Chapter 9

General Discussion
Mental retardation is the most common cause of developmental disabilities in many countries; chromosomal abnormalities being the major cause for such disorder. Over the past several years, genome wide subtelomeric screening is routinely carried out for diagnosis of IMR [Harada et al., 2004]. In the present investigation, ~31% MR cases had chromosomal abnormalities of which DS was the most common (85% of all chromosomal abnormalities). FISH with telomere specific probe had revealed MR cases with Xq terminal deletion. Among IMR cases, only ~3% had chromosomal abnormalities, which were mainly deletion, duplication and aneuploidy in nature. Data collected from various studies on MR have revealed a wide variation in reported percentage of chromosomal abnormalities [Rio et al., 2002; De Vries et al., 2003; Ravnan et al., 2006; Ahn et al., 2007]. Amongst Indians, ~28% of MR cases were reported to have chromosomal abnormalities, of which ~95% of were DS [ICMR, 1991]. A hospital-based study in Delhi [Verma et al., 2003] reported nearly 15% of cases with delayed developmental milestone and chromosomal abnormalities. In the present investigation, 6.25% of non DS MR cases revealed presence of chromosomal abnormalities.

In case of metabolic factors in association with MR, several studies have shown that Hcy is a potent neurotoxic component both under in-vivo and in-vitro conditions [Lipton et al., 1997; Bleich et al., 2004]. Increased brain Hcy concentration is found to be associated with change in neuronal plasticity, neurodegenerative disorders, altered cognitive behavior and mental retardation [Watanabe et al., 1995; Mudd et al., 2001; Mattson and Shea, 2003; Streck et al., 2003; Streck et al., 2004; Algaidi et al., 2005]. Elevated Hcy concentration along with decreased folate level following decreased SAM/SAH ratio leads to DNA hypomethylation [Kruman et al. 2000] which is considered as a predisposing factor for chromosomal non-disjunction [James et al., 1999].

The main metabolic pathways that regulate Hcy concentration are trans-sulfuration and trans-methylation [Mudd et al., 2001]; the enzymes CBS and MTHFR play crucial role in the transsulfuration and transmethylation pathway respectively to
maintain normal plHcy concentration. Decreased activity of CBS and MTHFR may increase plHcy concentration that could possibly lead to various neurological malformations [Mudd et al., 2001].

More than hundred CBS mutations have been reported to be associated with more or less severe phenotype. A pathogenic T833C in the CBS exon 8 was reported to be located at the surface of CBS enzyme that interacts with the solvent and was considered to be pyridoxine-responsive [Meier et al., 2001]. The T883C transition and certain other mutations probably affect the conformation and folding of the enzyme thereby affecting the stability of CBS. However, in vitro experiments in yeast construct had shown that the effect was corrected by certain mutations in the C-terminal domain or by deletion of this domain [Shan et al., 2001].

In the present investigation, the T833C variant always co-occurred with the 68bp insertion variant; the insertion variation restores the effect of this SNP in the protein sequence [Tsai et al., 1996; Romano et al., 2002]. Co-segregation of the CBS T833C/844ins68bp polymorphism is definitely suggestive of a strong LD between the two variations in the studied group. Distribution of this polymorphism was extremely heterogeneous in nature; highest in sub-Saharan population ~66% [Dutta et al., 2005; Romano et al., 2008]. Moreover, a micro-heterogeneity was also observed in distribution frequency of this allele [Giusti et al., 1999; Dutta et al., 2005]. It has been hypothesized that this common neutral polymorphism may be a source for pathogenic 833C mutation by mechanisms that probably involved meiotic gene conversion [Vyletal et al., 2007]. Phylogenetic analysis had shown that this insertion variation was conserved among gorillas, chimpanzees and bonobos who were homozygous for the 68bp insertion variation [Romano et al., 2008]. However, gorillas and bonobos carried the wild type allele “T” at 833 position where as chimpanzees contained the human haplotype. These genetic evidences suggest that the 68bp insertion at 844 position might have happened nearly 6-8 million years ago and the 833T>C substitution occurred within the allele carrying insertion during evolution [Romano et al., 2008].
No significant correlation was observed between plHcy concentration and the T833C/844ins68bp polymorphism. A recent report has shown that 844ins68bp variation in combination with apparently deleterious genotype of MTHFR 677TT reduces ~24% plHcy concentration as compared to individuals having 677TT genotype without the insertion variation [Summers et al., 2008]. However, this genotypic combination was not observed in the present study and the T833C/844ins68bp variant under heterozygous condition reduced the plHcy concentration in IMR cases, as compared to the homozygous, which was not significant.

Other genetic polymorphisms studied in the CBS gene i.e. G919A, C1105T, G1316A and G1330A were not polymorphic in the eastern Indian population studied.

Another polymorphism in the CBS, known as 31bp VNTR was reported to be located at exon-intron 13 junction. With increase in VNTR repeats [higher than 18 repeats allele] a reduction in CBS enzymatic activity was observed in fibroblast cell lines that may indirectly support formation of splice variants [Lievers et al., 2001]. A positive association between increase in plHcy concentration (post-methionine load) and higher VNTR repeats was also observed. This study indicated the presence of altered CBS that lead to suboptimal enzymatic activity in fibroblast cell lines [Leivers et al., 2001]; however, a controversy was raised against this comment [Afman et al., 2003]. In another study, higher VNTR alleles along with T833C/844ins68 polymorphism were shown to be risk factor for Alzheimer's disease after the age of 75yrs, and the 21st repeat VNTR allele was speculated as a risk factor for Alzheimer's disease after 64yrs [Beyers et al., 2004; Beyers et al., 2005]. In a previous study in the same eastern Indian population (n=219), no significant difference in allelic as well as genotypic frequencies were observed between MR cases and controls, nor any positive association between higher repeats VNTR allele and fasting plHcy concentration was noticed [Dutta et al., 2007b]. However, in the present study, significant difference in A5 (21 repeat) allele frequency was observed between male IMR cases (n=116) and
controls which may indicate a risk of IMR in association with higher repeat of the VNTR.

Investigations on \textit{MTHFR} polymorphisms and IQ score in different populations revealed contradictory observations [Visscher \textit{et al.}, 2003; Gueant \textit{et al.}, 2005; Krajinovic \textit{et al.}, 2005]. Lack of association between intellectual impairment and \textit{MTHFR} polymorphisms as well as normal cognitive aging and \textit{MTHFR} polymorphisms have been observed in Canadian [Krajinovic \textit{et al.}, 2005] and British populations [Visscher \textit{et al.}, 2003]. The 677T allele was found to be positively associated with low IQ in French DS patients [Gueant \textit{et al.}, 2005]. However, till date, no such association of this polymorphism with MR was observed, except in Hispanic male children [Shaw \textit{et al.}, 2007]. Investigations in association with MR and \textit{MTHFR} polymorphisms were much explored in DS patients. \textit{MTHFR} C677T and A1298C polymorphisms were reported to be associated with abnormal folate and methionine metabolism and were speculated to lead to DNA hypomethylation and abnormal chromosomal segregation [James \textit{et al.}, 1999]. In vitro studies on human cells lines showed that limitation of serum folate was associated with uracil miss-incorporation into DNA and DNA damage [Duthie and Hawdon, 1998; Fenech, 2005]. Chromosomal aneuploidy was also detected in tumors and other diseases associated with folate deficiency [Wang \textit{et al.}, 2004; Beetstra \textit{et al.}, 2005]. These polymorphisms were also hypothesized as maternal risk factor for having a DS child in various populations [Coppede \textit{et al.}, 2006; Martinez-Frias \textit{et al.}, 2006; Rai \textit{et al.}, 2006; Scala \textit{et al.}, 2006; Biselli \textit{et al.}, 2008]. Positive correlation was observed for neural tube defect along with these polymorphisms [van der Put \textit{et al.}, 1998]. In the present investigation, \textit{MTHFR} C677T and A1298C polymorphisms were explored in association with different levels of cognitive impairment and from the current study on eastern Indo-Caucasoid population, it may be inferred that these polymorphisms are not contributing to the etiology of IMR. For both polymorphisms, wild type alleles were present at higher frequency in IMR cases and their parents as compared to the control population. Moreover, earlier investigations also failed to show any association of
these polymorphisms with risk of having DS child or with MR in this particular population group [Dutta et al., 2007a; Dutta et al., 2008].

The present investigation had failed to reveal any significant effect of MTHFR polymorphisms on pLHcy concentration of IMR subjects. The “CT” genotype in C677T polymorphisms revealed increased pLHcy concentration over the “CC” genotype; however, the difference was statistically not significant.

A significant difference in pLHcy concentration was observed between male IMR cases and sex matched controls; however, no such difference was observed in sex-matched analysis of female. Further, no correlation could be drawn between pLHcy concentration and the CBS and MTHFR polymorphisms. Therefore, the observed significant difference in pLHcy concentration in male IMR cases, which was not high enough to be considered as hyperhomocysteinemia, could be due to some other factors that influence the Hcy concentration. Recently it has been shown that even reduced urinary excretion increased pLHcy concentration in psychiatric patients [Ipcioglu et al., 2008].