Chronic granulomatous disease (CGD) & Mendelian susceptibility to mycobacterial diseases (MSMD)

Tandakha Nd DIEYE
Prof, PhD,
University Cheikh Anta Diop, Dakar
IPIC, 2019, Madrid, 7\textsuperscript{th} November
OUTLINE

- INTRODUCTION
- PATHOGENESIS
- CLINICAL FEATURES
- DIAGNOSIS
- TREATMENT
- CONCLUSION
Introduction

- Chronic-granulomatous disease (CGD) is a rare inherited primary immunodeficiency syndrome affecting 1 in 250,000 individuals.

- Differents genetic Mutations are found in five structural genes to any of the subunits of NADPH oxidase.

- CGD is a genetically heterogeneous disease with an X-linked recessive (XR-CGD) form caused by mutations in the CYBB gene, and an autosomal recessive (AR-CGD) form caused by mutations in the CYBA, NCF1, NCF2, or NCF4 genes.
Introduction

Structural subunits of the NADPH Enzyme

NADPH oxidase system/superoxide formation

Rider, N. L. Journal of the Pediatric Infectious Diseases Society, 2018
The X-linked CGD (74% of cases) is caused by cytochrome b-245 beta chain (CYBB) gene mutations affecting \( \text{gp91}^{\text{phox}} \) or Nox2.

Defects in the neutrophil cytosolic factor 1 (NCF1) gene (20% of cases) affecting the \( \text{p47}^{\text{phox}} \) subunit is the most common form of autosomal recessive CGD.

Defects in the neutrophil cytosolic factor 2 (NCF2) affect the \( \text{p67}^{\text{phox}} \), those in the NCF4 gene affect the \( \text{p40}^{\text{phox}} \) and Mutations in the CYBA genes affect \( \text{p22}^{\text{phox}} \) account for the rarer (<5%) of all cases of CGD.
Introduction

A Genetic defect in CGD is particularly related to MSMD

More than 650 mutations in the CYBB gene have been found to cause chronic granulomatous (CGD)

Published: October 29, 2019

Three (3) forms of XR-CGD have been described, based on X91 protein levels: X91° (no protein), X91– (low levels of protein) and X91+ (normal levels of protein)
Introduction

A Genetic defect in CGD is particularly related to MSMD

- **Mutation gene CYBB**: Q231P, T178Q

---

**Figure**: MSMD causing mutations of CYBB: The exons of each gene are shown, designated by roman numerals. Q231P and T178Q: recessive mutation associated with partial deficiency function

- **Inheritance for MSMD**: X-linked recessive

- **Selective functional impairment** of superoxide production limited to mono-derived macrophage and B cells
Mutation gene **CYBB**: a de novo mutation in CYBB that results in the single-amino acid substitution C126R in the gp91phox protein.

- **X-linked CGD** in a patient with recurrent pneumonia and **regional BCGitis** who had no family history

---

A New Genetic defect in CGD in exon 5 is *de novo mutation*

de Albuquerque JAT et al, Front Pediatr. 2018
Introduction

Genetic etiologies of MSMD

- Rare PID caused by “inborn errors of IFN-y immunity”

From 1996, 13 genes (IFNGR1, IFNGR2, IL12RB1, IL12RB2, IL23R, IL12B, ISG15, STAT1, TYK2, IRF8, CYBB, NEMO and SPPL2A)

- From 1996 discovery of the first genetic etiology MSMD has been described in 501 individuals, 356 kindreds, from 57 countries, in Five continents (see figure)

Introduction

Genetic etiologies of MSMD

- 23 different genetic disorders
  - 15 autosomal recessive
  - 6 autosomal dominant
  - 2 X-linked recessive *(NEMO)*, and CYBB in CGD
  - Impact of the mutation, mode of transmission, affecting expression/function or both.

- Controlling production or response to IFNγ
# Overview of diseases underlying MSMD

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Defect</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL12RB1</td>
<td>AR</td>
<td>C</td>
<td>E-</td>
</tr>
<tr>
<td>IL12B</td>
<td>AR</td>
<td>C</td>
<td>E+</td>
</tr>
<tr>
<td>ISG15</td>
<td>AR</td>
<td>C</td>
<td>E-</td>
</tr>
<tr>
<td>SPPL2A</td>
<td>AR</td>
<td>C</td>
<td>E- or E+</td>
</tr>
<tr>
<td>IRF8</td>
<td>AD</td>
<td>P</td>
<td>E+</td>
</tr>
<tr>
<td>TYK2</td>
<td>AR</td>
<td>C</td>
<td>E-</td>
</tr>
<tr>
<td>IFNGR1</td>
<td>AR</td>
<td>C</td>
<td>E+</td>
</tr>
<tr>
<td>IFNGR2</td>
<td>AR</td>
<td>C</td>
<td>E-</td>
</tr>
<tr>
<td>IFNGR2</td>
<td>AR</td>
<td>P</td>
<td>E+ of mutant protein</td>
</tr>
<tr>
<td>IFNGR2</td>
<td>AR</td>
<td>P</td>
<td>E+ of WT protein</td>
</tr>
<tr>
<td>STAT1</td>
<td>AD</td>
<td>P</td>
<td>E+P-</td>
</tr>
<tr>
<td>STAT1</td>
<td>AD</td>
<td>P</td>
<td>E+B-</td>
</tr>
<tr>
<td>STAT1</td>
<td>AD</td>
<td>P</td>
<td>E+P-B-</td>
</tr>
<tr>
<td>NEMO (IKBKG)</td>
<td>XR</td>
<td>P</td>
<td>E+</td>
</tr>
<tr>
<td>CYBB</td>
<td>XR</td>
<td>P</td>
<td>E+</td>
</tr>
</tbody>
</table>
Selective susceptibility to weakly virulent mycobacteria (Bacille Calmette Guerin BCG vaccine strains, Environmental mycobacteria EM, Non Tuberculosis mycobacteria NTM)

- Localized to disseminated; One or more mycobacterial species; May or may not recur
- Severe disseminated forms of tuberculosis
- Invasive infections by other intra-macrophagic bacteria & fungi, Virus & Parasites.
How Chronic granulomatous disease (CGD) can be related to Mendelian susceptibility to mycobacterial diseases (MSMD)?

- Geographic Distribution
- Inborn defects of the IL-12/IFN-γ pathway
- CYBB (gp91phox) mutation in MSMD & in CGG
- Recurrent bacterial and fungal infections
- Invasive microorganisms including mycobacteria
Proteins for which mutation of the corresponding gene has been recognized to cause **solely MSMD** (IFN-γ R1, IFN-γ R2, SPPL2a, NEMO, gp91^{phox}, IL12p40, IL12Rβ1, ISG15) are depicted in black, those responsible for **syndromic MSMD** (JAK1, ROR-γ) are depicted with vertical lines, those that can cause either MSMD or syndromic MSMD (IRF8, STAT1, TYK2) are depicted with cross lines.
Estimated TB incidence rate in 2016
Pathogenesis

NADPH oxidase complex

CYBA

CYBB

Mycobacteria

IL-12/IFN-γ signaling pathway (figure)

Li, X, et al, Seminars in immunopathology 2008
Pathogenesis

IL-12/IFN-γ signaling pathway in MSMD

Autosomal

- IFN-γR1
- IFN-γR2
- IL-12R-β1
- IL-12 p40 (IL12B)
- STAT1
- interferon regulatory factor 8 (IRF8)
- interferon-stimulated gene 15 (ISG15)
- tyrosine kinase 2 (TYK2)
- the zinc-finger transcription factor GATA2 (GATA2)

XR

- IKBKG (encodes nuclear factor kappa B essential modulator [NEMO])
- CYBB (encodes gp91phox)

autosomal recessive (AR) or autosomal dominant (AD)
OUTLINE

- INTRODUCTION
- PATHOGENESIS
- CLINICAL FEATURES
- DIAGNOSIS
- TREATMENT
- CONCLUSION
Clinical features

**Complete defects**

- Onset early childhood
- Disseminated diseases

**Partial defects or less severe defects**

- Adolescence
- Milder recurrent infection
In CGD

- **Infections occur due to** pyogenic bacteria most commonly *Staphylococcus aureus* and the *Burkholderia cepacia* complex is characteristic.

- **Fungal infections** with *Aspergillus* spp., *Candida* spp. are also common.

- Other infective agents include intracellular organisms *Mycobacterium tuberculosis* and *Serratia marcescens*.

- Clinical presentations include recurrent cutaneous and deep seated abscesses such as hepatic abscesses, osteomyelitis and, septicemia *(Zhou Q, J Clin Immunol. 2018)*

Clinical features

In CGD

- **Pneumonia** is the most common pulmonary infection in **CGD**, and patients may also have complications as: lung abscesses, empyema, and hilar lymphadenopathy.

- **CGD patient** may develop a type of **fungal pneumonia**, called mulch pneumonitis, which causes **fever and shortness of breath** after exposure to decaying organic materials.

- **CGD Patients** are also predisposed to frequent and atypical infections *(Chiriaco et al. 2015)*.
Clinical features

In CGD

- Patients with characteristic **chronic granuloma formation** throughout the body caused by **recurrent infection** and **gastrointestinal inflammation** (Marciano et al. 2004)

- **Granulomas** can be particularly dangerous if they **obstruct and inflame** the gastrointestinal tract (Song et al. 2011).

- **Granulomas** cause **symptomatic inflammation** throughout the body (Bagaitkar et al. 2015; Chiriaco et al. 2015)
Clinical features

CGD and mycobacterial diseases

A retrospective analysis of 71 cases on Mycobacterial diseases in patients with chronic granulomatous disease by CONTI et al, 2016

- 44% of patients had tuberculosis,
- 75% presented with adverse effects of BCG vaccination;
- 18% had both tuberculosis and BCG infections.
- None of these patients displayed clinical disease caused by environmental mycobacteria, Mycobacterium leprae, or Mycobacterium ulcerans.

Clinical features

CGD and mycobacterial diseases

A retrospective analysis of 71 cases on Mycobacterial disease in patients with chronic granulomatous disease by Conti et al, 2016

- Most patients (76%) also had other pyogenic and fungal infections,
- 24% presented solely with mycobacterial disease.

In summary: Mycobacterial disease is relatively common in patients with CGD living in countries in which tuberculosis is endemic, BCG vaccine is mandatory, or both

Conti et al, Journal of Allergy and Clin Immuno 2016
## Clinical features

<table>
<thead>
<tr>
<th>In MSMD</th>
<th>Disease onset</th>
<th>BCG</th>
<th>Samonella</th>
<th>other possible infections</th>
<th>Granuloma</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STAT1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>Infancy (die early without HSCT)</td>
<td>Yes</td>
<td>No</td>
<td>Tuberculosis, fulminant viral infection (mainly herpes)</td>
<td>Yes</td>
<td>Poor</td>
</tr>
<tr>
<td>Partial</td>
<td>Infancy/early childhood/adolescence</td>
<td>Yes</td>
<td>Yes (50%)</td>
<td>Severe, curable viral infection (mainly herpes)</td>
<td>No report</td>
<td>Favourable</td>
</tr>
<tr>
<td><strong>IRF8</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>Infancy</td>
<td>Yes</td>
<td>No</td>
<td>CMC</td>
<td>Poorly formed</td>
<td>Poor</td>
</tr>
<tr>
<td>AD</td>
<td>Late infancy</td>
<td>Yes</td>
<td>No</td>
<td>No report</td>
<td>Yes</td>
<td>Favourable</td>
</tr>
<tr>
<td><strong>IRF8</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>Infancy</td>
<td>Yes</td>
<td>Yes</td>
<td>No report</td>
<td>No report</td>
<td>Favourable</td>
</tr>
<tr>
<td><strong>ISG15</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>Infancy</td>
<td>Yes</td>
<td>No</td>
<td>Invasive Hib infection, tuberculosis</td>
<td>Yes</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>NEMO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XR</td>
<td>Early to late childhood</td>
<td>Yes</td>
<td>No</td>
<td>Tuberculosis</td>
<td>Yes</td>
<td>Fair</td>
</tr>
<tr>
<td><strong>CYBB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Clinical features

*MSMD resulting from mutations in the IL-12/23 or IFN-γ receptors or STAT1 protein*

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>GATA2 deficiency</td>
<td>GATA2</td>
<td>Dendritic Cell, Monocyte, B and NK Cell deficiency; Viral and Fungal Infections; Myelodysplasia, Leukemia, EBV associated smooth muscle tumor; Erythema nodosum, arthritis and lupus like syndrome&lt;sup&gt;22&lt;/sup&gt;</td>
</tr>
<tr>
<td>WHIM Syndrome</td>
<td>CXCR4</td>
<td>Warts, Hypogammaglobuliemia, Recurrent bacterial Infections and retention of neutrophils in the bone marrow termed as Myelokathexis&lt;sup&gt;23&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anti-cytokine Autoantibody</td>
<td>Auto-antibodies targeted to IFN-γ and/or TNF-α</td>
<td>Common in East Asian population Recurrent Salmonella Infections Reactivation of latent varicella zoster&lt;sup&gt;24,25&lt;/sup&gt;</td>
</tr>
<tr>
<td>NEMO Deficiency</td>
<td>NF-kB</td>
<td>Ectodermal dysplasia, invasive pneumococcal disease incontinencia pigmenti, Mendelian Susceptibility to mycobacterial diseases</td>
</tr>
<tr>
<td>X-linked Recessive Chronic Granulomatous Disease</td>
<td>CYBB</td>
<td>Isolated susceptibility to mycobacterial infections unlike the more common type of CGD&lt;sup&gt;26,27&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mendelian Susceptibility to Mycobacterial Diseases&lt;sup&gt;*&lt;/sup&gt;</td>
<td>IL12B, IL12RB1, IFNγR1, IFNγR2, STAT1</td>
<td>Susceptibility to mycobacterial and viral infections; STAT1 deficiency is generally mild compared to others&lt;sup&gt;28&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Clinical features

CYBB deficiency and Mycobacterial infection

- Normal production of IL-12 and interferon-gamma

- Three forms have been described, based on X91 protein levels: X91° (no protein), X91− (low levels of protein) and X91+ (normal levels of protein)

- Hypomorphic CYBB mutations are more deleterious in monocyte-derived macrophages (MDMs) than in peripheral phagocytes and have been shown to underlie Mendelian susceptibility to mycobacterial disease (MSMD) (Bustamante J, et al. Nat Immunol. 2011).
Clinical features

CYBB deficiency and Mycobacterial infection

- Isolated susceptibility to *Mycobacterium tuberculosis* unlike the more common types of CGD
- Limited BCG susceptibility phenotypes

- But **BCG disease** has been reported from many countries in which Since 2007

- BCG disease and Tuberculosis cases was reported in many CGD patients from countries with routine BCG administration

OUTLINE

- CHRONIC GRANULOMATOUS DISEASE (CGD)
- MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASES (MSMD)
- PATHOGENESIS
- CLINICAL FEATURES
- DIAGNOSIS
- TREATMENT
- CONCLUSION
Diagnosis

- Guided by the clinical presentation

- **Assays** that rely on *superoxide production* include direct measurement of superoxide production, ferricytochrome c reduction, chemiluminescence, NBT reduction, or the flow cytometry-based test dihydorhodamine (DHR) oxidation

- **Nitroblue tetrazolium (NBT)** to measure intracellular hydrogen peroxide in Neutrophils

- **DHR** is quantitative measures of respiratory burst are increasingly determined by *flow cytometry*, using dihydorhodamine (DHR) expression to confirm the diagnosis

- **Next Generation Sequencing** including WES & WGS boost the discovery of novel genetic disorders underlying MSMD.
Treatment

- **Stem cell therapy (HSCT)** is a potential cure for CGD.

- **IFN-γ therapy** can be considered as a natural treatment (complete lack of cellular response to cytokines)

- **Antibiotics**

- Hematopoietic or induced pluripotent stem cells of CGD patients have been manipulated by gene transfer, viral vectors, and CRISPR-Cas9 system to give rise to healthy phagocytes, which are able to perform respiratory burst (*Becker et al. 1998; Chiriaco et al. 2014; Flynn et al. 2015*).

- More research into the safety and efficacy of these treatments may allow the clinical application of gene therapy to cure CGD.
Conclusion

- MSMD is linked to **defects in the INF-γ/ IL-12 pathway** and or phagocytes function (NADPH pathway/superoxide formation).

- Such defects are manifested either as a selective susceptibility to **mycobacterial and other intra-cellular organisms** or as manifestations of other PIDs.

- The most common genetic defect of **CGD (CYBB)** is associated with a **functional defect of macrophage** function that leads to **MSMD**.

- **CYBB defects** predispose to an X-L susceptibility to BCGiosis and other **MSMD (2% of MSMD)**.
“A man is never as big as when he is on his knees to help a child.”
— Pythagoras

THANKS

- Dr Nahla Erwa
- Dr Indou Deme
- Dr Awa Kane
- Dr Mame Sokhna Gueye
- Pr. Ahmed Aziz BOUSFIHA