Introduction
1.1 HISTORY

Mucopolysaccharidoses (MPS) are a family of heritable disorders, caused by the excessive intralysosomal accumulation of glycosaminoglycans (acid mucopolysaccharides) in virtually every nucleated cell in the body. Several reviews detail the early history of MPS [Dorfman and Matalon, 1976; Sly, 1980; Galjaard, 1980].

In 1917, Hunter presented [Hunter, 1917] a clear description of two brothers who appeared to suffer from an X-linked MPS, which bears his name and also is referred to as MPS II. Two years later, Gertrude Hurler described [Hurler, 1919], two unrelated boys with coarse facial features, multiple skeletal abnormalities, corneal clouding, hepatosplenomegaly and cardiac involvement. This detailed description of what was presumed to be Hurler syndrome or MPS I served as a prototype for the description of MPS that followed. Earlier this group of disorders were collectively referred by the unfortunate title "gargoyleism" [Ellis et al., 1936] because of the supposed resemblance of the patients to the gargoyles of certain cathedrals. The term "mucopolysaccharidosis" was later introduced [Brante, 1952] based on the finding of excessive glycosaminoglycans (GAGs) accumulation in the liver of patients with Hurler syndrome. These patients also excreted excessive mucopolysaccharides in their urine [Dorfman and Lorincz, 1957] and this was misinterpreted to indicate that the syndrome resulted from exaggerated synthesis of GAGs. In course of time MPS IV [Morquio, 1929; Brailsford, 1929], MPS III [Harris, 1961], MPS VI [Maroteaux et al., 1963] and MPS VII [Sly et al., 1973] were reported.
Interest was stimulated in this field when the distinguishing clinical features and classification of MPS was provided [McKusick, 1972]. This was aided by the report that urinary GAG analysis can help in distinguishing the MPS patients [Terry and Linker, 1964].

The evidence for a biochemical defect in these disorders came from the histochemical examination of cultured cells from these patients which showed "metachromasia" [Danes and Bearn, 1965]. Soon, using in vitro radiolabelling technique, it was shown that MPS were not disease of overproduction, but defective degradation of GAGs [Fratantoni et al., 1968]. Further exciting and dramatic series of investigations [Neufeld, 1974; Neufeld et al., 1975] showed that MPS could be grouped into complementation classes based on their ability to "cross correct" in vitro. It was also shown that the corrective factors could be isolated from normal tissue culture medium or urine of normal subjects and purified. Ultimately it was shown to be a single degradative enzyme, the absence of which lead to a mucopolysaccharidosis.

The isolation and characterisation of each new enzyme defined the basis of one more mucopolysaccharide storage disorder and at the same time defined a step in the degradation of GAGs. These studies were in for some surprises. It was observed that two clinically quite different disorders, the Hurler and Scheie syndromes were non complementary suggesting that they might be due to allelic mutations and ultimately were shown to be due to the deficiency of the same enzyme α-L-iduronidase. Another kind of genetic heterogeneity was observed with Sanfilippo syndrome. The fibroblasts from these patients fell into two complementation groups by in vitro cross correction
test, suggesting that two different enzymatic defects produced an indistinguishable clinical phenotype.

To date 10 enzyme deficiencies leading to six major types of MPS have been identified (Table 1). They share many clinical features, though in variable degrees and exhibit a wide spectrum of clinical severity within any one enzyme deficiency [Brooks, 1993]. Except for MPS II, which is an X-linked recessive disorder, all other MPS types are transmitted in an autosomal recessive manner [Neufeld and Muenzer, 1995].

1.2 CLINICAL MANIFESTATIONS

Mucopolysaccharidoses are progressive disorders, although the onset of the early symptoms, rate of progression and the severity of the different disorders differ considerably [Sly, 1980; Spranger, 1983; Gieselmann, 1995]. Within a single enzyme deficiency, a spectrum of clinical phenotypes ranging from near normal to severe and debilitating can be recognised [McKusick and Neufeld, 1983; Brooks, 1993]. However, it is difficult to offer prognosis based on biochemical analysis of a patient or even to relate a particular phenotype to a specific measurement of enzyme activity [Brooks, 1993].

These overlapping clinical features seen in MPS patients are due to different combinations of GAGs stored in different tissues. For example, storage of GAGs in connective tissues, produces connective tissue laxity in most of these disorders, manifested by inguinal and umbilical hernias. Connective tissue thickening also occurs, owing in part to GAG storage and in
part to excessive collagen deposition. This combination leads to coarse facial features, peripheral hernia entrapments, thickened meninges that may lead to cord compression, hydrocephalus and thickened joint capsules. Connective tissue deposition in valve, produces symptomatic heart disease, a common cause of death in MPS patients, to which coronary valvular insufficiency also contributes.

Glycosaminoglycan storage in central nervous system produces progressive mental retardation, especially in disorders involving impaired degradation of heparan sulfate [MPS I, II, III & VII]. Corneal clouding and visual handicap result from storage of partially degraded GAGs in corneal stroma due to impaired degradation of dermatan sulfate and keratan sulfate [MPS I, VI, VII & IV]. Hepatomegaly is common in these patients and may be massive but is rarely important clinically.

Almost all the enzyme deficiencies produce skeletal anomalies collectively referred as "dysostosis multiplex". Varying severity of dysostosis multiplex is the general bony manifestations of MPS but special appearance may occur in particular types. Hurler syndrome is the prototype of MPS. Hunter and Sanfilippo syndromes have moderate to mild dysostosis multiplex. Morquio syndrome shows distinctive bony changes. The skeletal changes associated with severe Maroteaux-Lamy are similar to radiographic findings of Hurler syndrome. Patients with Sly syndrome have moderately severe dysostosis multiplex. Although clinical presentations and the hall marks of bony changes help in the classification of MPS, definitive diagnosis depends on preliminary and enzyme analysis [Chen et al., 1996].
1.3 GENETICS

The genes responsible for most of the MPS types have been identified, localised and extensive structural studies have been carried out [Gieselmann, 1995]. These studies have aided in the mutational analysis of MPS genes and have resulted in a high degree of application in prenatal diagnosis and carrier detection.

1.4 BIOCHEMISTRY

1.4.1 Distribution and function of GAGs

Glycosaminoglycans are important and widespread constituents of connective tissue and ground substance. They are found in relatively large amounts in skin, cartilage, bone, cornea, blood vessels, heart valves and tendons. Smaller quantities occur in liver, brain, leukocytes and mast cells.

Hyaluronate is present in all connective tissues and at very low amount in skin and aorta. The highest content of chondroitin sulfate is present in cartilage and intervertebral disc. Dermatan sulfate is present in fibrous connective tissues like tendon, skin, aorta, sclera and joint capsule and also in cornea. Heparan sulfate is present on the surface of many cells, and often isolated from lung or intestine. Skeletal keratan sulfate is found in tissues related to cartilage but not in fibrous connective tissues. Corneal keratan sulfate which has an entirely different linkage to the core protein compared to the skeletal keratan sulfate forms more than 50% of GAGs in the cornea of the eye. [Heinegard and Paulsson, 1984; Kennedy and White, 1988].
The functional role of proteoglycans and GAGs has been obtained from studies on cartilage [Comper and Laurent, 1978]. The major role of proteoglycans and GAGs is to provide elastic stiffness to cartilage via the presence of a large number of charged groups. As a result of the large volume they occupy, proteoglycans and GAGs provide a selective barrier function to prevent large molecules from entering tissues. The principle function of connective tissue, composed mainly of collagen and aided by GAGs to form a three dimensional matrix, is to support and bind together organs and bones of the body [O'Brink et al., 1975]. In cornea, proteoglycans organise and orient the collagen fibres into the correct array to allow light at visible wavelengths to enter the eye [Kennedy and White, 1988].

1.4.2 Structure of GAGs

Glycosaminoglycans are long chain, linear, polyanionic polysaccharides present in tissues in covalent linkage with protein cores, forming proteoglycans [Roden, 1970; Gottschel, 1972]. The GAGs may represent 50-95% of the molecular weight of proteoglycans and consist of about 50 disaccharide repeat units. A single proteoglycan can contain more than one type of GAG [Heinegard and Paulsson, 1984]. The GAGs and their subunits have been extensively studied [Brimacombe and Webber, 1964; Balaza, 1970]. The repeating disaccharide units of each GAG contain one hexuronic acid and an amino sugar or hexosamine moiety. Keratan sulfate is exceptional wherein galactose replaces the hexuronic acid.
1.4.3 Metabolism of GAGs

1.4.3.1 Biosynthesis of GAGs

Proteoglycan synthesis is initiated with the synthesis of the protein core. This is followed by glycosylation of the serine residue to form the trisaccharide linkage region. This precursor now acts as an acceptor for the two monosaccharide transferases involved in the biosynthesis of GAGs. The two transferases catalyse the alternate addition of the hexuronic acid and hexosamine moieties. The nonsulfated precursor formed is sulfated at positions 4 and 6 by sulfotransferases which use PAPS as a sulfate group donor. Initiation of sulfation has been shown to occur well before chain termination. Eventhough considerable advances have been made in understanding the biosynthetic process underlying the formation of proteoglycans, still a complete and clear cut picture is not available.

1.4.3.2 Catabolism of GAGs

Understanding the normal pathway of GAG catabolism has been closely tied to the elucidation of enzyme deficiencies in MPS. Much of the research has concentrated on the abnormal, diseased state rather than the normal healthy state. The data available on normal state indicates that the cartilage proteoglycans are gradually degraded by proteases with near neutral pH optima [Barret, 1981]. The resulting fragments which contain chondroitin sulfates attached to a peptide are too small to be retained in the collagen matrix, and hence defuse from the matrix. Similar process are thought to occur in other tissues although much less is known about the actual process
involved. The proteoglycan fragments are degraded further by proteases with more acid pH optima to give GAG peptides which can be absorbed into the cells [Truppe and Kresse, 1978]. Here they are further degraded, in the lysosomes, by a series of enzymes which initially remove sulfate groups, followed by N-acetylation prior to sequential depolymerisation by the alternating action of specific L-iduronidase or D-glucuronidase and α-N-acetylglucosaminidase or β-hexosaminidase.

1.5 DIAGNOSIS OF MPS

The common feature of MPS is the deficiency of a lysosomal enzyme that is part of a catabolic pathway. The deficient enzymes are exohydrolases acting in sequence in the degradation of GAGs which form important constituents of connective tissue and the ground substance. Thus the deficiency of a single enzyme causes the blockage of the entire pathway leading to the accumulation of undegraded and partially degraded GAGs in the lysosomes, causing cell, tissue and organ dysfunction. The excess GAGs are excreted in the urine [Sly, 1980; Neufeld and Muenzer, 1995; Gieselmann, 1995].

The earliest biochemical procedure that was available for the diagnosis of MPS was the identification of GAGs in the urine. Numerous methods ranging from semiquantitative spot tests to precise qualitative and quantitative measurements were devised [Pennock, 1976]. Though spot tests were quick and inexpensive, they were subjected to both false positive and false negative results [Dembure and Roesel, 1991]. Later more sophisticated methods like the urinary GAG isolation [Whiteman and Young, 1977],
quantitation [Whiteman 1973; Gold, 1979, 1981] and the qualitative analysis by cellulose acetate membrane electrophoresis (CAME) [Cappelletti et al., 1979; Hopwood and Harrison, 1982; Elango et al., 1998] and sequential thin layer chromatography [Dembure and Roesel, 1991] were developed. These methods which helped in the preliminary analysis of urinary GAGs can only discriminate between broad classes of MPS, but cannot distinguish their subtypes.

The definitive diagnosis of MPS and classification of patients to different types and subtypes is possible only by the assay of lysosomal enzymes. Cultured fibroblasts are used to diagnose all MPS types, leukocytes for most and serum and plasma for Hurler, Hunter, Sanfilippo B and Sly syndromes. Chromogenic, fluorogenic or radioactive substrates are used for the assay [Hall et al., 1978]. Except arylsulfatase B, which is deficient in Maroteaux-Lamy syndrome, all sulfatases relevant to Hunter, Sanfilippo A & D and Morquio A syndromes as well as the acetyl transferase of Sanfilippo C syndrome require radioactive substrates for their estimation. But recently a convenient fluorimetric assay is made available to estimate the enzyme deficient in Morquio A [van Diggelen et al., 1990]. Though fluorimetric and radioactive methods are available for the assay of arylsulfatase B, the simple method using chromogenic substrate is highly reliable and can differentiate both arylsulfatases A and B [Fluharty and Edmond, 1978; Shapira et al., 1989].
1.6 Prenatal Diagnosis

Prenatal diagnosis is possible for all MPS and is routine for Hurler and Hunter. All enzyme assays developed for cultured fibroblasts can be used on cells grown from amniotic fluid. Except for Morquio syndrome, measurement of radiolabelled $[^{35}\text{S}]-\text{GAG}$ accumulation can also be used for the prenatal diagnosis [Fratantoni et al., 1969]. Chorionic villus biopsies are rich in enzymes of GAG degradation and are easy to use for the rapid diagnosis of MPS. Care should be taken in chorionic villus biopsies that the villi are not of maternal origin. In the case of Hunter syndrome, the sex of the fetus should always be determined since the X-linked mode of inheritance results in mosaicism in heterozygous females. The female fetuses can occasionally show enzyme activity almost as low as an affected male, due to clonal origin of the cells [Kleijer et al., 1979; Cooper et al., 1991; Besley et al., 1992].

1.7 Carrier Detection

Carrier testing is the service most often requested by MPS families, second only to demands for effective therapy. Heterozygote identification for the autosomal recessive MPS has been based primarily on measurement of the relevant enzymes in leukocytes or serum. The results are most reliable when the enzyme activity is in the lower range and least reliable when the activity is in the normal range, due to overlaps between the normal and heterozygote values [Neufeld and Muenzer, 1995].
DNA analysis has opened up a new approach with immediate practical application in the area of carrier testing, since it can provide unambiguous information on the carrier status. There are heterogeneity of mutations in each MPS, and hence, it is necessary to identify the mutant allele in the family prior to undertaking carrier detection. This can be done by testing for specific mutations or polymorphic markers [Gal et al., 1992] in the family.

1.8 THERAPY AND MANAGEMENT

Earlier it was believed that MPS should be treatable with exogenous enzymes, which would reach lysosomes by the process of endocytosis [Baudhuin et al., 1964]. But attempts to administer enzymes to MPS patients were unsuccessful, because of insufficient quantities of available enzymes and lack of appreciation of receptor mediated mechanisms, for uptake and delivery of the enzymes to lysosomes. Later the availability problem was solved by recombinant enzymes, modified for uptake by the affected cells. [Barton et al., 1991; Kakkis et al., 1993; Bielicki et al., 1993]. But the improvements if any, seen were only transient. Later allogenic bone marrow transplantation [BMT] was attempted. This showed only partial success but provided an impetus for the development of somatic gene therapy, using retroviral vectors to introduce cDNA encoding the missing lysosomal enzyme into the patient's own bone marrow cells [Karlsson, 1991]. To date BMT is the only procedure in which clinical changes have occurred. In BMT, somatic disease, except for the skeleton, is generally improved but intelligence and neurologic outcomes vary considerably.
Since no specific treatment is available, management of MPS patients consists of supportive care and treatment of complications. The progressive nature of organ involvement dictates the necessity for constant evaluation of the clinical status. Systematic evaluation of hearing, vision, and joint function, coupled with treatment can lead to improved quality of life, by minimizing the handicapping effects of the diffuse systemic disease.

1.9 ANIMAL MODELS

Several animal models of human MPS have become available. Clinical manifestations in the affected animals are similar but not identical to those in human patients. Availability of animal models is of particular value for testing new therapies such as bone marrow transplantation, enzyme replacement and gene transfer.

1.10 OBJECTIVES OF THE PRESENT STUDY

Mucopolysaccharidoses are a group of rare genetic disorders and epidemiological data are scarce [Neufeld and Muenzer, 1995]. The incidence of all MPS types put together has been estimated to be 1 in 16,000 to 30,000 live births, but relatively fewer studies have been published regarding the true incidence [Muenzer, 1986]. A more recent estimate [Danos and Heard, 1995] reported the incidence of MPS to be 1 in 15,000. In general, MPS appears to be a pan-ethnic disorder with the exception of an increased incidence of Hunter syndrome in the Jewish population in Israel and of Morquio syndrome in French - Canadians [Muenzer, 1986]. In India no systematic study of MPS has
been carried out. Most of the available data are on clinical and radiological features. Hence the present project was undertaken with the following objectives.

1. To contact different hospitals in Madras City which cater to patients from all over South India and record the incidence and distribution of MPS in our population.

2. To isolate and quantitate urinary GAGs from normal and clinically diagnosed MPS children with a view to identify the patients.

3. To qualitatively analyse the urinary GAGs and relate it to group the patients to different MPS types.

4. Based on the preliminary qualitative analysis, to choose a high prevalent and a low prevalent MPS type in our population for further study.

5. To estimate the activity of the pertinent lysosomal enzymes in normal children and adults with a view to ascertain the normal ranges of these enzymes in our population.

6. To confirm the MPS type and subtype of the study group by lysosomal enzyme assays.
7. To analyse the clinical features, available radiological data and family background of the confirmed MPS patients to see whether any special information pertaining to this population emerges.

8. To assay the pertinent enzymes in the parents of the affected children with a view to detect the carrier level of these enzymes in our population which will help in carrier detection.

9. To analyse these enzyme levels in the sibs of the patients to identify carriers, if any.

10. To undertake family studies of the affected children to get an insight into the relationship between the disease and consanguinity.

The two MPS types chosen for the present study, based on the preliminary analysis are, Morquio (high prevalent) and Maroteaux-Lamy (low prevalent) syndromes.