DISCUSSION

6 DISCUSSION

Gaucher disease is found to be the commonest LSD in India (Sheth et al., 2013; Verma et al., 2012). In the present study, 50 patients confirmed with GD were subjectsed for molecular study and further genotype-phenotype correlation. It was found that 43 (86%) patients were of Type-I GD, 3 (6%) of Type-II GD and 4 (8%) of Type-III GD that is in accordance with the earlier reported cases in International CollaborativeGaucher Group Gaucher Registry (Kaplan et al., 2006).The most common signs and symptoms noted by Kaplan et al. were splenomegaly (95%), hepatomegaly (87%), radiologic bone disease (81%), thrombocytopenia (50%), anemia (40%), growth retardation (34%), bone pain (27%), and bone crisis (9%). Similar observations was also seen in our GD patients were splenomegaly (94%), hepatomegaly (86%), pancytopenia (56%), bone abnormalities like osteomyelitis of femur, thinning of limbs, severe osteoporosis, erlenmeyer flask deformities, thinning of legs, avascular necrosis, bone infiltration found in 10(20%).

Plasma chitotriosidaseshows direct correlation with macrophage activation and demonstrates marked increased activity in42 (84%) of patients with GD. This is in accordance to our earlier study reported by Sheth et al. In 2 (4%) patients it was showing undetectable activity and in another 6 (12%) patients activity was found to be normal. This can be explained by the fact that 5-6% of the population who lack this enzyme as a result of genetic deficiency due to an expressional mutation in the human chitotriosidase gene.Approximately one-third of patients with GD are heterozygous for this null allele resulting in reduced or normal activity of Chitotriosidase in plasma (Boot et al., 1998, Aerts and Hollak, 1997).

β-Glucosidase activity in leucocytes or fibroblasts is the confirmative test for GD followed by mutation identification in *GBA* gene (Pastores and Hughes, 1993). In our study, all patients had shown significantly reduced activity 13-29% of the enzyme β -glucosidase except one patient with high residual activity, where skin fibroblasts have shown less than 10% of normal activity 8.1 nmol/hr/mg.protein (NR: 118-401.5nmol/hr/mg protein). Two patients were on ERT when referred to us with elevated chitotriosidase activitybut β –glucosidase was not known in these

patients.With the availability of the diagnostic enzyme test, there is no indication to histologically examine the bone, liver or spleen for diagnosis. Bone marrow examination may be needed if the splenomegaly does not regress on treatment or patient develops enlarged lymph nodes or symptoms to suggest development of a lymphoma (Nagral, 2014).

Enzyme activity in heterozygote carriers and normal individuals may show overlap and therefore enzyme analysis by itself cannot be used to differentiate carriers from normal individuals.Mutation analysis confirms the diagnosis and can prognosticate the natural course of the disease.

We have observed that L444P (c.1448T>C)mutant allelewas the most common among all patients under investigation, whereas the other common mutations N370S (c.1226 A>G), IVS2+1G>A (c.115 + 1 G>A)and 84GG were not detected in any of our patient. This is partly in disagreement with the previous study from India in 24 patients, where L444P(c.1448T>C), N370S (c.1226 A>G), IVS2+1G>A(c.115 + 1 G>A), D409H and 55Del mutant allele were observed in ~50% of the GD patients with L444P (c.1448T>C) as the most frequently identified, followed by D409H (1342G>C), (Bisariya et al., 2011). This is likely to be because of different ethnic group of northern origin withcomparatively less number of patients, whereas ours is mainly consisting of western origin with fairly large number of patients.In our study, 84GG was not found which is in accordance with earlier reports (Horowitz et al., 1993), Turkish (Emre et al., 2008), Romanian (Drugan et al., 2002) and Czech and Slovak patients (Hodanova et al., 1999).Among Japanese patients with GD, neither N370S (c.1226 A>G) nor c.84-85insG wereseen, but L444P(c.1448T>C) and F213I (c.754T>A) were found to be relativelycommon, lending further support to the founder-effect theory (Kawame et al. 1993; Eto and Ida 1999). None of our patients had shown N370S(c.1226 A>G) or 84GG. In study, 9 patients mutations are not found as exon sequencing of *GBA* gene was carried out but if we do the entire *GBA* gene sequencing then the mutations can be found in these patients. As it is previously reported (Hruska et al., 2008) that mutations in the intronic region are also present. Our study clearly shows that mutation spectrum in Indian GD patients seem to be more resembling to Japanese patients (Koprivica et al., 2000).

Our study also demonstrate that L444P (c.1448T>C) isthe most common genotype

that is in accordance with the Taiwanese population with homozygous L444P(c.1448T>C) mutation prevalent in 52.6% followed by Rec NciI(23.7%) (Wan et al., 2006), whereas German, Spanish and Portuguese patients were shown to have low prevalence of this mutation and higher prevalence of N370S (c.1226 A>G)mutation. L444P(c.1448T>C) mutation was identified in 60% of mutant allele in Thai patients (Tammachote et al., 2013). Study by Ida et al. (Ida et al., 1999) had shown L444P (c.1448T>C)mutation as the second most common in GD among non-Jewish patients, accounting for 37% of the total mutations surveyed. L444P (c.1448T>C)mutation was also found to be associated with all three groups of GDinRomanian (Drugan et al., 2002) population, which is also seen in our patients. Aforementioned mutation was also demonstrated in neuronopathic phenotype in Swedish, Pole, Ashkenazi Jewish and other Caucasian population, whereas in non-neuronopathic form in Taiwanese–Chinese (Wan et al., 2006) that is in agreement with present study. The heterogeneous expression of mutant allele on phenotype in different population could be because of effect of modifier gene on mutant allele as demonstrated by Alfonso et al. in Spanish population (Alfonso et al., 2007). It is also likely that many of our type 1 GD patients with L444P(c.1448T>C) genotype may develop neurological symptoms at the later stage as has been observed in Turkish population (Emre et al., 2008).

R463C (c.1504 C>T) mutation was observed with two (4%) in type 1 GD patients that is similar to that observed in Turkish population (Emre et al., 2008). This mutation was previously reported in 3.57% of non-Jewish population and not observed in Jewish population. Although the severity of this mutationis unknown but is reported to be associated with type 1 and type 3 GD (Horowitz et al., 1993). R463C (c.1504 C>T)/Rec Nci I mutation was also reported in one type 3 GD patient from India (Chauhan et al., 2013). R395C(and R359Q(c.1193 G>A) are considered to be the mild mutation associated with non-neuronopathic type 1 GD patients with lesser frequency, which has been previously observed in Brazilian (Rozenberg et al., 2006) and Spanish (Cormand et al., 1996) type 1 GD patient. G355D (c.1181 G>A) mutant allele was observed in one GD patient where patient was on ERT and other clinical phenotypes are not available, whereas the same was mutation observed in type 2 Taiwanese children. This could be owing to ethnic diversity and some effect of modifier gene on the mutant allele (Tsai et al., 2001). V352M (c.1171G>A) mutation

was observed in one type 1 GD patient that was previously reported from Russia (Bukina et al., 2007).Y220C (c.766 A>G) mutation was previously observed in type-I Brazilian patient that is in accordance with our patient (Rozenberg, 2006). R496C (c.1603C>T)(Kawame et al, 1992) was previously reported in one Japanese type-I GD patients,(Ida et al., 1997), which is similar as observed in our one type-I GD patient.

Presence of S356F (c.1184 C>T) mutant allele was seen with severe phenotype type 1 except neuronal involvement in Turkish (Emre et al., 2008) patient, and in our case also this mutation was found with severe phenotype in one patient (3.03%). This has been reported earlier in one patient from Northern India also (Verma et al., 2012).G202R(c.721G>A) mutation was considered to be severe and found in type-II patients which is similar to that reported previously (Beutler et al., 1994). The F213I (c.754T>A)which was found in 1 type-I GD patient was previously reported in types 1, 2, and 3 GD, thought to be a rare allele in Caucasian populations, was recently described as the second most common allele among Chinese (Choy et al., 2007; Park et al., 2003; He et al., 1992).

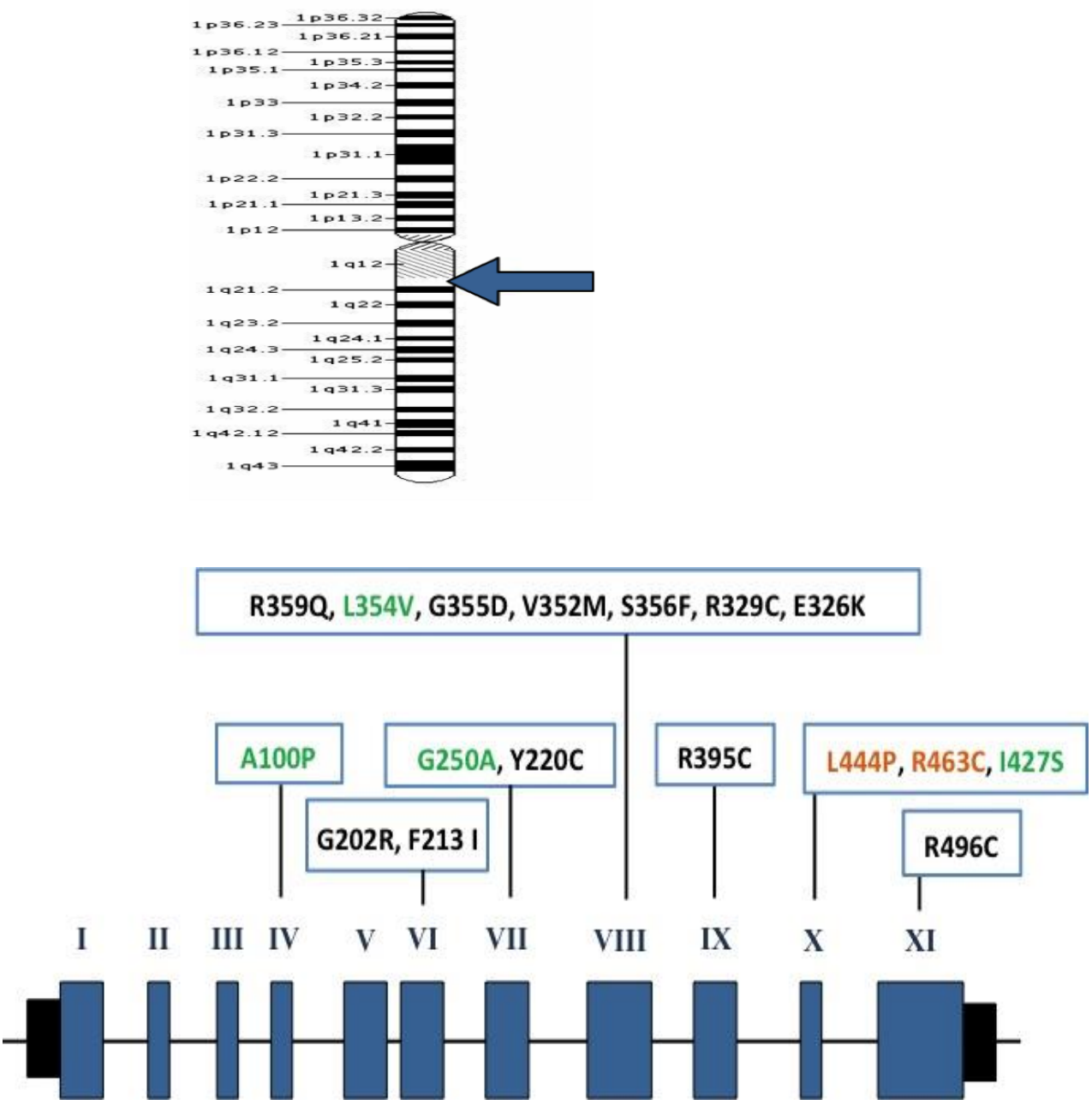
The most variable of all the symptoms attributed to GD is that of skeletal involvement such as bone abnormalities like osteomyelitis of femur, thinning of limbs, severe osteoporosis, erlenmeyer flask deformities, thinning of legs, avascular necrosis, bone infiltration found in 10(20%) of GD patients ranges from asymptomatic disease, with or without radiological signs, to symptomatic disease, which can be severe and engender considerable pain and disability. In our study, L444P(c.1448T>C), L444P(c.1448T>C/R329C(c.1102C>T), R463C (c.1504C>T), E326K (c.1093G>A)and R359Q (c.1193G>A) mutations in type 1 GD patients are found to be associated with bone involvement like osteoporosis, osteonecrosis or AVNin one each. In one patient, L444P(c.1448T>C) genotype was found to be associated with Erlenmeyer flask deformityof distal femora, pulmonary congestion and severe hepatosplenomegaly, and another patient with L444P (c.1448T>C) /R329C (c.1102C>T) genotype had shown phenotype of avascular necrosis and hepatosplenomegaly. R329C was previously reported in type-I Brazilian patients (Rozenberg, 2006).Although none of our patients with severe bone disease had shown N370S (c.1226 A>G) as has been observed earlier (Zimran, 1997).

The four novel mutations in the *GBA* coding region were analyzed using three web-

based tools, PolyPhen (http://genetics.bwh.harvard.edu/pph) (Ramensky et al., 2002), Mutation T@ster and SIFT (http://sift.bii.a-star.edu.sg) (Kumar et al., 2009) in order to assess their potential pathogenicity. Similar web based tools were previously used in Brazilian patients for novel mutations (Siebert et al., 2012).

Expression studies have shown that some residues in exons 5, 8, 9, and 10 of the gene are critical for catalytic activity (Grabowski, 1993; Grace et al., 1994). Consequently, it is not surprising that more than 50%of the disease causing mutations have been located in the region of exons 8 to 10 (Beutler and Gelbart, 1993; Horowitz and Zimran, 1994). The most wide spread missense mutations responsible for GD were N370S (c.1226 A>G), L444P(c.1448T>C) and R463C (c.1504 C>T) occur in residues at exons 9 and 10 which have been considered critical for the formation of the active site (Mistry and Cox, 1993) (Figure 6.1). Similar observation was seen in our patients with 59% of the disease causing mutations have been located in the region of exons 8 and 10.

GBA gene figure with mutations in Exons



Green: Novel mutations
Orange: Common mutations

Figure 6.1: Mutation spectrum of *GBA* gene in Indian GD patients

The evaluation of individuals homozygous for the mutations that are common in different ethnic groups provides a clearer picture of genotype-phenotype relationships in GD (Koprivica et al., 2000).Furthermore, our overall findings in Indian patients show that even when the large majority of DNA mutations are known, phenotypic differences among patients cannot be explained by genotype alone. Modifier genes, contiguous genes, transporter proteins, activator proteins, and environmental factors may contribute significantly to phenotype, as has been demonstrated in other genetic disorders (Koprivica et al., 2000; Emre et al., 2008) as shown in Figure6.2.

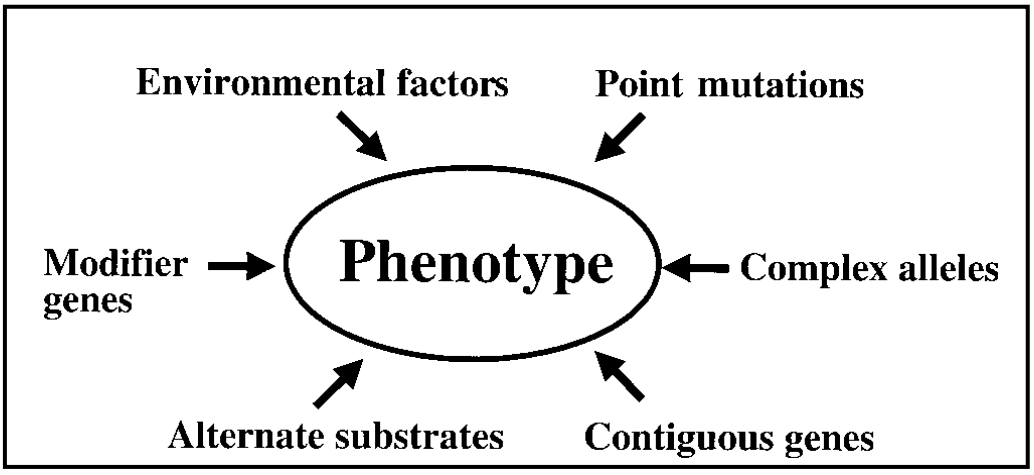


Figure 6.2: Factors which may contribute to phenotype in patients with GD(Koprivica et al., 2000)

Thus, present study provides an insight into the molecular bases of GD in Indian patients. The distribution of mutant alleles and the frequency with which particular genotypes were encountered displayed some specific particularities that may be related to the ethnic characteristics of our population. The identification of mutant alleles is crucial for advancing knowledge of the worldwide GBA mutation spectrum, and will contribute to a better understanding of the molecular basis of the disease. Such information will help in establishing genotype–phenotype correlations as well as in genetic counseling and/or in customized molecular analyses for families at risk(Ankleshwaria et al., 2014).

Currently, the gold standard for GD treatment is enzyme replacement therapy (ERT) (Sechi et al., 2016).Many studies have demonstrated the efficacy ofERT in reducing mortality, improving/normalizing hematological parameters and organ volumes, and improving or stabilizing bone pathology in GD (Bembi et al., 1994; Wenstrup et al., 2007; Zimran et al., 2015; Pastores et al., 2014). But due to its high molecular weight, the enzyme does not cross the blood–brain barrier; therefore, patients with neurological features only benefit from the effect of ERT on visceral, hematological, and skeletal features (Sechi et al., 2014). ERT is usually well- tolerated and except for possible anaphylactic reactions, side effects are usually mild and include nausea, abdominal pain, rash, fatigue, and headache (Weinreb et al., 2013).

SRT is an alternative treatment strategy; based on the administration of small molecules that are able to reduce the biosynthesis of the substances that accumulate in the disease- affected organs, restoring the balance between the rate of production and the rate of degradation (Platt and Jeyakumat, 2008).Eliglustat tartrate is a newly developed SRT for GD, which is created to specifically inhibit GCS, with the aim to reduce substrate accumulation with limited side effects.

The main limitation for both ERT and SRT is their effect only on the consequence of the disease and not on the disease itself. To completely counteract the disease, other approaches have been tried, such as bone marrow transplantation, or are under study, such as gene therapy or combined gene therapy and stem cell transplantation. Bone marrow transplantation has been performed in severe affected GD patients. However, it requires human leukocyte antigen-matched donors and its correlated morbidity and mortality are still high (Carriero et al., 2008). Gene therapy has the advantage of being a one-off procedure, cur- ing the origin of the disease. Unfortunately, several attempts in the past failed to find safe gene vectors. However, the use of self-inactivating lentiviral vectors carrying the *GBA1* gene under the control of human promoters has recently been successfully experimented with in mice and a study on humans is required to confirm the safety of this approach (Dahl et al., 2015).

The present study will help in monitoring the disease response on treatment. As abnormal macrophage activation is considered as one of the key GD pathways. In fact, chitotriosidase, a product of the macrophage activation that is usually elevated in GD plasma, and since its levels correlate with disease severity, it is commonly used to

monitor the disease response on treatment (Hollak et al., 1994). Molecular analysis carried out in present study will provide information of genotype that is associated with type of GD and it will help this information in ERT treatment. Thus, present study provides the information for deciding treatment of ERT and for monitoring response to treatment.