Primary Immunodeficiency: A Decade of Experience, Genetic Study and Clinical Cases

Presenter

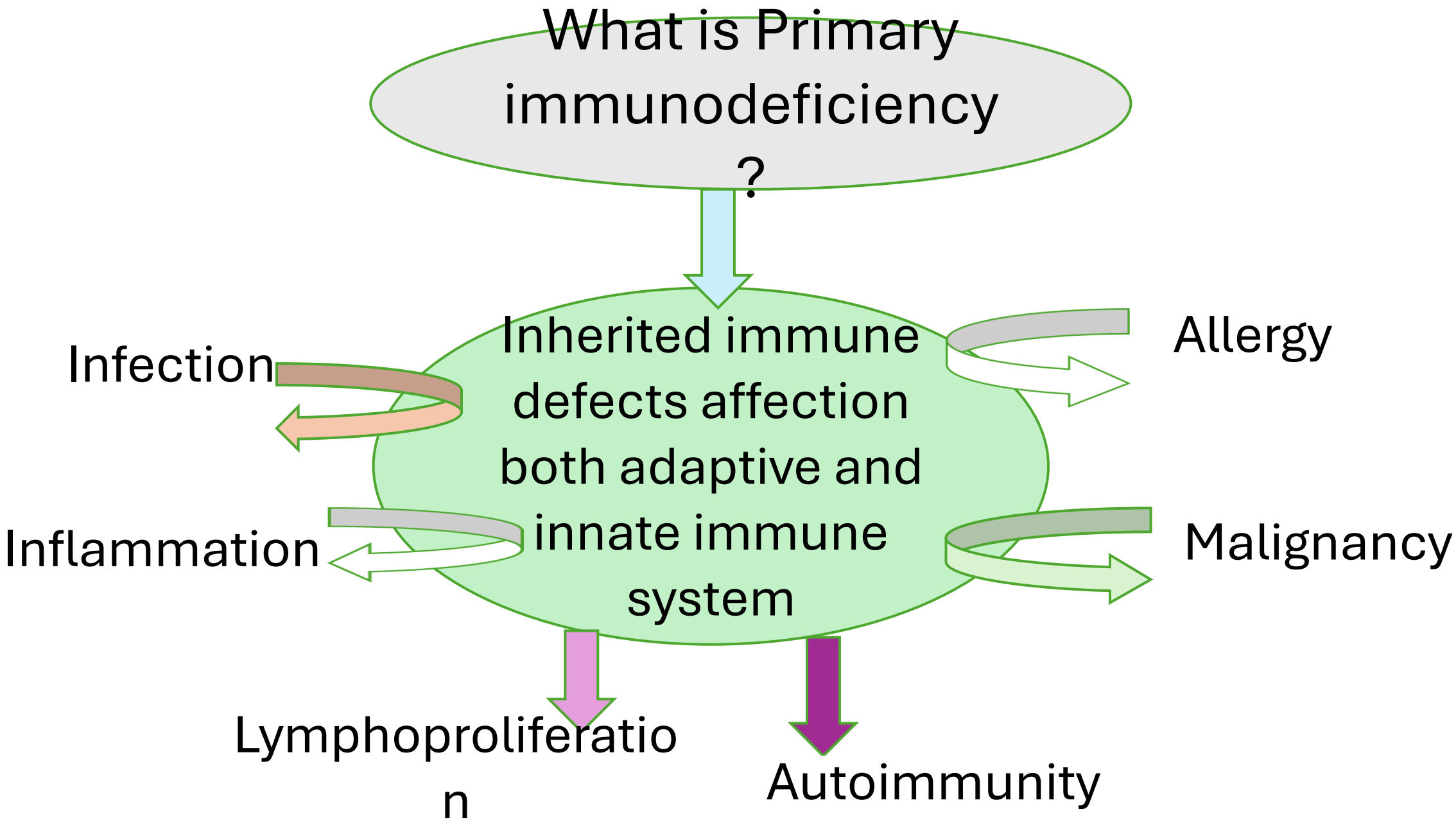
Dr. Sudipta Roy

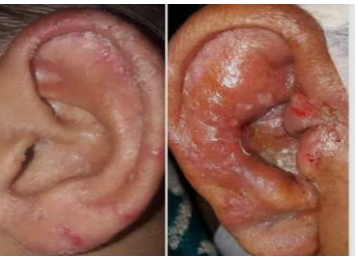
FCPS (Pediatrics)
Advanced training in Paed. Pulmonology & Bronchoscopy (AIIMS, New-Delhi)
Fellowship in Paed. Clinical Immunology (PGIMER, Chandigarh)

Associate Professor
Dept. of Pediatrics
AD-din Medical College Hospital

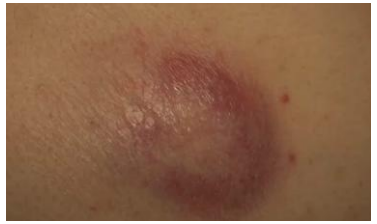
Discussion Points

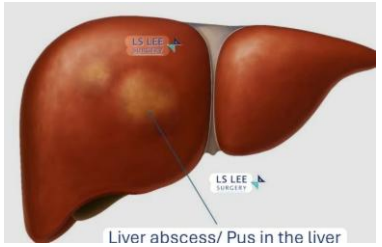
1. A brief overview of primary immunodeficiency
2. A decade of experience and evolution from basic immunology to genetics
3. A brief overview of genetic studies
4. Clinical cases discussion


What is Primary immunodeficiency ?
Inherited immune defects affection both adaptive and innate immune system
Infection
Allergy
Inflammation
Malignancy
Lymphoproliferation
Autoimmunity

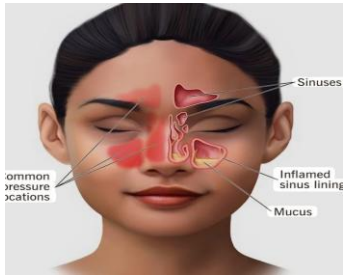


4 or more new ear infections (1yr)

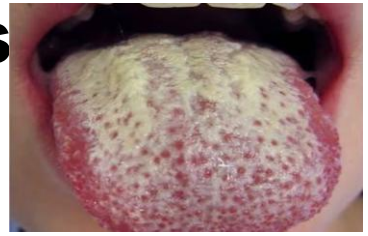




Recurrent deep skin/organ abscess

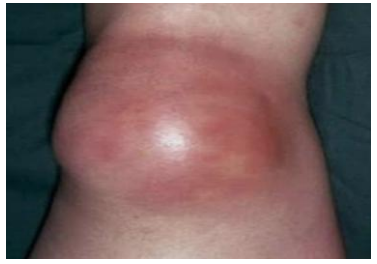


2 or more serious sinus infection(1yr)

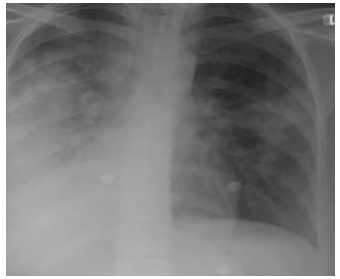


Persistent oral thrush/fungal infection (skin)

2 or more months on antibiotics



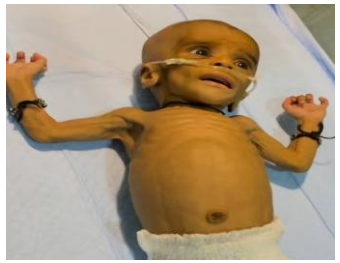
2 or more deep seated infections



2 or more pneumonias(1 yr)

Need for IV antibiotics to clear infections

Jeffrey Model Foundation



Failure to thrive

Family H/O PID

10 warning signs of PIDs

Updated classification of Inborn Errors of Immunity 2024(IUIS)
Covers ~550+ disorders caused by ~500+ genes

1. Immunodeficiency affecting cellular & humoral immunity
2. Combined immunodeficiencies with syndromic features
3. Predominantly antibody deficiencies
4. Congenital defects of phagocyte number or function
5. Complement deficiencies
6. Diseases of immune dysregulation
7. Defects in intrinsic and innate immunity
8. Autoinflammatory disorders
9. Bone marrow failure / DNA repair defects
10. Phenocopies of IEI

Basic Diagnostic Evaluation of PID / IEI

Initial Screening

Complete blood count (CBC) with differential
Peripheral blood smear ESR / CRP

Basic Immunology Tests

1. **Serum immunoglobulins** (IgG, IgA, IgM, IgE)
   IgG subclasses (if needed)
2. **Vaccine antibody response** (tetanus, pneumococcal)

3. **Lymphocyte subset analysis** (T, B, NK cells) by flow cytometry

4. **Functional assay** : Neutrophil function test (NBT / DHR assay)
5. **Complement screening** (CH50, AH50)
5. **Infection & Baseline Workup**: Culture / PCR as indicated -CXR / imaging

Genetic Testing (Definitive Diagnosis)

-Targeted gene panel (IEI/PID panel)

-Whole exome sequencing (WES)

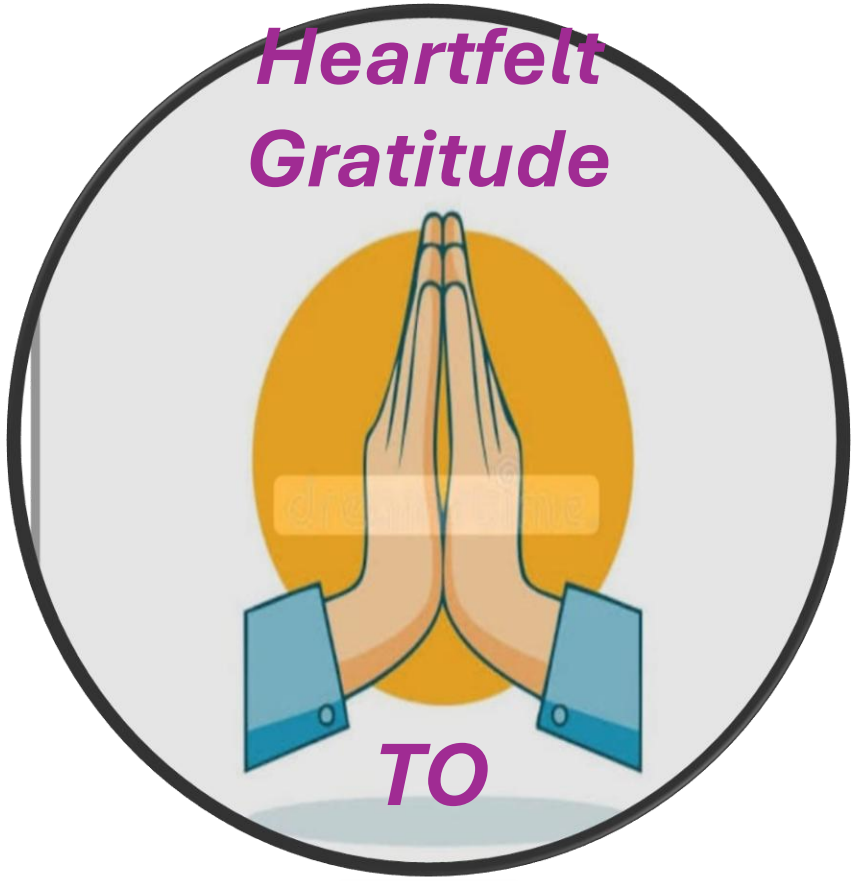
-Whole genome sequencing (WGS) (selected cases)

-Sanger sequencing (variant confirmation)

-Family studies (segregation analysis)

A Journey of Primary Immunodeficiency: 2017-2026

A Journey of Primary Immunodeficiency:2017-2026



PROF. ARM LUTHFUL KABIR SIR

Review Article
Bangladesh J Child Health 2018,
VOL 42(2):86-93

Pulmonary Manifestations of Primary Immunodeficiencies in Children –A review

ARM Luthful Kabir, Sudipta Roy
Correspondence: Dr. Sudipta Roy

Pulmonary manifestations of PIDs

5th Pulmocon-2018

6th Pulmocon-2020

BPA conference-2021

Scientific congress of APSID, 2020 PRIMER, Chandigarh

Prof. Yu-Lung Lau
Founder of APSID

Title: Clinical and Immunological Profile of Primary Immunodeficiency Diseases

Research Grant Achievement, 2022

Integrated Health Science Research & Development Fund Activity

Ministry of Health and Family Welfare, Government of Bangladesh

Recipient of competitive national research grant

Project: Molecular genetic approach to diagnose primary immunodeficiencies in children attending major hospitals in Dhaka city

Advanced Training, Pediatric Respiratory Unit, 2023

AIIMS, New Delhi

Prof. SK Kabra

At AIIMS, experienced the management of different PIDs with complicated respiratory issues

Difficulties Faced During the Study

1.Interpretation of flow cytometry and genetic reports
2.Lack of accurate clinical approach
3. Limited understanding of PID-related immune dysregulation (autoimmunity, autoinflammation, lymphoproliferation, malignancy)
4. Challenges in management plan
5.Long-term follow-up planning

MedGenome Labs Ltd. Bengaluru, India
(Subsidiary of Medgenome Inc., Foster City, CA, USA)
Email: customersupport@medgenome.com, Toll Free No: 1800 296 9696
Website: https://diagnostic.medgenome.com

MEDGENOME

DNA TEST REPORT - MEDGENOME LABS

Full Name / Ref No:	**ADIL- [33626]**	Order ID/Sample ID:	**1039868/8685839**
Gender:	Male	Sample Type:	Blood
Date of Birth / Age:	2 months	Date of Sample Collection:	29th August 2024
Referring Clinician:	Dr. Sudipta Roy, Genetics Solutions, Bangladesh, Dhaka	Date of Sample Receipt:	1st September 2024
		Date of Order Booking:	2nd September 2024
		Date of Report:	25th September 2024
Test Requested:	**Whole Exome Sequencing**		

CLINICAL DIAGNOSIS / SYMPTOMS / HISTORY

Baby *Adil*, born of a non-consanguineous marriage, presented with clinical indications of delayed development, persistent pneumonia, persistent fever and extensive candidiasis. On Examination, wheeze, failure to thrive present. Laboratory investigation showed decreased Serum Ig A, increased CD3+, CD3+ CD8+, CD19+ and CD3+ CD4+. Bronchoscopy showed tracheomalacia and right upper lobe bronchus stenosis. ECHO showed Small ASD and VSD. HRCT showed focal consolidation and inflammation of both lungs, Partial collapse of right upper lobe with stenosis of rt upper lobe. Baby *Adil* is suspected to be affected with Agammaglobulinemia or B cell deficiency or Hypogammaglobulinemia and has been evaluated for pathogenic variations.

RESULTS

VARIANT OF UNCERTAIN SIGNIFICANCE RELATED TO THE GIVEN PHENOTYPE WAS DETECTED

SNV(s)/INDELS

Gene[#] (Transcript)	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification[$]
NLRP3 **(+)** (ENST00000336119.8)	Exon 8	c.2684C>A **(p.Thr895Lys)**	Heterozygous	Familial cold inflammatory syndrome 1 (OMIM#120100); CINCA syndrome (OMIM#607115)	Autosomal dominant	Uncertain Significance **(PM2)**

Parental testing is recommended, and classification of the variant(s) may change based on segregation analysis.

COPY NUMBER VARIANTS CNV(s)

No significant CNVs for the given clinical indications that warrants to be reported was detected.

VARIANT INTERPRETATION AND CLINICAL CORRELATION

VARIANT (*NLRP3 gene* gene):

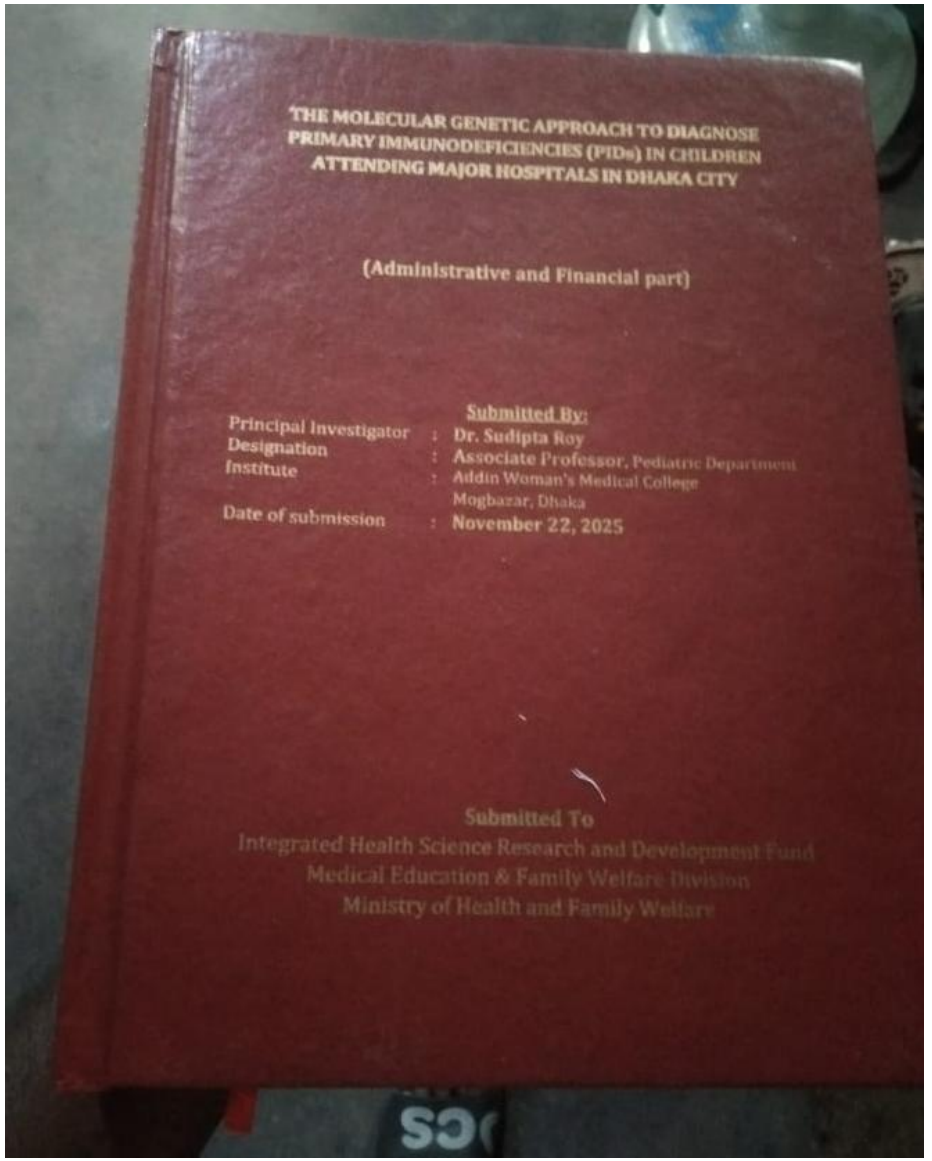
CAP ACCREDITED

Fellowship in Clinical Immunology, Center of Excellence, PGIMER, Chandigarh, India Funded by APSID, 2025

Outcomes of APSID Fellowship

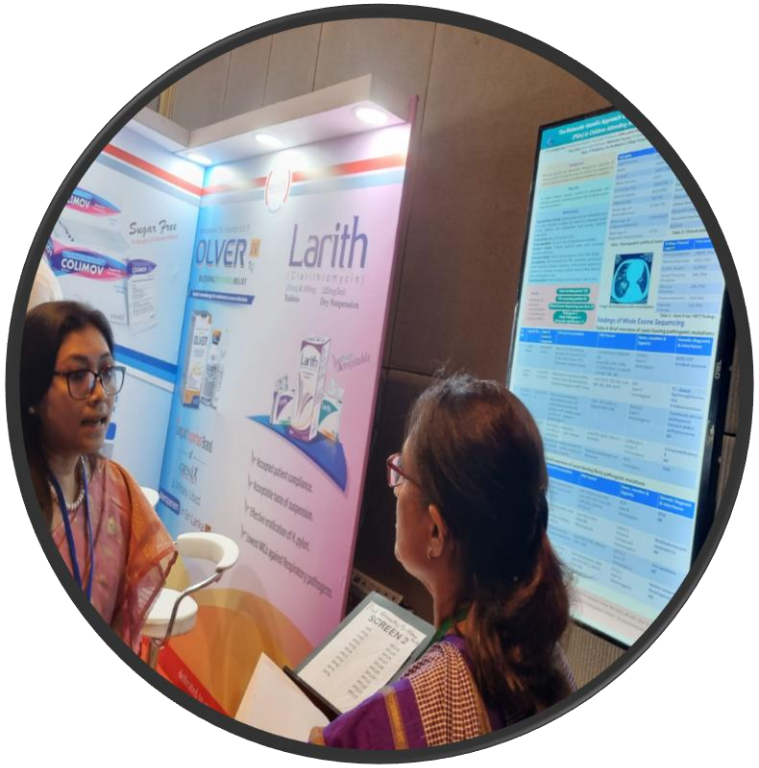
1. Improved interpretation of flow cytometry and genetic data
2. Strengthened clinical approach
3. Better investigation planning
4. Enhanced understanding of PID immune dysregulation spectrum
5. Improved treatment and follow-up strategies
6. Collaboration opportunity with PGIMER

Final project report submitted to Health Science Research,2025

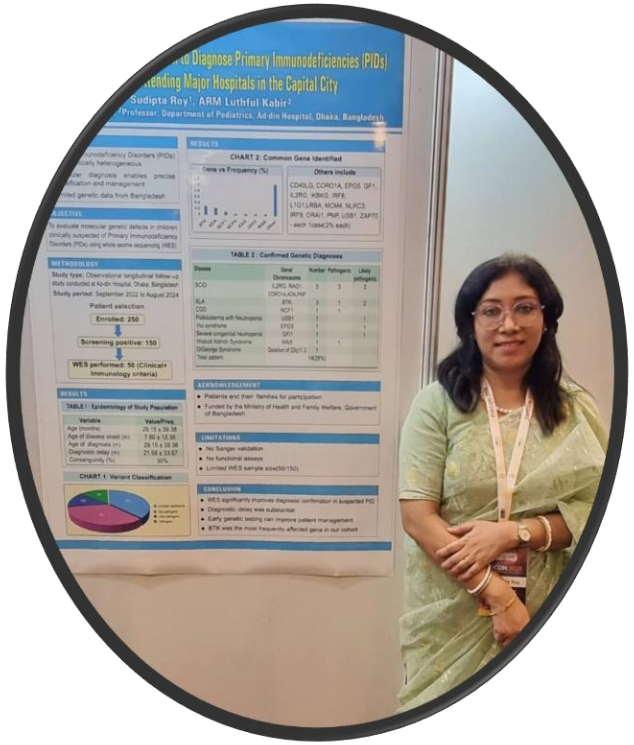


Molecular Genetic Approach to Diagnose Primary Immunodeficiency in Children Attending Major Hospital in Dhaka City

APSID-PID conference- Hong-Kong 2023



SAPA conference, Sri lanka- 2024



PIDCON conference- Kolkata - 2026

Genetic Study on Primary Immunodeficiency

The Molecular Genetic Approach to Diagnose Primary Immunodeficiencies (PIDs) in Children Attending Major Hospitals in Dhaka Cities

Authors

Sudipta Roy, Mahmuda Hassan, Marium Begum, Md. Mahbubul Hoque, Tania Islam, Farhana Rahman, Rahat Bin Habib, Zannatul Ferdous Sonia, ARM Luthful kabir

Funded by: Integrated Health Science Research & Development Fund Activity, MHFW,GOB

INTRODUCTION

- Primary Immunodeficiency Disorders (PIDs) are genetically heterogeneous
- Overall prevalence: 1in 1000 to 1in 2000, worldwide> 6 million people may be affected
- Molecular diagnosis enables precise classification and targeted management
- Limited genetic data from Bangladesh

OBJECTIVE

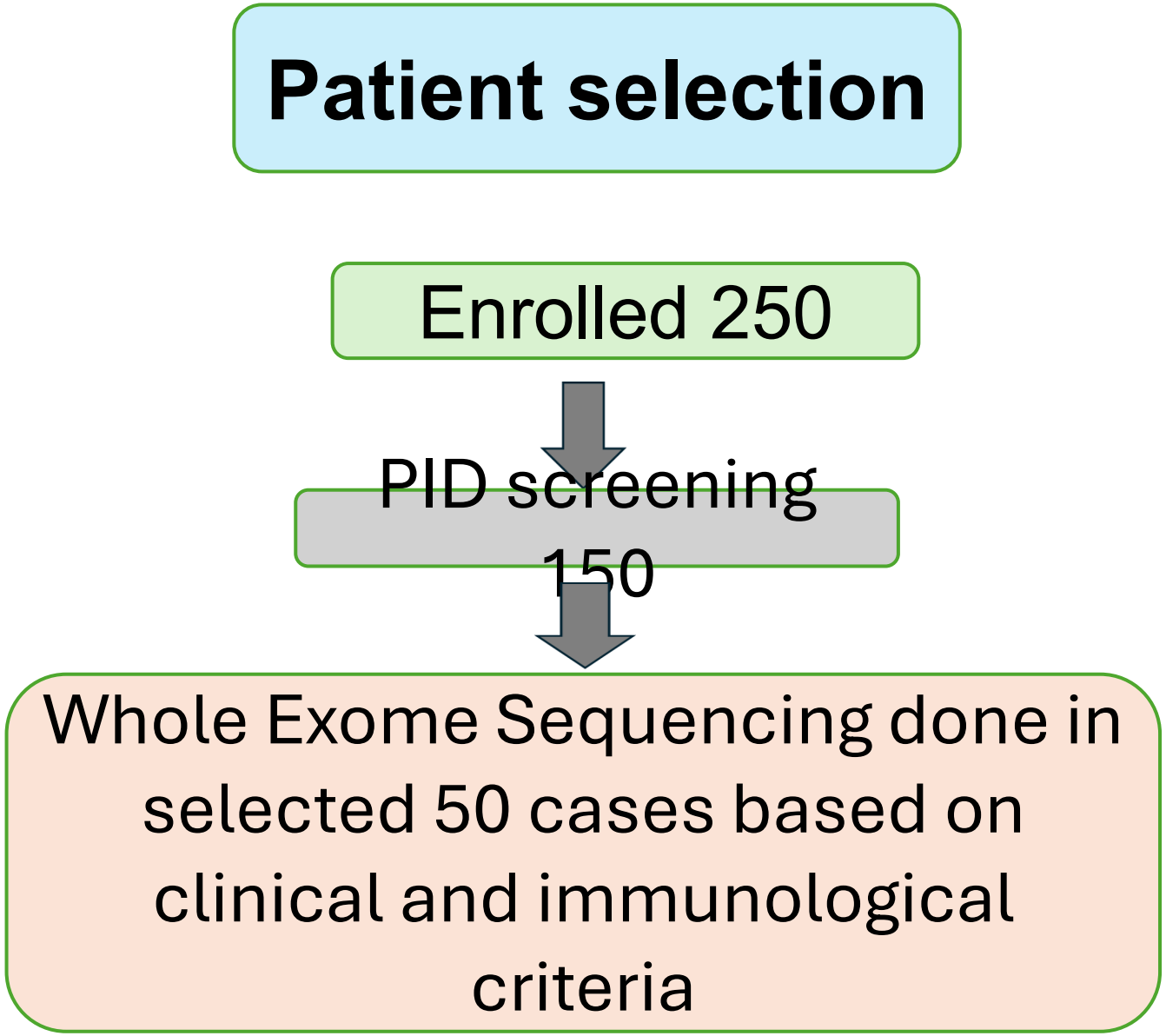
To evaluate molecular genetic defects in children clinically suspected of Primary Immunodeficiency Disorders (PIDs) using whole exome sequencing (WES)

METHODOLOGY

Study type: Observational longitudinal follow-up study conducted at five major hospitals
Study Population: 50
Study period: September 2022 to August 2024

Place of Study

1.Ad-din Women's Medical College Hospital
2. Basundhara Ad-din Medical College Hospital
3.Ad-din Barrister Rafiq-Ul-Huq Hospital
4.Bangladesh Shishu Hospital
5.Institute of Child and Mother Health


Patient selection
Enrolled 250
PID screening
150
Whole Exome Sequencing done in selected 50 cases based on clinical and immunological criteria

Inclusion criteria

Children under 18 years with recurrent or persistent infections (3 or more) like recurrent/persistent pneumonia, recurrent wheeze, otitis media, sinusitis, skin lesions, abscess, recurrent loose motion, etc (**Based on Jeffrey Modell Foundation criteria for warning signs)**

Exclusion criteria

-Children with HIV infection
-Chronic steroid ingestion
-Immunosuppressive condition (PEM, NS, Leukemia, Lymphoma),
-Any other severe or life-threatening disease/disorder

Investigations

a) PID screening tests

Complete blood count

Lymphocyte subset analysis--T lymphocytes (CD3+, CD4+, CD8+), B lymphocyte (CD19+), Natural killer cells (CD56+)

Serum Immunoglobulins- (IgG, IgA, IgM, IgE)

Complement levels (C3, C4)- in selected cases

b) Infection screening

CXR, CT scan chest, sputum examination, and gastric lavage, MT, Blood and urine C/S, x-ray paranasal sinuses, stool R/E, C/S,HIV tests, etc

Genetic study

Whole exome sequencing or clinical exome sequencing
No: selected 50 cases
Place: Medgenome, Bangaluru, India
Facilitated by: Genetic solution, Bangladesh

RESULTS

RESULTS

TABLE 1: Epidemiology Of Study Population

Variable	Frequency
Age (months) mean ±SD	29.15 ± 39.38
Age of disease onset	7.60 ± 12.36
Age of diagnosis	29.15 ± 39.38
Diagnostic delay	21.58 ± 33.57
Consanguinity	30%
M: F	1.6: 1

Table-2:Clinical manifestations

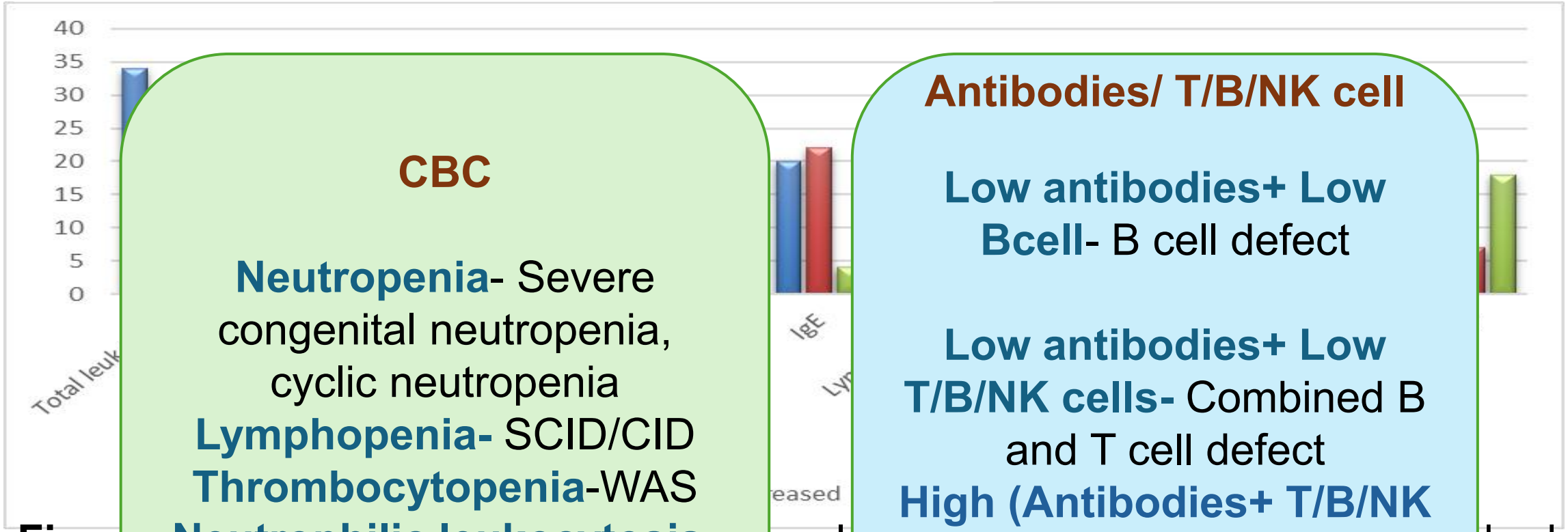
Clinical manifestations	Frequency (%)
Failure to gain weight	37 (74%)
Recurrent pneumonia	33(66%)
Recurrent wheeze	30(60%)
Recurrent candidiasis	27 (54%)
Recurrent prolong fever	27(54%)
Recurrent diarrhoea	13 (26%)
Recurrent otitis media	10(20%)
Persistent pneumonia	12 (24%)
Bronchiectasis	6(12%)
Absent tonsils	6(12%)
Recurrent sinusitis	2(4%)

Other manifestations: ITP, lymphoma, HLH, BCGoma, BCGosis, Liver/Lung abscess, hypocalcemia, neonatal seizure, Autoimmune hemolytic anaemia, poikiloderma

CXR/HRCT findings	Frequency (%)
Consolidation (Lobar)	**28(56%)**
Ground glass opacities	13(26%)
Multifocal patchy opacities	11(12%)
Bronchiectasis	**7(14%)**
Collapse	3(6%)
Pleural effusion/Empyema	3 (6%)
Multi cystic lesions	3(6%)
Small thymic shadow	2(4%)
Fibrotic change	2(4%)

Table-3:CXR /HRCT Chest findings

Figure-1: CBC, Antibodies, LSA



CBC

Neutropenia- Severe congenital neutropenia, cyclic neutropenia
Lymphopenia- SCID/CID
Thrombocytopenia-WAS
Neutrophilic leukocytosis- LAD, CGD, Severe infection

Antibodies/ T/B/NK cell

Low antibodies+ Low Bcell- B cell defect

Low antibodies+ Low T/B/NK cells- Combined B and T cell defect
High (Antibodies+ T/B/NK cell)- CGD
High IgE- Hyper IgE syndrome

Figur rmal had leuko **peni** lymph **2%).** s follow 12) wh 22 (44%) cases. Among the cases 50% had low Gated cell (CD45+) lymphocyte count, low CD3+ (52%), CD3+ CD8+ (60%), CD19+ (68%), CD65+ (36%), CD3+CD8+ (24%) and altered CD4+CD8+ was 56%.

Figure 2: Immunological diagnosis among the study

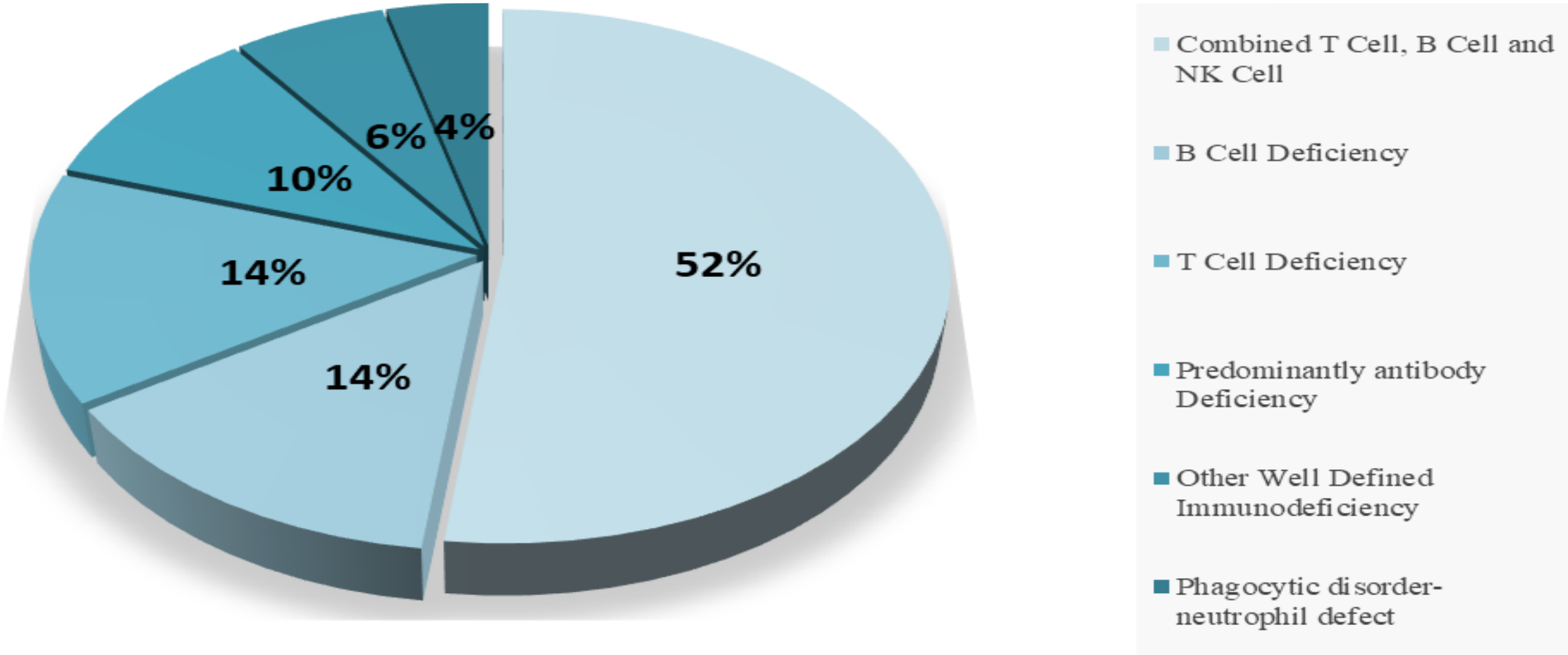


Figure-2:Combined T cell, B cell and NK cell deficiency (52%) followed by T cell deficiency (14%), B cell deficiency (14%), Predominantly Antibody deficiency (10%), Other well defined immunodeficiency syndrome (6%) and Phagocytic disorders- neutrophil defect (4%).

Figure-3: Affected Gene

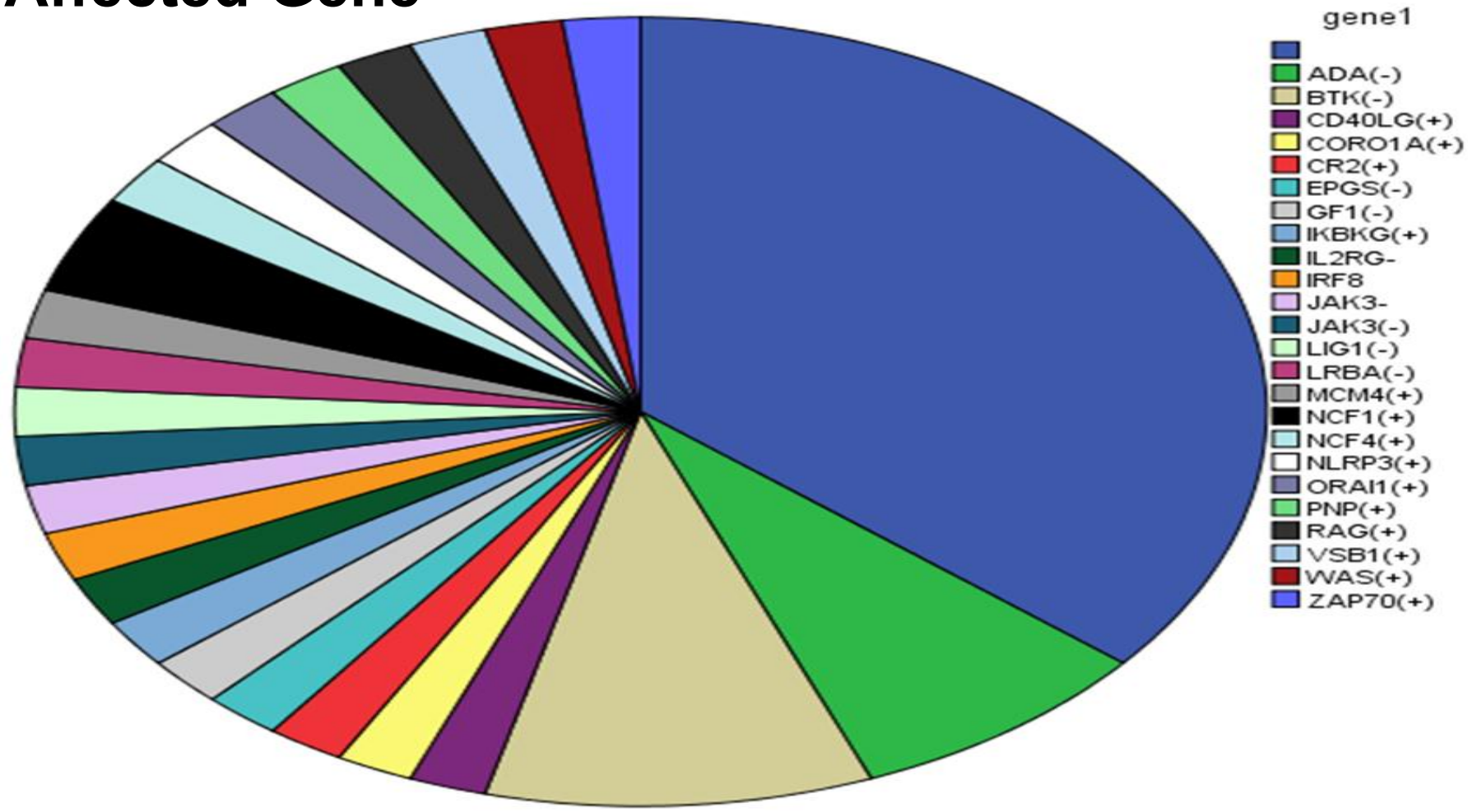
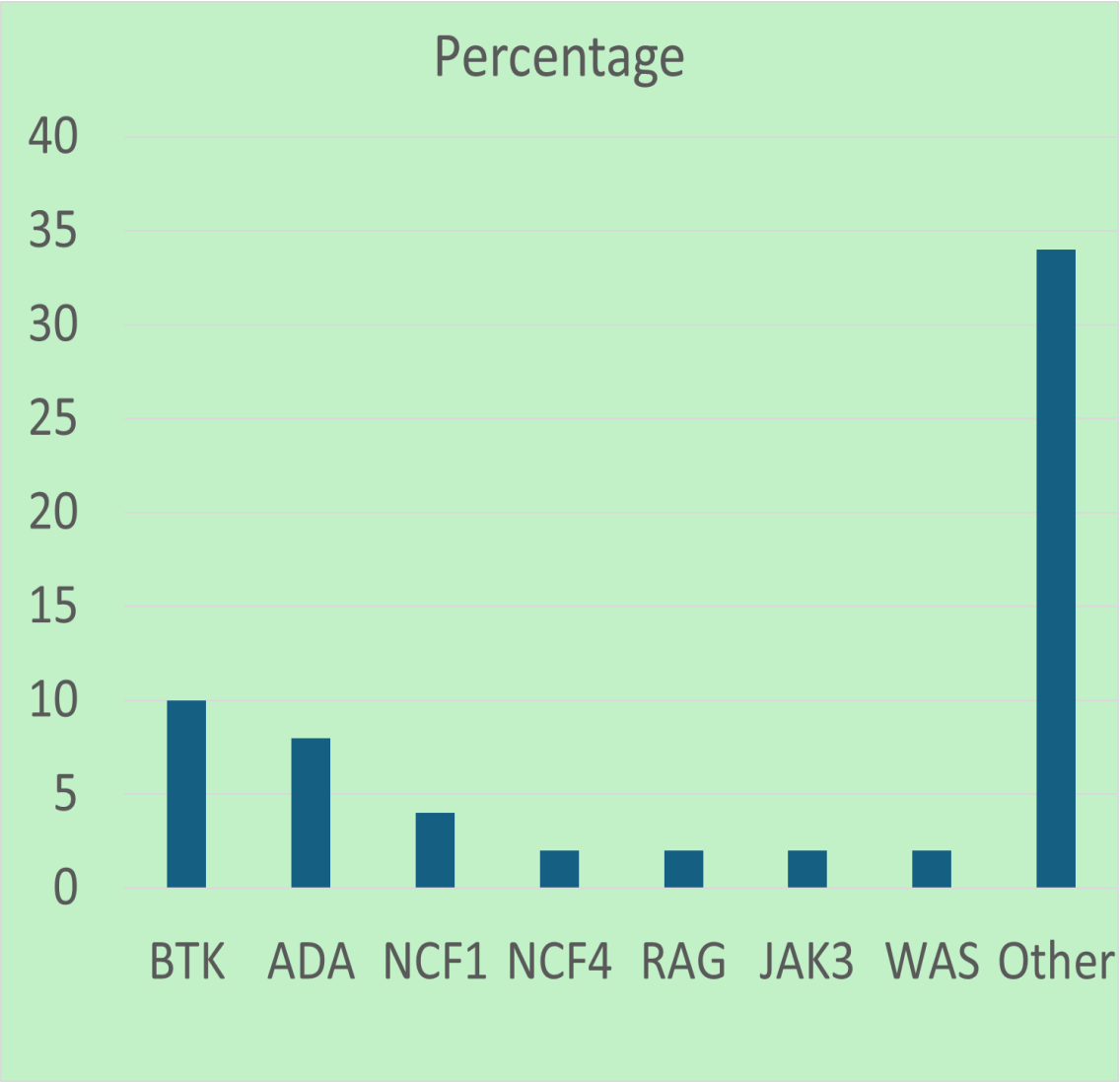


Figure-3:Whole exome sequencing revealed genetic mutations in 32 cases, accounting for 64% of the total. The most frequently identified mutations included BTK (-) in 10% of cases, ADA (-) in 8%, and NCF (+) in 4%. Other mutations observed were CD40LG (+), CORO1A (+), CR2 (+), EPGS (-), GF1 (-), IKBKG (+), IL2RG (-), IRF8, JAK3 (-), LIG1 (-), LRBA (-), MCM4 (+), NCF4 (+), NLRP3 (+), ORAI1 (+), PNP (+), RAG (+), VSB1 (+), WAS (+), and ZAP70 (+), each present in 2% of cases.

Figure-4: Common gene identified



Others include: CD40LG, CORO1A, EPGS, GF1, IL@RG, IKBKG, IRF8, L1G1,LRBA, MCM4, NLRP3, IRF8, ORA11, PNP, VSB1, ZAP70,each 1case(2% each)

Figure-5: Genetic diagnosis(N-50)

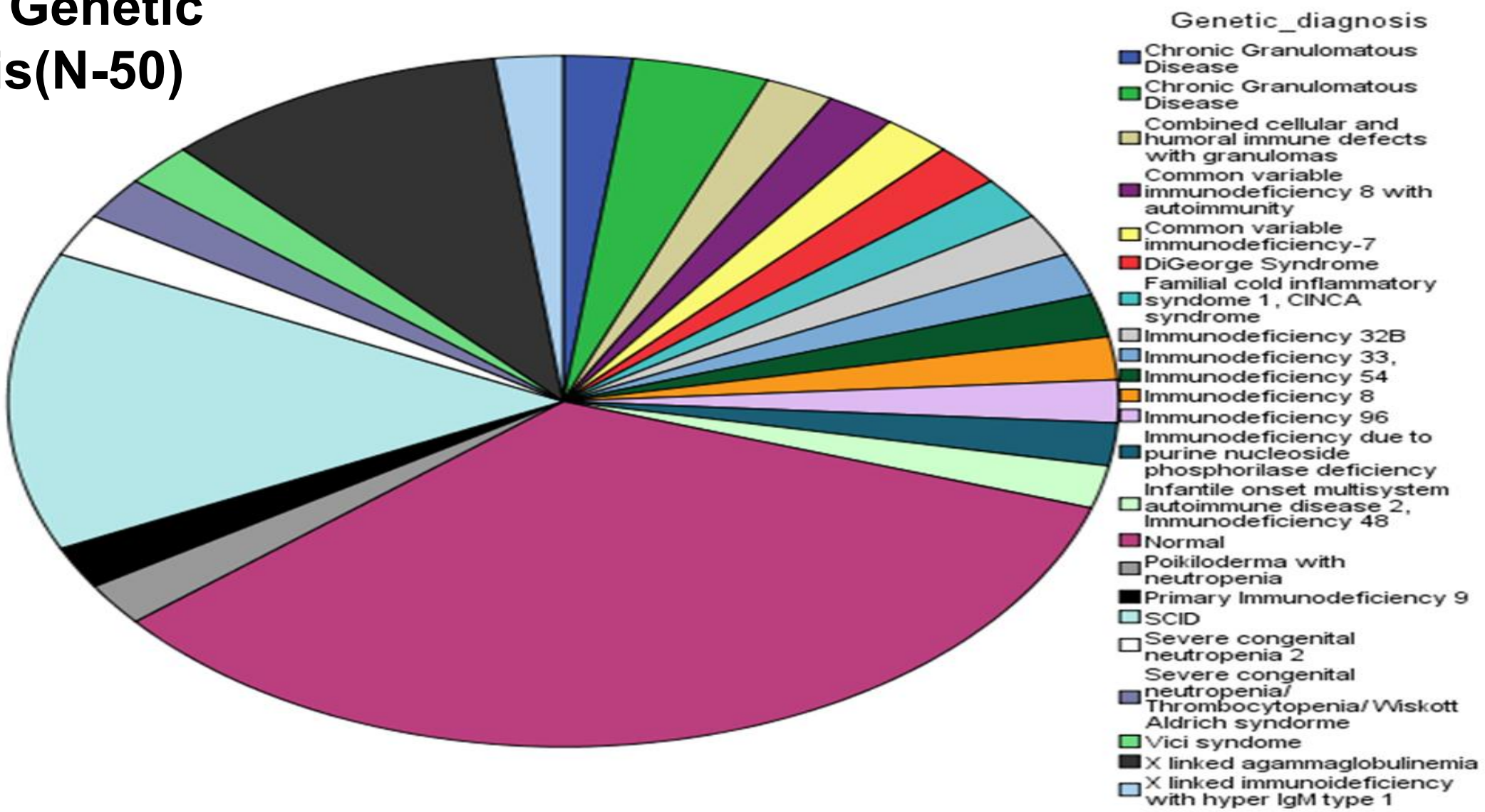
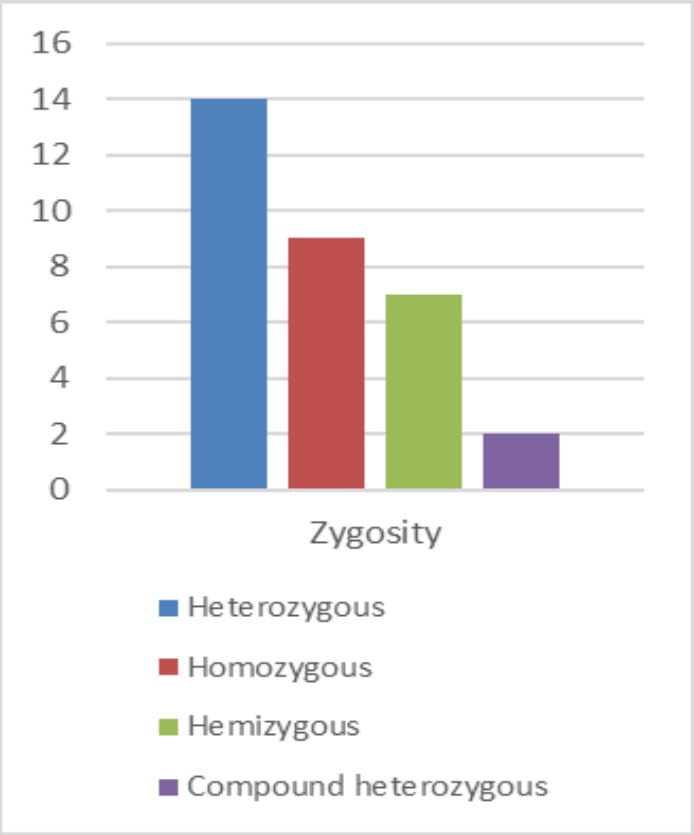
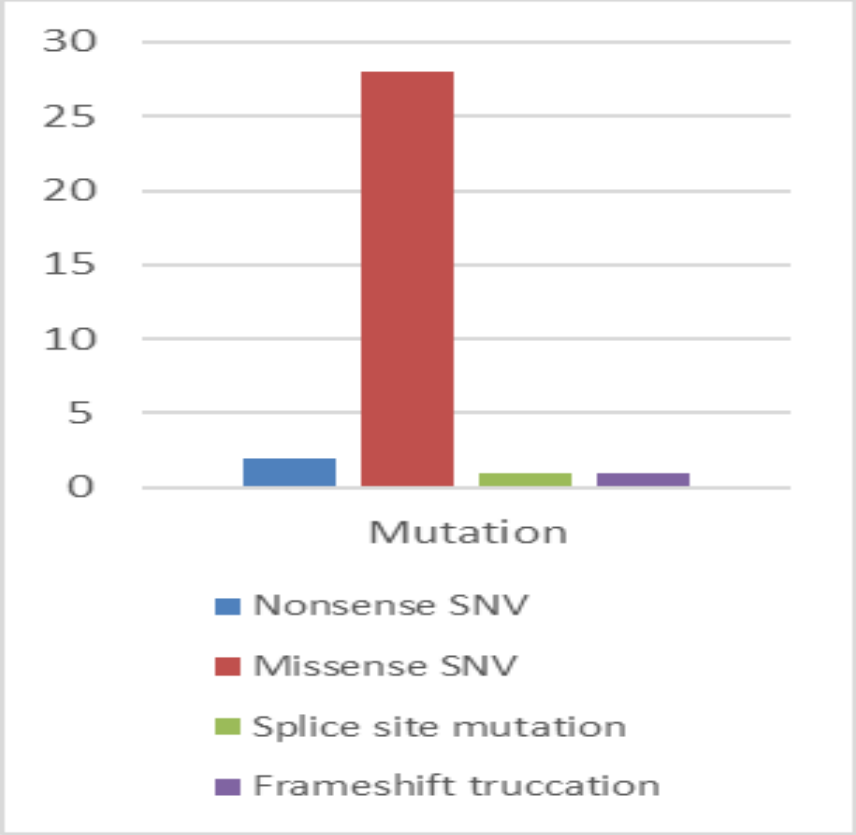


Figure-5: Severe Combined Immunodeficiency (SCID) was identified in 20% of the cases, with X-linked agammaglobulinemia occurring in 10%. Chronic granulomatous disease was noted in 6% of the cases, while DiGeorge syndrome, Wiskott-Aldrich syndrome, Severe congenital neutropenia, Poikiloderma with neutropenia, Vici syndrome were observed in 2% each.

Figure-6: Zygosity, Variant types, Mode of inheritance





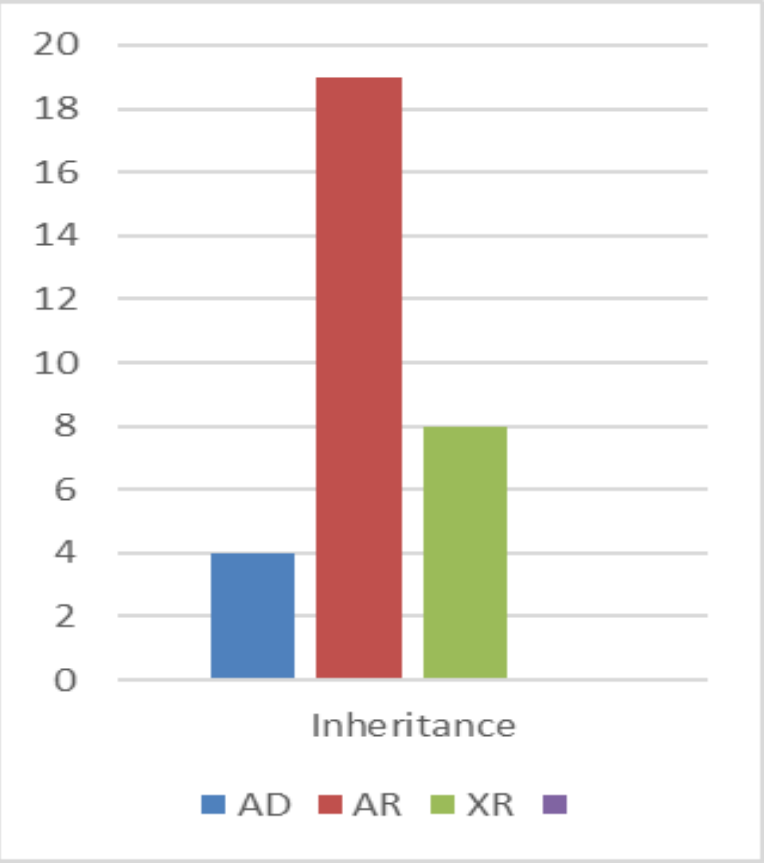


Figure 6: Most of the study cases had AR (34%) inheritance with missense SNV (52%) and had homozygosity(16%) while 28% had heterozygosity.

Figure-7: ACMG variant classification

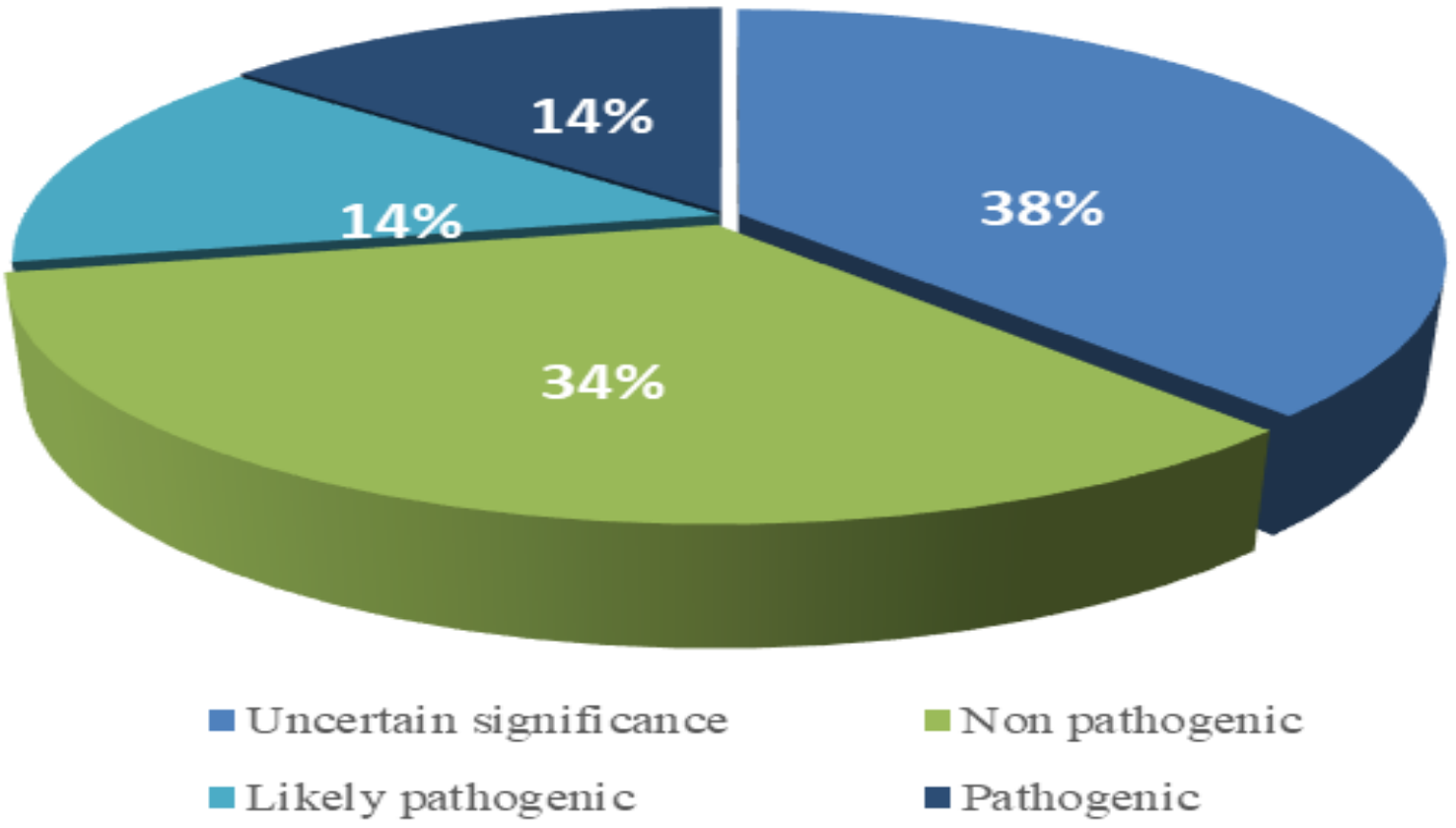


Figure 7: Pathogenic mutation was detected in 14%, likely pathogenic 14%, uncertain significant 38%, non-pathogenic 34%.

Table-4: Confirmed genetic diagnosis

Disease	Gene/Chromosome	Number	Pathogenic	Likely pathogenic
SCID	IL2RG, RAG1,CORO1A, ADA,PNP	5	3	2
XLA	BTK	3	1	2
CGD	NCF1	1	1	
Poikiloderma with Neutropenia	USB1	1		1
Vici syndrome	EPG5	1		
Severe congenital neutropenia	GFI1	1		1
Wiskott Aldrich Syndrome	WAS	1	1	
Di George Syndrome	Deletion of 22q11.2	1		
Total patient		14 (28%)		

LIMITATION

- Sanger sequencing not done
- Functional assay- not available
- Limited sample size(50/150)

CONCLUSION

- WES improves diagnostic confirmation in suspected PID
- Diagnostic delay was significant
- Early genetic testing can improve patient management
- BTK was the most frequently affected gene in our cohort.
- SCID was the most common diagnosis.

ACKNOWLEGEMENT

- Patient and family for participation
- Ministry of Health and Family Welfare, Government of Bangladesh
- AWMCH,BAMCH,ABRH, ICMH, BSH
- Microbiology lab, BMU
- Genetic solution, Bangladesh
- Medgenome, India
- Co-Investigators
- Dr. Turzo Mallik
- Md. Liakat Hossain
- Dr. Dipa Saha

Brief overview of important clinical cases

Severe Combined Immunodeficiency

Gene-IL2RG

Age:1 year, Male
Onset: 5 M

C/F: R. pneumonia, diarrhoea, **BCGosis,** dermatitis, FTT, fever,

Dissiminated BCG Disease

Antibodies: all very low

ACMG classification: Pathogenic X-LR

Gene-RAG-1

Age-3.5 yrs, Male
Onset: 1.5 years

CF: R. pneumonia,

Autoimmunity

splenomegaly

LSA: Low CD4

Antibodies: Normal

ACMG: Pathogenic AR

Gene-CORO1A

Age- 3 years M
Onset-2 M

CF- R. pneumonia,

ab

Malignancy

from lymphoma

LSA: T-B-NK+

Antibodies: Normal

ACMG: pathogenic AR

Gene-ADA

Age : 7 m, Male
Onset-3 M

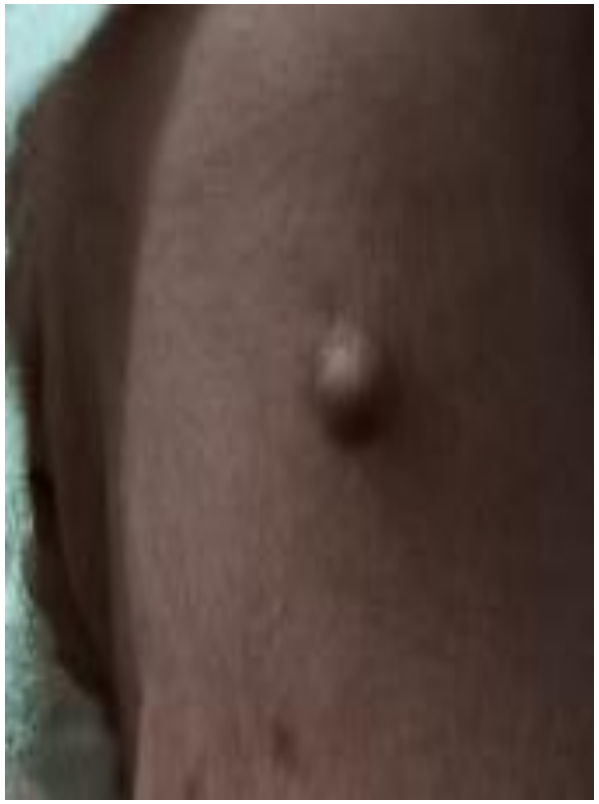
CF: R. pneumonia, diarrhoea, oral thrush, FTT

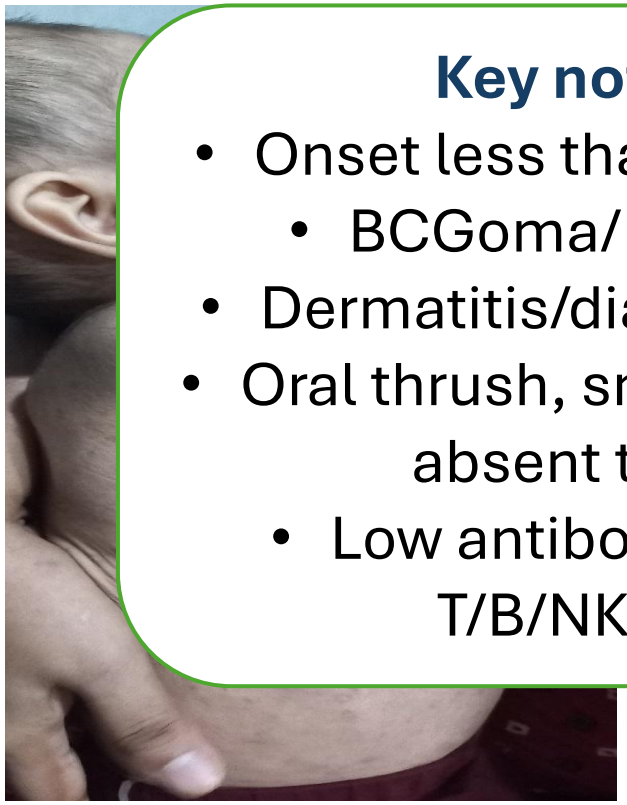
LSA- T-B+NK-

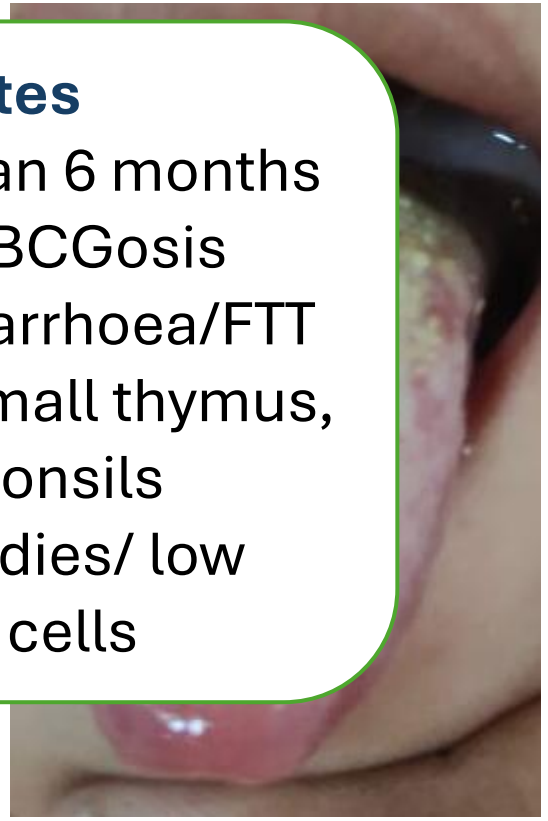
Antibodies: Normal

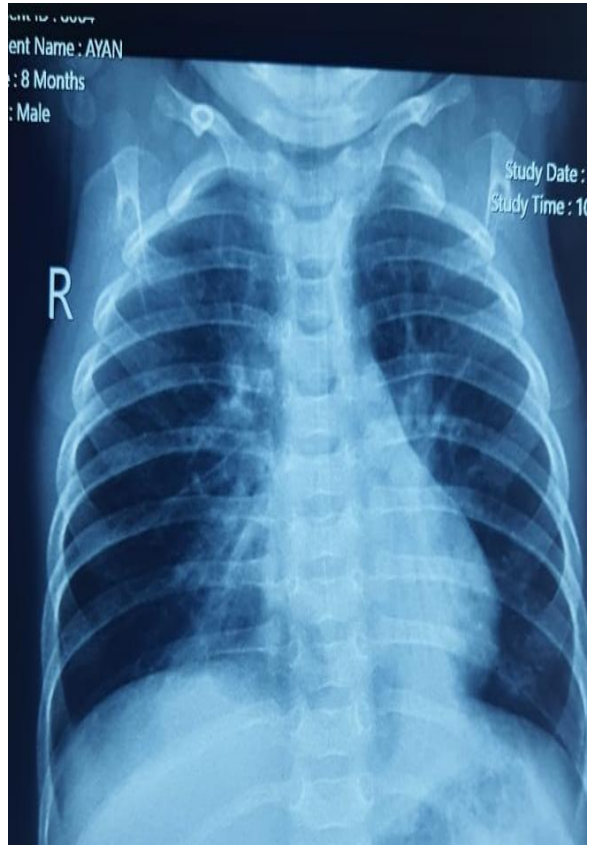
ACMG: Pathogenic AR

Severe Combined Immunodeficiency









Key notes

- Onset less than 6 months
- BCGoma/BCGosis
- Dermatitis/diarrhoea/FTT
- Oral thrush, small thymus, absent tonsils
- Low antibodies/ low T/B/NK cells

BCG site abscess- T cell

Chronic dermatitis- T cell

Oral thrush- T cell

Small thymic shadow-T cell

X-Linked Agammaglobulinaemia-B cell defect

Gene-BTK

Age: 8yrs/ Male
Onset:1.5 yrs
CF: Rec. pneumonia, diarrhoea, OM, skin abscess, FTT
LSA: CD19-4(o.1%)
Antibodies: low

ACMG: Likely pathogenic
X-LR

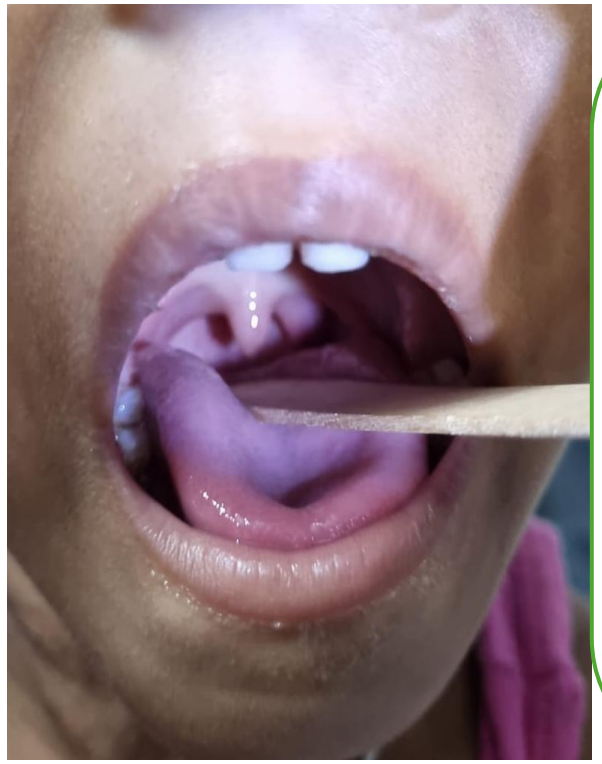
Gene BTK

Age-8yrs, Male
Onset:6months
CF: Rec. pneumonia, bronchiectasis, otitis media, FTT
LSA-CD19-0.1%
Antibodies- low
ACMG: Pathogenic
X-LR

Gene BTK

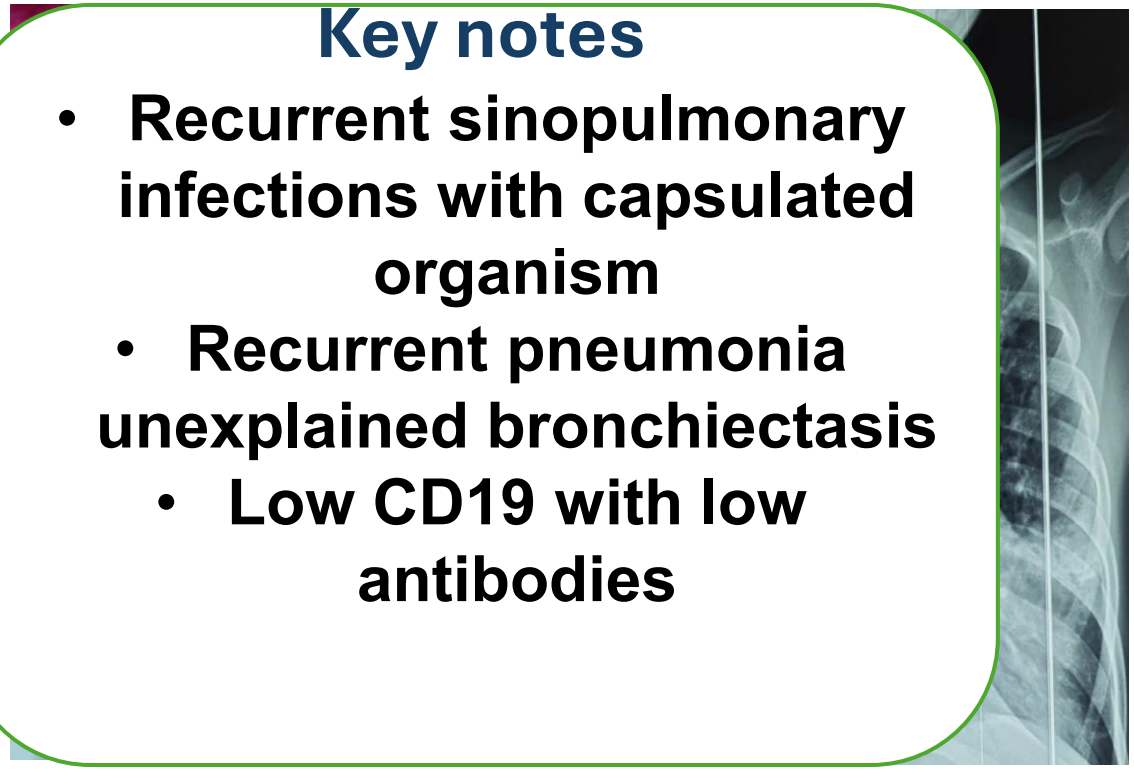
Age-16 yrs, M
Onset:6yrs
CF: Recurrent pneumonia, **Bronchiectasis,** FTT
LSA- CD19-0.5%
Antibodies-Low
ACMG- Likely pathogenic
X-LR

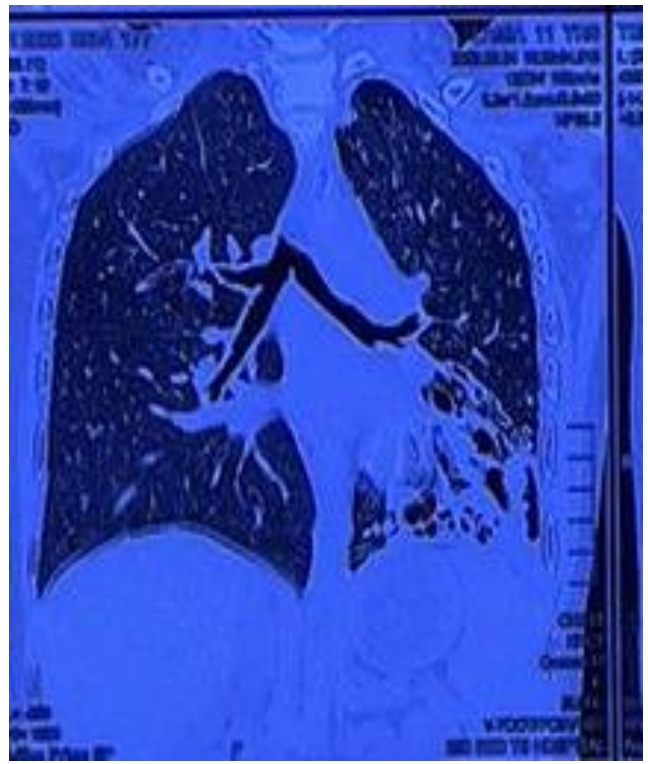
X-Linked Agammaglobulinaemia



Key notes

- Recurrent sinopulmonary infections with capsulated organism
- Recurrent pneumonia unexplained bronchiectasis
- Low CD19 with low antibodies





Absent tonsils-B cell

Scar from old skin abscess

Collapse consolidation

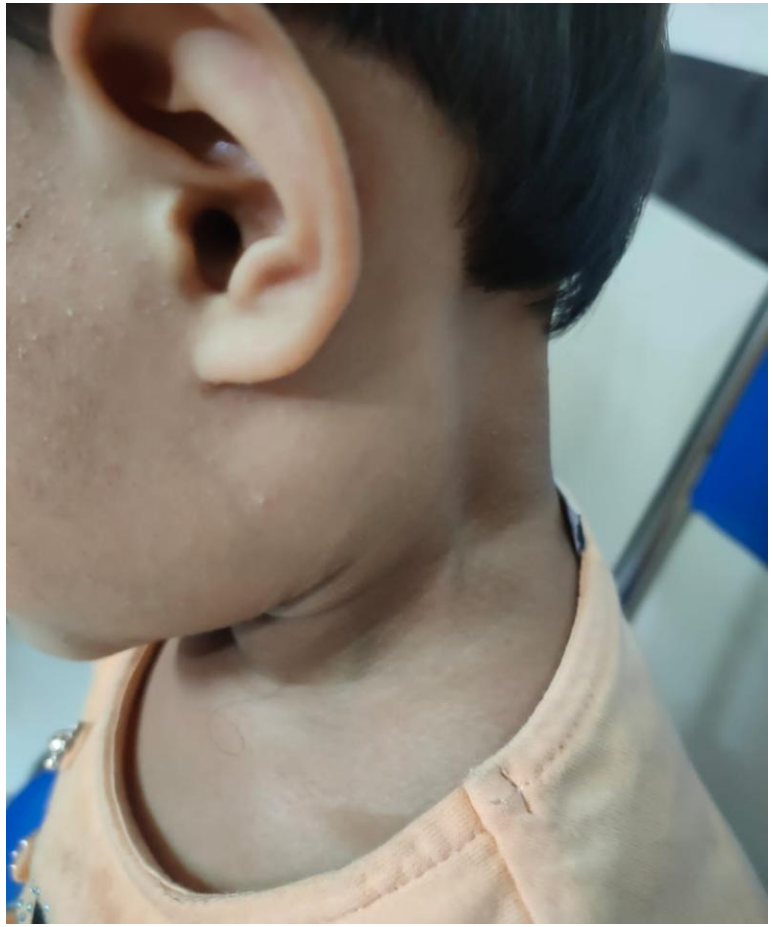
Bronchiectasis

Chronic Granulomatous Disease

Age-3y
CF: Rec
bronchi stent
lympha
Misdiag ed
ATT for
Lymph n a
Antibodies: S.IgA, IgG- High
ACMG- Pathogenic , AR

Key notes

- **Rec. pneumonia/ bronchiectasis**
- **Lymphadenopathy /hepatosplenomegaly**
- **Organ abscess**
- **Non-caseating granuloma**
- **High- neutrophil, leukocyte, antibodies, subsets**



Cervical Lymphadenopathy

Wiskott- Aldrich syndrome

Gene-WAS

Age-7 years, Male

Onset- 1

CF- Rec. ic skin rash, dia Rec. URTI, or skin abscess,

-Misdiag c ITP

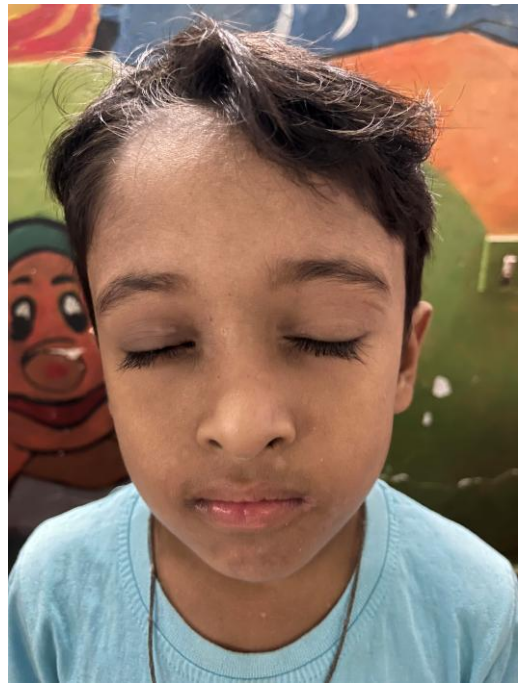
CBC- persistent thrombocytopenia

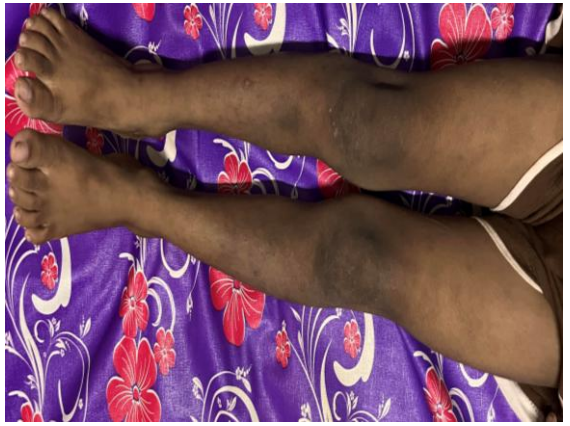
High-IgA,IgE, Low-IgM

ACMG- Pathogenic X-LR

Key message

Persistent thrombocytopenia with small platelets + systemic features- Exclude WAS





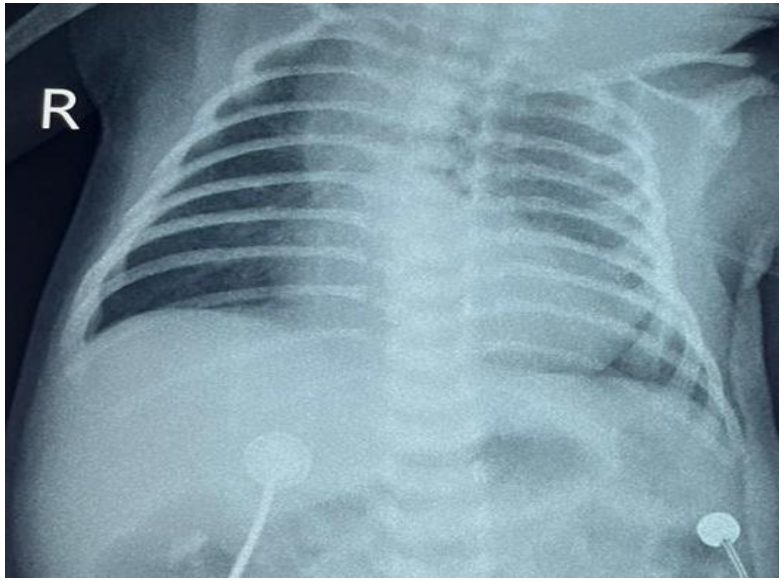
Hyper-pigmented Skin, Dermatitis

Digeorge Syndrome

Deletion of 22q11.2

Age -1 month, Male,
Onset-D1
CF- Neona
CHD(VSD
sided aorti
Low- Calc
Antibodie
LSA:Low- CD4. Low-CD19
FISH: Deletion of 22q11.2

Key message
Hypocalcemic tetany with CHD, recurrent infection- Exclude Digeorge syndrome



Poikiloderma with neutropenia

Gene-USB1 (+)

Age-7 years, Female,Onset-1.5 months

CF: F

Bron

Fever

lesion

Low-

Low-

Antibodies- Normal

Skin biopsy- Poikiloderma

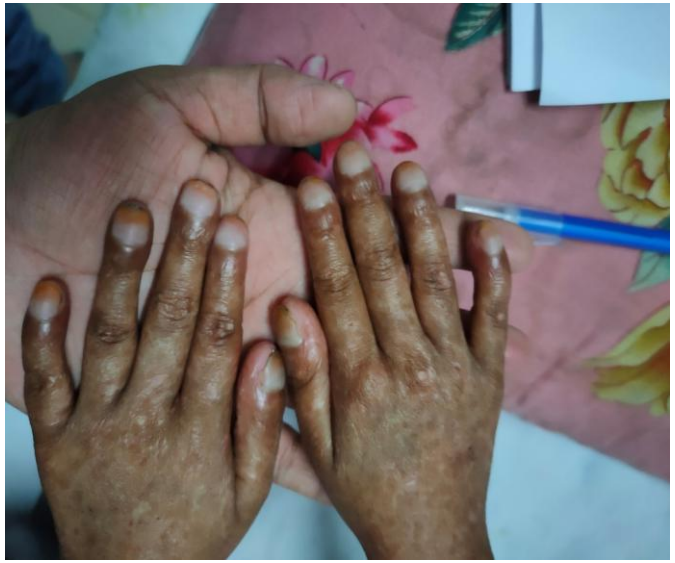
ACMG- Likely pathogenic, AR

Key notes

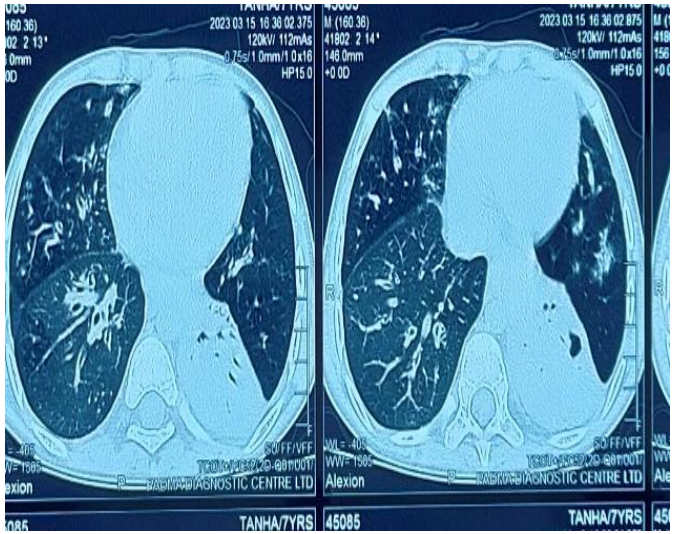
-We should practice thorough systemic evaluation

-Multisystem involvement is the key to suspect PID

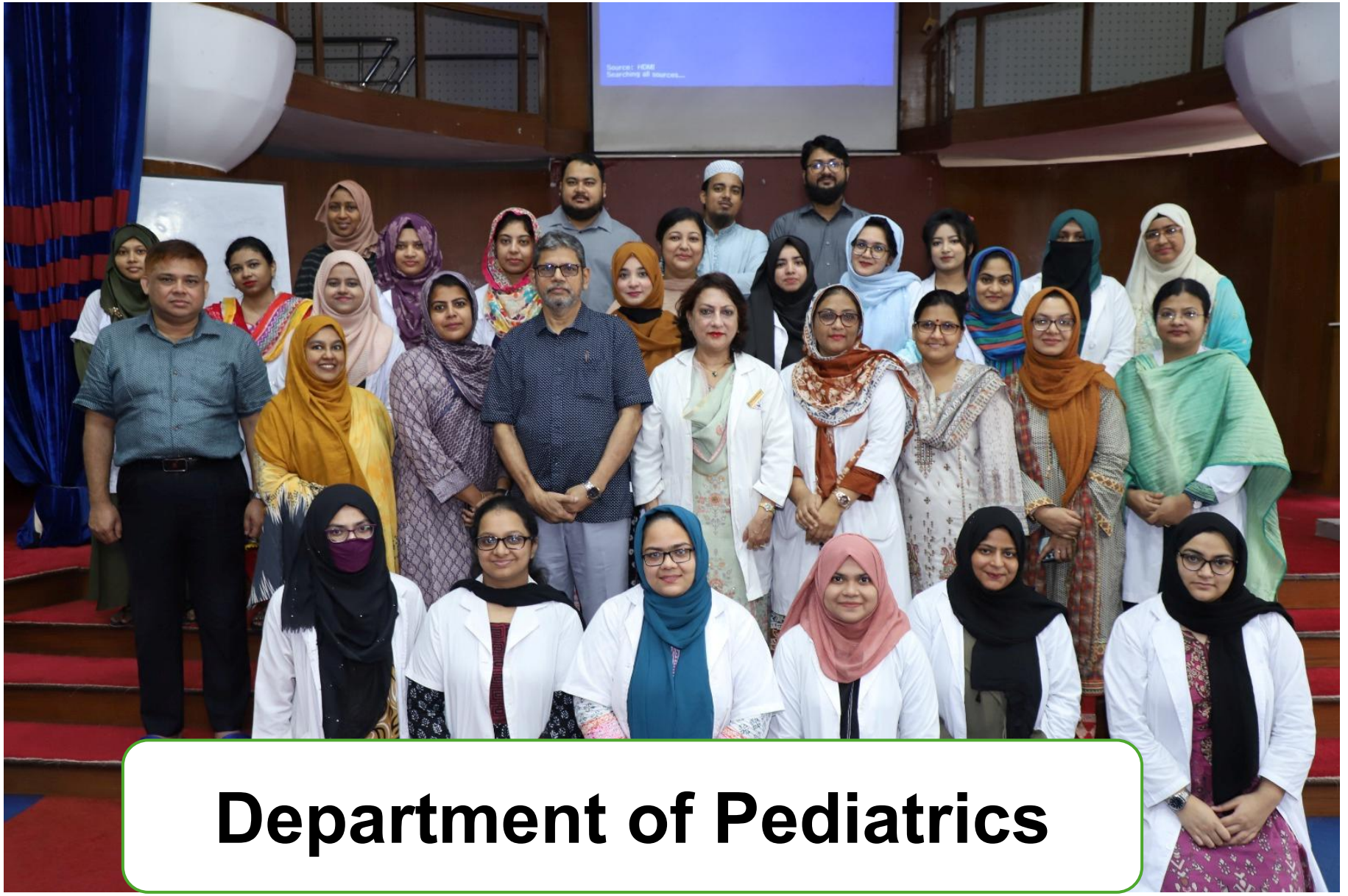
-WES- helpful to establish early diagnosis in rare PIDS



Clubbing/Poikiloderma



Bronchiectasis



Department of Pediatrics

Acknowledgement

- Prof. Sayeeda Anwar
- Prof. ARM Luthful Kabir
- Prof. Mahmuda Hassan
- Prof. Nahid Yasmin
- Dr. Vignesh Pandiarajan(PGIMER)
- Prof. Surjit Singh(PGIMER)
- Prof. SK Kabra(AIIMS)
- Microbiology dept. BMU
- AWMCH
- Pulmonology unit, AIIMS, New Delhi
- Immunology Unit, PGIMER, Chandigarh


thank
you