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Positive association between PPARD rs2016520 polymorphism and coronary heart disease in a Han Chinese population

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ABSTRACT. PPARD encodes peroxisome proliferator-activated receptor delta, which has been shown to play an important role in controlling lipid metabolism and atherosclerosis. In this case-control study, we explored the relationship between PPARD rs2016520 polymorphism and coronary heart disease (CHD) in a Han Chinese population. A total of 657 CHD cases and 640 controls were included in the association study. rs2016520 polymorphism genotyping was performed using the melting temperature-shift polymerase chain reaction method. The PPARD rs2016520-G allele reduced CHD risk by 17.9% (χ^2 = 5.061,

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P = 0.025, OR = 0.821, 95%CI = 0.692-0.975). Furthermore, a significant difference in CHD risk was observed for the PPARD rs2016520 polymorphism in the dominant model (AG + GG vs AA: χ^2 = 4.751, degrees of freedom (df) = 1, P = 0.029, OR = 0.784, 95%CI = 0.631-0.976). Analysis by age suggested that the G-allele decreased CHD risk by 14.8% in ages greater than 65 years (χ^2 = 4.446, P = 0.035, OR = 0.852, 95%CI = 0.684-1.060). In contrast, meta-analysis of PPARD rs2016520 among 3732 cases and 5042 controls revealed no association between PPARD rs2016520 and CHD (P = 0.19). We found that the PPARD rs2016520-GG genotype decreased CHD risk in a Han Chinese population. Moreover, we found an association between serum high-density lipoprotein cholesterol level and PPARD rs2016520 in senior individuals aged ≥ 65 years. The meta-analysis revealed no association between PPARD rs2016520 and CHD, suggesting ethnic differences in the association between the PPARD locus and CHD.

Key words: Coronary heart disease; Meta-analysis; PPARD; Polymorphism; rs2016520

INTRODUCTION

Coronary heart disease (CHD) has become a main risk factor of death in both developed and developing countries (Lopez et al., 2006). As a direct cause of CHD, atherosclerotic lesions are formed by blood lipid deposition or inflammation in the original smooth endarterium (Kroupis et al., 2010). CHD is a complex disease related to multiple environmental factors and multiple genes (Zhang et al., 2013). The risk factors of CHD include tobacco (Sacar et al., 2005), excessive alcohol consumption (Wakabayashi, 2013), and unhealthy diet (Ma et al., 2010). These environmental factors may lead to CHD through their impact on epigenetic changes in CHD-related genes (Jiang et al., 2013). In addition, recent studies have revealed that genetic components are the main risk factors of CHD (Zhou et al., 2012; Huang et al., 2013; Lian et al., 2013; Zhang et al., 2013).

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily, which are ligand-activated nuclear transcription factors. PPARs are dietary lipid receptors (Tyagi et al., 2011) that are important in lipid and lipoprotein metabolism, fat formation, insulin sensitivity regulation, and inflammation (Duval et al., 2002). PPARD is 1 of the 3 PPAR subtypes (Berger and Moller, 2002) including PPARA, PPARY and PPARD. PPARD is distributed in nearly every part of the body (Dongiovanni and Valenti, 2013). PPARD plays a key role in the regulation of multiple important biological processes, including lipid metabolism, insulin sensitivity, and atherosclerosis formation (Ehrenborg and Skogsberg, 2013). PPARD agonists can increase the levels of plasma high-density lipoprotein cholesterol (HDL-C) (Oliver et al., 2001), HDL-C is known to protect CHD in humans (Wilson et al., 1980). PPARD agonists can also decrease atherosclerotic lesions (Graham et al., 2005) by increasing the levels of anti-inflammatory molecules in human endothelial cells (Graham et al., 2005). PPARD rs2016520-GG carriers were found to have lower HDL-C concentration and higher risk of CHD than rs2016520-AA carriers (Skogsberg et al., 2003). Similar findings were observed in a study of a Turkish population (Yilmaz-Aydogan et al., 2012). Thus, the goal of our

study was to evaluate the contribution of the PPARD rs2016520 polymorphism to the risk of CHD in a Han Chinese population.

MATERIAL AND METHODS

Study population

We collected 1297 samples between 2010 and 2014 from Ningbo Lihuili Hospital, Ningbo Yinzhou People's Hospital, and Zhejiang Second Hospital. According to the criteria used in our previous studies (Zhou et al., 2012; Xu et al., 2013a), we divided the samples into 657 CHD cases and 640 non-CHD controls. All samples contained in this study were unrelated Han Chinese without congenital heart disease, cancer, or severe liver or kidney disease. This study was approved by the ethical committees of Ningbo Lihuili Hospital, Ningbo Yinzhou People's Hospital, and Zhejiang Second Hospital. All subjects signed informed consent forms.

Genotyping

Genomic DNA was extracted from whole blood using a nucleic acid extraction automatic analyzer (Lab-Aid 820, Xiamen, China). Genotyping was performed using the melting temperature (Tm)-shift polymerase chain reaction (PCR) approach (Wang et al., 2005; Yuan et al., 2012), which used 2 allele-specific primers (5'-gcgggcCGCAGATGGACCTCTACAG Ga-3' and 5'-gcgggcagggcggcCGCAGATGGACCTCTACAGGg-3'), and 1 common primer (5'-CTGTCTTCTCCTCTGCCCAGGC-3'). The PCR program consisted of 30 s of initial denaturation at 95°C, followed by denaturation at 95°C for 30 s, annealing at 59°C for 30 s, and extension at 72°C for 30 s for 40 cycles, with a final extension at 72°C for 30 s. PCR was performed on the ABI GeneAmp® PCR System 9700 96-Well Sample Block Module (Applied Biosystems, Foster City, CA, USA). Melting curve analysis was conducted on the Roche LightCycler 480® fluorescence quantitative PCR instrument (Roche, Basel, Switzerland). The melting curve analysis program was 95°C for 15 s, 60°C for 30 s, and then the temperature was increased by 0.11°C per s up to 95°C with fluorescence signal continuous acquisition. Melting curve data obtained using the Air borne software provided by Roche used automatic clustering based on fluorescence intensity analysis (Yuan et al., 2012).

Meta-analysis

Publication search and data extraction for the meta-analysis were collected after a search from 2000-2014 of online databases (PubMed, Embase, Web of Science, Wanfang Database, and China National Knowledge Infrastructure) The key words included "coronary artery disease" or "coronary heart disease" or "myocardial infarction" or "arteriosclerosis" or "coronary stenosis", together with "PPARD polymorphism" or "+294T/C polymorphism" were searched. As described in our previous studies (Chen et al., 2013; Xu et al., 2013b), we collected information in current meta-analysis, including the first author's name, year of publication, ethnic group, number of genotypes or allele, and total number of cases and controls. In addition, according to the method described in our previous study (Ye et al., 2013), we determined the genotype based on Wellcome Trust Case Control Consortium data (Ye et al., 2013). The procedures used in this meta-analysis are shown in Figure 1 .

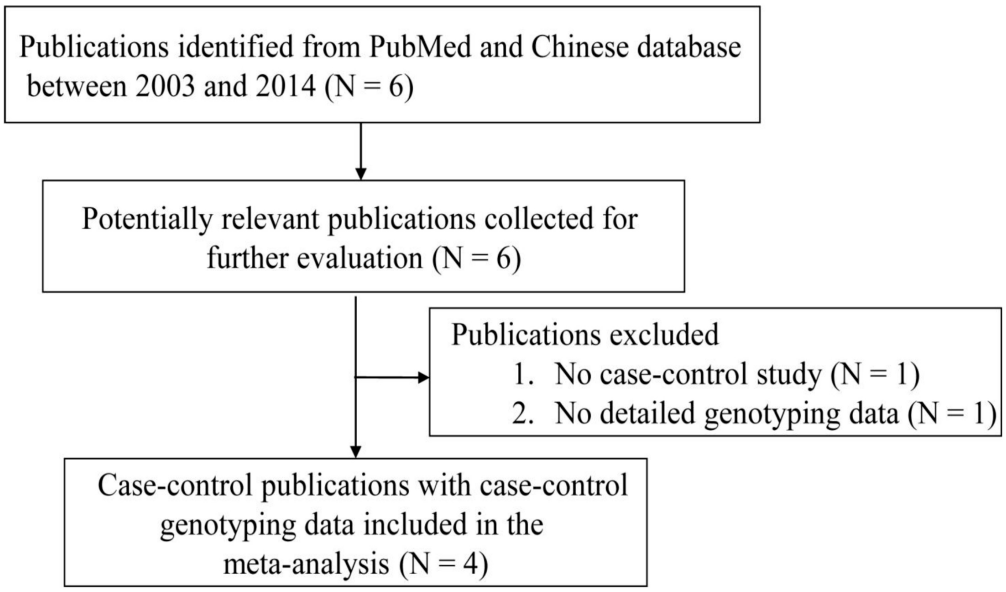

Figure 1. Flow chart for the meta-analysis of PPARD rs2016520.

Statistical analysis

Hardy-Weinberg equilibrium analysis was performed using the Arlequin program (version 3.5) (Excoffier and Lischer, 2010). Differences in genotype and allele frequencies between cases and controls were identified using the CLUMP22 software with 10,000 Monte Carlo simulations (Sham and Curtis, 1995). The OR with 95%CI were determined using an online program (http://faculty.vassar.edu/lowry/odds2x2.html). Heterogeneity in our meta-analyses was assessed using Cochran's Q and the inconsistency index (I2) statistic (Yin et al., 2012). An I2 < 50% indicated no heterogeneity among the studies in the meta-analyses (Yin et al., 2012). The combined ORs and corresponding 95%CIs in the meta-analysis were calculated by applying either the fixed-effect or random-effect method (Zhang et al., 2011; Du et al., 2013). A funnel plot was used to evaluate publication bias in the meta-analysis. A correlation test was performed using the SPSS software 18.0 (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

The genotype distribution of the PPARD rs2016520 polymorphism in both cases and controls were in Hardy-Weinberg equilibrium (Table 1). Our results showed that the PPARD rs2016520-G allele decreased the risk of CHD by 17.9% ($\chi^2 = 5.061$, $P = 0.025$, OR = 0.821, 95%CI = 0.692-0.975, Table 1). A further test in dominant model identified a significant difference of the CHD risk between G-allele carriers and AA genotype carriers (Table 2, AG + GG vs AA: $\chi^2 = 4.751$, df = 1, $P = 0.029$, OR = 0.784, 95%CI = 0.631-0.976).

Table 1. Genotype and allele distribution for PPARD rs2016520 in cases and controls.

	Genotype (counts)			χ^2	P (df = 2)	HWE	Allele (counts)		χ^2	P (df = 1)	OR (95%CI)
rs2016520	GG	AG	AA				G	A			
Cases (N = 657)	47	247	361			0.611	341	969			
Controls (N = 640)	58	268	314	5.108	0.078	0.925	384	896	5.061	0.025	0.821 (0.692-0.975)

Table 2. Genetic association of PPARD rs2016520 with CHD under the dominant and recessive models.

	Dominant		χ^2	P (df = 1)	OR (95%CI)	Recessive		χ^2	P (df = 1)	OR (95%CI)
rs2016520	GG	AG+AA				GG+AG	AA			
All cases	47	608				294	361			
All controls	58	582	1.547	0.214	0.776 (0.519-1.159)	326	314	4.751	0.029	0.784 (0.631-0.976)

Because gender and age often interact with the occurrence and development of CHD, we performed breakdown analyses by gender and age. Our results identified a strong association between rs2016520 and CHD in senior individuals aged 65 years or older (χ^2 = 4.446, P = 0.035, OR = 0.852, 95%CI = 0.684-1.060, Table 3). In addition, we were unable to identify a positive association between cases and controls in males and females (P > 0.05; Table 4). There was no association between rs2016520 and CHD in the stratification test by gender and age under the recessive and dominant models (P > 0.05; **Table S1**).

Table 3. Age-stratified association of PPARD rs2016520 with CHD.

		Genotype (counts)			χ^2	P (df = 2)	HWE	Allele (counts)		χ^2	P (df = 1)	OR (95%CI)
Age (years)	rs2016520	GG	GA	AA				G	A			
≤ 55	cases (N = 145)	15	54	76			0.311	84	206			
	controls (N = 220)	20	92	108	0.792	0.673	1.000	132	308	0.090	0.764	0.952 (0.687-1.318)
55-65	cases (N = 224)	16	89	119			1.000	121	327			
	controls (N = 242)	18	104	120	0.593	0.743	0.534	140	344	0.424	0.515	0.909 (0.683-1.211)
≥ 65	cases (N = 282)	16	104	162			1.000	136	428			
	controls (N = 174)	18	70	86	4.739	0.094	0.478	106	242	4.446	0.035	0.725 (0.538-0.978)

Table 4. Gender-stratified association of PPARD rs2016520 with CHD.

Gender		Genotype (counts)			χ^2	P (df = 2)	HWE	Allele (counts)		χ^2	P (df = 1)	OR (95%CI)
	rs2016520	GG	AG	AA				G	A			
Male	Cases (N = 466)	32	179	255			0.905	243	689			
	Controls (N = 350)	31	143	176	2.073	0.355	0.798	205	495	2.072	0.150	0.852 (0.684-1.060)
Female	Cases (N = 191)	15	70	106			0.460	100	282			
	Controls (N = 290)	27	125	138	2.884	0.237	1.000	179	401	2.454	0.117	0.794 (0.600-1.060)

A significant correlation was found between serum HDL-C level and rs2016520 in subjects older than 65 years in the CHD cases (r = -0.162, P 0.008, Figure 2). However, there was no association between rs2016520 and serum levels of HDL-C and low-density lipoprotein-cholesterol in the total samples and remaining subgroups by gender and age (P > 0.05, data not shown).

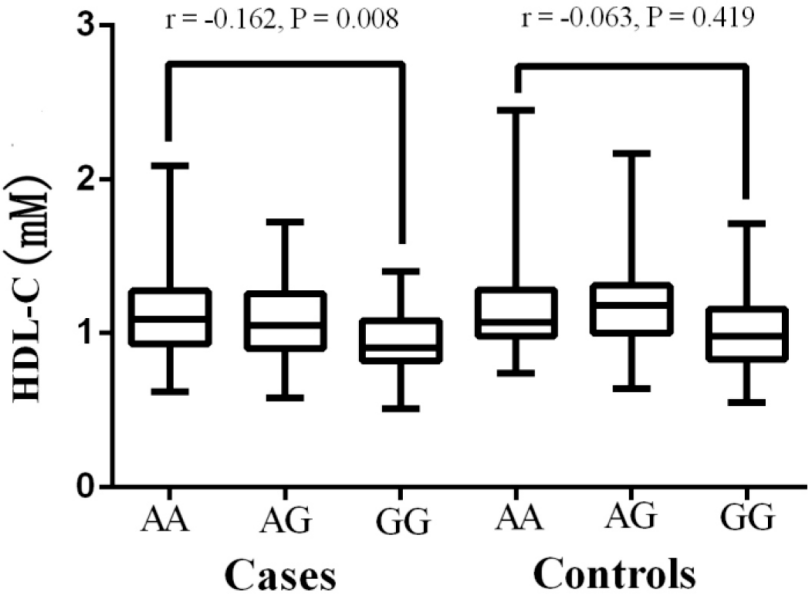

Figure 2. Breakdown correlation of HDL in individuals aged 65 years or older.

We performed a meta-analysis of PPARD rs2016520 in CHD. Our search for studies examining CHD and PPARD rs2016520 retrieved 6 articles from PubMed and the Chinese databases (China National Knowledge Infrastructure Wanfang) from 2000-2014. After excluding the publications with no detailed genotype data and those that were not case-control studies, 4 retrieved publications remained. Four case-control studies, 1 imputed dataset of Wellcome Trust Case Control Consortium, and our case-control study of 3732 cases and 5042 controls were included in the current meta-analysis. However, we observed no significant association between rs2016520 and CHD (P = 0.19; Figure 3). There was no publication bias in the meta-analysis (Figure 4). In addition, significant heterogeneity in the meta-analysis was observed (P < 0.0001, I2 = 82%; Figure 3), suggesting an ethnic difference in the association of this locus with CHD .

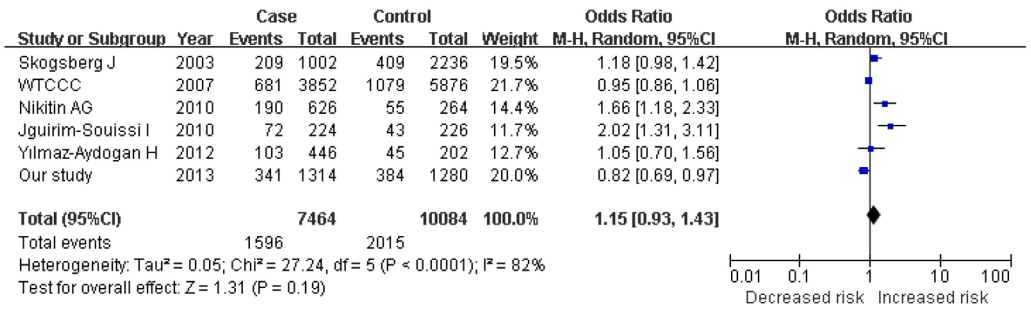

Figure 3. Meta-analysis of PPARD rs2016520 in the random-effect model.

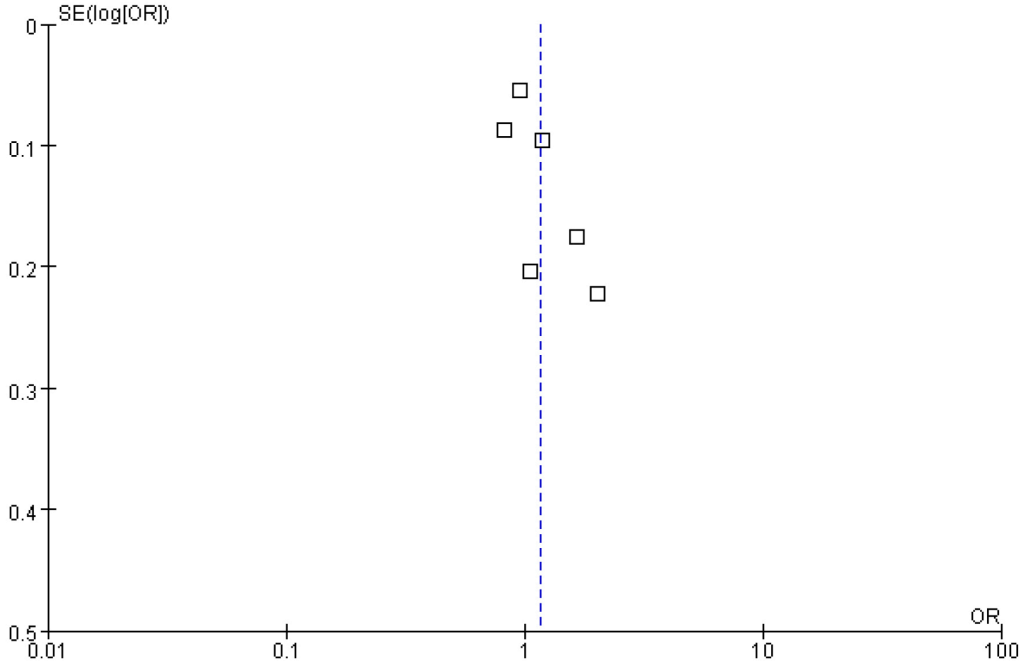

Figure 4. Funnel plot of PPARD rs2016520 in the random-effect model.

DISCUSSION

PPARD encodes a receptor of peroxisome proliferators such as hypolipidemic drugs and fatty acids. The PPARD protein is highly expressed in the myocardium and is an important transcription factor regulating lipid and glucose metabolism (Skogsberg et al., 2003; Nikitin et al., 2010; Jguirim-Souissi et al., 2010; Yilmaz-Aydogan et al., 2012). In the present study, we investigated the relationship between the PPARD rs2016520 polymorphism and CHD in Han Chinese.

The single-nucleotide polymorphism rs2016520 is located in the 5'-untranslated region of PPARD. A number of case-control studies have indicated that PPARD rs2016520 is associated with CHD. A previous study in Russians found a significant association between the PPARD rs2016520 polymorphism and the risk of CHD by modulating lipid levels (Nikitin et al., 2010). A study performed in Tunisians indicated that the minor allele of PPARD rs2016520 was associated with CHD (Jguirim-Souissi et al., 2010; Chehaibi et al., 2013). However, another study was unable to confirm this association in a British population (Skogsberg et al., 2003). Furthermore, our case-control study suggested that PPARD rs2016520 was significantly associated with CHD in Han Chinese. Specifically, the G-allele of PPARD rs2016520 decreased the risk of CHD by 17.9% (χ^2 = 5.061, P = 0.025, OR = 0.821, 95%CI = 0.692-0.975). Further breakdown analysis by age in our study suggested that the G-allele decreases CHD risk by 14.8% individuals older than 65 years (χ^2 = 4.446, P = 0.035, OR = 0.852, 95%CI = 0.684-1.060).

Moreover, PPARD rs2016520 showed a significant relationship between the G-allele and lower plasma HDL-C concentration in the female subgroup (Aberle et al., 2006).

They also found a clear association between the minor allele with CHD and body mass index (Aberle et al., 2006). PPARD rs2016520 showed no association with CHD, but was significantly associated with cholesterol metabolism in a Scottish study (Skogsberg et al., 2003). The PPARD rs2016520 polymorphism was associated with serum lipid levels and the risk of CHD in Russians (Nikitin et al., 2010). PPARD rs2016520 increased the effect of low-density lipoprotein-cholesterol on the pathogenesis of CHD in a Turkish population (Yilmaz-Aydogan et al., 2012). In addition, the G-allele of PPARD rs2016520 was associated with increased low-density lipoprotein-cholesterol levels in the serum in CHD patients (Yilmaz-Aydogan et al., 2012). However, the rs2016520 polymorphism had no influence on plasma lipoprotein concentrations in Tunisians (Jguirim-Souissi et al., 2010, Chehaibi et al., 2013). Furthermore, we found that the A-allele was associated with increased HDL-C concentration in subjects older than 65 years among CHD patients in our study ($r = -0.162$, $P = 0.008$).

In previous studies in Russian (Nikitin et al., 2010), Tunisian (Jguirim-Souissi et al., 2010; Chehaibi et al., 2013), Scotsmen (Skogsberg et al., 2003), and Turkish (Yilmaz-Aydogan et al., 2012) populations, the G-allele was consistently observed to be a risk factor of CHD. However, we found that the G-allele was a protective factor in the development of CHD in Han Chinese. The Wellcome Trust Case Control Consortium study in a European population that included 1926 cases and 2938 controls showed that the G-allele was a protective factor against CHD (OR = 0.92). In addition, the significant heterogeneity ($I2 = 82\%$) in the current meta-analysis indicated an ethnic difference in CHD risk. This ethnic difference may be explained by the different linkage disequilibrium (LD) patterns among various populations. The LD patterns in the HapMap database revealed large differences in the LD patterns between Africans (HapMap-YRI) and other populations (HapMap-CEU, HapMap-CHB/JPT) (**Figure S1**). Thus, the discrepancy of the contribution of the PPARD rs2016520 G-allele to CHD indicates that this polymorphism was not a causal polymorphism but in high LD with the causal polymorphism. The causal polymorphism in the PPARD locus should be identified in future studies.

There are several limitations to this study. First, all samples were patients that were differentiated into cases and controls based on the results of coronary angiography. Therefore, there may be selection bias in the case-control study, and the subjects may not be representative of the randomized population. Second, most of the subjects included in our study used lipid-lowering drugs, which may have potential associations between genotype and serum lipid. Third, articles selected for the meta-analysis were published only in English or Chinese, and publications in other languages were not searched. Fourth, there were 2332 polymorphisms in the PPARD locus. Our findings of PPARD rs2016520 may not represent the contribution of other polymorphisms. The discrepancy in the contribution of the PPARD rs2016520-G allele to CHD indicates ethnic heterogeneity in the PPARD locus. A careful screening for the causal polymorphism of PPARD should be conducted in future studies.

In conclusion, we found that the PPARD rs2016520 G-allele was a protective factor against CHD, particularly in subjects older than 65 years. The difference between our study in Chinese and studies in other populations suggested ethnic heterogeneity in the PPARD locus.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

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