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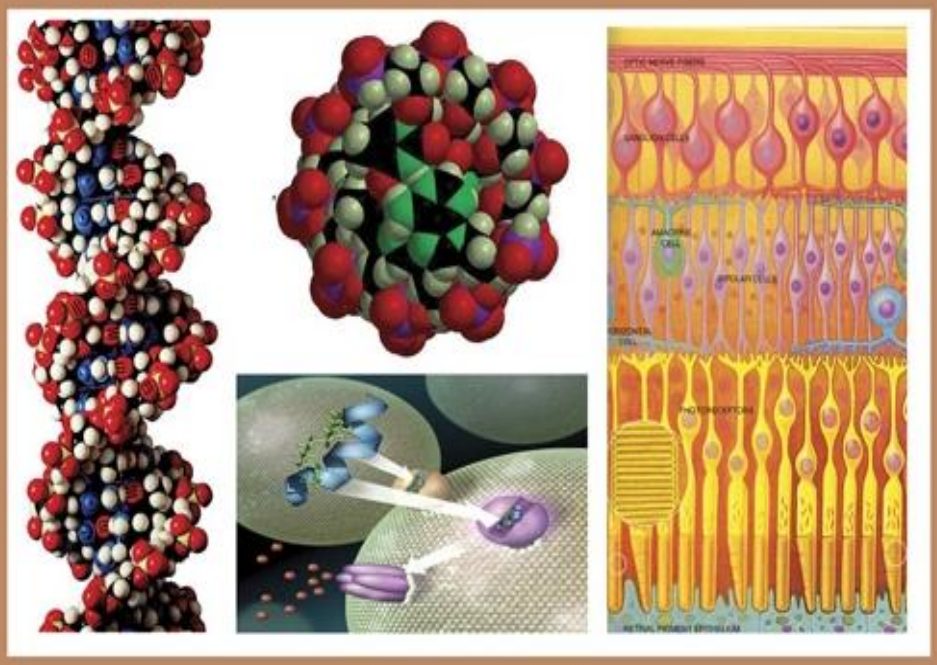

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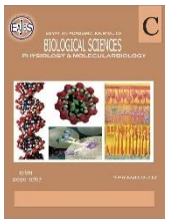

# Polymorphism in Long Non-Coding RNA- HOXA Transcript at The Distal Tip Among Rheumatoid Arthritis Patients

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**ABSTRACT**

Rheumatoid arthritis (RA), an autoimmune condition, is the most prevalent inflammatory arthropathy worldwide. Egypt's RA phenotypic spectrum varies from region to region, with a progressive shift in the F:M ratio. Limited studies have explored the correlation between lncRNA HOTTIP single nucleotide polymorphisms (SNPs) and RA. This study aimed to evaluate the genetic association of single nucleotide polymorphism rs3807598 (C:G) of HOTTIP among rheumatoid arthritis patients. Method: The study included 120 subjects from 2 groups: 60 RA patients and 60 healthy controls. Real-time PCR with TaqMan allelic discrimination assay was used to perform the genotyping. The odds ratios (ORs) models and 95% confidence interval (CIs) were used to test the associations. Results: The results indicated that lncRNA HOTTIP SNP rs3807598 (C:G) exhibited a statistically nonsignificant association with the risk of RA. Conclusion**:** Up-to-date, the role of HOTTIP SNPs in RA risk is still unknown, It is important to consider how epigenetic modifications including non-coding RNAs polymorphism and expression affect the RA risk and how well it responds to treatment. Further studies, with a larger sample size, could highlight more significant relations between SNPs of non-coding RNAs and RA.

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoinflammatory disease affecting connective tissue, characterized by progressive joint damage and specific systemic disorders (Jiang *et al.,* 2023; Chaudhari *et al.,* 2016). The main clinical symptoms of RA are symmetrical pain, stiffness, and swelling in one or more joints; the joints affected are those in the hands, wrists, feet, and knees. Without active clinical therapy, RA can result in severe disability and, eventually, early mortality. As the RA progresses, it can cause damage to the bones and cartilage as well as extra-articular disorders such as cardiovascular disease and organ damage (Han *et al.,* 2022; Conforti *et al.,* 2021). Rheumatoid arthritis affects approximately 1% of the world's population and disproportionally affects the female population.

According to estimates, depending on both sexes, the prevalence varies from 0.4% to 1.3% of the population (women are affected two to three times more often than men) (Littlejohn and Monrad, 2018).

Single nucleotide polymorphisms (SNPs) are genetic variants that only contribute 0.1% of population differences. About 50% of the SNPs in the coding area are silent or synonymous, while 25% are missense SNPs. By establishing or destroying miRNA sites, non-coding SNPs can influence the stability of mRNA and the activity of its promoter, altering how a gene expresses itself and ultimately affecting whether a gene is up- or down-regulated. For the proper understanding of the molecular pathways involved, the relevance of these polymorphisms in relation to RA risk needs to be investigated. SNP analysis will aid in the creation of new RA medications in addition to helping to understand the underlying disease process (Akhtar *et al.,* 2021). Multiple therapy modalities are necessary to improve the clinical result in RA, despite the fact that current management guidelines may still favour a "one-size-fits-all" treatment approach. Early disease-modifying anti-rheumatic drug (DMARD) treatment is the norm, although many patients eventually become disabled and experience significant morbidity (Gheita *et al.*, 2023).

Long non-coding RNAs (lncRNAs) are a class of RNA transcripts that are more than 200 nucleotides (nt) in length and have no/ limited ability to code for proteins. These RNAs may regulate the cell cycle, promote cell growth and differentiation, and inhibit apoptosis, among other biological activities. A number of tumours have also been found to express lncRNAs abnormally, where they act as tumour suppressor genes or oncogenes (Lv *et al.*, 2019). According to estimates, there are more than 100,000 lncRNAs in humans ( Zhao, *et al.*, 2016 ). lncRNAs are often categorized by their position relative to nearby protein-coding genes, i.e., intergenic, antisense, intronic, or bidirectional (Smith and Mattick, 2017). lncRNAs have been extensively researched in the context of cancer ( Silva *et al.*, 2015), inflammation (Chew, et al., 2018), and innate and adaptive immunity (Fitzgerald and Caffrey, 2014).

HOXA transcript at the distal tip, or LncRNA HOTTIP, is an enhancer-like lncRNA of the human HOXA locus. In several disorders, HOTTIP has a significant potential to function as an essential regulator. SNPs, the most prevalent kind of genetic variation, modify the secondary structure of lncRNAs or the expression of their coding genes, which affects how well they perform their tasks. Recent research on the lncRNA HOTTIP gene did in fact find a number of SNPs with significant implications on diseases risk (Zheng *et al.*, 2020) However, critical lncRNA HOTTIP gene SNPs to RA risk haven't yet been documented.

Up until recently, the genetic causes of a sizable fraction of familial RA have remained unknown. It is crucial to identify RA risk categories in various populations as well as the potential prognostic usefulness of specific genetic variants for the onset, progression, and management of the disease. This study aimed to evaluate the genetic association of single nucleotide polymorphism rs3807598 of HOXA transcript at the distal tip (HOTTIP) among rheumatoid arthritis patients.

## MATERIALS AND METHODS

The study included 60 RA patients and 60 healthy controls. All participants gave written informed consent prior to the study. RA patients were diagnosed and a validated score for established RA was used as a measure of disease activity. The observed controls had no signs of RA, including morning joint stiffness, citrulline antibody, positive rheumatoid factor (RF), or the findings of rheumatoid nodules. Furthermore, the patients with other inflammatory disorders or autoimmune diseases unrelated to RA were not included, also the exclusion criteria include patients

with arthralgia, heart failure, renal failure, and other autoimmune diseases or inflammatory conditions.

DNA was extracted from whole blood using QIAamp DNA extraction kit (Qiagen, USA) according to the manufacturer's instructions protocol. The extracted DNA samples were subjected to DNA quantitation and purity assessment using the NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA). Genotyping was performed using real-time PCR with TaqMan allelic discrimination assay (Applied Biosystems, USA). A pre-designed primer/probes sets for the two genotypes were used (Applied Biosystems, USA). the assessment of the genetic polymorphisms rs3807598(C/G) was carried out according to qRT-PCR protocol (Qiagen, Valencia, CA, USA).

**Statistical Analysis:**

Hardy–Weinberg equilibrium (HWE) test was performed for rs3807598 C>G in controls using a $\chi 2$ test. The differences in clinical characteristics in cases and controls were tested using a $\chi 2$ test. Multivariable logistic regression analysis was used to test for an association between SNP rs3807598 C>G and the risk of RA. Such association was quantified by odds ratios (ORs) and 95% confidence intervals (CIs). Two-sided $P < 0.05$ indicates differences were statistically significant.

## RESULTS

The genotype frequencies of HOTTIP gene among rheumatoid arthritis patients compared to control subjects are provided in Table 1. The genotype frequencies for rs3807598 C>G were 36% (CC), 38% (CG) and 26% (GG) in RA cases and 62% (CC), 30% (CG) and 8% (GG) in controls. After adjustments for age and gender (GG vs CC: adjusted OR=1.64, 95% CI=0.31-8.38; CG vs CC: adjusted OR=1.78, 95% CI=0.56-5.68; GG/CG vs CC: adjusted OR=1.81, 95% CI=0.60-5.40; and GG vs CC/CG: adjusted OR=1.41, 95% CI=0.31-6.37). According to the genetic association models, no significant evidence of the association between rs3807598 C>G and rheumatoid arthritis risk (Table 2).

**Table 1:** Genotype distribution and allele frequencies of HOTTIP gene among rheumatoid arthritis patients compared to control subjects.

| SNP | Group | Genotype distribution n (%) | | | *p*-HWE | *p*-value | Allele frequency (%) | | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| ***HOTTIP* n.342C>G (rs3807598)** | | **CC** | **CG** | **GG** | | | **C** | **G** | |
| | Control | 31 (62) | 15 (30) | 4 (8) | 0.126 | 0.013 | 77 | 23 | <0.001 |
| | Cases | 18 (36) | 19 (38) | 13 (26) | 0.644 | | 55 | 45 | |

Data are expressed as frequencies (percentages). SNP: Single nucleotide polymorphism, HOTTIP: HOXA transcript at the distal tip, *p*-HWE: *p*-value of Hardy-Weinberg Equilibrium.

**Table 2:** HOTTIP variants with rheumatoid arthritis risk according to the genetic association models.

| | Homozygous codominant model | Heterozygous codominant model | Dominant model | Recessive model |
|---|---|---|---|---|
| ***HOTTIP* n.342C>G (rs3807598)** | **GG *vs* CC** | **CG *vs* CC** | **GG/CG *vs* CC** | **GG *vs* CG/CC** |
| **†Adjusted OR (95%CI)** | 1.640 (0.312-8.380) | 1.789 (0.563-5.687) | 1.813 (0.608-5.401) | 1.418 (0.316-6.374) |
| ***p*-value** | 0.552 | 0.325 | 0.285 | 0.649 |

HOTTIP: HOXA transcript at the distal tip, OR: Odds ratio, 95% CI: 95% Confidence interval. †: Adjusted for sex and age.

## DISCUSSION

Inflammation, synovial hyperplasia, and cartilage erosion are the main symptoms of rheumatoid arthritis, a chronic inflammatory disease with an uncertain aetiology. To fully understand the precise mechanisms by which long noncoding RNAs (lncRNAs) may mediate signaling pathway molecules and alter the pathophysiology of RA, more research is required. Long noncoding RNAs and abnormal human disorders, such as cancer, mental illnesses, autoimmune diseases, and others, are intimately associated.

Till now, the role of lncRNA HOTTIP SNPs in RA risk remains unknown. Herein the current study aimed to assess the genetic correlation between rheumatoid arthritis and the single nucleotide polymorphism rs3807598 (C:G) of the HOXA transcript at the distal tip. Single nucleotide polymorphisms hold a significant role in the pathogenesis of the disease (Akhtar *et al.,* 2021). Clinical polymorphism, which manifests as a wide range of variations in symptoms, clinical presentations, and progression rates, is a clear characteristic of RA. Rheumatoid arthritis is seen as a complex disease that is brought on by both environmental and genetic triggers. Only 1% of the genome's coding region has SNPs; the majority of them are found in non-coding DNA. Near the noncoding areas of 40–50 genes, single RA-related nucleotide changes have been found. By activating tissue-specific super-enhancers, at least some of them are thought to raise the risk of RA (Mikhaylenko *et al.,* 2020).

The initial reason for the altering of the downstream functions was due to single nucleotide polymorphism in the lncRNAs. Long noncoding RNAs are known to affect cell activity by regulating a variety of target genes (Subramanian *et al.,* 2013) *via* serving as Wnt signalling pathway modifiers. Among lncRNAs, "HOTTIP", takes a role in cell growth and invasion. Though, the relationship between HOTTIP and RA is still unknown. It is well-recognized that secreted frizzled-related proteins (SFRPs) contribute to the pathogenesis of RA. In fibroblast-like synoviocytes, overexpression of SFRP4 was found to block b-catenin, C-myc, and CCND1, the main genes of the canonical Wnt signalling pathway, which in turn slowed the progression of adjuvant arthritis (Miao *et al.,* 2017). This is consistent with earlier research. According to several reports, paricalcitol reduces RA by inhibiting the Wnt/-catenin signalling pathway (Yolbaş *et al.,* 2018).

Numerous studies have revealed that the lncRNA HOTTIP is markedly increased in a variety of human malignancies, making it a useful diagnostic tool for screening and a significant therapeutic target for cancer treatment. The HOTTIP SNPs (rs3807598, rs2067087, and rs17427960) were associated with enhanced colorectal cancer risk (Lv *et al.,* 2019). Also, the risk-associated SNPs rs3807598 and rs2067087 are anticipated to contribute to gastric carcinogenesis by upregulating the expression of mature HOTTIP in recent years, according to Wang *et al.* (2020). Similarly, the overexpression of HOTTIP affects the risk of a variety of tumours, including hepatocellular carcinoma, gastric cancer, pancreatic cancer, lung cancer, prostate cancer, and osteosarcoma. On the other hand, Zheng *et al.* (2020) reported a negative association between lncRNA HOTTIP rs3807598 C>G and Hirschsprung disease.

Regarding HOTTIP and Rheumatoid arthritis patients, Huang *et al.* (2021), indicated that HOTTIP was highly expressed in rheumatoid arthritis synovial fibroblasts (RASFs) and that inhibiting it could reduce RASF proliferation and increase apoptosis. RASFs inflammatory responses may be inhibited by the loss of HOTTIP, which may also slow the development of RA. In addition, the finding that HOTTIP inhibited the expression of SFRP1 by encouraging SFRP1's promoter methylation through the recruitment of Dnmt3b is significant because

SFRP1 was previously described as a Wnt signalling pathway inhibitor (Miao *et al.*, 2021). In consequence, HOTTIP activates the Wnt Signalling pathway and induces inflammatory responses in RASFs by Binding to Dnmt3b. Consistently, a recent study found that HOTTIP knockdown inhibited the proliferation of hand synovial fibroblasts and induced apoptosis, which aided in the prevention of synovial hyperplasia and arthritis in RA (Bertoncelj *et al.*, 2018) Similar conclusions have been drawn on how inhibiting HOTTIP can stop OA from progressing (Kim *et al.*, 2013).

In conclusion, given the critical role of specific HOTTIP gene SNPs in several malignancies and the significance of HOTTIP in rheumatoid arthritis, the study reported a negative association between HOTTIP rs3807598 C:G and RA. Further validation studies with a larger sample size could be conducted and research into the impact of HOTTIP SNPs in RA risk is crucial.

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