Challenges in diagnosis of Genetic and metabolic disorders in newborn

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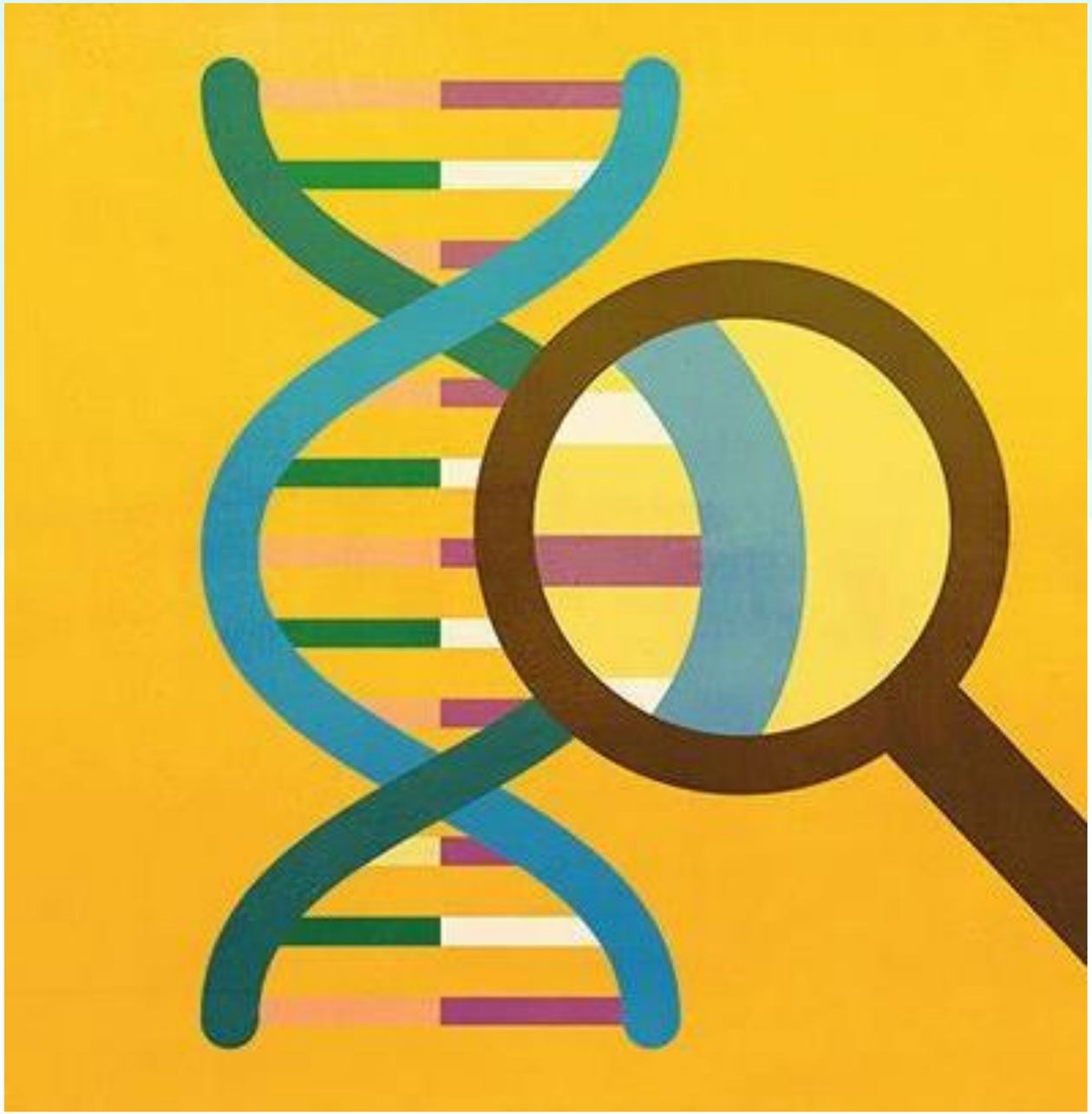


Part A :
Genetic disorders in newborn

Introduction : genetic testing, why it is done ?

- **Done for different reason’s :**
- **Preimplantation testing - embryos are screened**
- **Prenatal testing - cell free DNA testing - Down syndrome**
- **Newborn screening - hypothyroidism or PKU**
- **Pre symptomatic testing- risk of developing colorectal CA**
- **Carrier testing - family history of genetic disorders**
- **Diagnostic testing -Cystic fibrosis or Huntington’s disease**

Type of Genetic testing :

1. Cytogenetic tests	2. Molecular tests	3. Biochemical test
A. Karyotyping	A. Single gene study	A. HPLC
B. FISH-Fluorescence in situ hybridisation	B. Gene panel study	B. Gas chromatography
C. CMA-Chromosomal MicroArray	C. Exome/Whole genome sequencing	C. Mass spectrometry

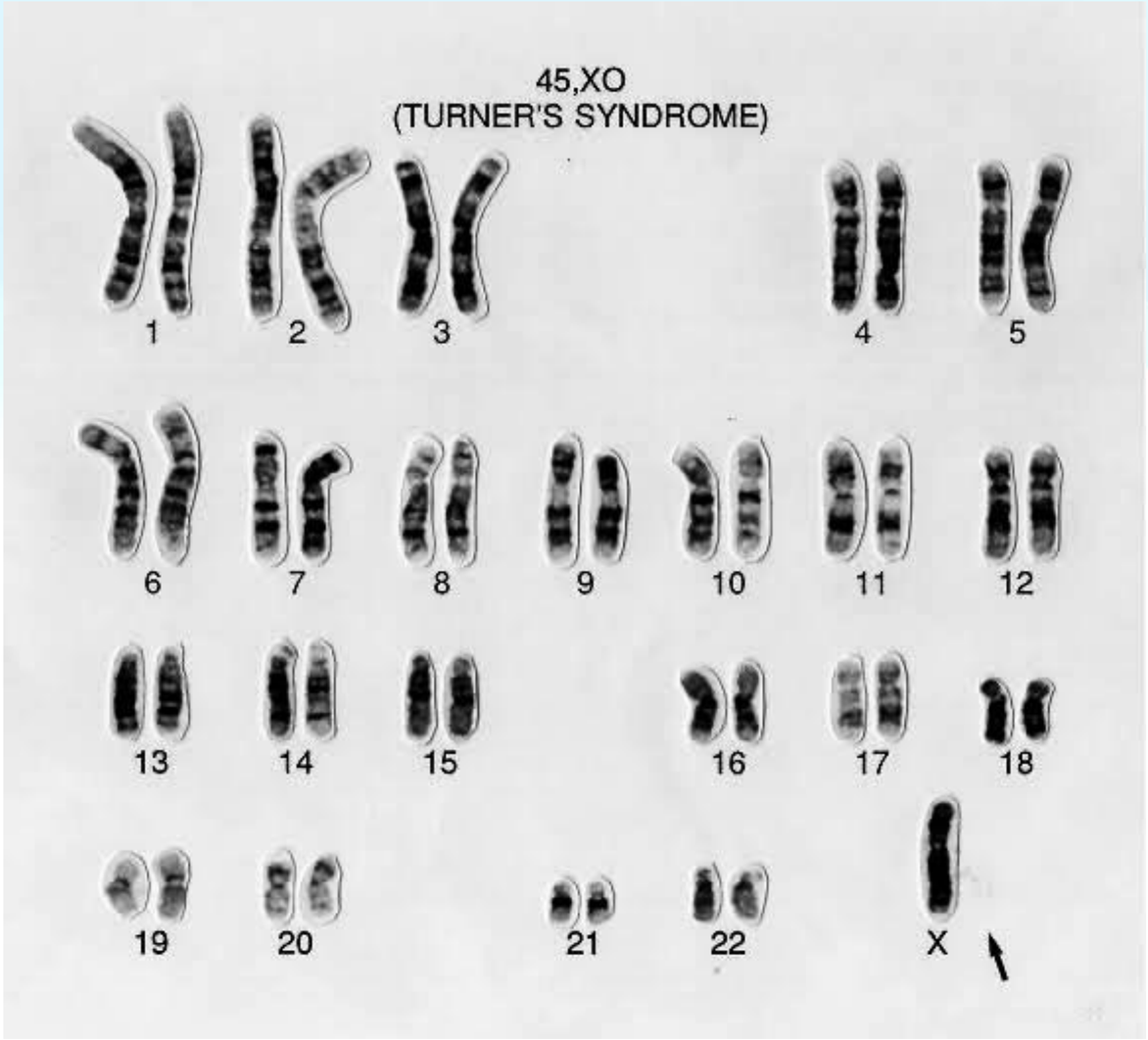
A. Karyotyping :

- Involves detection of number and composition of metaphase chromosomes based on examination in light microscope.
- Banding technique : G-banding
- used in cytogenetics to produce a visible karyotype by staining condense chromosome
- Two regions obtained :

1. Heterochromatic region - [AT - rich and Gene poor] - take dark stain
2. Euchromatic region - [GC - rich and transcriptionally active] - take light stain

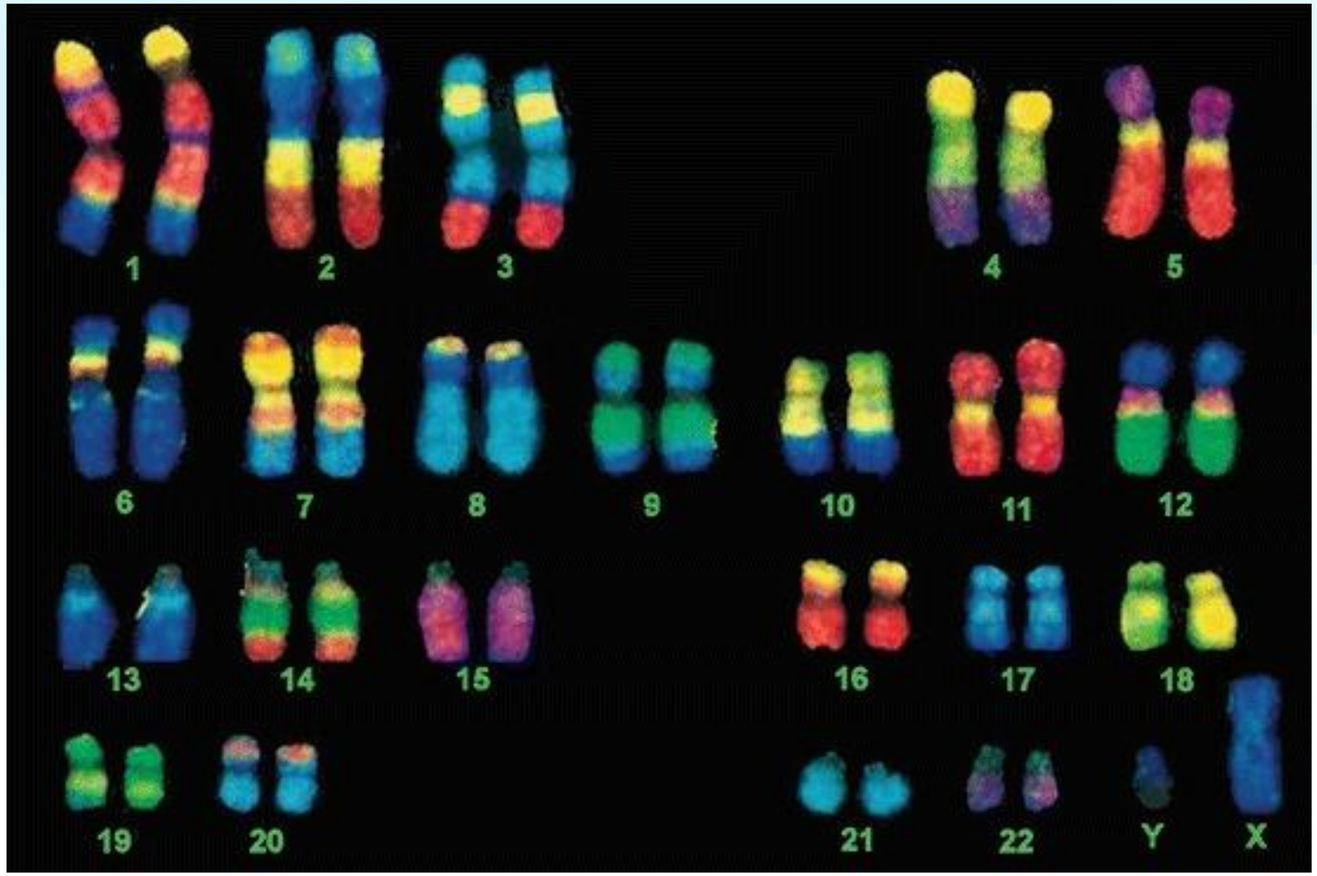
Disease detected by karyotyping :

1. Turner syndrome [45,X0]
2. Klinefelter syndrome [47,XXY]
3. Down syndrome [Trisomy 21]
4. Edwards syndrome [Trisomy 18]
5. Patau syndrome [Trisomy 13]
6. Cri du chat [5p-deletion]
7. Angelman and Prader-willi syndrome [15q-deletion]



B. FISH : it bridged molecular and cytogenetic analysis

- DNA probes specific to regions of particular chromosomes are attached to fluorescent markers and hybridised with a chromosome spread.
- This make it easy to detect segmental deletions and translocation among chromosome.



Difference :

Karyotype	FISH
Low Resolution and Sensitivity	High resolution and sensitivity
Cell division required- Metaphase stage	Not required
Balanced lesion / rearrangement (translocation,insertion,inversion) can be detected but not all	All balanced lesions can be detected precisely (specific chromosomal location)
Large structural change detected >5-10Mb	Small structural changes detected upto 100Kb
Screening of unknown defect possible	Not possible : commercially available probes are limited

C. Chromosomal Microarray [CMA]

- **CMA is a technology used for detection of clinically significant micro deletions or duplications, with a high sensitivity for submicroscopic aberrations.**
- **It is able to detect changes as small as 5-10Kb in size i.e resolution upto 1000 times higher than conventional karyotyping.**
- **CMA is used for uncovering copy number variants (CNVs) thought to play an important role in the pathogenesis of variety of disorders primarily….**

Disorders, primarily….

1. Developmental Delay [DD]
2. Intellectual Disability [ID]
3. Autism Spectrum Disorder[ASD]
4. Multiple Congenital Anomaly [MCA]

- **Note : Available evidence support CMA as first-tier cytogenetic diagnostic test for patients with DD/ID/ASD or MCA whereas Karyotype should be reserved for patients with obvious chromosomal syndrome, family history of chromosomal rearrangements, or h/o multiple miscarriage.**

Types of CMA and Difference :

1. aCGH - Array based Comparative Genomic Hybridisation	2. SNP - Single Nucleotide Polymorphism array
Single sequence oligonucleotide of ~ 60bp	Two 20~60bp oligonucleotide of different sequence
Two labelled DNA's patient and control are required per hybridisation	Only patient DNA which is labelled and Hybridised
Resolution down to size of oligonucleotide exon by exon	Resolution limited by SNP distribution and signal to background
No detection of uniparental disomy or consanguinity	Can be detected along with homozygosity and low level mosaicism
Detect a gene level Copy number variants (CNV's)	Detect larger CNV's or multi-gene CNV's

D. MLPA : Multiple Ligation dependant Probe Amplification

- **Principle :**

- **Multiplex PCR assay that utilise upto 40 probes, each specific for a different DNA sequence (exon of specific gene of interest, to evaluate the relative copy number of each DNA sequence.**

- **Application :**

- **Neuromuscular Disorders**
- **SHOX gene analysis**
- **Prenatal Diagnosis**
- **DNA Methylation Study**

Disorders :

DISEASES	GENE	APPLICATION	MLPA Advantage
Duchenne muscular dystrophy/ Becker muscular dystrophy	**DMD GENE**	Diagnosis	All 79 exons of DMD-gene analysed in two reactions and detection of deletion and heterozygous duplication.
Spinal muscular atrophy	**SMN-1 SMN-2**	Diagnosis	Detection of heterozygous SMN-1 loss and discriminates SMN-1 deletion and conversion to SMN-2.
Idiopathic short stature	**SHOX**	Diagnosis	Analyse SHOX gene coding as well as enhancer region and detection of partial gene deletion and duplication.
Aneuploidies of 13, 18, 21, X and Y chromosome	**-**	Diagnosis	A single probe mix for the detection of several aneuploidies
Prader-Willi syndrome Angelman syndrome	**15q11-13**	Diagnosis	A single MS-MLPA set is used for the analysis of both disease

Limitations of MLPA :

- **Sensitivity to sample used for DNA extraction (blood or buccal swab) 100-200ug of DNA sample required.**
- **MLPA cannot be used to investigate single cell as compared to FISH**
- **Mosaicism can go undetected as presence of normal cell mask the abnormal cell**
- **Unable to detect balance rearrangement since patient DNA is compared with control DNA**

E. Next Generation Sequencing or Massively parallel sequencing

- **Two type :**

A. Targeted gene panel

B. Exome sequencing

C. Targeted gene panel :

- **Focus on small group of gene to look for disease predisposing or disease causing variants.**
- **It analyses between 2 to 500 gene with different diagnostic strategies depending on phenotype, differential diagnosis and genetic heterogeneity of phenotype.**
- **Advantage :**
- **Higher depth sequencing**
- **Guaranteed 100% coverage of gene.**

B. Exome sequencing :

- 85% disease causing variants are concentrated in 1-2% of genome that is protein coding (exonic region) and collection of all exons are called exome.
- The exonic region are selectively captured and sequenced to yield new cause of genomic disease or identification of known genetic disease.
- Exome sequencing should be first line test for neurodevelopmental disorders : is called Genotype first approach.

Exome sequencing:

A. Isolation of genomic DNA

B. Unspecific fragmentation

C. End Repair

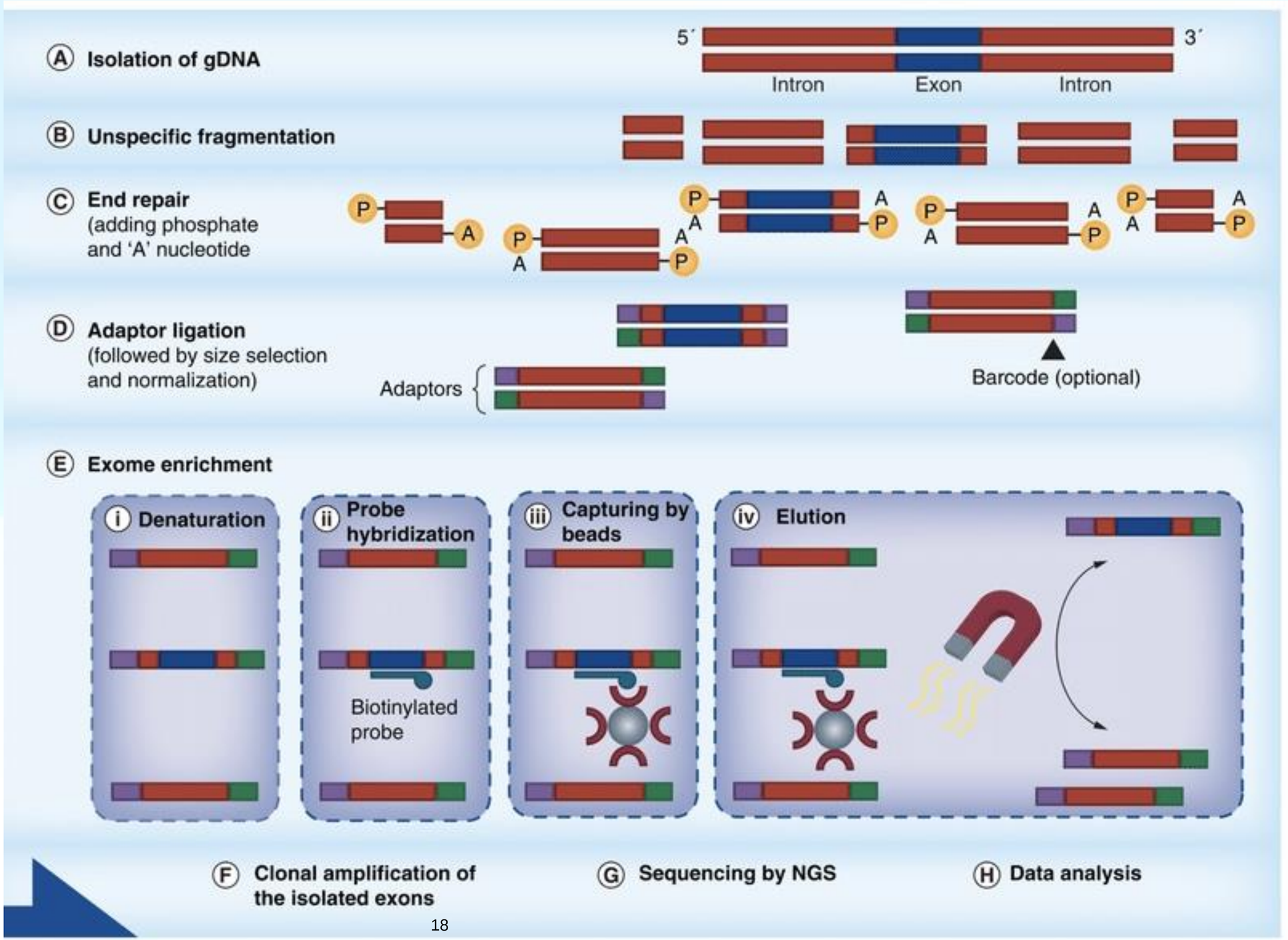
D. Adaptor ligation

E. Exome enrichment

F. Clonal amplification of isolated exons

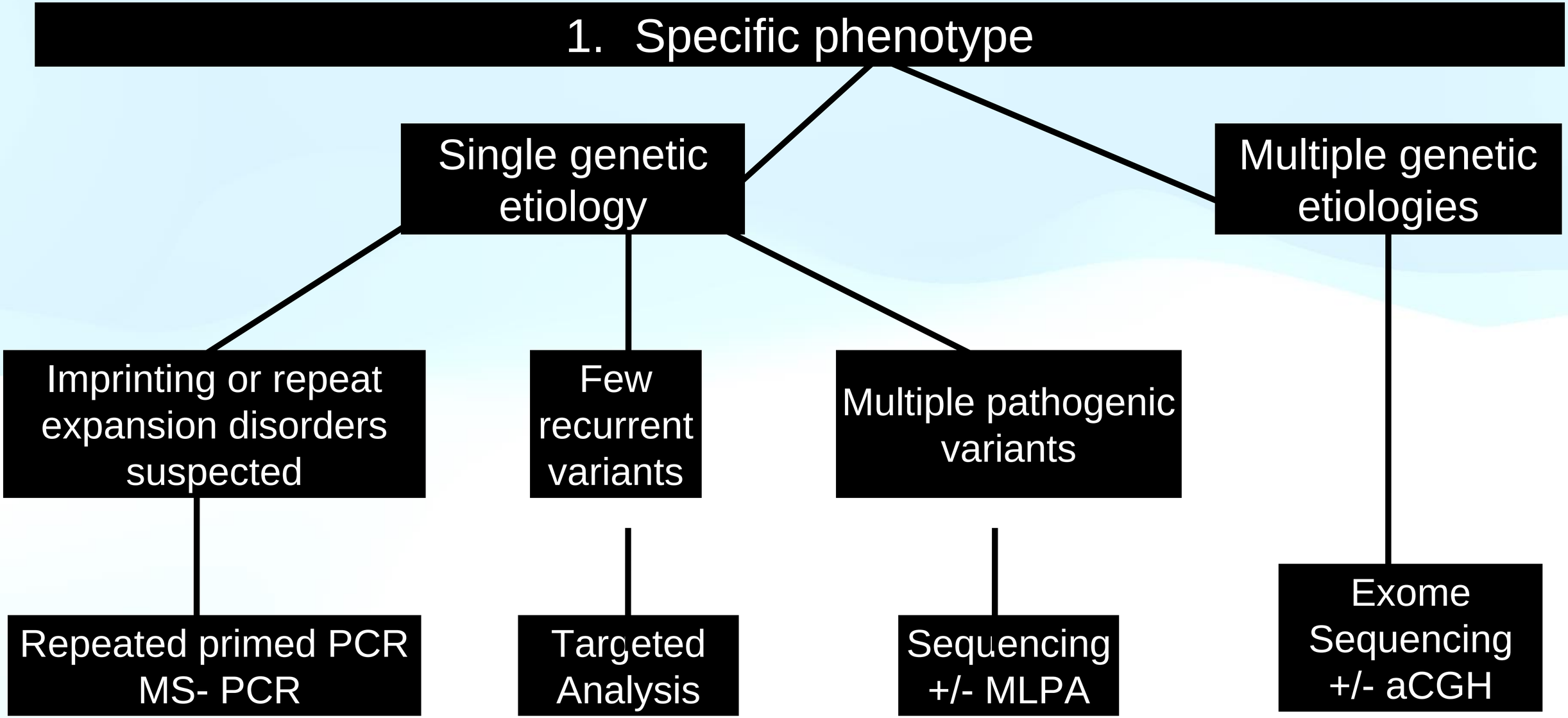
G. Sequencing by NGS

H. Data Analysis



✤ Approach to Genetic testing :

A. General considerations in determining appropriate technology used for diagnosis :



MS-PCR :Methylation specific PCR, MLPA:Multiple ligation dependant probe amplification, aCGH - array based Comparative Genomic Hybridisation

2. Broad phenotype

- Possible Mitochondrial syndrome
- Unrecognisable syndrome

Exome Sequencing +/- aCGH

mtDNA sequencing

3. Chromosomal Disorders

- Aneuploidy suspected
- NIPT follow-up
- Specific phenotype
- Family history
- Infertility

Cytogenetic study

- DD/ID/MCA
- Broad phenotype
- Phenotype specific for del/dup syndrome

CMA

aCGH- Array based Comparative Genomic Hybridisation, mtDNA- mitochondrial DNA , DD- Developmental delay , ID- Intellectual disability, MCA- Multiple congenital anomaly, del- deletion, dup- duplication, CMA - Chromosomal Micro Array , NIPT - Non invasive prenatal Testing

B. Diagnostic workflow for various genetic syndromes :

1. Baby with NIPT positive for Turner syndrome

Karyotype

FISH or CMA for sex chromosome mosaicism and /or structural changes

2. Child with suspected Prader-Willi syndrome

Methylation specific PCR – Not Prader-Willi syndrome

+

MLPA for CNVs

–

UPD analysis

–

ID analysis

3. Isolated hearing loss

- Long range PCR for STRC gene
- aCGH for exon level CNVs

FISH - Fluorescent insitu hybridisation
CMA - Chromosomal microArray
UPD - Uniparental disomy
ID - Intellectual disability
aCGH- array based Comparative Genomic Hybridisation
CNVs - Copy Number Variants

Part - B
Metabolic Disorder in newborn :

Introduction : Metabolic testing, why it is necessary?

- The combination of low specificity of presenting symptoms and low prevalence of metabolic disorders make diagnosis difficult.
- Genetic counselling and prenatal diagnosis : Most of the IEM are single gene defects, inherited in an autosomal recessive manner, with a 25% recurrence risk.
- Therefore when the diagnosis is known and confirmed in the index case, prenatal diagnosis can be offered, wherever available for the subsequent pregnancies.

Clinical clues suggesting IEM :

- **High index of suspicion will go long way in clinching the diagnosis of IEM**
- **IEM should be considered in differential diagnosis of any sick neonates along with common acquired cause such as sepsis, HIE, duct dependent cardiac lesion, CAH, congenital infection.**

✦ **In Newborn :**

- **Nonspecific, unexplained features such as poor feeding ,lethargy, vomiting, hypotonia, faliure to thrive, respiratory abnormalities, hiccups, apnea, bradycardia and hypothermia with normal sepsis screen.**

In Children :

- **Sudden and rapid illness in a previously normal baby precipitated by fever, vomiting or fasting.**
- **Rapidly progressive encephalopathy of unknown etiology.**
- **Persistent or recurrent hypoglycaemia, intractable metabolic acidosis, unexplained leukopenia or thrombocytopenia.**
- **Hyperammonemia , Organomegaly , Developmental regression**
- **Family history of unexplained neonatal death or progressive neurological disease, HELLP Syndrome in mother.**

- **Peculiar odour**
- **Parental consanguinity : most acutely presenting metabolic disorders are autosomal recessive.**

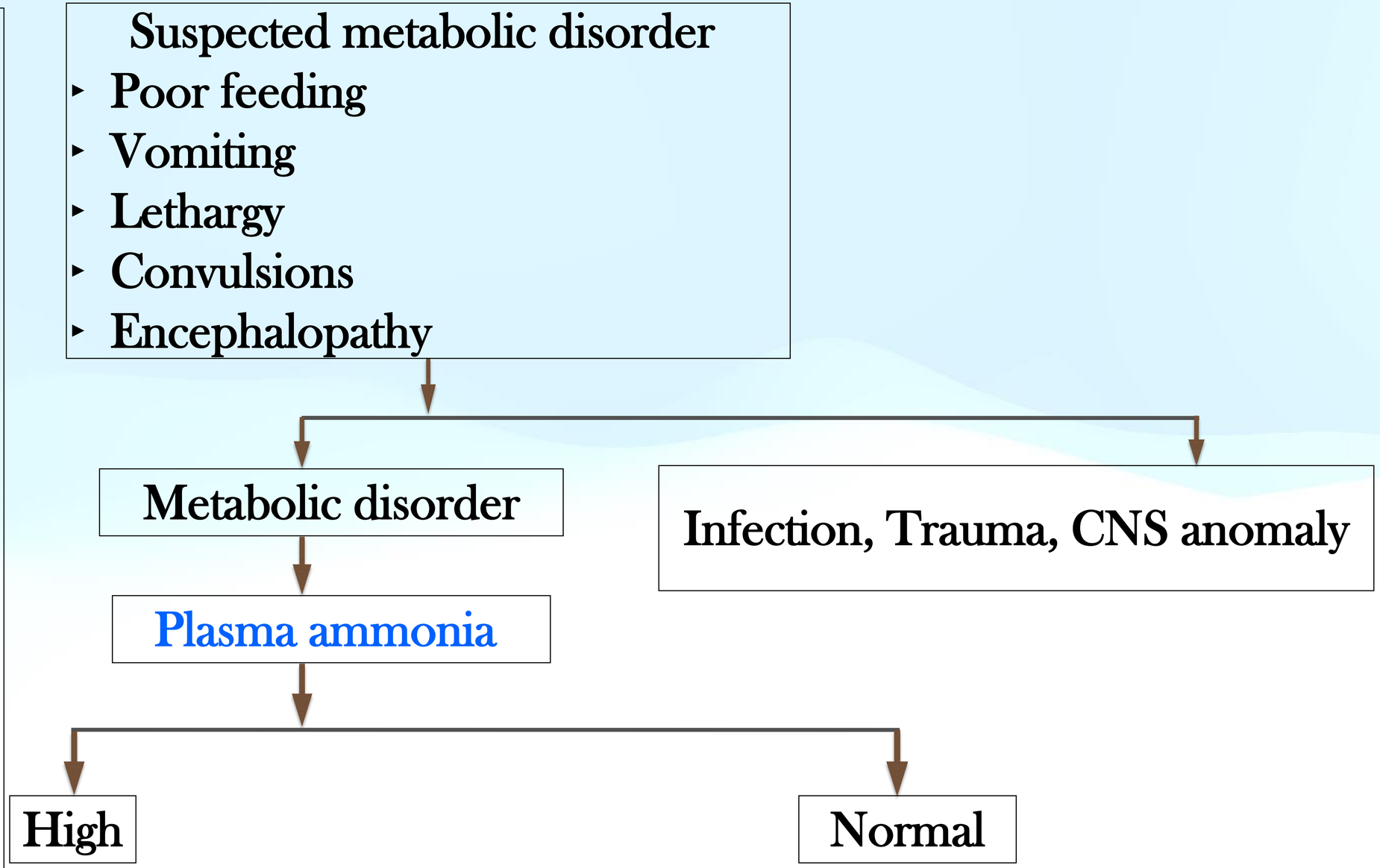
Odour	Diseases
Musty	Phenylketonuria
Cabbage like	Tyrosinemia
Maple syrup like	MSUD
Sweaty feet like	Isovaleric acidemia Glutaric acidemia type 2
Cat urine like	Multiple carboxylase deficiency

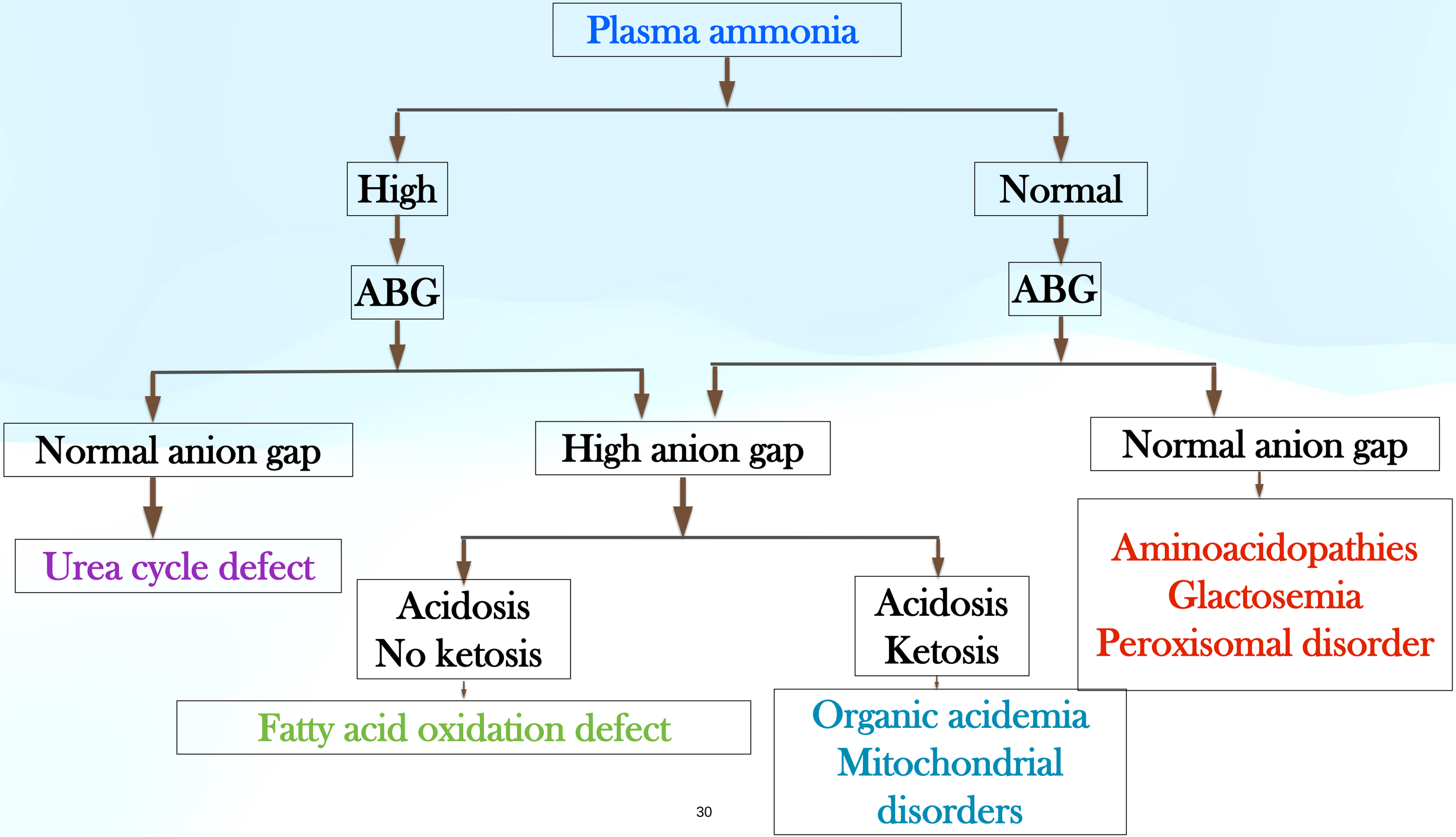
Laboratory investigations :

- First line investigation (metabolic screening) :

1. Blood Metabolic screening	CBC Glucose Lactate pH Bicarbonate Electrolyte Ammonia ↓ ABG
2. Urine metabolic screen	pH Ketones Reducing substance

☑ Approach to a child with a suspected metabolic disorders

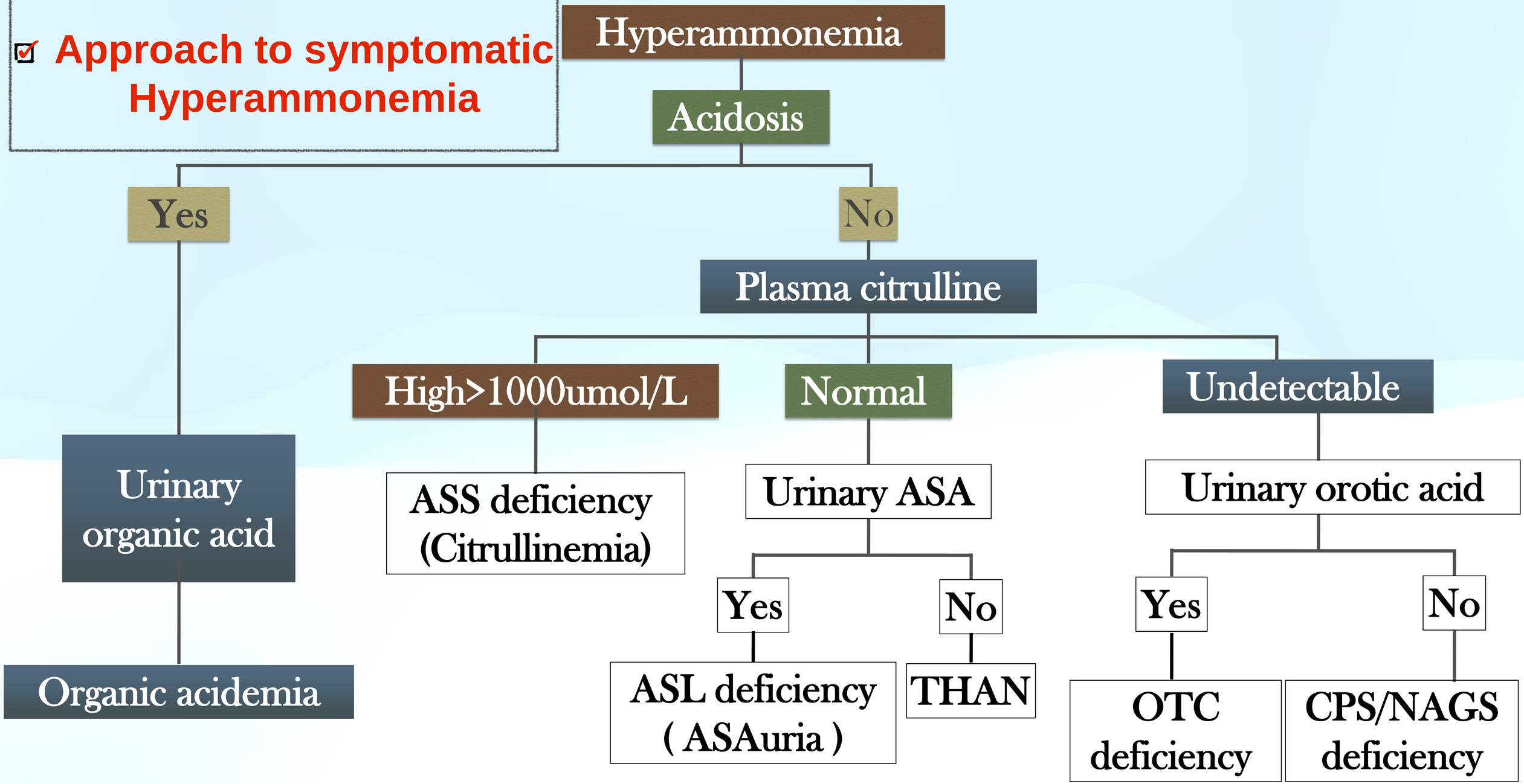



Plasma ammonia
High
Normal
ABG
ABG
Normal anion gap
High anion gap
Normal anion gap
Urea cycle defect
Acidosis
No ketosis
Acidosis
Ketosis
Aminoacidopathies
Glactosemia
Peroxisomal disorder
Fatty acid oxidation defect
Organic acidemia
Mitochondrial
disorders

☑ Differential diagnosis of metabolic disorders :

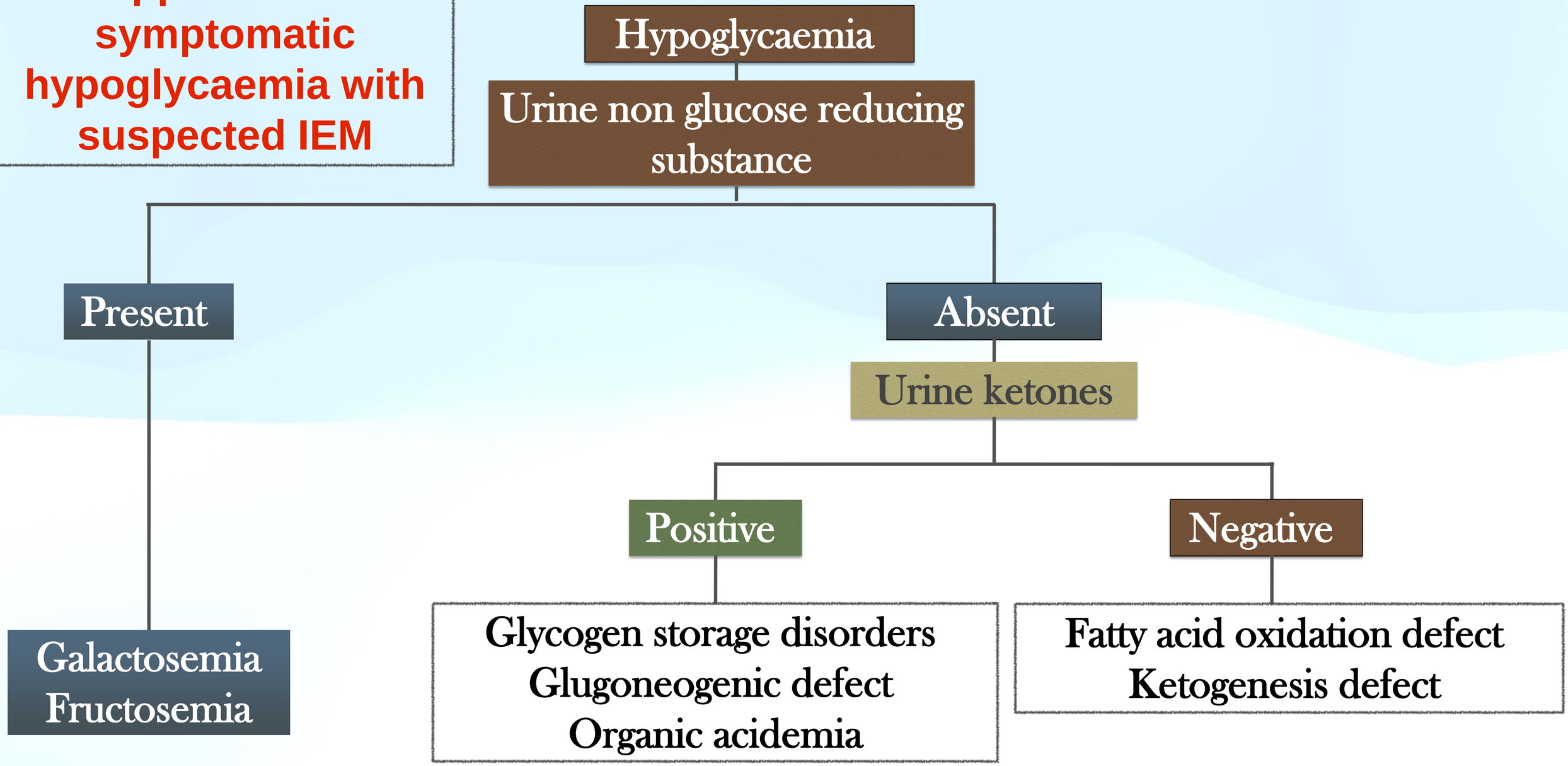
Diagnosis	Acidosis	Ketosis	Plasma Lactate	Plasma Ammonia	Plasma Glucose
Aminoacidopathies	+/-	+	N	N	N
Organic Acidemia	+++	+++	Inc.	Inc.	Dec.
Mitochondrial disorders	+	+/-	+++	N	N
Urea cycle disorders	N	N	N	+++	N
Fatty acid oxidation defect	+/-	N	+/-	-	Dec.

☑ Approach to symptomatic Hyperammonemia



ASS-Arginosuccinate synthetase , ASL-Arginosuccinate Lyase, OTC - Ornithine transcarbamoylase , THAN- Transient Hyperammonemia of Newborn
CPS- Carbamoyl phosphate synthetase , NAGS- N-Acetyl Glutamate synthetase, ASAuria - Arginosuccinic aciduria

☑ Approach to symptomatic hypoglycaemia with suspected IEM



☑ Second line investigation (ancillary and confirmatory test) :

1. **Gas chromatography mass spectrometry (GCMS) of urine - for diagnosis of organic acidemias.**
2. **Plasma amino acids and acyl carnitine profile : by tandem mass spectrometry (TMS) - for diagnosis of organic acidemia ,urea cycle defect, Aminoacidopathies, fatty acid oxidation defect.**
3. **High performance liquid chromatography (HPLC) : for quantitative analysis of amino acids in blood or urine ; required for diagnosis of organic acidemia and Aminoacidopathies.**

4. **Lactate/pyruvate ratio - in case of elevated lactate.**

5. **Urinary orotic acid - in case of hyperammonemia for classification of urea cycle defect.**

6. **Enzyme assay: Biotinidase assay and GALT (Galactose-1-P uridyltransferase) assay**

7. **Neuroimaging : MRI**

8. **Magnetic resonance spectroscopy : lactate peak in mitochondrial disorders and leucine peak in MSUD.**

9. **EEG , Plasma Very long chain FA , Mutation analysis, CSF aminoacid analysis : these tests are also employed.**

Principles of Management :

- **Specific treatment is directed towards reversing the basic pathophysiological process causing the disease. It includes :**

- **Reduction of substrate accumulation for a deficient enzyme**
- **Reduce accumulated toxic metabolites**
- **Replace deficit enzyme**
- **Residual enzyme activity enhancement**

Take home message : Genetic disorders

- **Genomic diagnostic testing has evolved from low resolution (e.g., karyotype) and single locus (e.g. Sanger, FISH, PCR) analysis to high resolution genome testing (e.g. CMA, ES, GS).**
- **Challenges in how to rapidly and accurately interpret the staggering number of single nucleotide and copy number variants that exist in the human genome.**
- **Education and counselling for both non-genetics clinicians and patients are required.**
- **Informed consent prior to undertaking genetic studies is essential.**

Take home messages : Metabolic disorders

- Again high index of suspicion coupled with a rational use of resources through critical metabolic thinking is the vehicle for the accurate diagnosis
- Newborn metabolic screening helps both parents and society as a whole.
- Genetic counselling and prenatal diagnosis should be given importance.

THANK YOU