2007-10

Interleukin 12B gene polymorphism and apparent resistance to HCV infection

Hegazy, DM

http://hdl.handle.net/10026.1/10096

10.1002/hep.22022
Hepatology

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.
Polymorphic differences in the SOD-2 gene may affect the pathogenesis of nephropathy in patients with diabetes and diabetic complications

Annwyne Houldsworth a,⁎, Andrea Hodgkinson a, Steve Shaw b, Ann Millward a,c, Andy G. Demaine a

Article info

Article history:
Received 25 October 2014
Accepted 2 April 2015
Available online xxxx

Keywords:
SOD-2
Diabetes mellitus
Nephropathy
Antioxidants
Oxidative stress

Abstract

The effective treatment of diabetes and the prevention of diabetic complications may be improved by a better understanding of the antioxidant function of intracellular defences against oxidative stress. Polymorphisms in antioxidant genes may determine cellular oxidative stress levels as a primary pathogenic role in diabetes and/or in its complications. SOD-2 was investigated in patients with type 1 diabetes mellitus (T1DM) to ascertain if specific genotypes have any protective influences in the pathogenic mechanisms in diabetes and/or in several different complications, including retinopathy, nephropathy and diabetic controls compared to normal healthy controls.

Method: 278 (136M:142F) T1DM patients and 135 (72M:63F) normal, healthy controls were investigated for SOD-2 polymorphism in the mitochondrial targeting sequence with Ala/Val (C-9T) substitution.

Results: A significant difference in the C-9-T genotype was observed between patients and normal controls but not between diabetic controls and patients with complications. There were significantly more of the diabetic control (DC, n = 62) group (11.3%) than the patients with diabetic nephropathy (DN, n = 73) (1.4%) with the CC genotype (p = 0.03 and χ² = 4.27, OR = 9.16 (1.08 < OR < 20.04)). Further significance was found between normal healthy controls (11.4%) and patients with nephropathy (1.4%) with the genotype CC (p = 0.03, χ² = 4.68, OR = 0.11 (0.00 < OR < 0.87)).

No significant differences were found between these groups for the allelic frequency or between the different complication groups after correction for the number of groups.

All groups were in Hardy Weinberg equilibrium.

Conclusion: The SNP in SOD-2 results in a substitution of C to T, which causes an amino acid change from alanine to valine. The variation in the SOD-2 leader signal affects the processing efficiency of the enzyme. A significantly greater proportion of the diabetic control group had the CC genotype suggesting antioxidant protection against diabetic nephropathy. The healthy control group also had a higher incidence of the protective genotype, which may suggest protective influences from the antioxidant gene in the CC form.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Effective treatment of diabetes and the prevention of microvascular complications in both type 1 and type 2 diabetes may be improved by a better understanding of the antioxidant function of intracellular defences against oxidative stress (OS) (Baynes, 1991; Michels, 1994; Naudi et al., 2012). The generation of dangerous reactive intermediates, known as reactive oxygen species (ROS), is a by-product of normal aerobic metabolism and plays an important role in the pathogenesis of the long-term complications of diabetes. All the hyperglycaemia-induced pathways produce ROS that in turn act as intercellular signals to continue the pathway cycles (Brownlee, 2001; Brownlee, 2005; Salahudeen et al., 1997). Hyperglycaemia induced metabolic imbalances, involve ROS and cause OS (Cohen et al., 2012). Further, increase in the cytosolic ratio of free NADH/NAD+ results in pseudohypoxia, which play important roles in mediating early neural and vascular dysfunction. There are similar mechanisms involved in true hypoxia (Guiligano et al., 1996).

It has been reported that there is an early molecular event involving an increase in mitochondrial mass and mtDNA content in response to exogenous and endogenous oxidative stress (Lee et al., 2000). ROS from mitochondria induce cyclo-oxygenase gene expression and suggests a potential role of ROS in diabetic nephropathy (Kiritsoti et al., 2003).
Enzymic systems are part of a cell’s line of defence against the lethal or mutagenic damage caused by OS by removing ROS from the cell and involve enzymes such as catalytic superoxide dismutases (SOD): copper/zinc-dependent SOD (CuZnSOD) in the cytosol, manganese-dependent SOD (MnSOD) in the mitochondria and catalase (CAT) in the cytosol and peroxisomes (Michiels et al., 1994).

SOD catalyses the dismutation of hydrogen peroxide and superoxide into oxygen, enabling cell repair and reducing the damage inflicted by OS. Hydrogen peroxide is further broken down to water by catalase or peroxidase. ROS induces this antioxidant enzyme expression in tissues but defective production or action could result in OS and ROS tissue damage ultimately leading to cell death (Beckman and Ames, 1998).

Decreased levels of SOD-2 may contribute to the development of certain diseases. Mice without the gene that encodes SOD-2 die 10 days after birth with cardiomyopathy and lipid accumulation in the liver and skeletal muscles (Li et al., 1995a). In animal cells decreased SOD-2 and catalase levels were observed in breast cancer, adenomas and leukaemia (Sun et al., 1993). A polymorphism in SOD-2 (Ala16Val) was shown to modulate the import of human SOD-2 into rat liver mitochondria (Sutton et al., 2003).

Differences in antioxidant expression may explain a predisposition of a patient with diabetes to diabetic complications such as nephropathy, neuropathy, cardiovascular disease or retinopathy. ROS are increasingly formed in diabetes mellitus by the auto-oxidation of glucose and glycosylated proteins. Hyperglycaemia leads to the activation of the polyol pathway and contributes to the formation of triose phosphate and its auto-oxidation which results in α-oxaldehyde and H2O2 (Negre-Salvyare et al., 2008). Defective antioxidant expression may be partly due to polymorphic differences in the genes encoding the antioxidant enzymes. SOD-2 expression was decreased in patients with T1DM and patients with T2DM and was determined by the SOD-2 genotype (Flekac et al., 2008). There is growing evidence to suggest that polymorphisms in the promoter region of the aldose reductase gene (ALR2) are associated with susceptibility to nephropathy, retinopathy and neuropathy and differing levels of the gene’s expression (Demaine et al., 2000; Heesom et al., 1998). Antioxidant responses to hyperglycaemia have shown that SOD-2 responses did not change between diabetic patient complication groups or in normal controls (Hodgkinson et al., 2003). Although SOD-2 is involved in controlling dioxyn toxicity in an organelle of extreme oxidative load, the mitochondria, decreased levels of SOD-2 may contribute to the development of certain diseases. Normalising mitochondrial superoxide production blocks three pathways of hyperglycaemic damage (Nishikawa et al., 2000). Antioxidant therapy has proved promising in preventing the onset of diabetic heart disease (Wold et al., 2005).

Various animal studies have shown the effects of deficient antioxidant function and mice without the gene that encodes SOD-2 die 10 days after birth with cardiomyopathy and lipid accumulation in the liver and skeletal muscles (Sentman et al., 2006). In other animal cells, decreased SOD-2 and catalase levels were observed in breast cancer, adenomas and leukaemia (Shimizu et al., 2010; Salvemini et al., 1999). In mouse models with heart muscle tissue specific Mn-SOD conditional knockout mice that displayed no complications after a 20 year duration of T1DM.

Treatment of diabetic animals with SOD/catalase mimetics prevents the diabetes induced oxidative inactivation of ENOS and aortic prostacyclin synthase (both antiatherogenic enzymes), which are usually induced during ROS overproduction during hyperglycaemia (Tiwari et al., 2009; Vojtková et al., 2013).

The SOD-2 targeting signal sequence polymorphism has been identified on chromosome 6q25 and may be in linkage with the susceptibility genes IDDM5 (6q22) and IDDM8 (6q27), discovered by Todd when screening the human genome for T1DM related genes (Todd and Farrall, 1996). A polymorphism in the mitochondrial targeting signal sequence could affect the transport of the enzyme through the mitochondrial membrane and a defect may alter the membrane receptor recognition site resulting in less of the enzyme protein entering the cell thus lowering the antioxidant response to oxidative stress.

Ala/Ala homozygotes for a polymorphism in the SOD-2 mitochondrial targeting sequence (Ala-9 Val substitution) has been found to be significantly lower for patients with diabetic nephropathy (DN) than patients without nephropathy whereas the Val/Val genotype was significantly higher in the DN group in a Russian cohort (Chistyakov et al., 1999). Different results have been observed in different populations and ethnic differences have been observed with this polymorphism (Van Landeghem et al., 1999). A recent study shows that impaired oxidative balance may have a prognostic significance on disease activity and determines the severity or the disease outcome in chronic HCV patients (Houldsworth et al., 2014).

Manganese superoxide dismutase (SOD-2) is found in the mitochondria in nearly all cells and with a molecular mass of 40,000 kDa it consists of four subunits each of which probably contains a manganese atom. The localisation of SOD-2 to chromosome 6 (6q25) (Todd and Farrall, 1996) and some of the features of the SOD-2 gene are typical of housekeeping genes (Church et al., 1992; Dynan et al., 1986).

Mutations in the SOD-2 gene have also been associated with idiopathic cardiomyopathy (IDC), sporadic motor neuron disease, and cancer (Muller et al., 2007; Li et al., 1995b; Van Remmen et al., 2003). The role of mitochondrial oxidative stress by ROS is associated with the pathogenesis of target organ damage in hypertension and the role of mitochondrial antioxidants such as SOD-2 shows promising strategies to reduce the development of endothelial dysfunction, cardiac hypertrophy, and renal and cerebral damage in hypertension (Rubattu et al., 2014).

2. Materials and methods

2.1. Patients included

Type 1 diabetic cases were included for the SOD-2 polymorphism analysis. Cases were subdivided into groups according to the presence or absence of microvascular complications in long-term diabetes.

Patients were recruited from the Diabetic Centre at Derriford Hospital, Derriford, Plymouth and from Kings College Hospital.

Patients with T1DM diagnosed using the WHO criteria for 10 years or more. Diabetic controls must have been diagnosed for 20 years without further complications such as nephropathy, neuropathy, retinopathy or cardiovascular disease. There were 278 patients with T1DM available for the study (see Table 1).

2.2. Patient with diabetes and complications

Nephropathy (DN) was described in patients with more than 10 years of diabetes and proteinuria at least 3 × in 12 months. Retinopathy (DR) was defined in patients with more than 5 dots or blots per eye, hard or soft exudates, new vessel formation of maculopathy or vitreous haemorrhage. Short duration (SD) patients had been diagnosed with diabetes for less than 10 years whereas uncomplicated (DC) patients displayed no complications after a 20 year duration of T1DM.

2.3. Controls

135 ethnically matched controls were studied. Control DNA was obtained from the cord blood of European Caucasian subjects collected sequentially after normal obstetric delivery from the Obstetric Department, Derriford Hospital (Plymouth, UK).

Ethics committee approval and patient consent were obtained for all studies performed.

Please cite this article as: Houldsworth, A., et al., Polymorphic differences in the SOD-2 gene may affect the pathogenesis of nephropathy in patients with diabetes and diabetic compli..., Gene (2015), http://dx.doi.org/10.1016/j.gene.2015.04.006
2.4. Genetic studies

Genomic DNA was extracted conventionally from whole blood using a ‘salting out’ method. The restriction site was found at $-9$ on the mitochondrial targeting sequence of the manganese superoxide dismutase, which is a $C$ to $T$ substitution resulting in an amino acid change of alanine to valine.

Two primers were used to amplify SOD-2 (ala $-9$, val), the forward primer was 5'-AGC GCC GTC GTA GAC-3' and the reverse primer 5'-TAC TTC TCC TCG GTG AGC-3' (Fig. 1).

The restriction enzyme used to digest the amplified fragment was BsaW1 (Bacillus stearothermophilus W1718 Chen) (5’A^GCGG A3’; 3’T GCCG^T 5’). The C183T polymorphism in the mitochondrial sequence of MnSOD was digested by BsaW1 in a water bath at 60 °C for 90 min produced two fragments of 82 and 164 bp. Samples where the cut site was abolished due to the presence of the ‘T’ allele produced fragments of 246 bp. The migration of the DNA was visualised under ultra violet light and the bands were photographed using a transilluminator linked to specialist computer software. In order to genotype the samples, the position of the bands was determined relative to the DNA ladder fragments.

2.5. Statistical analysis

The frequency of the alleles and genotypes in both patient subgroups and controls were compared for significance, using contingency tables and chi-squared test with Yates correction where appropriate (Table 2). P values of $<0.05$ were considered to be significant. We also included odds ratios with 95% confidence limits where appropriate.

2.6. Hardy–Weinberg distribution

In a non-selected population, the relative frequencies of different alleles tend to be constant and are described by a single equation. Several factors may distort the gene frequencies in a population. The patients infected with diabetes were recruited from the South West of England as were the healthy control subjects. A high frequency may indicate the possibility that evolutionary forces are in operation and applying selective pressure in favour of heterozygotes or homozygotes for mutant genes that cause the more common inherited disorders. We tested for Hardy Weinberg distribution in the population of the genotypes that were studied using the calculation ($p^2 + 2pq + q^2 = 1$). The frequency of $C = p$ and the frequency of $T = q$. This tested for genetic variation of a population in equilibrium (Emigh, 1980).

3. Results

3.1. SOD-2 genotype for patients with T1DM

We determined the genotype frequency of 278 patients with T1DM for a C/T substitution polymorphism on the mitochondrial targeting sequence of the manganese superoxide dismutase gene.

Patients with T1DM diagnosed using the WHO criteria for 10 years or more. Diabetic controls must have been diagnosed for 20 years without further complications such as nephropathy, neuropathy, retinopathy or cardiovascular disease. Non-diabetic controls were without any of the above complications. The patients with T1DM (n = 278, 136M:142F) available for the study had a mean age of 45.6 years $\pm$ 1.08 SEM with a mean duration of diabetes of 30.9 years $\pm$ 1.01 SEM. The normal controls (n = 135, 72M:63F) were obtained from the cord blood of European Caucasian subjects collected sequentially after normal obstetric delivery from the Obstetric Department, Derriford Hospital (Plymouth, UK). The subjects had no family history of T1DM and were not infected with HCV. All differences were corrected using the Yates correction.

Patient complications were nephropathy (DN) which was defined as $>10$ years of diabetes and proteinuria at least three times in 12 months (n = 73, 31M:42F) an average age of 47.8 $\pm$ 1.96 SEM and a mean duration of disease of 33.0 years $\pm$ 1.64 SEM. Diabetic retinopathy (DR) was defined as $>5$ dots or blot or per eye, hard or soft exudates, new vessels evidence of maculopathy or vitreous haemorrhage and the group had a mean age of 62.8 years $\pm$ 2.49 SEM (n = 15, 7M:8F). The uncomplicated diabetic controls (DC) had no diabetic complications after 20 years of duration of T1DM and an average age of 52.0 $\pm$ 2.02 SEM (n = 62, 27M:35F). There was also a group of patients that had only been diagnosed for a short period of time (SD) with a mean age of 30.0 years $\pm$ 2.16 SEM (n = 33, 18M:15F) (see Table 1).

There were significantly more of the diabetic control (DC) group (11.3%) than the patients with diabetic nephropathy (DN) (1.4%) with the CC genotype ($p = 0.03$ and $\chi^2 = 4.27$, OR $= 9.16$ (1.08 $< OR < 204.03$)). Further significance was found between normal healthy controls (17.0%) and patients with nephropathy (1.4%) with the genotype CC ($p = 0.03$, $\chi^2 = 6.08$, OR $= 0.11$ (0.00 $< OR < 0.87$)).

4. Discussion

The role of free radical reactions in protein oxidation, DNA damage and lipid peroxidation is strongly debated in relation to human disease and has been implicated in many disease states. It is not clear whether ROS are solely a major cause of tissue damage in disease or if they need to be accompanied by other factors as well as the tissue injury. It is clear that free radical reactions occur more readily than normal in diseased or damaged tissues and this may exacerbate disease. Increased oxidisability of damaged tissues can be due to the inactivation or leakage of antioxidants from cells.

Proliferative cells that are exposed to sub-cytotoxic OS such as H$_2$O$_2$, UV, ethanol, etc. display mitochondrial DNA deletions, cell morphology, histochemistry changes, cell cycle regulation and gene expression differences (Sozou and Kirkwood, 2001). Polymorphic genetic differences may change the antioxidant gene expression in a way similar to these somatic mutations caused by OS.

The single nucleotide polymorphism results in a substitution of C to T, which causes an amino acid change from alanine to valine. The variation
Manganese Superoxide Dismutase Mitochondrial Targeting Sequence

AGCCAGCAGCT GCGTAGACX CAGATGTGTA GCCGGGCCAGT GTGCGGCACC
AGCAGGCAAGC TGGCTCGGAT TTTGGGGTAT CTGGGCTCCA GGCAAGAAGCA
CAGCCCTCCC GACCTGGCCT ACGACTACGG GCCTGGAGA CTTCCATCA
ACGGCGAGAT CATGCAGCTG CACCACAGCA AGCACCAGGC GGCCTAGCTC
ACCGAGGAGT AGTA

Primers
Forward 5′ AGC CCA GCC TGC GTA GAC −3′
Reverse CGT CAC CGA GGA GAA GTA
5′ TAC TTC TCC TCG GTG ACG −3′

Fig. 1. Manganese superoxide dismutase mitochondrial targeting sequence.

Table 2
The % frequency of the SOD-2 genotypes and alleles for a population of patients with diabetes and without diabetic complications.

<table>
<thead>
<tr>
<th>Genotype frequency</th>
<th>Diabetic patients</th>
<th>Diabetic controls</th>
<th>Diabetic with nephropathy</th>
<th>Diabetic with retinopathy</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases (n)</td>
<td>n = 278</td>
<td>N = 62</td>
<td>n = 73</td>
<td>n = 15</td>
<td>n = 135</td>
</tr>
<tr>
<td>C/C</td>
<td>13M:142F</td>
<td>27M:35F</td>
<td>31M:42F</td>
<td>7M:8F</td>
<td>72M:63F</td>
</tr>
<tr>
<td></td>
<td>10.1% (28)</td>
<td>11.3% (7)</td>
<td>14.1% (1)</td>
<td>6.7% (1)</td>
<td>17.0% (23)</td>
</tr>
<tr>
<td></td>
<td>65.1% (181)</td>
<td>64.5% (40)</td>
<td>76.7% (56)</td>
<td>60.0% (9)</td>
<td>60.0% (81)</td>
</tr>
<tr>
<td></td>
<td>24.8% (69)</td>
<td>24.2% (15)</td>
<td>21.9% (16)</td>
<td>33.3% (5)</td>
<td>23.0% (31)</td>
</tr>
<tr>
<td>C/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Allelic frequency

<table>
<thead>
<tr>
<th>Number of alleles or chromosomes</th>
<th>Diabetic patients</th>
<th>Diabetic controls</th>
<th>Diabetic with nephropathy</th>
<th>Diabetic with retinopathy</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases (n)</td>
<td>n = 556</td>
<td>n = 124</td>
<td>n = 146</td>
<td>n = 30</td>
<td>n = 270</td>
</tr>
<tr>
<td>C</td>
<td>41.6% (237)</td>
<td>43.5% (54)</td>
<td>39.7% (58)</td>
<td>36.7% (11)</td>
<td>47.5% (128)</td>
</tr>
<tr>
<td>T</td>
<td>57.4% (319)</td>
<td>56.5% (70)</td>
<td>60.3% (88)</td>
<td>63.3% (19)</td>
<td>52.5% (142)</td>
</tr>
</tbody>
</table>

All patients and control groups were in Hardy Weinberg equilibrium.
No other significant differences were found.

a Significant difference between diabetic controls (11.3%) and patients with diabetic nephropathy (1.4%), p = 0.04, χ² = 4.27 for CC, OR = 9.16 (1.08 < OR > 204.03).
b Significant difference between normal controls (11.4%) and patients with diabetic nephropathy (1.4%), p = 0.03, χ² = 4.68 for CC, OR = 0.11 (0.00 < OR < 0.87).

Please cite this article as: Houldsworth, A., et al., Polymorphic differences in the SOD-2 gene may affect the pathogenesis of nephropathy in patients with diabetes and diabetic complication., Gene (2015), http://dx.doi.org/10.1016/j.gene.2015.04.006
Other findings support the protective effect of Ala1 at rs4800 against the damaging effects of oxidative stress and suggest that distant linkage equilibrium may exist with another true disease causing gene variant (McKnight et al., 2012). A recent meta-analysis suggested that C allele of C47T polymorphism in SOD2 gene has protective effects on risk of DMI, diabetic nephropathy and diabetic retinopathy (Möllsten et al., 2011). A full understanding of the mechanisms is still unclear and further research is required to clarify this and to consider if SOD2-2 therapy may be advantageous to patients with diabetes (Salvemini et al., 1999).

References


