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Original article

Murine Double Minute 2 Gene (*MDM2*) rs937283A/G variant significantly increases the susceptibility to breast cancer in Saudi Women

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ABSTRACT

Breast cancer is predominant causes of mortality in women worldwide. Genetic polymorphisms have a significant role in breast cancer aetiology. *TP53* and its inhibitor the murine double minute *2 (MDM2)* genes encode proteins that have crucial functions in the DNA damage response. The allelic variations within these genes could influence the susceptibility to breast cancer. *MDM2* promotor polymorphism rs937283A/G has a role in susceptibility to cancer and modifies the promoter activity. In the present case-control study, the association of *MDM2* rs937283A/G polymorphism and breast cancer susceptibility in Saudi women with samples of 137 breast cancer patients, and 98 healthy controls were explored. *MDM2 gene* polymorphism rs937283A/G was genotyped by polymerase chain reaction restriction fragment length polymorphism and confirmed by sequencing. The results revealed that rs937283A/G variant is significantly increases the risk of breast cancer in Saudi women (p-value = 0.0078). Moreover, rs937283A/G polymorphism was associated with high risk of breast cancer in estrogen positive breast cancer patients (p-value = 0.0088), progesterone positive breast cancer patients (p-value = 0.0043), human epidermal growth factor receptor 2 negative breast cancer patients (p-value = 0.0026), and triple negative breast cancer patients where (p-value = 0.0003). Positive association between increased breast cancer risk and rs937283 variant in premenopausal Saudi women, below 50 years of age, was demonstrated (p-value = 0.0023). Collectively, *MDM2* rs937283A/G polymorphism could act as a possible biomarker for breast cancer susceptibility in Saudi women.

1. Introduction

Breast cancer (BC) has an enormous effect on women's health around the world. It is one of the serious and predominant cancer in Saudi Arabia (Saggu et al., 2015). It was reported that BC is the second commonest malignancy in women of Saudi Arabia (Al-Qahtani, 2007). The Saudi Cancer Registry (SCR) reported that breast cancer accounted for 28.7% of all the cancer types diagnosed in women in 2014 (https://nhic.gov.sa/eServices/Documents/2014.pdf). Recent study showed that breast cancer incidence rates among women treated in the Armed Forces Hospital Southern Region, from the period of January 2010 to December 2017, ranged

Abbreviations: CI, Confidence interval; OR, odds ratio; PCR, polymerase chain reaction; RFLP, Restriction fragment length polymorphism; *TP53*, Tumor Protein 53 gene.

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between three to eight confirmed cases for every 1000 patients (Asiri et al., 2020).

TP53 in non-cancerous cells is maintained at minimal levels by murine double minute 2 (MDM2). MDM2 and p53 form a negative-feedback loop. The core mechanism might be related to that MDM2 and TP53 protein form a complex, followed by attenuation of the TP53 activity. MDM2 is TP53 ubiquitin E3 ligase, which subsequently leads to TP53 degradation (Moll and Petrenko, 2003). Furthermore, increased *MDM2* expression occurs in a various human cancer and is related to cancer development (Shaikh et al., 2016). *MDM2* gene encodes a protein inhibits apoptosis. It has been demonstrated to play a substantial function in a different pathological and physiological mechanisms (Mendoza et al., 2014). Breast cancer onset and progression may be modified by genetic polymorphisms within oncogenes (Howell et al., 2014). Interestingly, rs937283 is a genetic polymorphism which resides in *MDM2* promoter region P1. Transition of A to G at rs937283 is known to enhance *MDM2* transcription, which subsequently rises *MDM2* mRNA and protein levels of expression, which promotes TP53 degradation and therefore increases cancer susceptibility (Jiao et al., 2016). Recent survey that studied rs937283A/G variant and its association with BC, revealed that G allele was confer high risk to BC in Asian (Chinese) population (Chen et al., 2018). There was no study examined the role of *MDM2* rs937283A/G polymorphism with susceptibility to breast cancer in the Saudi population. In the present study, the association of rs937283A/G polymorphism with BC risk in Saudi women was studied and the association of rs937283A/G variant was assessed within presence or absence of estrogen (ER), progesterone (PR) hormones, human epidermal growth factor receptor 2 (HER2) and at different menopausal status.

2. Material and methods

2.1. Patients and controls

The study was endorsed by the ethical committees of Taibah University, College of Dentistry and King Fahad Hospital in Al Madinah Al Munawarah. Archive paraffin embedded tumour and benign tissue samples were obtained for this study from Department of Histopathology, at King Fahad Hospital in Al Madinah Al Munawarah, where diagnosis was occurred. A total of 235 anonyms samples, consisted of 137 patients with breast cancer and 98 from healthy individuals who diagnosed with benign tumours, were included. The data of different clinicopathological characteristics such as the age at diagnosis, type of breast cancer, histologic grade, hormonal status of malignant breast tumours and the laterality of tumour was obtained.

2.2. The Genotyping of MDM2 rs937283A/G variant

Archives formalin-fixed, paraffin-embedded (FFPE) tissues samples (10-μm thick sections) were collected into 1.5 ml tubes. Genomic DNA was extracted using heating out-phenol–chloroform method. PCR-RFLP technique was carried out to genotype *MDM2* gene rs937283A/G polymorphism. PCR carried out using forward primer: 5′ GTGTGTCGGAAAGATGGAGC 3′ and reverse primer: 5′ CAGTACCTGCTCCTCACCAT 3′. The size of the amplified PCR products was 264 bp. *MboI* restriction enzyme was used, according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA USA). The Genotyping of MDM2 rs937283 A/G variant digested DNA fragments (The PCR products) were separated by electrophoresis in 3% agarose gel which stained with ethidium bromide. Genotypes were AA (115, 87, and 62 bp), AG (202, 115, 87, and 62 bp), or GG (202 and 62 bp). Six selected PCR products with the three different genotypes were shipped to Macrogen, Inc. (Korea) for DNA sequencing.

2.3. Statistical genetics analysis

Allele and genotype frequencies of rs937283A/G variant were calculated from data generated from the genotype of individuals. Departure from Hardy-Weinberg equilibrium was tested. Possible associations between rs937283A/G variant within *MDM2* gene and breast cancer susceptibility, in overall data and stratified groups, were tested. All statistical analyses were conducted using AssociatORRR software (https://www.genecalculators.net/associatorrr-cc.html). Odd ratio (OR) with 95% confidence intervals (CI) were used to determine association. P-value < 0.05 was considered as statistically significant.

3. Results

3.1. Patient characteristics

Demographic data of breast cancer cases are presented in Table 1. There was preponderance (80%) of invasive (infiltrating) ductal carcinoma. The cancer cases with grade II and III were 41% and 40% respectively. Bilateral BC was found in one patient. Breast cancer cases exhibited a higher rate of positive estrogen receptor (53%). There was no difference in frequencies (43%) of positive and negative progesterone receptor cases. The frequency of HER2 negative breast cancer patients was 58%. A slight difference was observed between premenopausal < 50 and postmenopausal ≥ 50 patients, which they were found to be 45% and 49% respectively.

Table 1
Demographic Characteristics of Saudi Breast Cancer Patients.

Variable		Cases (N = 137)
Histological subtypes	DCIS	12 (9%)
	Intracystic ductal carcinoma in situ	1 (0.7)
	Invasive (infiltrating) ductal carcinoma	110 (80%)
	Invasive lobular carcinoma	6 (4%)
	Medullary carcinoma	2 (1.5%)
	Mucinous carcinoma	4 (3%)
	Invasive squamous cell carcinoma	2 (1.5%)
Histological grades	Grade I	6 (4.4%)
	Grade I to II	4 (3%)
	Grade II	56 (41%)
	Grade II to III	10 (7%)
	Grade III	54 (40%)
	Unreported	7 (5%)
Laterality	Unilateral (Right Breast)	70 (51%)
	Unilateral (Left Breast)	66 (48%)
	Bilateral	1 (0.7%)
Hormonal status		
ER status	ER positive	73 (53%)
	ER negative	46 (34%)
	Unreported	18 (13%)
PR status	PR positive	59 (43%)
	PR negative	59 (43%)
	Unreported	19 (14%)
HER2 status	HER2 positive	38 (28%)
	HER2 negative	80 (58%)
	Unreported	19 (14%)
Age (years)	Premenopausal < 50	62 (45%)
	Postmenopausal ≥ 50	67 (49%)
	Unreported	8 (6%)

DCIS, Ductal carcinoma in situ; ER, Estrogen; PR, Progesterone; HER2, Human epidermal growth factor receptor 2.

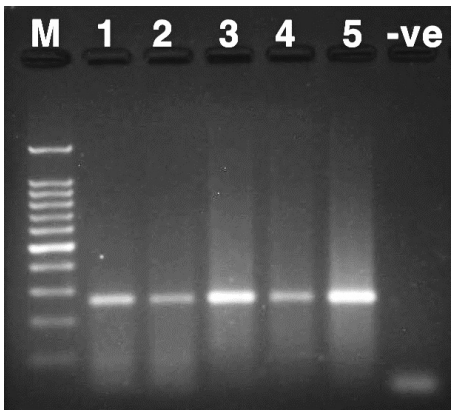

Fig. 1. 1% stained agarose gel electrophoresis showing PCR result of the rs937283 A/G polymorphism. M represents the 100 bp molecular-weight marker. Lanes 1, 2, 3, 4, and 5: 264 bp PCR product. Lane -ve: Negative control (no template).

3.2. rs937283 genotype distribution.

Fig. 1 shows the PCR amplification of the single nucleotide polymorphism rs937283 A/G with an expected PCR fragment length of 264 bp.

PCR-amplified and digested DNA samples were examined by DNA sequencing, and the results were concordant (Fig. 2)

A: 3% Stained agarose gel showing PCR-RFLP results: M represents the 50 bp molecular-weight marker. Lane 1: Undigested PCR product. Lanes 2, 4, 5, 6, 8, 10, 11, and 12: Homozygous wild type for A/A with 115 bp, 87 bp, and 62 bp fragments. Lanes 3, 7, 9, and 13: Heterozygous for A/G genotype with 202 bp, 115 bp, 87 bp, and 62 bp fragments. Lanes 14 and 15: Homozygous mutant for G/G genotype with 202 bp and 62 bp fragments.

B: Results of DNA sequence analysis, Homozygous wild type for A/A, Heterozygous A/G and Mutant G/G genotypes of rs937283A/G polymorphism are indicated with arrows

3.3. Genetic association

The allele and genotype distributions of rs937283A/G variant in cancer patients and controls are shown in Table 2. The current study shows that the genotype and allele frequencies of *MDM2* rs937283A/G polymorphism were in Hardy Weinberg equilibrium ($p < 0.05$). There was significant difference in genotype frequencies between cases and controls (p-value = 0.013). The minor G allele of *MDM2* gene rs937283 variant was associated with an increased BC susceptibility in Saudi women p = 0.004. Furthermore, stratification analyses by hormonal and menopausal status were conducted to explore the impact of these factors in susceptibility to breast cancer. It was found that *MDM2* rs937283A/G allele and genotype distributions were different between BC patients and controls in ER + patients (p-value = 0.007 and 0.006, respectively), PR + patients (p-value = 0.004 and 0.003, respectively) and HER2- patients (p-value = 0.002 and 0.004, respectively). Moreover, the risk of breast cancer was significantly higher in patients with triple

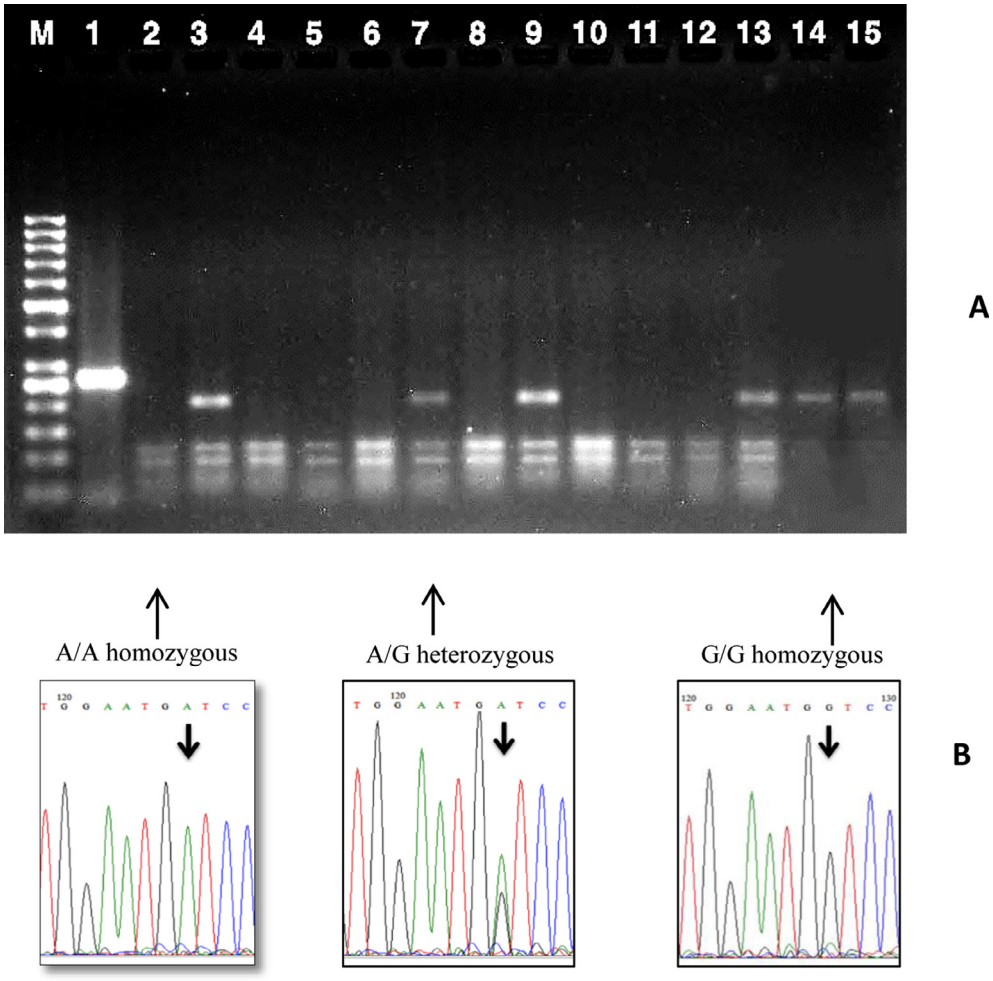

Fig. 2. Results of polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) and sequence analysis carried out for rs937283A/G polymorphism.

Table 2
Allele and Genotype Distributions of *MDM2* rs937283A/G variant and Association with Breast Cancer Susceptibility.

Polymorphism group	rs937283	Cases	Controls	P-value[1]	OR (95% CI)
Breast cancer	A	166 (0.606)	144 (0.735)	0.004	1.80 (1.21–2.69)
	G	108 (0.394)	52 (0.265)		
	AA	45 (0.328)	51 (0.52)	0.013	
	AG	76 (0.555)	42 (0.429)		
	GG	16 (0.117)	5 (0.051)		
ER+ breast cancer	A	87 (0.596)	144 (0.735)	0.007	1.88 (1.19–2.97)
	G	59 (0.404)	52 (0.265)		
	AA	21 (0.288)	51 (0.52)	0.006	
	AG	45 (0.616)	42 (0.429)		
	GG	7 (0.096)	5 (0.051)		
ER− breast cancer	A	56 (0.609)	144 (0.735)	0.04	1.78 (1.05–3.01)
	G	36 (0.391)	52 (0.265)		
	AA	18 (0.391)	51 (0.52)	0.448	
	AG	20 (0.435)	42 (0.429)		
	GG	8 (0.174)	5 (0.051)		
PR+ breast cancer	A	68 (0.576)	144 (0.735)	0.004	2.04 (1.26–3.30)
	G	50 (0.424)	52 (0.265)		
	AA	15 (0.254)	51 (0.52)	0.003	
	AG	38 (0.644)	42 (0.429)		
	GG	6 (0.102)	5 (0.051)		
PR− breast cancer	A	74 (0.627)	144 (0.735)	0.058	1.65 (1.01–2.69)
	G	44 (0.373)	52 (0.265)		
	AA	24 (0.407)	51 (0.52)	0.486	
	AG	26 (0.441)	42 (0.429)		
	GG	9 (0.152)	5 (0.051)		
HER2+ breast cancer	A	50 (0.658)	144 (0.735)	0.233	1.44 (0.81–2.55)
	G	26 (0.342)	52 (0.265)		
	AA	17 (0.447)	51 (0.52)	0.839	
	AG	16 (0.421)	42 (0.429)		
	GG	5 (0.132)	5 (0.051)		
HER2− breast cancer	A	92 (0.575)	144 (0.735)	0.002	2.05 (1.31–3.20)
	G	68 (0.425)	52 (0.265)		
	AA	22 (0.275)	51 (0.52)	0.004	
	AG	48 (0.6)	42 (0.429)		
	GG	10 (0.125)	5 (0.051)		
TNBC	A	17 (0.425)	144 (0.735)	0.0002	3.75 (1.86–7.56)
	G	23 (0.575)	52 (0.265)		
	AA	2 (0.1)	51 (0.52)	0.004	
	AG	13 (0.65)	42 (0.429)		
	GG	5 (0.25)	5 (0.051)		
PreM BC	A	69 (0.556)	119 (0.763)	0.0003	2.564 (1.54–4.28)
	G	55 (0.444)	37 (0.237)		
	AA	20 (0.322)	46 (0.59)	0.017	
	AG	29 (0.468)	27 (0.346)		
	GG	13 (0.21)	5 (0.064)		
PostM BC	A	87 (0.649)	25 (0.625)	0.851	0.90 (0.43–1.87)
	G	47 (0.351)	15 (0.375)		
	AA	23 (0.343)	5 (0.25)	0.426	
	AG	41 (0.612)	15 (0.75)		
	GG	3 (0.045)	0 (0)		

Abbreviations: CI, confidence interval; OR, odds ratio; PreM, premenopausal; PostM, postmenopausal; TNBC, Triple negative breast cancer; [1]Fisher exact. Two tailed p value.

negative breast cancer (p = 0.0002 and 0.004, respectively). In contrast, borderline association (p-value = 0.04) and no association (p = 0.448) between rs937283A/G variant and ER- breast cancer patients. No association was found in PR- and HER2 + breast cancer patients. Regarding the analysis of menopausal status of Saudi women at age range < 50 for premenopausal group vs. ≥ 50 years for postmenopausal group, there was a statistically significant difference in the allelic and genotypic frequencies between premenopausal breast cancer patients and controls, conferring a significantly increased risk in the premenopausal breast cancer patients (p-value = 0.0003 and 0.017, respectively). On the other hand, no significant difference was found for postmenopausal group.

4. Discussion

Breast cancer has been a worldwide health problem. Despite the tremendous progress in breast cancer treatment recently, advanced stages with distant metastasis persist with poor prognosis (Masters et al., 2015). Thereby, for early diagnosis and risk identification, finding of the genetic variants have a role in susceptibility to BC will be useful. Several studies showed an association between various gene variants with susceptibility to breast carcinoma. *MDM2* gene is an oncogene, a negative regulator of TP53, it has adverse effect on repair of DNA double strand break. It binds directly TP53 protein in absence of pressure (Boersma et al., 2006; Zhao et al., 2012). *MDM2* gene contains functional single nucleotide polymorphisms (SNPs), which are associated with tumour growth, DNA repair, angiogenesis, and apoptosis. Recently, the rs937283A/G, was recognized as *MDM2* gene promoter functional polymorphism. Transition of allele A to allele G significantly increases *MDM2* transcriptional level, which elevated MDM2 mRNA and protein, resulted in TP53 pathway attenuation (Jiao et al., 2016). Indeed, *MDM2* rs937283A/G variant in this study was significantly increased breast cancer risk in Saudi women.

The evaluation of the rs937283A/G genetic polymorphism showed an increased frequency of the minor allele G among breast cancer patients relative to that of controls in Saudi women.

Consistent with the present research, positive association was reported between the *MDM2* rs937283A/G polymorphism and breast cancer in Asian (Chinese) population (Chen et al., 2018), thus providing confirmation of rs937283A/G as potential risk for breast cancer. Significant different in rs937283A/G allele /genotype distributions was found between cases and controls in Asian and Saudi populations, where p-value = 0.003/0.008 and 0.0038/0.0078 respectively. The association between rs937283A/G polymorphism within *MDM2* gene and susceptibility to BC could be attributed to the following reasons: First, compelling evidence that the *TP53* is important gene in preservation genomic integrity and prevention of tumorigenesis. The overexpression of *MDM2* lead to loss of TP53 activity and therefore, promoting tumour growth (Wade et al., 2013; Gansmo et al., 2016). Second, the studied polymorphism within the *MDM2* gene has functional outcomes. The findings by Chen et al (2018) suggested that *MDM2* promoter SNP rs937283A/G variant could have a substantial consequence on breast cancer risk, which indicates the importance of MDM2 levels in carcinogenesis. In addition, other *MDM2* promoter polymorphisms may have a vital role.

Regarding rs937283A/G polymorphism and the risk of breast cancer with hormonal and menopausal status. The present study revealed positive association between rs937283 and risk in ER+ breast cancer patients. Consistent with these findings, many studies revealed that MDM2 mRNA and protein were high in breast tumours that express ER (Sheikh et al., 1993; Gudas et al., 1995; Brekman et al., 2011). In contrast, all the ER-negative breast tumours were reported to express significantly less MDM2 mRNA and protein (Gudas et al., 1995). Moreover, association between rs937283A/G polymorphism and BC risk in PR+ breast cancer patients has been confirmed. This association was strengthened by the fact that PR positive breast cancers showed *MDM2* overexpression (Haupt et al., 2017). *MDM2* overexpression in BC patients may be related to ER and PR activation. A significant association with susceptibility to BC in HER2 negative breast cancer patients was found in this study, which suggested that *MDM2* rs937283A/G variant may have greater effect in breast tumour, where *HER2* expression is absence. Many human breast cancers overexpress *MDM2*. Notably, it has been demonstrated that *MDM2* expression is the highest in TNBC subtype; this further validates the reliability of this results. The expression of *MDM2* in TNBC can lead to different tumour consequences; *MDM2* can promote TNBC metastasis (Gao et al., 2019). Treatment of triple negative breast cancer patients by targeting the MDM2 signalling axis could lead to new method in combination therapy (Tonsing-Carter et al., 2015). Pre-menopausal BC patients, at the time of diagnosis, had a higher frequency of rs937283 G allele, these findings suggested that this polymorphism may affect susceptibility to BC in Saudi women at pre-menopausal age.

Genetic testing can distinguish individuals with high risk for diseases including BC. The current results can be extended in clinical application. Early detection of women at-risk of BC could delay the disease progression during personalized medication. In addition, environmental factors could be detected, and lifestyle could be changed to minimize the risk of BC. However, this study included some constrains, first, hospital-based case control design was performed. Hence, the possible bias from selection should be considered. Second, the groups in stratified analysis could limit detection of the interactions between each variable and *MDM2* rs937283A/G in BC patients. Third, the exact molecular mechanism for controlling rs937283A/G transcriptional action is not clear, which requires to be executed in forthcoming functional studies. Finally, the present study included only Saudi population; additional confirming studies in other populations are required.

5. Conclusion

This study was reported first assessment of the association of SNP rs937283A/G with breast cancer in the kingdom of Saudi Arabia. This was the first analysis of the association of SNP rs937283 according to the hormone receptors status in breast cancer patients and age of onset. The results showed that *MDM2* gene rs937283A/G polymorphism increases the risk of breast cancer in a sample of Saudi women. *MDM2* rs937283A/G variant may act as an important risk factor and diagnostic biomarker in Saudi women with breast cancer patients.

CRediT authorship contribution statement

Weam Talal Yehya Shebli: Methodology, formal analysis, original draft writing. **Mohammad Kdaimes H. Alotibi:** Conceptualization, Supervision, Validation, Writing-reviewing and editing. **Rawya Ibrahim AL-Raddadi:** Methodology. **Razan Jamaan Alamri:** Methodology. **Emad Ibrahim Yagoub Fallatah:** Resources, Methodology. **Ahmed Safar Alhujaily:** Resources, Methodology. **Hiba Salaheldin Mohamed:** Conceptualization, Supervision, Validation, Writing-reviewing and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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