsatellite alleles,

only the Z-2 allele was considered as a risk allele, whereas the Z+2 allele was considered a protective allele in some eligible studies. To address the ambiguities raised by the inconsistencies among molecular genetic studies, it is recommended to apply statistical analyses-mediated meta-analyses. Herein, we examined whether the aggregate evidence across all available studies provides evidence of statistical significance. Thus, to evaluate the putative association between diabetic nephropathy and the *ALR2* gene (AC)_n repeat allele, we applied meta-analysis to all available and eligible case-control studies.

Materials and methods

Eligible criteria

We considered all studies that examined the association of the *ALR2* polymorphism with diabetic nephropathy. Sources included Medline and Embase (last search update was performed in April 2007). The search strategy was based on combinations of the terms diabetic nephropathy, aldose reductase, polymorphism, allele and genetics. The references cited in the retrieved publications were also screened (the final update was April 2007). The eligible criteria included case-control study that determined the distribution of *ALR2* genotypes in diabetic nephropathy cases as well as in a concurrent control group of microvascular complication-free subjects with diabetes using a molecular method for genotyping. We excluded studies or subgroups thereof with familial linkage designs.

Data extraction

Two investigators independently extracted the data and reached consensus on all items. In all these identified studies, diabetic subjects with nephropathy were considered as 'cases' while those subjects with long-term diabetes without nephropathy served as 'controls'. In addition, the following information was sought from each study: authors, journal and year of publication, country of origin, number of cases and controls, racial descent of the study population and frequencies of alleles. For studies where subjects of different racial descent were included, data were extracted separately for each race. Furthermore, we examined whether matching had been used, whether there was specific mention of blinding of the personnel who performed the genotyping to the clinical status of the subjects and whether the genotyping method had been validated. For publications that did not contain complete information on the distribution of genotypes in cases and controls, we obtained the pertinent data directly from primary study investigators.

Meta-analysis

In the meta-analysis, diabetic nephropathy patients were compared with diabetic controls without nephropathy for the frequencies of the Z-2 allele (reported as a risk allele) and the frequencies of the Z+2 allele (reported as a protective allele) in some studies, including both type 1 and 2 diabetic subjects. Because the case-control studies were involved, the random effects odd ratio (OR) was used as the metric of choice. For each typical contrast, we estimated the between-study heterogeneity across all eligible comparisons using χ^2 -based *Q* statistics. Heterogeneity was considered significant for $P < 0.05$. Data were combined using both fixed effects (Mantel-Haenszel algorithm) and random effects (Der Simonian and Laird algorithm). Random effects incorporate an estimate of the between-study variance and tend to provide wider confidence intervals (CIs), when the results of the constituent studies differ among themselves. In the absence of between-study heterogeneity, the two methods provide identical results. Random effects are more appropriate when heterogeneity is present.

Inverted funnel plots were used to evaluate whether the magnitude of the observed association was related to the variance of each study. We also performed cumulative meta-analysis to evaluate whether the summary OR and its 95% CIs of the frequency of the Z-2 and Z+2 alleles changed over time as more data accumulated. Analyses were conducted using the SPSS, version 10.0, software (SPSS, Inc., Chicago, IL, USA), and Review Manage (version 4.2; Oxford, England). All *P* values are two tailed.

Results

Eligible studies on diabetic nephropathy in type 1 diabetes mellitus

Eight studies (Heesom *et al.* 1997, Shah *et al.* 1998, Dyer *et al.* 1999, Isermann *et al.* 2000, Moczulski *et al.* 2000, Neamat-Allah *et al.* 2001, Ng *et al.* 2001, Lajer *et al.* 2004) evaluated the association between the *ALR2* gene (AC)_n polymorphism and the occurrence of diabetic nephropathy in type 1 diabetic subjects. In the study by Shah *et al.* (1998), one subgroup from the US and the other from Italy were included. Neamat-Allah *et al.* (2001) also included two distinct racial descents, one from North Ireland and the other from the UK. Thus, subjects in seven out of eight studies were all Caucasians, and in the study conducted by Moczulski *et al.* (2000), 90% of the subjects were Caucasians. Specific matching for age at diagnosis or examination and duration of diabetes was described in six comparisons (Heesom *et al.* 1997, Shah *et al.* 1998, Moczulski *et al.* 2000, Neamat-Allah *et al.* 2001, Lajer *et al.* 2004),

and in the other four comparisons (Dyer *et al.* 1999, Isermann *et al.* 2000, Neamat-Allah *et al.* 2001, Ng *et al.* 2001), this information was not mentioned. Detection of albumin in urine was used to diagnose for diabetic nephropathy in all of the eligible studies. Appropriate molecular methods for genotyping were used in all of the eligible studies and the allele assignment was verified by an independent observer in the majority of the eligible studies.

Eligible studies about diabetic nephropathy in type 2 diabetic subjects

Eight studies (Maeda *et al.* 1999, Moczulski *et al.* 1999, Neamat-Allah *et al.* 2001, Liu *et al.* 2002, Park *et al.* 2002, Wang *et al.* 2003, Zhao *et al.* 2004, Wolford *et al.* 2006) about the relationship between the ALR2 gene (AC)_n polymorphism and diabetic nephropathy susceptibility were identified. The study conducted by Neamat-Allah *et al.* (2001) contained subjects of two different ethnic descents. Five comparisons involved Asian subjects, including Chinese (Liu *et al.* 2002, Wang *et al.* 2003, Zhao *et al.* 2004), Japanese (Maeda *et al.* 1999) and Korean (Park *et al.* 2002), two of them involved Pima Indian (Neamat-Allah *et al.* 2001, Wolford *et al.* 2006) and the other two involved Caucasian (Moczulski *et al.* 1999, Neamat-Allah *et al.* 2001). Age at diagnosis or examination and duration of diabetes were matched in five comparisons (Maeda *et al.* 1999, Moczulski *et al.* 1999, Park *et al.* 2002, Zhao *et al.* 2004, Wolford *et al.* 2006), and in three comparisons (Neamat-Allah *et al.* 2001, Liu *et al.* 2002, Wang *et al.* 2003), the subjects with nephropathy were older and/or had longer duration of diabetes than their controls. Information of age and diabetic duration in the Pima Indian group from the US (Neamat-Allah *et al.* 2001) was not mentioned. In four studies (Liu *et al.* 2002, Park *et al.* 2002, Wang *et al.* 2003, Zhao *et al.* 2004), subjects with nephropathy exhibited a higher proportion of hypertension or average level of blood pressure than non-nephropathy controls. Except for the study by Zhao *et al.* (2004) in which autopsy was used, diabetic nephropathy was diagnosed via the detection of the level of albumin in urine. Appropriate molecular methods for genotyping were used in all of the eligible studies and the allele assignment was verified by an independent observer in the majority of the eligible studies.

Meta-analysis databases

A database was established according to the extracted information from each eligible study. Table 1 lists the characteristics of the eligible studies involving type 1 diabetic subjects, and Table 2 lists the characteristics of the eligible studies involving type 2 diabetic subjects.

Table 1 Characteristics of eligible studies for diabetic nephropathy in type 1 diabetic patients

References	Country (region)	Racial descent	Number of subjects		Duration of diabetes (years)		Z - 2 frequency		Z + 2 frequency	
			DN (+)	DN (-)	DN (+)	DN (-)	DN (+)	DN (-)	DN (+)	DN (-)
Heesom <i>et al.</i> (1997)	UK	Caucasian	75	43	29.3 ± 9.6	31.1 ± 9.4	46/150	16/86	18/150	23/86
Shah <i>et al.</i> (1998)	USA	Caucasian	31	27	18.3 ± 10.4	17.3 ± 10.5	45/60	24/54	2/60	1/54
Shah <i>et al.</i> (1998)	Italy	Caucasian	21	25	25.3 ± 7.7	25.4 ± 7.4	26/42	10/50	-	-
Dyer <i>et al.</i> (1999)	UK	Caucasian	211	129	Not mentioned	Not mentioned	150/422	96/258	54/422	31/258
Isermann <i>et al.</i> (2000)	Germany	Caucasian	47	42	≥ 10	≥ 20	41/94	27/84	15/94	13/84
Moczulski <i>et al.</i> (2000)	USA	90% Caucasian	221	193	24.9 ± 7.7	23.3 ± 6.4	168/442	111/386	75/442	71/386
Neamat-Allah <i>et al.</i> (2001)	Ireland	Caucasian	126	116	Not mentioned	≥ 20	75/202	85/220	25/202	41/220
Neamat-Allah <i>et al.</i> (2001)	England	Caucasian	77	85	26.3 ± 8.1	29.0 ± 9.9	45/134	59/156	23/134	22/156
Ng <i>et al.</i> (2001)	Australia	Caucasian	30	49	< 15	≥ 15	9/30	26/98	6/30	15/98
Lajer <i>et al.</i> (2004)	Denmark	Caucasian	431	468	27.9 ± 8.7	26.8 ± 9.9	336/862	341/936	96/862	157/936

DN (+), patients with diabetic nephropathy and DN (-), patients without diabetic nephropathy.

Table 2 Characteristics of eligible studies for diabetic nephropathy in type 2 diabetic patients

References	Country (region)	Racial descent	Number of subjects		Duration of disease (years)		Z-2 frequency		Z+2 frequency	
			DN (+)	DN (-)	DN (+)	DN (-)	DN (+)	DN (-)	DN (+)	DN (-)
Moczulski et al. (1999)	Poland	Caucasian	295	179	16.8±5.4	14.2±6.5	201/590	124/358	46/590	30/358
Maeda et al. (1999)	Japan	Japanese	216	123	13.8±9.0	12.4±6.6	112/432	66/246	96/432	43/246
Liu et al. (2002)	China	Chinese	136	128	10.5±7.0	8.6±6.6	72/274	45/256	60/274	94/256
Neamat-Allah et al. (2001)	USA	Pima Indian	182	145	Not mentioned	≥ 10	96/348	66/282	26/348	20/282
Neamat-Allah et al. (2001)	England	Caucasian	85	148	12.6±8.1	8.9±8.7	63/324	103/548	19/324	34/548
Park et al. (2002)	Korea	Korean	48	38	16.0±6.6	14.4±4.2	31/96	19/76	16/96	18/76
Wang et al. (2003)	China	Chinese	280	458	8.1±6.3	4.2±4.7	131/560	213/916	166/560	277/916
Zhao et al. (2004)	China	Chinese	135	51	10.6±1.2	12.1±3.0	80/270	31/102	-	-
Wolford et al. (2006)	USA	Indian	104	104	20.4±7.1	20.7±5.5	61/208	57/208	-	-

DN (+), patients with diabetic nephropathy and DN (-), patients without diabetic nephropathy.

Up to ten alleles of the 5'-end ALR2 locus have been detected in different populations. Because of the low frequencies of the variant ALR2 microsatellite alleles, only the Z-2 allele was considered as a risk allele and the Z+2 allele was considered as a protective allele in some eligible studies. There were a total of ten subjects with type 1 diabetes mellitus with nephropathy case-control comparisons, including 1270 cases and 1177 controls. Nine comparisons consisting of 1481 cases and 1374 controls were about the relationship between the gene polymorphism and nephropathy in type 2 diabetes.

Quantitative synthesis

There was a significant between-study heterogeneity in association of the Z-2 allele with nephropathy among the studies on type 1 diabetic ($\chi^2=32.80$, $P=0.0001$). Four individual comparisons had positive results and the remaining six had negative results. The summary OR for association of Z-2 allele with diabetic nephropathy by random effects was 1.40 and was statistically significant (95% CI (1.07, 1.84), $P=0.01$). The result of the meta-analysis on the association between the Z+2 allele and diabetic nephropathy demonstrated a fixed effects summary OR of 0.77 (95% CI (0.65, 0.91), $P=0.002$) without between-study heterogeneity ($\chi^2=14.52$, $P=0.07$; Fig. 1). Conversely, in studies involving type 2 diabetic subjects, there was no significant association between the Z-2 allele and susceptibility of diabetic nephropathy. Although the subjects were from different ethnic groups, there was no significant between-study heterogeneity among individual studies ($\chi^2=6.94$, $P=0.54$). The fixed effects combined OR of Z-2 to susceptibility was 1.09 (95% CI (0.96, 1.22), $P=0.18$), and the random effects combined OR of Z+2 allele was 0.88 (95% CI (0.67, 1.15), $P=0.35$) with significant heterogeneity among individual studies ($\chi^2=15.56$, $P=0.02$; Fig. 2).

Potential bias

Cumulative meta-analysis for the Z-2 allelic frequency showed that the pooled OR of Z-2 to susceptibility to diabetic nephropathy in type 1 diabetic subjects decreased from 3.08 at the end of 1998, 2.05 at the end of 1999, 1.94 at the end of 2000 and to 1.50 at the end of 2001. In type 2 diabetic subjects, the pooled OR of Z-2 to susceptibility to diabetic nephropathy was stable at a fixed level of around 1.0 (0.97 at the end of 1999, 1.04 at the end of 2001, 1.13 at the end of 2002, 1.09 at the end of 2003 and 1.08 at the end of 2004). The inverted funnel plots are shown in Figs 3 and 4.

Figure 1 Meta-analysis of the association between the *ALR2* gene (AC)_n polymorphism and susceptibility to diabetic nephropathy in type 1 diabetic subjects. Each study is shown by a point estimate of the OR and the accompanying 95% CI. (A) Z-2 allele and (B) Z+2 allele. DN, diabetic nephropathy.

Discussion

ALR2 is a monomeric-reduced NADPH-dependent enzyme and a member of aldo-keto reductase superfamily. The enzyme was first described in 1956 by Hers (1956) to exhibit a glucose-reducing activity. It is the first enzyme in the polyol pathway and converts glucose to sorbitol, which is subsequently transformed to fructose by sorbitol dehydrogenase. It has been demonstrated that during a hyperglycaemic event, the elevated glucose level enhances ALR2 activity, thereby increasing the glucose flux through the polyol pathway that impacts other NADPH-dependent enzymes shown to play a key role in long-term diabetic onset complications such as neuropathy and nephropathy (Hodgkinson *et al.* 2001). Furthermore, in peripheral blood monocytes from patients with diabetic nephropathy, the ALR2 mRNA and ALR2 protein levels were significantly elevated (Shah *et al.* 1997). Therefore, inhibition of ALR2 expression may offer patients suffering from diabetes mellitus a viable therapeutic measure against the debilitating pathologies associated with chronic hyperglycaemia. Recently, some genetic variations in the *ALR2* gene located on chromosome 7q35 were reported, including two common

polymorphisms such as an (AC)_n dinucleotide repeat microsatellite and a C(-106)T SNP, and examined for their putative association with diabetic nephropathy in several populations (Demaine 2003).

Here, we conducted a meta-analysis and showed that a small, but statistically significant association exists between the occurrence of diabetic nephropathy and (AC)_n microsatellite polymorphism at the 5'-end of ALR2 in type 1 diabetic subjects. This association was statistically significant in four out of ten individual eligible case-control comparisons. The Z-2 allele is likely to be a genetic risk factor (OR=1.40), while the Z+2 allele might exhibit some protective role for susceptibility to diabetic nephropathy in type 1 diabetic patients (OR=0.77). However, there has been some heterogeneity about Z-2 allele among the results of various studies in type 1 diabetic subjects ($\chi^2 = 32.80$, $P = 0.0001$). Furthermore, the meta-analysis of nine case-control comparisons in type 2 diabetic subjects demonstrated no relationship between diabetic nephropathy and the gene polymorphism of ALR2. Neither did the Z-2 allele seem to exhibit a risk factor nor did the Z+2 allele appear to play a role protective to susceptibility of the microvascular complication in type 2 diabetic subjects. There was no evidence for

Figure 2 Meta-analysis of the association between the *ALR2* gene (AC)_n polymorphism and susceptibility to diabetic nephropathy in type 2 diabetic subjects. Each study is shown by a point estimate of the OR and the accompanying 95% CI. (A) Z-2 allele and (B) Z+2 allele. DN, diabetic nephropathy.

heterogeneity of the OR across eligible studies about type 2 diabetic subjects.

Although the exact mechanism underlying the effect of the genetic polymorphism on the increased susceptibility for nephropathy is yet not well understood, some studies have demonstrated that genetic polymorphisms were associated with an elevated ALR2 transcriptional activity and ALR2 protein level in different populations. For example, Shah *et al.* (1998) reported that the Z-2 allele was associated with a significantly higher ALR2 mRNA level as well as enzyme activity in subjects with nephropathy compared with subjects with neutral or

protective allelic combinations in a Caucasian subject population, while Zou *et al.* (2000) reported similar observations in a population of Chinese subjects with type 2 diabetes. With respect to the C(-106)T SNP, increased expression of ALR2 has been demonstrated in human retinal pigment epithelial cell lines that are homozygous C-106 alleles as compared with those with the T-106 or heterozygous for these alleles (Stevens *et al.* 2000). Some of the results of linkage disequilibrium between the (AC)_n dinucleotide repeat microsatellite and the C(-106)T SNP were conflicting. Recently, Yang *et al.* (2003) reported that a haplotype

Figure 3 Funnel plot of frequencies of allele by their s.e.m. in eligible studies about type 1 diabetes. (A) Z-2 allele and (B) Z+2 allele.

Figure 4 Funnel plot of frequencies of allele by their s.e.m. in eligible studies about type 2 diabetes. (A) Z-2 allele and (B) Z+2 allele.

consisting of the Z-2 and C alleles corresponded to the highest relative transcriptional activity as compared with other haplotypes in HepG2 cells, suggesting that these haplotypes may have functional properties that increased the susceptibility to microvascular complications.

We estimated between-study heterogeneity using χ^2 -based Q -statistics and the study magnitude was expressed by study weight (inverse of variance). It was observed that the heterogeneity among the individual studies should lead to extra caution in the meta-analysis of studies on type 1 diabetic patients. With the exception of one study (Moczulski *et al.* 2000), the ethnicity of 10% of subjects (about 40 subjects) was not mentioned. The remaining subjects that participated in the eligible studies analysed herein were all Caucasian. Consequently, the impact of ethnicity was minor. It was noticeable that three earlier comparisons completed in 1997 and 1998 were relatively smaller in size with a study weight of 8.4, 6.8 and 5.5% respectively, but the pooled OR for those studies was relatively higher than those obtained in more recent studies. The significant differences in the genetic effects observed in large versus small studies or in initial versus more recent studies appeared to be common in same genetic association studies. Recently, Ioannidis *et al.* (2003) examined 55 meta-analyses involving genetic association studies and found that only 16% of the meta-analyses were subsequently replicated with formal statistical significance without heterogeneity or bias. Typically, large studies and subsequent research suggested weak or no association at all, compared with strong associations proposed by smaller and/or initial studies. Publication bias or time-lag bias should be considered. In this respect, small studies that show a statistically significant result tend to be published more easily and in a shorter time frame than other studies reporting 'negative' results and similar sample size and quality.

Given the wide ethnicity range of type 2 diabetic subjects, there was no heterogeneity in type 2 diabetic

case-control comparison studies. When the results of the two largest individual studies reported by Moczulski *et al.* (1999; weight=20.03%) and Wang *et al.* (2003; weight=24.37%) were compared, it was observed that the ORs of Z-2 allele to the susceptibility of diabetic nephropathy in Polish Caucasians and Hong Kong Chinese were similar to each other, but none of those were statistically significant. The overall data demonstrate that the Z-2 allele in ALR2 (AC)_n microsatellite polymorphism is not a genetic risk factor in the progress of nephropathy in type 2 diabetic subjects. The present meta-analysis generated a conflicting result in regard to the association between diabetic nephropathy and the gene polymorphism of ALR2 in different types of diabetes, which can be explained as follows. First, although hyperglycaemia is the common clinical characteristic of both type 1 and 2 diabetes, both types of diabetes represent a distinct disease process. Type 1 diabetes is mainly characterized by the destruction of islet β -cells by the immune system, a process determined by the activity of major histocompatibility complex (MHC)-restricted T lymphocytes. The MHC locus (human leukocyte antigen (HLA) region) is the major susceptibility interval of the disease (Park & Eisenbarth 2001). Type 2 diabetes represents a complex and heterogeneous group of disorders, which has genetic heterogeneity underlying the common phenotypic endpoint of elevated blood glucose level, likely involving a large number of contributing genes (Tusié Luna 2005). Second, the variability in subject selection criteria is a possibly important confounding factor in studies of the two types of diabetes. In the case-control comparisons of type 1 diabetic subjects, the main clinical characteristics, including age at diagnosis or examination and the duration of diabetes, were well matched among each other. However, in some studies involving type 2 diabetic subjects, the subjects with nephropathy were older and the respective duration of diabetes was longer than their counter partners. Third, other causes, including the level of blood pressure and

lipid levels, in addition to hyperglycaemia may contribute to the level of protein urea in type 2 diabetic patients. In several studies, either the average level of blood pressure or proportion of patients with hypertension in cases with nephropathy was obviously higher than those measured in the control subjects without nephropathy.

Furthermore, limitations of retrospective case-control studies should be considered to explain our observations. Olmos *et al.* (2000) reported in a progressive study that subjects with the Z-2 allele of the (AC)_n repeat had 8.9 times faster progression of another microvascular complication, retinopathy, than subjects with other alleles. In another progressive study (Olmos *et al.* 2006), it was observed that the other locus C(-106)T polymorphism in ALR2 has a similar association with retinopathy. This strongly suggests a higher proportion of microvascular complications, including end-stage renal disease and even a higher mortality at the earlier stage of diabetes, in patients with risk genes as compared with those with protective genes. Niskanen *et al.* (1996) reported that during a 10-year follow-up study, there was a markedly increased cardiovascular mortality among type 2 diabetic patients with albuminuria. As most of the eligible studies included in the current meta-analysis selected subjects with a long-term duration of diabetes, it is likely that the association between the gene polymorphism and nephropathy is underestimated and some information might be overlooked. It is therefore advisable to conduct new studies on the association between genetic alterations and the occurrence of diabetes employing larger subject populations with different ethnicities.

In conclusion, the present meta-analysis suggested an association between the (AC)_n microsatellite polymorphism at the 5'-end of the ALR2 gene and nephropathy in Caucasian subjects with type 1 diabetes mellitus. In addition, it was observed that the Z-2 allele is a genetic risk factor for diabetic nephropathy, while the Z+2 allele exhibits some protective effect in the process of the complication. There was no association when the same relationships were considered in type 2 diabetic subjects of various ethnic groups.

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