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The Relationships between SORD -888G>C Gene Polymorphism and Diabetic Retinopathy in a South Sumatran Malay Population

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ABSTRACT

The sorbitol dehydrogenase (SORD) enzyme has 2 promoter regions with polymorphisms at -1214 C>G (rs2055858) and -888 G>C (rs3759890), which have a complete imbalance, related to gene expression in retinal cells of diabetic patients, and more likely to have a role in diabetic retinopathy (DR) pathogenesis. The SORD -888G>C genotype occurs more often in Japanese DR patients than in patients without complications. A relationship between DR and the G allele in -888G>C polymorphism is observed in Diabetes Mellitus (DM) type-2 patients. This study aims to elucidate the relationship between the SORD -888 G>C gene polymorphism to the occurrence of DR cases in Malay population in South Sumatra. This is a case-control study. Malay DM type-2 patients who undergo direct ophthalmoscopy and fundus imaging in the Dr. Mohammad Husein Central General Hospital eye clinic in Palembang, South Sumatra, Indonesia were the subjects of this study. Patient DNA was isolated from blood samples, PCR analyzed and sequenced in order to determine the polymorphism frequencies of the SORD -888 G>C gene. The results showed that every subject had GG genotype in SORD -888 G>C gene promoter. There was no observable polymorphism in SORD -888G>C gene in the authentic Malay population in South Sumatra.

Keywords: SORD-888 G>C polymorphism, Malay population, South Sumatera

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INTRODUCTION

Diabetic retinopathy (DR) has the potential to cause blindness in diabetes mellitus (DM) patients. The probability of this complication is escalating along with the increasing duration of DM in patients. [1,2,3] This complication prevalence in DM patients is 28.5% with a threat to eye vision capability for about 30%, with 15% blind. [2,3] The *Diab Care Asia* report in 2008, involving 1758 DM patients in 18 primary and secondary health services in Indonesia, revealed that 42% of DM patients will get retinopathy complication, in which about 6.4% is proliferative DR. [4]

Blindness caused by DR is related to the obstruction and damage in capillaries in the retina. Chronic hyperglycemia conditions trigger the cascade of physiological and biochemical alterations that lead to micro vascular damage and retinal disfunction. [5] *The Diabetes Control and Complications Trial* (DCCT) and the *United Kingdom Prospective Diabetes Study* (UKPDS) found that there is a strong correlation between chronic hyperglycemia and DR, but the mechanism is not clear. [5,6] Some of the biochemical pathways between hyperglycemia and DR have been investigated. Under hyperglycemic conditions, glucose flux will increase via polyol pathway, where aldose

reductase (AR) enzyme will deplete the glucose supply as it is converted to sorbitol, and finally, to fructose by sorbitol dehydrogenase (SORD). [5] A connection of AR gene polymorphisms with DR was reported in several populations, which revealed the significance of AR polymorphisms in DR cases (*P = 0.009).* [7–11]

Quick conversion of accumulated sorbitol to fructose by SORD triggers osmotic damage in retinal endothelial cells and pericyte through the activation of advanced glycation end products (AGEs), oxidative-nitrosative stress, Protein Kinase C (PKC) pathway activation, inflammation, and the imbalance of growth factors. The osmotic damage eventually leads to DR. [5,6] Some studies have revealed some important connection between DR and SORD, which has an important role in the second part of polyol pathway. [5,12]

SORD was overexpressed in mammalian pericyte culture after exposure to high doses of glucose and eventually stimulated reactive oxygen species generation. AR inhibitor and antioxidants significantly block the bad effects of excess SORD by preventing the loss of pericyte and vascular hyperpermeability, which were the initial characteristic of DR in streptozotocin-induced DM rats. [13]

SORD has two promoter regions, containing polymorphisms at -1214 C>G (rs2055858) and -888 G>C (rs3759890), which have a completely imbalanced connection and are related to the gene expression of retinal cells in DM patients. It is suggested that SORD polymorphisms have a role in DR pathogenesis. [13,14] The SORD -888 G>C genotype occurs more frequently in DR patients than in DM patients without complications in Japanese populations. The connection between the DR and the G allele in -888 G>C polymorphism has already observed in DM type-2 diabetes. [15] Another study in Brazilian people showed no observable connection between SORD-888 G>C promoter polymorphism with DR cases. [14]

MATERIALS AND METHODS

This was a case-control study. DM type-2 patients who had the direct ophthalmoscopy and fundus imaging in Dr. Mohammad Husein Central General Hospital eye clinic in Palembang was selected as subjects in this study. Inclusion factor for DR group subjects was: (1) had DR based on ETDRS criteria and confirmed by direct ophthalmoscopy and fundus imaging; (2) aged 45–65 years old; (3) and authentic Malay ethnic from South Sumatra. Authentic Malay DM type-2 patients without any DR symptoms after direct ophthalmoscopy were considered as control. Every group had 20 subjects and their age, gender, and the DM duration were recorded.

Patient blood samples, 3 mL for each patient, were collected from a median cubital vein and stored in an ethylene diamine tetraacetic acid (EDTA)-coated tube for DNA and PCR analysis. DNA isolation was based on the Chelex-100 DNA isolation method. Isolated DNA was then PCR analyzed using these primers: forward, 5'-GTCAGGCTGGTCTCGAACTC-3'; and reverse, 5'-CTGCCTGAGGGTCCATATTC-3' using i-cycle PCR machine (Biorad). PCR-amplified DNA was then purified and sequenced by direct sequencing of the ABI Sequencer DNA (Applied Biosystems, Inc). The sequence data was analyzed by BioEdit VII software by matching the data with reference sequence from Gene Bank. Gene ID NT010194 was used as the reference sequence.

The data was analyzed using SPSS 16.0 for Windows to assess the distribution and gene frequency of SORD-888 G>C promoters in both subject groups. The relationship between SORD promoter polymorphisms and DR cases was analyzed with a 95% confidence interval.

RESULTS

There was no significant difference in subjects with DR and subjects in control group in gender, mean age, DM duration, cholesterol level and blood pressure (Table 1).

Every subject in the DR, and control group, had the GG genotype in their SORD -888G>C gene promoter based on the DNA analysis (Table 2). The allele distribution in SORD -888G>C gene promoter analysis showed that every subject, DR, and control, had the G allele or the wild-type allele (Table 3).

DISCUSSION

SORD gene expression was initially investigated in streptozocin-induced diabetic rats. It showed vascular dysfunction due to elevated oxidation from the process of sorbitol to fructose conversion. SORD has more significant effect in sorbitol metabolism than AR. [17]

Table 1. Subject's general characteristic in DR group and control group.

Characteristic	DR group (%)	Control group (%)	p
Gender			
• Male	11 (55)	10 (50)	0,89*
• Female	9 (45)	10 (50)	
Age (year)	55,95 ± 6,78	55,85 ± 5,95	0,96**
DM duration			
• ≥ 5 years	17 (85)	19 (95)	0,76*#
• < 5 years	3 (15)	1 (5)	
Cholesterol level			
• > 200 mg/dL	11 (55)	8 (40)	0,34*
• ≤ 200 mg/dL	9 (45)	12 (60)	
Blood pressure			
• Hypertension	12 (60)	11 (50)	0,75*
• Normotension	8 (40)	9 (45)	

*Chi-square test, *# Fisher exact test, ** Unpaired T-test

Table 2. Distribution frequency of SORD-888 G>C promoter gene

Genotype	DR group (%)	Control group (%)
GG	20 (100)	20 (100)
GC	0 (0)	0 (0)
CC	0 (0)	0 (0)

Table 3. G and C allele distribution frequency of SORD-888 G>C gene promoter

Allele	DR group (%)	Control group (%)
G (wild type)	40 (100)	40 (100)
C (polymorphic)	0 (0)	0 (0)

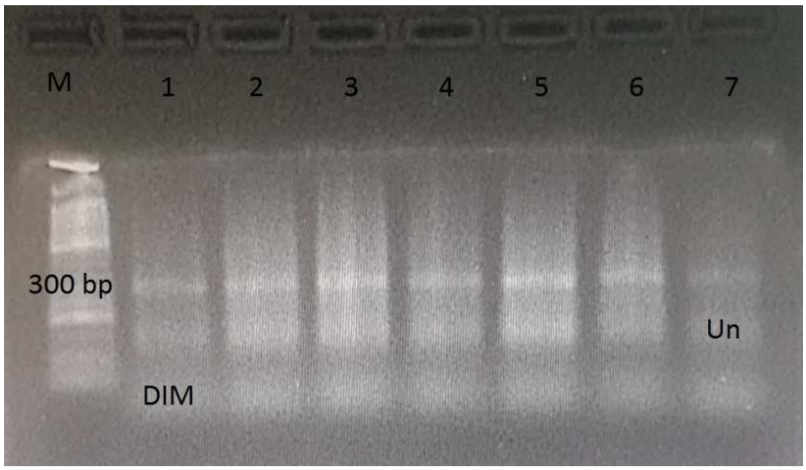

Figure1. Gel Electrophoresis of SORD -888 G>C amplicon (300 bp)

Legend:
M = Marker
1 – 7 = Sample number
Un = Unspecific band
DIM = Dimer

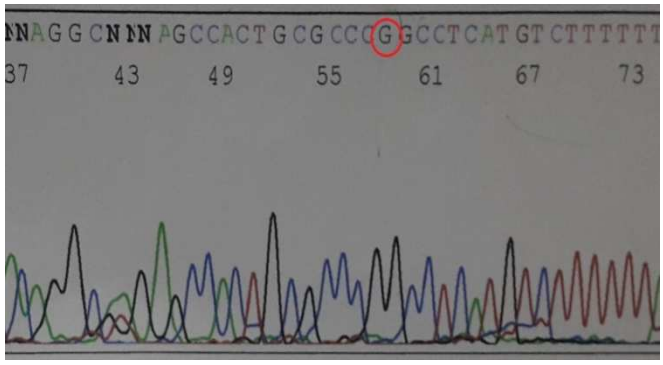

Figure 2. Sample PCR-Sequencing Electroforegam

```
Query  45    AGCCACTGCGCCCGGCCTCATGTCTTTTTTATACTTAAGCACCAGGCCCATGTAATGATG
104

Sbjct  1220  AGCCACTGCGCCCGGCCTCATGTCTTTTTTATACTTAAGCACCAGGCCCATGTAATGATG
1279

Query  105   CACGAATTCATTTACTCCTACTCGTGCCAGGCCCTTTCCTGGGGGCTGGAGACAAAGGGC
164

Sbjct  1280  CACGAATTCATTTACTCCTACTCGTGCCAGGCCCTTTCCTGGGGGCTGGAGACAAAGGGC
1339

Query  165   CGACGTGGACGCTGCGTCATGGTAGCACCTGTCTCGCAAAGTGCACACCGTGCTCCACCC
224
```

Legend: Sbjct = Gene Bank reference
Query = Sample sequencing result

The polymorphisms of SORD in Schwann cells diabetic rat and found that polymorphisms mostly occurs at -1535 C>G and -888 G>C. The -888 GC genotype has higher occurrence frequency (65%) in DR group than in a not-DR group. There was no significant difference in age, DM duration, HbA1c level, and blood pressure between DR and non-DR group. [13] The diabetic-induced cows showed that SORD was overexpressed and caused glucose toxicity in pericytes. Those events have an important role in DR pathogenesis. [18] The SORD polymorphism in Japanese patients with DR, especially the -888 G>C polymorphism. The result showed that the G allele has a higher frequency in DR patients than the control; the connection was strong but not significant ($p < 0.09$). [13]

SORD gene promoter polymorphisms at -1214 C>G and -888 G>C was found in Polish populations and no observable correlations in the -1214 C>G gene polymorphism with the DR cases and found weak correlations in -888 G>C polymorphism (OR 1.73, 95% CI 1.06–2.38). [15] The study in Caucasian-Brazilian population that showed same genotype frequency in DR group and non-DR group ($p = 0.963$). It showed that there was no relathioships between SORD -888 G>C polymorphism and the DR cases in Caucasian-Brazilian populations. [14]

In accordance with the previous studies, all 40 Malay subjects in South Sumatra (100%) have G allele with GG genotype, without any C polymorphic alleles. This result indicates no mutation occurred in the SORD gene and there is no observable connection between the SORD polymorphism in its promoter gene with the DR cases in South Sumatra.

This study result and previous studies suggest a weak role of genetic factors in DR cases. It is also suggested that epigenetic factors may play a more significant role. Subsequent studies should analyze epigenetic factors in sorbitol metabolism pathway, as some studies have found the significant epigenetic effects in such pathways.

CONCLUSION

There is no observable polymorphism in SORD -888 G>C gene promoter in Malay population in South Sumatra.

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Conflict of Interest
No conflict of interest

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