Appendix
Appendix I

Sample size calculation

Sample size was estimated using QUANTO software Version 1.0 (http://hydra.usc.edu/gxe) for each genetic marker. Frequency of rare allele occurring in the healthy controls was obtained from literature. As there was no report on North Indian population, frequency as reported for the Caucasians population was used. Desired power of our study was set at 80% with a significance level of 0.05 in a two-sided test. By means of QUANTO program, our sample was considered to adequate the study all nine polymorphisms.
Appendix II

Proforma for evaluation of patients

1 Name and C.R. No.:

2 Date of Birth:

3 Age/Sex:

4 Contact Address:

5 Educations: (I) Illiterate (II) Primary (III) Matric (IV) Graduate (V) postgraduate

6 Occupations: (I) Skilled (II) Unskilled (III) Office Govt. (IV) School/College teacher (V) Farming (VI) Unemployed

7 Marital Status: (I) Married (II) Unmarried (III) Widowed (IV) Divorced

8 Referred by: (I) Self (II) Doctor (III) Spouse (IV) Family & friends

9 Medical History: Currently having any medical problems or symptoms

(I) Nil (II) Recurrent fever (III) Weight loss (IV) Cough (V) Diarrhea

(VI) STI’s (VII) TB (VIII) OI’s (IX) Others

10 Currently on treatment: ........................................................................................................

11 Do you think that you are at risk of HIV?

(I) No risk (II) Perinatal (from mother to child) (III) Needle stick injury (IV) Unprotected sex (vaginal/ anal) with males/ females/ CSW

(V) Contaminated blood through – (A) Blood transfusion (B) IDU (C) Organ transplant

12 Tested before for HIV: (A) How many times? _____ (B) Last test (month/year): ____ (C) Result ____________

13 If positive for HIV do you have CD4 count done? ____________
Appendix III

Common reagents

**LYSIS BUFFER**
For 500 ml
0.32M Sucrose-54.8 gms
1% Triton X - 5ml
1mM MgCl₂ - 0.51 gms
12 mM Tris – 0.73 gms

**PROTEINASE K BUFFER**
For 500ml
0.375 M MgCl₂ 11.0 gms
0.12 M EDTA 22.4 gms
Adjust the pH with NaOH. Complete dissolution occurs only when the pH is close to 8.0

**SODIUM DODECYL PHOSPHATE (10%; pH 7.2)**
For 1000ml
SDS 100 gms
Water 900 ml

**TRIS ACETATE EDTA (TAE) BUFFER (50X)**
For 1000ml
Tris base 242 gms
Glacial acetic acid 57.1 ml
EDTA (0.5M) 100 ml
The stock solution was 50X and was diluted to 1X at the time of use.

**TRIS BORATE EDTA (TBE) BUFFER (10X)**
For 1000ml
EDTA (0.5M; pH 8.0) 40ml
Boric acid 55 gms
Appendix

Tris Base 108 gms
The stock solution was prepared 10X and was diluted to 1X at the time of use. All the reagents were prepared in Milli Q water

**DOUBLE DYE (BROMOPHENOL BLUE & XYLENE CYANOL) (6X)**
Bromophenol blue 0.25%
Xylene Cyanol 0.25%
Sucrose in water (w/v) 40%
Store at 4°C

**TRIS SATURATED PHENOL**
Tris saturated phenol 800ml
Chloroform 200 ml
The water-saturated phenol was washed with Tris buffer at pH 8.0 repeatedly until the pH of the wash fluid was 8.0. It was then layered with Tris at a pH 8.0. The solution was then stored in dark bottles. Only the lower layer of the Tris saturated phenol was used.

**ETHIDIUM BROMIDE (EtBr) (10 mg/ml)**
Add 1 gm of ethidium bromide to 100ml water. Stir on a magnetic stirrer for several hours. Wrap in an aluminum foil and store in the dark bottle at 4°C.
Working ethidium bromide (0.5ug/ml)

**BROMOPHENOL BLUE (6X)**
Bromophenol blue 0.25%
Sucrose in water (w/v) 40%, store at 4°C

**ETHYLENE DIAMINE TETRA ACETIC ACID (EDTA)**
For 1000ml (0.5M; pH 8.0)
EDTA 186.1 gms
Water 800 ml (approx.)
Adjust pH 8 with NaOH (20g of NaOH pellets) make up the final volume and autoclave.
ACRYLAMIDE (30%)
Acrylamide 29gms
N,N'-methylene-bis-acrylamide 1gms
Water to 100ml

POLYACRYLAMIDE GEL (15%)
Total volume 25 µl
30% Acrylamide mix 12.5 ml
TBE Buffer (10X) 2.5 ml
Water 9.78 ml
10% Ammonium per sulfate (APS) 200µl
TEMED 20µl

POLYACRYLAMIDE GEL (20%)
Total volume 25 µl
30% Acrylamide mix 16.6 ml
TBE Buffer (10X) 2.5 ml
Water 5.61 ml
10% Ammonium per sulfate (APS) 200µl
TEMED 20µl