You are requested to participate in our study entitled “Genetic variations in the host innate and adaptive immune response in tuberculosis: A search for risk loci in North Indians.” The aim of the study is to find certain genetic loci present in the variants of innate and adaptive immune factors that might be predisposing or making a certain person susceptible for tuberculosis disease in North Indian population. This would be achieved by means of DNA extraction, genotyping and cytokine profiling of Pulmonary Tuberculosis and Lymph node tuberculosis patients.

**WILLING PARTICIPATION:** Your participation in the study is of your own free will. The response to any of your queries will be provided by the investigator. You have the right to opt out of the study at any point of time without giving any reason to do so. If you don’t participate in this study, your treatment for illness in any way won’t be compromised. All the expenses shall be borne by the investigator and no extra expense will be incurred upon you.

**METHODOLOGY:** Blood samples will be drawn. It is a safe procedure when conducted by a skilled investigator or technician. The collected samples will be addressed by their laboratory numbers only. The papers will be used for scientific purpose only.

**CONSENT**

I __________________________________________ S/D/O ____________________ R/O ________________________, Certify that I have been told about the study “Genetic variations in the host innate and adaptive immune response in tuberculosis: A search for risk loci in North Indians”. I give my full, free and voluntary consent to participate in the study for collection of required samples. I am also aware of my right to opt out of the study at any time point without having to give a reason for such action. I have been informed that my treatment will not be affected if I don’t participate in this study.

Signature of the patient:

Signature of witness:

Signature of the investigator:

Date:
रोगी भागीदारी सूचना फार्म सहमति पत्र

आपसे अनुरोध है कि हमारे अध्यनकार्य शीर्षक "जेनेतिक भरिएसोनस इन द होस्ट इननेट अण्ड अदाप्तीव इम्म्युन रेस्पोन्स इन तुबेरकुलोसिस: अ सेर्च फ़ोर् रिस्क लोसी इन नोरत् इन्दिअन्स" में भाग ले। जिसका उद्देश्य अध्ययन के विषय में हमें विस्तार से पता चलना है। इसके लिए हम अपने पूरा काम करेंगे।

स्वाच्छिक भागीदारी: स्वेच्छिक भागीदारी का इस शोध कार्य में आपकी भागीदारी स्वेच्छिक होगी। इस अध्ययन में सम्बंधित आपके परीक्षण के उन्नत अनुसंधान द्वारा दिए जाने वाले उपचार के बारे में आपके अधिकार को पूरा करने के लिए हम आपके मुख्य रूप से सभी अध्ययन से बाहर होने का पूरा अधिकार देंगे।

सहमति: मैं, _______________ पुत्र/पुत्री _______________ नवाबी परमाणुत्तम करता/करती हूँ की मुझे इस अध्ययन शीर्षक "जेनेतिक भरिएसोनस इन द होस्ट इननेट अण्ड अदाप्तीव इम्म्युन रेस्पोन्स इन तुबेरकुलोसिस: अ सेर्च फ़ोर् रिस्क लोसी इन नोरत् इन्दिअन्स" के बारे में समझ दाखिल नहीं होगा। मैं स्वेच्छा से इस अध्ययन में हमें अपने विषय में समझ दाखिल नहीं करेंगे। मैं इसके बारे में अपने नमूनों को एकत्र करने के लिए आपको सहमति देंगे।

अन्वेषक के हस्ताक्षर: _______________
साक्षी के हस्ताक्षर: _______________

दिनाकः
PATIENT PROFORMA

1. Name of Patient :
2. Age / Sex :
3. Date of birth :
4. Father’s name :
5. Mother’s name :
6. Patient’s hospital file no. :
7. Permanent address :
8. Telephone no. :
9. Ethnicity:
10. Place of living : ( rural / urban / not sure )
11. Language spoken :
12. Socio economic status :
13. Educational status : (illiterate / primary school / high school / graduate / PG )
14. Employment status : ( student / employed / unemployed )
15. Type of employment :
16. Smoking : ( smoker / ex smoker / non smoker )
17. Alcohol use : ( alcoholic / ex alcoholic / non alcoholic / occasional )
18. Chewing tobacco : ( yes / no )
19. BCG vaccinated : (yes / no)
20. Type of diet :

Detailed history

1. Type of TB : Pulmonary TB / Lymph node TB
2. Family history of TB : ( yes / no )
3. If yes, relation of the patient with the affected person (s) :
4. Time since first diagnosed with TB infection :

5. History for pulmonary TB
   a. X – ray report : involvement of lung ( unilateral / bilateral / cavitary / diffused )
   b. Sputum status :
   c. Body weight (kg) : ( reduced / stable )
   d. Blood report : Hb / TLC / DLC / ESR
e. Mantoux report: Yes / No (If yes, size of induration)
f. HIV status: known (+ve / -ve) / not known
g. Any other disease: Diabetes / COPD / asthma / any other
h. Eye color:
i. Hair color:

6. Additional information for lymph node TB
   a. Location of lymph node: neck / axilla / groin / any other
   b. No. of nodes:
   c. Size of largest node:
   d. Consistency: soft / firm / hard
   e. Tenderness:
   f. Matted / unmated
   g. Suppurating / non suppurating
   h. Any other associated disease:
Appendix – 1B

Preparations:

TAE (50 X Stock Solution) (Per litre)

- Tris base: 242.0 gm
- 0.5 M EDTA (pH 8.0): 100 ml
- Glacial acetic acid: 57.1 ml

TBE (5 X Stock Solution) (Per litre)

- Tris base: 54 gm
- 0.5 M EDTA (pH 8.0): 20 ml
- Boric acid: 20 ml

EDTA (0.5M, pH 8.0) / litre

- H₂O: 800 ml
- EDTA: 186.1 gm

Stir vigorously on a magnetic stirrer

Adjust pH to 8.0 with NaOH pellets (20 gm / litre)

Srelize by autoclaving (TAE, TBE, EDTA,PBS).

Store at RT.

Phosphate buffered Saline (PBS):

- NaCl: 8 gm
- KCl: 0.2 gm
- Na₂HPO₄: 1.44 gm
- KH₂PO₄: 0.24 g

Adjust pH to 7.4-7.8 (Using HCl)

Bromophenol Blue (Loading Dye):

- BPB: 0.025 gm
- Xylene Cyanol: 0.025 gm
- Glycerol: 3 ml

TE:

- Tris-Cl: 10mM(pH 7.5)
- EDTA: 10mM (pH 8.0)

Add approximately 800ml distilled water and mix.

Adjust the pH to 8.0 with concentrated HCl

Make up to a final volume of 1 liter

Autoclave
Appendix 1 C Software

PLINK V 5.0  
Haploview V3.3  
Graph Pad Prism V 5.0  
SPSS 16.0  
MedCalc  
GeneRunner V 3.01  
Bioedit  
EIGENSOFT V 3.0  
MassArray Assay designer software V 4.0  
PS : Power and Sample size Program V 3.0.43

Purcell lab, Broad Institue, USA  
Daly lab, Broad Institute USA  
Graph Pad Software, San Diego, CA USA  
IBM SPSS Data Collection  
MedCalc Software, Broekstraat, Mariakerke, Belgium  
Hastings Software, Inc.  
Tom Hall, Ibis Biosciences  
Reich Lab, Broad Institute, USA  
Sequenom, Inc.  
Dale Plummer,Vanderbilt University

All used versions were for Windows 7, except for EIGENSOFT which runs on LINUX.

Appendix 1D Online Resources

BLAST  
dbSNP  
Genetic Power Calculator  
HapMap  
PubMed  
IGVDB  
PRIMER 1

www.ncbi.nlm.nih.gov/BLAST  
pngu.mgh.harvard.edu/~purcell/gpc/  
www.hapmap.org  
www.igvdb.res.in  
http://cedar.genetics.soton.ac.uk/public_html/primer1.html
Epilogue

The presented work in this thesis clarifies the genetic contribution in susceptibility to tuberculosis in north Indians. This study has also tried to work out the “cause” and “effect” by correlating the serum cytokine levels with the SNPs detected in relevant genes. Many successful associations explored for the first time in tuberculosis were identified here that define the genetic profile of north Indians and their proneness to develop or resist tuberculosis. The quest that was started in by framing the aims and objectives of this thesis is complete but the understanding and information that has emerged needs to be validated in large and different cohorts. A GWAS on tuberculosis from India is highly warranted and the regions of most association obtained in the study could serve as hotspots to proceed.

Also, the identified variants and their contribution can be mapped to provide us mechanistic insights to the complex nature of biological interactions in tuberculosis. Also, the biomarkers identified for LNTB, could be validated in prospective studies and can serve as an important aid in diagnosis of LNTB.

The differential immune response in pulmonary and lymph node TB identified in this study could be probed to understand the different clinical manifestations of tuberculosis. The questions answered in this thesis have come a long way but still has a longer path to traverse. Further, systematic efforts would facilitate better understanding of the genetic basis of resistance or susceptibility to TB which could be translated into targeted immunotherapy as a preventive measure as well as an effective adjunct to multidrug therapy for TB.