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HUMAN DNA LIGASE I (*LIGI*) GENE AND RISK OF CERVICAL CANCER IN NORTH INDIAN WOMEN

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***Aim*: DNA repair genetic polymorphisms may affect cancer susceptibility as genetic variations in DNA repair genes may influence DNA repair capacity. In the present study, the association of polymorphic forms of DNA repair gene, DNA ligase I (*LIGI*) was examined with the risk of cervical cancer in case of North Indian women. *Materials and Methods*: Polymorphism was determined by polymerase chain reaction — restriction fragment length polymorphism (PCR-RFLP) method and risk of cervical cancer was evaluated by calculating odds ratios (ORs) and 95% confidence interval (CI) using a multivariate logistic regression analysis. *Results*: No association was found between variant forms (AC, AA) of *LIGI* gene and risk of cervical cancer (OR — 0.8, 95% CI 0.46–1.53 and OR — 1.0, 95% CI 0.51–2.06, respectively). However, increased but statistically non-significant risk of adenocarcinoma was observed for cervical cancer patients having AC (OR — 4.6, 95% CI 0.62–33.82) and AA (OR — 5.0, 95% CI 0.63–39.58) genotypes. *Conclusion*: It can thus be concluded that there is no association between LIGI polymorphisms and cervical cancer risk. However, they may be playing an important role in modulating the risk of cervical adenocarcinoma in North Indian women. Further investigations in larger studies need to be carried out for more analysis.**

***Key Words*: cervical cancer, risk, *LIGI*, North Indian women, PCR-RFLP, genetic polymorphism.**

Cancer can be initiated by DNA damage caused by ultra violet (UV) ionizing radiation and environmental chemical agents [1]. To safeguard the genome against these agents, human beings have developed a set of complex DNA repair systems. Dysfunction in these systems thus plays a critical role in cancer development [2].

Human DNA ligase I plays an essential role in DNA replication, recombination, and repair by catalyzing the formation of phosphodiester bonds between adjacent 5'-phosphoryl and 3'-hydroxyl termini at single breaks in duplex DNA molecules [3–5]. The strongest evidence of role in DNA replication comes from the human 46 BR cell line in which a mutation in the DNA ligase I gene correlates with a delay in the joining of the Okazaki fragments [6]. Some studies [7–9] suggest that DNA ligase I also play important role in DNA repair. It is involved in both nucleotide excision repair [10] and long-patch base excision repair [11]. Taken together, these studies suggest that DNA ligase I is involved in different aspects of DNA metabolism [5].

DNA ligase I is responsible for the majority of DNA ligase activity in proliferating cells, whereas most of the ligase activity in resting cells is due to other DNA ligases [4]. Sun *et al.* [12] measured DNA ligase I level by Western immune-blot assay in various human malignant tumor specimens and benign tissues obtained from patients, in peripheral blood lymphocytes obtained from healthy donors, and in human tumors grown in nude mice. They reported that the amount of DNA ligase I enzyme in malignant tumors was considerably higher than that in benign normal tissues and peripheral blood lymphocytes. The level of DNA ligase I in human tumors grown in nude mice was also very high, and the expression of DNA ligase I was constitutive during *in vivo* tumor development. This suggest that DNA ligase I is potentially an important target for the design of new anticancer agents, and there is a strong possibility of achieving selective inhibition against rapidly proliferating tumor cells. Antisense oligonucleotides (ODNs) targeting the mRNA of DNA ligase I have been designed and tested [12] and were found to inhibit the expression of DNA ligase I without affecting that of DNA ligase III.

Polymorphic variations in genes involved in DNA repair have been found to be widely associated with cancer susceptibilities. Two DNA ligase I (*LIGI*) variants have been identified in HeLa cells as well as human tissues [13]. In the first one, there is a single nucleotide polymorphism in which either A or C is found at a site in exon 6. In the other variant, there is a complex GT repeat at the 5' end of intron 6, consisting of a 48–50 nucleotide polypurines. The *LIGI* exon 6 A→C polymorphism does not cause amino acid change. The biological relevance of this variant and whether it is in linkage disequilibrium with functional polymorphisms at other sites is unknown. Shen *et al.* [14] investigated the role of this polymorphism in the etiology of lung cancer, but did not find any association, suggesting that it might not be playing an important role in susceptibility to lung cancer. We don't know about published studies that have explored the relationship between *LIGI* polymorphism and cervical cancer risk. Therefore the objective of the present study was to analyze this association in the North Indian women.

Sample collection. A total of 150 blood samples were obtained from histologically confirmed cervical cancer patients attending Outpatient department (OPD) at the Gynecology Department of Post Graduate Institute of Medical Education and Research, Chandigarh and Mohan Dai Oswal Cancer Hospital, Ludhiana. None of the patients had received radio- or chemotherapy. The control group consisted of 150 individuals who were free of any malignancy and well matched according to age and ethnicity. Written consent was obtained from all the research participants and the study was approved by the ethical

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committees of both institutes. Genomic DNA of blood samples was isolated by SDS/proteinase K and phenol chloroform method.

Genotyping of DNA ligase I. The human DNA ligase I *Hae III*/exon 6 polymorphism was determined by using PCR-RFLP method [14]. 25 μl of reaction mixture was prepared, which contained 50 ng of genomic DNA, 6.25 pmol of each of primers (F-ATGCCCTGTAGGTTCAATGG, R-TGGAGGTCTTTAGGGGCTTG), 0.1 nM of each of dNTPs, IX PCR buffer (50 mM KCl, 10 mM Tris-HCl and 0.1% Triton X-100), 1.5 mM $MgCl_2$ and 1U of Taq polymerase (MBI, Fermentas). Amplification was carried out, using an initial denaturation at 95 °C for 5 min; 35 cycles of melting at 95 °C for 30 s, annealing at 58 °C for 35 s, and extension at 72 °C for 40 s; followed by a final extension step at 72 °C for 10 min. The PCR product was of 165 bp size (Fig. 1). PCR product (10 μl) was then digested with 5 unit *Hae III* (MBI, Fermentas) at 37 °C for 3 h and analyzed by electrophoresis on 2.5% agarose gel. AA homozygotes were identified by the presence of only 165 bp fragment, AC heterozygote by the presence of 165, 100 and 65 bp fragments and CC homozygotes by 100 and 65 bp fragments (Fig. 2).

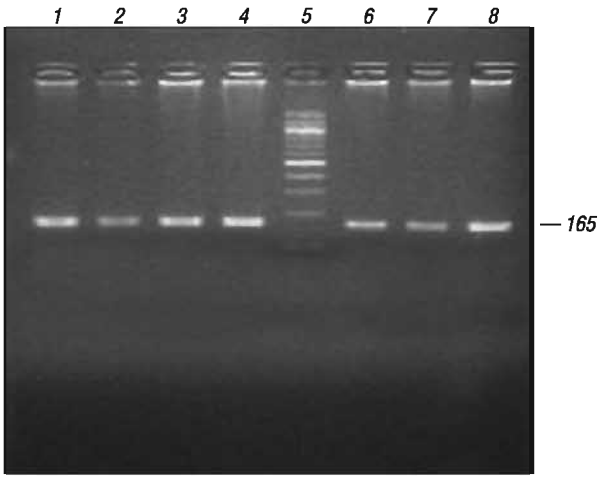

Fig. 1. PCR amplified product of *LIGI*. Lane 1–4, 6–8 PCR Product (165 bp). Lane 5 = 100 bp DNA Marker

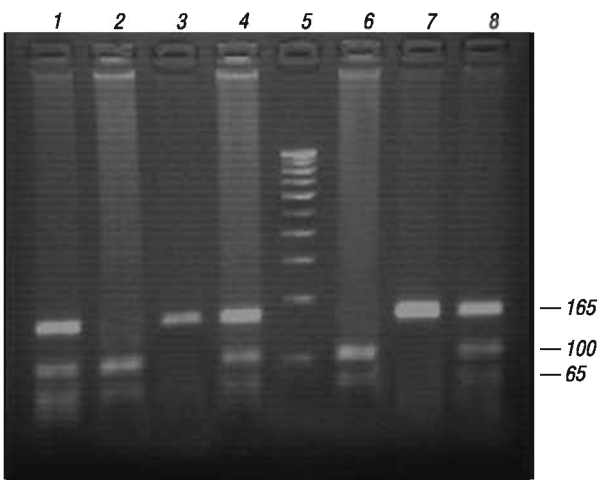

Fig. 2. RFLP analysis of *LIGI*. Lane 1, 4, 8 — Heterozygous mutant (AC heterozygote) (165, 100 and 65 bps). Lane 2, 6 — Homozygous wild (CC homozygotes) (100, 65 bps). Lane 3, 7 — Homozygous mutant (AA homozygotes) (165 bp). Lane 5 — 100 bp DNA marker

Statistical analysis. The association between different polymorphic forms of *LIGI* gene with the risk of cervical cancer was estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) using SPSS version 10.0 and Epical Version 3.2. The probability level of less than 0.05 was used as the criterion of significance. χ^2 test was performed to check whether the genotype distribution in the population was according to Hardy — Weinberg equilibrium or not.

When classified histologically, most cervical cancers are either squamous cell carcinomas or adenocarcinomas. In the current study out of 150 cervical cancer cases, 131 (87.3%) were squamous cell carcinoma and 19 (12.7%) were adenocarcinoma. Distribution of *LIGI* genotypes among cases and controls is given in Table 1. There were no significant differences in the distribution of polymorphic forms of *LIGI* between cases and controls. The *LIGI* CC, AC and AA genotype frequencies were 24.7; 48.7 and 26.6% in cervical cancer cases and 22.7; 53.3 and 24.0% in controls, respectively. When classified histologically the frequency of CC genotype was higher in case of patients with squamous cell carcinoma (27.5%). However, AC and AA genotypes were more prevalent in patients with adenocarcinoma (63.1 and 31.6%, respectively). The genotype distribution for both cases and controls was in Hardy — Weinberg equilibrium.

Table 1. Distribution of *LIGI* genotypes among cases and controls

	n[c]	CC (%)	AC (%)	AA (%)
Controls	150	34 (22.7)	80 (53.3)	36 (24.0)
Cases	150	37 (24.7)	73 (48.7)	40 (26.6)
Squamous cell carcinoma	131	36 (27.5)	61 (46.6)	34 (25.9)
Adenocarcinoma	19	1 (5.3)	12 (63.1)	6 (31.6)

Notes: n[c] = number of case/controls.

On analyzing the relationship between variant forms of *LIGI* with the risk of cervical cancer, no association was observed between *LIGI* genotypes and overall cervix cancer risk. However, when divided into histological subtypes, increased risk of adenocarcinoma was observed for those having AC (OR — 4.6, 95% CI 0.62–33.82), AA (OR — 5.0, 95% CI 0.63–39.58) and AC + AA (OR — 4.7, 95% CI 0.65–34.02) genotypes, as compared to those with CC genotype (Table 2).

Table 2. OR and corresponding 95% CI of *LIGI* genotypes and risk of cervical cancer

Genotypes	All OR (n=300)	Squamous cell carcinoma	Adenocarcinoma
CC	1.0 (ref)	1.0	1.0
AC	0.8 (0.46–1.53)	0.7 (0.39–1.33)	4.6 (0.62–33.82) p=0.07
AA	1.0 (0.51–2.06)	0.9 (0.44–1.83)	5.0 (0.63–39.58) p=0.08
AC+AA	0.9 (0.51–1.58)	0.8 (0.43–1.38)	4.7 (0.65–34.02) p=0.06

Common polymorphisms in DNA repair genes may alter protein function and an individual's capacity to repair damaged DNA, therefore defect in repair capacity may lead to genetic instability and carcinogenesis [15]. As a result, genes coding for DNA repair molecules have been proposed as candidate cancer-susceptibility genes [16, 17]. Based on a review of epidemiologic studies, Berwick and Vineis [18] suggested that reduced DNA repair capacity was associated with increased risk of cancer. Li et al. [19] reported that

genetic variations in DNA repair genes may act alone or in combination with other risk factors in modifying a patient's risk for pancreatic cancer.

In the present study DNA repair gene, *LIGI* did not show significant association with risk of developing cervical cancer among the North Indian women. Similar results have been reported by Shen et al. [14] in case of lung cancer and Lee et al. [20] in case of head and neck cancer. Sobti et al. [21] also reported no association between DNA ligase I and risk of lung cancer in North Indian population.

Our study has several merits as well as limitations. One major limitation is the number of cases and controls, which limits the power of the study to detect significant associations. For example, in our study, an increased risk of adenocarcinoma was observed for individuals having AC, AA and AC + AA genotypes but the associations were not strong enough to reach statistical significance. Despite this limitation, the current study, is the first, to report that inheritance of variant forms of *LIGI* might modify the risk of adenocarcinoma of cervix. A major strength of this study was that the controls were selected from the same population as that of cervical cancer cases; which therefore, reduced an important potential source of selection bias. The findings of this study, may shed a new light on association between *LIGI* and cervical cancer risk and pave the path for further studies and help in understanding whether inheritance of polymorphic forms of *LIGI* affects the development of cervical cancer or not.

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