Anthropological genetics has emerged as a useful tool for studying various multifactorial complex diseases since the knowledge of human variations and its underlying genetic and environmental factors specific to populations is the primary goal of anthropological geneticists. Moreover, recent advancements in science and technology facilitated the use of genomic markers for studying intra and inter population variations. Such studies intensively helped in understanding the human evolution and migrations. Moreover, understanding genetic variation in disease risk and mapping candidate genes associated with the complex disease which are reported to be population specific may provide new insight into the disease etiology which could lead to genetic screening programmes to identify the populations at higher risk. Recent advancements in science and technology facilitated the use of genomic markers for studying intra and inter population variations. Such studies intensively helped in understanding the human evolution and migrations. Moreover, understanding genetic variation in disease risks and mapping candidate genes associated with the complex disease which are reported to be population specific may provide new insight into the disease etiology which could lead to genetic screening programmes to identify the populations at higher risk. Thus, anthropological genetics faced a paradigm shift from understanding genetic variation for evolutionary purpose to understanding genetic variations in relation to various non-communicable complex disorders.
One of the best examples of complex disease, where the phenotypic expression is the interplay of gene-gene and gene-environment interaction is *alcoholism/alcohol dependence*. Here, both gene and environment (social and cultural) act importantly in developing the disease profile (Li et al. 2000). Prevalence of alcohol dependence is widely different across the world. Alcohol dependence is one of the leading health risks and is likely to become world’s third largest risk factor for disease and disability (WHO, 2011). In light of the above, the present study not only attempts to understand the etiology of alcohol dependence (environment and genetic factors) and also the effect of alcohol consumption/dependence on health with special reference to anthropometric, physiological and biochemical variables among a Mendelian population. Thus, the present study hypothesized that variation/mutation in the selected genes are expected to be more common among the alcohol dependent cases as compared to controls influencing the susceptibility to alcohol dependence and the effect will be more pronounced under certain environmental conditions like early age at onset of alcohol consumption, smoking, lower educational level and unemployment.

The present study was conducted among the *Meitei* community of Manipur, a northeastern state of India. Four hundred and eighty four (484) males aged 25-75 years, unrelated up to 1st cousin and belonging to East Asian ancestry were recruited from four districts of Manipur namely Imphal East, Imphal West, Thoubal and Bishnupur, where *Meitei* community is predominantly inhabited. Of them, 155 individuals were AD cases, recruited either from de-addiction center or through population based household survey. In both ways, diagnosis of alcohol dependence was determined using the DSM-IV criteria (American Psychiatric Association, 1994, 2000). Age matched controls (329) without any history of substance dependence was randomly recruited from the same community through household survey. The selected control group included individuals consuming alcohol who were not categorized as alcohol dependence as per DSM-IV criteria, rather they were alcohol abusers i.e. occasional drinkers (ODs) and moderate drinkers (MDs). Information pertaining to occupation, education, smoking and age at first use of alcohol was also collected for both AD cases and controls. Moreover, anthropometric measurements such as height, weight,
waist circumference, hip circumference and physiological variable (SBP and DBP) were also collected from all the subjects.

Intra venous blood samples (5mL) were collected with prior informed written consent, of which 2mL was used in analyzing biochemical variables like TC, TG, LDL, VLDL, fasting glucose and two liver enzymes (AST and ALT) through outsourcing (CECIL Medical Laboratory, Manipur). Remaining 3mL were used for DNA isolation and further molecular analysis like mutation detection of selected eight SNPs (ADH1B Arg47His [rs1229984]; ADH1C Ile349Val [rs698]; ALDH2 Glu487Lys [rs671]; DRD2 TaqI B [rs1079597], TaqI D [rs1800498] and -141C Ins/Del [rs1799732]; ANKK1 TaqI A [rs1800497] and DRD1 -48A/G [rs4532]) in the Molecular Anthropology Laboratory of Department of Anthropology, University of Delhi following the standard protocols. Appropriate statistical softwares were used to analyze the generated data. Present study was approved by ethical committee of Department of Anthropology, University of Delhi, India.

Of the 462 individuals recruited in the present study, 72.08% were found to be alcohol consumers and 27.92% were absolute non-drinkers indicating higher occurrence of alcohol consumption in the studied Meitei population. Moreover, AD cases were more common in urban setting whereas ODs and MDs were more frequent in rural setting. AD cases and controls did not differ significantly with respect to age indicating no important role of age on alcohol dependence. However, with respect to age at onset of alcohol consumption AD cases differ significantly from controls. More than 50% of the AD cases were found to start consuming alcohol below 20 years. Whereas, maximum number of control individuals (29.5%) were found to start consuming alcohol after 20 years suggesting that individuals who started drinking at early age are more likely susceptible to developed alcohol dependence in later age. Moreover, other environmental factors like lower level of education/illiteracy, smoking and unemployment are found to be significantly higher among AD cases as compared to controls possessing more than one fold increased risk for alcohol dependence. This indicates that individuals who start consuming alcohol at an early age, those who smoke, less educated and belong to lower grade occupational (unemployment) group are more likely to develop alcohol dependence in later years.
Regarding genetic variables, all 484 individuals could not be analyzed for the selected molecular markers due to unsuccessful results and hence number of samples varies for each genetic marker. Genotypes and allele frequencies of the eight selected SNPs were calculated and compared among AD cases and controls.

Concerning molecular risk factors with respect to alcohol metabolism, of the total 484 samples, only 445 samples (AD cases=143 and controls=302) could be genotyped for ADH1B Arg47His polymorphism, 447 individuals (AD cases=137 and controls=310) could be genotyped for ADH1C Ile349Val polymorphism and 455 individuals (AD cases=140 and controls=315) could be genotyped for ALDH2 Glu487Lys polymorphism. All the selected genetic markers were found to be polymorphic in both AD cases and controls. Moreover, both AD cases and controls were found to be in Hardy Weinberg Equilibrium with respect to the selected alcohol metabolizing genetic markers except for ALDH2 Glu487Lys polymorphism.

In the present study, both common and variant homozygous genotypes of ADH1C Ile349Val polymorphism were found to be higher among controls as compared to the AD cases. However, heterozygous ADH1C*1/*2 genotype was found to be higher among AD cases as compared to controls, though difference was not found to be statistically significant. This could possibly be due to the more or less similar representation of common and variant allele among AD cases and controls.

The heterozygote genotypes ADH1B*1/*2 and ALDH2*1/*2 was found to be higher among controls as compared to AD cases. However, difference was found to be statistically significant with respect to ALDH2 polymorphism only. Moreover, the mutant homozygous genotypes of both ADH1B Arg47His and ALDH2 Glu487Lys polymorphisms were found to be absent in both the groups i.e. AD cases and controls. Odd ratio revealed that individuals carrying ADH1C*1, ADH1B*2 and ALDH2*2 allele of ADH1C Ile349Val, ADH1B Arg47His and ALDH2 Glu487Lys polymorphism respectively showed decreased risk for alcohol dependence, though the risk was found to be statistically significant with respect to ALDH2 Glu487Lys polymorphism only. Hence, it could be inferred that variant allele of this polymorphisms is likely to confer protection from alcohol dependence.

With regard to neurocognitive functions related with alcohol, of the total 484 recruited, 451 individuals (AD cases=143 and controls=318) could be genotyped for
DRD1 -48A/G polymorphism and only 415 samples (AD cases=129 and controls=286) could be genotyped for DRD2 (-141C Ins/Del, TaqI B and TaqI D) and ANKK1 TaqI A polymorphisms. Above genetic markers were found to be polymorphic in both AD cases and controls and were also found to be in Hardy Weinberg Equilibrium with respect to the selected SNPs related with neurocognitive function except for DRD1 -48A/G polymorphism.

Of all the considered SNPs related with neurocognitive function, ANKK1 TaqI A polymorphism was found to be significantly associated with alcohol dependence. The minor allele frequency was found to be higher among the AD cases as compared to controls in both heterozygous and homozygous conditions with respect to TaqI A and -141C Ins/Del polymorphism. However, the difference was significant only in TaqI A site of ANKK1 gene whereas a borderline significance of DRD2 -141C Del allele was observed with alcohol dependence. However, DRD2 (TaqI B and TaqI D) and DRD1 -48A/G polymorphisms seem to have no such association with alcohol dependence. Odds ratio revealed that individuals carrying TaqI A1 allele showed more than 1 fold significant increased risk for alcohol dependence in both dominant and recessive genetic models. However, odds ratio of both DRD2 -141C Ins/Del and DRD1 -48A/G polymorphism showed more than one fold increased risk for alcohol dependence among individuals carrying mutant allele, though the risk was not found to be statistically significant. Risk of TaqI A1 allele for alcohol dependence and increase of -141C Del allele among AD cases was further observed in haplotypic association analysis. Haplotypes (A1-D2-Ins and A1-D2-Del) containing A1 allele was found to be significantly associated with alcohol dependence. Moreover, haplotype (A1-D2-Del) containing both A1 and Del allele contributed 3.24 fold significant increased risk for alcohol dependence signifying potential role of A1 allele in interaction with Del allele in the development of alcohol dependence.

In multivariate regression analysis, only ANKK1 TaqI A polymorphism of the selected eight SNPs was found to be independently associated with alcohol dependence after controlling all the potential confounding environmental variables like smoking, educational level, occupational status and age at onset of alcohol consumption.

An attempt was made to understand how gene-gene interaction and genetic factors combine with (or are shaped by) environmental factors to influence the
development/causation of alcohol dependence. Gene-gene interaction analysis revealed that individuals who have certain combinations of genotypes are likely to become alcohol dependent conferring gene-gene interaction in the pathogenesis of alcohol dependence. It is evident from interaction of ALDH2 Glu487Lys polymorphism with most of the other SNPs included in the present study posed more than one fold significant increased risk for alcohol dependence (except with ADH1C Ile349Val and DRD2 TaqI D polymorphism). ANKK1 TaqI A polymorphism was found to be significantly interacting with other two sites of DRD2 gene (DRD2 -141C Ins/Del and TaqI D) posing more than one fold significant increased risk for alcohol dependence. Moreover, DRD2 -141C Ins/Del polymorphism is found to significantly interact with one of the alcohol metabolising candidate genes (ADH1B Arg47His) considered in the present study.

As evident from gene-environment interaction analysis, certain genotypes of selected genetic markers when exposed to particular environment (early age at onset of alcohol consumption, lower education level, unemployment and smoking status) are found to be more prone to alcohol dependence. All the considered environmental variables were found to interact with genetic factors posing more than one fold increased risk for alcohol dependence. Moreover, significant increased risk was found among all the selected eight SNPs with early age at onset of alcohol drinking and smoking. However, interaction of DRD1 -48A/G polymorphism with occupational status; DRD2 (TaqI D and -141C Ins/Del) and ADH1C Ile349Val polymorphisms with occupational status and educational level was not found to be statistically significant. This is indicative of the fact that individuals who have certain combinations of genetic variations are likely to become alcohol dependent and this likelihood is further increased in the presence of adverse environmental factors.

In order to understand the effects of alcohol consumption on health, comparative analysis of various anthropometric, physiological and biochemical parameters was performed in the present study. In general, the mean values for anthropometric (BMI & WHR) and physiological (SBP & DBP) variables are found to be slightly higher than the respective normal values whereas the mean values for waist circumference (WC) fell in their respective normal ranges. Comparative analysis of anthropometric and physiological revealed that mean values of BMI, WC and SBP were found to be
significantly higher among controls as compared to AD cases. But no significant difference was observed between AD cases and controls with respect to WHR and DBP. Furthermore, while analyzing sub-categories of controls (NDs, ODs and MDs) and AD cases, mean values of anthropometric and physiological parameters showed an increasing trend from NDs to ODs to MDs with an abrupt drop among AD cases. Individuals with high BMI, high WC, high WHR and hypertension were found to be significantly higher among combined controls than the AD cases. Further, while comparing among sub-categories of control (NDs, ODs and MDs) and AD cases, high BMI, high WC and hypertension were found to be more frequent among ODs and MDs as compared to NDs. However, difference was found to be statistically significant with respect to hypertension only. Conversely, AD cases showed lower frequencies of individuals with high BMI, high WC, high WHR and hypertension as compared to NDs, with significant difference in high BMI and hypertensive cases. Individuals with low BMI (underweight) were found to be significantly higher among the AD cases as compared to controls. Moreover, AD cases showed more than 6 fold increased risk for low BMI (underweight). Therefore, alcohol dependence is associated with the risk of low BMI (underweight) among the Meiteis of Manipur thereby resulting in overall health deterioration of the individuals. Of different hypertension types, pre-hypertension cases were found to be more frequent in NDs and AD cases. However, stage I and stage II were found to be lower among AD cases whereas more frequent among ODs and MDs as compared to NDs. Odds ratio revealed more than one fold increased risk of overall hypertension and stage II hypertension for ODs and MDs, though the risk was found to be statistically significant with respect to overall hypertension only.

Regarding mean values of biochemical variables, all the parameters considered for lipid profile were fell in their respective normal ranges. However, mean values of TC, TG, LDL, VLDL, TC/HDL and fasting glucose level were found to be significantly higher among controls as compared to AD cases whereas, HDL was found to be relatively higher among AD cases than controls. While comparing among different sub-groups of controls and AD cases, an increasing trend from NDs to ODs to MDs with an abrupt drop among AD cases was observed for mean values of TC and LDL. But, TG and VLDL show decreasing trend from NDs to AD cases with slight increase
SUMMARY AND CONCLUSION

only among the ODs. Individuals with high TC, high TG, high LDL, high VLDL and high fasting glucose level were found to be higher among combined controls as compared to AD cases. However, difference was found to be statistically significant for LDL, VLDL and borderline significance for TG. While analyzing among sub-categories of control (NDs, ODs and MDs) and AD cases, ODs was found to have an overall unfavourable lipid profile. Odds ratio revealed more than one fold increased risk of high TC, high TG, high LDL and high VLDL for ODs, though the risk was not found to be statistically significant. Individuals with low HDL, high TC/HDL and high LDL/HDL were found to be higher among AD cases as compared to controls. However, difference was found to be statistically significant with respect to low HDL only. This indicates that low HDL, is likely an effect/result of heavy alcohol consumption or of alcohol dependence in those individuals who otherwise have a favourable lipid profile with respect to TC, TG, LDL and VLDL. This hints towards the vital role of low HDL as an atherogenic marker among alcohol dependence in the present studied population that might predispose them to future cardiovascular risk. Moreover, in the present study alcohol dependence show a trend towards decreased glucose level (hypoglycaemia) which may also further increase their risk for future cardiovascular events. The observed variation in different patterns of alcohol consumption with respect to biochemical variables (like lipid profile and fasting glucose level) could also be attributed to the socio-cultural conduct related to alcohol consumption in the society. Individuals who consume alcohol occasionally and moderately are usually accepted by the society, family and the peer group unlike the alcohol addicts who are usually neglected.

Liver enzyme analysis reveals that abnormal mean values of both liver enzymes (AST and ALT) were significantly higher among AD cases as compared to controls. While analyzing among different sub-categories of controls (NDs, ODs and MDs) and AD cases, an increasing trend in mean values was observed from ODs to MDs to AD cases with non-drinkers slightly higher than ODs. This could possibly be attributed to the smaller sample size in the considered ODs category (N=6). Further, individuals with high AST and high ALT levels were found to be significantly higher among AD cases than controls. Odd ratio revealed more than 2 fold significant increased risk of AST and ALT for AD cases. Therefore, elevation of liver enzyme (AST and ALT)
levels could be one of the adverse effects of alcohol consumption as compared to the lipid profile parameters among *Meitei* population. Assessment of future 10 year cardiovascular risk with respect to TC, HDL, SBP and smoking status further supported the findings of present study. Occasional drinkers showed the maximum risk (>15%) for future 10 year CVD risk score followed by AD cases. Cardiovascular risk variables like DBP, WC, LDL and LDL/HDL ratio were also found to increase significantly from individuals with low risk score to individuals with high risk score, suggesting their significant contribution to increasing risk of future cardiovascular events. This is indicative of the negative health impact of individuals consuming alcohol, even occasional drinkers, which is usually accepted at society, peer and family levels.

In conclusion, alcohol consumption is found to be very frequent in the presently studied *Meitei* population. Smoking, low education and unemployment (or lower grades) are likely to increase the preponderance for alcohol dependence. Moreover, individuals consuming alcohol at early age are more susceptible to develop alcohol dependence in later age. Gene which is involved in alcohol metabolism, particularly ALDH2 *Glu487Lys* polymorphism, was found to have significant protective role against the causation/development of alcohol dependence in the studied population. However, individuals possessing both ANKK1 *TaqI A1* and DRD2 -141C Del alleles might be at higher risk for alcohol dependence; the risk being more pronounced in individuals homozygous for *TaqI A1* allele. Moreover, ANKK1 *TaqI A* polymorphism was also found to be independently associated with alcohol dependence. Individuals who have combinations of genetic variations/mutations in the selected 8 SNPs such as ADH1B *Arg47His*, ADH1C *Ile349Val*, ALDH2 *Glu487Lys*, ANKK1 *TaqI A*, DRD1 -48A/G and DRD2 (*TaqI B*, *TaqI D* and -141C Ins/Del) when exposed to adverse environment like early age at onset of alcohol consumption and smoking are likely to become alcohol dependent conferring gene-environment interaction in the pathogenesis of alcohol dependence. In the present study, low BMI (underweight) and two liver enzymes (AST and ALT) were found to be significantly associated with alcohol dependence, and hence could be important outcomes of alcohol dependence among this population. The respective mean values for biochemical variables fell under normal range among all the different patterns of
alcohol drinking. As the data indicates, it seems that alcohol dependence results in favourable lipid profile thereby giving an impression that heavy drinking might be good for health. But, if we look at the other side of the coin, AD cases show a quite high risk of future cardiovascular events and also they have an overall deteriorated health (as evident from their low BMI and high frequency of low HDL). Usually heavy drinking is not accepted at society, peer group and family levels and such individuals are therefore neglected by everyone and are left in the world of solitude and loneliness unlike occasional and moderate drinkers who are accepted by the society. This social acceptance among ODs and MDs might be a cause of development of future adverse cardiovascular events in later age. The population thus needs to be counseled regarding the ill effects of alcohol consumption not only in early ages of life specifically in school days and also when people opt for occasional and moderate drinking. Better employment opportunities with improved economic status are expected to bring down the alcohol consumption in the population. Therefore, the results of the present study need to be replicated and validated in large population/community specific studies in order to develop right interventional strategies and therapeutics.