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Tuberculosis risk in P2X7 1513A/C polymorphism of the tribes of Jhargram, West Bengal

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Abstract
Increase of tuberculosis disease depends on the interaction of human host and mycobacterial stain. The adenosine triphosphate mediated purinergic P2X7 receptors kill the intracellular *Mycobacterium tuberculosis* and cell death occur of the infected macrophage. Only a single nucleotide polymorphism at the exon 13 of P2X7 1513A/C gene can change the function of this receptor and increased the risk of tuberculosis in tribal population of Jhargram. The P2X7 1513A/C gene polymorphism has been studied on TB infected (n=56) and non-TB (n=60) individuals and the CC genotype has significantly vast chance to occur tuberculosis (P<0.05). Consequences of this research plan established that CC genotype of P2X7 polymorphism is related with susceptibility to TB in contaminated patients of that.

Keywords: P2X7 receptor, 1513 A/C polymorphism, tuberculosis, tribal population

1. Introduction
India records for an expected one third (32%) of all tuberculosis (TB) cases around the world, with the most elevated number of occurrence cases [1]. It is evaluated that 33% of the total population is contaminated with *Mycobacterium tuberculosis* [2, 3]. Among this individuals who are contaminated, just roughly 5– 10% will develop clinical disease [4]. The result of tuberculosis is regulated by the environment or bacterial, beside it many investigations have confirmed that host genetic elements are concerned within the disease [5-14].
Resistance capacity of a host depends on immune system. In immune system, macrophage is the primary and crucial cell for mycobacterial infection. Additionally, extracellular ATP induces the bactericidal action of macrophages through activation of the P2X7 purinergic receptor; this leads to apoptosis of the macrophage, which play a critical role in host defence mechanism against *M. tb* infection [15].
Different types of gene polymorphisms of the P2X7 receptor have been identified which influence its function and have been related with various diseases including tuberculosis [7, 16, 17]. Several genetic polymorphisms have been studied in association with TB including P2X7 in Indian population [3, 12, 18].
P2X7 is a ligand-gated cationic channel with trimeric structure expressed on human cells, including membranes of cells in the immune system especially macrophage and hemotopoietic system, and is activated by adenosine triphosphate (ATP) [19, 20]. The human P2X7 1513A/C gene is situated on chromosome 12q24.31 and consists of 13 exons, with exon 12 and 13 coding for the C-terminal tail of this molecule [5-7, 17, 21]. The current study presents the association of P2X7 1513A/C polymorphism with tuberculosis susceptibility in the tribal people of Jhargram district of West Bengal, India.

2. Material & Methods
2.1 Sample Collection
Hundred sixteen individuals from Jhargram District were recruited for this study. Among them fifty six individuals are patient of pulmonary tuberculosis (pTB) and sixty individuals are healthy non-tuberculosis people. Informed written consent was taken from all the participants and this study was approved by the ethical committee of Vidyasagar University, Paschim Medinipur, West Bengal.

2.2 DNA Isolation
Peripheral blood samples were collected in EDTA coated vials (Himedia, India). Genomic DNA was extracted by utilizing the Blood Genomic Isolation kit (Himedia, India), and then the purified DNA was kept in -20°C. Quantity and purity were determined by spectrophotometer.

2.3 Assay
Genotyping was performed by 3-step Polymerase Chain Reaction (PCR) in Applied Biosystem thermal-cycler and it is followed by Restriction Fragment Length Polymophism (RFLP) and then 2% Agarose gel electrophoresis was done.
One milliliter of peripheral blood was taken, and genomic DNA was isolated by Blood Genomic Isolation kit (Himedia, India). The P2X7 1513A/C is a 316-bp fragment. This gene was genotyped by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism with the following primers: forward 5′-AGACCTACGATGGACTTCACAG-3′; reverse 5′-AGCGCCAGCAAGGGCTC-3′. Four PCR conditions included initial 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 45s and a final extension at 72°C for 5 min. The PCR product was digested at 37°C for 6–8 h with the restriction endonuclease *Hae*II (New England Biolabs Inc, USA). The digested product

was run on 2% agarose gel pre-stained with ethidium bromide and visualized under ultraviolet light (Biorad Gel Doc). Samples with single 316-bp band were identified as A/A, samples with 3 bands of 316, 200, 119 were distinguish as heterozygous A/C and 2 bands with 200,119 as C/C polymorphism [22].

Table 1: Details of the primers used for the P2X7 polymorphisms studied. This conditioned for PCR along with the target amplicon size and restriction enzymes used.

Gene and Polymorphism	Primer	Fragment length	Annealing temperature	Restri-ction enzyme used	Polymorphic fragments (bp)
P2X7 1513 A/C	5′AGACCTACGATGGACTTCACAG3′ 5′AGCGCCAGCAAGGGCTC3′	316-bp	57°C	Hae II	316,200, 119

2.4 Statistical Analysis

All data were analyzed by SPSS 20.0 software (IBM Corporation, Somers, NY, USA). Data were expressed as chi-square test was done for the homogeneity distribution of these tuberculosis and non-tuberculosis group. Genotype frequencies comparisons between groups were presented as odds ratio (OR) and 95% confidence interval (CI). All p values were two-sided, and the level of significance was set at $p < 0.05$.

3. Result

Genotyping of P2X7 1513A/C polymorphism; an uncut PCR product of 316bp for AA genotype, while 200 and 119bp fragments indicated CC genotype and heterozygote had all three bands(AA, AC, CC) are visualizing in 2% agarose gel electrophoresis. Agarose gel of genotypes of the P2X7 1513 A/C receptor polymorphism shows three type of banding pattern among the study population (Figure1). All samples were amplified by specific and constant forward (FO) reverse (RO) primers.

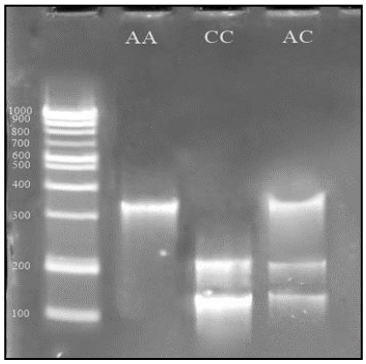

Fig 1: The 2% agarose gel electrophoresis of P2X7 1513 A/C gene after restriction endonuclease digestion and Polymerase Chain Reaction

The genotype and allele frequencies for the 1513A/C polymorphism in patients and controls are shown in Table 2 and 3; in addition it is showing the odd ratio and confidence interval in these same tables. The chi-square between the pTB patient and healthy non-TB group was significant ($p<0.05$).

Table 2: The P2X7 1513A/C genotype number and frequency of TB infected and non-TB individuals

P2X7 1513 A/C	Tuberculosis patients (n=56)	Healthy tuberculin negative individuals (n=60)	p- value	OR	95% CI
	n (frequency)	n (frequency)			
AA	26 (0.46)	36 (0.60)		1	
AC	18 (0.32)	21 (0.35)	0.677	1.186	0.53 to 2.66
CC	12 (0.21)	3(0.05)	0.014	5.538	1.42 to 21.62

Table 3: The P2X7 1513A/C gene number and frequency of TB infected and non-TB individuals

P2X7 1513A/C	Tuberculosis patients (n=56)	Healthy tuberculin negative individuals (n=60)	p- value	OR	95% CI
	n (frequency)	n (frequency)			
A	70(0.62)	93(0.77)		1	
C	42(0.37)	27 (0.23)	0.013	2.067	1.16 to 3.67

Our analysis showed that the C allele of P2X7 1513A/C was strongly associated with active tuberculosis patients (0.37% vs. 0.23%, p=0.013) in comparison to the healthy tuberculosis negative group. Additionally, C allele was associated with increased risk development of tuberculosis (Odds Ratio (OR) = 2.067, 95% Confidence Intervals (CI) =1.16-3.67).

It is also found that the CC genotype of P2X7 1513A/C was significantly higher in tuberculosis group than healthy non-TB individuals (OR= 5.54, CI= 1.42-21.63, P=0.01). But the heterozygote AC genotype is not so significantly different in tuberculosis and non-tuberculosis groups.

4. Discussion

The C allele of P2X7 was more common to the tuberculosis patients compared the healthy non-TB individuals; it is showing in the table (Table 2 and 3). So, it can be revealed that the C allele is responsible for the progression of TB infection. Our result shows strong evidence that the allele C and genotype CC of P2X7 1513A/C are associated with increased risk of tuberculosis disease to the tribal people of Jhargram. This data shows significant level of homogeneity chi-square value at $p<0.05$ significant levels. The similar result of P2X7 1513C allele susceptibility to TB also found in

Punjabi population [12]. The 1513C allele increases risk of TB also shows the Caucasian population [1]. Ge *et al.* (2016) demonstrate that the P2X7 1513A/C polymorphism with prevalence of pulmonary tuberculosis among Indian populations [23]. Wu *et al.* (2014) also shows the 1513C allele is the risk factor for pulmonary tuberculosis [24].

The P2X7 receptor is expressed by haemotopoietic stem cells and can mediate cell death, killing of infective micro-organisms and inflammatory response regulation [25, 26]. The purinergic receptor P2X ligand-gated ion channel 7 (P2X7) gene has been reported to be considerably associated with the possibility of development of active tuberculosis [27]. Prior discoveries have demonstrated that ATP plays an essential part in activating anti-mycobacterial action in human macrophages, plays out its activities through P2X7 cell surface receptor [28]. A sequence of intracellular events occurs that include the opening of cation channels and pores, and the beginning of nuclear factor-kB which is activated by the P2X7 receptor [29, 30]. Its activation additionally enhances the downstream signalling phenomenon, such as activation of caspases which leads to apoptosis, that activates the fusion of phagosome and lysosome and trigger to the killing of mycobacterial cells [31]. After ATP activation the P2X7 receptor opens a channel that permits a cascade of intracellular downstream events such as stimulation of cascade of caspases which lead to the apoptosis of the target cell. Earlier studies have described that apoptosis is induced by ATP and the related killing of microorganism (the causative pathogen is *Mycobacterium tuberculosis*) were inhibited when the pathogen survive and replicate within the phagosome of host macrophage and inhibit the phagosome-lysosome fusion [32-34]. The P2X7 gene is highly polymorphic, with many single nucleotide polymorphisms that influence the function of this receptor. Among them 1513A/C polymorphism which is common, found in exon 13 which changes over glutamic acid to alanine at position 496, responsible for the loss of receptor function [5, 12]. The expression of this receptor is further up-regulated by Interferon-gamma (IFN-γ), an important cytokine playing a major role in the inflammatory process seen in TB infection [5-7, 17].

Development and progression of TB is a consequence of complicated host-pathogen interactions including numerous components in the innate with acquired immune systems [12]. The P2X7 gene is exceptionally polymorphic and an variety of non-synonymous SNPs have been reported for which were involved in alternation of receptor function or expression, especially 1513A/C [12, 35]. The heterozygote AC genotype also show half of the effectiveness of cell function than wild type AA genotype [7]. The homozygous CC genotype of P2X7 1513A/C polymorphism reduce the receptor function on macrophage surface and it decrease the response of P2X7 to ATP and reduce the chance of ATP mediated bacterial killing [36]. In the condition of CC homozygous containing macrophage, the P2X7 expression decrease 12 folds than wild type macrophage [37]. When the deficiency occurs in P2X7 mediated resistance of mycobacterial infection within macrophage of pulmonary system, it may spread extrapulmonary sites.

In the study on the tribal people of Jhargram, there is higher frequency (37%) of homozygous C allele for TB patient rather than homozygous C allele for non-TB individuals (23%). Therefore, this polymorphism of P2X7 1513A/C shows significant TB susceptibility to tribes of Jhargram. This study clearly shows that the homozygous C allele of P2X7 results in loss of function of receptor and play an important role in elevate the susceptibility to tuberculosis.

5. Conclusion

This will be extremely critical on knowing the hereditary and epigenetic association in the tuberculosis infection with creating the detection, conformity, and control for this illness. The identification for hereditary polymorphisms will be extremely crucial for TB susceptibility. Not only this P2X7 1513 A/C polymorphism is responsible for tuberculosis susceptibility, also some other genetic polymorphisms responsible for the same. But known to all that it is not very easy process to detect the genetic condition of every person of a country. So, precaution should be taken before the disease occurrence if the host remain distant from such type of communicable pathogens.

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