UNIVERSITY OF RWANDA

MOLECULAR DIAGNOSIS OF FRAGILE X SYNDROME AMONG RWANDAN CHILDREN WITH NEURODEVELOPMENTAL DISORDERS

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MOLECULAR DIAGNOSIS OF FRAGILE X SYNDROME AMONG RWANDAN CHILDREN WITH NEURODEVELOPMENTAL DISORDERS

By

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DECLARATION

I, Laurence MIZERO, declare that this dissertation entitled **"Molecular diagnosis of fragile X syndrome in Rwandan children with neurodevelopmental disorders"** is my original scholarly work, and has not been disseminated before. Passages, words, or concepts from the dissertation that have been quoted from other sources have been explicitly referenced.

Laurence MIZERO

Signature:  Date: July 31st 2025

DEDICATION

First and foremost, I extend my deepest gratitude to the Almighty God, who is the source of our life and whose guidance has enabled us to reach this milestone.

To my beloved husband, Martinien Havugimana, my children, and my entire family, for their unwavering and unreserved love and support throughout my academic career. Additionally, I dedicate this work to my classmate for their interactions with me during this project.

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I say, “May our Almighty God bless you”.

Laurence MIZERO

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LIST OF ABREVIATIONS

ASD: Autism Spectrum Disorder

CGG: cytosine-guanine-guanine

DNA: Deoxyribonucleic acid

FM: Full Mutation

FMR1: Fragile X Messenger Ribonucleoprotein 1

FMRP: Fragile X Mental Retardation Protein

FXPOI: Fragile X associated Primary Ovarian Insufficiency

FXS: Fragile X Syndrome

FXTAS: Fragile X-Associated Tremor/Ataxia Syndrome

ID: Intellectual Disability

mPCR: Methylation Polymerase Reaction

NDDs: Neurodevelopmental Disorders

NNPTH: Ndera Neuropsychiatric Teaching Hospital

PCR: Polymerase chain Reaction

PM: premutation

UTR: Untranslated Region

ABSTRACT

Background: Fragile X syndrome is among the commonest causes of neurodevelopmental disorders, including autism spectrum disorder, intellectual disability and global developmental delay. However, there is no information on the prevalence of Fragile X Syndrome among children with Neurodevelopmental Disorders in Rwanda. This research aimed to determine the prevalence rate of Fragile X Syndrome and the carrier status of mothers among Rwandan children with Neurodevelopmental Disorders, the methylation status of the *FMR1* gene and the clinical characteristics of the Fragile X Syndrome children in Rwanda.

Methods: This quantitative cross-sectional study used a purposive sampling method to analyze the presence of Fragile X Syndrome among thirty children (aged 2 – 17years) who visited Kigali University Teaching Hospital and Ndera Neuropsychiatric Teaching Hospital**,** Rwanda for diagnosis of Neurodevelopmental Disorders between November 2023 and May 2025.

Results: Among the 30 children with Neurodevelopmental disorder, males predominated (70%), with most children (46.7%) aged between 2 and 5 years. Most of the children had autism spectrum disorder (70%), followed by Global Development Delay (16.7%), Intellectual Disability (10%), and a combination of both autism spectrum disorder and Intellectual Disability (3.3%). Only 3 (10%) males were identified as positive with Full Mutation (>200CGG repeat), Out of which two had autism spectrum disorder, while the third child had Intellectual Disability. They presented with macroorchidism (33%), speech delay (33%), prominent ear (67%), and long face (100%). Furthermore, mental retardation was moderate in two of them (67%) but severe in the third patient (33%). All the Fragile X Syndrome children had complete methylation, with levels above 80%. Lastly, two mothers of the children tested positive for Fragile X Syndrome with premutation status, confirming maternal transmission.

Conclusion: About 10% of Rwandan children with Neurodevelopment Disorders have Fragile X Syndrome with full mutation, which is transmitted from their mothers. Screening for Fragile X Syndrome in patients with Neurodevelopmental Disorders is highly recommended to enhance clinical understanding of the genetic etiology of Neurodevelopmental Disorders in Rwanda.

Keywords: Fragile X Syndrome, Neurodevelopmental Disorders, Autism spectrum Disorder, Intellectual Disability and Global Developmental Disorder

TABLE OF CONTENT

CHAPTER I: INTRODUCTION

1.1 Background of the study:

Fragile X Syndrome (FXS) is a hereditary condition resulting from a mutation in *FMR1* gene (Fragile X Messenger Ribonucleoprotein 1) located on the X chromosome and is known to be a leading cause of inherited intellectual disability globally. The core cause of the disorder is attributed to the pathological amplification of the cytosine-guanine-guanine (CGG) trinucleotide repeat located within the 5′ untranslated region of the *FMR1* gene, If the expansion exceeds 200 copies, it causes the DNA methylation of the *FMR1* promoter region which turn off *FMR1* gene, preventing translation of Fragile X mental retardation protein 1 (FMRP) (Le & Mazzocco, 2000). The *FMR1* gene was identified in 1991 by Verkerk (Nolin *et al*., 2003). FXS has an X-linked dominant inheritance pattern that is linked to a variety of clinical characteristics and can be very difficult to diagnose clinically (Elhawary *et al*., 2023). Phenotypic abnormalities observed in individuals with FXS encompass craniofacial dysmorphisms, decreased muscle tone, macroorchidism, which is predominantly evident in affected males (Crawford *et al*., 2020). The global prevalence of FXS is about 1/4 000 in males and 1/8 000 in females worldwide (Essop & Krause, 2013), in the individual with CGG repeat pre-mutation of the *FMR1* gene the prevalence is approximately 1 in 200 among females and 1 in 450 among males (Jacquemont *et al*.,2004).

The *FMR1* gene encodes the FMRP, a selective RNA-binding protein that is critically involved in the regulation of synaptic plasticity and higher-order neurocognitive functions, including the modulation of learning, memory consolidation, and behavioral control. Transcriptional silencing of the *FMR1* gene leads to a significant decrease or complete loss of FMRP in children with FXS. This genetic mutation impairs the maturation and functional connectivity of neuronal circuits by perturbing synaptogenesis and activity-dependent signaling pathways, Extensive neurogenetic research has demonstrated that children with FXS have severe cognitive developmental delays, increased anxiety, hyper-sensory hypersensitivity and impaired social communication (Razak *et al., 2020)*.

These signs tend to coincide with autism spectrum disorder and intellectual disability, which makes it difficult to diagnose and treat. Longitudinal studies also show that loss of FMRP in critical periods of development can lead to structural changes in brain morphology and functionality that remain beyond childhood, which explains the significance of early diagnosis and tailored therapeutic approaches (Quintin *et al*., 2016).

Globally, multiple studies have highlighted the importance of molecular diagnostics in identifying FXS among children with neurodevelopmental disorders (NDDs). A population-based study in Canada by Hunter *et al*. (2014) reported that FXS accounts for approximately 2–6% of intellectual disability cases, with PCR-based screening proving effective in detecting CGG repeat expansions in the *FMR1* gene. In 2019, a diagnostic cohort analysis in South Korea demonstrated that methylation-specific PCR combined with capillary electrophoresis significantly improved the detection rates of full mutations in children presenting with unexplained developmental delay. These findings underscore the critical role of molecular tools in early identification and intervention for FXS, particularly in settings where clinical features alone may not be sufficient for accurate diagnosis(Hunter *et al*., 2014; Yim *et al*., 2008).

Recent research conducted in Cameroon, a Sub-Saharan African country, revealed that among 46 individuals tested in a rural cascade screening study, 21.1% males with clinical intellectual disability were confirmed to have a full mutation in the *FMR1* gene, confirming that they had Fragile X Syndrome. Additionally, 14.8% and 37% females with full mutation and pre-mutation were respectively presented (Kamga *et al*., 2020). These findings highlight the presence of FXS and Fragile X-associated conditions in Sub-Saharan Africa, although comprehensive prevalence estimates across the region are still lacking. A broader review also emphasizes the scarcity of epidemiological data and the urgent need for expanded genetic services and awareness campaigns to improve diagnosis and care for affected individuals (Mbachu *et al*., 2024)

Despite advances in molecular diagnostics worldwide, data on Fragile X Syndrome in sub-Saharan Africa, particularly Rwanda, remain limited. Understanding the genetic basis of Fragile X Syndrome in Rwandan children with neurodevelopmental disorders is crucial for determining the prevalence and diagnostic significance of FMR1 gene mutations in this population. The findings are vital for improving early intervention strategies, guiding genetic counseling, and shaping policies for neurodevelopmental disorder screening. In this study, we analyzed the molecular profile of FXS by examining CGG repeat expansions and methylation status in the FMR1 gene using methylation PCR in children with neurodevelopmental disorders, aiming to provide essential data for future diagnostic and research efforts in Rwanda.

1.2 Problem statement

The FXS is the most prevalent cause of significant NDDs, including monogenic autism spectrum disorder, hereditary intellectual disability (ID) and global developmental delay (GDD). The FXS arises from the expansion of the CGG trinucleotide sequence situated proximal to the promoter region of the *FMR1* gene, which encodes the polysome-associated RNA-binding protein FMRP, and plays key roles in neuronal development (Kraan *et al*., 2019). Normal individuals have fewer than 45 repeats, whereas the FM is characterized by an expansion of over 200 CGG repeats, which induces hypermethylation of the *FMR1* gene promoter, leading to transcriptional silencing. This results in the absence of FMRP, a crucial regulator of synaptic plasticity and cognitive development (Borch *et al*., 2020). Molecular diagnostic methods, such as PCR-based CGG repeat sizing and methylation-sensitive assays, are crucial for the precise identification and classification of *FMR1* gene alleles.

However, there is limited data on the prevalence and molecular diagnosis of FXS in sub-Saharan Africa, including Rwanda. Most children with ID, ASD, and GDD are diagnosed clinically, with limited access to genetic testing. Therefore, children with FXS mutations or carriers are not diagnosed until an older age due to the absence of suggestive phenotypic features or family history. In addition, currently, molecular diagnostics method for confirming the disorder are either unavailable or inaccessible in many low and middle-income countries. Therefore, the diagnostic procedures are based on clinical presentation and family history, which are not always accurate. Understanding the burden of FXS through molecular diagnosis could improve diagnostic precision and early detection, family counseling, and access to tailored intervention.

1.3 Study objectives

1.3.1 General objective

The overall aim of this study was to conduct a molecular investigation of the FXS in Rwandan children with NDDs.

1.3.2 Specific objectives

The specific objectives of the study were to:

1. Determine the prevalence of the FXS among Rwandan children with Neurodevelopmental Disorder and the carrier status of their mothers

2. Characterize the methylation status of the FMR1 gene in Rwandan children with Fragile X syndrome.

3. Assess the clinical and demographic characteristics associated with confirmed FXS cases in the study cohort.

1.4 Research Questions

1. What is the prevalence of FXS among Rwandan children with NDD and the carrier status of their mothers?
2. What is the methylation status of FXS among fully-mutated Rwandan children with FXS?
3. What clinical and demographic characteristics are significantly associated with confirmed Fragile X Syndrome cases among Rwandan children diagnosed with neurodevelopmental disorders?

1.5 Significance of the study

The FXS is the most common inherited disorder that causes NDD. Despite its clinical significance, FXS remains underdiagnosed in many parts of the world, including Rwanda. This study provided essential data on the molecular diagnosis of FXS in Rwanda. The confirmed diagnosis of FXS, through identification and quantification of *FMR1* CGG repeat expansions and methylation status facilitates precise categorization. The results will support better clinical management, guide educational and therapeutic planning, and inform genetic counseling services. Additionally, the findings will contribute to the plan of the screening program for voluntary genetic check-up before marriage and early molecular testing for FXS in Rwanda.

1.6 Definition of Key Terms

Fragile X Syndrome: A hereditary neurodevelopmental condition that results in transcriptional silence and FMRP absence due to an increase of the CGG trinucleotide repeat (>200 repetitions) in the 5′ untranslated region of the FMR1 gene (Ciaccio *et al*., 2017).

Autism Spectrum Disorder: ASD is a neurodevelopmental condition that impairs social interaction, communication abilities, cognitive processing, and behavioral regulation (Hodges *et al., 2020)*.

Global Developmental Delay: is described as a considerable delay in at least two areas of development, such as daily living activities, motor skills, cognitive abilities, speech and language, and personal or social skills (Habibullah *et al*., 2019).

FMRP (Fragile X Mental Retardation Protein): an RNA-binding protein encoded by the FMR1 gene

Hypermethylation: An epigenetic change defined by the excessive addition of methyl groups to cytosine residues in DNA, especially at CpG dinucleotides within gene promoter regions (Ehrlich, 2019).

Mosaicism: Mosaicism refers to the presence of two or more distinct genetic profiles within the cells of a single individual.

CHAPTER II: LITERATURE REVIEW

2.1 Introduction

This chapter presents an overview of existing studies and scholarly work on Fragile X Syndrome in a global perspective and in the African context. The chapter provides key insights and diagnostic practices related to FXS in children with NDDs.

2.2 Theoretical review

Neurodevelopmental disorders (NDDs) include ID, GDD and ASD, a group of conditions that result from inadequate brain development, and which typically occur during early childhood and affect cognitive, motor, language, and social domains. The etiology of these disorders is complex, including genetic mutations, chromosomal and epigenetic dysregulations, which are frequently exacerbated by environmental exposures in prenatal and perinatal stages (Rodan *et al*., 2025). ID is defined as the disruption of intellectual functioning and adaptive behavior resulting from interrupted synaptic signaling and neuronal connectivity. GDD is associated with extensive developmental delays in multiple domains that may be caused by underlying genetic or metabolic malfunctions. while ASD is characterized by impaired neural circuitry, social cognition, sensory integration, and communication, often linked to dysregulation of synaptic functions and an abnormal excitatory-inhibitory balance (Prasad *et al*., 2025).

Fragile X Syndrome (FXS) is the most prevalent inherited cause of intellectual disability (ID) and a major contributor to autism spectrum disorder (ASD) and global developmental delay (GDD). It arises from a CGG trinucleotide repeat expansion in the 5′ untranslated region of the *FMR1* gene, which triggers hypermethylation and transcriptional silencing. The resulting absence or deficiency of FMRP, a modulator of synaptic plasticity impairs the transport and local translation of mRNAs at neuronal synapses, culminating in cognitive deficits, autistic phenotypes, and delayed neurodevelopment (Tabolacci *et al*., 2022). These disorders clinically present themselves with inconsistent severity, ranging from severe to mild learning problems up to severe intellectual deficiency and behavioral dysregulation. The diagnosis is based on DSM-5 criteria, developmental examinations and molecular diagnostics involving chromosomal microarray and FMR1 testing, helping in the diagnosis of FXS in individuals with unexplained ID, GDD, or ASD (Carter *et al*., 2023). Early identification of FXS not only informs prognosis and recurrence risk but also enables tailored interventions and genetic counseling, particularly in resource-limited settings where diagnostic equity remains a challenge.

In mammals, cytosine methylation commonly takes place along the linear DNA strand, where DNA methyltransferases catalyze the addition of methyl groups to cytosine residues that are positioned next to guanine bases in the 5′ to 3′ orientation. DNA demethylation involves the conversion of 5-methylcytosine residues back into cytosine within the DNA sequence. DNA methylation constitutes a key epigenetic mechanism involved in the regulation of transcriptional activity. Hypermethylation of the CpG island associated with the FMR1 gene leads to transcriptional silencing of the gene (Kim & Costello, 2017).

2.2.1 Structural Features and Biological Relevance of the FMR1 Promoter and Intron 1

FMR1 transcription is controlled by the FMR1 promoter, a stretch of DNA upstream of the transcription start site. In FXS, the FMR1 gene is silenced due to methylation of 52 CpG dinucleotides within its CpG island, leading to loss of gene expression. FMR1 silencing in the FXS disease happens about 11 weeks of pregnancy, and the FM repeat expansions set off an epigenetic switch that includes changes to histone proteins and elevated DNA methylation (Pietrobono *et al*., 2002).

2.2.2 Molecular epigenetic landscape of FMR1 disorders and its implications for downstream cellular pathways

Different epigenetic changes that affect gene expression and clinical outcomes are characteristics of FMR1-related disorders, which are FXS, Fragile X-associated Tremor/Ataxia Syndrome (FXTAS), and Fragile X-associated Primary Ovarian Insufficiency (FXPOI) (Kraan *et al*., 2019).

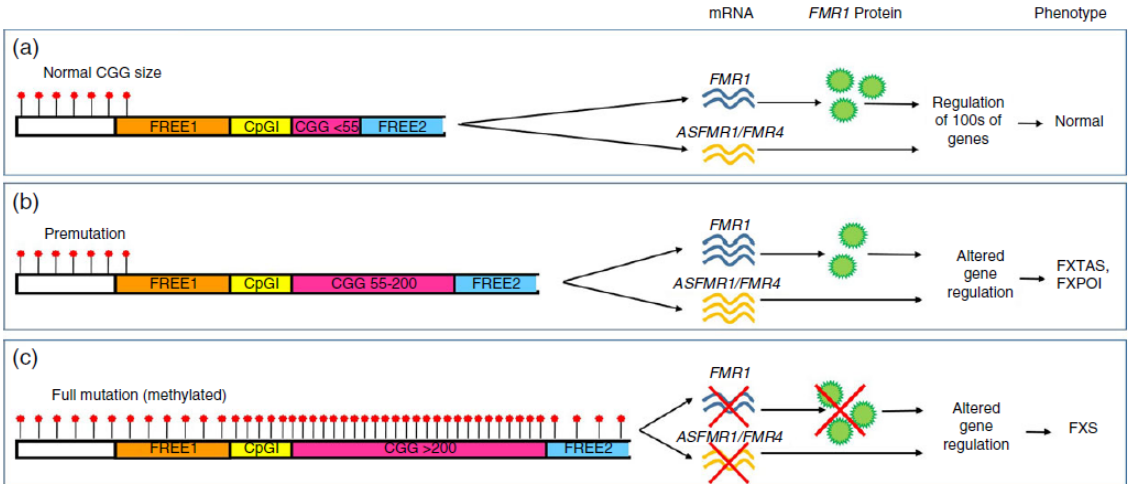

Figure 1: Correlation between epigenetic modifications and clinical manifestations in FMR1-related conditions

(a) Normal FMR1 gene, with normal size of CGG repeat are associated with unmethylated promoter and FMR1 is transcribed at normal rate, FMRP is translated and regulate many genes associated with normal neurodevelopment (b) Premutation alleles (55–200 CGG repeats) are typically unmethylated at the promoter, resulting in elevated FMR1 mRNA levels; however, FMRP production is reduced. (c) Full mutation CGG repeats (>200 repeats) are associated with methylation of the FMR1 promoter, which silences transcription of FMR1 mRNA, resulting in the absence of FMRP production. Adapted from Kraan *et al*,2019.

2.2. 3 History and Physical characteristic of FXS

Newborns with FXS usually do not exhibit early clinical signs, as their head circumference, weight, and height measurements are generally within the normal limits. Physical and developmental symptoms typically manifest in early childhood. In most cases, FXS has been diagnosed retrospectively, following the onset of observable symptoms, resulting in considerable delays in diagnosis. This delay frequently results in families having more children before becoming aware of the heightened recurrence risk associated with the syndrome (Bailey et al., 2008; Raspa *et al*., 2023).

2.2. 4 Fragile X syndrome heritability

Inheritance of FXS results in its typical non-Mendelian pattern of inheritance and is determined by the number of CGG trinucleotide repeats in the promoter region of the FMR1 gene. During maternal transmission of a Pre mutation (PM) allele on the X chromosome, an expansion event of the allele leads to the conversion to a Full Mutation (FM) allele in the offspring. This somatic expansion mechanism is responsible for the variable penetrance and expressivity of the FXS phenotype among affected families (Tabolacci *et al*., 2022).

Persons affected with FXS have both FM and abnormal DNA methylation. A female premutation carrier has a mutated X chromosome and a normal functioning X chromosome with 50% chance of passing the mutated X chromosome to the offspring. The passage from premutation to FM status occurs only with a transmission from the mother (IJsselmuiden & Faden, 1992). Women who carry the Fragile X premutation face an elevated risk of developing Fragile X-associated Primary Ovarian Insufficiency (FXPOI), while both male and female premutation carriers are susceptible to neurodegenerative disorders such as Fragile X-associated Tremor/Ataxia Syndrome (FXTAS).

2.2.5 Primary Ovarian Insufficiency

Primary ovarian insufficiency refers to the early termination of menstrual cycles occurring before the age of 40. It is a unique phenotype among women who are carriers of the premutation. However, it is not reported in females with the FM. *FMR1* premutation carriers (55–200 CGG repetitions) are susceptible to FXPOI. About 20% of female carriers suffer from early menopause, overall ovarian dysfunction and Primary Ovarian Insufficiency which is significantly higher when compared with only 1% in the general population (Sullivan *et al*., 2005).

2.2.6 Fragile X-associated tremor/ataxia syndrome (FXTAS)

Fragile X-associated Tremor/Ataxia Syndrome is a progressive neurological disorder linked to the Fragile X premutation, and is marked by symptoms including tremors and impaired coordination (Cabal-herrera et al., n.d.). The disorder is more frequently observed in older premutation carriers than in younger individuals. The likelihood of developing FXTAS rises with advancing age, affecting approximately 40% of male and 16% of female who carry the mutation (Crawford *et al*., 2020). Males who inherit the mutation will be affected, and they will transmit the mutation to all their daughters. Female with the Fragile X premutation may exhibit varying levels of intellectual disability or behavioral challenges, and have a 50% chance of passing the mutation to each of their offspring (Kamga *et al*., 2020).

2.2.7 Molecular Basis and Diagnostic Techniques

Over the past two decades, the molecular diagnosis of FXS has evolved significantly. Conventional cytogenetic techniques have been largely replaced by DNA-based methodologies, which provide higher sensitivity and specificity. The first line diagnostic method with a few exceptions, is PCR-based screening for CGG repeat length within *FMR1* gene. To assess the methylation status of the adjacent CpG island and confirm the presence of a full mutation, methylation-specific PCR or Southern blot analysis is commonly employed (Ciaccio *et al*., 2017). Capillary electrophoresis and fragment analysis using platforms such as the ABI 3500 Genetic Analyzer allow precise sizing of CGG repeats and differentiation between normal, premutation, and full mutation alleles.

2.3 Empirical Literature Review

Over 99% of Fragile X syndrome cases result from an increased number of CGG repeats in the 5' untranslated region of the *FMR1* gene. The alternative etiology involves a spectrum of genetic alterations, predominantly comprising large-scale deletions and duplications, disruptions in regulatory elements and point mutations (Lyons *et al*., 2015). FXS is associated with the *FMR1* gene located at locus Xq27.3 and encodes the FMRP. According to the prevalence rates most cases are male and about 50% of females with the whole mutation are affected by ID (Le & Mazzocco, 2000).

A study was done in southern Brazil among 90 patients out of which women were 64 and men were 26, The results showed that 42 women (65.6%) were normal, 2 women (3.2%) were intermediate/gray zone, 15 women (23.4%) were premutation, and 5 women (7.8%) were full mutation. Among men, 6 (23.1%) were normal, 15 (57.7%) were with FM , and 5 (19.2%) were with mosaic permutation (Ramos *et al*., 2020). FXS prevalence in Indonesia's population with ID ranging between 0.9 to 1.9%, however it was higher (6.15%), showing an increased number of FXS among patients with ASD. A genetic screening and cascade testing initiative carried out in a rural community on Java Island revealed a notably high prevalence of 45% among individuals with intellectual disability, indicating the potential presence of a localized genetic cluster (Sihombing *et al*., 2021). A study conducted in South Africa found that among 2,239 unrelated individuals diagnosed with intellectual disability and/or fragile X syndrome (ID/FXS), 128 (5.7%) tested positive for the *FMR1* full mutation (FM), including 5.2% of male probands and 0.5% of female probands. The FM was identified in 4.8% of white patients, 5.2% of Black patients, 8.1% of patients of mixed ancestry, and 9.9% of Indian patients (Essop & Krause, 2013).

According to the study done in Cameroon, 46 participants underwent the FXS carrier testing, among whom 60.87% were normal (with CGG repeats < 55). Out of the 19 male participants, four were found to carry a full mutation (FM) defined by CGG repeats exceeding 200, and each was diagnosed with severe intellectual disability (ID). Remarkably, one of these individuals came from a family with three daughters, hinting at a degree of social acceptance and tolerance of disability within that specific environment. Furthermore, among the 27 female participants, 10 had a premutation, while 4 were identified with a full mutation. (Kamga *et al*., 2020). The FXTAS is a neurodegenerative disorder caused by a pre-mutation of 55 to 200 CGG repeats in the 5′ untranslated region (UTR) of the *FMR1* gene (Wilson *et al*., 2001).

The clinical features of this condition include intention tremor and ataxia. In contrast to FXS, where expanded FM alleles (>200 CGG repeats) result in hypermethylation and silencing of the *FMR1* gene, *FMR1* mRNA levels are higher in FXTAS carriers, although FMRP, the protein that *FMR1* encodes, is expressed at a slightly lower level. *FMR1* mRNA levels are higher in FXTAS carriers, although FMRP, the protein that *FMR1* encodes, is expressed at a slightly lower level (Tassone *et al*., 2007) The risk of developing FXTAS increases with age in both males and females, but it is significantly higher in men: approximately 17% to 75% of male premutation (PM) carriers are affected, compared to only 8% to 16% of female PM carriers over the age of 50 (Seltzer *et al*., 2012).

The FXPOI is a primary disorder associated with FXS; and is defined as hypergonadotropic hypogonadism before age 40 years, affected women presents with early infertility, irregular menstrual periods and premature menopause with high risk of osteoporosis and heart disease. Its incidence in women with a premutation allele is 20% while the incidence of 1% was reported in the general population (Allen *et al*., 2007).

CHAPTER III: METHODOLOGY

3.1 Study setting

The study was conducted in two hospitals, namely Kigali University Teaching Hospital (CHUK) and Ndera Neuropsychiatric Teaching Hospital (NNPTH). CHUK is the tertiary referral hospital located in Kigali the capital of Rwanda. CHUK is the largest referral hospital in the country, with a capacity of 483 beds. It provides quality healthcare to the population, as well as training, clinical research, and technical support to district hospitals. It mainly receives patients transferred from district hospitals, in particular patients requiring a specialist consultation, especially in in pediatrics department /genetic service.

NNPTH is the Rwanda's only referral and teaching hospital for psychiatry and neurology, and receives a high number of patients with intellectual disability and autism spectrum disorder.

3.2 Study Design

This was a quantitative cross-sectional study aimed at identifying molecular characteristics of FXS in children clinically diagnosed with NDDs. A molecular analysis technique (methylation PCR) was used.

3.3 Study population, sampling strategy, and sample size calculation

The population of this study was all children aged between 2 years to 17 years old, who required a diagnosis of neurodevelopmental disorders whom genetic disease was suspected, which prompted FXS testing. These children were recruited from CHUK and NNPTH from November 2023 to May 2025. Our population was comprised of 30 children, including 21 males and 9 females.

In this study, a purposive sampling method was used. Purposive sampling is a non-probability sampling method in which researchers deliberately choose participants based on particular features or criteria related to the study.

Sample size calculation was done for estimated prevalence of 2% as follow:

$$\mathbf{n=Z^2 \cdot P \cdot (1-P)/d^2}$$

n: Required sample size.

Z: Z-score corresponding to the desired confidence level (e.g., 1.96 for 95% confidence).

P: Estimated prevalence of FXS in the population 2% for males

d: Desired precision or margin of error (5%)

N= $(1.96)^2 * 0.02 * (1-0.02) / (0.05)^2$= **30.2**

3.4 Inclusion criteria and exclusion criteria

3.4.1 Inclusion criteria

- Children diagnosed with neurodevelopmental disorders and suspected of having an underlying genetic condition
- Age: 2-17 years old
- Informed consent signed by parents or guardians

3.4 2 Exclusion criteria

- Children with confirmation of a known chromosomal etiology of NDDs

3.5. Data collection

3.5.1. Data Collection tool

To achieve the objectives of the study, we designed a data collection tool that includes demographic information, prenatal and neonatal history, personal and family medical history, detailed physical examination, and types of NDDs.

3.5.2. Data collection procedure

The data collection began with my presentation to the participants and explaining them the purpose of the study. Participants or their parents signed the consent form, and the samples were collected at CHUK and NNPTH.

3.6 Laboratory methods and analysis

3.6.1 Genomic DNA extraction and quantification

Genomic DNA was isolated from whole blood samples using the QIAamp® DNA Blood Midi Kit. About 2 mL of whole blood was used. DNA quantification was performed to ensure optimal genomic DNA input (20ng/μL). After DNA extraction and quantification each sample was aliquoted into two portions; one portion was sent to University of Liege for FMR1 gene analysis and the remaining was kept as backup.

3.6.2 DNA methylation

The next step was methylation-sensitive PCR, a technique that uses a methylation-sensitive restriction enzyme (HpaII) to facilitate enzymatic digestion of genomic DNA. First, a mix control containing a

digestion control and a CGG size control was combined with genomic DNA, and then, each sample passed through two distinct enzymatic restriction digestions (methylation sensitive and insensitive) in parallel (QIAGEN, 2015).

3.6.3 Amplification of DNA sequences

The principle of the AmplideX mPCR FMR1 kit is based on amplification by using two sets of primers: one labeled with the FAM fluorophore for standard CGG repeat analysis (Forward primer: 5'CGCCATTTTTGCCTCAACCA3'& Reverse primer: 5'ACAGCTCGTCACTGTTCTGG3') and another labeled with the HEX fluorophore for methylation-specific PCR (Forward primer:5'AAAGCGAGACTGAGTGGTGG3' & Reverse primer:5'GATACAGCCCCAGCAGGTTT3'). After PCR amplification of the *FMR1* gene, the PCR products are loaded onto a capillary electrophoresis on the 3500 Genetic Analyzer. Undigested DNA fragments labeled with FAM were identified in the FAM channel and served to assess the CGG repeat number. The HEX-labeled PCR products, representing digested DNA treated with methylation-sensitive restriction enzymes, were detected in the HEX channel and used to assess the methylation status of the *FMR1* gene (Ana, 2021).

3.6.4 Data analysis

The Amplidex mPCR Kit, the ABI 3500 Genetic Analyzer, and Gene Mapper software were used to conduct molecular analysis of *FMR1* CGG repeats expansion and to evaluate methylation status.
Gene Mapper software, which provides accurate fragment sizing and fluorescence quantification, was used to analyze peak data. After normalizing using the corresponding reference peak within the same channel, the ratio of peak heights measured in Relative Fluorescence Units of the digested sample (HEX channel) to the undigested sample (FAM channel) was calculated to determine the methylation status.

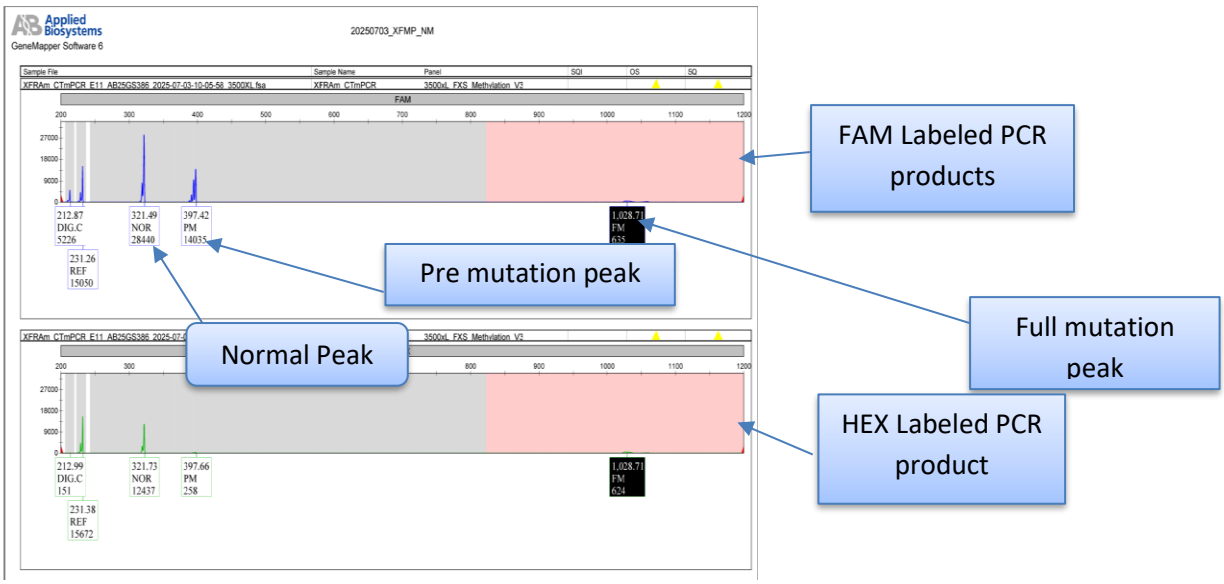

Figure 2: Electropherogram report for Fragile X syndrome

Electropherogram report for Fragile X syndrome and methylation analysis by mPCR kit using ABI 3500 Genetic Analyzer and Gene Mapper software. FAM-labeled PCR products indicate the presence of un-methylated DNA, whereas HEX-labeled PCR products correspond to methylated DNA. DIC: Digestion control, REF: Reference peak, NOR: normal, PM: Pre-mutation and FM: Full Mutation

Methylation status calculation

$$\%Me_i = 100 \times \frac{[Peak_i/Peak_{REF}]_{HEX}}{[Peak_i/Peak_{REF}]_{FAM}}$$

%Mei: Percentage of methylation

Peaki: The fluorescence intensity (RFU) of the target allele peak

Peak REF: The fluorescence intensity of the reference peak

HEX: Digested DNA channel, indicating methylated alleles.

FAM: Undigested DNA channel, indicating total allele presence

Methylation Status Interpretation

Methylation status	Methylation percentage	Interpretation
Unmethylated	≤20%	No methylation abnormality detected
Partial methylation	20 – 80%	No methylation abnormality detected
Complete methylation	≥80%	presence of a methylation abnormality

3.7 Data Entry and Statistical Analysis

Data were cleaned using Excel and then imported in SPSS v25 for further statistical analysis. Descriptive statistics were used to summarize the prevalence of CGG repeats and categorize them into normal, intermediate, premutation, or FM. Chi-square and Fisher's exact tests were used to assess associations between molecular results and clinical features.

3.8 Ethical Considerations

3.8.1 Ethical approval

The research was carried out in strict compliance with the ethical standards of the Declaration of Helsinki, which provides the international framework for conducting research involving human subjects. Before the study began, I received ethical approval Nº 298/CMHS IRB/2023 from the Institutional Review Board of the College of Medicine and Health Sciences (CMHS) and Nº EC/CHUK/1/171/2023, 045/NNPTH/EC/2023 from CHUK and NNPTH hospitals. At the hospital, I explained clearly to the

participants the aim of the study, procedures, potential risks and benefits, and their right to withdraw at any time without any inconvenience. Consent to participate in the study was signed by the parents or legal guardians

3.8.2 Confidentiality

Data were anonymized by a code number corresponding to identification and stored securely on a personal password-protected computer.

CHAPTER IV: RESULTS AND DISCUSSION

This chapter presents the findings of our study according to the research objectives. The results are presented in figures and tables.

The demographic distribution of the 30 children recruited for molecular analysis of *FMR1* CGG repeat expansions and methylation status. The males were predominant (70%), with most children (46.7%) aged between 2 and 5 years. Most of the children had autism spectrum disorder (ASD) (70%), followed by Global Developmental Delay (16.7%), Intellectual Disability (ID) (10%), and a combination of both ASD and ID (3.3%).

Table 1: Demographic characteristics of the study participants

Variables	Frequency(n)	Percentage (%)
Gender		
Female	9	30
Male	21	70
Age		
2-5	14	46.7
6-11	10	33.3
12-18	6	20
Type of neurodevelopmental disorder		
Intellectual Disability	3	10
Autism Spectrum Disorder	21	70
Global Developmental Disorder	5	16.7
Autism Spectrum Disorder and Intellectual Disability	1	3.3

By using SPSS, we found that most of the patients were normal at 90% with CGG repeats ranging 5-44; 10% were positive with greater than 200 CGG repeats

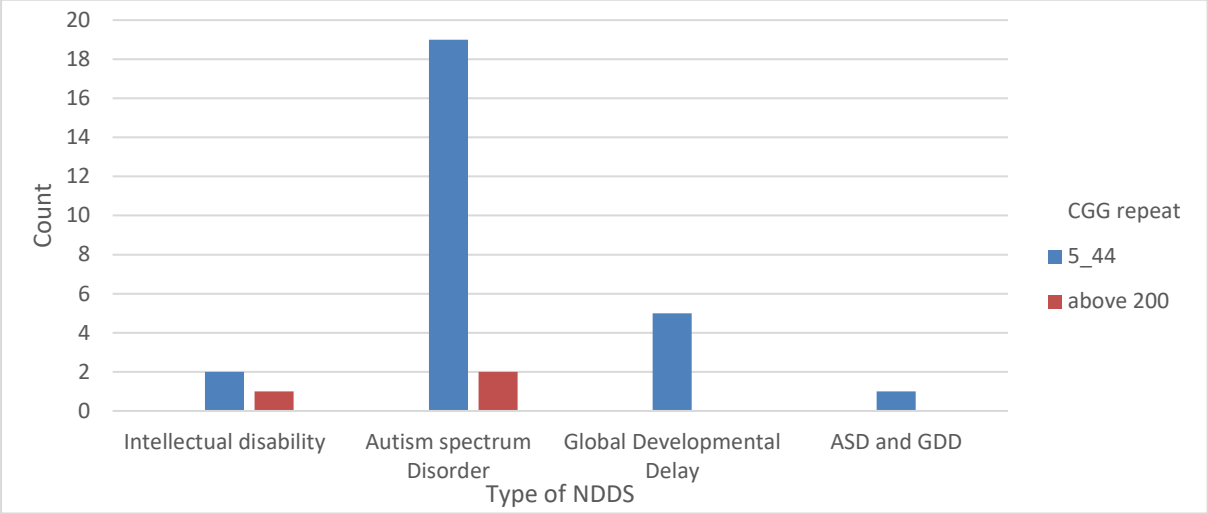

Figure 3: CGG repeat distribution among Rwandan children with neurodevelopmental Disorders

By analyzing the prevalence of FXS among tested participants out of the 30 children, 3 cases (10%) were identified as positive for full mutation (FM) with CGG repeats exceeding 200, while the remaining 27 children (90%) exhibited normal CGG repeat counts ranging from 5 to 44

Table 2: Prevalence of Fragile X Syndrome

Gender	Positive for FXS Full-Mutation	Negative for FXS Normal	Total
Female	0	9(30%)	9(29%)
Male	3(10%)	18(60%)	21(71%)
Total	3(10%)	27(90%)	30(100%)

With the ABI 3500 Genetic Analyzer, capillary electrophoresis produced distinct electropherogram peaks that matched fragment sizes and allele calls. Successful amplification and precise sizing were validated by the CGG repeat region, and the fluorescence intensity supported dependable detection (Figure 4).

Figure 4: Electropherogram for full mutated patient

Electropherogram representing control peaks both for digestion and for Reference and full mutation peak in both FAM and Hex channel confirming full mutation for FXS.

The table presents the distribution and prevalence of FXS genotypes among male and female participants. Normal alleles were the most frequent, while full mutations were detected in 10% of males. The majority of cases (90%) fell within the normal CGG repeat.

Table 3: Prevalence of Fragile X syndrome Genotype

Gender	Allele type	Frequency(n)	Prevalence (%)
Male	Normal	18	60
	Full mutation	3	10
Female	Normal heterozygous	2	6.7
	Normal Homozygous	7	23.3
	Full mutation	0	0
CGG repeats categories	Normal CGG repeats (5 – 44 repeats)	27	90
	Gray or Intermediate zone (45 – 54 repeats)	0	0
	Pre-mutation (55 – 200 repeats)	0	0
	Full Mutation (> 200 repeats)	3	10

Clinical characteristics of the male children with FM were identified. It was noted that they presented with macroorchidism (33%), speech delay (33%), prominent ear (67%) and long face (100%). Furthermore, mental retardation was moderate in two of them (67%) but severe in the third patient (33%).

Table 4: Major clinical characteristics of Male Children with FXS

Category	Clinical Features	Prevalence%
Physical	Macroorchidism	33%
	Long face	100%
	Prominent ear	67%
	Speech delay	33%
Severity of mental retardation		
	Moderate	67%
	Severe	33%

Among the Fragile X syndrome Patients with FM, the methylation status was investigated. It was noted that all of them had complete methylation, being above 80%

***Table 5*: Methylation status of full-mutated children**

Patients ID	methylation percentage	Interpretation
Patient1	124%	Complete methylation
Patient2	125%	Complete methylation
Patient3	97%	Complete methylation

Lastly, the carrier status of the mothers of FXS children with FM was investigated by studying the two peaks (normal and pre-mutated alleles) in both FAM and Hex channels (**Figure 5**). It was observed that two mothers, including one with two children affected by FXS tested positive for FXS premutation status, confirming maternal transmission.

***Table 6*: Identification of carrier mothers**

Patient ID	CGG repeats	Diagnosis	Mother testing	Carrier status
Patient1	>200	Fragile x syndrome	Yes	Premutation
Patient2	>200	Fragile syndrome	Yes	Premutation
Patient3	>200	Fragile x syndrome	Yes	Premutation

Electropherogram showing fluorescence peaks from FAM and HEX channels, indicating fragment sizes and signal intensities for CGG repeat analysis (Figure 5).

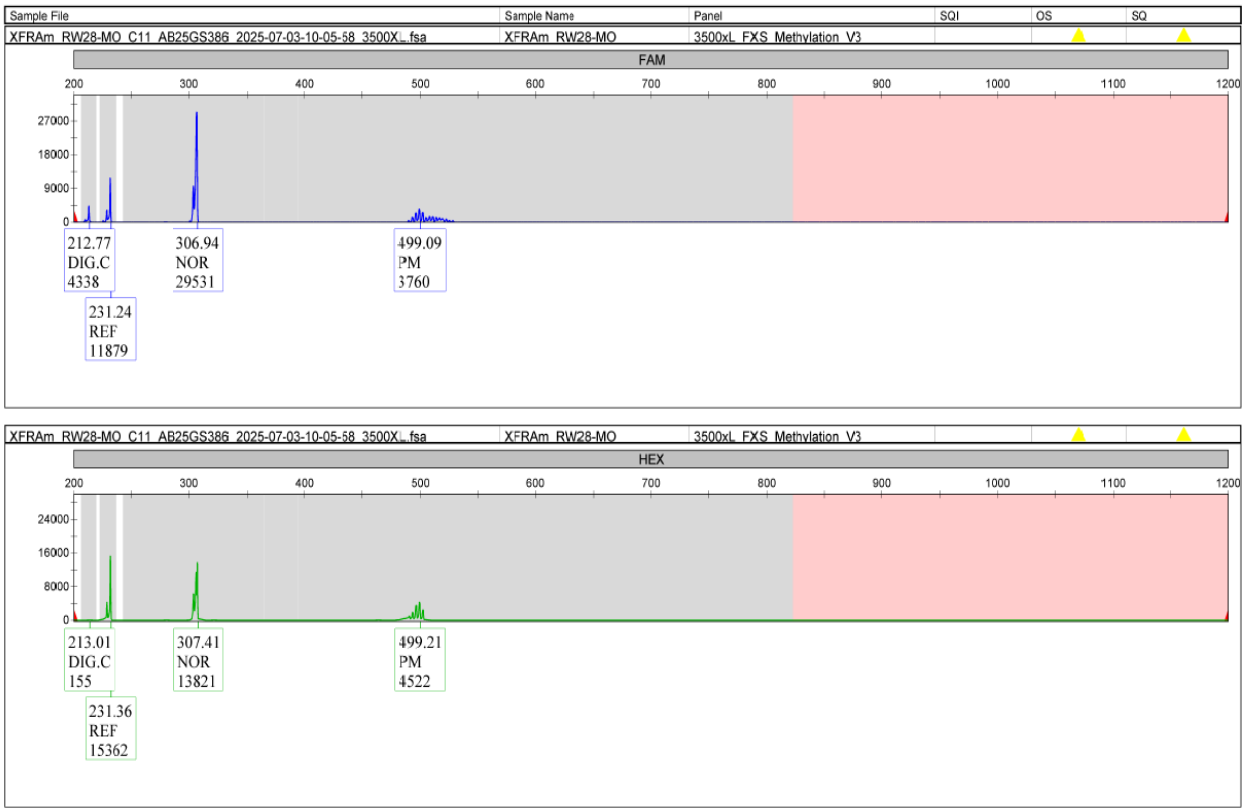

***Figure 5*: Electropherogram for pre-mutated mother**

Electropherogram representing carrier mother with two peaks (normal and pre mutated allele in both FAM and Hex channel confirming carrier status.

CHAPTER IV: DISCUSSION

Fragile X Syndrome constitutes the most prevalent inherited etiology of mild to severe intellectual disability and is recognized as the foremost monogenic cause of autism spectrum disorder, it caused by the expansion of a DNA segment (CGG repeats) within the Fragile X Messenger Ribonucleoprotein 1 gene, leading to a silencing of the gene and hindering the synthesis of the FMRP protein, which is essential for brain development, this insufficiency of Fragile X Mental Retardation Protein interrupts the normal development and functioning nervous system, result in different disorder like Autism Spectrum Disorder, Intellectual Disability, and Global Developmental Delay. This study aimed to identify children with FXS, determine their methylation status, and explore the clinical and demographic characteristics of affected children. This research uncovered the existence of FXS in Rwandan children with Neurodevelopmental Disorders and emphasized the crucial role of molecular diagnostics for early identification and precise categorization. The identification of maternal carrier status underscores the importance of implementing family-centered genetic testing and counseling strategies.

In the present study, the prevalence was 10% male children who were diagnosed with Fragile X Syndrome, all exhibiting full methylation (>80%) of the Fragile X Messenger Ribonucleoprotein 1 gene indicative of full mutation. Moreover, two carrier mothers were identified with mosaicism, presenting both normal and premutation CGG repeat alleles, these findings are comparable to global data, except slight differences in prevalence rates. A study done in South Africa reported a prevalence of FXS in males with intellectual disability at approximately 2.5%. another substantial retrospective population study crossing two decades and involving more than 2,000 individuals with intellectual disabilities in urban South Africa revealed that the prevalence rate of FXS among African participants was 5.2%(Mbachu et al., 2024). A different population study focusing on intellectually disabled adolescent and adult males in institutions in rural South Africa found a prevalence rate of 6.1% for FXS among the participants examined (Goldman *et al*., 1997).

Our report regarding the methylation status of the Fragile X Messenger Ribonucleoprotein 1 gene in children with Fragile X syndrome showed that all three confirmed cases exhibited complete methylation, with percentages between 97% and 125%. This indicates transcriptional silencing of the Fragile X Messenger Ribonucleoprotein 1 gene and aligns with classical Fragile X Syndrome pathology. The methylation profile was consistent for both genetic and epigenetic in contrast with other reported studies they are variability in Fragile X Messenger Ribonucleoprotein 1 alleles. Patients with FXS and methylation mosaicism

exhibit a milder intellectual disability, higher Intelligence Quotient and adaptive behavior, compared to those with completely methylated Full Mutation. The mosaic males with unmethylated *FMR1* and Full Mutation alleles do not exhibit behavioral phenotypes because smaller alleles are responsible for producing the *FMR1* protein while those individuals have elevated risk of developing Fragile X-associated tremor/ataxia syndrome (Annear & Frank Kooy, 2023).

The association between Fragile X Syndrome and neurodevelopmental disorders including Autism Spectrum Disorder, Intellectual Disability, and Global Developmental Delay is attributable to the underlying molecular pathogenesis of the *FMR1* gene. Fragile X Syndrome is predominantly caused by an expansion of the CGG trinucleotide repeat exceeding 200 copies within the 5′ untranslated region (5′ UTR) of the *FMR1* gene, which is located on the Xq27.3 locus of the X chromosome. The expansion of CGG repeats lead to hypermethylation and transcriptional silencing, this result in the absence or severe reduction of Fragile X Mental Retardation Protein (FMRP), which is crucial in neurodevelopment (Razak *et al*., 2020).

FXS is the most prevalent known inherited single gene disorder with an estimated 1% to 6% of ASD cases. Autism Spectrum Disorder is defined by deficits in social interaction and communication, accompanied by restricted, repetitive behaviors, interests, or activities. Our findings showed that among identified 3 cases of fragile X syndrome, 66% were having Autism Spectrum Disorder which is quit similar with the study done using the gold standard criteria for diagnosing autism, the study were involved males and females with FXS and found that 30% to 54% and 16% to 20% met the diagnostic criteria for autism through direct assessment respectively (Clifford *et al*., 2007).

Fragile X Mental Retardation Protein (FMRP), an RNA-binding protein, modulates the translation of hundreds of mRNAs that play roles in synaptic development, plasticity, and neuronal signaling. Its reduction or absence compromise several crucial molecular pathways including glutamatergic and GABAergic signaling, resulting in a disruption of excitatory and inhibitory neurotransmission, a hallmark of autism spectrum disorder and other Neurodevelopmental Disorders. The deficiency of FMRP alters dendritic spine architecture, leading to the presence of elongated, immature spines. These structural abnormalities compromise synaptic connectivity and neural signaling, thereby contributing to Intellectual Disability and Global Developmental Delay (Hagerman *et al*., 2009; Razak *et al*., 2020).

Furthermore, Fragile X Syndrome (FXS) is classified as a syndromic subtype of autism spectrum disorder (ASD), with approximately 60% of affected males and 20% of affected females meeting established

diagnostic criteria for ASD. People with FXS frequently show heightened anxiety, sensitivity to sensory stimuli, repetitive actions, and difficulties with social communication a key characteristic of ASD. The similarity in observable traits highlights common molecular processes, especially the disruption of FMRP-related pathways like mGluR5 signaling, which are involved in both Fragile X Syndrome (FXS) and idiopathic autism (Annear & Frank Kooy, 2023).

When looking on physical feature for identified FXS children most constant characteristic was a long face (100%), followed by prominent ears (67%), while macroorchidism and speech delay were observed in one third of the cases. Intellectual disability was moderate in 67% and severe in 33% of the affected males. In Japanese cohort reported similar physical features: long face and large ears were present in 71% of patients, All individuals exhibited behavioral disorders, with ASD diagnosed in 86%, indicating a comparable neurodevelopmental profile *(Okazaki et al*., 2021).

3.10 Strengths and limitations of the study

3.10.1 Strengths

This study was the first research to investigate the molecular characteristic of FXS in children with neurodevelopmental disorders in Rwanda, addressing a significant effective diagnosis gap. The findings of the study will contribute in medical management, educational planning, genetic counseling and for improving care for affected families and shaping future plan for early detection for fragile x syndrome. The strengths of the study also include no selection bias.

3.10.2 Limitations

This study was conducted using purposive sampling, with participants recruited from hospitals offering diagnostic services for neurodevelopmental disorders. While these clinical settings provided valuable insights, the sample size was limited by the duration of the study. As a result, the small number of participants may not adequately reflect the genetic and clinical diversity of the broader Rwandan population. Limited availability of local molecular testing facilities may constitute outsourcing some laboratory analyses, affecting cost and timely work. No other research was conducted on Fragile X Syndrome here in Rwanda, the literature resources in the region is very limited.

CHAPTER V: CONCLUSION AND RECOMMENDATION

Molecular screening for FXS among Rwandan children presenting with NDDs confirmed three male patients harboring the full mutation of the *FMR1* gene, all exhibiting complete methylation consistent with transcriptional silencing. Additionally, two carrier mothers were identified. These findings underscore the diagnostic utility of FXS testing in children with NDDs and highlight the critical importance of integrating genetic counseling into routine clinical practice.

We recommend that future research involve larger cohorts and screen all children with NDDs of unknown genetic origin. Such studies should include both female patients and extended family members to enhance understanding of genotype phenotype correlations and inheritance patterns. Furthermore, efforts should focus on strengthening molecular diagnostic infrastructure, increasing awareness, and providing targeted training for healthcare providers and families to reduce diagnostic delays and optimize clinical management and support for affected individuals.

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APPENDICES

Appendix A:Urupapuro rwo kwemera kugira uruhare mubushakashatsi/ababyeyi

ARISE-GENNEURWA

Urupapuro rwo kwemera kugira uruhare mu bushakashatsi- Umubyeyi

Izina ry' ubushakashatsi : Gusuzuma Uturemangingo Twose Tw'umubiri N'impamvu Ziterwa N'ibidukikije mu bana bavukanye uburwayi bw'ubwonko mu Rwanda .

Mwaramutse,
Nitwa Profeseri Annette UWINEZA,nkaba ndi muganga ukurikirana indwara z'uruhererekane mu miryango mu bitaro bikuru bya Kigali (CHUK),nkaba n'umwarimu muri Kaminuza Nkuru y'u Rwanda, nkaba ndimo gukora ubushakashatsi mfatanyije n'abandi bashakashatsi b'abanyarwanda ndetse n'abo mu Bubiligi. Ubushakashatsi bwacu bugamije kumenya ubwoko bw'uturemangingo tudakunze kuboneka dutera kudakura cg kudakora neza kw'ubwonko hifashishijwe uburyo bwo gusoma utunyabugingo twose tw'umuntu.
Bityo rero, watoranijwe mu kugira ngo witabire ubu bushakashatsi. Ubu bushakashatsi bwemejwe na komite ishinzwe kurengera abakorwaho ubushakashatsi y'ishuri ry'ubuvuzi muri Kaminuza y'u Rwanda, ibitaro bya kaminuza bya Kigali (CHUK) na Butare (CHUB), ibitaro bya Gisirikare by'u Rwanda n'ibitaro by'indwara zo mu mutwe byigisha bya Ndera .

A. Ubu bushakashatsi bugamije iki ?
Intego y'ubu bushakashatsi n'ugukusanya amakuru yose ku cyaba gitera kuvukana uburwayi bw'ubwonko biturutse ku ndwara z'uruhererekane , kugira ngo ayo makuru abashe gukoreshwa mu kumva neza ibimenyetso byazo kandi no kumenya amavu n'amavuko y'izo ndwara, kugirango abaganga babashe kurushaho kuzisobanukirwa no kubasha kuzivura.
Kubera iyo mpamvu dushobora kubasaba gusuzuma idosiye yanyu yo kwa muganga (kureba imiterere yanyu) no kubafatira amaraso. Ibizava muri ubu bushakashatsi bizabikwa mu gitabo kandi bizakoreshwa gusa mu gusuzuma indwara z'uruhererekane.

B. Kubera iki twaguhisemo kugirango winjire muri ubu bushakashatsi ?
Watoranijwe kugira uruhare muri ubu bushakashatsi kubera ko wowe cyangwa umuntu wo mu muryango wawe afite uburwayi bw'ubwonko yavukanye akaba ashobora kuba yarabutewe n'indwara y'uruhererekane. Namwe mwerekanye ko mwifuza kurushaho kumva icyaba cyarateye iyo ndwara kandi ubumenyi buzavamo buzatuma murushaho kumva amavu n'amavuko y'iyo ndwara.
Ubushakashatsi bugirwamo uruhare gusa n'ubyifuza niyo mpamvu tubasaba gufata umwanya mukabitekerezaho binashobotse mukabiganira n'abo mu muryango wanyu. Kugira uruhare muri ubu bushakashatsi nta gahato karimo kandi uburenganzira bw'ikiremwa muntu buzubahirizwa.

C. Ni iki nasabwa gukora igihe nemeye kugira uruhare muri ubu bushakashatsi ?
Tuzakorana nawe amasaha abiri tugusaba kuduha amakuru akenewe muri ubu bushakashatsi. Uzatwemerera kandi tugufate amaraso. Ubusanzwe uburyo ubu bushakashatsi buzakorwa bimeze nk'ibyo wakorerwa ugiye kwisuzumisha cyangwa gusuzumisha umwana wawe kugirango avurwe

izo ndwara z'uruhererekane. Kandi kutagira uruhare muri ubu bushakashatsi ntibyabuza ko wavurwa cyangwa umwana wawe yavurwa igihe aje kwa muganga. Nugira uruhare muri ubu bushakashatsi tuzagufatira ibipimo nk'ibyo umuntu uje kwivuza bisanzwe. Iyo uje kwivuza cyangwa kuvuzwa tureba imiterere yanyu, tukabafatira amafoto (kugirango tubashe kwerekana neza imiterere yanyu, turabasuzuma, tukababaza indwara mwarwaye , n'ibindi byabaranze kuva mu buto bwanyu, tukababaza ibibazo k' umuryango wanyu (abana mufite, niba hari abafite ibibazo nk'ibyanyu, ...) tukabafatira amaraso, nibiba ngombwa dushobora gufatira n'ababyeyi banyu.

D. Amaraso bazamfatira azamara iki ?

Amaraso muzafatirwa azakoreshwa kugirango dukuremo utunyangingo fatizo (ADN) kugirango tubone uko dusuzuma izo ndwara z'uruhererekane. ADN ni ijambo ry'igifaransa rikoreshwa risobanura utunyabuzima tuba mu mubiri wacu dufite amakuru y'ibyo tuba twarakuye ku babyeyi bacu cyangwa kubandi bantu tugira icyo dupfana kw'isano y'amaraso. Iyo ADN ifite utwo tunyabuzima bita « gènes » dutuma tumera uko turi nko kuba muremure, mugufi, inzobe, igikara , etc, ariko dushobora gutuma tugira indwara zimwe na zimwe nk'aho usanga abantu bo muryango umwe bafite indwara zimwe twatanga urugero aho usanga mu muryango bafite ubumuga bw'uruhu, cyangwa ubugufi bukabije. Iyo ADN yanyu izasuzumwa kugirango tubashe kubona izo ndwara z'uruhererekane.

E. Ni igihe kingana gute nzaguma muri ubwo bushakashatsi ?

Ubushakashatsi bwinshi ku ndwara z'uruhererakane, busuzuma ADN bushobora kumara igihe kirekire. Nibyo kubera hagenda havumburwa ubundi buryo buhanitse bwo kubona izo ndwara dushobora kongera gukoresha ADN yanyu. Muri ubu bushakashatsi tuzabafatira amaraso inshuro imwe gusa , tuzohereza ADN yanyu mu bindi bigo by'ubuvuzi bifite ikoranabuhanga riteye imbere.

F. Ni iyihe nyungu iri mu kwitabira ubu bushakashatsi?

Kugira uruhare muri ubu bushakashatsi bigufitiye inyungu zikurikira: Ubu bushakashatsi buzafasha gusuzuma neza uburwayi hanyuma buguhe amakuru y'ukuri yateye indwara umwana wawe afite. Amakuru azavamo azafasha kandi abaganga kumenya uko bita ku mwana wawe bityo bibafashe kuzamura imibereho ye. Ku babyeyi bizabafasha kumenya ingaruka zishobora kuba ku bandi bana muzabyara. Bizagirira akamaro kandi n'abandi barwayi muhuje indwara batari muri ubu bushakashatsi.

G. Ni izihe ngaruka zishoboka zijyanye n'ubu bushakashatsi ?

Ingaruka kuri wowe nk'uwitabiriye ubu bushakashatsi ni nke. Mu ikusanyamakuru, ushobora gusabwa kudusangiza amakuru wumva yihariye kuri wowe. Ibibazo bimwe na bimwe bishobora kugukomeretsa/kugukoza isoni, ndetse singombwa ko usubiza ibibazo ibyo ari byo byose utifuza gusubiza. Bishobora kubaho ko nanone ibibazo bimwe na bimwe biguhungubanya mu mutima no mu ntekerezo. Ushobora gufata akaruhuhuko tugakomeza nyuma.Na none mu gufatirwa amaraso

ushobora kubabara, kubyimba, ariko hazafatwa ingamba kugira ngo ibyo byavuzwe haruguru bitabaho.

H. Ninde uzagira uburenganzira bwo kubona no gukoresha amakuru yanjye?
Amaraso yawe azakusanyirizwa mu ducupa twabugenewe kandi amazina yawe ntazaba ariho, kuburyo ntamuntu uri gupima amaraso ushobora kukumenya nk'umuntu ku giti cye. Tugusezeranyije mu buryo busesuye kudatangaza ibyawe. Abashakashatsi bonyine barebwa n'ubu bushakashatsi nkuko byavuzwe haruguru nibo bashobora kugera ku mubare w'ibanga uhuye n'amakuru ku buzima bwawe. Tukwijeje ko ibisubizo byawe bizafatwa mu ibanga risesuye no mu buryo butekanye ndetse n'amazina yawe ntazigera agaragara aho ariho hose mu gihe cyo gutangaza ibyavuye mu bushakashatsi kandi nta muntu numwe bizasangizwa keretse abashakashatsi bari muri uyu mushinga w'ubushakashatsi.amaraso yanyu n'ibiyagize ntibizakoreshwa mu nyungu zindi zitari izatangajwe mbere.

I. Ese nshobora kwitabira ku bushake no kwikura mu bushakashatsi
Kwitabira kwawe ni ku bushake, ushobora guhitamo kutitabira ubu bushakashatsi cyangwa kubwikuramo igihe icyo aricyo cyose, ukabimenyesha gusa ikipe y'ubushakashatsi. Turifuza kukumenyesha nanone ko niba uhisemo kutitabira ubu bushakashatsi, nta ngaruka bizakugiraho nko kudahabwa ubuvuzi usanzwe uhabwa kandi wari ubwemerewe. Niba ufashe icyemezo cyo kwikura muri ubu bushakashatsi, abashakashatsi bazakubaza niba amakuru yari yamaze gukusanywa kuri wowe ashobora gukoreshwa.

J. Ni gute nzabona amakuru ku byavuye mu bushakashatsi ?
Muri uyu mushinga w'ubushakashatsi, tugamije gusesengura ibizami by'amaraso n'amakuru, bishobora gufata amezi 6 mbere yuko tumenya ko ibyavuyemo bifite igisobanuro icyo ari cyo cyose. Niba tubonye ibisubizo ku turemangingo bifite igisobanuro cyihariye kandi kijyanye n'ubuzima bwawe, ibi bisubizo uzabihabwa tumaze kugirana ikiganiro gikwiye kirebana n'igisobanuro cyabyo.

K. Nzahemberwa kwitabira ubu bushakashatsi?
Nta gihembo kigenewe abazitabira ubu bushakashatsi. Cyakora itsinda ry'ubushakashatsi rizagenera buri mubyeyi wese waje amafaranga ibihumbi bitanu (5000 Frw) agenewe igihe yamaranye n'umushakashatsi.

L. Ni nde nahamagara igihe bibaye ngombwa?
Niba ufite ikibazo icyo aricyo cyose kijyanye n'ubu bushakashatsi, ntuzagire impungenge zo guhamagara abayobozi b'uyu mushinga w'ubushakashatsi Prof. Annette UWINEZA (+250)788741577),Dr Norbert DUKUZE (+250781268520), or Olivier HAKIZIMANA (+250788622969) cyangwa umuyobozi mukuru wa komite ishinzwe kurengera abakorerwaho ubushakashatsi muri Kaminuza y'u Rwanda ishami ry'ubuvuzi Prof. Stefan Jassen

(+250)784575900 cyangwa ugahamagara umunyamabanga wa komite Mr Sunday Francois Xavier + 250 788563311.

Kwemera kwitabira ubushakashatsi kubushake

Ibibazo byanjye kuri ubu bushakashatsi byasubijwe na----------
Nasomye(cyangwa nasomewe) ndetse numvise uruhare rwanjye muri ubu bushakashatsi, kandi nanumvise ko igihe icyo ari cyo cyose nakenera kwikuramo nabikora nta bisobanuro kandi nta ngaruka byagira mu kwitabwaho mu by'ubuzima. Kandi nemeye ko umwana wanjye yinjira mu bushakashatsi.

Ahagenewe umukono n'amakuru k'uwitabiriye ubushakashatsi

1.....
Amazina y'umwana

2..... .....
Amazina y'umubyeyi cyangwa umurera Umukono, itariki, ahantu

3. ....
Amazina y'Umushakashatsi Umukono, itariki, ahantu

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Appendix B: Urupapuro rwokwemera kugira uruhare mu bushakashatsi/Abana

ARISE-GENNEURWA

Urupapuro rwo kwemera kugira uruhare mu bushakashatsi ku bana

Izina ry' ubushakashatsi : Gusuzuma Uturemangingo Twose Tw'umubiri N'impamvu Ziterwa N'ibidukikije mu bana bavukanye uburwayi bw'ubwonko mu Rwanda .

Mwaramutse,
Nitwa Profeseri Annette UWINEZA,nkaba ndi muganga ukurikirana indwara z'uruhererekane mu miryango mu bitaro bikuru bya Kigali (CHUK),nkaba n'umwarimu muri Kaminuza Nkuru y'u Rwanda, nkaba ndimo gukora ubushakashatsi mfatanyije n'abandi bashakashatsi b'abanyarwanda ndetse n'abo mu Bubiligi. Ubushakashatsi bwacu bugamije kumenya ubwoko bw'uturemangingo tudakunze kuboneka dutera kudakura cg kudakora neza kw'ubwonko hifashishijwe uburyo bwo gusoma utunyabugingo twose tw'umuntu.
Bityo rero, watoranijwe mu kugira ngo witabire ubu bushakashatsi. Ubu bushakashatsi bwemejwe na komite ishinzwe kurengera abakorwaho ubushakashatsi y'ishuri ry'ubuvuzi muri Kaminuza y'u Rwanda, ibitaro bya kaminuza bya Kigali (CHUK) na Butare (CHUB), ibitaro bya Gisirikare by'u Rwanda n'ibitaro by'indwara zo mu mutwe byigisha bya Ndera .

A. Ubu bushakashatsi bugamije iki ?
Intego y'ubu bushakashatsi n'ugukusanya amakuru yose ku cyaba gitera kuvukana uburwayi bw'ubwonko biturutse ku ndwara z'uruhererekane , kugira ngo ayo makuru abashe gukoreshwa mu kumva neza ibimenyetso byazo kandi no kumenya amavu n'amavuko y'izo ndwara, kugirango abaganga babashe kurushaho kuzisobanukirwa no kubasha kuzivura.
Kubera iyo mpamvu dushobora kubasaba gusuzuma idosiye yanyu yo kwa muganga (kureba imiterere yanyu) no kubafatira amaraso. Ibizava muri ubu bushakashatsi bizabikwa mu gitabo kandi bizakoreshwa gusa mu gusuzuma indwara z'uruhererekane.

B. Kubera iki twaguhisemo kugirango winjire muri ubu bushakashatsi ?
Watoranijwe kugira uruhare muri ubu bushakashatsi kubera ko wowe cyangwa umuntu wo mu muryango wawe afite uburwayi bw'ubwonko yavukanye akaba ashobora kuba yarabutewe n'indwara y'uruhererekane. Namwe mwerekanye ko mwifuza kurushaho kumva icyaba cyarateye iyo ndwara kandi ubumenyi buzavamo buzatuma murushaho kumva amavu n'amavuko y'iyo ndwara.
Ubushakashatsi bugirwamo uruhare gusa n'ubyifuza niyo mpamvu tubasaba gufata umwanya mukabitekerezaho binashobotse mukabiganira n'abo mu muryango wanyu. Kugira uruhare muri ubu bushakashatsi nta gahato karimo kandi uburenganzira bw'ikiremwa muntu buzubahirizwa.

C. Ni iki nasabwa gukora igihe nemeye kugira uruhare muri ubu bushakashatsi ?
Tuzakorana nawe amasaha abiri tugusaba kuduha amakuru akenewe muri ubu bushakashatsi. Uzatwemerera kandi tugufate amaraso. Ubusanzwe uburyo ubu bushakashatsi buzakorwa bimeze nk'ibyo wakorerwa ugiye kwisuzumisha cyangwa gusuzumisha umwana wawe kugirango avurwe

izo ndwara z'uruhererekane. Kandi kutagira uruhare muri ubu bushakashatsi ntibyabuza ko wavurwa cyangwa umwana wawe yavurwa igihe aje kwa muganga. Nugira uruhare muri ubu bushakashatsi tuzagufatira ibipimo nk'ibyo umuntu uje kwivuza bisanzwe. Iyo uje kwivuza cyangwa kuvuzwa tureba imiterere yanyu, tukabafatira amafoto (kugirango tubashe kwerekana neza imiterere yanyu, turabasuzuma, tukababaza indwara mwarwaye , n'ibindi byabaranze kuva mu buto bwanyu, tukababaza ibibazo k' umuryango wanyu (abana mufite, niba hari abafite ibibazo nk'ibyanyu, ...) tukabafatira amaraso, nibiba ngombwa dushobora gufatira n'ababyeyi banyu.

D. Amaraso bazamfatira azamara iki ?

Amaraso muzafatirwa azakoreshwa kugirango dukuremo utunyangingo fatizo (ADN) kugirango tubone uko dusuzuma izo ndwara z'uruhererekane. ADN ni ijambo ry'igifaransa rikoreshwa risobanura utunyabuzima tuba mu mubiri wacu dufite amakuru y'ibyo tuba twarakuye ku babyeyi bacu cyangwa kubandi bantu tugira icyo dupfana kw'isano y'amaraso. Iyo ADN ifite utwo tunyabuzima bita « gènes » dutuma tumera uko turi nko kuba muremure, mugufi, inzobe, igikara , etc, ariko dushobora gutuma tugira indwara zimwe na zimwe nk'aho usanga abantu bo muryango umwe bafite indwara zimwe twatanga urugero aho usanga mu muryango bafite ubumuga bw'uruhu, cyangwa ubugufi bukabije. Iyo ADN yanyu izasuzumwa kugirango tubashe kubona izo ndwara z'uruhererekane.

E. Ni igihe kingana gute nzaguma muri ubwo bushakashatsi ?

Ubushakashatsi bwinshi ku ndwara z'uruhererakane, busuzuma ADN bushobora kumara igihe kirekire. Nibyo kubera hagenda havumburwa ubundi buryo buhanitse bwo kubona izo ndwara dushobora kongera gukoresha ADN yanyu. Muri ubu bushakashatsi tuzabafatira amaraso inshuro imwe gusa , tuzohereza ADN yanyu mu bindi bigo by'ubuvuzi bifite ikoranabuhanga riteye imbere.

F. Ni iyihe nyungu iri mu kwitabira ubu bushakashatsi?

Kugira uruhare muri ubu bushakashatsi bigufitiye inyungu zikurikira: Ubu bushakashatsi buzafasha gusuzuma neza uburwayi hanyuma buguhe amakuru y'ukuri yateye indwara umwana wawe afite. Amakuru azavamo azafasha kandi abaganga kumenya uko bita ku mwana wawe bityo bibafashe kuzamura imibereho ye. Ku babyeyi bizabafasha kumenya ingaruka zishobora kuba ku bandi bana muzabyara. Bizagirira akamaro kandi n'abandi barwayi muhuje indwara batari muri ubu bushakashatsi.

G. Ni izihe ngaruka zishoboka zijyanye n'ubu bushakashatsi ?

Ingaruka kuri wowe nk'uwitabiriye ubu bushakashatsi ni nke. Mu ikusanyamakuru, ushobora gusabwa kudusangiza amakuru wumva yihariye kuri wowe. Ibibazo bimwe na bimwe bishobora kugukomeretsa/kugukoza isoni, ndetse singombwa ko usubiza ibibazo ibyo ari byo byose utifuza gusubiza. Bishobora kubaho ko nanone ibibazo bimwe na bimwe biguhungubanya mu mutima no mu ntekerezo. Ushobora gufata akaruhuhuko tugakomeza nyuma.Na none mu gufatirwa amaraso

ushobora kubabara, kubyimba, ariko hazafatwa ingamba kugira ngo ibyo byavuzwe haruguru bitabaho.

H. Ninde uzagira uburenganzira bwo kubona no gukoresha amakuru yanjye?

Amaraso yawe azakusanyirizwa mu ducupa twabugenewe kandi amazina yawe ntazaba ariho, kuburyo ntamuntu uri gupima amaraso ushobora kukumenya nk'umuntu ku giti cye. Tugusezeranyije mu buryo busesuye kudatangaza ibyawe. Abashakashatsi bonyine barebwa n'ubu bushakashatsi nkuko byavuzwe haruguru nibo bashobora kugera ku mubare w'ibanga uhuye n'amakuru ku buzima bwawe. Tukwijeje ko ibisubizo byawe bizafatwa mu ibanga risesuye no mu buryo butekanye ndetse n'amazina yawe ntazigera agaragara aho ariho hose mu gihe cyo gutangaza ibyavuye mu bushakashatsi kandi nta muntu numwe bizasangizwa keretse abashakashatsi bari muri uyu mushinga w'ubushakashatsi.amaraso yanyu n'ibiyagize ntibizakoreshwa mu nyungu zindi zitari izatangajwe mbere.

I. Ese nshobora kwitabira ku bushake no kwikura mu bushakashatsi

Kwitabira kwawe ni ku bushake, ushobora guhitamo kutitabira ubu bushakashatsi cyangwa kubwikuramo igihe icyo aricyo cyose, ukabimenyesha gusa ikipe y'ubushakashatsi. Turifuza kukumenyesha nanone ko niba uhisemo kutitabira ubu bushakashatsi, nta ngaruka bizakugiraho nko kudahabwa ubuvuzi usanzwe uhabwa kandi wari ubwemerewe. Niba ufashe icyemezo cyo kwikura muri ubu bushakashatsi, abashakashatsi bazakubaza niba amakuru yari yamaze gukusanywa kuri wowe ashobora gukoreshwa.

J. Ni gute nzabona amakuru ku byavuye mu bushakashatsi ?

Muri uyu mushinga w'ubushakashatsi, tugamije gusesengura ibizami by'amaraso n'amakuru, bishobora gufata amezi 6 mbere yuko tumenya ko ibyavuyemo bifite igisobanuro icyo ari cyo cyose. Niba tubonye ibisubizo ku turemangingo bifite igisobanuro cyihariye kandi kijyanye n'ubuzima bwawe, ibi bisubizo uzabihabwa tumaze kugirana ikiganiro gikwiye kirebana n'igisobanuro cyabyo.

K. Nzahemberwa kwitabira ubu bushakashatsi?

Nta gihembo kigenewe abazitabira ubu bushakashatsi. Cyakora itsinda ry'ubushakashatsi rizagenera buri mubyeyi wese waje amafaranga ibihumbi bitanu (5000 Frw) agenewe igihe yamaranye n'umushakashatsi.

L. Ni nde nahamagara igihe bibaye ngombwa?

Niba ufite ikibazo icyo aricyo cyose kijyanye n'ubu bushakashatsi, ntuzagire impungenge zo guhamagara abayobozi b'uyu mushinga w'ubushakashatsi Prof. Annette UWINEZA (+250)788741577),Dr Norbert DUKUZE (+250781268520), or Olivier HAKIZIMANA (+250788622969) cyangwa umuyobozi mukuru wa komite ishinzwe kurengera abakorerwaho ubushakashatsi muri Kaminuza y'u Rwanda ishami ry'ubuvuzi Prof. Stefan Jassen

ETHICS COMMITTEE
CHUK

(+250)784575900 cyangwa ugahamagara umunyamabanga wa komite Mr Sunday Francois Xavier + 250 788563311.

Kwemera kwitabira ubushakashatsi kubushake

Ibibazo byanjye kuri ubu bushakashatsi byasubijwe na----------
Nasomye(cyangwa nasomewe) ndetse numvise uruhare rwanjye muri ubu bushakashatsi, kandi nanumvise ko igihe icyo ari cyo cyose nakenera kwikuramo nabikora nta bisobanuro kandi nta ngaruka byagira mu kwitabwaho mu by'ubuzima.

Ahagenewe umukono n'amakuru k'uwitabiriye ubushakashatsi

1.....
Amazina y'umwana

2..... .....
Amazina y'umubyeyi cyangwa umurera Umukono, itariki, ahantu

3. ....
Amazina y'Umushakashatsi Umukono, itariki, ahantu

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Appendix C: Data collection tool

Confidential

Identification

Record ID	_____
Participant Study ID	_____ (Participant Study ID)
Father's Participant ID	_____ (Father Participant Study ID)
Mother's Participant ID	_____ (Mother's Participant ID)
Date of Birth	_____
Gender	◯ Female ◯ Male ◯ Not reported
Where is your primary home location? (Confirm from medical records) Country:	◯ Rwanda ◯ Burundi ◯ DRC ◯ Other
Specify if other country of residence	_____
Province	◯ Kigali ◯ Eastern ◯ Western ◯ Southern ◯ Northern

District	◯ Bugesera ◯ Burera ◯ Gakenke ◯ Gasabo ◯ Gatsibo ◯ Gicumbi ◯ Gisagara ◯ Huye ◯ Kamonyi ◯ Karongi ◯ Kayonza ◯ Kicukiro ◯ Kirehe ◯ Muhanga ◯ Musanze ◯ Ngoma ◯ Ngororero ◯ Nyabihu ◯ Nyagatare ◯ Nyamagabe ◯ Nyamasheke ◯ Nyanza ◯ Nyarugenge ◯ Nyaruguru ◯ Rubavu ◯ Ruhango ◯ Rulindo ◯ Rusizi ◯ Rutsiro ◯ Rwamagana
Sector	_____
Telephone number	_____
Site of consultation	_____
Date of consultation	_____ (date of consultation)

Prenatal and Neonatal Period

What is the chief complaint ?	_____
Did the mother have any problem during the pregnancy?	◯ Yes ◯ No (Probleme during pregnancy)
Which problem did you have during pregnancy?	_____
Birth weight (in gm)	_____ (Birth Weight)
What was the APGAR Score ?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10 ◯ Unknwon
Mode of delivery	◯ normal delivery ◯ forceps ◯ vacuum ◯ cesarean section (mode of delivery)
Maturity at birth	◯ Premature ◯ Term ◯ Post-term.
Place of Birth	◯ Home ◯ Health center ◯ Hospital
Which health Center/hospital ?	_____
Did he/she cry immediately after birth?	◯ Yes ◯ No ◯ Don't know
if no, after how long he cried?	_____
Did he/she need resuscitation ?	◯ Yes ◯ No ◯ Don't know

If yes, why?	_____
Did he/she have hypotonia ?	◯ Yes ◯ No ◯ Don't know
Did he/she have jaundice ?	◯ Yes ◯ No ◯ Don't know
Did he/she have seizure ?	◯ Yes ◯ No ◯ Don't know
Did he/she have fever ?	◯ Yes ◯ No ◯ Don't know
Provide any additional information of the neonatal period	_____

Personal Medical History

Did he/she have a normal psychomotor development?	◯ Yes ◯ No
At which age did he/she sit ?	_____
At which age did he/she crawl ?	_____
At which age did he/she walk ?	_____
Did he/she have any disease during childhood?	◯ Yes ◯ No
If yes, which one?	_____
Did he/she have epilepsy?	◯ Yes ◯ No
If yes, which type of epilepsy ?	_____
Did he/she have history of head trauma ?	_____
Did he/she have history of any surgery ?	◯ yes ◯ No
If yes , what type of surgery ? At which age ?	_____

Family History

Age of the mother at the time of birth	_____
Age of the father at the time birth	_____
Parents have any consanguinity	◯ Yes ◯ No
If yes which degree?	◯ 1st degree ◯ 2nd degree ◯ 3rd degree ◯ Other
What is the other degree of consanguinity ?	_____
Mother's history of miscarriage	◯ Yes ◯ No ◯ Don't know
If yes, how many miscarriages ?	_____
Any history of previous genetic disease in the family	◯ Yes ◯ No ◯ Don't know
Which genetic disease ? who was/were affected in the family?	_____
Any history of congenital malformation in the family?	◯ Yes ◯ No ◯ Don't know
Which congenital malformation ? who was/were affected in the family?	_____
Pedigree (Three generations)	

Physical examination

Weight (kg):	_____
Height (cm):	_____
Head Circumference (cm):	_____ (Head Circumference)
Presence of dysmorphic features	◯ Yes ◯ No
If yes , describe dysmorphic features	_____
Presence of any associated anomaly ?	◯ Yes ◯ No
If yes, does the patient have following cardiovascular diseases?	☐ Unknown cyanotic congenital heart disease ☐ Unknown acyanotic congenital heart disease ☐ Atrial Septal Defect ☐ Atrioventricular septal defect ☐ Coarctation of aorta ☐ Dextrocadia ☐ Diaphragmatic hernia ☐ Hypoplastic left heart syndrome ☐ Patent Ductus Arteriosus ☐ Pulmonary valve atresia ☐ Pulmonary valve stenosis ☐ Situs invertus ☐ Tetralogy Of Fallot ☐ Total anomalous pulmonary venous connection ☐ Transposition of the Great Arteries ☐ Ventricular Septal Defect ☐ Other cardiovascular anomaly
What type of other cardiovascular anomaly	_____
If yes does he/she have face or head anomaly?	☐ Anencephaly ☐ Anophthalmos ☐ Anotia (absent ear)/Microtia (incompletely formed ear) ☐ Choanal Atresia ☐ Cleft palate and/or lip ☐ Congenital cataract ☐ Craniosynostosis ☐ Encephalocele ☐ Holoprosencephaly ☐ Hydrocephalus ☐ Microcephaly ☐ Microphthalmos ☐ Porencephaly ☐ Other anomaly of face/head

Other anomaly of face/head	_____
if yes Endocrine system abnormality	☐ Hypothyroidism ☐ Other endocrine anomaly
Other endocrine anomalies	_____
Reproductive system	☐ Indeterminate sex (ambiguous genitalia) ☐ Other reproductive anomaly
Other reproductive anomaly	_____
Gastrointestinal system anomaly	☐ Atresia of bile ducts (biliary atresia) ☐ Atresia of oesophagus with tracheo-oesophageal fistula ☐ Atresia of oesophagus without fistula ☐ Congenital absence, atresia or stenosis of anus ☐ Congenital absence, atresia or stenosis of small intestine ☐ Gastroschisis ☐ Omphalocele ☐ Prune belly syndrome ☐ Stenosis of the colon or intesine ☐ Other GI anomaly
Other GI anomaly	_____
Presence of Urinary system anomaly	☐ Exstrophy of urinary bladder ☐ Hydroureter ☐ Hypospadias ☐ Multicystic renal dysplasia ☐ Polycystic kidneys ☐ Posterior urethral valves ☐ Renal agenesis/hypoplasia ☐ Other urinary system anomaly
Other urinary system anomaly	_____
Musculoskeletal system	☐ Achondroplasia ☐ Arthrogryposis multiplex congenita ☐ Club foot (e.g. Talipes equinovarus ☐ Osteogenesis imperfecta ☐ Polydactyly ☐ Syndactyly ☐ Unspecificied congenital malformation of limb(s) ☐ Other MSK anomaly
Other MSK anomaly	_____
Nervous system anomaly	☐ Neural tube defects ☐ Other nervous system anomaly

Other NS anomaly	_____
Other Anomalies	_____
Degree of Intellectual disability/	◯ Mild ◯ Moderate ◯ Severe Proufound ◯ No intellectual disability
Degree of Developmental delay	◯ Mild ◯ Moderate ◯ Severe ◯ Profound ◯ Normal development
Type of Neurodevelopmental disorder:	◯ Global developmental delay isolated. ◯ Global developmental delay isolated and congenital anomalies ◯ intellectual disability isolated ◯ intellectual disability isolated and congenital anomalies ◯ Microcephaly ◯ Autism spectrum disorders ◯ Others
Other type of NDD	_____
Genetic investigation	☐ Karyotype ☐ Array_CGH(microarry) ☐ Sanger sequencing ☐ Whole exome sequencing ☐ Whole genome sequencing ☐ X-fragile detection
Karyotype result /or other genetic result	_____
Other investigations	☐ Cardiac ultrasound ☐ Brain CT Scan ☐ MRI ☐ EEG ☐ EMG ☐ Biology (specify if abnormal) ☐ Other
Give the result of the investigation if available	_____

Appendix D: Ethical Approval from the University Teaching Hospital of Kigali

CENTRE HOSPITALIER UNIVERSITAIRE
UNIVERSITY TEACHING HOSPITAL

Quality Health Care
Training & Research

Ethics Committee / Comité d'éthique

13th Feb,2025 *Ref.:EC/CHUK/1/171/2023*

Review Approval Notice

Dear Annette UWINEZA,

Your research project: ***"Genomic and Environmental factors of neurodevelopmental disorders in Rwandan Children: (GENNEURWA) "***

During the meeting of the Ethics Committee of University Teaching Hospital of Kigali (CHUK) that was held on 13th Feb,2025 to evaluate your request for ethical approval of the above mentioned research project, we are pleased to inform you that the Ethics Committee/CHUK has approved your renewal to this research project.

You are required to present the results of your study to CHUK Ethics Committee before publication by using this link:www.chuk.rw/research/fullreport/?appid=1010&&chuk.

PS: Please note that the present approval is valid for 12 months.

Yours sincerely,

Dr Emmanuel Rusingiza Kamanzi
The Chairperson, Ethics Committee,
University Teaching Hospital of Kigali

ETHICS COMMITTEE
CHUK

Scan code to verify.

" University teaching hospital of Kigali Ethics committee operates according to standard operating procedures (Sops) which are updated on an annual basis and in compliance with GCP and Ethics guidelines and regulations "

Web Site : www.chuk.rw ; B.P. 655 Kigali- RWANDA Tél.: 00 (250) 252575462. E-Mail: chuk.hospital@chuk.rw

Appendix E: Ethical Approval from Ndera Neuropsychiatric Referral Hospital

NDERA NEURO-PSYCHIATRIC TEACHING HOSPITAL
BROTHERS OF CHARITY
P.O Box 423 Kigali - Rwanda; Tel: +250 788 827 364 / +250 781 447 928 / Toll free : 2575
Website: www.caracsnderahospital.rw E - mail : ndera.hospital@moh.gov.rw

NDERA NEURO-PSYCHIATRIC TEACHING HOSPITAL ETHICS COMMITTEE

Ndera, October 20, 2023
N° 045/NNPTHEC/2023

Principal Investigator: Assoc .Prof. Annette UWINEZA, MD, PHD
Associate professor in Human genetics, CMHS-UR

Your research progress report regarding your project **"Genomic and environmental factors of neuro-developmental disorders of children in Rwanda: (GENNEURWA)".**

Members of Ethics Committee - NNPT Hospital			Involved in the decision		
N°	Names	Position	Yes	Absent	Withdrawn from the proceeding
1.	Dr. Fidele SEBERA	Director of Medical & Allied Health Sciences Services / Neurologist – NNPTHEC *Chairperson*	x		
2.	Josiane UMWIRINGIRWA	Research assistant – NNPTHEC *Secretary*	x		
3.	Alain NYAMWASA	Data Manager	x		
4.	Israel IRAZIRIKANA	Legal Advisor		x	
5.	Emmanuel KAGABO	Mental health nurse	x		
6.	Ernestine RUDASINGWA	Mental health nurse		x	
7.	Clemence UWAMAHORO	Mental health nurse-Icyizere center	x		
8.	Dr Jean Petit HABONIMANA	Medical doctor	x		
9	Emmanuel NTAKIYISUMBA	Senior mental health nurse in charge of education, research, CPD and coaching	x		

After reviewing your progress report and related documents, presented during the Ndera Neuro-psychiatric Teaching Hospital Ethics Committee. (NNPTHEC) meeting of October 10, 2023 where quorum was met, **we hereby provide a renewed approval for the above-mentioned protocol.**

Please note that the approval of protocol is valid for **12 months.**

You are responsible for fulfilling the following requirements:

1. Changes, amendments and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of changes.
2. Only approved consent form to be used in the enrollment of participants.
3. All consent forms signed by subjects should be retained on file, the NNPTHEC may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to NNPTHEC in a timely fashion and before expiry of this approval.
5. Failure to submit a continuing review application will result in termination of the study.
6. Notify Ndera Neuro-psychiatric Teaching Hospital Ethics Committee once the study is finished.
7. Submission of **a final copy of research findings** to the hospital is **mandatory**.

Date of Issue: **October 20, 2023**
Expiration date: **October 19, 2024**

Sincerely,

NDERA NEUROPSYCHIATRIC Teaching Hospital

Dr.Fidele SEBERA

Chairperson, Ndera Neuro-psychiatric Teaching Hospital Ethics Committee

C.C:

- Director General of Ndera Neuro-psychiatric Teaching Hospital
- Director of Education, Research, CPD and Quality Improvement

Appendix F: Ethical Approval from the University of Rwanda

UNIVERSITY of RWANDA

COLLEGE OF MEDICINE AND HEALTH SCIENCES

DIRECTORATE OF RESEARCH & INNOVATION

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 27th /June/2023

Prof. Annette Uwineza
School of Medicine and Health Sciences, CMHS, UR

Approval Notice: No 298/CMHS IRB/2023

Your Project Title ***"Genomic and Environmental factors of neurodevelopmental disorders in Rwandan Children"*** has been evaluated by CMHS Institutional Review Board.

Name of Members	Institute	Involved in the decision		
		Yes	No (Reason)	
			Absent	Withdrawn from the proceeding
Assoc. Prof. Stefan JANSEN	UR-CMHS	X		
Assoc. Prof. Donatilla MUKAMANA	UR-CMHS	X		
Dr Danilo Melanes ZAMBRANO	UR-CMHS	X		
Prof Peace UWAMBAYE	UR-CMHS	X		
Dr Nuhu ASSUMAN	UR-CMHS	X		
Dr Moussa HAKIZIMANA	UR-CMHS	X		
Dr. Oliva BAZIRETE	UR-CMHS	X		
Dr. Judith MUKAMULIGO	UR-CMHS	X		
Dr. Eugene RUTAYISIRE	UR-CMHS	X		
Dr Innocent HAHIRWA	UR-CMHS	X		
Assoc. Prof. Eugene RUTEMBESA	UR-CMHS	X		
Dr Isiaka ABDULLATEEF	UR-CMHS	X		
Assoc. Prof. Aimable MUSAFIRI	UR-CMHS	X		
Mr Sunday Francois Xavier	UR-CMHS	X		

After reviewing your protocol during the IRB meeting of where quorum was met and revisions made on the advice of the CMHS IRB submitted on 26th June 2023, **Approval has been granted to your study.**
Please note that approval of the protocol and consent form is valid for **12 months**.

Email: researchcenter@ur.ac.rw P.O Box 3286 Kigali, Rwanda www.ur.ac.rw

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 27th /June /2023

Expiration date: The 27th /June /2024

Prof Stefan JANSEN
Ag. Chairperson Institutional Review Board,
College of Medicine and Health Sciences, UR

Cc:
- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate Studies, UR