ANNEXURES

CASE RECORD FORM

MOLECULAR AND ENZYMATIC STUDIES IN CHILDREN WITH GAUCHER DISEASE

Date: _____ CRF No: _____

FRIGE Reference No: _____

Patient's Name: _____

Address: _____

_____

Tel: No: _____

Native Place address: _____

_____

Referred By: _____

Self/relatives/other patients/family doctor/Pediatrician/Gynecologist/Neurologist/ any other specialist (please specify)

Age: _____Yrs. Sex: M/F

Body Weight (In Kg): _____ Height (cms): _____

Upper Segment/ Lower Segment Ratio (cms): _____

Head Circumference (cms): _____ Chest Circumference (cms): _____

Mid Arm Circumference (cms): _____

Age of Onset of Symptoms:

At Birth	Birth to six months	Six months to one year	one year to 3 years	later

Presenting Symptoms:

Delayed milestones	
Convulsions	
Coarse features	
Growth retardation	
Skeletal abnormality	
Family history of LSD	

Family history of LSD	
Any Other [Please specify]	

Diagnosis and Complications (If Any):

Sickness/ Symptoms	Date of diagnosis	Current status (Controlled/ Uncontrolled)	Current Medications (Drug/Dose/Duration

Family History:

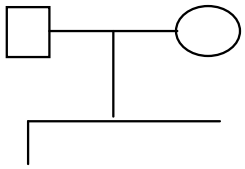
Name of the Mother: Age: Yrs

Name of the Father: Age: Yrs

Religion and Caste: _____

Original Native place if known: _____

How common is consanguinity in the community: _____



ASSESMENT OF SYMPTOMATOLOGY

I. FACIAL FEATURES:

Coarse:_____ Mild coarse: _____ Normal: _____

II. CNS FUNCTIONS:

Mental Retardation:		Present/Absent	
a. Severe MR b. Moderate MR c. Mild MR			
Regression of milestones		Yes/No	
Hypotonia		Yes/No	
Deep and superficial reflexes		Yes/No	
Power		Yes/No	
Myoclonal jerks		Yes/No	
Seizures	Yes/No	Since how long	Months/Yrs
Cranial Nerves		Yes/No	
Signs of cord compression		Yes/No	
Hyperaccusis		Yes/No	
Hearing Status		Yes/No	
Aggressive Behavior		Yes/No	
Any Other [Please specify]		Yes/No	

III. SKELETAL ABNORMALITIES:

Dysostosis Multiplex	Present/Absent
Short Stature	Present/Absent
Bone crisis/Osteonecrosis	Present/Absent
If present please specify the Signs.	
Status of joints and posture	

IV. SKIN/HAIR FINDINGS:

Hypertrichosis	Present/Absent
Skin papules	Present/Absent
Telangiectasia	Present/Absent
Angiokeratomas	Present/Absent
Alopecia	Present/Absent
Any other (Please specify)	

V. EYE FINDINGS:

Normal	
Cherry Red Spot	Yes/No
Corneal Clouding	Yes/No
Cataract	Yes/No
Visual Blindness	Yes/No
Any other (please specify)	

VI. CARDIOVASCULAR SYSTEM FINDINGS:

ECG changes	
Cardiac failure	
Cardiomyopathy	
ECHO findings	

VII. HEPATOSPLENOMEGALY:

Hepatospleenomegaly	Present/Absent
Mild	
Moderate	
Severe	

VIII. HEMATOLOGICAL STUDY:

Haemogram	
Blood/Bone marrow	
Vacuolated Lymphocytes	Present/absent
Specific Findings:	

ADDITIONAL INVESTIGATIONS:

CT scan/MRI:	
EEG:	
EMG/MCV:	
USG:	
X-Ray	
Others (BERA/ERG):	

SCORE: 0 → Absent
+ → Present

ND= Not done
NA= Not Available

Investigations:

Screening test for common LSDs:

Plasma/Serum Chitotriosidase **(Screening for Gaucher and NPD)**	
I-cell Screening **(Screening for Mucolipidosis-II/III)**	
Azure A test (MPS spot) **(Screening for MPS)**	
GAG Quantitative **(Screening for MPS)**	
GAG Qualitative **(Screening for MPS)**	

Enzyme Study (Lymphocytes and/or Plasma)

Sr. No.	Enzymes (Disease name)	Proband	Father	Mother
Mucopolysaccharidosis				
1.	α-iduronidase **(Hurler Syndrome, MPS-I)**			
2.	α-iduronate Sulphate (from Plasma) **(Hunter Syndrome, MPS-II)**			
3.	Heparan Sulphamidase **(Sanfilippo Syndrome type A, MPS IIIA)**			
4.	N- acetyl-α-glucosaminidase (from Plasma) **(Sanfilippo Syndrome type B, MPS IIIB)**			
5.	β-galactosidase-6-Sulphate-Sulphatase **(Morquio Syndrome type A, MPS IVA)**			
6.	β-galactosidase **(Morquio Syndrome type B, MPS IVB)**			
7.	Arylsulfatase – B **(Maroteaux- Lamy Syndrome, MPS VI)**			
8.	β-glucuronidase **(Sly Syndrome, MPS VII)**			
Defects in degradation of Glycolipids				
9.	β-galactosidase **(GM1 gangliosidosis)**			
10.	Hexosaminidase-A **(Tay-Sach's disease - GM2 gangliosidosis)**			

11.	Hexosaminidase-T **(Sandhoff disease - GM2 gangliosidosis)**			
12.	β-glucosidase **(Gaucher disease)**			
13.	Sphingomyelinase **(Niemann Pick Disease A & B)**			
14.	Acid Lipase **(Wolman disease)**			
Defects in degradation of sulphatides				
15.	Aryl – A **(Metachromatic Leucodystrophy, MLD)**			
16.	β-galactocerebrosidase **(Krabbe disease)**			
Defects in degradation of Glycogen				
17.	α-1-4-glucosidase **(Pompe disease, GSD II)** **With Acarbose:** **Without Acarbose:** **Ratio:**			
18.	Debrancher enzyme **(GSD-III)**			
Defects in degradation of Glycoproteins				
19.	α-fucosidase **(Fucosidosis)**			
20.	α- mannosidase **(Mannosidosis)**			
Defects in degradation of Globotriaosylceramide				
21.	α-galactosidase **(Fabry disease)**			
Defects in protein degradation (NCL)				
22.	Palmitoyl Protein Thioseterase (PPT) **(Batten disease, NCL-I)**			
23.	Tripeptidyl Peptidase-I (TPP-1) **(Batten disease, NCL-II)**			
Defects in lysosomal trafficking proteins				
24.	filipin stain (Cultured fibroblast) **(Niemen-Pick disease C)**			
Defects in lysosomal transporters				
25	N-Acetyl-Neuraminic acid (NANA) (Urine) **(Sialic acid storage disorders)** Free NANA (Urine) Total NANA (Urine)			

Molecular Analysis:

Table-1	**Variations previously reported for the phenotype in literature or databases and are recognized cause of clinical phenotype**					
Patients Name	Gene Strand	Genomic position	cDNA position (Ref. Sequence Number)	Amino acid change	Exon/ Intron no.	Mutation status (Homozygous/ Heterozygous)
Proband						
Mother						
Father						
Other (please specify)						

Table-2	**Variations previously unreported for the phenotype in literature or databases and are of the type that is expected to be the cause of the clinical phenotype**					
Patients Name	Gene Strand	Genomic position	cDNA position (Ref. Sequence Number)	Amino acid change	Exon/ Intron no.	Mutation status (Homozygous/ Heterozygous)
Proband						
Mother						
Father						
Other (please						

specify)						

Table-3	**Variations previously unreported for the phenotype and are of the type which may or may not be causative of the clinical phenotype**					
Patients Name	Gene Strand	Genomic position	cDNA position (Ref. Sequence Number)	Amino acid change	Exon/ Intron no.	Mutation status (Homozygous/ Heterozygous)
Proband						
Mother						
Father						
Other (please specify)						

***Insilico* analysis:**

Location (Exon)	**Codon number**	**Codon change**	**Amino acid change**	**Mutation T@ster score**	**SIFT Score**	**Polyphen2 Score (sensitivity, specificity)**

- The Mutations T@ster score is taken from an amino acid substitution matrix (Grantham Matrix) which takes into account the physico-chemical characteristics of amino acids and scores substitutions according to the degree of difference between the original and the new amino acid scores may range from 0.0 to 6.0.
- The SIFT score is the normalized the probability that the amino acid change is tolerated and ranges from 0 to 1. The amino acid substitution is predicted damaging is the score id <=0.05, and tolerated if the score is >0.05.
- The Polyphen2 score is the naïve Bayes posterior to probability that this mutation is damaging and thus ranges from 0 to 1.

Clinical Photographs of Patient:

Conclusion:

Foundation for Research In Genetics & Endocrinology (FRIGE: E – 13237) | ISO 9001: 2008

INSTITUTE OF HUMAN GENETICS

GENETICS CENTRE Reg. No. : 648

FRIGE HOUSE, Jodhpur Gam Rd., Satellite, Ahmedabad-380015. Gujarat. INDIA

INFORMED CONSENT FOR GENETIC STUDIES (Enzymes and Molecular test)

We the undersigned parents of _____agree to investigate our child for suspected Lysosomal storage disorders.

We understand that:

1. The sample (Blood / DNA) analysis being performed is specific for the disease being tested and in no way guarantees absence of other disorders.
2. In some cases it is necessary to do an indirect test that does not identify a specific disease causing deficiency of enzyme/mutation. If I am to have an indirect test, my health care provider has discussed these issues with me. I understand that in most cases, a negative that result does not necessarily rule out a genetic condition.
3. Results of genetic testing should be considered with the results of other types of testing and clinical evaluation.
4. Lack of all needed family members may compromise the quality or decrease the accuracy of the result obtained.
5. No clinical tests other than those authorized will be performed; however, any remaining sample may be used for quality control purposes or research, provided the analysis is carried out anonymously.
6. Despite the highly accurate nature of Enzymes/ Molecular Genetic testing and laboratory quality control measures, errors (False positives and false negatives) may occur at a frequency estimated to be about 2%.
7. Generally, Enzymes/Molecular Genetic tests are relatively new and are being improved and expanded continuously. The testing is often complex so that there is always some possibility the test will not work properly or that an error will occur. There is a low but finite error rate, which is estimated to be about 2% in direct tests
8. The results will be reported to me only, or to my physician or to the person I nominate.
9. My signature below acknowledges my voluntary participation in this study, appreciating the above limitations

Date _____ Signature _____

Witness:
Name & Address: Signature _____

ALTERNATE INFORMED CONSENT: Physicians / Counselor's statement:

I have explained the benefits and drawbacks of Molecular Genetic studies to this individual. I have addressed the limitations outlined above, answered this person's questions and I have obtained verbal consent to order the above test.

Date _____ Signature _____

Name/ Address/ Fax/ Email of Physician/ Counselor