Discussion
We found that smoking was not associated with NAFLD or elevated liver enzymes in subjects with and without NAFLD. This finding was in agreement to Norberto et al, 2006. Suzuki et al, 2005 demonstrated the association of smoking with increased levels of ALT; however we did not observed any such association in the present study. Currently, smoking is known to be associated with several metabolic disturbances that are considered risk factors for NAFLD. In large study cohorts, smoking increases the prevalence of diabetes mellitus, and subjects who smoke have greater IR (Foy et al, 2005), which is considered to be the hallmark of NAFLD (Mendez et al, 2004).

NAFLD is a common problem in the USA, Asia – Pacific region, Europe, and the Middle East. Most of our patients were aged 18-50 years, which is considered as a young age group. This may reflect the changing diet of young Asian Indians to caloric rich food, which has increased the number of people with obesity in India. Angulo et al, 2002 reported that the prevalence of NAFLD increases by a fraction of 4.6 in patients with obesity, with up to 74% of such patients having NAFLD. In previous studies, 30% to 80% of patients diagnosed with NAFLD were found to have obesity (Ludwig et al, 1980; Powell et al, 1990; Falck et al, 2001). Our results are in agreement with these studies, with a mean BMI of 28.1± 3.2. On the other hand, fatty liver is associated with mild to moderate enlargement of the liver. Hepatomegaly is commonly the sole physical finding in most patients with NAFLD, which was also seen in our patients. Similar results have been found in other related studies (Ludwig et al, 1980; Powell et al, 1990; Falck et al, 2001).

Data regarding the prevalence of non-alcoholic fatty liver disease (NAFLD) in North Asian Indians are limited. It is important to note that all the associations of NAFLD; such as with obesity, abdominal obesity, diabetes, hypertriglyceridemia, and insulin resistance are highly prevalent in urban Asian Indians, and may be of significance in pathogenesis of NAFLD. Recently we have observed that obese with NAFLD have significantly higher insulin resistance than those without NAFLD (Misra et al, 2009). Bajaj et al, 2009 had proposed that the prevalence of NAFLD in India would approximate prevalence of the MS since most of the metabolic co-variates of NAFLD are highly prevalent in Asian Indians. As evidenced by other investigators in India, profile of various chronic diseases
remains largely similar (Misra et al., 2002, vikram et al., 2006, Mohan et al., 2007, Gupta et al., 2003).

Buffalo hump, classically seen in Cushing’s syndrome and HIV-associated lipodystrophy after treatment with protease inhibitors, is a rather prominent physical sign. In contrast with moderate to severe deposition of dorsocervical fat seen in above conditions, our previous experience showed that some “healthy” subjects have slight deposition of fat in dorsocervical area which may not be detected unless specifically looked for. Many of these subjects have IR and the MS, which prompted us to define this “mild” buffalo hump (Misra et al., 2006) and initiate the current study. Whether this mild buffalo hump signifies abnormal corticosteroid metabolism in Asian Indians is not clear. In the present study we observed that buffalo hump, skin tags, arcus and double chin were significantly associated with NAFLD (P<0.05). This is particularly important since Asian Indians with the MS have raised serum cortisol levels (Ward et al., 2003). Further, only one study has shown that the ectopic fat deposition below chin is significantly associated with diabetes (Haque et al., 2003). However, no such study characterizing significance of double chin has been carried out in apparently healthy subjects.

We also evaluated the clinical usefulness of hs-CRP in the diagnosis of NAFLD disease. There are limited number of studies implying that serum CRP is elevated in NAFLD (Haukeland et al., 2006, Yoneda et al., 2007, Riquelme et al., 2009). CRP has short life around 18 hours and the elevation of serum CRP usually reflects its synthesis in response to a pathological process (Kao et al., 2006). CRP is therefore considered as a useful nonspecific biochemical marker of chronic inflammation (Kao et al., 2006). Recent data also show that hs-CRP is a biomarker for NAFLD in some ethnic groups [(Japanese), (Uchihara et al., 2006)] while no association has been shown by others [(Europeans), (Haukeland et al., 2006)]. Our study confirmed that the increase in circulating CRP levels could be by itself a marker of the presence of NAFLD. The CRP response alone however no diagnostic specificity. Due to its low specificity, CRP values can really only be interpreted when all other clinical and laboratory information is available. We therefore suggest that serial measurements of CRP can be helpful in clinical management and follow up of NAFLD patients. It is also well known that CRP concentration is elevated in severely obese patients but this elevation is moderate and not related to metabolic...
syndrome, diabetes, and more importantly to steatohepatitis (Anty et al, 2006). Since both the case and control group were obese in our study, elevated CRP probably was related to adiposity. Some recent reports have suggested that CRP may be an independent risk factor for progression of NAFLD (Park et al, 2004 & 2006; Hanley et al, 2005) and reported the clinical usefulness of the measurement of hs-CRP for diagnosis of NASH (Yoneda et al, 2007). Patients with NAFLD are well known to have metabolic syndrome with increased CRP levels (Patel et al, 2006, Ridker et al, 2003). Elevated levels of inflammation markers, particularly hs-CRP, may play a key role in the development of atherosclerosis as well. As stated previously, Asian Indians have higher hs-CRP levels and high hepatic fat content as compared to white Caucasians (Misra et al, 2009; Petersen et al, 2006). In combination, these factors may lead to increased risk of atherosclerosis in Asian Indians. Conversely, it could be speculated, but remains to be investigated whether reduction in hepatic triglycerides and hs-CRP levels may help in preventing both the progression of NAFLD to NASH and development of CHD. Our study indicated that subjects with elevated CRP levels were at 1.96 times higher risk for development of NAFLD.

Asian Indians manifest insulin resistance and the metabolic syndrome at a younger age and of a higher magnitude than many other ethnic groups (Misra et al, 2007 & 2004)). Possible determinants of insulin resistance in Asian Indians are: excess overall adiposity, in particular abdominal adiposity, excess truncal subcutaneous adipose tissue (SCAT) and low skeletal muscle mass (Misra et al, 2004, 2007). Marchesini et al, 1999 have reported that IR as assessed by HOMA is higher in NAFLD subjects as compared to controls largely due to increased insulin concentration with normal or near normal glucose levels. An Italian study of 46 adults with NAFLD showed IR to be the strongest predictor of NAFLD, with fasting insulin levels nearly twice as high in patients with NAFLD as in controls. (Marchesini et al, 1999, Kawasaki et al, 1997). Seung et al, 2004 reported that IR plays the most important role, independent of obesity, in the development of NAFLD. They also considered that IR accompanied by systemic inflammatory response is of key importance for inducing NAFLD, particularly in apparently healthy non-obese men. However, our data indicated that subjects with elevated insulin levels were at 12.9 times higher risk for development of NAFLD.
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NAFLD is also associated with elevated serum triglycerides (Park et al, 2005, Bellantani et al, 2000), low HDL-C (Park et al, 2005), and central adiposity (Park et al, 2005), all features of the metabolic syndrome. In our study, all subjects had evidence of elevated serum TG, low HDL, central adiposity and IR, which may explain the high rates of NAFLD even in those subjects without the NAFLD. Our study suggests that components of metabolic syndrome, especially obesity as measured by BMI and waist circumference, are associated with superimposed NAFLD and NASH. Additionally, age, hypertension, and dyslipidaemia, are also associated with the presence of superimposed NAFLD. Furthermore, serum fasting blood glucose levels seem to be higher in patients with NAFLD (P<0.05). Multivariate analysis showed that BMI independently increased the NAFLD risk (p=0.03). This factor has been mentioned in a study (Mofrad et al, 2003). Our results indicated that individuals with higher BMI (28.1±3.2) were at 1.85 times higher risk to develop NAFLD. Several studies have shown that ALT is related to features of the MS and NAFLD (Hanley, et al 2005, Ioannou et al, 2005, Ruhl et al, 2003, Alper et al, 2008, Fraser et al, 2009). Accordingly, ALT concentration was significantly correlated with all components of the MS (FBG, TG, TC, HDL-C, LDL-C, VLDL, SBP, and DBP) in our study. Among the MS components, elevated TG concentrations in this cohort were strongly associated with severity of NAFLD and presence of NASH. Elevated TG and low HDL levels are characteristically found in subjects with IR, T2DM, and the MS (Defronzo et al, 2004, Kashyap et al, 2007) and these conditions have been closely related to progression of NAFLD to NASH (Marchesini et al, 2005). Our study represent that lipid parameters were significantly associated with NAFLD. The impressive association between circulating TG concentrations and NASH underscores the importance of fatty acid delivery to the liver in the pathogenesis of NAFLD, in addition to the development of IR (Kashyap et al, 2003). Free fatty acids are the primary drivers of VLDL secretion in the liver (Lewis et al, 1995). Thus, the same process that results in increased VLDL-TG secretion by the liver and extensive-lipolysis of adipose tissue is likely responsible in large measures for NAFLD development. Our results indicated that high systolic and diastolic blood pressures, high serum level of TG, TC, LDL, VLDL and low HDL were significantly associated with NAFLD(P<0.05),
which also agrees with the results in most studies. Previous reports suggested that
the role of TG is more important than TC in the pathogenesis of MS and NAFLD
(Bedogni et al., 2005; Adams et al., 2005; Marchesini et al., 2003; Klatskin et al., 1961).

The effects of extreme obesity on BMD have not been well examined. Although previous
epidemiologic studies have shown a positive correlation between bone mass and body
weight (Reid et al., 2002), the association between extremely high body fat and BMD
remains controversial, partially because of the secular trend in obesity. Obese individuals
generally have increased BMD compared with lean subjects (Wafaa et al., 2009).

Several mechanisms have been proposed regarding the association between BMD and
estimates of lean mass and fat mass. Increased fat mass or lean mass implies an increased
mechanical load on bone. In the present study, lean mass, total BMD, total mass and total
body fat are significantly increased in NAFLD subjects (p<0.04). Fox et al., 2000
concluded that BMD is associated more with lean mass than with fat mass, while
increased body fat and lean tissue mass are the main determinants of bone mass. Several
studies reported that increase in lean mass indicates a higher level of habitual physical
activity and is associated with higher bone strength as a consequence of greater forces
acting on the bone (Inomoto et al 2008 and Hingorjo et al 2008). These conflicting
results in the literature regarding the association of body composition with BMD may be
due to differences between studies in skeletal regions measured, bone mass parameters or
body composition parameters. Alternatively, body composition may affect BMD
differently according to the race (Nakaoka et al 2000).

Body composition and age have been associated with BMD. Lean mass and fat mass play
a positive role in relation to bone mass. The association between lean mass and bone
mass may be due to mechanical load forces on bone. Moreover, fat mass is metabolically
active, and increases BMD through hormonal metabolism of adipocytes, thereby
intervening in osteoblast function. Jonathan et al., 2005 reported that increased BMI and
BMD in White women, while a slight but significant decrease in BMD occurs in African
American women (Jonathan et al, 2005). In the present study we observed that NAFLD
males and females had higher BMI and total BMD.
Several physiological factors that are associated with fat distribution are also associated with BMD. These include age, gender, heredity, parity, menopausal status, physical activity, smoking and alcohol consumption, and hormones such as sex steroids, glucocorticoids, growth hormones, insulin, leptin, and adiponectin (Reid et al, 2008; Lenchik et al, 2003). Studies on the relationship between body fat distribution and BMD have yielded conflicting results. Most of the positive associations we found between BMD and fat distribution are indeed explained by increased BMI. In addition, reduced BMD in HIV-infected patients was associated with post glucose load hyperglycemia and central adiposity, independent of age and BMI. The association of increased abdominal visceral fat and reduced spine BMD had been previously reported in a cross-sectional study of 41 HIV-infected patients using quantitative computerized tomography to measure both BMD and abdominal adiposity (Huang et al, 2001). In the present study using a stepwise logistic regression model most significant factor associated with the development of NAFLD were BMD, Trunk tissue and left arm tissue.

Genetic Analysis of single-nucleotide polymorphisms (SNPs)

Genetic factors are important in the development of NAFLD, and recent advances in SNP genotyping methods have enabled the detection of genetic variations associated with increased susceptibility to NAFLD. There have been a few reports of genetic variations that are associated with NAFLD. The selection of a case and control group is one of the key issues in obtaining correct results in comparative genetic research. At the same time, selection of sex and age matched groups of healthy people may cause mistakes due to their inadequately reflecting the population’s genetic structure. In the present study, we propose most relevant genes for association with NAFLD.

**Sterol regulatory element-binding protein-2 (G1784C) gene polymorphism**

The important finding of this study in the genotype and allele frequency of this SNP of cases and controls differed significantly. The frequency of C allele in these subjects (0.21) was slightly lower than those found in normolipidemic subjects from Switzerland.
Studies of association between SREBP-2 (1784G>C) gene polymorphisms and lipids are fewer. Previous studies had been shown significant effect on serum lipid levels (Peggy et al, 2003 & Muller et al, 2002). 1784G/C SNP of SREBP-2 gene was reported to be significantly associated with the elevated levels of serum TC and LDL in Chinese population (Duan et al, 2004). A study from China reported that SREBP-2 gene was associated with high LDL-C. SREBP1 and SREBP2 are transcription factors for the LDL receptor and also very important in the regulation of synthesis of fatty acid which is a key player in metabolic syndrome and insulin-resistance. We did not observe any association of SREBP-2 (1784G>C) gene polymorphism with most of the lipid parameters in the present study.

Interestingly TG levels were significantly increased in G/G genotype in NAFLD subjects in the present study. Horton et al, 1998 reported that overexpression of SREBP tends to favor the synthesis of cholesterol over triglyceride in liver of transgenic mice.

CRP is a strong marker for atherosclerotic disease and cardiovascular events (Pepys et al, 2003 and Danesh et al, 2004), and its levels are elevated in the metabolic syndrome and with visceral adiposity (Rexrode et al, 2003). Furthermore, new data suggest that CRP may not be just a marker of inflammation and cardiovascular risk but also a contributor to vascular damage and cardiovascular events (Clapp et al, 2005). The relation between SREBP-2 and CRP levels has not been assessed till date. The findings of our study of elevated CRP in NAFLD subjects with G/C genotype suggests a possible association of SREBP-2 polymorphism and CRP in NAFLD which need to be explored further.

Transgenic mice overexpressing SREBPs produced massive fatty livers owing to increased accumulation of cholesteryl esters and triglycerides (Shimano et al, 1996). Fatty liver of obese mice with insulin resistance is caused by elevated SREBP levels, thereby increasing lipogenic gene expression, enhances fatty acid synthesis, and accelerates triglyceride accumulation in the liver (Shimomura et al, 1999). In addition, increasing evidence has demonstrated chronic inflammation could promote NAFLD. Shimano et al, 1997 indicated that an increase in the amount of SREBP-2 in the liver, and this was associated with an overproduction of cholesterol. In the present study we showed
that fasting blood glucose and liver enzyme (ALT) were significantly higher in CC genotype of SREBP-2(1784G>C) gene, which may be IR and over production of SREBP in the liver, but not confirmed.

Duan *et al.*, 2005 reported that 1784G>C genotype of SREBP-2 gene may be good genetic marker for hypercholesterolemia. The 1784G>C polymorphism was also reported to have a significant impact on total cholesterol level in the hypercholesterolemic subjects from Switzerland and Israel population (Miserez *et al.*, 2002). However, this effect was restricted to hypcholesterolemic subjects, suggesting a gene--gene or gene environment interaction with little effect in these populations. We did not observe any significant correlation of this polymorphism with hypercholesterolemia.

Significant association of SREBP-2 -(1784G>C) gene polymorphism has been reported with avascular necrosis in the Korean population (Kim *et al.*, 2008). Robinet *et al.*, 2003 reported that SREBP-2 (1784G>C) is a good candidate gene for a role in genetic predisposition to atherosclerosis. Our observation showed that C allele of SREBP-2 (1784G>C) gene is significantly associated with NAFLD. The present study need to confirm in a large sample size.

**Peroxisome proliferator-activated receptor γ (Pro12Ala) gene polymorphism**

The frequency of the common Pro12Ala polymorphism of *PPAR γ* in south Indians living in Chennai was found to be 19 percent while that in South Asians in Dallas was 18 percent and in Caucasians in Dallas it was 20 percent (Radhat *et al.*, 2007). The Caucasian diabetic subjects had significantly lower Ala allele frequency when compared with the Caucasian non diabetic subjects (20 vs. 9%, *P* =0.006). Our study indicated that the frequency of Pro12Ala in NAFLD and controls subjects was 23.4 % vs. 16.7% respectively. Therefore the frequency was high in North Indians as compared to that in South Indians population. The low frequency of the Ala allele 0.14 observed by us in NAFLD subjects is similar to that observed in other Asian populations, especially in Chinese (Liu *et al.*, 2008) and Northern French population (0.867 and 0.133), Caucasians [(12.0%) (Altshuler *et al.*, 2000)]. However, this frequency was significantly higher than that in other Asian populations from Taiwan (4.0%), Korea [(4.1%), (Oh *et al.* 2000)], Malaysia [(3.2%), (Tai *et al.* 2004)] and Japan [(3.0%) (Mori *et al.*, 1998)] in obese and
T2DM subjects. The present study analysed the \textit{PPAR}\textsubscript{\textgamma} Pro12Ala polymorphism using a recessive and dominant model. In recessive model Pro/Pro+Pro/Ala genotypes were more prevalent than Ala/Ala genotype (p=0.2) in both cases and controls. In dominant model Pro/Pro genotype was more prevalent in cases and controls (OR 1.52, p=0.12). There was no association between \textit{PPAR}\textsubscript{\textgamma} Pro12Ala polymorphism and NAFLD.

In contrast to our sample which is representative of general north Indian population, previous reports on \textit{PPAR}\textsubscript{\textgamma} gene polymorphism in Asian Indians have been reported in mixed populations from South India, consisting of patients with T2DM, and individuals with the metabolic syndrome and non-diabetic subjects (Radha \textit{et al}, 2006; Haseeb \textit{et al}, 2009). A study from South India showed no difference in fasting and 2-hr insulin levels between Asian Indians with T2DM with Pro12Ala and wild genotype, as compared to lower insulin levels in white Caucasians with Pro12Ala genotype (Radha \textit{et al}, 2006).

As compared to obesity or IR, the MS consists of a cluster of clinical and biochemical variables, which may be not only influenced by multiple lifestyle factors but also myriad genetic influences. We did not observe any association of \textit{PPAR}\textsubscript{\textgamma} (Pro12Ala) gene polymorphism with the MS in the present study which is similar to observations of a study on Chinese population (Yang \textit{et al}, 2009). Haseeb \textit{et al}, 2009 reported no association of Pro12Ala with the MS, T2DM and obesity, suggesting lack of protectiveness of this polymorphism for T2DM in Asian Indians in South India. In Pima Indians, the Pro12Ala was not associated with T2DM, but was modestly associated with BMI (Yunhua \textit{et al}, 2003). Also, in our case study Pro12Ala genotype has not associated with high BMI in NAFLD and obese subjects. Li \textit{et al}, 2008 reported that \textit{PPAR}\textsubscript{\textgamma} Pro12Ala affected abdominal fat storage of diabetic Ugyurs and Kazaks subjects. Our study also indicated that Pro/Ala genotype had associated with abdominal obesity in NAFLD but not in obese subjects.

Ereqat \textit{et al}, 2009 observed that Ala/Ala genotype has associated with lower fasting plasma glucose but had no influence on blood pressure, BMI, or other metabolic parameters in diabetic subjects. Our observation showed that TG levels were higher in Ala/Ala genotype in NAFLD subjects, but post parandial blood levels were higher in obese subjects, which has not been showed by other investigators. Previous study has linked the Pro/Ala with better insulin sensitivity in Caucasians (Abate \textit{et al}, 2005).
However, these studies have often been confounded by the presence of decreased BMI in the Pro/Ala, and no body composition studies with direct measures of body fat content and distribution were available. In the present study we did not find any insulin sensitivity but BMI was increased in obese subjects with Pro/Pro genotype. We have previously shown that Asian Indians have relatively higher truncal and abdominal fat mass as compared to white Caucasians and black populations (Misra et al, 2004). Importantly, in this study we showed that truncal abdominal fat mass could also be genetically influenced by Pro/Ala genotype. An association of the $PPAR_\gamma$ Pro12Ala polymorphism with ALK in NAFLD subjects is not investigated. However, as the ALK levels were significantly higher in NAFLD subjects with Ala/Ala genotype, this showed that Ala/Ala genotype may be a risk factor of NAFLD. Previous reports indicated that BMD values did not show significant differences between the genotypes and the effect of lean mass on BMD was no longer observed when BMD was expressed as a ratio to height, reflecting the function of body size. Ogawa et al, 1999 reported that an association of $PPAR$ (Pro/Ala) gene and BMD with postmenopausal osteoporosis in Japanese women. The data in this study thus suggest that the obese subjects with Ala12Ala genotype in the $PPAR_\gamma$ gene had significantly increased trunk BMD & spine BMD.

**Peroxisome proliferator-activated receptor $\gamma$ (C161T) gene polymorphism**

In this present study, we observed low frequency of C allele 0.12, similar to that observed in Japanese population [(0.14), (Ogawa et al, 2005). In dominant model C/C genotype was more prevalent in cases and controls OR was 1.81 genotype (p=0.05). The C/T genotype demonstrated a risk factor for NAFLD in Dominant model (p= 0.05). Dongxia et al, 2008 reported that the presence of a T allele of the C161T polymorphism seems to affect body weight and BMI. The CT/TT genotype contributed to higher BMI and higher serum triglyceride and HbA1c levels in Japanese T2DM patients (Maeda et al, 2004). In our study, no association with body weight and BMI were observed.
A study from China reported that the T allele had lower fasting insulin and HOMA-IR levels (Dongxia et al., 2008). In our study we found no significant association between fasting insulin and HOMA-IR levels did not associate with T allele.

One study on Brazilian patients with T2DM reported that TC and TG were decreased in subjects with C161T genotype (Tavares et al., 2005), but in our observation TC was significantly increased in obese subjects with T/T genotype; TG and hemoglobin was significantly increased in cases with T/T genotype.

Orio et al., 2003 observed that there was no association between C161T in PPARγ gene and metabolic parameters in patients with PCOS. Our findings were similar with this study in NAFLD and controls subjects. Previous study indicated that PPARγ C161T genotype was not associated with obesity in CAD subjects (Wang et al., 1999). Our observations were similar with this study in case and control subjects. A previous study reported that CRP did not change with the C161T genotype of PPARγ gene in Carotid Atherosclerosis subjects (Chao et al., 2007) and our findings are similar in this study.

Two previous studies have examined this polymorphism in the context of bone. In the first study of 394 postmenopausal Japanese women, an association of TT genotype of C161T genotype of PPAR γ gene and increased total body BMD was observed (Ogawa et al., 1999). In the second study of 138 premenopausal and 125 postmenopausal Korean women showed no association with this SNP (C161T) and any marker of bone formation, bone resorption, or BMD at the spine or hip, with the exception of serum osteoprotegerin [(OPG), (Rhee et al., 2005)]. In our study we found that the values of leg BMD were significantly higher in cases with T/T genotype and total BMD was significantly increased in controls with C/T genotypes (p=0.006).

Shi et al., 2008 indicated that hepatic steatosis associated with elevated liver enzymes HBsAg-positive chronic hepatitis patients. Our observation of high mean value of ALK and GGT levels was significantly increased in cases and controls with T/T genotype (p<0.05). It suggested that these liver enzymes may be significantly associated with T/T genotype in obesity and NAFLD, because of abdominal obesity.
We examined the influence of the gene variants and haplotypes of the \textit{PPAR\gamma} gene in a case-control study, and found that, in the analysis of individual Pro/Ala and C161T SNPs of \textit{PPAR\gamma} gene significantly associated with the disease, Ala allele of Pro12Ala genotype and T allele of C161T genotype carriers being more likely to have NAFLD in comparison with controls. Xiao \textit{et al}, 2009 reported that the frequencies of haplotypes Ala/T ($P < 0.0001$) and Ala/C ($P < 0.05$) were significantly higher, while the frequency of Pro/T ($P < 0.0001$) was significantly lower in the Tajik population than that in the Han population. Jing \textit{et al}, 2005 showed a significant difference in haplotype frequencies between cases and controls. We observed the Pro/C haplotype frequency in cases was significantly lower than controls ($p=0.004$). The Ala/C and Pro/T haplotypes frequencies were significantly higher in cases as compared to controls ($p= 0.04$). Moreover, we observed that \textit{PPAR\gamma} (Pro/Ala and C161T SNPs) gene variant haplotypes frequencies in NAFLD subjects significantly differed from those in control subjects, indicating that the Ala/T haplotype increased in the risk of NAFLD, indicating that subjects carrying this haplotype are more than twice as likely to have NAFLD in comparison with controls. Additionally, we evaluated the role of the gene variants in disease severity and observed a significant association between the clinical and biochemical spectrum of NAFLD. Cheuk \textit{et al}, 2008 reported that Pro12Ala and C161T polymorphisms of the PPAR-\gamma gene are important predictors of cardiovascular event in patients with diabetic nephropathy. Another study reported an association between psoriatic arthritis and a known coding SNP of the \textit{PPAR \gamma} gene in a Caucasian population (Christopher \textit{et al}, 2010). Hap Map data (2003), which show an $R^2$ value of 0.08 for Han Chinese and 0.31 for Caucasians. In contrast we observed $R^2$ value lower in NAFLD subjects (0.02). Our observation showed that strong LD observed for Pro12Ala and C161T SNPs in the present study, which also seen LD between these two SNPs was different in cases ($D1 = 0.1$; $p= 0.006$) and controls ($D1 = 0.07$; $p= 0.1$). Statistically significant difference ($p= 0.006$) in the prevalence of the specific combination of \textit{PPAR\gamma} variants in this study.

The Pro12Ala polymorphism may not be functional, but it may be in LD with the causal mutation. This LD could vary between populations, being higher in those in which the
association between the Pro12Ala polymorphism and diabetes is greater. Additional support for the existence of another causal mutation in the PPAR\textgamma \text{ gene comes from the work of Muller et al, 2003 in Pima Indians. Muller et al, 2003 have identified a functional SNP in the promoter region of } PPAR\textgamma \text{ in high LD with the Pro12Ala polymorphism. }

This SNP, positioned within a putative E2 box, significantly altered transcriptional activity from a luciferase reporter construct. However, the in vitro functionality of the Pro12Ala mutation has also been demonstrated by Masugi et al, 2002), who reported decreased transactivation capacity and reduced stimulation of PPAR\textgamma \text{ target genes for the Ala12 variant compared with the wild-type protein. Conversely, Kolehmainen et al, 2003 reported that the Pro12Ala polymorphism has only minor influence on the mRNA expression of PPAR\textgamma \text{ target genes in adipose tissue of obese subjects. In summary, the data might indicate that both a relevant functional variant [such as the newly identified PPAR\textgamma\text{2 promoter SNP (Muller et al, 2003) or a mutation in the PPAR\textgamma\text{1 protein] and Pro12Ala contribute to the PPAR\textgamma\text{2-related phenotypes. }

**Myostatin (A55T) gene polymorphism**

Overall, the allele frequency of T allele was 0.036 in Caucasians subjects of general population, 0.12 in African American (Ferrell et al, 1999) and 0.02 in Belgium population (Martine et al, 2004). Neither of the common polymorphisms had a significant impact on muscle mass response to strength training in either Caucasians or African Americans. These allelic variants provide markers for examining association between the Myostatin gene and inter individual variation in muscle mass and differences in loss of muscle mass with aging (Ferrel et al, 1999). We observed the frequency of T allele of Myostatin (A55T SNP) gene was 0.21 for cases and 0.10 for controls.

The increase in skeletal muscle mass is attributed to both hyperplasty (increase in muscle fiber number) and hypertrophy (increase in muscle fiber size). Muscle homozygous null mice also have reduced storage of adipose tissue (McPherron et al, 2002). Furthermore, heterozygous myostatin mice have a 25% increase in body weight, which is lesser than that seen in the homozygous mice, suggesting a dose dependant effect of myostatin protein levels on skeletal muscle mass. Naturally occurring myostatin mutations in cattle and human have subsequently been shown to lead to pronounced hyper muscularity.
Marchitelli et al, 2003 & McPherron et al, 2002). Body weight is a robust predictor of bone mass and density in humans (Reid et al, 2002), but there is considerable debate about whether muscle mass or fat mass are more important determinants of peak bone mass and density. Importantly, we report important associations of body weight with A/T genotype of Mayostatin (A55T SNP) gene in NAFLD subjects.

One study from Italy, suggests that myostatin might represent an important regulator of skeletal muscle size also in conditions of food restriction in obese subjects (Milan et al, 2004), but our study does not supporte this criteria. Gonzalez et al, 1998 showed that HIV patients were leads to heavy muscle wasting, increased level of myostatin was observed in circulation.

As skeletal muscle is the principle area of glucose disposal, increasing muscle bulk would increase insulin sensitivity, perhaps due to improved muscle physiology and vascularity. Analyses of body composition and bone densitometry in patients with DMD show that the low BMD observed with DMD is highly correlated with a significant decrease in lean body mass (Palmieri et al, 1996). Myostatin is highly conserved across species and when functionally inactivated, results in profound increases in skeletal muscle mass in cows, dogs, sheep, mice and humans (Lee et al, 2007). Our observation showed that total lean body mass was significantly associated with A/T genotype of Myostatin (A55T SNP) gene in NAFLD subjects. Rush et al, 2009 reported that fat distribution, muscularity, bone mass and leg length may contribute to ethnic-specific relationships between body fatness and BMI.

Adipose tissue is also the principal source of estrogen in postmenopausal women (Grodin et al, 1973), so it is not surprising that fat mass is significantly correlated with BMD and hip fracture in older women. High fat mass is also associated with hyperinsulinemia, and insulin increases indexes of bone formation when administered in vivo (Reid et al, 2002). Myostatin gene polymorphisms have effects on multiple traits. Mutations in myostatin regulatory regions have been shown to be associated with abdominal fat weight, abdominal fat percentage, birth weight, breast muscle percentage and breast muscle weight in chickens (Xianghai et al, 2006). Our observation showen that total tissue percentage and total body fat percentage was significantly associated with T/T genotype.
of Myostatin (A55T SNP) gene in NAFLD subjects, which has not been showed by other investigators.

The mice with deleted myostatin gene had much less body fat and 30 percent lower fasting blood sugar and 80% lower fasting insulin levels, showing a reduction in obesity and a strong resistance to developing diabetes. They also had 50 percent lower LDL-C and 30 to 60% lower levels of TC and triglycerides (fats in the blood), respectively. These results indicate protection against the development of atherosclerosis (Aaron et al., 2009). In the present study we found that TC was more prevalent in NAFLD subjects with T/T genotype of Myostatin (A55T SNP) gene.

**Gene interaction**

**PPARγ** (Pro12Ala and C161T) gene

Braissant et al., 1996 reported that PPARγ expressed in adipose tissue such as liver or small intestine. PPARγ polymorphism, Pro12Pro replacement of alanine for proline (Opkin et al., 2001) has allelic frequency of nearly 15% in some Caucasian populations (Lthsuler et al., 2000 & Tumvoll et al., 2002). Pro/Pro and C/C genotypes were significantly higher in controls (74%) as compared to cases (61 %, p= 0.008), whereas the measurement of the biochemical parameters revealed significantly higher GGT levels in the patients as compared to the controls. Bellentani et al., 2000 showed that PPARγ is important gene in fatty liver. PPARγ (Pro12Ala) Pro allele and PPARγ (C161T) C allele correlated with higher GGT levels, both polymorphisms showed a significant association with GGT, but failed to show an association with MS, IR and body fat distribution. The divergence from Pro to Pro (Pro12Ala) and C to T (C161T) alleles increased ALT levels, respectively. It is an important finding, which showed that the GGT levels play causative role in regulation of biochemical levels.

In the current study we observed no correlation between Ala/Ala, G/G; Ala/Ala, G/C; Ala/Ala, C/C genotypes of Pro12Ala and C161T SNPs of PPAR γ gene in cases and controls. Previous study showed a strong association between 161T and CAD subjects (Xin X et al., 1999). Dongxia et al., 2008 reported that those with an A allele of Pro12Ala and a T allele of the C161T polymorphism had a higher BMI (Dongxia et al., 2008). In our study we could not found any significant association of PPARγ gene with BMI in
case and control subjects. Wang et al, 1999 reported that the C161T polymorphism of the PPAR\(_\gamma\) gene associated with a decreased CHD risk. We could not confirm this finding in our study population. One explanation for these divergent findings could be that our study was exclusively performed in patients with obesity.

**PPAR \(\gamma\) (Pro12 Ala) and SREBP-2(1784G>C) genes**

The genotype distribution and allele frequency for the Pro/Pro genotype of PPAR\(\gamma\) (Pro12Ala) and G/G genotype of SREBP-2 (1784G>C) gene was significantly increased in controls (63%) as compared to cases (47%). The measurements of the liver enzymes (ALT, AST and GGT) were significantly higher in cases as compared to controls (p>0.05).

Lemoine et al, 2006 reported that the expression of SREBP and PPAR\(\gamma\) contribute to the pathogenesis of steatosis and fibroin necrotic changes in insulin-resistant lipodystrophic patients. This is an important finding showing that the ALT and GGT associated Pro/Pro, G/C and Pro/Pro, C/C conversions played a causative role in the regulation of two liver enzymes. Shimomura et al, 1999 showed that fatty liver of obese mice with insulin resistance is caused by elevated SREBP levels, thereby increasing lipogenic gene expression. Furthermore the combination of Pro/Ala of PPAR\(\gamma\) gene and G/G genotypes of SREBP-2 gene was significantly increased in cases than the controls. Klopotek et al, 2006 reported that PAR\(\gamma\) activation by troglitazone lowered the cholesterol synthesis in HepG2 and Caco-2 cells by reducing the concentration of nuclear SREBP-2 and successive downregulation of its target genes involved in cholesterol synthesis, but in our study we did not observe any association of the PPAR\(\gamma\) genotypes with cholesterol levels.

**PPAR\(\gamma\) (Pro12 Ala) and Myostatin (A55T) genes**

Pro/Pro genotype of PPAR\(\gamma\) (Pro12Ala) and A/A genotype of Myostatin (A55T) gene were significantly increased in controls (73%) as compared to cases (56.1%), since most of the wild type genotype was found in controls as compared to cases.

Martine et al, 2004 reported that an association study of myostatin polymorphisms with base line muscle strength or responsiveness to strength in humans, but presently no study is available in relation with PPAR\(\gamma\) (Pro12Ala) and Myostatin (A55T) gene.
polymorphism. Liver-targeted gene transfer of a myostatin inhibitor is a valuable tool for preclinical investigation of myostatin blockade and provides novel insights into the long-term effects and shortcomings of myostatin inhibition on striated muscle in mouse model (Morine et al, 2010). Karunaratne et al, 2007 reported that PPARγ and myostatin may play a role in regulation of muscle versus connective tissue in the intralitter variation model of prenatal nutrition in an animal model. We also found, subjects having the Pro/Pro (Pro12Ala) and A/A (A55T) genotype combination with highest ALT levels in cases than the controls. Pro/Pro (Pro12Ala) and A/A (A55T) genotype combination associated with significantly increased GGT levels in cases as compared to controls.

**PPAR γ (C161T) and SREBP-2 (1784G>C) genes**

In our study, the gene-gene interaction with two polymorphisms of C/C genotype of PPARγ (C161T) and G/G genotype of SREBP-2 (1784G>C) gene was significantly increased in controls (73%) as compared to cases (56.1%). Hui et al, 2008 reported that CT genotype of PPARγ, TG, waist hip ratio, hypoadiponectinaemia and HOMA-IR were the risk factors for NAFLD. We observed AST levels in correlation to C/T genotype of PPAR γ (C161T) and C/C genotype of SREBP-2 (1784G>C) were significantly increased in case group, also subjects having the C/C, G/G and T/T, G/G genotype combination had highest GGT levels in the case group. It is known that patients with normal liver enzymes may harbor advanced stages of NAFLD; on the other hand, a decline in ALT levels may reflect improvement in histology (Hickman et al, 2004). Ohlson et al, 1988 found elevated ALT in nondiabetic Swedish men to be a risk factor for T2DM, independent of obesity, body fat distribution, plasma glucose, lipid, AST, bilirubin concentrations, and family history of diabetes. Further, the small number of patients in each group may have resulted in lack of significance.

**PPARγ (C161T) and Myostatin (A55T) genes**

In the present study, no genotype combination of Myostatin (A55T) and PPARγ (C161T) genes was found in case and control subjects. ALT levels were significantly higher in C/T, A/A and C/T, T/T genotypes in case group as compared to control group. C/T and T/T genotypes were associated with significantly increased AST levels in cases than the
controls. McPherron and Lee et al., 2002 suggested that myostatin was involved in the regulation of adiposity as well as myogenesis. In this sense, it is quite interesting that a greater expression of myostatin and \textit{PPARy2} genes was observed at 16 Japanese Black Cattle. These results strongly support that myostatin may regulate fat metabolism through activation of \textit{PPARy2}. The response of \textit{PPARy2} might have been more distinct by using Japanese Black Cattle because of the sensitivity of adipogenesis. Lin et al., 2005 reported that increased hepatic fatty acid $\beta$-oxidation and mitochondrial biogenesis with Myostatin disruption may prevent the development of hepatic steatosis that was observed in the mouse model. Shibata et al., 2006 reported increased expression of the myostatin and the \textit{PPARy} genes. These parallel increases suggested a possible contribution to marbling formation during the fattening period in Japanese Black Cattle. The American breed similar to Japanese Black Cattle was reported to have greater ability to accumulate intramuscular adipose tissue than Angus cattle (Lunt et al., 1993).

\textit{Myostatin (A55T) and SREBP-2 (1784G>C) gene}

The increased frequency of A/A (A55T) and G/G (G1784C) genotype homozygotes in cases can be explained as a result of gene interactions between Myostatin and \textit{SREBP-2} (1784G>C) genes. We conclude that the interaction of the Myostatin (A55T) and \textit{SREBP-2} gene polymorphisms can be related to some conditions that contribute to NAFLD, despite the absence of a single gene associated with the developing disease. David et al., 2008 reported that expression of Myostatin and its associated binding proteins (SREBPs) can be modulated in adipose tissue and skeletal muscle by chronic obesity and suggest that alterations in their expression may contribute to the changes in growth and metabolism of lean and fat tissues occurring during obesity. A/T genotype of \textit{Myostatin} (A55T) gene and G/G genotype of \textit{SREBP-2} (1784G>C) gene was significantly increased in cases as compared to controls. A/A genotype of Myostatin (A55T) gene and G/G genotype of \textit{SREBP-2} (1784G>C) gene was significantly increased in controls (65.9%) as compared to cases (40.0%). A/T and G/G genotypes were also significantly higher in cases as compared to controls.
Summary & Conclusion
A total of 335 subjects were recruited from different areas of Delhi in North India. Among these, 162 (129 males & 33 females) subjects who were diagnosed to have fatty liver were categorized as cases while 173 (109 males and 64 females) subjects who had normal abdominal ultrasound were identified as controls. Males and females were 238 (71%) and 97 (29%) respectively.

In cases, 108 (67%) were grade one, 46 (28%) grade two and 8 (5%) grade three fatty liver (NAFLD) patients.

Hypertension was present in 11.2% cases and 8.7% of controls. Liver disease was present in 0.62 % in cases.

Family history of obesity was more prevalent in controls (13.8%) as compared to cases (11.7%). Family history of diabetes was higher in cases (30.8%) as compared to controls (21.9%). Hypertension was present in 20.3% cases and 23.6% of controls. Family history of heart disease was more prevalent in cases (12.1%) as compared to controls (5.7%).

Percentage of regular tobacco users in cases (16.05%) was higher as compared to controls (12.14%).

Total percentage of regular alcohol users was 1.50% (cases, 1.86% & controls, 1.16%).

DBP (p=0.003), weight (p=0.02) and BMI (p=0.006) were significantly higher in cases as compared to controls.

Significantly higher values of clinical parameters (pulse rate, SBP, DBP, weight and BMI ) and clinical examination (buffalo hump, skin tags, xanthelasma, double chin and arcus) were found in cases as compared to controls (p<0.05).

Most of anthropometric parameters were more prevalent in cases (p<0.05)

Cases had significantly higher level of serum lipids (TG, TC, LDL and VLDL), ALT, and GGT than control subjects (p<0.05).

Median range of insulin, HOMA-IR and CRP levels were significantly increased in case than control subjects (p<0.05).

Trunk BMD, pelvis BMD, spine BMD and total BMD were significantly higher in cases as compared to controls (p<0.003).
Total tissue, total fat, total lean mass and total body mass were significantly higher in cases as compared to controls (p<0.05).

Most of the clinical, biochemical, anthropometric and DEXA parameters were significantly higher in grade-3 NAFLD subjects (p<0.05).

Using a step-wise logistic regression model for assessment of the risk factors of NAFLD, the most significant factors associated with the development of NAFLD were insulin (OR 12.9), total BMD (OR 10.03), trunk tissue (OR 3.26) and TG (OR 1.13).

**SREBP-2 G1784C SNP**

The observed allelic frequency of G allele of SREBP-2 (G1784C) gene was 0.79 & that of C allele was 0.21. The SREBP-2 (G1784C) genotype frequency follow Hardy Weinberg equilibrium (chi value=5.41). Frequency of C/C genotype was higher in cases as compared to controls (11.1% vs. 5.87%). In cases 57.41% were G/G homozygous, 31.48% G/C heterozygous and 11.11% were homozygotes C/C. In control subjects 75.14% were G/G homozygous, 19.08% G/C heterozygous and 5.78% were homozygotes C/C. SREBP-2 1784G>C gene polymorphism was significantly associated with NAFLD (P= 0.003)

The mean values of CRP and total body mass was significantly higher in cases with G/C genotype as compared to controls (p<0.05). Weight, fasting blood glucose, ALT and hemoglobin were significantly increased in cases with C/C genotype than in controls (p<0.05).

**Myostatin A55T SNP**

Overall observed allelic frequency of Myostatin (A55T) gene of T allele was 0.84 & C allele was 0.15. The Myostatin (A55T) genotype frequency followe Hardy Weinberg equilibrium (chi value=5.87). In cases 56.4 % were A/A homozygous, 25.7% A/T heterozygous and 8.6% were homozygous for the variant allele is T/T.
control subjects 83% were A/A homozygous, 12.1% A/T heterozygous and 4.05% were homozygous for the variant allele is T/T. Frequency of T/T genotype was higher in cases as compared to controls.

- *Myostatin* (A55T) gene polymorphism, higher body weight and TG were most prevalent in cases with A/T genotype than in controls (p<0.05). TC, ALK, total tissue percentage, total lean mass and total body fat percentage were significantly increased in cases with T/T genotype as compared to controls (p<0.05).

**PPAR-γ Pro12 Ala SNP**

- Overall observed allelic frequency of Pro allele was 0.88 & Ala allele was 0.12. About 80% subjects were Pro/Pro homozygous, 15.5% were Pro/Ala heterozygous and 4.5% were Ala/Ala homozygous. *PPAR-γ* genotype frequency did not follow Hardy Weinberg equilibrium (chi value=9.7). The frequency of Ala/Ala genotype was higher in cases as compared to controls (6.0% vs. 3.5%).

- In addition to single genotype influence, we also investigated the genotypes by the models. In case control study of *PPAR-γ* Pro12 Ala SNP, we analyzed by two models. In recessive model Pro/Pro + Pro/Ala genotypes were more prevalent than Ala/Ala genotype OR 1.83 (95% CL 0.65-5.15, Chi2, 1.34, P=0.2) in both cases and controls. In dominant model Pro/Pro genotype was more prevalent in cases and controls OR was 1.52 (95% CL, 0.88-2.61).

- BMI was more prevalent in controls with Pro/Pro genotype as compared to cases (p=0.004). Mean value of post parandial blood glucose, MTC, trunk BMD and spine BMD was significantly higher in controls with Ala/Ala genotype (p<0.05). Mean values of TG, serum bilurubin, WHR, ALK was significantly higher in cases with Ala/Ala genotype as compared to controls (p<0.05). The values of WC, HC, arm BMD, left leg fat and trunk total mass were significantly increased in cases with Pro/Ala genotypes as compared to control genotypes (p>0.05).
Overall observed allelic frequency of C allele was 0.88 & T allele was 0.12 with genotype frequencies of cases and controls (85.3 % wild type, 11.0% heterozygous and 4.1 % variant) respectively. PPAR-γ C161T genotype frequencies did not follow Hardy Weinberg Equilibrium (chi value=10.5).

In addition to single genotype influence, we also investigated the genotypes by the models. In recessive model C/C + C/T genotypes were more prevalent than T/T genotype OR 1.07 (95% CI: 0.36-3.12, P=0.90). In dominant model C/C genotype was more prevalent in cases and controls OR was 1.81 genotype (p=0.05). The C/T genotype demonstrated a risk factor for NAFLD in Dominant model (95% CI; 0.98-3.3; p= 0.05).

The mean values of TG, hemoglobin, neutrophiles and leg BMD were significantly increased in cases with T/T genotype as compared to controls (p<0.05). ALK and GGT levels significantly increased in cases and controls with T/T genotype (p<0.05). In control subjects, TC was significantly increased in T/T genotype as compared to case genotypes (p=0.002). Total BMD was significantly increased in controls with C/T genotypes (p=0.006).

Haplotype and Linkage disequilibrium analysis of PPAR γ (Pro12Ala & C161T) gene

Overall haplotype frequencies of Pro/C, Ala/C, Pro/T and Ala/ T genotypes were 0.79, 0.113, 0.086 and 0.01 respectively. Ala/T haplotype was found to be at a lower frequency. The Pro/C haplotype frequencies in cases were significantly lower than those in controls (p=0.004). The Ala/C and Pro/T haplotype frequencies were significantly higher in cases as compared to controls (p= 0.04 & p= 0.03).

LD between these two SNPs was different in cases (D1 = 0.1; p= 0.006) and controls (D1 = 0.07; p= 0.1). Statistically significant difference (p= 0.006) in the prevalence of the specific combination of PPAR γ variants by ethnic group was noted.
Gene-gene interaction of *PPAR y* Pro12Ala and C161T polymorphisms indicated that, Pro/Pro and C/C genotypes were significantly higher in controls (74%) as compared to cases (61%, p= 0.008). AST levels were increased in cases with Pro/Pro and C/T genotype combination (p=0.04). Pro/Pro and C/C genotype combination had higher GGT levels in cases as compared to controls (p=0.004).

In the interaction of *PPAR y* (Pro12Ala) and *SREBP-2* (G1784C) genes shown, Pro/Pro genotype of *PPAR y* and G/G genotype of *SREBP-2* gene were significantly increased in controls (63%) as compared to cases (47%, p= 0.002). Subjects having the Pro/Pro, G/C and Pro/Pro, C/C genotypes combination had significantly increased ALT levels in cases (p>0.05). Pro/Pro, G/G; Pro/Pro, G/C and ProAla, G/G genotype combination associated with significantly increased GGT levels in cases (p<0.04).

Pro/Pro genotype of *PPAR y* (Pro12Ala) and A/A genotype of *Myostatin* (A55T) gene interaction were significantly higher in controls (73%) as compared to cases (56.1%, p= 0.009). Subjects having the Pro/Pro, A/A genotype combination had highest ALT levels in cases as compared to controls (P= 0.001). Pro/Pro and A/A genotype combination associated with significantly increased GGT levels in cases as compared to controls (p<0.003).

The combination of C/C genotype of *PPAR y* C161T and G/G genotype of *SREBP-2* gene was significantly increased in controls (73%) as compared to cases (56.1%, p= 0.01). AST levels of C/T genotype of *PPAR y* C161T and C/C genotype of *SREBP-2* were significantly increased in case group (p= 0.007). Subjects having the C/C, G/G and T/T, G/G genotype combination had highest GGT levels in cases as compared to controls (p=0.008).

The combination of *PPAR y* C161T and *Myostatin* (A55T) gene indicated that C/C genotype of *PPAR y* and A/A genotype of *Myostatin* gene was increased in controls.
(73.8%) as compared to cases (51.2%, p=0.08). ALT levels were significantly higher in C/T, A/A and C/T, T/T genotypes in case group (p= 0.004). The C/T and T/T genotypes was associated with significantly increased AST levels in cases as compared to controls (p=0.009). C/C, A/T; C/T, A/A and T/T, A/A genotype combination had highest GGT levels in cases as compared to controls (p= 0.005).

- The combination of A/A genotype of *Myostatin* (A55T) gene and G/G genotype of *SREBP-2* (G1784C) gene was significantly increased in controls (65.9%) as compared to cases (40.0%, p= 0.01). The A/T and G/G genotypes also were significantly higher in cases as compared to controls (p=0.02). The A/T, G/G; A/T, C/C and T/T, C/C genotypes were associated with higher AST levels had in cases as compared to controls (p<0.04). The A/A, G/G; T/T, C/C genotypes was significantly associated with increased GGT levels in cases (p<0.05).

**Our study suggested that**

- Most of clinical, biochemical and anthropometric parameters were associated with NAFLD.
- Total BMD and body fat mass was associated with NAFLD.
- High Insulin levels, total BMD, trunk tissue and triglyceride were found to be independent predictors of NAFLD.
- *SREBP-2* 1784G>C gene polymorphism was significantly associated with NAFLD.
- The haplotype analysis of *PPARγ* gene (Pro/Ala and C161T), Ala/C and Pro/T haplotype frequencies were significantly higher in NAFLD subjects.
- Linkage disequilibrium between these two SNPs was different in cases (D1=0.1, p= 0.006).
- The above findings need to be confirmed by increasing the sample size.


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