Chapter 3

Results and Discussion
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The advancement in the technologies to analyze the human genome have led to the discovery of copy number variation (CNV), represented as one of the significant class of human genetic variation. The CNVs are larger (greater than 1 kb) segment of DNA which are present at variable copy number as compared to the reference genome contributing to a significant fraction in the human genome (Feuk, Carson and Scherer 2006). Several genes are found to exhibit copy number variations within them. Several studies have highlighted the importance of CNV in human diseases and advancement in the technology have allowed for the analysis of CNVs in thousands of disease and healthy individuals (Girirajan, Campbell and Eichler 2011; Lee and Scherer 2010; Stankiewicz and Lupski 2010; Zhang et al. 2009). This has led to an exploration of the functional aspects of these variants contributing to disease susceptibility and pathogenesis.

The current research work aims to evaluate the copy number variable genes and their correlation to diseases susceptibility in Indian population. Secondly, the ethnic variability among different population was also analyzed. The list of the genes selected for CNV analysis is depicted in Table 3.1.

Table 3.1: List of the copy number variable genes selected and studied for its association to disease susceptibility in Indian population

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>CNV type</th>
<th>Association to disease studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial tumor suppressor 1 (MTUS1)</td>
<td>8p22</td>
<td>Deletion (exon-specific)</td>
<td>Breast cancer and Head and neck cancer</td>
</tr>
<tr>
<td>Late cornified envelope 3B and 3C (LCE3B and LCE3C)</td>
<td>1q21.3</td>
<td>Deletion (multigene)</td>
<td>Psoriasis</td>
</tr>
<tr>
<td>Glutathione S- transferase mu 1 (GSTM1)</td>
<td>1p13.3</td>
<td>Deletion</td>
<td>Asthma</td>
</tr>
<tr>
<td>Glutathione S- transferase theta 1 (GSTT1)</td>
<td>22q11.23</td>
<td>Deletion</td>
<td>Asthma</td>
</tr>
<tr>
<td>CC chemokine ligand 3-like 1 (CCL3L1)</td>
<td>17q21.2</td>
<td>Segmental duplication</td>
<td>HIV-1 infection</td>
</tr>
<tr>
<td>Fc fragment of IgG, low affinity IIIb,</td>
<td>1q23.3</td>
<td>Segmental</td>
<td>-</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>receptor (CD16b) ( (FCGR3B) )</th>
<th>duplication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cytosolic sulfotransferase 1A1 ( (SULT1A1) )</td>
<td>16p11.2</td>
</tr>
</tbody>
</table>

3.1 Quantitative and Qualitative analysis of human genomic DNA

For all the selected genes a case-control approach was used in order to analyze the copy number variants. The isolation of the genomic DNA was performed from healthy control individuals and patient blood samples and was analyzed quantitatively and qualitatively. Human genomic DNA was isolated from the human blood as mentioned in Section 2.3.1. The samples were checked for purity and yield by measuring the absorbance at wavelengths 260 nm and 280 nm, using spectrophotometer. On an average, 80 – 150 µg of DNA was isolated from 5 ml of blood. The absorbance \( (A_{260}/A_{280}) \) ratio ranged from 1.6 – 1.8, which indicated good purity of the isolated DNA samples. The samples were analyzed by agarose gel electrophoresis (0.7%). The DNA appeared as a smear and the size of the largest band was greater than 14 kb (Figure 3.1).

Figure 3.1: Agarose gel (0.7%) of the human genomic DNA. Lane M shows the marker \( \lambda \) DNA/BstE II digest. Lanes 1 – 11 show isolated genomic DNA from different individuals.
3.2 Copy number analysis for the selected genes

Copy number variations (CNVs) are well studied as important feature of human genetic variation in disease susceptibility studies. Many researchers have well cited and explored the CNV association to human disease pathogenesis. However, the consequences of CNV are not well understood yet (Girirajan, Campbell and Eichler 2011; Lee and Scherer 2010; Stankiewicz and Lupski 2010; Zhang et al. 2009).

After the isolation of the genomic DNA from healthy control individuals and patient blood samples, the selected genes were studied and evaluated based on three perspectives:

I. Frequency distribution of the allele and genotype of the variants
II. Ethnic variability among populations, and
III. Correlation of variants with disease susceptibility

There are several methodologies developed till date to detect copy number changes in the human genome (Dhawan and Padh 2009; Feuk, Carson and Scherer 2006). Advances in the detection platform have allowed us to screen these variants at higher resolution. Currently, genome-wide array based approach is been used by several researcher which is described in detail in Section 1.5.2. However, for the present study we have selected different PCR-based detection platforms to study the copy number changes as described in detail in Section 2.3.3. The methodologies that were used in the current study for assessing CNV included mainly conventional PCR, long range PCR, real-time PCR and PRT assays.

3.2.1 Mitochondrial tumor suppressor 1 (MTUS1) gene

Mitochondrial tumor suppressor gene 1 (MTUS1) has been identified as a candidate copy number variant gene which maps on chromosome 8p22. It comprises of a common deletion encompassing the entire coding exon 4. The CNV in the MTUS1 is found to be associated with decreased risk to cancer (Frank et al. 2007).
3.2.1.1 Results

3.2.1.1 A. Detection of MTUS1 exon-specific deletion by conventional PCR

Variants of MTUS1 were detected by conventional PCR method as described in detail in Section 2.3.4.1. The polymorphic sites screened during the analysis were identified based on the presence or absence of the bands in Reaction A and B (Table 2.7 and 2.8). This led to the identification of the individuals genotype. The gel analysis of the PCR reactions is shown in Figure 3.2.

Figure 3.2: Agarose gel (2\%) of the PCR products for MTUS1 gene.

Lane description: Lane M shows the marker O’ Gene ruler 50 bp ladder. Lane 1, 3, 5 represents reaction specific for exon 4 specific deletion while lane 2, 4, 6 represents reaction specific for gene presence. Lane 1, 2 (sample 1) – homozygous wild-type genotype (PCR product: 133 bp), Lane 3, 4 (sample 2) and lane 5, 6 (sample 3) – heterozygous genotype (PCR products: 133 bp and 271 bp).
3.2.1.1. B. Allele and genotype frequency for MTUS1

The exon-specific deletion in MTUS1 was assessed in 41 head and neck cancer patients, 15 breast cancer patients and 280 healthy individuals. The allele frequency of MTUS1 for head and neck cancer patients, breast cancer patients and healthy individuals is shown in Figure 3.3. The genotype frequency of MTUS1 for healthy individuals, head and neck cancer patients and breast cancer patients is shown in Figure 3.4. The homozygous wild-type (wt/wt) genotype was observed at a frequency of 41.5%, 73.3% and 42.9% in head and neck cancer patients, breast cancer patients and healthy individuals, respectively. The heterozygous (wt/Del) genotype was observed at a frequency of 58.5% and 26.7% and 57.1% in head and neck cancer patients, breast cancer patients and healthy individuals, respectively. However, no homozygous deletion (Del/Del) genotype was observed either in head and neck cancer patients, breast cancer patients or healthy individuals.

Figure 3.3: Allele frequency of MTUS1 in Indian healthy individuals and cancer patients (head and neck cancer and breast cancer).
3.2.1.1. C. Ethnic variation in MTUS1 genotype frequencies

The observed genotype frequencies for MTUS1 were compared to those reported in German population (Frank et al. 2007). A graphical representation of the comparison between the populations is shown in Figure 3.5. The MTUS1 genotype frequencies in healthy control individuals differed significantly from that reported in the German population. The wt/wt genotype was observed at a frequency of 42.9% in the Indian population (our data) as compared to 91.3% in German population. The wt/Del genotype was observed at a frequency of 57.1% in the Indian population (our data) as compared to 8.6% in German population. However, the Del/Del genotype was not detected in our population while a frequency of 0.1% was reported in German population. There was a statistically significant difference ($p< 0.0001$) between the Indian population (our data) and Germans.
Figure 3.5: Comparison of genotype frequency of *MTUS1* deletion variant in Indian and German healthy individuals and cancer patients.
3.2.1.1. D. Association of MTUS1 CNV (exon-specific deletion) with cancer

The association between MTUS1 exon-specific deletion and susceptibility to cancer was studied in 41 head and neck cancer patients, 15 breast cancer patients and a control group of 280 healthy individuals.

**MTUS1 and head and neck cancer susceptibility**

The observed genotype frequencies in Indian head and neck cancer patients and healthy individuals were compared to evaluate the protective role of MTUS1 exon-specific deletion in head and neck cancer risk. A graphical representation of the comparison between the two groups is shown in Figure 3.

The frequency of MTUS1 deletion variant (wt/De + Del/De) was found to be similar in head and neck cancer patients (58.5%) and healthy individuals (57.1%). Thus, no statistically significant difference between the two groups ($\chi^2 = 0.028; p = 0.8671$) was observed. The odds ratio (OR) that we observed for head and neck cancer patients with wt/wt genotype compared to wt/De and Del/De genotype was 1.06 (95% CI 0.85–1.33, $p = 0.8671$) (Table 3.2).

**Table 3.2: Genotype frequencies of MTUS1 deletion variants in cancer patients (breast cancer and head and neck cancer) and control (healthy) subjects, ORs with 95% CI and $p$ values in Indian population.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Genotypes</th>
<th>Cases</th>
<th>Control</th>
<th>OR (95% CI), $p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>MTUS1</td>
<td>8p22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Head and Neck cancer</td>
<td>wt/wt</td>
<td>17</td>
<td>41.5</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wt/De</td>
<td>24</td>
<td>58.5</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Del/De</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>41</td>
<td></td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>Breast cancer</td>
<td>wt/wt</td>
<td>11</td>
<td>73.3</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wt/De</td>
<td>4</td>
<td>26.7</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Del/De</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>15</td>
<td></td>
<td>280</td>
</tr>
</tbody>
</table>

wt/wt, homozygous wild-type genotype; wt/De, heterozygous genotype; Del/De, homozygous deletion genotype; OR, odds ratio (Del/De + wt/De) versus (wt/wt); CI, confidence interval; $p$, significance values.
**MTUS1 and breast cancer susceptibility**

The observed genotype frequencies in Indian breast cancer patients and healthy individuals were compared to evaluate the protective role of *MTUS1* exon-specific deletion in breast cancer risk. A graphical representation of the comparison between the two groups is shown in Figure 3.4. The frequency of *MTUS1* deletion variant (wt/Del + Del/Del) was found to be 26.7% in breast cancer patients as compared to 57.1% in healthy individuals. A significant association of the deletion variant with a decrease risk to breast cancer (OR 0.27, 95% CI 0.08–0.87, *p* = 0.0207) was found in Indian population (Table 3.2).

### 3.2.1.1. E. Association of *MTUS1* CNV and cancer risk in different populations

The association of the small deletion (1128 bp deletion lacking the complete coding exon 4) in *MTUS1* with a decreased risk of familial and high-risk breast cancer was first analyzed in German population by Frank et al. (2007). The break point of the deleted region was redefined and PCR assay was developed to investigate the deletion CNV in a case-control study design comprising of 593 familial breast cancer patients and 732 control individuals. The deletion variant was found to be associated with a decreased risk for both familial (*p* = 0.01) and high-risk familial breast cancer (*p* = 0.003). In previous studies the expression pattern of *MTUS1* was found to be markedly reduced in pancreatic cancer tissue and the pancreatic cancer cell line MIA PaCa-2. In contrast, recombinant expression of MTUS1 in MIA PaCa-2 cells inhibited proliferation (Seibold et al. 2003). Thus, it was been hypothesized that the deletion of *MTUS1* exon 4 might increase the tumor suppressor activity. However, functional studies and validation of the results in larger sample size is necessary for better conclusion. Further, the role of *MTUS1* CNV in cancer susceptibility has been analyzed so far in only German population.

### 3.2.1.2 Discussion

*MTUS1*, the tumor suppressor gene encompass a CNV region characterized by 1,128 bp deletion leading to a variant lacking the complete coding exon 4 (162 bp/54 amino acids). Its role in cancer susceptibility was assessed in a large German case-control population (Frank et al. 2007).
The association of the deletion variant with a decreased risk for both familial ($p = 0.01$) and high-risk familial ($p = 0.003$) breast cancer was reported (Frank et al. 2007).

The association of the *MTUS1* deletion variant with decreased risk to head and neck cancer and breast cancer was analyzed in our population: healthy individuals ($n = 280$), head and neck cancer patients ($n = 41$) and breast cancer patients ($n = 15$) using conventional PCR technique. The genotyping method was validated by sequencing the PCR products of randomly selected samples (*Appendix – III; page number 145*). The genotype frequencies in the control samples were not in the agreement with the Hardy-Weinberg equilibrium. The genotype frequency of deleted variant (wt/Del + Del/Del) was found to be 57.1% in healthy individuals, 58.5% in head and neck cancer patients and 26.7% in breast cancer patient. A significant association with deletion variant with a decrease risk to breast cancer (OR, 0.27; 95% CI 0.08–0.87, $p = 0.0207$) was found in Indian population. However, a comparison with head and neck cancer (OR, 1.06; 95% CI 0.85–1.33, $p = 0.8671$) did not find a significant association. Further, functional studies and validation of the findings in larger sample size is required to verify these results for any future conclusion.

In order to understand the genetic diversity of Indian population, the observed genotype frequency of healthy individuals was compared with that reported in German control population. A significant difference ($p<0.0001$) highlighting ethnic diversity among population was observed.

### 3.2.2 Late cornified envelope 3B and 3C (*LCE3B* and *LCE3C*) genes

The members of LCE gene cluster, *LCE3B* and *LCE3C* maps on human chromosome 1p21.3. The deletion comprising both *LCE3B* and *LCE3C* genes, involved in skin barrier defense, are found to be associated with psoriasis susceptibility (de Cid et al. 2009).
3.2.2.1 Results

3.2.2.1. A. Detection of LCE3B and LCE3C gene deletion by conventional PCR

Deletion comprising LCE3B and LCE3C genes (LCE3C_B-del) was (Table 2.9) detected by conventional PCR method described in detail in Section 2.3.4.2. The polymorphic sites screened during the analysis were identified based on the presence or absence of the bands in Reaction A and B (Table 2.9 and 2.10). This led to the identification of the individuals genotype. The gel analysis of the PCR reactions is shown in Figure 3.6.

![Figure 3.6: Agarose gel (1.5%) of the PCR products for LCE3B and LCE3C gene.](image)

**Lane description:** Lane M shows marker pCAMBIA digested with EcoRV. Lane 1, 3, 5, 7, 9 represents reaction specific for gene deletion while lanes 2, 4, 6, 8, 10 represents reaction for gene presence. Lane 1, 2 and 5, 6 (sample 1 and 3) – heterozygous (LCE3C_B/LCE3C_B-del) genotype (PCR product: 1044 bp and 778 bp), Lane 3, 4 and 7, 8 (sample 2 and 4) – homozygous wild-type (LCE3C_B/ LCE3C_B) genotype (PCR product: 778 bp), Lane 9, 10 (sample 5) – homozygous deletion (LCE3C_B-del/ LCE3C_B-del) genotype (PCR product: 1044 bp).
3.2.2.1. B. Allele and genotype frequency for LCE3B and LCE3C

The *LCE3B* and *LCE3C* gene deletion (LCE3C_B-del) was assessed in 11 psoriasis patients and 203 healthy individuals. The allele and genotype frequency of *LCE3B* and *LCE3C* for psoriasis and healthy individuals is shown in Figure 3.7 and Figure 3.8 respectively. The homozygous wild-type (LCE3C_B/LCE3C_B), heterozygous (LCE3C_B-del/LCE3C_B) and homozygous deletion (LCE3C_B-del/ LCE3C_B-del) genotype was observed at a frequency of 18.2%, 45.4% and 36.4% in psoriasis patients and 55.2%, 33.5% and 11.3% in healthy control individuals respectively.

![Figure 3.7: Allele frequency of LCE3B and LCE3C in Indian healthy individuals and psoriasis patients.](image-url)
Results and Discussion

3.2.2.1. C. Ethnic variation in LCE3B and LCE3C genotype frequencies

The observed genotype frequencies of LCE3C_B-del was compared to those reported in European ancestry population [Finland, France, Germany, Ireland, Italy, Spain, The Netherlands, UK, and US (US-California: US-CA, and US-Michigan: US-MI)] and Asiatic origin population [(China, Mongolia and Japan) (Riveira-Munoz et al. 2011) and Northern Chinese (Harbin) (Xu et al. 2011)]. A graphical representation of the comparison between these populations is as shown in Figures 3.9 and 3.10.

The observed genotype frequencies of LCE3C_B-del in the Indian population (our data) were quite different from that reported in European ancestry and Asiatic origin populations. Upon analysis, a statistically significant difference between Indians (our data) and Finnish ($p< 0.0001$), French ($p< 0.0001$), German ($p< 0.0001$), Irish ($p< 0.0001$), Italian ($p< 0.0001$), Spanish ($p< 0.0001$), Dutch ($p< 0.0001$), UK ($p< 0.0001$), US-CA ($p< 0.0001$), US-MI ($p< 0.0001$),
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**Figure 3.9:** Comparison of genotype frequency of *LCE3B* and *LCE3C* gene deletion (homozygous for the deleted allele (LCE3C_B-del)) in healthy individuals among Indian and European ancestry populations.
Figure 3.10: Comparison of genotype frequency of \textit{LCE3B} and \textit{LCE3C} gene deletion [homozygote for the deleted allele (LCE3C\_B-del)] in healthy individuals among Indian and other Asian populations.
Chinese ($p<0.0001$), Chinese (Shanghai) ($p<0.0001$), Mongolian ($p=0.002$) Japanese ($p<0.0001$) and Spanish Caucasians (Asturias) ($p<0.0001$) was observed. However, a comparison of the LCE3C_B-del to that reported in northern Chinese population (Harbin) showed a non-significant difference between populations.

Further the frequency distribution of the $LCE3B$ and $LCE3C$ gene deletion reported in different populations across the globe was plotted on the world map as depicted in Figure 3.11. The graphical representation showed that the frequency of the LCEC_B-del variant was found to be highest in European ancestry and American populations as compared to different region of the world (Riveira-Munoz et al. 2011; Xu et al. 2011).

3.2.2.1. D. Association of $LCE3B$ and $LCE3C$ CNV (multigene - deletion) with psoriasis

The association between $LCE3B$ and $LCE3C$ gene deletion and susceptibility to psoriasis was studied in 11 psoriasis patients and a control group of 203 healthy individuals. The frequency of $LCE3B$ and $LCE3C$ homozygous deletion was found to be 36.4% in psoriasis patients as compared to 11.3% in healthy individuals. There was significant difference in the frequency of the $LCE3B$ and $LCE3C$ deletion between psoriasis patients and healthy controls (OR 4.47, 95% CI 1.22–16.43, $p = 0.0151$) (Table 3.3).

3.2.2.1. E. Association of $LCE3B$ and $LCE3C$ CNV with psoriasis in different populations

The role of $LCE3B$ and $LCE3C$ gene deletion as a susceptibility factor for psoriasis is well established and studied across different populations (Coto et al. 2010; Li et al. 2011; Riveira-Munoz et al. 2011; Xu et al. 2011). In a study, association of the CNV encompassing $LCE3B$ and $LCE3C$ genes was studied in 2,831 samples from European ancestry population (Spain, Netherlands, Italy and the United States). It was observed that the individual carrying the LCE3C_LCE3B-del allele were at an increased risk for psoriasis (OR = 1.30–1.50). Also, the LCE3C_LCE3B-del allele frequency was found to be higher in individuals with psoriasis (68%) compared with controls (59%). Thus, the absence of $LCE3B$ and $LCE3C$ (LCE3C_LCE3B-del)
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Figure 3.11: Worldwide map of the frequency distribution of LCE3B and LCE3C deletion variant.
Table 3.3: Genotype frequencies of *LCE3B* and *LCE3C* gene deletion in patients (psoriasis) and control (healthy) subjects. ORs with 95% CI and *p* values in Indian population.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Polymorphism</th>
<th>Genotypes</th>
<th>Cases</th>
<th>Control</th>
<th>OR (95% CI), <em>p</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>LCE3B</em></td>
<td>1p21.3</td>
<td>Gene deletion</td>
<td>LCE3C_B/LCE3C_B</td>
<td>2</td>
<td>112</td>
<td>1.00</td>
</tr>
<tr>
<td>and <em>LCE3C</em></td>
<td></td>
<td></td>
<td>LCE3C_B/LCE3C_B-del</td>
<td>5</td>
<td>68</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LCE3C_B-del/LCE3C_B-del</td>
<td>4</td>
<td>23</td>
<td>4.47 (1.22–16.43), 0.0151</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>11</td>
<td>203</td>
<td></td>
</tr>
</tbody>
</table>

LCE3C_B/LCE3C_B, homozygous wild-type genotype; LCE3C_B/LCE3C_B-del, heterozygous genotype; LCE3C_B-del/LCE3C_B-del, homozygous null genotype; OR, odds ratio (LCE3C_B⁺⁺) versus (LCE3C_B⁺⁺⁺ + LCE3C_B⁺⁺); CI, confidence interval; *p*, significance values.
was found to be significantly associated \((p = 1.38 \times 10^{-08})\) with risk of psoriasis (de Cid et al. 2009). Finally, to further investigate the potential role of \(LCE3B\) and \(LCE3C\) CNV in psoriasis, the expression analysis studies of \(LCE3C\) in normal skin and in lesional and uninvolved psoriatic epidermis were carried out. The expression of \(LCE3C\) and \(LCE3C\) was found to be low in all normal skin samples, irrespective of their \(LCE3C\_LCE3B\)-del genotype and thus, a significant correlation was found (Pearson’s \(r = 0.88, \ p = 2.0 \times 10^{-06}\)) between the normalized \(LCE3C\) expression and copy number (de Cid et al. 2009).

### 3.2.2.2 Discussion

Psoriasis is a chronic inflammatory disease of the skin with the varied prevalence worldwide, being more frequent in Europeans as compared to less common among Asian ancestry population (Bowcock 2005). To date, genetic polymorphism at several genetic loci are reported to be associated with risk to psoriasis. Among these, CNV encompassing \(LCE3B\) and \(LCE3C\) genes was found to be associated with risk of psoriasis in European ancestry population (de Cid et al. 2009). \(LCE3B\) and \(LCE3C\), members of the late cornified envelope (LCE) gene cluster genes are found to be located in the epidermal differentiation complex and involved in susceptibility to psoriasis (de Cid et al. 2009).

The role of \(LCE3B\) and \(LCE3C\) gene deletion (LCE3C_B-del) as a risk factor for psoriasis in Indian population was analyzed in 11 patients with psoriasis and 203 healthy controls individuals using conventional PCR technique. The genotyping method was validated by sequencing the randomly selected PCR products (Appendix – III; page number 145). The frequency distribution of the \(LCE3B\) and \(LCE3C\) gene deletion differed between the controls (11.3%) and psoriasis patients (36.4%). Thus, the results revealed a statistically significant difference \(LCE3B\) and \(LCE3C\) deletion frequency between the patients and healthy controls (OR 4.47, 95% CI 1.22–16.43, \(p = 0.0151\)). However, the observed genotype frequencies were not in agreement with the Hardy-Weinberg Equilibrium for control population. Further, the samples size is very small therefore the findings need to be validated in larger sample size for future conclusion. However, indicative correlation of \(LCE3B\) and \(LCE3C\) gene deletion with susceptibility to
psoriasis was found in Indian population. This indicates that the LCE gene cluster might be crucial susceptibility gene in the pathogenesis of psoriasis.

The frequency of LCE3C_3B-del in Indian healthy individuals was compared to multiple population data reported. A significant ($p < 0.0001$) difference was observed among the European ancestry and Asiatic origin population, which highlighted that the frequency of the variant differs with ethnicity, which may be attributed by several factors and thus, can be taken in account during investigations of susceptibility variants for common diseases. The world map depicting the frequency of the $LCE3B$ and $LCE3C$ gene variant across globe highlighted that the LCE3C_B-del variant involved in psoriasis susceptibility was found to be highest in European and American populations. This aspect can be further analyzed from clinical point of view on one hand and on the other hand, evolution of the disease variant loci can also be of concern.

### 3.2.3 Glutathione S-transferase mu 1 ($GSTM1$) gene

The human $GSTM1$, a member of Glutathione S-transferases (GSTs) superfamily maps on chromosome 1p13.3. The CNV (gene deletion) in $GSTM1$ is found to be associated with asthma susceptibility (Iwaschenko, Sideleva and Baranov 2002).

#### 3.2.3.1 Results

**3.2.3.1. A. Detection of $GSTM1$ gene deletion by long range PCR**

$GSTM1$ deletion was detected by two reaction PCR and long range PCR method as described in detail in Section 2.3.4.3. The polymorphic sites screened during the analysis were identified based on the presence or absence of the bands in reaction A and reaction B (Table 2.11 and 2.12). This led to the identification of the individual’s genotype. The gel analysis of the PCR reactions is shown in Figure 3.12.
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Figure 3.12: Agarose gel (1%) of the PCR products for GSTM1 gene.

**Lane description:** Lane M1 shows the marker \( \lambda \) DNA/BstE II digest; Lane M2 shows the marker O’ Gene ruler 100 bp ladder. Lane 1, 3, 5 represents reaction specific for gene deletion while lane 2, 4, 6 represents reaction for gene presence. Lane 1, 2 (sample 1) – homozygous wild-type (GSTM1\(^{+/+}\)) genotype (PCR product: 420 bp), Lane 3, 4 (sample 2) – heterozygous (GSTM1\(^{+/0}\)) genotype (PCR products: 4748 bp and 420 bp), Lane 5, 6 (sample 3) – homozygous deletion (GSTM1\(^{0/0}\)) (PCR product: 4748 bp).

### 3.2.3.1. B. Allele and genotype frequency for GSTM1

The GSTM1 gene deletion was assessed in 34 asthma patients. The GSTM1 alleles and genotypes were analyzed using PCR and long-range PCR methods (Section 2.3.4.3). The allele and genotype frequency of GSTM1 for asthma patients and healthy individuals (Dhawan, PhD. Thesis, Nirma University, 2010) is shown in Figure 3.13. The homozygous wild-type (GSTM1\(^{+/+}\)), heterozygous (GSTM1\(^{+/0}\)) and homozygous deletion (null genotype - GSTM1\(^{0/0}\)) genotype was observed at a frequency of 35.3%, 26.5% and 38.2% in asthma patients respectively.
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3.2.3.1. C. Ethnic variation in GSTM1 genotype frequencies

The observed null genotype frequency of GSTM1 (Dhawan, Ph.D. thesis, Nirma University, 2010) was compared to those reported in Caucasian population (France, Italy, Netherlands, Sweden, UK) (Hanene et al. 2007), African and American population [(African American (AA), Brazil (non-whites), Brazil (whites), Cameroon, Egypt, Mexico (Amerindian), Namibia, Canada, Whites (USA))] (Hanene et al. 2007; Mishra et al. 2004; Piacentini et al. 2011), Arabic population (Iran, Saudi Arabia) (Piacentini et al. 2011), and Asian population (North India, South India, China, Japan, Mongolia) (Mishra et al. 2004; Piacentini et al. 2011). A graphical representation of the comparison between these populations is shown in Figure 3.14 and 3.15.

The observed genotype frequencies of GSTM1 homozygous null genotype (GSTM1^0^0 or GSTM1 null) in the Indian population (our data) were quite different from that reported in Caucasian, African/American, Asian and Arabic populations.
Figure 3.14: Comparison of the distribution of $GSTM1$ null genotype among Indian (our data) and other Asian and Caucasian populations.
Results and Discussion

Figure 3.15: Comparison of the distribution of $GSTM1$ null genotype among Indian (our data) and Arabic and African/American populations.
Results and Discussion

Upon analysis, a statistically significant difference between Indians (our data) and French ($p<0.0001$), Italians ($p=0.014$), Dutch ($p=0.0007$), Swedish ($p<0.0001$) and UK ($p<0.0001$) among Caucasian population was observed.

There was a statistically significant difference between Indians (our data) and Chinese ($p=0.0004$), Japanese ($p=0.001$) and Mongolian ($p=0.0251$). However, a non-significant association was observed between Indian (our data) and North India ($p=0.685$), South India ($p=0.292$) among Asian populations.

There was a statistically significant difference between Indians (our data) and Iranian ($p=0.0031$), Saudi Arabian ($p<0.0001$) among Arabic populations.

There was a statistically significant difference between Indians (our data) and Brazilian whites ($p=0.003$), Egyptian ($p<0.0001$), Amerindian Mexican ($p<0.0001$), Namibian ($p<0.0001$), Canadian ($p=0.0004$) and Whites (USA) ($p=0.0002$). However, a non-significant association was observed between Indian (our data) and African American ($p=0.142$), Brazilian non-whites ($p=0.900$), Cameroon ($p=0.2059$) among African and American populations.

Further, the frequency of the $GSTM1$ null genotype reported in different population was then plotted on the world map as depicted in Figure 3.16. The graphical representation showed a wide range of variability in the $GSTM1$ null frequency within different populations. The frequency distribution of the $GSTM1$ null phenotype was found to be similar among European, American and Asian populations as compared to African population (Ada, Suzen and Iscan 2004; Garte et al. 2001; Hanene et al. 2007; Mishra et al. 2004; Piacentini et al. 2011).
Results and Discussion

Figure 3.16: Worldwide map of the frequency distribution of GSTM1 genotype.
**3.2.3.1. D. Association of GSTM1 CNV (gene deletion) with asthma susceptibility**

The association between *GSTM1* gene deletion and susceptibility to asthma was studied in 34 asthma patients and a control group of 161 healthy individuals (Dhawan, PhD. thesis, Nirma University, 2010). The frequency of *GSTM1* homozygous null genotype was found to be 34.8% in asthma patients and 38.2% in healthy individuals. No statistically significant difference in the frequency of null genotype was detected between the two groups ($\chi^2 = 0.146; p = 0.7024$). Further, there was 1.16 fold increased risk of asthma observed in individuals with the *GSTM1* null genotype (OR 1.16, 95% CI 0.53–2.53, $p = 0.7024$), but this increase was not found to be significant in Indian population (*Table 3.4*).

**3.2.3.1. E. Association of the GSTM1 CNV with asthma susceptibility in other ethnic groups**

The role of *GSTM1* gene copy number has been studied in asthma susceptibility in different population. The CNV encompassing the whole gene deletion leads to a complete loss of function of the enzyme causing increased asthma susceptibility in individuals.

In a Russian case-control study comprising of 109 patients with atopic bronchial asthma and 90 healthy individuals, *GSTM1* CNV was analyzed. The frequency of *GSTM1* gene deletion was found to be higher in asthmatic patients (76.1%) as compared to controls (47.8%). Further, a significant association between individual carrying the *GSTM1* gene deletion at a higher risk (3.5-fold) of developing asthma was found (Ivaschenko, Sideleva and Baranov 2002). Similar positive association between *GSTM1* genetic polymorphism and increased asthma susceptibility was found in Tunisian (Hanene et al. 2007) and Turkish population (Ada, Suzen and Iscan 2004) as well. However, in contrast Freidin et al. demonstrated lack of significant association between GST and bronchial asthma severity (Freidin et al. 2002).
Table 3.4: Genotype frequencies of GSTM1 gene deletion in patients (asthma) and control (healthy) subjects, ORs with 95% CI and p values in Indian population.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Chromosomal Location</th>
<th>Polymorphism</th>
<th>Genotypes</th>
<th>Cases</th>
<th>Control</th>
<th>OR (95% CI), p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GSTM1&lt;sup&gt;++&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1</td>
<td>1p13.3</td>
<td>Null genotype</td>
<td>12</td>
<td>35.3</td>
<td>35</td>
<td>21.7</td>
</tr>
<tr>
<td>GSTM1</td>
<td></td>
<td></td>
<td>GSTM1&lt;sup&gt;-/0&lt;/sup&gt;</td>
<td>9</td>
<td>26.5</td>
<td>70</td>
</tr>
<tr>
<td>GSTM1</td>
<td></td>
<td></td>
<td>GSTM1&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>13</td>
<td>38.2</td>
<td>56</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
<td>N</td>
<td>34</td>
<td>161</td>
<td></td>
</tr>
</tbody>
</table>

GSTM1<sup>++</sup>, homozygous wild-type genotype; GSTM1<sup>-/0</sup>, heterozygous genotype; GSTM1<sup>-/-</sup>, homozygous null genotype; N = total number of samples in the respective group; n = number of samples, OR, odds ratio (GSTM1<sup>-/-</sup>) versus (GSTM1<sup>++</sup> + GSTM1<sup>-/0</sup>); CI, confidence interval; p, significance values.
3.2.3.2 Discussion

GSTM1 encodes for enzyme belonging to the glutathione S-transferase (GST) super family involved in the phase II detoxification system. The association of genetic polymorphism in GST genes and asthma development is well reported in several studies (Ivaschenko, Sideleva and Baranov 2002). Mainly the deletion of the entire GSTM1 gene leading to the nonfunctional null allele is reported to play a role in disease development. The null genotype leads to the inability to detoxify the ROS, which is produced as a result of the oxidative stress due to inflammation which is a characteristic symptom of asthma (Ivaschenko, Sideleva and Baranov 2002; Kabesch et al. 2004). Thus, the association of the GSTM1 (GSTM1<sup>0/0</sup>) null genotype in asthma susceptibility was assessed in our population: healthy individuals (n = 161) (Dhawan, PhD. Thesis, Nirma University, 2010), asthma patients (n = 34). The observed genotype frequencies were in agreement with the Hardy-Weinberg Equilibrium for control population. The GSTM1 null genotype (GSTM1<sup>0/0</sup>) frequency was found to be similar in asthmatic patients (38.2%) and healthy individuals (34.8%). There was a 1.16-fold increased risk of asthma in individuals with the GSTM1 null genotype (OR = 1.16; 95% CI, 0.53–2.53) when compared to the control group. But no significant association (p = 0.7024) of the GSTM1 gene deletion with increased risk to asthma was found in Indian population. Thus, a validation of the above findings in larger sample size is necessary for better conclusion. Apart from this, intra- and interethnic differences in the GSTM1 gene deletion frequencies in control individuals of different population are well documented (Garte et al. 2001). The frequency distribution of the GSTM1 null genotype in our control population was then compared to those reported in different populations. The frequency of GSTM1 null genotype in our healthy controls (34.8%) differed significantly from that reported in Caucasian, African/American, other Asian and Arabic populations except for African American (p = 0.142), Brazilian non-whites (p = 0.900) and Cameroon (p = 0.2059) among African/American population and North India (p = 0.685), South India (p = 0.292) among Asian populations (Ada, Suzen and Iscan 2004; Garte et al. 2001; Hanene et al. 2007; Mishra et al. 2004; Piacentini et al. 2011).
The distribution of *GSTM1* null frequency reported across different populations was then plotted on the world map. The *GSTM1* null frequency differed significant among Europeans, American, Africans and Asians, highest in European, American and other Asian populations. Thus, it can be interpreted that the heterogeneity in the frequencies of *GSTM1* gene polymorphism found is may be under the influence of environmental factors, exposure to toxins, geographic location, differential susceptibility to certain diseases and ethnicity. However, this data can have unequal ethnic and geographic coverage and difference in sample sizes in each population can attribute to these differences as well Therefore, detailed studies on the geography of GST variants could therefore increase knowledge about the relationship between ethnicity and the prevalence of certain diseases (Piacentini et al. 2011).

3.2.4 Glutathione S-transferase theta 1 (*GSTT1*) gene

The human *GSTT1*, a member of Glutathione S-transferases (GSTs) superfamily maps on chromosome 22q11.2. The CNV (gene deletion) in *GSTT1* is found to be associated with asthma susceptibility (Ivaschenko, Sideleva and Baranov 2002).

3.2.4.1 Results

3.2.4.1. A. Detection of *GSTT1* gene deletion by long range PCR

*GSTT1* deletion was detected by two reaction PCR and long range PCR method as described in detail in Section 2.3.4.4. The polymorphic sites screened during the analysis were identified based on the presence or absence of the bands in reaction A and reaction B (*Table 2.13 and 2.14*). This led to the identification of the individual’s genotype. The gel analysis of the PCR reactions is shown in *Figure 3.17*. 
Figure 3.17: Agarose gel (1%) of the PCR products for GSTT1 gene.

Lane description: Lane M1 shows the marker Lambda DNA/BstE II digest; Lane M2 shows the marker pUC19 DNA/Msp I ladder. Lane 1, 3, 5 represents reaction specific for gene deletion while lane 2, 4, 6 represents reaction specific for gene presence. Lane 1, 2 (sample 1) - homozygous wild-type (GSTT1+/+) genotype (PCR product: 264 bp), Lane 3, 4 (sample 2) - heterozygous (GSTT1+/0) genotype (PCR product: 3106 bp & 264 bp), Lane 5, 6 (sample 3) - homozygous deletion (GSTT10/0) genotype (PCR product: 3106 bp).

3.2.4.1. B. Allele and genotype frequency for GSTT1

The GSTT1 gene deletion was assessed in 29 asthma patients. The GSTT1 alleles and genotypes were analyzed using PCR and long-range PCR methods (Section 2.3.4.4). The allele and genotype frequency of GSTT1 for asthma patients and healthy individuals (Dhawan, PhD. thesis, Nirma University, 2010) is shown in Figure 3.18. The homozygous wild-type (GSTT1+/+), heterozygous (GSTT1+/0) and homozygous deletion (null genotype - GSTT10/0) genotype was observed at a frequency of 20.7%, 65.5% and 13.8% in asthmatic patients respectively.
3.2.4.1. C. Ethnic variation in GSTT1 genotype frequencies

The observed genotype frequencies of GSTT1 (Dhawan, Ph.D. thesis, Nirma University, 2010) was compared to those reported in Caucasian population (France, Italy, Netherlands, Sweden, UK) (Hanene et al. 2007), African/American population (African American (AA), Brazil (non-whites), Brazil (whites), Cameroon, Egypt, Mexico (Amerindian), Namibia, Whites (USA), Canada) (Hanene et al. 2007; Mishra et al. 2004; Piacentini et al. 2011), Arabic population (Iran, Saudi Arabia) (Piacentini et al. 2011), and Asian population (North India, South India, China, Japan, Mongolia) (Mishra et al. 2004; Piacentini et al. 2011). A graphical representation of the comparison between these populations is shown in Figures 3.19 and 3.20. The observed genotype frequencies of GSTT1 homozygous null genotype in the Indian population (our data) were quite different from that reported in Caucasian, African and American, Asian and Arabic populations.
Results and Discussion

**Figure 3.19:** Comparison of the distribution of *GSTT1* null genotype among Indian (our data) and other Asian and Caucasian populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>GSTT1 Null</th>
<th>GSTT1 Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian</td>
<td>8.1</td>
<td>91.9</td>
</tr>
<tr>
<td>North Indian</td>
<td>18.4</td>
<td>81.6</td>
</tr>
<tr>
<td>South Indian</td>
<td>16.8</td>
<td>83.2</td>
</tr>
<tr>
<td>Chinese</td>
<td>46</td>
<td>54</td>
</tr>
<tr>
<td>Japanese</td>
<td>44.3</td>
<td>55.7</td>
</tr>
<tr>
<td>Mongolian</td>
<td>25.6</td>
<td>74.4</td>
</tr>
<tr>
<td>Dutch (n=419)</td>
<td>22.9</td>
<td>77.1</td>
</tr>
<tr>
<td>French (n=512)</td>
<td>16.8</td>
<td>83.2</td>
</tr>
<tr>
<td>Italian (n=273)</td>
<td>19</td>
<td>81</td>
</tr>
<tr>
<td>Swedish (n=423)</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td>UK (n=922)</td>
<td>20.5</td>
<td>79.5</td>
</tr>
</tbody>
</table>
**Results and Discussion**

**Figure 3.20:** Comparison of the distribution of *GSTT1* null genotype among Indian (our data) and Arabic and African/American populations.
Results and Discussion

Upon analysis, a statistically significant difference between Indians (our data) and French \( (p = 0.0058) \), Italians \( (p = 0.002) \), Dutch \( (p < 0.0001) \), Swedish \( (p < 0.0001) \) and UK \( (p = 0.0001) \) among Caucasian populations.

There was a statistically significant difference between Indians (our data) and North India \( (p = 0.021) \), South India \( (p = 0.0006) \), Chinese \( (p < 0.0001) \), Japanese \( (p < 0.0001) \) and Mongolian \( (p < 0.0001) \) among Asian populations.

There was a statistically significant difference between Indians (our data) and Iranian \( (p < 0.0001) \) and Saudi Arabian \( (p < 0.0001) \) among Arabic populations.

There was a statistically significant difference between Indians (our data) and African American \( (p = 0.009) \), Brazilian whites \( (p = 0.00001) \), Brazilian non-whites \( (p < 0.0001) \), Cameroon \( (p < 0.0001) \), Egyptian \( (p < 0.0001) \), Namibian \( (p < 0.0001) \), Whites (USA) \( (p = 0.012) \) and Canadian \( (p = 0.0014) \). However, a non-significant association was observed only between Indian (our data) and Mexican Amerindian \( (p = 0.2506) \) among African and American populations.

Further, the frequency of the \textit{GSTT1} null genotype reported in different population was then plotted on the world map as depicted in \textit{Figure 3.21}. The graphical representation showed a wide range of variability in the \textit{GSTT1} null frequency within different populations. The frequency distribution of the \textit{GSTT1} null phenotype was found to be highest in Chinese and Japanese among Asian population and Cameroon among African population. However, in other populations the \textit{GSTT1} null frequency distribution differed significantly (Ada, Suzen and Iscan 2004; Garte et al. 2001; Hanene et al. 2007; Mishra et al. 2004; Piacentini et al. 2011).
Figure 3.21: Worldwide map of the frequency distribution of $GSTT1$ genotype.
3.2.4.1. D. Association of GSTT1 CNV (gene deletion) with asthma susceptibility

The association between GSTT1 gene deletion and susceptibility to asthma was studied in 29 asthma patients and a control group of 171 healthy individuals (Dhawan, Ph.D. thesis, Nirma University, 2010). The frequency of GSTT1 null genotype was found 13.8% in asthma patients and 8.1% in healthy individuals. No statistically significant difference in the frequency of null genotype was detected between the two groups ($\chi^2 = 0.951; p = 0.3294$). Further, there was 1.79-fold increase risk of asthma observed in individuals with the GSTM1 null genotype (OR 1.79, 95% CI 0.47–6.76, $p = 0.3294$) when compared to the control group, but this increased risk was not found to be significant in Indian population (Table 3.5).

3.2.3.1. E. Association of the GSTT1 CNV with asthma susceptibility in other ethnic groups

The role of GSTT1 gene copy number has been studied in asthma susceptibility in different population. The CNV encompassing the whole gene deletion leads to a complete loss of function of the enzyme causing increased asthma susceptibility in individuals.

There are several studies where the association between asthma and glutathione-S transferase gene polymorphisms has been reported. In a Russian case-control study comprising of 109 patients with atopic bronchial asthma and 90 healthy individuals, GSTT1 CNV was analyzed. The frequency of GSTT1 gene deletion was found to be higher in asthmatic patients (67%) as compared to controls (23.3%). Further, a significant association between individual carrying the GSTT1 gene deletion at a higher risk (6.6-fold) of developing asthma was found (Ivaschenko, Sideleva and Baranov 2002). Similar positive association between GSTT1 genetic polymorphism and increased asthma susceptibility was found in Tunisian (Hanene et al. 2007) and Turkish population (Ada, Suzen and Iscan 2004) as well. However, in contrast, Freidin et al. demonstrated lack of significant association between GST and bronchial asthma severity (Freidin et al. 2002).
Table 3.5: Genotype frequencies of \textit{GSTT1} gene deletion in patients (asthma) and control (healthy) subjects, ORs with 95\% CI and $p$ values in Indian population.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Polymorphism</th>
<th>Genotypes</th>
<th>Cases</th>
<th>Control</th>
<th>OR (95% CI), $p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>\textit{GSTT1}</td>
<td>22q11.23</td>
<td>Null genotype</td>
<td>\textit{GSTT1}$^{++}$</td>
<td>6</td>
<td>20.7</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>\textit{GSTT1}$^{+}$</td>
<td>19</td>
<td>65.5</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>\textit{GSTT1}$^{0}$</td>
<td>4</td>
<td>13.8</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>29</td>
<td></td>
<td>171</td>
</tr>
</tbody>
</table>

\textit{GSTT1}$^{++}$, homozygous wild-type genotype; \textit{GSTT1}$^{+}$, heterozygous genotype; \textit{GSTT1}$^{0}$, homozygous null genotype; N = total number of samples in the respective group; n = number of samples, OR, odds ratio (\textit{GSTT1}$^{0}$) versus (\textit{GSTT1}$^{++}$ + \textit{GSTT1}$^{+}$), CI, confidence interval, $p$, significance value.
3.2.4.2 Discussion

GSTT1 encodes for enzyme belonging to the glutathione S-transferase (GST) super family involved in the phase II detoxification system. The association of genetic polymorphism in GST genes and asthma development is well reported in several studies (Ivaschenko, Sideleva and Baranov 2002). Mainly the deletion of the entire GSTT1 genes leading to the nonfunctional null allele are reported to play a role in disease development. The null genotype leads to the inability to detoxify the ROS, which is produced as a result of the oxidative stress due to inflammation which is a characteristic symptom of asthma (Ivaschenko, Sideleva and Baranov 2002; Kabesch et al. 2004). Thus, the association of the GSTT1 (GSTT1<sup>0/0</sup>) null genotype in asthma susceptibility was assessed in our population: healthy individuals (n = 171) (Dhawan, PhD. Thesis, Nirma University, 2010), asthma patients (n = 29) for GSTT1. The observed genotype frequencies were not in agreement with the Hardy-Weinberg Equilibrium for control population. The GSTT1 null genotype (GSTT1<sup>0/0</sup>) frequency was found to be higher in asthmatics patients (13.8%) than the healthy individuals (8.1%) and 1.7 folds increased risk for asthma was seen in individuals carrying the GSTT1 null genotype, but this difference was found to be non-significant (p = 0.3294). Thus, a validation of the above findings in larger sample size is necessary for better conclusion.

The intra- and inter-ethnic differences are well reported in the GSTT1 gene deletion frequencies in control populations (Garte et al. 2001). The frequency distribution of the GSTT1 null genotype in our healthy control sample was compared with those reported in other control populations. A significant difference between Indian population (our data) and Caucasian, African/American, Asian and Arabic populations except Swedish (p = 0.0972) among Caucasian population and Mexican Amerindian (p = 0.2506) among African/American population (Ada, Suzen and Iscan 2004; Garte et al. 2001; Hanene et al. 2007; Mishra et al. 2004; Piacentini et al. 2011).

The distribution of GSTT1 null frequency reported across different population was then plotted on the world map. The GSTT1 null frequency differed significant among Europeans, American, Africans and Asians, highest in Chinese, Japanese and Cameroon population. Thus, it can be
interpreted that the heterogeneity in the frequencies of \textit{GSTT1} gene polymorphism found is may be under the influence of environmental factors, exposure to toxins, geographic location, differential susceptibility to certain diseases and ethnicity. However, this data can have unequal ethnic and geographic coverage and difference in sample sizes in each population can attribute to these differences as well. Therefore, detailed studies on the geography of GST variants could therefore increase knowledge about the relationship between ethnicity and the prevalence of certain diseases (Piacentini et al. 2011).

3.2.5 Chemokine (C-C motif) ligand 3-like 1 (\textit{CCL3L1}) gene

\textit{CCL3L1}, a natural ligand for HIV-1 coreceptor CCR5 maps on human chromosome 17q21.1 and is found to be variable in copy number. The CNV resulting in the segmental duplication in \textit{CCL3L1} has been found to be involved in HIV-1/AIDS susceptibility by limiting the HIV-1 to entry the cells (Gonzalez et al. 2005).

3.2.5.1 Results

3.2.5.1. A. Detection of \textit{CCL3L1} segmental duplication by real time PCR

\textit{CCL3L1} copy number was measured (\textit{Table 2.15 and 2.16}) using TaqMan real-time PCR as described in detail in Section 2.3.5.1. All the samples were assayed in duplicate and averaged to determine (or score) the copy number based on the Ct values obtained for endogenous control (\textit{RNase P}) and target gene (\textit{CCL3L1}).

3.2.5.1. B. Distribution of \textit{CCL3L1} gene copy number

We assessed the distribution of \textit{CCL3L1} copy number (CN) in 64 HIV-1 patients (HIV\textsuperscript{+}) and 138 healthy control individuals (HIV\textsuperscript{−}). The \textit{CCL3L1} copy number ranged from 1 – 6 copies per diploid genome and 0 – 5 copies per diploid genome, with an average gene dose of two copies in healthy individuals and HIV-1 patients respectively. The frequency distribution of the \textit{CCL3L1} copy number is shown in \textit{Figure 3.22}. The \textit{CCL3L1} copy number distribution in HIV-1 patients and healthy individuals was found to be CN <2, 25%; 2, 39.1%; >2, 35.9% and CN <2, 11%; 2, 43%; >2, 46%, respectively.
Results and Discussion

3.2.5.1. C. Ethnic variation in CCL3L1 gene copy number

The observed copy number distribution for CCL3L1 gene was compared to those reported in several human populations. A graphical representation of the comparison between these populations is shown in Figure 3.23.

The observed median copy number (CN = 2) in Indian healthy (HIV\textsuperscript{−}) population (our data) was quite different from that reported in Japanese (Nakajima et al. 2008), African and non-African (Europe, Middle East, East Asia, Oceania, America) populations (Gonzalez et al. 2005). Upon analysis, a statistically significant difference was found between Indians (our data) and Japanese (Nakajima et al. 2008), African \((p < 0.0001)\), European \((p = 0.001)\), Middle East \((p = 0.0325)\), East Asian \((p = 0.001)\), Oceanic \((p = 0.0017)\), Americans \((p = 0.001)\), European Americans (EA) \((p = 0.0017)\), African Americans (AA) \((p = 0.0001)\) and Hispanic American (HA) \((p = 0.001)\). However, a non-significant association was observed between Indian (our data) and Indian
Figure 3.23: Distribution of *CCL3L1* gene copy numbers among human populations. Colored vertical bars represent the mean copy number in the study population (red: Indian subjects (our data), blue: Human genome diversity cell line panel (HGDP-CEPH) cohort, Wilford Hall Medical Center (WHMC) HIV – subjects, non-WHMC HIV – subjects), error bars indicate the standard deviation. The numbers above the bars indicate the inter-quartile range of *CCL3L1* gene copy number (Gonzalez et al 2005).
results and discussion

Samples from AIIMS (Nakajima et al. 2008) \( (p = 0.0185) \) and Central/South Asians (Gonzalez et al. 2005) \( (p = 0.1119) \).

3.2.5.1. D. Association of CCL3L1 CNV (segmental duplication) with HIV-1 susceptibility

The association between \( CCL3L1 \) gene copy number and susceptibility to HIV/AIDS was studied in 64 HIV\( ^+ \) and 138 HIV\( ^- \) individuals. The median \( CCL3L1 \) copy number in both HIV\( ^+ \) and HIV\( ^- \) individuals was two (range from 0 to 5, HIV\( ^+ \); 0 to 6 HIV\( ^- \)). The distribution of the \( CCL3L1 \) copy numbers between HIV\( ^+ \) and HIV\( ^- \) individuals was significantly different \( (\chi^2 = 6.72; p = 0.0095) \).

The frequency of < 2 copies of \( CCL3L1 \) was found to be higher in HIV\( ^+ \) (25.0 %) than HIV\( ^- \) (10.86%) individuals as compared to \( \geq 2 \) copies in HIV\( ^+ \) (75.0 %) and HIV\( ^- \) (89.14%) individuals. The association of \( CCL3L1 \) gene copy number and risk of acquiring HIV was then determined. The population-specific median copy number (CN = 2) in healthy control group was chosen to be the reference point for calculating the risk of HIV as summarized in Table 3.6. There was 2.7-fold increase risk to HIV observed in individuals with < 2 copies (OR 2.7, 95% CI 1.25–5.96, \( p = 0.0095 \)) when compared to \( \geq 2 \) copies in Indian population.

3.2.5.1. E. Association of the CCL3L1 CNV with HIV-1 susceptibility in other ethnic groups

\( CCL3L1 \) gene copy number has been reported to influence HIV-1 infection and pathogenesis (Gonzalez et al. 2005) in several population groups. It has been reported that \( CCL3L1 \) copy numbers lower than the average for each ethnic group evaluated was found to be associated with increased susceptibility to HIV-1 infection, presumably due to lower expression of \( CCL3 \) (MIP-1a) and reduced capacity to block or interfere with HIV-1 binding to CCR5 (Gonzalez et al. 2005; Shao et al. 2007). These findings have been confirmed by several independent studied but not in all subsequent independent cohort studies.

The association of \( CCL3L1 \) gene copy number has also been evaluated in several autoimmune diseases like rheumatoid arthritis, Crohn’s disease, psoriasis or systemic lupus erythematosus. The association of the \( CCL3L1 \) copy number higher than two was found to be a risk factor for
### Table 3.6: Association of CCL3L1 copy number and risk of acquiring HIV. ORs with 95% CI and \( p \) values in Indian population.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Polymorphism</th>
<th><strong>CCL3L1</strong> gene copies</th>
<th><strong>Cases</strong> (n = 64)</th>
<th><strong>Control</strong> (n = 138)</th>
<th>OR (95% CI), ( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>CCL3L1</td>
<td>17q21.1</td>
<td>Segmental duplication</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>15</td>
<td>14</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>25</td>
<td>59</td>
<td>42.8</td>
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<td>3</td>
<td>13</td>
<td>38</td>
<td>27.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>7</td>
<td>17</td>
<td>12.3</td>
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<td>3</td>
<td>8</td>
<td>5.8</td>
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<td></td>
<td></td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Risk of acquiring HIV relative to the population–specific median \([(\text{odds ratio (OR)} = 1)], \text{ CI, confidence interval; } p, \text{ significance values; } n, \text{ number of samples; ND, not determined.}

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rheumatoid arthritis in large New Zealand cohort, however in the same study this association was not replicated in a smaller UK cohort suffering from rheumatoid arthritis (McKinney et al. 2008). Similar interaction of CCL3L1 CNV and CCR5 genotypes has been suggested in SLE pathogenesis as well (Mamtani et al. 2008). Further, in another independent study, the association of CNV in CCL3L1 was assessed with three autoimmune diseases (like Crohn’s disease, rheumatoid arthritis and psoriasis). However, negative association between disease and CCL3L1 copy number was reported (Carpenter et al. 2011).

3.2.5.2 Discussion

The CC chemokine ligand-3–like-1 (CCL3L1) is among the several cytokines genes which are clustered on chromosome 17q12, a hotspot for segmental duplication (Gonzalez et al. 2005). CCL3L1, a HIV-suppressive chemokine is a natural ligand for the HIV-1 coreceptor CC chemokine receptor 5 (CCR5) (Nakajima et al. 2008). The role of increased CCL3L1 gene copy number has been reported to be associated with HIV-1/AIDS susceptibility in several studies. Individuals possessing a CCL3L1 copy number below the ethnic group median were found to be at an increased risk of acquiring HIV. However there are several reports which do not support this observation. Apart from this, the role of CCL3L1 gene copy number and susceptibility to other complex diseases has been explored as well.

The distribution of CCL3L1 gene copy number have been found be varied among people of various geographical ancestries (Ahuja et al. 2008; Bhattacharya et al. 2009; Dolan et al. 2007; Gonzalez et al. 2005; He et al. 2009; Modi 2004). In our present study the distribution of CCL3L1 gene copy number was assessed in HIV/AIDS patients (HIV+) (n = 64) and healthy individuals (HIV-) (n = 138). The median CCL3L1 copy number was found to be CN = 2 for both the study groups; range 0 – 6 copies for HIV− and range 0 – 5 copies for HIV+ individuals per diploid genome. The frequency of CCL3L1 CN < 2 was found to be significantly higher (p = 0.0095) in HIV+ (25.0 %) than HIV− (10.86%) individuals on comparison with CN> 2 in both the groups. Further, the association between CCL3L1 copy number and risk of acquiring HIV was assessed in our study. Compared to CCL3L1 CN = 2, the individuals possessing less than
two copies had significantly higher risk of acquiring HIV while the individuals possessing more than two copies had lower risk of acquiring HIV. Thus, a significant association of the \textit{CCL3L1} CNV with susceptibility to HIV-1 infection was found in Indian population. A lower copy number was found to be linked with an increased risk of HIV-1 infection, while a higher copy number with reduced risk for acquiring HIV-1 in our study population.

The median copy number in the healthy control individuals was then compared with those reported in other population. There was a significant difference between Indians (our data) and Japanese, Africans, Europeans, Middle East, East Asians, Oceanics, Americans, European Americans (EA), African Americans (AA) and Hispanic American (HA). However, a non-significant association was observed between Indians (our data) and Indian samples from AIIMS (Nakajima et al. 2008) and Central/South Asians (Gonzalez et al. 2005). Thus, an inter-ethnic variation with respect to \textit{CCL3L1} gene copy number is demonstrated among populations (Gonzalez et al. 2005).

3.2.6 \textbf{Fc fragment of IgG, low affinity IIIb, receptor (CD16b) (\textit{FCGR3B}) gene}

\textit{FCGR3B} gene, maps on chromosome 1q23 is found to be involved in regulation of inflammatory and immune responses. The CNV (segmental duplication) at human Fc gamma receptors (\textit{FCGR3B}) is associated with conferring susceptibility to a range of autoimmune disorders (Schaaschl, Aitman and Vyse 2009).

3.2.6.1 Results

3.2.6.1. \textit{A. Detection of FCGR3B segmental duplication by real time PCR}

\textit{FCGR3B} copy number (CN) was measured (\textit{Table 2.17 and 2.18}) using TaqMan real-time PCR as described in detail in \textbf{Section 2.3.5.2}. All the samples were assayed in duplicate and averaged to determine the copy number based on the Ct values obtained for endogenous control (\textit{RNase P}) and target gene (\textit{FCGR3B}).
3.2.6.1. B. Distribution of FCGR3B gene copy number

We assessed the distribution of FCGR3B copy number (CN) in 100 healthy individuals. The FCGR3B CN ranged from 1 – 3 per diploid genome, with an average gene dose of two copies in healthy individuals. The frequency distribution of the FCGR3B copy number is shown in Figure 3.24. The FCGR3B copy number distribution in healthy individuals was found to be CN 1, 21%; 2, 77%; 3, 2%.

![Figure 3.24](image1)

**Figure 3.24:** Distribution of FCGR3B gene copy number in Indian healthy individuals.

3.2.6.1. C. Ethnic variation in FCGR3B gene copy number

The observed copy number distribution for FCGR3B gene was compared to those reported in several human populations. A graphical representation of the comparison between these populations is shown in Figures 3.25 and 3.26.

The observed FCGR3B CN < 2 in Indian (our data) was compared to that reported in Caucasian (Australia) (Graf et al. 2012), European Caucasian descents (New Zealand, UK, Netherlands, Breunis) (McKinney et al. 2010), African/American [(Afro-Caribbean (Trinidadian), Kenya, West Africa (UK), Yoruba (Ibadan, Nigeria), Colombia] (Hollox, Detering and Dehnugara 2009;
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Mamtani et al. 2010; Molokhia et al. 2011; Niederer et al. 2010) and Asian [Hong Kong Chinese, Japan (Tokyo), Vietnam (Kinh)] (Hollox, Detering and Dehnugara 2009; Niederer et al. 2010) populations.

Upon analysis, a statistically significant difference between Indians (our data) and Australian ($p = 0.0003$), New Zealanders ($p < 0.0001$), UK ($p < 0.0001$), Dutch ($p < 0.0001$), Breunis ($p < 0.0001$) among Caucasian, and European Caucasian descents respectively. However, a non-significant difference was observed between Indians (our data) and Afro-Caribbean ($p = 0.2774$), Kenyan ($p = 0.5023$), West African (UK) ($p = 0.1976$), Yoruban ($p = 0.3865$) among African population. There was a statistically significant difference between Indians (our data) and Colombian ($p = 0.0026$), Hong Kong Chinese ($p = 0.0006$), Japanese ($p = 0.0554$) and Vietnamese ($p = 0.0012$) among American and Asian populations respectively.

Further, the distribution of $FCGR3B$ gene copy number across different populations was plotted on the world map as depicted in Figure 3.27. The frequency of $FCGR3B$ copy number $< 2$ was found to be comparatively higher in our population (Indians), Americans and Africans as compared to European Caucasians and other Asian populations.
Figure 3.25: Comparison of the distribution of *FCGR3B* gene copy number among Indian (our data) and European Caucasian descents.
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**FCGR3B gene copy number distribution in different populations**

<table>
<thead>
<tr>
<th>Population</th>
<th>&lt;2 copy</th>
<th>≥2 copy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian (our data) (n = 100)</td>
<td>21</td>
<td>79</td>
</tr>
<tr>
<td>Chinese (Hong Kong) (n = 989)</td>
<td>9.8</td>
<td>90.2</td>
</tr>
<tr>
<td>Japanese (Tokyo) (n = 32)</td>
<td>6.3</td>
<td>93.7</td>
</tr>
<tr>
<td>Vietnamese (Kinh) (n = 885)</td>
<td>10.1</td>
<td>89.9</td>
</tr>
<tr>
<td>West African (n = 93)</td>
<td>29</td>
<td>71</td>
</tr>
<tr>
<td>Kenyan (n = 833)</td>
<td>18.2</td>
<td>81.8</td>
</tr>
<tr>
<td>Yoruban (Nigeria) (n = 35)</td>
<td>14.3</td>
<td>85.7</td>
</tr>
<tr>
<td>Colombian (n = 409)</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Afro-Caribbean (Trinidad) (n = 479)</td>
<td>16.5</td>
<td>83.5</td>
</tr>
</tbody>
</table>

**Figure 3.26:** Comparison of the distribution of FCGR3B gene copy number among Indian (our data) and other Asian and African/American populations.
Results and Discussion

Figure 3.27: Worldwide map of the frequency distribution of $FCGR3B$ gene copy number.
3.2.6.2 Discussion

The Fc gamma receptor 3B (FCGR3B) gene codes for the low-affinity Fcγ receptor, FcγRIIIb (CD16b), which is exclusively expressed by human neutrophils and tightly linked to the surface of neutrophils by the glycosylphosphatidyl-inositol (GPI) anchor (Bournazos et al. 2011). CNV in the Fc gamma receptor region is well characterized and the association of FCGR3B CNV with several autoimmune diseases like systemic lupus erythematosus, systemic vasculitis, rheumatoid arthritis (RA) has been reported (Aitman et al. 2006; Fanciulli et al. 2007; Schaschl, Aitman and Vyse 2009; Willcocks et al. 2008).

In our present study the distribution of FCGR3B gene copy number was assessed in healthy control individuals (n = 100). A range of 1 – 3 copies per diploid genome of FCGR3B was seen in Indian population (our data). The frequency of FCGR3B CN = 2 (77%) was found to be highest.

The distribution of FCGR3B CN < 2 and ≥ 2 in our healthy individuals (Indian population) were then compared to healthy control samples reported in other populations. There was a significant difference between Indians (our data) and Caucasian, European Caucasian descents, Americans and Asians. However, a non-significant association was observed between Indians (our data) and Africans. Thus, an inter-ethnic variation with respect to FCGR3B gene copy number is demonstrated among populations. The world map depicting the frequency distribution of the FCGR3B CNV highlighted the same observation and thus, the observation can then be analyzed further from clinical point of view and migratory patterns can also be analyzed.

3.2.7 Human cytosolic sulfotransferase 1A1 (SULT1A1) gene

SULT1A1 which maps on human chromosome 16p12.1 is involved in the sulfate conjugation of variety of natural and synthetic compounds (Hebbring et al. 2007). CNV (gene deletion/duplication) in SULT1A1 has been reported which can have potential role as a risk factor for disease (Hebbring et al. 2007).
3.2.7.1 Results

3.2.7.1. A. Detection of SULT1A1 gene duplication/deletion by PRT assay

*SULT1A1* copy number (CN) was measured (*Table 2.19 and 2.20*) using PRT assay as described in detail in *Section 2.3.6.1*. The ratio of the peak heights of test gene (*SULT1A1*) and control gene (*SULT1A2*) was calculated to score the copy number of the samples.

3.2.7.1. B. Distribution of SULT1A1 gene copy number

We assessed the distribution of *SULT1A1* copy number (CN) in 157 healthy individuals. The *SULT1A1* CN ranged from 1 – 6 copies per diploid genome. The frequency distribution of the *SULT1A1* copy number is shown in Figure 3.28. The *SULT1A1* copy number distribution in healthy individuals was found to be CN 1, 3.8%; 2, 64.9%; 3, 21.7%; 4, 5.1%; >4, 4.5%.

![Figure 3.28: Distribution of SULT1A1 gene copy number in Indian healthy individuals.](image-url)
3.2.7.1. C. Ethnic variation in SULT1A1 gene copy number

The observed copy number distribution for SULT1A1 gene was compared to those reported in African American and Caucasian American populations (Hebbring et al.) and South African Tswana population (Mbongwa et al. 2011). A graphical representation of the comparison between these populations is shown in Figures 3.29.

The observed SULT1A1 copy number distribution in Indian population (our data) was quite different from that reported in African American and Caucasian American. SULT1A1 gene deletion, i.e. one copy was reported at a frequency of 4.7% in Caucasian American and 0.7% in South African (Tswana) as compared to 3.8% in Indians (our data). However, none of the African American subjects carried a gene deletion. Further, 69.6%, 37.4% and 39.7% of Caucasian Americans and African Americans and South African (Tswana) respectively had two copies, while 25.7%, 62.6% and 59.7% had three or more copies respectively as compared to 64.9% (CN = 2) and 30.9% (CN > 2) in Indians (our data).

Upon analysis, a statistically significant difference in the distribution of SULT1A1 copy number was observed between Indians (our data) and African American (AA) \( (p = 0.0001) \) and South African (Tswana) \( (p < 0.0001) \) population. However, a non-significant association was observed between Indian (our data) and Caucasian Americans \( (p = 0.3741) \).
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**$SULT1A1$ gene copy number distribution in different populations**

<table>
<thead>
<tr>
<th>SULT1A1 copy number</th>
<th>Indian (n = 157)</th>
<th>Caucasian-American (n= 362)</th>
<th>African-American (n = 99)</th>
<th>South African (Tswana) (n = 459)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 copy</td>
<td>3.8</td>
<td>4.7</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>2 copy</td>
<td>64.9</td>
<td>69.6</td>
<td>37.4</td>
<td>39.7</td>
</tr>
<tr>
<td>3 copy</td>
<td>21.7</td>
<td>21</td>
<td>37.4</td>
<td>37.9</td>
</tr>
<tr>
<td>4 copy</td>
<td>5.1</td>
<td>3.6</td>
<td>21.2</td>
<td>18.5</td>
</tr>
<tr>
<td>&gt; 4 copy</td>
<td>4.5</td>
<td>1.1</td>
<td>4</td>
<td>3.3</td>
</tr>
<tr>
<td>&gt; 2 copy</td>
<td>31.2</td>
<td>25.7</td>
<td>62.6</td>
<td>59.7</td>
</tr>
</tbody>
</table>

Figure 3.29: Comparison of the distribution of $SULT1A1$ copy number among Indian and Caucasian-American, African-American and South African (Tswana) populations.
3.2.7.2 Discussion

Human cytosolic sulfotransferase (SULT) enzymes catalyze the sulfate conjugation of many drugs, xenobiotics, neurotransmitters, and steroid hormones (Hebbring et al. 2007). Sulfate conjugation catalyzed by SULT1A1, is one of the major drug metabolism pathways in humans (Hebbring, Moyer and Weinshilboum 2008). SULT1A1 gene maps on short arm of chromosome 16 and is reported to have variation in copy number. The functional implication of the SULT1A1 was assessed in a study where the variation in the SULT1A1 gene copy number was correlated to the level of SULT1A1 enzyme activity (Hebbring et al. 2007; Hebbring, Moyer and Weinshilboum 2008).

In our present study, the distribution of SULT1A1 gene copy was assessed in healthy control individuals (n = 157). A range of 1 to > 4 copies, with the frequency of SULT1A1 CN = 2 (64.9%) to be highest was observed in our (Indian) population.

The varied frequency distribution of SULT1A1 gene copy number is well reported in populations with different ethnicity (Hebbring et al. 2007). The distribution of SULT1A1 copy number in Indian healthy individuals (our data) was then compared to those reported in other populations. There was a significant difference observed between Indians (our data) and African American (AA) and South African (Tswana) population. However, the frequency SULT1A1 copy number distribution was found to be similar among Indians (our data) and Caucasian Americans. Thus, it can be concluded that multiple copies of SULT1A1 gene are found in several population, but the frequency distribution vary with ethnicity.
3.4 SUMMARY

Increasing our understanding of the role of these genetic factors in determining human susceptibility to the various diseases has become a major research goal in elucidating the complexity and pathogenesis of any disease. In the present study, we assessed the frequency distribution of the copy number variants in several genes and their involvement in the susceptibility to diseases like cancer, psoriasis, asthma and HIV-1/AIDS in Indian population using a case-control approach.

For the study of the frequency distribution in the selected copy number variants genes, a range of 100 – 280 healthy Indian control individuals were analyzed using PCR-based methods. The variant frequency observed in the healthy Indian control samples were then compared to those reported in several populations worldwide in order to highlight the ethnic variability (intra- and inter-individual differences) between different populations.

The exon-specific deletion variant for MTUS1 was analyzed in 280 healthy individuals. The frequency distribution of the deletion variant (wt/Del + Del/Del) was found to be 57.1 % in Indian population. Further, the frequency of deletion variant of MTUS1 was found to be significantly ($p< 0.0001$) different among Indian (our data) and German population.

The gene deletion comprising both LCE3B and LCE3C, members of late cornified envelope (LCE) gene cluster was analyzed 203 healthy Indian individuals. The frequency of the LCE3B and LCE3C gene deletion was found to be 36.4% in healthy control individuals. Further, the observed LCE3C_B-del frequency in Indian population was compared to those reported of European ancestry and other Asiatic origin populations. A significant ($p< 0.0001$) difference was observed among Indians and European ancestry as well as with other Asiatic origin populations, which highlighted the ethnic variability among different populations across the globe.

The distribution of CCL3L1 CNV (segmental duplication) was analyzed in 138 healthy control individuals. A range of 1 – 6 copies per diploid genome was found in Indian population. Further, a significant ($p< 0.005$) inter-ethnic variation in the frequency distribution of CCL3L1 gene copy
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number was demonstrated among Indians (our data) and Japanese, Africans, Europeans, Middle East, East Asians, Oceanics, Americans, European Americans (EA), African Americans (AA) and Hispanic American (HA). However, a non-significant difference was observed between Indians (our data) and Central/South Asians \( (p = 0.1119) \).

In case of \( FCGR3B \), 100 healthy control individuals were analyzed for assessment of segmental duplication in \( FCGR3B \) gene. \( FCGR3B \) copy number ranged from 1 – 3 copies per diploid genome in Indian population. The frequency distribution for \( FCGR3B \) gene copy number differed significant \( (p< 0.005) \) among Indians (our data) and Caucasian, European, European Caucasian, American and other Asian populations. However, Afro-Caribbean \( (p = 0.2774) \), Kenyan \( (p = 0.5023) \), West African (UK) \( (p = 0.1976) \) and Yoruban \( (p = 0.3865) \) among African population had similar frequency distribution pattern of \( FCGR3B \) gene copy number as compared to Indians (our data).

For \( SULT1A1 \), 157 healthy control samples were analyzed to evaluate the frequency distribution of \( SULT1A1 \) gene copy number in Indian population. The \( SULT1A1 \) copy number ranged from 1 – 6 copies per diploid genome. The frequency distribution of \( SULT1A1 \) gene copy number was found to similar among Indians (our data) and Caucasian Americans \( (p = 0.3741) \). But, a significant difference between Indians (our data) and African American (AA) \( (p = 0.0001) \) and South African (Tswana) \( (p< 0.0001) \) was observed in our analysis.

In order to examine, if there is any correlation of the copy number variants studied in the selected gene with respective disease susceptibility or protection in Indian population, a pilot case-control study was designed.

The role of CNV (exon-specific deletion) in a mitochondrial tumor suppressor 1 \( (MTUS1) \) gene on cancer risk was investigated in 15 breast cancer patients, 41 head and neck cancer and 280 healthy individuals. Upon analysis, a significant \( (p = 0.0207) \) association of the deletion variant with decrease risk for breast cancer and a lack of significant \( (p = 0.8671) \) correlation with head and neck cancer was found in Indian population. The association of the common deletion comprising \( LCE3B \) and \( LCE3C \) genes in susceptibility to psoriasis was evaluated in 11 psoriasis
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patients and 203 healthy control individuals. A significant ($p = 0.0151$) correlation of the LCE3C_B-del as a risk factor for psoriasis was observed in Indian population, indicating to be an important factor in the pathogenesis of psoriasis. The role of GSTM1 and GSTT1 gene deletion in asthma susceptibility was assessed in 34 and 29 asthma patients and 161 and 171 healthy control individuals, for respective genes. A 1.16-fold increased risk of asthma was observed in individuals with GSTM1 homozygous deletion, but this increase was not significant ($p = 0.7024$) in Indian population. Similarly, 1.79-fold increased risk of asthma was observed in individuals with GSTT1 homozygous deletion, but this increase was not significant ($p = 0.3294$) in Indian population. The association of the CCL3L1 copy number in HIV-1/AIDS susceptibility was also assessed in 64 HIV patients (HIV$^+$) and 138 healthy individuals (HIV$^-$). Compared to CCL3L1 copy number CN = 2, the individuals possessing less than two copies or more than two copies had significantly higher or lower risks respectively, of acquiring HIV-1 infection in Indian population. The sample size in the above mentioned association studies is very small thus, the results needs to be further validated in large sample size for future conclusion.

The study was initiated with purpose of elucidating the frequency distribution of copy number variations in Indian populations. CNVs are well recognized as one of the important source of genetic variation contributing to disease susceptibility and pathogenesis. The study aims to analyze the known CNVs and also to ascertain correlation with known disease susceptibility or protection. This study will pave a way to analyze and apply the CNV analysis in future genetic studies, in molecular diagnostics and personalized medicine. The future prospect is to validate the results in larger sample for further conclusion.