# PREVALENCE AND TYPES OF INBORN ERRORS OF METABOLISM AMONG CHILDREN AND ADOLESCENTS WITH AUTISM SPECTRUM DISORDER AT KENYATTA NATIONAL HOSPITAL.

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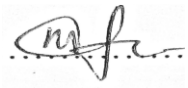
## DECLARATION

This dissertation proposal is my original work and has not been presented for the award of any degree in any other university.

**Signature**:  **Date:** 5th/05/2023

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**COLLABORATING INSTITUTIONS**

1. UNIVERSITY OF NAIROBI
2. KENYATTA NATIONAL HOSPITAL

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**LIST OF ABBREVIATIONS**

| | |
|---|---|
| ADI-R: | Autism Diagnostic Interview-Revised |
| AR: | Autosomal Recessive |
| ASD: | Autism Spectrum Disorder |
| ASK: | Autism Society of Kenya |
| CARS: | Childhood Autism Rating Scale |
| DSM IV: | Diagnostic Statistical Manual-Fourth |
| ID: | Intellectual Disability |
| IEM: | Inborn Errors of Metabolism |
| KNH: | Kenyatta National Hospital |
| PHE: | Phenylalanine |
| PKU: | Phenylketonuria |
| UoN: | University of Nairobi |
| MBChB: | Bachelor of Medicine and Bachelor of Surgery |

**DEFINITION OF TERMS**

**Inborn Errors of Metabolism:** genetic metabolic disorders that occur due to lack of specific enzymes that the body requires to process different molecules such as carbohydrates, fats, proteins, vitamins, and so on

**Autism Spectrum Disorder:** is a Neurodevelopmental disorder, with onset in early childhood, characterized by deficits in social communication and the presence of restricted interests and repetitive behaviour, as per the diagnostic statistical manual- fifth (DSM-5)

**Intellectual disability:** is a neurodevelopmental disorder characterized by limitations in both intelligence and adaptive skills, affecting at least one of three adaptive domains; conceptual, social, or practical.

**Genetic Mutation**: an alteration in the nucleotide sequence of the genome of an organism, which may or may not produce detectable changes in the observable characteristics of an organism

**Genotype**: the total of the genetic information contained on the chromosomes which determines an individual's phenotype.

**Autosomal recessive:** a pattern of inheritance whereby for the trait or disease to be displayed, two copies of the gene for the trait or disorder need to be present. The gene is located on a non-sex chromosome.

**Heterozygous**: Having different alleles for one or more genes in homologous chromosome segments

**Homozygous**: having identical alleles of the gene are present on both homologous chromosomes.

**Phenotype**: the observable characteristics or traits of an organism produced by the interaction between the organism's genotype and the environment in which it finds itself

**Consanguinity:** a relationship between two or more persons, characterized by the sharing of common ancestors; with an implication of having similar genetic material and characteristics passed unto offspring. It increases the probability of inheritance of transmissible genotypes.

**Dysmorphic:** an abnormality in the development of form or structure, which results in the abnormal shape of a body structure

**Metabolism**: these are chemical reactions that occur in the living organisms that aim to sustain life e.g. respiration, digestion

**Intellectual disability (also known as mental retardation):** is a neurodevelopmental disorder characterized by limitation in intellectual function (intelligence) and adaptive functioning of varying severity, presenting before 18 years of age.

**Intellectual functioning** refers to one's general mental ability including reasoning, planning, problem-solving, abstract thinking, and learning.

**Adaptive function:** refers to one's ability concerning everyday life skills or tasks that the average person can complete.

**Convulsions (Seizure**): is a clinical expression of abnormal, excessive, or synchronous discharges of neurons residing primarily in the cerebral cortex. The outward effects of which vary from uncontrolled shaking movements involving most of the body; to shaking movements involving only one part of the body, to a subtle momentary loss of awareness and with varying degrees of awareness of ones surrounding. It may last for seconds to a few minutes.

**Cataracts:** refers to an opacity of the lens of the eye, which can cause partial or total blindness

**Contracture** is the shortening and hardening of a muscle or a joint due to increased tone of a given group of muscles or when normally elastic tissues (muscles or tendons) are replaced by inelastic fibrous tissues.

**Hepatomegaly:** refers to the enlargement of the liver beyond the normal size

**Splenomegaly:** refers to the enlargement of the spleen beyond the normal size

**Palpitations:** refers to abnormal awareness of the heartbeat such as loud, fast, not regular

**Orthopnea:** refers to being short of breath when lying flat (on a couch or bed)

**Edema:** refers to fluid build-up and retention in the body tissues, commonly presenting with swelling and hardness of the legs/feet, arms, and face when they are affected. Other body tissues may be affected

**Developmental delay:** Failure to attain the developmental skills expected for the age and sex

**Encephalopathy:** clinical features that occur due to damage to the brain structure or function

**Microcephaly/Macrocephaly:** refers to a smaller (microcephaly) or lager (macrocephaly) size of the head than expected for age

**Nystagmus:** repetitive, uncontrolled movements of the eyes

**Ptosis:** Drooping of the upper eyelids

**ABSTRACT**

**Background**: Autism affects about 1 in every 160 children globally. Genetic defects are associated with the development of Autism and about 10 to 20 % of these patients have genetic metabolic abnormalities. Involvement of the central nervous system in patients with Inborn Errors of Metabolism, result in neuropsychiatric manifestations including autism. Thus early detection and treatment of inborn errors of metabolism in patients with Autism is important for better management and improved outcomes.

**Study objectives:** To determine the prevalence of Inborn Errors of Metabolism (IEMs) in children and adolescents with Autism spectrum disorder at Kenyatta National Hospital (KNH); to determine types of IEMs, and to describe the clinical characteristics of Autism Spectrum Disorder patients with Inborn Errors of Metabolism.

**Study design:** This was a descriptive Cross-sectional study.

**Methodology:** The study population comprised patients aged 18 months to 25 years with a clinical diagnosis of autism, on follow-up at the occupational therapy, neurology, and Psychiatry clinics at KNH. Consecutive sampling technique was used to recruit 78 study participants. Data was collected using an interviewer-administered questionnaire. A medical history and physical examination were done for each participant to obtain data on disease symptoms, treatment they are undergoing and relevant family history. Blood samples were then drawn and sent to Centogene Laboratory in Germany for genetic testing

**Data analysis**: SPSS software was used for data analysis Continuous data was summarised using medians and Interquartile range, and categorical data summarized using frequencies and percentages. The prevalence of inborn errors of metabolism was calculated as a proportion and Binary logistic Regression analysis was used to assess for associations. p value < 0.05 was used to define significant associations.

**Results:** Prevalence of Inborn errors of metabolism among children and adolescents with autism spectrum disorder at KNH was 14.1% 95%CI (7.3 - 23.8%). Metabolic disorders identified included Hunter syndrome 45.5% (n =5), Phenylketonuria 36.3% (n =4), Glycogen storage disorder 9.1% (n =1), and Methylmalonic aciduria with homocysteinemia at 9.1% (n =1). Clinical characteristics associated with presence of genetic mutations linked to Inborn Errors of metabolism were macrocephaly (OR =9.19, 95%CI:1.72 – 48.98, p =0.009), family history of autism (OR =2.71, 95%CI:1.34 – 14.32, p<0.001), and mental retardation (OR =4.13, 95%CI:2.11 – 15.74, p<0.001)were significantly associated with IEM. Genetic disorders such as autosomal dominant susceptibility to autism, Cornelia

de Lange syndrome type 1 (CDLS), Intellectual developmental disorders, Bardet-Biedl syndrome type 19, Coffin-Siris syndrome-12 among others, were also found.

**Conclusion**: There is high prevalence of IEMs among children and adolescents with Autism Spectrum Disorders at KNH and Hunter syndrome is the most common IEM reported among these children. Positive Family history of ASD, intellectual disability, macrocephaly, overweight, and musculoskeletal abnormalities showed a positive association with the presence of IEM among patients with ASD. Macrocephaly, family history of autism, mental retardation and seizures were associated with genetic mutations for IEMs among children.

**Recommendations:** There is need to integrate early testing for enzyme deficiencies among children with autism spectrum disorders to guide better treatment approaches for improved outcomes

## 1.0. INTRODUCTION

### 1.1. BACKGROUND

Autism spectrum disorder (ASD) refers to a neurodevelopmental disorder, with onset in early childhood, and in which the affected individuals have impaired social communication, restricted interests, and repetitive behavior (1). According to World Health Organization (WHO), there are approximately 1 in 160 children with Autism globally, with 10 to 15 per 10,000 of the adult population affected worldwide. In the United States, 1 in every 54 persons has Autism, with 4 males being affected for every 1 female(2). The prevalence of Autism in Sub-Saharan Africa and Kenya remains largely unknown. It is estimated to be about 2.3% of the population in Nigeria (3), and about 6.8 per 1000 persons in Uganda(4). In Kenya, Autism is estimated to affect about 4% of the population, by the Kenya autism society.

Genetic and environmental factors are implicated in the development of autism by disrupting neuronal development and connectivity, myelination, or by impairing the function of synapses and neurotransmitters (5). A significant number of individuals with autism have genetic metabolic abnormalities that cause enzyme deficiencies (6). Inborn errors of metabolism occur due to single gene defects that result in a deficiency of a specific enzyme, its cofactor, or a transport protein (6). Enzyme deficiencies affect different pathways of neurodevelopment resulting in neurological dysfunction in untreated individuals. Autism occurs as one of the neuropsychiatric manifestations in patients with enzyme deficiencies (6) (7) (8)

The common enzyme deficiencies found in patients with autism include Phenylketonuria, Isovaleric acidemia, acyl CoA dehydrogenase deficiency, and mitochondrial disorders. Others include Niemann pick disease, Lesch Nyhan syndrome, and Smith Lemli Opitz syndrome (7) (9).

Individuals with autism manifest with deficits in social communication and interaction which presents as impaired social-emotional reciprocity or impaired nonverbal communication. They also have repetitive movements and speech, ritualized patterns of behavior, and fixated interests (10). Enzyme deficiencies on the other hand may manifest with acute symptoms of vomiting, feeding intolerance, lethargy, seizures, or respiratory disturbances (11). Chronic manifestations include neuropsychiatric symptoms, and behavioral disturbances such as inflicting self- injuries, aggression(12). Intellectual disability, seizures, hyperkinetic movements, dystonia, chorea, and hearing loss may also occur. Other patients will have dysmorphic features; developmental delay; failure to thrive; gastrointestinal, cardiac, or skin manifestations (11) (13).

While the diagnosis of Autism spectrum disorder is made clinically, based on the diagnostic statistical manual V criteria, the diagnosis of Inborn Errors of Metabolism (IEM) relies upon an assessment of the symptoms and signs, as well as genetic testing. Findings that may indicate an underlying enzyme

deficiency include unexplained lethargy, recurrent vomiting, early-onset seizures, dimorphic features, intellectual disability; history of siblings with similar features, or family history of early childhood deaths due to neurologic, or cardiac disease. Genetic analysis is then carried out by whole genome or exome sequencing, to confirm the diagnosis (5) (10) (11)

As some of the patients presenting with autism spectrum disorder may have underlying, treatable enzyme deficiencies, there is a need to identify these enzyme deficiencies to help develop a proper management plan. Diagnosis majorly begins with a high index of suspicion, based on the patient's symptoms and signs which may be the first indicators of an underlying Inborn Error of Metabolism (13). Clinical features which may suggest the presence of a metabolic disease include unexplained, recurrent lethargy, recurrent vomiting, early-onset seizures, dysmorphic features, and intellectual disabilities (ID). Other features include delayed development and milestone acquisition, family history of consanguinity, other siblings/ family members with similar features, or history of early childhood deaths among siblings due to neurologic, cardiac, or hepatic disease. (11)

The common genetic analysis approaches that may be conducted to confirm the diagnosis of Inborn Errors of Metabolism include karyotyping, chromosomal microarray, or single-gene sequencing**.** Tandem Mass Spectrometry analysis done on a dry blood spot sample, has also been applied in numerous settings especially in developed countries to diagnose different forms of Inborn Errors of Metabolism (14) (15)

### 1.2 CAUSES OF AUTISM SPECTRUM DISORDER

Genetic and environmental factors play a role in the development of autism, by interfering with normal neurodevelopment. Genetic defects are found in a significant number of patients with autism spectrum disorder (ASD), and there is a higher concordance rate of autism of up to 70% in identical twins. (16)(1)

Genetic mutations that cause autism may be inherited or may occur spontaneously, as genetic material deletion, duplication, or sequence change. Some of these mutations also result in disorders of metabolism or mitochondrial function, as well as specific syndromes such as tuberous sclerosis, fragile X syndrome, down syndrome, among others.

Environmental factors associated with an increased risk of autism include toxins, teratogens, prenatal infections such as rubella, cytomegalovirus; maternal drug use such as valproic acid during pregnancy. (17)(18)

Several inborn errors of metabolism (IEM) thus have been associated development of the clinical autism spectrum. Mutations in genes that code for specific metabolic enzymes result in individual IEM and, ASD phenotype if not treated. Common enzyme deficiencies associated with ASD include

Phenylketonuria, Biotinidase Deficiency, Isovaleric Acidemia, Disorders of Mitochondrial function, Glutaric Acidemia, Lesh Nyhan Syndrome, among others. The different metabolic insults result in disruption of neuronal development and connectivity, impaired myelination, and impaired synaptic function and neurotransmission. (9)(16)

### 1.3 OVERVIEW AND CLASSIFICATION OF INBORN ERRORS OF METABOLISM

Inborn errors of Metabolism refer to genetic metabolic disorders that occur due to single gene defects that result in the deficiency of a specific enzyme, its cofactor, or a transport protein. In such a case, the body is not able to properly metabolize, break down or synthesize different substrates. Because of this dysfunction, some substrates accumulate in the body to toxic levels; or the body is not able to make important molecules like amino acids or support some metabolic pathways. The mode of inheritance of Inborn errors of metabolism (IEM) is mainly autosomal recessive (AR). (19) (6)

Disorders of metabolism can be grouped according to the metabolic process affected, or according to the underlying pathophysiologic process that results from the enzyme deficiency. (11)

**Table1: Classification of IEM Based on metabolic process affected:**

| **Main Category** | **Sub-types** |
|---|---|
| **Disorders of Intermediary Metabolism** | Disorders of Amino acid metabolism and transport<br>Disorders of Fatty acid metabolism<br>Disorders of carbohydrate metabolism<br>Disorders of Mitochondrial energy metabolism<br>Disorders of Vitamin metabolism such as folate, cobalamine<br>Others: disorders of peptide and mineral metabolism |
| **Disorders of synthesis and breakdown of complex molecules** | Disorders of Purine and Pyrimidine metabolism<br>Disorders of Lysosomal storage<br>Disorders of peroxisomes<br>Disorders of Heme and bile acid metabolism<br>Others: disorders of Glycosylation, lipoproteins, and sterol metabolism |
| **Disorders of Neurotransmitter metabolism** | Disorder of serine and Glycine metabolism<br>Disorder of Gamma-aminobutyric metabolism<br>Others: a disorder of pterin and amine metabolism |

Disorders of Metabolism can also be classified based on underlying pathophysiologic processes, into the following 3 categories (11)

### 1.3.1. Disorders that give rise to Intoxication or Encephalopathy:

These include inborn errors of intermediary metabolism which lead to either acute or progressive intoxication because of the accumulation of toxic compounds proximal to the site of metabolic block. Examples are disorders of amino acid metabolism (such as phenylketonuria, homocystinuria, among others.), organic acidurias (such as Isovaleric aciduria, propionic aciduria), urea cycle defects, carbohydrate intolerance disorders (such as galactosemia, fructose intolerance). Other disorders that may cause intoxication include Wilson's disease, hemochromatosis, among others. Affected patients may have symptom-free periods which are then followed by episodes of acute intoxication with progression or recurrence of metabolic disturbances. (11)

### 1.3.2. Disorders Involving Energy Metabolism:

These include inborn errors of intermediary metabolism. Symptoms occur due to deficiency in energy production or utilization in various organs such as the liver, heart, muscle, brain, and other tissues. Examples include defects of Mitochondrial energy metabolism, fatty acid chain oxidation disorders, disorders of glycolysis, as well as disorders of gluconeogenesis and glycogen metabolism

Affected patients present with low blood sugar, high lactate, hepatomegaly, generalized hypotonia, myopathy, failure to thrive, heart involvement (with cardiomyopathy, heart failure), as well as neurological manifestations due to brain involvement. Some of these disorders interfere with embryo-fetal development and may cause dysmorphism and other congenital malformations. (11) (6)

### 1.3.3. Disorders of complex Molecule metabolism:

These include disorders of metabolism in different cellular organelles, which affect the breakdown of complex molecules and include lysosomal storage disorders, peroxisomal disorders, disorders of glycosylation, and cholesterol synthesis. Symptoms are usually progressive and permanent and are not associated with food intake or other factors. (11) (6)

The common types of Inborn Errors of metabolism are as listed below:

**Table 2: Common Inborn Errors Metabolism**

| | |
|---|---|
| Disorders of Amino Acid Metabolism | Phenylketonuria, Tyrosinemia |

| | |
|---|---|
| | Homocystinuria, Marple syrup disease, Alkaptonuria |
| Disorders of Organic Acid Metabolism | Isovaleric acidemia<br>Propionic acidemia<br>Methylmalonic acidemia<br>Glutaric acidemia |
| Disorders of Fatty Acid Metabolism | **Acyl-CoA Dehydrogenase Deficiency**: Very long-chain, Long-chain, Medium-chain, and Short-chain.<br>**Carnitine Transporter Deficiency (CTD)**<br>**Carnitine palmitoyltransferase IA deficiency (CPT1D)** |
| Disorders of carbohydrate metabolism | **Disorders of carbohydrate intolerance**: Galactosemia, Galactokinase deficiency<br>**Disorders of Glycogenolysis/ Glycogen storage disorders (GSD):** Glucose 6 phosphatase deficiency (GSD1), Lysosomal acid maltase deficiency (GSD II), GSD III, among others<br>**Disorders of Gluconeogenesis:** Fructose 1,6 biphosphate deficiency, Pyruvate carboxylase deficiency among others |
| Disorders of lysosomal metabolism | **Mucopolysaccharidoses**(MPS); MPS 1( Hurler disease), MPS II (Hunters disease), MPS III (Sanfilippo), MPS IV, MPS VI, MPS VII, PMS IX.<br>**Sphingolipidosea**: GM 1 gangliosidosis, GM2 gangliosidosis types 1 ( Tay Sachs disease) and type 2 (Sandhoff), Fabry's disease, Gaucher disease.<br>**Oligosaccharidoses**: Galacosialidosis, Mannnosidosis<br>**Mucolipidosis**: Mucolipidosis type I (sialidosis), type II, type II and type IV |

| | |
|---|---|
| Peroxisomal Disorders | **Disorders of peroxisome biosynthesis**: Zellweger syndrome, Infantile Refsum disease, Rhizomelic chondrodysplasia<br>**Deficiencies of single peroxisomal enzymes**: Refsum disease, X-linked adrenoleukodystrophy, Etc. |
| Disorders of purine/pyrimidines | Lesch Nyhan syndrome<br>Adenosine deaminase deficiency |
| Disorders of steroid metabolism | **Congenital Adrenal hyperplasia**<br>**Disorders of Aldosterone production**<br>**Defects in cholesterol metabolism**: Smith Lemli Opitz syndrome, lysosomal acid lipase deficiency |
| Disorder of mitochondrial function | Pyruvate carboxylase deficiency<br>Pyruvate dehydrogenase complex deficiency<br>Kearns Sayre syndrome<br>Mitochondrial encephalopathy lactic acidosis and stroke-like episodes(MELAS) |

### 1.3.4. Disorders of Amino acid metabolism

These occur due to defects in amino acid metabolism pathways. They result in abnormal accumulation of amino acids (amino academia) in blood and urine (aciduria). Affected patients may present in the newborn period with features of acute onset poor feeding and reduced activity especially after a protein feed, and this may progress to encephalopathy, coma, and even death, if untreated. Older children on the other hand present with developmental delays or regression. Biochemical findings include metabolic acidosis, high ammonia, or low blood sugar. Untreated disorders such as phenylketonuria are associated with irreversible manifestations including intellectual disability, seizures, behavioral abnormalities, autistic manifestations. Malformations such as microcephaly may occur. (11) (6)

### 1.3.5. Organic Acidemias

These disorders are characterized by the accumulation of toxic organic acid metabolites in blood and increased excretion of organic acids in urine. Most patients present in the newborn period and early infancy, with poor feeding, vomiting, and lethargy. If untreated, progression occurs, and the older

children may have developmental delays and regression. Patients also present with metabolic acidosis, high ammonia, hypoglycemia, and impaired liver function. (6)

### 1.3.6. **Urea cycle disorders**

Occur due to deficiency of enzymes involved in the urea cycle pathway such as ornithine transcarbamylase deficiency and citrullinemia. Newborn infants present with high ammonia following protein feeds with respiratory alkalosis, and sometimes, impaired liver function. (11)

### 1.3.7.0 Disorders of Carbohydrate metabolism

Occur due to deficiency of enzymes involved in glycogen, galactose, or fructose metabolism and include galactosemia and glycogen storage diseases. Patients present with hypoglycemia especially during times of decreased carbohydrate intake or fasting; lethargy, encephalopathy, liver dysfunction, myopathy, hepatomegaly, and cardiomyopathy. Biochemical findings may include hypoglycemia, ketosis, metabolic acidosis, liver dysfunction. (20) (11)

#### 1.3.7.1 Galactosemia

Is an autosomal recessive disorder that occurs due to a deficiency of enzymes involved in the conversion of galactose to glucose. Examples include Galactose 1 phosphate Uridyl Transferase (GALT) deficiency, Galactokinase (GALK) deficiency, Uridine diphosphate (UDP) deficiency, and galactose 4 epimerase (GALE). Galactose accumulates in the body.

GALT deficiency is the most common, and severe type. Early manifestation occurs with the introduction of milk-containing feeds and includes vomiting, poor feeding, jaundice, lethargy, diarrhea, sepsis due to increased susceptibility to infections: enlarged liver, failure to thrive. Encephalopathy and other neuropsychiatric manifestations may be present. Cataracts may be present at birth or within the first few weeks. Late manifestations include neurodevelopmental impairments and growth delay. Red blood cell enzyme assay and deoxyribonucleic acid testing can be used to confirm the diagnosis. (6)(11)

#### 1.3.7.2 Glycogen storage disorders (GSD)

Glycogen maintains glucose balance in the body and also provides energy for high-intensity muscle activity. Affected patients present with low blood sugar, high ketoacidosis during fasting, exercise intolerance with muscle pain, cramps, rhabdomyolysis, myoglobinuria. There may also be a progressive weakness of trunk and extremity muscles. These include Glycogen synthase 2 deficiency, GSD Ia, GSD Ib, GSD III, GSD IV, GSD VI, GSD IXa1, Glucose

transporter (GLUT2) deficiency; which mainly affect the liver. Muscle glycogen synthase deficiency (GSD 0b), GSD II, GSD IIb, GSD V, GSD VII, GSD X, GSD XI, GSD XII, GSD XIII, GSD XIV, GSD XV, on the other hand, affect skeletal muscles (11) (20)

**Glycogen storage disease I (also known as von Gierke disease)**

It occurs due to glucose-6-phosphatase deficiency (G6PD). GSD Ia results from the deficiency of glucose-6-phosphatase while GSD Ib is due to the Glucose 6 Phosphatase transporter deficiency. It is an autosomal recessive disorder. Affected patients present, at three to six months of age with an enlarged liver, episodes of hypoglycemia, lactic acidosis, and hyperuricemia (due to decreased renal clearance, a compensatory increase in production). some have recurrent bacterial infections, reduced muscular tone, growth failure, and delayed psychomotor development. Hematologic involvement with anemia reduced platelet, and white blood cells may also occur. Other manifestations include gastrointestinal symptoms (perioral, perianal infections, abscesses), impaired kidney function (proteinuria, hematuria, reduced creatinine clearance), and neurologic complications (such as convulsions, structural brain changes). diagnosis is confirmed by genetic testing. (11) (21)

**Glycogen storage disease II (also known as Pompe disease)**

Occurs due to Lysosomal acid alpha-glucosidase deficiency resulting in impaired degradation of glycogen. Glycogen accumulates within the lysosomes and cytoplasm in all tissues, causing tissue destruction. It is an autosomal-recessive disorder. The Infantile-onset phenotype is characterized by absent or minimal enzyme activity while in the late-onset phenotype, patients have reduced enzyme activity. Infantile onset disease presents in the first few months of life with cardiomyopathy, generalized muscular hypotonia, feeding difficulties, and failure to thrive. Patients with Late-onset disease present in late childhood with myopathy delayed gross-motor development and progressive weakness of limb-girdle muscles. Involvement of the diaphragm and disordered sleep breathing patterns can lead to respiratory failure and even death. Genetic sequencing is important for confirmation of the diagnosis. (19) (11) (22)

**Glycogen Storage Disease Type III**

Occur due to deficiency of Glycogen debrancher enzymes (amyl-1,6-glucosidase). It is an autosomal recessive disorder. Debrancher enzyme deficiency is classified into four subtypes:

- IIIa: lack of both glucosidase and transferase activity in liver and muscle;
- IIIb: lack of both activities in liver only;
- IIIc: selective loss of glucosidase activity;

- IIId: selective loss of transferase activity

Patients present with hypoglycemia, enlarged liver, ketosis, hyperlipidemia. Growth failure, muscle weakness, with distal muscle wasting may be present (23) (24).

**Glycogen Storage Disease Type V (also known as McArdle disease)**

Occur due to Myophosphorylase enzyme deficiency that is involved in glycogen breakdown. It is inherited as an autosomal recessive trait. As a consequence, contracting muscles are not able to mobilize adequate amounts of muscle glycogen during the anaerobic phase of exercise. Affected patients present with exercise intolerance, fatigue, myalgia, cramps, and muscle weakness. Genetic testing is efficient to confirm the diagnosis (13) (11) (22)

### 1.3.8 Disorders of Fatty acid oxidation

Fatty acid oxidation is a source of energy production, especially during fasting. Disorders of fatty acid oxidation occur due to deficiency acyl-CoA dehydrogenases, defects of beta-oxidation enzymes, or fatty acid transportation defects. Affected infants present with lethargy and encephalopathy during decreased carbohydrate intake or fasting. Other features include hypoglycemia, hyperammonemia, or metabolic acidosis. Diagnosis is confirmed through enzyme assay and deoxyribonucleic acid analysis. (11) (25)

### 1.3.9 Disorders of Mitochondrial metabolism

The mitochondria are involved in the metabolism of organic acids, fatty acids, and amino acids. These are metabolized to acetyl-CoA and enter the Krebs cycle as citric acid. Several organ systems are affected, including the heart, kidney, liver, skeletal muscle, or brain. Affected patients present with skeletal or visceral abnormalities, poor feeding, vomiting, myopathy, cardiomyopathy, liver failure, seizures, and developmental delay. Other individuals may present with blindness and deafness. Metabolic derangement includes metabolic acidosis, lactic acidosis, hypoglycemia, and ketosis. Enzyme assay and deoxyribonucleic acid analysis testing can be used to confirm the diagnosis. (11) (26)

### 1.3.10. Peroxisomal disorders

Different catabolic and anabolic metabolic processes occur in peroxisomes such as beta-oxidation of very-long-chain fatty acids, oxidation of dicarboxylic acids, synthesis of bile acids, among others. When the adrenal gland is involved, adrenal damage and insufficiency occur due to the accumulation of fatty acids within the peroxisomes, leading to primary adrenal insufficiency. Patients with impaired

peroxisome function may present with microcephaly, dysmorphic facial features, hepatomegaly, hypotonia, or neurologic dysfunction to a varying extent. (11) (6)

### 1.3.11. Lysosomal storage disorders

Lysosomes contain enzymes that break down mucopolysaccharides, sphingolipids, and glycoproteins. Lack of the specific enzymes involved in the breakdown of these substances leads to the accumulation of glycosaminoglycans, glycoproteins, or glycolipids within lysosomes of various tissues; leading to cell distention, and disruption of cellular function.

Lysosomal storage disorders are subdivided based on the compound and pathway involved and include Mucopolysaccharidoses, Sphingolipidoses, Glycoproteinoses, lysosomal enzyme transport disorder, Lysosomal membrane transport disorder, among others. Affected persons manifest variedly, depending on the organ site and extent of storage affected. Clinical features include progressive hepatomegaly, splenomegaly, neurologic regression, short stature, coarse facial features, limitation/restriction of small and large joint movement, peripheral neuropathy, or even ataxia. An enzyme assay is required to confirm the diagnosis. (11) (27)

### 1.3.12. Purine and pyrimidine disorders

Purine and pyrimidine nucleotides are essential components of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), energy compounds, and cofactors, including adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD). Affected patients may present with neurologic dysfunction, delayed physical and mental development, anemia, renal calculi, and immune deficiency. (11) (28)

### 1.3.13. Disorders of steroidogenesis and cholesterol synthesis

Steroidogenesis is the process by which cholesterol is converted to active steroid hormones. Cholesterol is important in human embryogenesis and development. Disorders of steroidogenesis occur due to defects in the synthesis pathway of glucocorticoids or mineralocorticoids leading to deficiency of cortisol or aldosterone.

On the other hand, disorders of cholesterol synthesis occur due to defects of specific enzymes in the cholesterol biosynthetic pathway. (6) (29)

**Smith-Lemli-Opitz syndrome** (SLOS) occurs due to the 7-dehydrocholesterol reductase enzyme deficiency, required in cholesterol biosynthesis. SLOS is an autosomal recessive disorder that occurs due to mutations in the 7-dehydrocholesterol reductase gene. Affected persons present with dysmorphic features (such as microcephaly, micrognathia, structural ear abnormalities, syndactyly), growth retardation (prenatal and postnatal), feeding difficulties, intellectual disability, and behavioral

abnormalities. Affected males may have ambiguous genitalia. A significant number of patients with Smith-Lemli-Opitz syndrome, develop autistic features including deficits in social interaction and communication, repetitive and stereotyped behaviors. (30) (31)

**Twenty-one (21)-hydroxylase deficiency** accounts for more than 95% of Congenital Adrenal Hyperplasia cases and is divided into classic salt-wasting, classic simple virilizing, and nonclassic adrenal hyperplasia. The classic salt-wasting sub type is the most common subtype and patients may present with a severe salt-wasting crisis in the neonatal period. Patients with virilizing congenital adrenal hyperplasia may present as toddlers with signs and symptoms of hyperandrogenism. On the other hand, patients with nonclassic congenital adrenal hyperplasia may manifest in childhood with precocious puberty (32)(33).

## 1.4. INBORN ERRORS OF METABOLISM FOUND IN PATIENTS WITH AUTISM SPECTRUM DISORDER

A significant number of patients with Autism have been found to have genetic metabolic abnormalities that cause enzyme deficiencies. These are higher among consanguineous families. Examples of enzyme deficiencies found in patients with Autism include Phenylketonuria, Isovaleric acidemia, acyl CoA dehydrogenase deficiency, and mitochondrial disorders, Lesch Nyhan syndrome, Smith Lemli Opitz syndrome and Niemann pick disease. (34)(9)

Individuals with IEM have involvement of the central nervous system, which occurs when the metabolic derangements are not identified and treated early. Mechanisms that result in the development of autistic manifestations in these patients include the abnormal levels of substrates and metabolites which disrupt normal neurodevelopment, as well as migration of neurons in the cerebral cortices. There may also be disruption of normal synapse and neurotransmitter formation and function, impaired formation of myelin and neuronal cytoskeleton, or neuron degeneration. The growth of different areas of the brain is impaired in disorders of amino acid, and lipid metabolism. Migrations of neurons in the cortices may be affected in individuals with amino acid, fatty acid, and peroxisomal disorders. (35) (30) (36) (37)
Disorders of amino acid metabolism result in abnormal levels of some neurotransmitters especially dopamine, noradrenaline, and serotonin, and this may also contribute to autistic behavior. (37)
Phenylketonuria is one of the most common genetic metabolic disorders found in individuals with ASD. This is inherited in an autosomal recessive pattern, and a significant number of untreated patients develop autism. A mutation of the Phenylalanine hydroxylase gene occurs, which results in a lack (or reduced levels) of phenylalanine hydroxylase, an enzyme that converts phenylalanine to tyrosine. If untreated Phenylalanine accumulates, and this causes damage to the developing brain. Reduction in

myelin, loss of neurons, impaired interneuron connections also. Reduction in levels of neurotransmitters dopamine and serotonin occurs and results in an imbalance between the brain excitation/ inhibition pathways. These contribute to autistic manifestations, and restriction of phenylalanine in the diet has been shown to improve autism manifestations. (12) (34) (38)

Individuals with Smith Lemli Opitz syndrome may exhibit autistic behavior, in up to half of the cases. The deficiency of steroids in the brain affects neuronal processes, neuroendocrine, and neurotransmitter function. Other manifestations include microcephaly, hypotonia, growth failure, and impaired motor movements. (31)

Disorders of mitochondria function, especially mutations in the mitochondrial gene that codes for aspartate and glutamate, may cause a reduction in body levels of pyruvate, carnitine, with high levels of lactate, alanine, ammonia. Affected individuals may also present with autism. (39)

Disorders of purine metabolism, such as Adenylsuccinase deficiency, result in the accumulation of succinyl purines in the brain, which may cause hypoplasia of the cerebellum. Majority of affected individuals present with autism spectrum disorder phenomena as well as developmental delay and growth failure, seizures. Biotinidase deficiency results in low brain biotin levels, and neurological manifestations, including autistic behaviour, occur. (9) (39)

### 1.5. CLINICAL FEATURES OF AUTISM SPECTRUM DISORDER

Autism is a spectrum of disease, with a wide range of manifestations and different levels of impaired function. The majority of patients with autism have different associated comorbid conditions including other developmental disorders, psychiatric disturbances, personality, and behavioral disturbances, as well as gastro intestinal, immune dysregulation, and sleep disorders. Autism may present as part of genetic disorders, hence some patients have features of underlying genetic syndromes such as fragile X syndrome, Retts syndrome, phenylketonuria; among others. (1) (2) (14)

Patients with autism have deficits in social communication and interaction that affect impaired social-emotional reciprocity and nonverbal communication. They also have repetitive movements and speech, ritualized patterns of behavior, as well as fixated interests. These features are as defined in the 5th diagnostic and statistical manual (DSM-V). (1) (40)

**Table 3: DSM V Criteria for ASD**:

| **Criterion** | **Symptoms** |
|---|---|

| | |
|---|---|
| A. Persistent deficits in social communication and social interaction as manifestation by all 3 of: | 1. Deficits in social-emotional reciprocity.<br>2. Deficits in nonverbal communicative behaviour used in social interaction.<br>3. Deficits in developing, maintaining, and understanding relationships. |
| B. Restricted, repetitive patterns of behaviour, interests, or activities: manifested by 2 or more of the following: | 1. Stereotyped or repetitive motor movements, or speech.<br>2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior.<br>3. Restricted, fixated interests of abnormal in intensity or focus.<br>4. Increased or decreases reactivity to sensory stimuli |
| C. Symptoms must be present in the early childhood | |
| D. Symptoms cause significant impairment in social, occupational, or other areas of functioning | |

Any associated conditions present, such as developmental, psychiatric, behavioural, and medical conditions are also specified.

Symptoms of autism can present in early life, including infancy; or later in life when social demands for peer interaction and group participation increase (such as preschool and school ages) (10). Cases with mild disease present in late childhood with behaviour disturbance, anxiety, or even hyperactivity. Common symptoms initially reported include delays in speech and language abilities, failure of the child to make eye contact, or a child with no interest to play and socializing with other children. Other children may get a regression of language, communication, and social skills that had previously been well attained. (10) (16)(41)

Children with autism have deficits of verbal and nonverbal skills that are required to share their thoughts, intentions, and feelings. Others may even lack the intent to communicate and socialize; while others are unable to recognize and understand the social communication behaviors by other people. They are unable to copy the play actions of peers and adults, fail to make eye contact when called by name, or have delayed or absent joint attention. There may be an impaired ability to use and interpret nonverbal behaviors such as eye gaze, facial expression, gestures, or body posture. They avoid eye contact or gaze at areas other than another person’s eyes. Some may fail to notice, or just misinterpret aspects of the nonverbal communication of others (such as pointing, waving, nodding, or shaking of the head). Such difficulties affect the patients' ability for social-emotional reciprocity. (1) (40)

Patients also have impaired pragmatic language ability (knowing what to say, how to say it, and when to say it). They may be unable to use language in communication and have difficulties initiating, responding to, or sustaining a conversation. Some are unable to choose the appropriate words or responses or topics for a given social context. (1) (40)

Autism patients have difficulties in forming and sustaining friendships, because of impaired social skills. They are unable to register, remember, process, and interpret social information, thus cannot successfully socialize and build friendships. They misunderstand the emotional responses of others, for instance to another's distress; may not be able to notice that a social partner is not interested in their preferred topic of conversation. They are also unable to understand the difference between acquaintances, friends, and intimate relationships and also have difficulties in inferring the intentions, beliefs, attitudes, or likely behaviors of others. They are also unable to form and maintain developmentally appropriate peer relationships. (1) (40)

Patients with autism also have a pattern of marked restricted and repetitive behaviors, activities, and interests; as well as hyper or hyposensitivity to sensory input. They may exhibit repetitive body movements such as hand flapping, finger-twisting, rocking, or swaying. Some line up an exact number of playthings repeatedly; repeat phrases heard from others. Head-banging, face or body slapping, self-biting, or self-pinching behavior may be present and may result in injuries to the child. Patients insist on sameness and resist change, like preferring to always eat particular foods in a specific order, or following the same route from one place to another. They also have difficulties in shifting their attention away from their preferred topic. (1) (10). Autistic individuals have abnormal responses to sensory stimuli (noises, touch, odors, tastes, visual stimuli). They may have a preoccupation with spinning objects, or lights, or odors. They may prefer eating only foods with certain tastes and textures; may sniff or lick nonfood objects, avoid being touched, or have increased sensitivity to certain kinds of touch (1) (10)

Several developmental, psychiatric, and medical disorders, are commonly present in patients with Autism spectrum disorder. Intellectual impairment is a common condition found in autistic patients and affects up to half. Patients may have limited cognitive skills and experience difficulties performing tasks that require higher-level conceptual processes, reasoning, interpretation, and understanding. Language impairments is also common, and some affected individuals prefer using physical gestures instead of words or using single words or phrases while communicating. (1) (2) (41)

Other neurodevelopmental, and behavioral disorders that are common in ASD such as Anxiety disorder, Attention deficit hyperactivity disorder; depression, and mood disorders, especially in adolescents and

adults. Aggression, self-injurious as well as suicidal behaviors may also be present. Learning difficulties, with poor school performance may be present. (1) (2) (41)

Epilepsy is also found frequently in patients with autism, especially in early childhood, and adolescents. Sleep problems have also been described, and these include bedtime resistance, sleep anxiety, and restlessness. Motor deficits with an abnormal gait, clumsiness, or hypotonia may be present. Dysmorphic features, such as macrocephaly or microcephaly may occur. Gastrointestinal problems such as recurrent constipation, gastrointestinal reflux, esophagitis have been noted. Children with autism may also have allergic and autoimmune disorders. (1) (2) (10)(41)

Autism may also occur as part of some genetic syndromes, whereby the underlying genetic abnormality that causes specific syndromes, result in autistic phenomena. Up to half of the patients with Tuberous sclerosis complex develop autism. A significant number of males with Fragile X syndrome have associated intellectual disability as well as ASD. Some patients with Angelman syndrome have also been shown to develop the clinical manifestations of autism, usually associated with severe ID, microcephaly, and movement disorders. Other syndromes associated with ASD include Retts syndrome Cohen syndrome, Cornelia de Lange syndrome, neurofibromatosis type 1, Down syndrome among others. (1) (2) (10)(41)

Depending on the severity of autism, patients require different levels of support with life functions. Patients with severe (level three) ASD, have severe deficits that markedly interfere with function in all aspects of life, and thus require very substantial support. Patients with moderate (level 2 of ASD) also have marked deficits and social impairments and also require substantial support. Patients with mild autism (level 1 of ASD), have deficits in social communication that are only more noticeable when there is no adequate support. (1) (2) (10)

## 1.6. CLINICAL PRESENTATION OF PATIENTS WITH INBORN ERRORS OF METABOLISM AND AUTISM SPECTRUM DISORDER

While autistic patients present with the clinical spectrum of autism, patients with inborn errors of metabolism (IEM) will present with features of the underlying enzyme deficiency.

Disorders of metabolism may manifest with acute symptoms of vomiting, feeding intolerance, lethargy, seizures, and respiratory disturbances. Chronic manifestations on the other hand include neuropsychiatric symptoms, and behavioral disturbances such as inflicting self-injuries, aggression, Intellectual disability, seizures, hyperkinetic movements, dystonia, chorea, and hearing loss are also common. Some patients will have dysmorphic features (such as microcephaly, macrocephaly), ptosis, cataracts, developmental delay, and failure to thrive. (6)(11)

Systemic manifestations may be present, and these include gastrointestinal manifestations such as (hepatomegaly, cirrhosis, splenomegaly), cardiomyopathy, renal disorders (renal failure, nephrotic syndrome), skin and hair abnormalities (dermatitis, blonde hair, alopecia), among others. (6)(11)

## 2.0 LITERATURE REVIEW

### 2.1. Introduction

Autism spectrum disorder (ASD) is a developmental disability that has detrimental effects on the individual's social, communication, and behavioral abilities and functions. A significant number of children, worldwide, have autism. Autism may be diagnosed in early childhood or later in life when clinical manifestations become more apparent. The abilities and needs of people with ASD vary significantly among individuals and over time. Some people with autism can live independently while others have severe disabilities and need lifelong care and support (16) (42).

The etiology of autism includes genetic and environmental factors. Genetic defects that cause autism spectrum disorder are found in a big number of autistic patients. Gene mutations that cause autism may be inherited or occur spontaneously and may also result in disorders of metabolism and mitochondrial function, or syndromes (such as tuberous sclerosis, fragile X syndrome, Down syndrome, Angelman syndrome) in which the patients also have autistic features (43)(44).

### 2.2. Prevalence and types of Inborn Errors of Metabolism in patients with autism spectrum disorder (ASD)

Inborn errors of metabolism (IEM) are majorly rare disorders, some having an incidence of about 1 per 100,000 births. However, their estimated collective incidence is higher, at about 1 per 800 to 1 in 2500 (45). Altimimi et al. conducted a study conducted in Iraq, to assess the prevalence of IEM. Up to 17.9% of the children were found to have IEM. Disorders of amino acid metabolism represented the majority of IEM (10 cases) in which phenylketonuria and maple syrup urine disease were the most common. Organic and fatty acid metabolism defects were found in five and two cases, respectively. IEM was also detected in a high proportion of children with unexplained developmental delay (46).

A systematic review conducted to assess the burden of IEM globally from 1980 to 2017 revealed that the pooled prevalence of IEM was highest in the Eastern Mediterranean region at 75.7 per 100,000 live births with an estimated case fatality rate of 33% in low- and middle-income countries, and about 23, 529 deaths per year globally. IEM also accounts for 0.4% of all child deaths worldwide (45).

Some studies have been done to assess the prevalence of IEM in patients with ASD, and several genetic metabolic disorders have been described (30). A study conducted in India by Kumar V. Suresh et al, to investigate underlying IEM among 90 children aged between 3 and 12 years with Autism, reported a 15% prevalence of IEM. Testing for metabolic markers was done using Gas Chromatography and Mass Spectrometry. The participants with underlying IEM had elevated metabolites such as Phenylacetate, Ketoglutarate, methylmalonate, Lactate, Propionate, Isovalerate, and were thus diagnosed to have Isovaleric acidemia, Tetracarboxylic acid cycle disorders, Methylmalonic acidemia,

Lactic acidosis, and Propionic academia (7). The findings from this study assert that it is imperative to develop a comprehensive strategy aimed at early detection and treatment of these disorders to improve the clinical outcome of children with these conditions

In another study conducted in China, by Haijie et al, to analyze for IEM in 277 children with ASD, amino acid and carnitine blood levels were checked using liquid chromatography. Verification was done using gas chromatography on urine samples. Blood samples were then drawn, and genetic testing was done using dry blood spot tandem mass spectrometry. The findings revealed a 6.9% prevalence of IEM. The identified types of IEM in the study included phenylketonuria, homocystinuria, glutaric acidemia, isovaleric acidemia, propionic, methylmalonic acidemia, argininemia, citrullinemia, and primary carnitine deficiency (47). The results from this study show that ASD and IEM occur together, and the application of the Tandem mass spectrometry is efficient in the diagnosis of the underlying IEM in children

In a study conducted by Spilioti et al., in Greece, to assess for the presence of manageable IEM in patients with ASD, a total of 187 children aged between 4 and 14 years were enrolled in the study. Assessment for autism was done based on the diagnostic statistical manual-Fourth (DSM IV) criteria. Results from the study found a 4% prevalence of IEM. The different types of enzyme deficiencies found included Lesch Nyhan syndrome, succinic semialdehyde dehydrogenase deficiency, Biotin deficiency, and phenylketonuria; and participants with inborn errors of metabolism IEM had elevated levels of hydroxyisovaleric acid, methylcitrate, and lactate. The study further evaluated different IEM treatment interventions, and 12 patients treated empirically with Biotin supplementation showed improvement of clinical features, as per Childhood Autism Rating Scale assessment, 6 months after initiation of treatment. Six other participants treated with a ketogenic diet also had significant clinical improvement (48).

Orozco et al. also conducted a case-control study in the United States, investigating IEM among children with ASD, idiopathic development delays, and down syndrome. In this study, forty-nine metabolites that included amino acids, organic acids, sugars, among other compounds, were elevated in all cases (49). Steiner et al did a case study, in Brazil, on the natural history and genotypes of patients who had been diagnosed with phenylketonuria and Autism. The study participants had hair hypopigmentation, microcephaly, severe mental retardation, and delayed verbal language in addition to other symptoms of Autism. Other signs found were a global developmental delay, seizures, spasticity, ataxia, aggression, and hyperactivity, but in varying proportions (43). Homozygous mutation for phenylalanine hydroxylase gene was found in all 3 patients, and at a newborn screening of a sibling to one study participant. The study concluded that failure to diagnose and treat Phenylketonuria in patients with

homozygous phenylalanine hydroxylase gene mutation is associated with the development of symptoms of Autism.

Saad et al (in 2013) did a study in Egypt to assess for autistic symptoms in children diagnosed to have phenylketonuria late. The study was done on 32 patients who had been diagnosed with PKU after the age of one year and were being followed up at the Assiut Pediatric University hospital. clinical features of ASD were assessed using DSM IV and CARS assessment tools, then diagnosis and classification were done. Autism was found in 8 (25%) of the 32 participants, and of these, 3 were females while males were 8. 2 participants had Severe autism while 6 had mild to moderate disease (12). The study concluded that if the diagnosis and management of PKU are delayed, it leads to a very high incidence of Autistic features, and ID; and therefore, recommended for neonatal screening for PKU.

Sabrina Baieli et al did a similar study, in Italy (in 2003. University of Catania); to evaluate for features of ASD, in patients that had been diagnosed with PKU. 243 patients were included in this study, and 97 had classical PKU of which 62 had been identified at neonatal screening but 35 had been diagnosed late. Family history, general physical examination neurodevelopmental assessment was done. Evaluation for Autism was done using both the Autistic Diagnostic Interview-Revised (ADI-R) and the Childhood Autism Rating Scale (CARS) tests. 2 out of the 35 patients (5.7 %) diagnosed late with PKU met the diagnostic criteria for autism (50). This study also concluded that PKU is one of the causes of autism.

Darryn M Sikora et al conducted a study to determine the prevalence of autism spectrum disorders (ASDs) in children with smith Lemli Opitz syndrome (SLOS). Fourteen children, aged 3-16 years were evaluated through a parental interview, direct observation, and behavior checklist, to assess for features of the autism spectrum, based on DSM-IV criteria. All the patients had features of autism spectrum disorder. 75 % of the (three-fourths) children with SLOS had ASD, 50% had Autistic Disorder, and the rest with pervasive disorder not otherwise specified (31). These results indicate that most children with SLOS develop ASD.

### 2.3. Clinical characteristics of autistic spectrum disorder patients with underlying Inborn Errors of Metabolism

The clinical characteristics of autistic patients with inborn errors of metabolism (IEM) vary from those with ASD alone. Age and sex distribution of IEM may vary from that of ASD. In a cross-sectional study conducted in France to assess the quality of life and associated factors in Japanese children with inborn errors, approximately 54% of children with IEM were aged below 12 years with a similar male to female distribution(42). Another study was conducted in Iraq, investigating developmental delays in children with IEM, found that younger age was a significant risk factor, (median age of 22 months),

but the gender of the child had no significant association (46). A study done in Egypt to assess for autistic symptoms in children diagnosed with late phenylketonuria found that 25% of the participants had Autism, and of these 37.5 5% were female and 62.5% were males. (12).

Autistic patients who have enzyme deficiencies will have autistic manifestations as well as features of the underlying IEM. Features of Autism occur, due to the neurodevelopmental impairments caused by metabolic dysfunction (19). Similarly, features of enzyme deficiencies may present acutely as metabolic emergencies or may be chronic.

The time at which inborn errors of metabolism manifest depends on whether there is marked rapid accumulation of toxic metabolites or significant lack of substrates or the enzyme (19). In patients with chronic forms of IEM, there is a risk of complications involving several body organs, due to impaired cellular metabolic processes, and degeneration of different organ systems.

Disruption of normal neurodevelopmental, as well as brain damage, occurs and these cause different central nervous system manifestations. Neuropsychiatric manifestations such as ASD may occur, as well as behavioral abnormalities (self-injuries, aggression, personality changes, psychosis); encephalopathy, and even movement disorders, among others. (12) (37). Individuals with the spectrum of Autism will manifest with deficits in social communication and interaction (such as deficits in social reciprocity; nonverbal communicative behaviors; or skills in developing, maintaining, and understanding relationships), restricted interests, and repetitive behavior or activities (10).

Other neurological manifestations that are found in patients with enzyme deficiencies include hyperkinetic movements, dystonia, chorea, most prevalent in patients with Lesch Nyhan syndrome. On the other hand, decreased and slow movements, rigidity may occur with Niemann Pick disease. Intellectual disability (ID), developmental delay, seizures or epileptic encephalopathy, or sensorineural hearing loss also occur (13) (35).

Argmann et al. found that some autistic patients with IEM may present with dysmorphic features, such as microcephaly, macrocephaly, nystagmus, ptosis, strabismus, cataracts among others. Others have failed to thrive and have short stature or gastrointestinal manifestations such as vomiting, hepatomegaly, cirrhosis, splenomegaly. Other manifestations are cardiomyopathy, renal disorders (renal failure, nephrolithiasis, nephrotic syndrome, renal tubular acidosis), skin and hair abnormalities (51).

Patients with underlying Biotinidase deficiency may also present with ataxia, seizures, hypotonia, skin rash, alopecia, and impaired vision or hearing. Phenylketonuria is associated with microcephaly, severe intellectual disability, spastic gait, skin hypopigmentation. Other than autistic features, patients with Adenylosuccinate lyase deficiency may present with developmental delay, encephalopathy, seizures, and growth retardation. Patients with cerebral creatine deficiency syndrome on the other hand have

expressive language developmental delay, Intellectual disability (ID), abnormal movements, and epilepsy. (52) (16)

### 2.4. Summary of findings from studies on IEM among children with ASD:

| **Study Title, Author, Year** | **Study design** | **Study population Sample size** | **Findings** |
|---|---|---|---|
| Orozco et al.<br>-Metabolomics analysis of children with Autism, Idiopathic Developmental Delays, and Down syndrome.<br>-United States. 2019. | Population-based case-control study | Children aged between 24 and 60 months with Autism, idiopathic developmental delays, and Down syndrome.<br>N = 442 | Forty-nine metabolites were elevated, including amino acids, organic acids, sugars, and other compounds |
| V. Kumar et al;<br>-Inborn Errors of Metabolism in patients with Autism Spectrum Disorders<br>-Tertiary Care Center. India<br>2014 | -Cross-section study.<br>-Metabolic screening was done on study participants | Children with autism; aged 3 to 12 years<br>N=90 | An abnormal elevation of several metabolites was including Isovalerate, Ketoglutarate, Lactate, Malate, Phenylacetate, Propionate, Methylmalonate<br>-Fifteen percent (15%) of the study participants were thus diagnosed with: Isovaleric acidemia, Tetra Carboxylic Acid cycle disorders, Methylmalonic acidemia, Lactic acidosis , Propionic acidemia |
| Haijie et al<br>-Analysis of inborn error metabolism in children with autism spectrum disorders<br>-Hainan, China. 2019 | -Cross-section study<br>-dry blood spot tandem mass spectrometry was carried out | -Children aged 24 to 60 months with autism spectrum disorder<br>n=277 | About seven percent (6.9%) of the study participants were found to have inborn errors of metabolism such as<br>-phenylketonuria, homocysteinemia, glutaric academia, isovaleric academia, propionic, methylmalonic acidemia, argininemia, citrullinemia, and primary carnitine deficiency |

| | | | |
|---|---|---|---|
| Spilioti et.al.<br>-Evidence for treatable inborn errors of metabolism in a cohort of patients with autism spectrum disorder.<br>-Greece, 2013. | -Cross-sectional study<br>-Tandem Mass Spectrometry was done | Greek patients aged 4 to 14 years, with an autism spectrum disorder.<br>N=187 | -Findings were 4% prevalence of IEM; including Lesch Nyhan syndrome, succinic semialdehyde dehydrogenase deficiency, Biotin deficiency, phenylketonuria |
| Altimimi et al.<br>-Inborn Errors of Metabolism in Children with Unexplained Developmental Delay.<br>-Misan, Iraq. 2019. | -Cross-sectional study | -Children aged one month and eight years old with unexplained developmental delay<br>(n =112) | - 17.9% of the children had inborn errors of metabolism.<br>-Disorders of amino acid metabolism represented the majority of the cases (10cases), and this included phenylketonuria, maple syrup urine disease<br>-identified risk factors for developmental delay were a median age of 22 months, while the gender of the child and consanguinity had no significant association. |

There is, however, limited data in the Kenyan setting as well as the whole of Sub-Saharan Africa, on the underlying types of enzyme deficiencies in patients with Autism even though specific treatment may be available.

## 2.5. Study Justification

Inborn errors of metabolism (IEM) cause significant detrimental effects on the health and well-being of individuals. Many body systems are affected in the disease process, and progressive, irreversible degeneration of organs occurs if they are not detected and treated early. IEM thus contributes to a high burden of childhood morbidity and mortality.

Autism, which is one of the neuropsychiatric manifestations of Enzyme deficiencies, is a debilitating disease, and most affected children and adolescents require constant help with different life functions. Thus, identification of IEM in patients with autism would guide targeted treatment interventions for better outcomes

There are no studies that have been done in Africa to quantify Inborn Errors of Metabolism in patients with autism. This study sought to bridge the gap in knowledge on the prevalence and types of IEM among ASD patients in our setting. The study findings would form a basis for reviewing the utility of metabolic testing in ASD patients.

## 2.6. Research question

What is the prevalence and what are the types of enzyme deficiencies associated with Inborn Errors of Metabolism among patients with Autism Spectrum Disorder at Kenyatta National Hospital?

## 2.7. Objectives

### 2.7.1. Primary objective

To determine the prevalence of Inborn Errors of Metabolism in patients with Autism spectrum disorder aged 18 months to 25 years, at Kenyatta National Hospital.

### 2.7.2. Secondary objective

i. To determine types of Inborn Errors of Metabolism among patients with Autism spectrum disorder aged 18 months to 25 years, at Kenyatta National Hospital.

ii. To describe the clinical characteristics associated with presence of Inborn Errors of Metabolism among patients with Autism Spectrum Disorder at Kenyatta National Hospital.

## 3.0: METHODOLOGY

### 3.1. Study Design

A descriptive cross-sectional design was adopted.

### 3.2. Study setting

The study was conducted at Kenyatta National Hospital Pediatric and adolescent Occupational Therapy, Neurological and Psychiatric clinics. Kenyatta National Hospital (KNH) is the main national referral hospital, located in Nairobi, Kenya. It also serves as a teaching hospital for the University of Nairobi, College of Health Sciences. KNH has specialists that attend to patients with IEM and ASD, including Neurologists, Psychiatrists, Endocrinologists, speech/hearing specialists, and Occupational therapists among others. The hospital serves patients within Nairobi County, and also receives referral patients from the peripheral hospitals countrywide, and as such has a high patient turnover. Patients with ASD are mainly seen at the following outpatient clinics: pediatric and adolescent Neurology, Psychiatry, and Occupational therapy clinics. Others may be admitted to the wards if they present with acute illnesses. Patients were recruited from all these clinics, into the study.

### 3.2 Study population.

The study population comprised patients with clinically diagnosed ASD, aged 18 months to 25 years, on follow-up at the Kenyatta National Hospital's Occupational Therapy, Neurology, and Psychiatry Paediatric and Adolescent clinics.

### 3.3. Inclusion and Exclusion Criteria

#### 3.3.1. Inclusion criteria.

Patients aged 18 months to 25 years, on follow-up at the paediatrics Occupational Therapy, Neurological, Endocrinology and Psychiatric clinics, with a diagnosis of Autism documented in their medical records, and whose caregivers gave informed consent. These were participants who meet the criteria for Autism as outlined in the DSM-5 as follows (40)

**Table 4: DSM 5 Criteria for ASD**:

| **Criterion** | **Symptoms** |
|---|---|
| A. Persistent deficits in social communication and social interaction and manifest with all 3 of: | 1. Deficits in social-emotional reciprocity.<br>2. Deficits in nonverbal communication used for social interaction.<br>3. Deficits in developing, maintaining, and understanding relationships. |

| | |
|---|---|
| B. Restricted, repetitive patterns of behaviour, interests, or activities which manifest with 2 or more of the following: | 1. Stereotyped or repetitive motor movements, speech, or use of objects.<br>2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behaviour.<br>3. Restricted, fixated interests that are abnormal in intensity or focus.<br>4. Hyper or hypo reactivity to sensory stimuli |
| C. Symptoms must be present in the early childhood | |
| D. Symptoms cause significant impairment in social, occupational, or other areas of functioning | |

The study population included both newly registered patients, and those who had been on repeat follow-up over the preceding 2 years.

### 3.3.2. Exclusion criteria

Patients whose caregivers declined consent to participate. Patients who did not meet the DSM V criteria were also excluded from the study.

## 3.4. Study period

The study was conducted over 8 months, from September 2021 to May 2022. This was after approval to conduct the study was obtained from Kenyatta national hospital (KNH), and University of Nairobi's ethics and research committee and permission granted by the Kenyatta national hospital administration to conduct the study.

## 3.5. Case definition

The cases that were enrolled in the study were children and adolescents with a diagnosis of autism spectrum disorder, as indicated in their medical records. The diagnosis was made based on presence of signs and symptoms that meet the Diagnostic and Statistical Manual (DSM) 5 autism criteria. See appendix I.

## 3.6. Sample Size Calculation

The sample size calculation was based on prevalence of IEMs among children with autism spectrum disorders obtained from the study by Kumar et al. (conducted in India, at a Tertiary Care Center, in 2014), who found the prevalence of inborn errors of metabolism to be 15%

Fischer's formula was used as follows**:**

$$n = \frac{Z^2 p(1-p)}{e*2}$$

where,

Z is the standard normal deviate of 95% confidence interval (1.96)

P was the proportion of participants we estimated to have underlying enzyme deficiencies (0.14)

1-p is the proportion of our participants we estimated not to have enzyme deficiencies (0.86)

e is the margin of error (0.05)

When replacing the figures in the formula

We had,

$n = 1.96^2*0.14(1-0.14)/0.05*0.05$

$n = 3.8416*0.14*0.86/0.0025$

$n = 0.4625/0.0025$

$n = 185$

Since the target population of children and adolescents with autism spectrum disorder was less than 5000; sample size correction using the Finite Population correction factor was done as follows:

Finite Population Correction ( FPC)

-N1=n/1+(n-1/N)

N1=sample size for population <5000. n=sample size for population >5000. N total size of the population from which sample size was to be drawn (about 100 patients).

Thus N1= 185/[1+(185-1/100)]

Therefore N1 =65.

Assuming a 10% non-response, the sample size was 71 + 7

= 78

Thus, a total of 78 patients with autism spectrum disorder, both new, and those who had been on at KNH, over the last 2 years, were recruited into the study.

### 3.7. Sampling technique

Consecutive sampling technique was utilized to recruit participants in the Occupational Therapy, Neurological and Psychiatric clinics. Participants were recruited into the study as they came into the clinics for their routine visits.

### 3.8. Research assistant recruitment

The principal investigator recruited two research assistants to help in the study data collection process. These research assistants possessed a diploma in clinical medicine and surgery, and thus were qualified and registered clinical officers, who had done clinical rotations at the paediatric wards and other departments, at KNH. They had at least 6 months post-internship clinical experience to ensure that they had interacted and taken care of patients with autism spectrum disorder at one of these service points.

The research assistants were thus be able to understand the type of patients (including patients with autism) usually seen at KNH's Psychiatry, Neurology, and Occupational Therapy clinics; and the forms of therapy that are offered to patients with autism at these clinics. They were able to identify the potential study participants with diagnoses of Autism spectrum disorder from the patient registers at these clinics. They were also able to assess and examine the different clinical symptoms and signs that these patients had.

The principal investigator trained the research assistants, through face-to-face teaching and discussion session, on the procedures for data collection and taking blood samples, specifically on:

- How to approach potential study participants and their parents or guardians, at the occupational, endocrinology, psychiatry, and neurology clinics.
- How to explain the study to be undertaken, the purpose and utility of the study, any risks and benefits thereof.
- The procedure for data collection and questionnaire administration
- How to obtain informed consent and assent
- How to take relevant clinical history and do physical examination of the participants
- How to take the blood samples, make dried blood sport and package the filter paper.

The principal investigator then prompted the research assistants to demonstrate back, what they had been taught, and clarify that they understood.

### 3.9. Screening and Enrolment

### 3.10. Data collection procedure

Data collection began after obtaining study approval from the KNH-UoN Ethics and Research Committee and permission from Kenyatta National Hospital administration to conduct the study within the institution. The principal investigator, with the help of research assistants, approached potential participants and their guardians on arrival at the clinics. The patient register was accessed daily to identify potential participants. The research team explained the study purpose to them including potential benefits and risks. Potential participants who consented to the study were recruited.

Socio-demographic details and symptoms were captured for each study participant. Detailed physical general and systemic examination was thereafter done. Anthropometric measurements were also taken (height, weight, head circumference). Weight was measured using either a pan-type or platform weighing scale depending on the participant's age, height was measured using a calibrated stadiometer, and head circumference using a calibrated tape measure. The recorded measurements were then be plotted in the respective WHO charts to determine if they are within normal range or not. Patient files were then accessed to obtain information on the criteria used for clinical diagnosis of autism and relevant medical history. To test for inborn errors of metabolism, 5mls of blood was drawn from each study participant, as described below, and dried blood spots (DBS) was made on filter papers. air dried filter papers were packaged and stored, then later shipped to Centogene laboratory in Germany for Genetic testing.

#### 3.10.1. Sample collection, storage and Transportation

Participants' particulars and brief clinical information were entered into the requisition forms, as well as onto the filter card. Blood from each study participant was collected into a sterile sample bottle, using aseptic technique. dried blood spots were made on filter cards, by saturating blood onto each circle in the filter paper. This procedure was repeated to fill all 10 required blood spots on each filter card. The card was then air-dried for about an hour.

The air-dried filter papers were stored in a sealed plastic bag at room temperature. Material Transfer approval was sought KHN-UON Ethics and Research Committee, and then samples transported via Dalsey, Hillblom, and Lynn (DHL) courier, to Centogen laboratory in Germany where genetic analysis (Whole Genome sequencing and Measure of enzyme activity) was done.

#### 3.10.2 Sample processing and Testing

Specimens received at Centogene laboratory were logged into a register and assigned a lab specimen number. Processing was then commenced. Library construction was performed using the Agilent Sure

Select XT Human all Exon V6 kit, and sequencing is performed in a NovaSeq 6000 sequencer using a 150bp read length protocol. Alignment of raw reads was performed with Burrows-Wheeler aligner (BWA) using Human Genome version 19 (hg 19) as a reference genome sequence. Variant calling was performed using the Genome Analysis Toolkit (GATK) recommended pipeline, and variant annotation was done using the SnpEff software. The results of genetic analysis were released after a wait period of 1 to 4 months, and these were communicated to the principal investigator via email.

### 3.11. Quality and infection control procedures

Centogene laboratory is accredited and adheres to laid down standard operating procedures.

Quality control measures and standard operating procedures were adhered to at all times during the research, specimen collection, and processing. Proper collection methods and storage of specimens was done, sterile containers and techniques was employed.

### 3.12. Data Variables

**Dependent variable**

Presence of Inborn errors of metabolism (Yes, No), and types of inborn errors of metabolism

**Independent variables**

Factors associated with presence of inborn errors of metabolism: Age, Gender, Area of residence, Weight, Head circumference, family history of autism, intellectual disability seizures, among others

### 3.13. Data management.

All the data was assembled, and the principal investigator inspected and cleaned raw data to ensure the correctness and completeness. Completed questionnaires was entered into a pre- designed data template using Epidata version 3.1 software, kept in a computer with a secured password, and backed up in a compact disc and an external hard drive.

#### 3.13.1. Data analysis

Data was analyzed using Stata version 14 software. Associations were considered significant if the p-value obtained was less than 0.05. Descriptive statistics were used to describe the socio-demographic characteristics of the study population such as gender, age, residence, weight, height, among others. Continuous variables were summarized using Means (SD) while categorical variables were summarized using Frequencies (n) and percentages (%).

Thus, to evaluate for the prevalence of Inborn Errors of Metabolism in patients with autism spectrum disorder, the following formula was used;

The % Prevalence $= \frac{a}{a+b} * 100$

Where a = Number of autism patients with enzyme deficiencies b = Number of autism patients without enzyme deficiencies.

Binary logistic regression analysis was conducted to determine factors associated with enzymes deficiencies among study participants

### 3.14. Ethical consideration

The principal investigator sought approval to conduct the study from the KNH-UoN Ethics and Research Committee. Permission to carry out the study at KNH hospital was then obtained from Kenyatta National hospital administration. Caregivers and guardians of participants recruited into the study gave written informed consent before enrolment. Those who did not consent were excluded from the study.

Strict patient confidentiality was observed during collection, storage and, processing data, and in the handling of the results. When the results of the genetic tests were received, they were reviewed by the investigating team, and then disclosed to the patients and family/guardians, and the participants' attending primary doctors. All patients found to have inborn errors of metabolism and other genetic disorders were referred to the appropriate specialist clinic for continued care.

The following measures were observed, by the study team and study participants, to minimize the risk of Covid-19 transmission (as per Kenya Ministry of Health / WHO guidelines):

- Observation of hand hygiene by hand washing or use of 70% alcohol-based sanitizers before and after the procedures.
- Using disposable gloves during sample collection.
- Wearing of scrubs or dust coats, by the research team, when examining participants.
- Putting on surgical masks, at all times, and N95 masks during the examination of participants suspected to have respiratory illnesses.
- Disinfection of commonly shared clinical equipment (tape-measure, stethoscopes, thermometers) before and after use on each of the participants.
- Maintaining social distancing of at least 1.5 meters, between participants and relatives; at the clinic.

### 3.15. Dissemination of findings

The study findings have been e presented to the UoN Department of Paediatrics and Child Health, as part of the requirements of the Master of Medicine Program, in both hard and soft copies. Hard copies of the results will be sent to the University of Nairobi repository for storage. We plan to share a summary of the findings with the Head of Pediatrics department at KNH, to help to improve patient care. The findings will be submitted for publication, in peer-reviewed scientific journals.

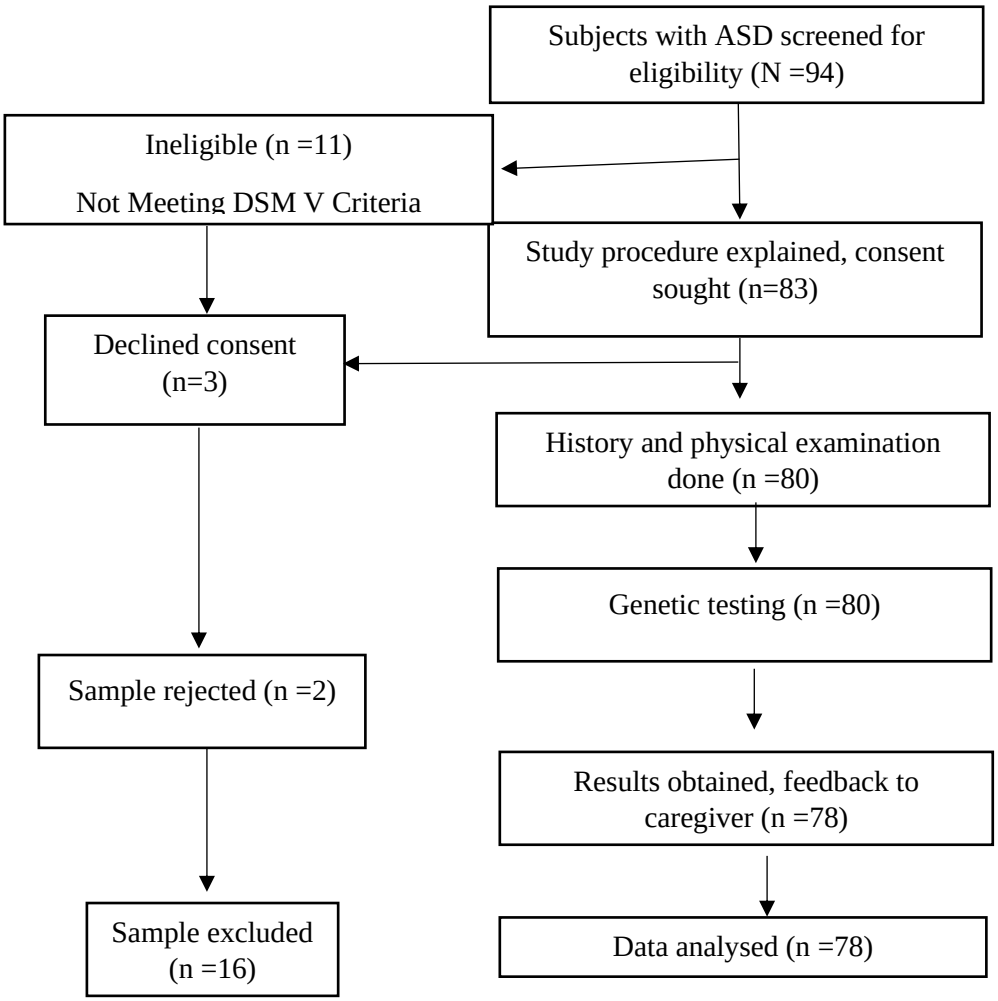
## 4.0. RESULTS

### 4.1.Introduction

This study investigated for the prevalence of Inborn Errors of Metabolism in patients with Autism spectrum disorder aged 18 months to 25 years, at Kenyatta National Hospital. Ninety-four (94) patients with autism were screen for eligibility to participate in the study, using the DSM V clinical diagnostic criteria for autism spectrum disorder (ASD). Eleven participants did not fulfil the criteria for ASD and were excluded from the study. The study procedure was explained to the eighty-three participants who met the DSM V criteria, and consent sought. Three caregivers declined to give consent and were excluded from the study. The remaining 80 participants gave consent, and participated in the study. After the blood samples were sent for genetic counselling, samples for 2 participants were rejected, and the testing had to be repeated at a later date. Patients attending these clinics, with diagnosis of other neurological disorders such as cerebral palsy, were also excluded from the study.

The study also assessed for the types of enzyme deficiencies associated with inborn errors of metabolism, as well as the and clinical profile of the participants with Inborn errors of metabolism.

### 4.1.1. Study Flowchart



## 4.2.Demographic and anthropometric characteristics of study participants

Study findings established that 80.8% (n =63) of the respondents were male. The average age of participants was 7.08 (SD3.2) years, with a range of 2 to 16 years, and 61.5% (n =48) were reside within Nairobi. The mean weight of participants was 17.9(SD5.5) kgs, and the mean height was 117.3(SD13.3) cm. The average head circumference was 47(SD3.2) cm while mean mid-upper arm circumference was 16.9(SD1.9) cm. Table 4.1 below summarises demographic characteristics of the study participants.

*Table 4.1: Demographic and anthropometric characteristics of study participants*

| **Patient characteristics** | **Frequency** | **Percent** |
|---|---|---|
| Gender | | |
| Male | 63 | 80.8 |
| Female | 15 | 19.2 |
| Age (Mean ±SD) | (7.08 SD3.2) | |
| <=5 years | 55 | 70.5 |
| 6 - 12 years | 18 | 23.1 |
| >12 years | 5 | 6.4 |
| Residence | | |
| Nairobi | 48 | 61.5 |
| Kiambu | 12 | 15.4 |
| Machakos | 8 | 10.3 |
| Others (Makueni, Kajiado, Tharaka Nithi, Muranga, Narok) | 10 | 12.8 |
| Weight | | |
| Underweight | 15 | 9.2 |
| Normal | 41 | 52.5 |
| Overweight | 22 | 28 |
| Head circumference | | |
| Microcephaly | 12 | 15.4 |
| Normal | 37 | 47.4 |
| Macrocephaly | 29 | 37.2 |

## 4.3.Clinical profile of the study participants

Majority of the respondents, 60.3% (n =47) had delayed speech; and 23% reported delay in other milestones. Comorbidities identified included seizure disorders at 14.1% (n =11), hyperactivity at 5.1%(n =4), diabetes at 2.6%(n =2) and ADHD at 1.3%(n =1). With regards to treatment, 47.4% (n =37) of the study participants were undergoing occupational therapy while 14.1% (n =11) were on

anticonvulsants. Thirteen participants (16.7%) had a positive family history of autism, with more than half, at 53.8% (n =7), reporting similar history in a sibling.

Some of the respondents, 11.5% (n =9) had history of recurrent respiratory and Ear, Nose and Throat (ENT) infections while 14.1% (n =1) had hearing difficulties or loss. Sixteen percent of respondents (n =13) had intellectual disability, Seizures were found in 11.5% (n=9) of the participants and Hyperactivity in 6.4 %(n=5). Dysmorphic features were present in 7.7% (n =6) while a similar proportion of the respondents had cataracts. About 3.8% (n =3) of the respondents had abnormal movement of limbs, while 7.7% (n =6) had difficulty walking and joint stiffness. Nine percent (n =7) of the respondents had hepatomegaly, while other respondents, 6.4% (n =5) had brown and thin hair. These findings are shown in Table 4.2.

*Table 4.2: Clinical profile of the study participants*

| **Clinical features, Treatment and family history** | **Frequency** | **Percent** |
|---|---|---|
| Milestone assessed | | |
| Delayed speech | 47 | 60.3 |
| Delay in other milestones | 14 | 17.9 |
| Delayed walking | 4 | 5.1 |
| No delay | 13 | 16.7 |
| Other associated medical conditions (n =19) | | |
| Seizure disorder | 11 | 14.1 |
| Hyperactivity | 4 | 5.1 |
| Diabetes | 2 | 2.6 |
| ADHD | 1 | 1.6 |
| Therapy/medications (n =64) | | |
| Occupational therapy | 37 | 47.4 |
| Anticonvulsants | 11 | 14.1 |
| Nutritional therapy | 4 | 5.1 |
| Physiotherapy | 3 | 3.8 |
| Family member with similar illness | | |
| Yes | 13 | 16.7 |
| No | 65 | 83.3 |
| Relationship of child with affected family member (n =13) | | |
| Sibling (sister, brother) | 7 | 53.8 |
| Mother | 4 | 30.8 |
| Cousin | 2 | 15.4 |

| | | |
|---|---|---|
| Recurrent ENT infection | 9 | 11.5 |
| Hearing difficulties or loss | 11 | 14.1 |
| Intellectual disability | 13 | 16.7 |
| Impaired vision | 13 | 16.7 |
| Dysmorphic features | 6 | 7.7 |
| Cataracts | 6 | 7.7 |
| Abnormal movements of limbs | 3 | 3.8 |
| Difficulty in walking | 6 | 7.7 |
| Hepatomegaly | 7 | 9 |
| Recurrent respiratory illness | 9 | 11.5 |
| Abnormal hair texture, colour (brown and thin) | 5 | 6.4 |

### 4.3.1. Severity of autism among participants: Ability to carry out daily activities among patients with autism spectrum disorder

Some of the respondents, 44% (n =34) were able to carry out most activities of daily living without help; while56% (n =44) had partial or total dependency and required to be assisted (shown in Figure 4.1).

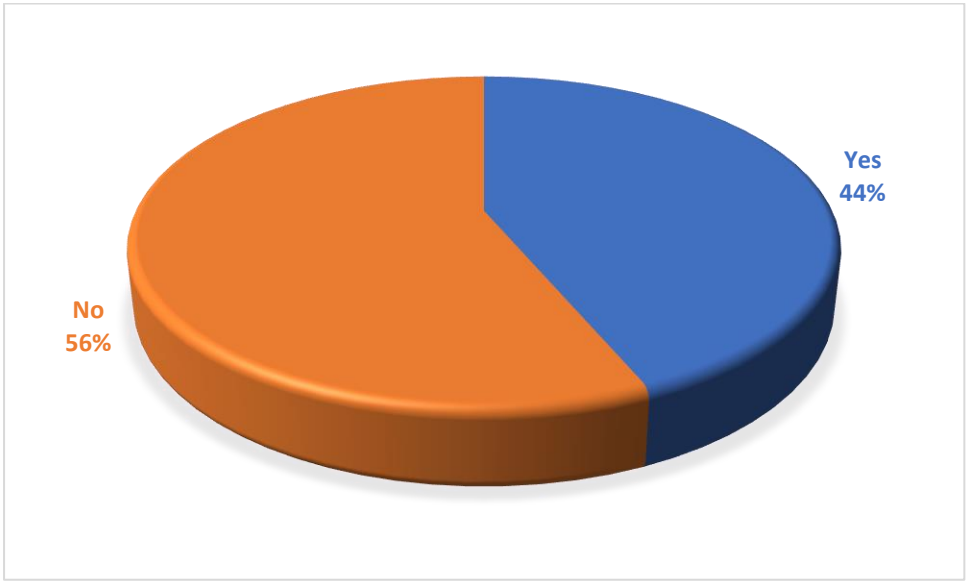



*Figure 4.1: Ability to carry out activities of daily living among patients with autism spectrum disorder*

### 4.4. Objective 1: Prevalence of Inborn Errors of Metabolism Among among children and adolescents with Autism spectrum disorder at Kenyatta National Hospital

The prevalence of inborn errors of metabolism among children and adolescents with autism spectrum disorder at KNH was 14.1% (n =11), 95%CI (7.3%, 23.8%).

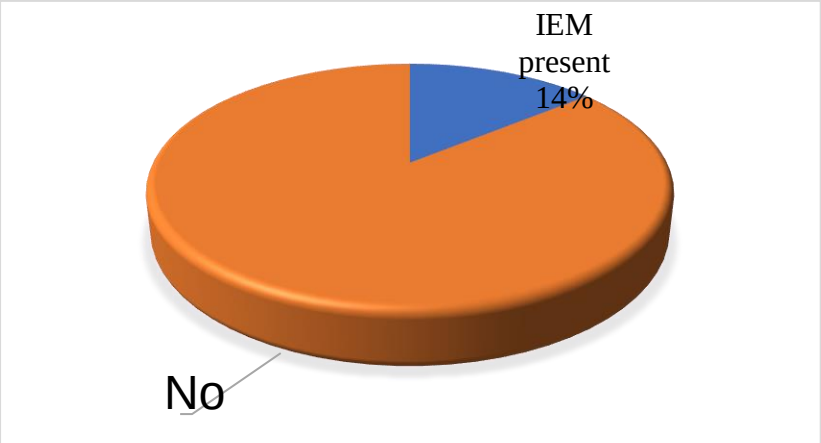



*Figure 4.2: Prevalence of Inborn errors of metabolism*

### 4.5. Objective IIA: Types of Inborn Errors of Metabolism detected among children and adolescents with Autism spectrum disorder, at Kenyatta National Hospital

Eleven study participants had Inborn Errors of Metabolism (IEM). Hunter syndrome was the most common at 45.5% of all identified IEM (n =5). Phenylketonuria was found in 4 participants, at 36.3% (n =4), while Glycogen storage disorder were found in 1 study participant. Methylmalonic aciduria and homocysteinemia was also present in 1 study participant. These findings are summarized in Table 4.3.

*Table 4.3: Types of Inborn Errors of Metabolism among children and adolescents with Autism spectrum disorder aged 18 months to 25 years.*

| **Type of IEM** | Frequency **n (%)** |
|---|---|
| Phenylketonuria | 4 (36.3%) |
| Glycogen storage disorder type 4 | 1 (9.1%) |
| Mucopolysaccharidosis type II (Hunter syndrome) | 5 (45.5%) |
| Methylmalonic aciduria and homocysteinemia | 1 (9.1%) |

### 4.5.1. Other Genetic Disorders found among the respondents with autism spectrum disorder

Other genetic disorders were found in 17.9% of participants (n=14). These include autosomal dominant susceptibility to autism, Cornelia de Lange syndrome type 1 (CDLS), Intellectual developmental disorders, Developmental and epileptic encephalopathy, and Bardet-Biedl syndrome type 19, among others. These genetic abnormalities are as shown in Table 12.

*Table 4.4:Genetic disorders Identified, among the Study Population*

| **Diagnosis** | **Frequency of Occurrence** |
|---|---|
| Autosomal dominant susceptibility to Autism | 1 |
| Cornelia de Lange syndrome type 1 (CDLS1 | 1 |
| Intellectual Developmental Disorder Syndromes | 3 |
| Developmental and Epileptic encephalopathy 11 (Early infantile epileptic encephalopathy type 11 / familial neonatal-infantile seizures) | 1 |
| Developmental delay with variable intellectual and behavioral impairment | 1 |
| Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation | 1 |
| Bardet-Biedl syndrome type 19 | 2 |
| Coffin-Siris syndrome-12/ neurodevelopmental syndrome | 1 |
| Glass syndrome /SATB2-associated syndrome (SAS) | 1 |
| *TUBB4A*-related leukodystrophy | 1 |
| *Chopra-Amiel-Gordon syndrome* | 1 |
| Incidental Finding: | |
| *Hereditary nonpolyposis colorectal cancer type 4* (Lynch syndrome) | 1 |

### 4.5.2. Objective IIB; Clinical characteristics associated with the presence of IEM among patients with ASD at KNH.

**Patient and Disease related characteristics associated with the presence of Inborn errors of metabolism among study participants:**

On univariate analysis, overweight and macrocephaly were significantly associated with presence of inborn errors of metabolism. Participants who were overweight were 10 times more likely to have IEM compared to those who had normal weight, crude odds ratio, COR= 10.37, 95%CI:1.22 – 88.02, p=0.032; while those who had macrocephaly were nine times more likely to have IEM compared to those with normal head circumference, COR =9.19, 95%CI:1.72 – 48.98, p =0.009. These was no

association between either age or gender with presence of IEM. Respondents who had a family member with ASD were 2.7 times more likely to have IEM compared to those with no affected family members, COR =2.71, 95%CI:1.34 – 14.32, p<0.001. Furthermore, the findings showed that participants who had intellectual dissability were four times more likely to have IEM, compared to those without, COR = 4.13, 95%CI:2.11 – 15.74, p<0.001. Participants with seizures, dysmorphic features, difficulties in ambulation were also more likely to have IEM as shown in Table 13.

*Table 4.5:Univariate analysis of the patient and Disease related characteristics and Presence of Inborn errors of metabolism among the Study Participants*

| **Parameter** | **Patients with IEM** | **Patients without IEM** | **Crude OR(95%CI)** | **P-value** |
|---|---|---|---|---|
| Age | | | | |
| <=5 years | 8(72.7) | 44(65.7) | Ref | |
| 6 - 12 years | 2(18.2) | 18(26.9) | 1.1(0.11 - 10.71) | 0.935 |
| >12 years | 1(9.1) | 5(7.5) | 1.8(0.13 - 24.16) | 0.657 |
| Gender | | | | |
| Male | 9(81.8) | 53(79.1) | 1.19(0.23 - 6.14) | 0.599 |
| Female | 2(18.2) | 14(20.9) | Ref | |
| Body weight | | | | |
| Underweight | 2(18.2) | 5(7.5) | 0.75(0.12 - 4.57) | 0.747 |
| Normal | 1(9.1) | 35(52.2) | Ref | |
| Overweight | 8(72.7) | 27(40.3) | 10.37(1.22 - 88.02) | 0.032 |
| Head circumference | | | | |
| Microcephaly | 2(18.2) | 9(13.4) | 1.97(0.34 - 11.57) | 0.453 |
| Normal | 2(18.2) | 42(62.7) | Ref | |
| Macrocephaly | 7(63.6) | 16(23.9) | 9.19(1.72 - 48.98) | 0.009 |
| Family members with ASD | | | | |
| Yes | 7(63.6) | 5(7.5) | 2.71(1.34 - 14.32) | <0.001 |
| No | 4(36.4) | 62(92.5) | Ref | |
| Delayed milestones | | | | |
| Yes | 8(72.7) | 57(85.1) | 0.46(0.11 - 2.07) | 0.265 |
| No | 3(27.3) | 10(14.9) | | |
| Mental retardation(Intellectual Disability) | | | | |
| Yes | 5(45.5) | 14(20.9) | 4.13(2.11 - 15.74) | <0.001 |
| No | 6(54.5) | 53(79.1) | Ref | |
| Seizures | | | | |
| Yes | 3(27.3) | 8(11.9) | 2.77(0.61 - 12.63) | 0.182 |
| No | 8(72.7) | 59(88.1) | Ref | |
| Impaired vision | | | | |
| Yes | 2(18.2) | 11(16.4) | 1.13(0.21 - 5.97) | 0.586 |
| No | 9(81.8) | 56(83.6) | Ref | |
| Dysmorphic features | | | | |
| Yes | 2(18.2) | 4(6.0) | 3.51(0.56 - 21.94) | 0.198 |
| No | 9(81.8) | 63(94) | Ref | |
| Abnormal movements of limbs | | | | |
| Yes | 2(18.2) | 1(1.5) | 4.67(0.21 - 15.41) | 0.125 |
| No | 9(81.8) | 66(98.5) | Ref | |

| | | | | |
|---|---|---|---|---|
| Recurrent respiratory illness | | | | |
| Yes | 2(18.2) | 7(10.4) | 1.91(0.34 - 10.64) | 0.372 |
| No | 9(81.8) | 60(89.6) | Ref | |

### 4.5.3. Multivariate analysis of factors associated with presence of IEM in patients with Autism Spectrum Disorders

The study established that participants who had macrocephaly were 10 times more likely to have IEM compared to those who had normal head circumference, AOR =10.2, 95%CI:2.31 – 23.67, p=0.024. Autism participants who had family member with autism were three times more likely to have inborn errors of metabolism (IEM) compared to those without, AOR = 2.85, 95%CI:1.23 – 23.67, p =0.011. Participants with intellectual disability were 14 times more likely to have IEM compared to those who did not have intellectual disability, AOR =14.16, 95%CI:3.22 – 25.32, p<0.001 as shown in Table

*Table 4.6: Multivariate Analysis of Factors Associated with Presence of IEM*

| Parameter | AOR (95%CI) | P-value |
|---|---|---|
| **Body weight** | | |
| Normal | Ref | |
| Underweight | 0.63(0.21 - 2.51) | 0.321 |
| Overweight | 1.67(0.34 - 3.22) | 0.289 |
| **Head circumference** | | |
| Normal | Ref | |
| Microcephaly | 1.23(0.41 - 3.22) | 0.655 |
| **Macrocephaly** | **10.20(2.31 - 23.67)** | **0.024** |
| **Family members with ASD** | | |
| Yes | **2.85(1.23 - 6.22)** | **0.011** |
| No | Ref | |
| **Intellectual disability** | | |
| Yes | **14.16(3.22 - 25.32)** | **<0.001** |
| No | Ref | |
| Reduced muscle tone | | |
| Yes | 2.75(0.33 - 2.78) | 0.321 |
| No | Ref | |

## 5.0: DISCUSSION

### 5.1. Demographic characteristics of Study Participants

The study found a male preponderance of autism spectrum disorder (ASD), with 81 percent of the respondents being males (male to female ratio of 4.2:1). These findings compare with those from previous study by Rachael et al which revealed that autism spectrum disorder was more common among male children as compared to female children and adolescents (53). However, a recent meta-analysis found that among patients with severe autism, the incidence is higher among females with ASD,with male to female ratio of 2:1.(54).

The average age of participants was 7 years, with 70 percent aged less than five years. Majority of the participants in our study had presented with initial symptoms from the age of 1 to 2 years. A study by Parmeggiani et al. found that majority of the children (41.9% of the cases) with ASD had their first symptoms between 7 and 12 months, 27.6% between 13 and 24 months, 6.7% between 25 and 36 months, and 1.9% between 37 and 51 months. (55). Another study conducted by Maarten et al. established that the mean age at diagnosis of ASD was 60.48 months (range: 30.90–234.57 months). The study sited factors that affect age at diagnosis of ASD to include type of autism spectrum disorder, additional diagnoses and comorbidities, as well as patients gender (56).

### 5.2. Prevalence of IEM

The prevalence of inborn errors of metabolism in this study population was 14.1 percent. Our findings are consistent with findings in the study done in India by Kumar et al which revealed that the prevalence of IEM was 14 percent (7). The findings from our present study are higher than those from other studies conducted in Greece and China (48)(49). In the study conducted by Spilioti et al in Greece, prevalence of IEM among children with autism was lower, at 4 percent (48). Haijie et al study, in China found the IEM prevalence in children with autism to be 6.9 percent. The higher prevalence of IEM in our study could be attributed to gap and high cost of diagnosis, and lack of treatment facilities. Most countries especially low- and middle-income countries do not have technical ability and resources to diagnose and treat IEMs early, thus untreated cases progress and develop clinical autism. A study conducted in Iraq by Altimimi et al found that the proportion of children with inborn errors of metabolism was 17.9 percent, among children with unexplained developmental delays (46). Family history of consanguinity was higher, in the study by Altimimi et al, and this may account for the higher prevalence of IEM in that study population. The difference could also be due to the fact that the target population in our study, included only children diagnosed with ASD while in the by Altimimi et al included all children with unexplained developmental delays.

### 5.3. Types of IEM

The current study revealed that Hunter Syndrome (45.5%) and Phenylketonuria (36.3%) were the common IEM types among the participants investigated. Other IEM identified in our study are Glycogen Storage Disorder type 4, and Methylmalonic aciduria with Homocysteinemia. In the study conducted in China by Haijie et al, the types of IEM identified included Phenylketonuria, Homocystinuria, Methylmalonic Acidemia, comparable to those found in our study. In addition, their study identified Glutaric acidemia, Isovaleric acidemia, Propionic, Argininemia, Citrullinemia, and Primary Carnitine Deficiency which were not present in the current study were (47). Other previous studies identified more metabolic abnormalities, among study participants with autism (45) (48) . The study conducted by Spilioti et al., in Greece, identified Phenylketonuria as well as Lesch Nyhan syndrome, Succinic Semialdehyde Dehydrogenase Deficiency, and Biotin Deficiency. The study by Kumar V. Suresh et al found Methylmalonic Acidemia and other disorders such as Isovaleric acidemia, Tetracarboxylic acid cycle disorders, Lactic acidosis, and Propionic academia (7).

Saad, in a study conducted in Egypt revealed that 25% of the participants with Phenylketonuria developed autism spectrum disorder (12).. Some studies have described autism spectrum among children with hunters syndrome(57)(58)(59). Hasan et al described a case of a younger child with Hunter Syndrome, who presented with the spectrum of autism, with features of restricted social communicative behaviors and interest, poor eye contact, unresponsiveness to his name being called, repetitive behaviors, and declined interaction with the peers (57). A study by Rumsey et al found that 62% of the study participants with Hunter Syndrome met the criteria for ASD (58). Eisengart et al described multiple neurobehavioral symptoms among children with hunter syndrome including difficulties in focus and attention, impulsivity, impaired emotional and behavioral function and social interaction (59).

### 5.4. The characteristics of Autism Spectrum Disorder patients with Inborn Errors of Metabolism.

Majority of the participants diagnosed with inborn errors or metabolism (IEM), 72%, were aged less than five years, with median age of two, and age range of 2 to 17 years. A study done in Iraq revealed that the median age of children with IEM was 22 months, lower compared to our present study. Furthermore, our study found that 4 in every 5 participants were males. A study by Hanna Alobaidy, in Libya found that 54% of children diagnosed with IEM were males, while the median age at diagnosis was eight months (range was 1 to 96 months), and 13% were diagnosed at six months of age or younger. Similarly, a study conducted in France revealed that majority of patients with IEM were male (60).

The findings from our study showed that the clinical profile of autism respondents with IEM included macrocephaly or microcephaly; delayed milestones, overweight, positive family history of family member affected with autism or neurological disorders; mental retardation/intellectual disability, history of seizures, and dysmorphic features. Other features noted were visual disturbance, recurrent respiratory tract infections, musculoskeletal abnormalities such as difficulties in ambulation, abnormal limb movements. These findings are comparable to findings in previous studies by Spilioti et al. and Kumar et al (48). (7).

In a study by Spilioti et al, respondents were found to have positive family history of neurological disease, with a 1st to 3rd degree relative with similar symptoms, epilepsy, ataxia, developmental delays, dysmorphic features such as low set ears, increased inter eye distance. The study by Spilioti further found a positive history of consanguinity among several parents of affected participants and brain abnormalities on magnetic resonance imaging (MRI) (48). There was no history of consanguinity in our study, and no MRI screening of participants was done.

In the study by Kumar et al; all autism participants identified to have IEM were males. This is higher compared to the findings in our study. Respondents were aged 2.6 years to 9 years and clinical findings noted include developmental delays. Clinical presentation of the respondents included developmental delays, behaviour problems (disinhibited, demanding attitude) poor eye contact, pigmentation of the skin, dermatitis, speech difficulties, metabolic crises, poor appetite, low muscle tone, vomiting, metabolic disorders; respiratory/gastrointestinal infections, mental retardation, seizures, metabolic acidosis (7).

Our study results are comparable to those from Saudubray & Garcia-Cazorla in Spain who revealed that patients with inborn errors of metabolism can present with diverse neurodevelopmental manifestations (34). In the study by Hanna Alobaidy, respondents with IEM were found to have positive history of consanguineous marriages (86%), unlike the findings in our study. Participants also had history of a family member with or who died of a similar illness, seizures (60). Another study by Saad revealed that 62.5% of phenylketonuria participants with autism were males. The respondents also had microcephaly, seizures, light skin as well as had skin lesions (12).

### 5.5 Other genetic disorders found

Our study found that 17.9 % the autism spectrum disorder without IEM had varied genetic disorders. These include Autosomal Dominant Susceptibility to Autism, Cornelia de Lange syndrome type 1 (CDLS), Intellectual Developmental Disorder syndrome, Developmental and Epileptic Encephalopathy, Bardet Biedl syndrome type 19, Microcephaly with or without Chorioretinopathy, Lymphedema, or Mental Retardation; Developmental Delay with variable Intellectual and Behavioral

Impairment, Coffin-Siris Syndrome-12, and Glass Syndrome. A systematic review and metanalysis by Richards et al found that several genetic disorders are associated with the spectrum of autism, and these included Rett's syndrome, Cohen's syndrome, Cornelia de Lange syndrome, Tuberous Sclerosis complex, Angelman's syndrome, Fragile X syndrome among others (61).

## 6.0 CONCLUSION AND RECOMMENDATIONS

### 6.1 Conclusion

1. Prevalence of Inborn Errors of Metabolism was high in our study population of children and adolescents with Autism Spectrum Disorder, at 14.1%.

2. Among the participants with Inborn Errors of Metabolism, hunter syndrome (45.5%), Phenylketonuria (36.3%) and Glycogen storage disorder (9.1%) were the commonly occurring types. Other types include Methylmalonic aciduria with homocysteinemia.

3. Positive Family history of ASD, intellectual disability, macrocephaly, overweight, and musculoskeletal abnormalities showed independent association with the presence of IEM among patients with ASD.

### 6.2 Recommendations

1. Patients with Autism Spectrum Disorder should be tested early for Inborn Errors of Metabolism, to guide treatment approaches for improved patient outcomes
2. Clinicians to have a high index of suspicion for possible underlying Inborn Errors of Metabolism in patients with a Positive Family history of ASD, or those with intellectual disability, macrocephaly, overweight or musculoskeletal manifestations.
3. Policymakers (Kenya Ministry of health) to avail genetic testing laboratory in Kenya, for IEM, and Subsidize cost.
4. Further studies to be done, to assess the outcome of autism patients with underlying inborn errors of metabolism.

## 7.0 STUDY STRENGTH AND LIMITATIONS

### 7.1 Study Strengths

1. This is the first study to provide an insight into the prevalence of Inborn Errors of Metabolism (IEM) in patients with autism in Kenya
2. The study offered information on the different types of Inborn Errors of Metabolism and the clinical features associated with the presence of IEM in patients with autism.
3. The study was able to identify other genetic disorders among autism patients

### 7.2 Limitations of the study

1. Some caregivers of eligible subjects declined to participate in the study which could have led to selection bias.

2. The sample size was too small to provide accurate data with regards to the secondary study objectives.

3.This was a hospital-based study conducted at a tertiary referral facility, and results may not be generalizable to the general population

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**APPENDICES**

**APPENDIX 1: DIAGNOSTIC AND STATISTICAL MANUAL -5TH EDITION. CRITERIA FOR AUTISM SPECTRUM DISORDER**:

| **Criterion** | **Symptoms** |
|---|---|
| A. Persistent deficits in social communication and social interaction manifesting with 3 of: | 1. Deficits in social-emotional reciprocity.<br>2. Deficits in nonverbal communicative behaviors used for social interaction.<br>3. Deficits in developing, maintaining, and understanding relationships. |
| B. Restricted, repetitive patterns of behaviour, interests, manifested by 2 or more of the following: | 1. Stereotyped or repetitive motor movements, speech, or use of objects.<br>2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior.<br>3. Highly restricted, fixated interests that are abnormal in intensity or focus.<br>4. Hyper or hyporeactivity to sensory stimuli |
| C. Symptoms must be present in early childhood | |
| D. Symptoms cause clinically significant impairment in social, occupational, or other areas of functioning | |

**APPENDIX II: CONSENT FORM**

- For children below the age of 6 years, the consent forms will be issued to the guardian and parents
- These forms shall also be issued to parents /guardians of children above the age of 8 years and below 18 years; in addition to the assent form to be filled by the child himself/herself
- Participants above 18 years will be issued with consent forms for signing.
- Swahili and English versions of the consent and assent forms shall be issued

**Appendix 1A: Consent form for participation in the study (English Version):**

**Study Title**: Magnitude and Types of Inborn Errors of Metabolism in patients with Autism Spectrum Disorder

**Name of Researcher**: Dr. Mercy Mapenzi Mwakubo

**Supervisors:** Dr. Lucy N. Mungai, Dr. Beatrice Mutai, Dr. Josephine Omondi

My name is Dr. Mercy Mapenzi Mwakubo, I'm a resident at Kenyatta National Hospital, and a postgraduate student at the University of Nairobi pursuing a Master of Medicine Degree in Pediatrics and Child Health.

I am conducting a study on the Magnitude and Types of Inborn Errors of Metabolism in patients with Autism Spectrum Disorder, who are on follow-up at KNH. This study is being conducted with the permission of Kenyatta National Hospital- University of Nairobi and Ethics and research committees.

Your son/daughter is being requested to participate in the study because she/ he meets the conditions required to be included in the study

The purpose of this consent form is to give you the information you will need to help you decide whether or not to agree to participate in the study.

Autism Spectrum Disorder is a neurodevelopmental disorder, with onset in early childhood, in which the affected individual has deficits in social communication, restricted interests, and repetitive behavior. Inborn Errors of Metabolism, on the other hand, are genetic metabolic disorders that occur due to single gene defects which result in the lack of an enzyme, its cofactor such that the body is unable to process substrates as carbohydrates, fats, proteins, vitamins, and so on. Enzyme deficiencies are found in a significant of patients with Autism. IEM can affect any organ system, and when the central Nervous system is affected, the neurological dysfunction can result in Autism spectrum disorder (ASD). Specific treatment of underlying IEM is available, for some cases, such as Enzyme replacement and correction of other metabolic abnormalities and this prevents worsening of the disease and may improve symptoms of Autism in some children.

**Purpose**: the participation of your in this study will help us confirm whether he/she has any underlying Inborn Error of Metabolism that may have resulted in the manifestation of Autism. If he/she is found to have an Inborn Error of Metabolism, he/she will be linked to our Endocrinology department, for specific management.

The results of this study will also help inform the need for Testing for Inborn Errors of Metabolism among patients with Autism spectrum disorder in Kenya.

**Procedure**:

Counseling shall be provided to you and your parent or caregiver, before the study begins, and after study test results are received. It shall be conducted by the principal investigator under the supervision of pediatric endocrinologist Dr. Lucy Mungai, who is part of the research team as a supervisor. During counseling, we shall provide you with details of

- The study Purpose, Utility
- Information on cause of autism, including Inborn errors of metabolism. information on the pattern of inheritance for Inborn Errors Metabolism shall be given, including the chance of the presence of such genes that cause these disorders among other family members of an affected individual. And how to reduce the risk of transmission of genes that cause such disorders, including avoiding consanguineous marriages.
- The steps of the study include clinical history taking, examination, as well as blood sample collection.
- Sample shipment to Germany, testing for enzyme deficiency that shall be conducted; storage, custody, and destruction of remaining sample, as well as authorization in case further research, is required on the same sample.
- Importance of carrying out genetic testing for inborn errors of metabolism.
- How you shall be contacted once the genetic test results are received, for disclosure of the results. The implication of any positive tests results, to you and your family, shall be explained.
- How if you are found to have treatable IEM, shall access care at the KNH endocrinology department

If you agree for your child(s) to participate in the study, I shall request you to sign the consent form. I will then proceed to write down Initials of your child's name and a number/code on the data collection form.

I will proceed to ask a series of questions on where you live, contact information, history of other family members with similar symptoms as yours. I will then do a full physical examination of your child. I will note your responses, and findings of the examination, in writing, in the questionnaire. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview.

I shall obtain a blood sample on a filter paper after a finger prick. The sample will be air-dried and packaged, and then sent to Germany for genetic analysis. The analysis will be made for any genetic mutation, for specific enzyme deficiency. I will inform you of the results of the test, and these too shall be kept results confidential. We expect to get the results back within 2 weeks to 1 month.

Your remaining blood sample shall be kept under custody CENTOGENE until the principal investigator confirms that the research team and the patient have received the test results; and that there is no need for further testing. During this time, the laboratory will preserve and store the sample. After confirmation is given by the investigator that there is no requirement for further testing, the laboratory shall proceed to destroy the sample filter papers by incineration. If further research is to be done on the same sample material, informed consent shall be sought from the guardian/parent.

To protect your privacy, all the information you and your child provide shall be held with utmost confidence, and shall not be shared with anyone outside the research team

**Kindly understand the following:**

1. **Participation** in the study is voluntary.
2. **Confidentiality** shall be maintained at all times. Any data provided by you or your child will be preserved in a password-protected computer data storage and will keep all of the paper records in a locked file cabinet.
3. **Refusal of any participation** in the study will not attract any penalties. Your child shall continue to receive treatment as required.
4. **Risks**: there are minimal risks in participating in this study. Mild pain shall be experienced during pricking when the blood sample is being collected.
5. **Benefits**: Free evaluation for Inborn Errors of metabolism in you and your child. Pre-test counseling will be offered to you. If you are found to have Inborn Errors of Metabolism you will be referred to the Endocrinology Clinic at Kenyatta National Hospital for Specific treatment and follow-up.

If you have further questions or concerns about your child participating in this study, please call or send a text message to the study staff: Dr. Mercy Mapenzi on **0726026685**. Supervisors DR Lucy Mungai: 0724654135; Email dr.lmungai@gmail.com; Dr. Beatrice Mutai, mobile phone number 0708552909; Email mutaibc@gmail.com; and Dr. Josephine Omondi, mobile phone number 0737562973, Email address josephinekumba@gmail.com. For more information about your child's rights as a research participant, you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. **2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke.**

**Right to participate or withdraw from the study:**

Your decision to have you or your child participate in this research is voluntary. You are free to decline or withdraw participation of you or your child in the study at any time without injustice or loss of

benefits. Just inform the study staff and the participation of you or your child in the study shall be stopped. You do not have to give reasons for your or your child's withdrawal if you do not wish to do so. Withdrawal from the study will not affect the services you or your child is otherwise entitled to in this health facility or other health facilities.

**CONSENT FORM (STATEMENT OF CONSENT)**

The person being considered for this study is unable to consent for him/herself because he or she is below the legal age of consent. You are being asked to give your permission to include your child in this study.

**Parent/guardian statement**

I have read this consent form or had the information read to me. I have had the chance to discuss this research study with the researcher/ research assistant. I have had my questions answered by him or her in a language that I understand. The risks and benefits have been explained to me. I understand that I shall be given a copy of this consent form after signing it. I understand that my participation and that of my child in this study is voluntary and that I may choose to withdraw it at any time. I understand that all efforts shall be made to keep information regarding me and my child's identity confidential. By signing this consent form, I have not given up my child's legal rights as a participant in this research study.

I voluntarily agree to my child's participation in this research study: Yes No

I agree to have my child undergo finger pricking to obtain a blood sample: Yes No

I agree to allow the collected blood sample to be stored by the principal investigator: Yes No

I agree to allow the collected blood sample to be shipped to Germany for genetic testing: Yes No

I agree to allow CENTOGENE laboratory to store the collected blood sample for as long as necessary until the principal investigator receives the results and confirms that no further testing is necessary: Yes No

I agree to provide contact information for follow-up and results sharing: Yes No

Parent/Guardian signature: _____ Date _____

Parent/Guardian printed name: _____

**Researcher's statement**

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given his/her consent.

Researchers Name: ……..... Signature: ……. Date: ……

Role in the study: ……..

Witness Name: …… Signature: ……. Date: …….....

**APPENDIX IIB: SWAHILI CONSENT FORMS**:

**Idhini ya kushiriki katika utafiti (wagonjwa wa Autism)**

**UTAFITI JUU YA**: Idadi na aina za magonjwa ya makosa ya kuzaliwa ya kimetaboliki (inborn errors of metabolism), kati ya wagonjwa wa usonji (autism)

**Jina la mtafiti:** Daktari Mercy mapenzi Mwakubo

Mimi ni mwanafunzi wa Uzamili katika Chuo Kikuu cha Nairobi; ninayesomea shahada ya afya na magonjwa ya watoto.

Ili kuhitimu shahada hii, ninafanya utafiti kusuhu Idadi na Aina za Magonjwa ya Makosa ya Kuzaliwa ya Kimetaboliki (Inborn Errors of Metabolism), kati ya Wagonjwa wa Usonji (Autism), Katika spitali kuu ya Kitaifa ya Kenyatta.

Utafiti huu unafanyika kwa idhini ya Kamati ya Maadili na Utafiti, ya Spitali ya kitaifa ya Kenyatta na Chuo Kikuu cha Nairobi.

**Kusudi la Utafiti huu:**

Ushiriki wako katika utafiti huu utasaidia kudhibitisha kama uko na ugonjwa wa Makosa ya Kuzaliwa ya Kimetaboliki (Inborn Errors of Metabolism), ambao umesababisha hali ya Usonji (Autism).

Ukipatikana na ugonjwa wa Makosa ya Kuzaliwa ya Kimetaboliki, utapokea huduma ya matibabu yapasayo, katika Cliniki ya Endocrinolojia (Endocrinology Clinic), ya Spitali kuu ya Kitaifa ya Kenyatta.

Matokeo ya utafiti huu, yatasaidia kueka mikakati ya vipimo vya kuchunguza magonjwa ya makosa ya Kuzaliwa ya Kimetaboliki (Inborn Errors of Metabolism), kati ya wagonjwa wenye maradhi ya Usonji. Na pia kuanzishwa kwa kipimo hicho hicho kati ya watoto wachanga wanapo zaliwa.

**Tafadhali maelezo yafuatayo niya muhimu:**

1. Ushiriki katika uchunguzi huu, ni kwa hiari yako.

2. Nitaitunza siri yako. Maelezo ya mtoto wako, na yako, yatahifadhiwa katika mashine ya kompyuta iliyo na neno la siri. Makartasi yenye maelezo hayo, yatahifadhiwa katika kabati ya siri.
3. Kukataa kushiriki katika utafiti hakutavutia adhabu yoyote. Mtoto wako ataendelea kupokea matibau yanayo stahili.
4. Hakuna hatari inayotarajiwa kwa kushiriki katika utafiti huu.
5. Faida kwako kupitia kushiriki katika utafiti huu:
   Kupata Huduma ya Uchunguzi wa magonjwa ya Makosa ya Kuzaliwa ya Kimetaboliki, bila malipo yoyote.
   Iwapo utapatikana na ugonjwa wa Makosa ya Kuzaliwa ya Kimetaboliki, utapokea huduma ya matibabu yapasayo, katika Cliniki ya Endocrinolojia (Endocrinology Clinic), ya Spitali kuu ya Kitaifa ya Kenyatta.
   Kupewa mawaidha yafaayo kuhusu magonjwa ya maumbile kwa familia nzima.
6. Hakuna fidia ya fedha kwa ajili ya kushiriki katika utafiti huu.
7. Uko na uhuru wa kukataa kuhusishwa katika utafiti huu wakati wowote kupitia. Na una haki ya kujiondoa wakati wowote.

**Utaratibu**:

Iwapo utakubali kushiriki kwenye utafiti huu, nita yaandika maelezo ya kukutambulisha wewe na mwanao. Maelezo yako, naya mtoto wako yatahifadhiwa vyema, na kwa siri.

Kabla ya kuanza utafiti, utapokea ushauri maalum, kutoka kwa mtafiti mkuu, chini ya uongozi wa Daktari wa Edocrinologia wa watoto, Daktari Lucy Mungai. Kupitia ushauri huu, tutakupa maelezo kuhusu

- Kusudi na umuhimu wa utafitu huu
- Vigezo vinavyoweza kusababisha ugonjwa wa usonji (autism), ikiwemo magonjwa ya makosa ya kuzaliwa ya kimetaboliki; jinsi magonjwa haya yanavyo rithiwa katika familia. Jinsi ya kuzuia urithi wa magonjwa haya, ikiwepo pamoja na kuzuia ndoa za watu wa ukoo mmoja.
- Taratibu ya utafiti huu, ikiwemo kuchukua historia ya ugonjwa , kufanya uchunguzi wa mwili, na kutoa sampuli za damu.
- Usafirishaji wa sampuli za damu hagi maabara ya CENTOGENE, ujerumani kwa uchunguzi wa magonjwa ya makosa ya kuzaliwa ya kimetaboliki. Kwamba sampuli itakayobakia baada ya vipimo hivi, itahifadhiwa na maabara, hadi wakati mtafiti mkuu atapata nakala ya majibu na kuyakuthibitisha. Kwamba baadaye, nakala za sampuli hizi zitateketezwa.

- Umuhimu wa kufanyiwa uchunguzi wa jeni katika kuchunguza magonjwa ya makosa ya kuzaliwa ya kimetaboliki.
- Jinsi tutakavyo wasiliana nawe wakati tutakapo pokea majibu ya kipimo hiki, ili kukujulisha majibu. jinsi ukupatika kua na magonjwa ya makosa ya kuzaliwa ya kimetaboliki, ita ashiria katika familia yako.
- Namna utaweza kupata matibabu yafaayo, japo utakau na ugonjwa wa makosa ya kuzaliwa ya kimetaboliki, kupitia kliniki ya endocrinologia ya spitali kuu ya Kenyatta

Halafu Nitaendelea kuliza maswali kuhusu dalili za ugonjwa wa mwanao. Na pia, maelezo kuhusu familia yenu. Alafu nitafanya uchunguzi wa kimwili, kwa mwanao. Maelezo haya nitayajaza katika fomu zetu za utafiti.

Kisha mtoto wako atatolewa damu chache, ambayo itahifadhiwa kwenye kartasi maalum. Sampuli hizi za damu zitatumwa kwenya maabara ya CENTOGENE, nchini Ujerumani ili zifanyiwe vipimo maalum.
Sampuli ya damu itakayo baki, baada ya kufanyiwa vipimo, itahifadhiwa na maabara hiyo, hadi wakati mtafiti mkuu atakapo pokea na kuthibitisha majibu ya kipimo. Kisha kartasi ya sampuli ya damu ita teketezwa na maabara.
Tuanatarajia kupata majibu ya vipimo hivi, baada ya wiki mbili au mwezi mmoja. Nitawajulisha matokeo ya vipimo, ambayo pia yatahifadhiwa kwa siri.

Kusudi kuu la kukupa habari hizi ni kukuwezesha kuamua iwapo utamruhusu mwanao kushirikishwa kwenye utafiti huu. Iwapo unakubali kushiriki, nitakuomba uweke sahihi kwenye fomu ya kibali. Una uhuru wa kukataa kujibu maswali ambayo hupo radhi nayo.
Majina ya mwanao, na yako, hayata andikwa katika fomu ambayo itatumwa pamoja na sampuli za damu, kwenye maabara.

**KAULI YA MZAZI:**
Nimeyasoma maagizo yote yaliyomo ndani ya fomu hii ya idhini na kuelewa.
Nimekuwa na fursa ya kujadili maelezo ya utafiti huu, na mtafiti / msaidizi wa utafiti. Nimeelezwa hatari na faida za kushiriki utafiti huu. Ninaelewa kuwa nitapewa nakala ya idhini hii nitakapoitia sahihi. Ninaelewa kuwa ushiriki wa mtoto wangu katika utafiti huu ni kwa hiari na ninaweza kukataa kuhusishwa kwenye utafiti huu, wakati wowote. Naelewa kuwa maelezo yangu, na ya mwanangu yatahifadhiwa kwa siri ipasavyo. Katika kusahihisha idhini hii sijasalimisha haki za sharia za mtoto wangu.

Nimekubali kwa hiari yangu, kushirikisha mtoto wangu kwa utafiti huu: Ndio La
Nimekubali mtoto wangu kutolewa damu: Ndio La
Nimekubali kuruhusu sampuli ya damu isafirishwe hadi Ujerumani, kufanyiwa

vipimo: Ndio La

Nimekubali kuiruhusu maabara ya CENTOGENE kuihifadhi sampuli yangu ya damu, hadi mtafiti atakapoyapokea na kuthibitisha majibu ya kipimo: Ndio La

Nimekubali kumpa mtafiti nambari ya simu, kwa ajili ya mealezo zaidi: Ndio La

**Jina la Mzazi/Mlezi: …… Sahihi ……** Tarehe ……

**KAULI YA MTAFITI**

Mimi kama mchunguzi, ninaye weka sahihi idhini hii, nimempa mzazi wa mshiriki huyu, maelezo muhimu kuhusu utafiti huu. Natumai kuwa ameelewa na kukubali kushirikishwa katika utafiti huu, kwa hiari.

Jina la Matifiti …… Sahihi: ……Tarehe: ……

Jina La Sashidi: ……...Sahihi: …… Tarehe: ……

Ukiwa na maswali zaidi au unahitaji maelezo zaidi kuhusu ushiriki katika utafiti, tafadhali piga simu au tuma ujumbe kwa Daktari Mercy Mapenzi Mwakubo, Idara ya Maabara ya Afya na Afya ya Watoto, Chuo Kikuu cha Nairobi. Nambari ya Simu ya Simu 0726026685. Barua pepe mercymwakubo@gmail.com. Wasimamizi wa Utafiti: Daktari. Lucy Mungai, Nambari ya simu; 0724654135; Barua pepe dr.lmungai@gmail.com. Daktari Beatrice Mutai, Nambari ya simu:0708552909 ; Barua pepe Email mutaibc@gmail.com. Dr. Josephine Omondi, nambari ya simu 0737562973; Barua pepe josephinekumba@gmail.com

Kwa habari zaidi kuhusu haki zako kama mshiriki wa utafiti, unaweza kuwasiliana na katibu / mwenyekiti, Hospitali ya Taifa ya Kenyatta - Chuo Kikuu cha Nairobi na kamati ya utafiti no.2726300 Ext. 44102. Barua pepe uonbi.ac.ke.

**APPENDIX II** C: ASSENT FORM FOR CHILDREN ABOVE 6YEARS AND BELOW 18 YEARS (ENGLISH VERSION)

My name is Dr. Mercy Mapenzi Mwakubo, I'm a resident at Kenyatta National Hospital, and a

postgraduate student at the University of Nairobi pursuing a Master of Medicine Degree in Pediatrics and Child Health.

I am conducting a study on the Magnitude and Types of Inborn Errors of Metabolism in patients with Autism Spectrum Disorder, who are on follow-up at KNH. This study is being conducted with the permission of Kenyatta National Hospital- University of Nairobi and Ethics and research committees.

You are being requested to kindly participate in the study because you meet the conditions to be included in the study

**Purpose**

Through the testing that will be done during this study, we shall be able to confirm whether you have any underlying Inborn Error of Metabolism that may have resulted in the manifestation of Autism. If you are found to have an Inborn Error of Metabolism, you will be linked to our Endocrinology department, for specific management.

The results of this study will also help inform policy formulation on routine Testing for Inborn Errors of Metabolism among patients who present with features of Autism spectrum disorder.

**Procedures**

If you accept to participate in the study, I will write down the initials of your name and a number/code in the data collection form. The information you provide will be kept safe and will not be shared with anyone else outside the research team.

Counseling shall be provided to you and your parent and caregiver before the study begins and after study test results are received. It shall be done by the principal investigator, under the guide of Pediatric endocrinologist Dr. Lucy Mungai; who is part of the research team as a supervisor. During counseling, we shall provide you with details of;

- The study Purpose, Utility
- Causes of autism, including Inborn errors of metabolism. information on the pattern of inheritance for Inborn Errors Metabolism shall be given, including the chance of presence of such genes that cause these disorders among other family members of an affected individual; and how to reduce the risk of transmission of genes that cause such disorder, including avoiding consanguineous marriages.
- The steps of the study include clinical history taking, examination, as well as blood sample collection.

- Sample shipment to Germany, testing for enzyme deficiency that shall be conducted; storage, custody, and destruction of remaining sample, as well as authorization, incase further research is required on the same sample.
- Importance of carrying out genetic testing for inborn errors of metabolism.
- How you shall be contacted once the genetic test results are received, for disclosure of the results. The implication of any positive tests results, to you and your family, shall be explained.
- How if you are found to have treatable IEM, shall access care at the KNH endocrinology department

I will proceed to ask a series of questions on your contact information, residence, whether there are other family members with Autism, as well as on your clinical symptoms. I will then do a full physical examination of your child. I will note your responses, and findings of the examination, in writing, in the questionnaire. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview.

I shall obtain a blood sample on a filter paper after a finger prick. The sample will be air-dried and packaged, and then sent to Germany for genetic analysis. The analysis will be made for any genetic mutation, for specific enzyme deficiency. I will inform you of the results of the test, and these too shall be kept results confidential. We expect to get the results back within 2 weeks to 1 month.

Your remaining blood sample shall be kept under custody CENTOGENE until the principal investigator confirms that the research team and the patient have received the test results; and that there is no need for further testing. During this time, the laboratory will preserve and store the sample. After confirmation is given by the investigator that there is no requirement for further testing, the laboratory shall proceed to destroy the sample filter papers by incineration. If further research is to be done on the same sample material, informed consent shall be sought from you and their guardian/parent.

To protect your privacy, all the information you and your child provide shall be held with utmost confidence, and shall not be shared with anyone outside the research team.

Participation in the study is voluntary. Refusal of any participation in the study will not attract any penalties. Your child shall continue to receive treatment as required.

**Benefits**:

Free evaluation for Inborn Errors of metabolism in you and your child. Pre-test counseling will be offered to you. If you are found to have Inborn Errors of Metabolism you will be referred to the Endocrinology Clinic at Kenyatta National Hospital for Specific treatment and follow-up. Counseling, for your family, shall also be offered on the inheritance pattern of inborn errors of metabolism that may

cause autism spectrum disorder; the possible presence of gene mutation in other family members of an affected individual, the importance of genetic testing of family members of an affected individual; how to reduce the risk of transmission of genes that cause such disorder, including avoiding consanguineous marriages.

**Risks, stress, and discomfort:**

The needle we use to take the blood may hurt. You might get a bruise on your arm. Sometimes you may develop swelling at the site as well. In case this happens, please call us so that we may help you.

If you have further questions or concerns about your child participating in this study, please call or send a text message to the study staff: Dr. Mercy Mapenzi on **0726026685**. Supervisors DR Lucy Mungai: 0724654135; Email dr.lmungai@gmail.com. And Dr. Beatrice Mutai, mobile phone number 0708552909; Email mutaibc@gmail.com. Dr. Josephine Omondi, mobile phone number 0737562973, Email address josephinekumba@gmail.com. For more information about your child's rights as a research participant, you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. **2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke.**

**Right to participate or withdraw from the study:**

Your decision to participate in this research is voluntary. You are free to decline or withdraw your participation in the study at any time without injustice or loss of benefits (Just inform the study staff and your participation shall be stopped). You do not have to give reasons for your withdrawal if you do not wish to do so. Withdrawal from the study will not affect the services you are otherwise entitled to in this health facility or other health facilities.

**Name of the Investigator:**

**Signature** **Date**

**Subject's statement:**

This research study has been explained to me. I agree to take part in this study. I have had a chance to ask questions. If I have more questions, I can ask the investigator.

**Name of the Subject:**

**Signature:** **Date:**

**Name of parent or legal guardian:**

**Signature:** **Date:**

**APPENDIX II D**: IDHINI YA KUSHIRIKI KATIKA UTAFITI, KWA WATOTO WENYE UMRI WA ZAIDI YA MIAKA 6 NA CHINI YA MIAKA 18

**UTAFITI JUU YA**: Idadi na aina za magonjwa ya makosa ya kuzaliwa ya kimetaboliki (inborn errors of metabolism), kati ya wagonjwa wa usonji (autism)

**Jina la mtafiti:** Daktari Mercy mapenzi Mwakubo
Mimi ni mwanafunzi wa Uzamili katika Chuo Kikuu cha Nairobi; ninayesomea shahada ya afya na magonjwa ya watoto.
Ili kuhitimu shahada hii, ninafanya utafiti kusuhu Idadi na Aina za Magonjwa ya Makosa ya Kuzaliwa ya Kimetaboliki (Inborn Errors of Metabolism), kati ya Wagonjwa wa Usonji (Autism), Katika spitali kuu ya Kitaifa ya Kenyatta.
Utafiti huu unafanyika kwa idhini ya Kamati ya Maadili na Utafiti, ya Spitali ya kitaifa ya Kenyatta na Chuo Kikuu cha Nairobi.

**Kusudi la Utafiti huu:**
Ushiriki wako katika utafiti huu utasaidia kudhibitisha kama uko na ugonjwa wa Makosa ya Kuzaliwa ya Kimetaboliki (Inborn Errors of Metabolism), ambao umesababisha hali ya Usonji (Autism).
Ukipatikana na ugonjwa wa Makosa ya Kuzaliwa ya Kimetaboliki, utapokea huduma ya matibabu yapasayo, katika Cliniki ya Endocrinolojia (Endocrinology Clinic), ya Spitali kuu ya Kitaifa ya Kenyatta.
Matokeo ya utafiti huu, yatasaidia kueka mikakati ya vipimo vya kuchunguza magonjwa ya makosa ya Kuzaliwa ya Kimetaboliki (Inborn Errors of Metabolism), kati ya wagonjwa wenye maradhi ya Usonji.
**Tafadhali maelezo yafuatayo niya muhimu:**

1. Ushiriki katika uchunguzi huu, ni kwa hiari yako.
2. Nitaitunza siri yako. Maelezo ya mtoto wako, na yako, yatahifadhiwa katika mashine ya kompyuta iliyo na neno la siri. Makartasi yenye maelezo hayo, yatahifadhiwa katika kabati ya siri.
3. Kukataa kushiriki katika utafiti hakutavutia adhabu yoyote. Mtoto wako ataendelea kupokea matibau yanayo stahili.
4. Hakuna hatari inayotarajiwa kwa kushiriki katika utafiti huu.

5. Faida kwako kupitia kushiriki katika utafiti huu:
   Kupata Huduma ya Uchunguzi wa magonjwa ya Makosa ya Kuzaliwa ya Kimetaboliki, bila malipo yoyote.
   Iwapo utapatikana na ugonjwa wa Makosa ya Kuzaliwa ya Kimetaboliki, utapokea huduma ya matibabu yapasayo, katika Cliniki ya Endocrinolojia (Endocrinology Clinic), ya Spitali kuu ya Kitaifa ya Kenyatta.
   Kupewa mawaidha yafaayo kuhusu magonjwa ya maumbile kwa familia nzima.
6. Hakuna fidia ya fedha kwa ajili ya kushiriki katika utafiti huu.
7. Uko na uhuru wa kukataa kuhusishwa katika utafiti huu wakati wowote kupitia. Na una haki ya kujiondoa wakati wowote.

**Utaratibu**:

Iwapo utakubali kushiriki kwenye utafiti huu, nita yaandika maelezo ya kukutambulisha wewe na mwanao. Maelezo yako, na yamtoto wako yatahifadhiwa vyema, na kwa siri.

Kabla ya kuanza utafiti, Daktari wa Edocrinologia Daktari Lucy Mungai; pamoja na mtafiti mkuu, watakupa ushauri maalum. Kupitia ushauri huu, tutakupa maelezo kuhusu

- Kusudi na umuhimu wa utafitu huu
- Hali zinavyoweza kusababisha ugonjwa wa usonji (autism), ikiwemo magonjwa ya makosa ya kuzaliwa ya kimetaboliki; jinsi magonjwa haya yanavyo rithiwa katika familia. Jinsi ya kuzuia urithi wa magonjwa haya, ikiwepo pamoja na kuzuia ndoa za watu wa ukoo mmoja.
- Taratibu ya utafiti huu, ikiwemo kuchukua historia ya ugonjwa , kufanya uchunguzi wa mwili, na kutoa sampuli za damu.
- Usafirishaji wa sampuli za damu hadi maabara ya CENTOGENE, ujerumani kwa uchunguzi wa magonjwa ya makosa ya kuzaliwa ya kimetaboliki. Kwamba sampuli itakayobakia baada ya vipimo hivi, itahifadhiwa na maabara, hadi wakati mtafiti mkuu atapata nakala ya majibu na kuyakuthibitisha. Kwamba baadaye, nakala za sampuli hizi zitateketezwa.
- Umuhimu wa kufanyiwa uchunguzi wa jeni katika kuchunguza magonjwa ya makosa ya kuzaliwa ya kimetaboliki.
- Jinsi tutakavyo wasiliana nawe wakati tutakapo pokea majibu ya kipimo hiki, ili kukujulisha majibu. Jinsi iwapo utupatika kua na magonjwa ya makosa ya kuzaliwa ya kimetaboliki, ita ashiria katika familia yako.
- Namna utaweza kupata matibabu yafaayo, japo utakau na ugonjwa wa makosa ya kuzaliwa ya kimetaboliki, kupitia kliniki ya endocrinologia ya spitali kuu ya Kenyatta

Halafu Nitaendelea kuuliza maswali kuhusu dalili za ugonjwa wa mwanao. Na pia, maelezo kuhusu familia yenu. Alafu nitafanya uchunguzi wa kimwili, kwa mwanao. Maelezo haya nitayajaza katika fomu zetu za utafiti.

Alafu utatolewa damu chache, ambayo itahifadhiwa kwenye kartasi maalum. Sampuli hizi za damu zitatumwa kwenya maabara, nchini Ujerumani ili zifanyiwe vipimo maalum.

Sampuli ya damu itakayo baki, baada ya kufanyiwa vipimo, itahifadhiwa na maabara hiyo, hadi wakati mtafiti mkuu atakapo pokea na kuthibitisha majibu ya kipimo. Kisha kartasi ya sampuli ya damu ita teketezwa na maabara

Tuanatarajia kupata majibu ya vipimo hivi, baada ya wiki mbili au mwezi mmoja. Nitakujulisha matokeo ya vipimo, ambayo pia yatahifadhiwa kwa siri.

Kusudi kuu la kukupa habari hizi ni kukuwezesha kuamua iwapo utashiriki kwenye utafiti huu. Iwapo unakubali kushiriki, nitakuomba uweke sahihi kwenye fomu ya kibali.

**Athari za Kushiriki katika utafiti:**

Sindano tunayotumia kuchukua damu inaweza kuumiza. Unaweza kupata mchibuko juu ya ngozi. Wakati mwingine unaweza kuendeleza uvimbe kwenye tovuti pia. Ikiwa hii itatokea, tafadhali piga simu ili tupate kukusaidia.

Una uhuru wa kukataa kujibu maswali ambayo hupo radhi nayo. Tutahifadhi maelezo yako kwa siri.

Ukiwa na maswali zaidi au unahitaji maelezo zaidi kuhusu ushiriki katika utafiti, tafadhali piga simu au tuma ujumbe kwa Daktari Mercy Mapenzi Mwakubo, Idara ya Maabara ya Afya na Afya ya Watoto, Chuo Kikuu cha Nairobi. Nambari ya Simu ya Simu 0726026685. Barua pepe mercymwakubo@gmail.com. Au kwa wasimamizi wa Utafiti: Daktari. Lucy Mungai, Nambari ya simu; 0724654135; Barua pepe dr.lmungai@gmail.com. Daktari Beatrice Mutai, Nambari ya simu:0708552909 ; Barua pepe Email mutaibc@gmail.com. Daktari Josephine Omondi, mobile; numbari ya simu 0737562973; Barua pepe, josephinekumba@gmail.com

Kwa habari zaidi kuhusu haki zako kama mshiriki wa utafiti, unaweza kuwasiliana na katibu au mwenyekiti, Hospitali ya Taifa ya Kenyatta - Chuo Kikuu cha Nairobi na kamati ya utafiti no.2726300 Ext. 44102. Barua pepe uonbi.ac.ke.

**Taarifa ya Somo:**

Utafiti huu umeelezewa kwangu. Nakubali kushiriki katika utafiti huu. Nimekuwa na nafasi ya kuuliza maswali. Ikiwa nina maswali zaidi, ninaweza kumuuliza daktari.

**Jina la mshiriki:** **Sahihi:** **Tarehe:**

**Jina la mzazi au mlezi wa kisheria:**

**Sahihi:** **Tarehe:**

**KAULI YA MTAFITI**

Mimi kama mchunguzi, ninaye weka sahihi idhini hii, nimempa mshiriki huyu maelezo yote muhimu kuhusu utafiti huu. Natumai kuwa ameelewa na kukubali kushirikishwa katika utafiti huu, kwa hiari.

**Jina la mtafiti:** **Sahihi:** **Tarehe:**

**Appendix III: DATA COLLECTION TOOL/ QUESTIONNAIRE:**

**Serial Number:** **Code Number:** **Date of Interview**:

**PART 1: DEMOGRAPHIC DATA:**

1. Age: year(s) month(s)
2. Gender: Male[ ] Female[ ]
3. Area of residence/County

**PART 2: CLINICAL DATA/CLINICAL FEATURES**

**A. Evaluate the participant for the presence of the following symptoms of Autism spectrum disorder.**

4. Does the patient have deficits in communication? Yes or NO
   If yes, briefly describe:
   State duration of these symptoms:
5. Does the patient have deficits in social interaction?
   If yes, briefly describe
   State duration of these symptoms
6. Does the participant exhibit Restricted interest and activities?
   If yes, briefly describe:
   State duration of these symptoms
7. Does the participant exhibit repetitive behavior, interest, and activities

**B. Assessment for Severity of autism:**

8. Is the participant able to carry out different social or occupational activities, at home, school, or work without help?

If not, briefly describe the type and level of the help:

**PART 3: *GROWTH AND DEVELOPMENT***

| **Parameter** | **Value** | **Normal, Below, or Above expected** |
|---|---|---|
| Weight | | |
| Height | | |
| Body mass index | | |
| Mid upper arm circumference | | |
| Head Circumference | | |
| Milestones attained | | |

**PART 4: EXAMINATION FINDINGS**

**A. General examination:**

9. Does the participant have any dysmorphic features:

   If yes, briefly describe those found

**B. Systemic examination;**

**10.** Describe any abnormality noted on systemic examination

Central Nervous system:

Respiratory system:

Cardiovascular system:

Gastrointestinal system:

Genitourinary system:

Musculoskeletal system:

**Part 5: Genetic Analysis Results**

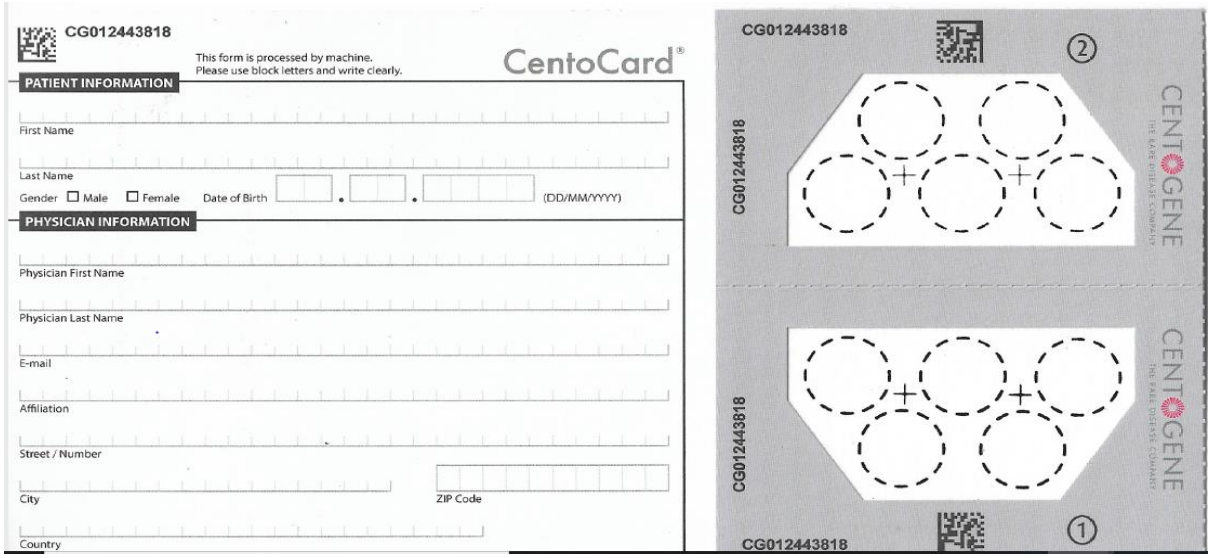
Date sample was taken:

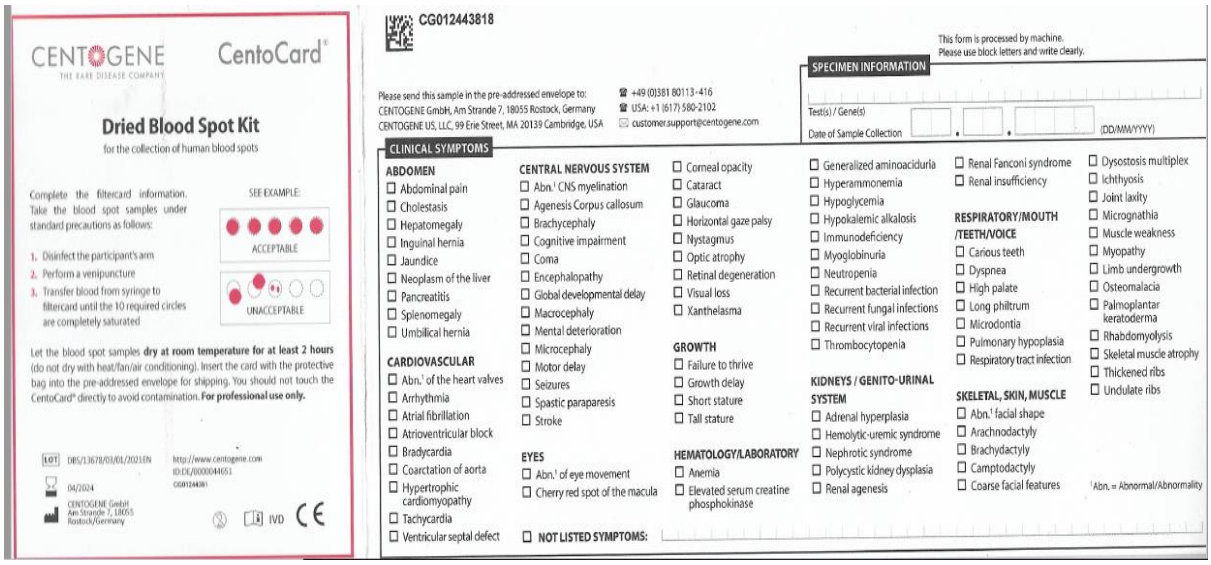
**Results**

| Mutation | Enzyme deficiency | Diagnosis (IEM) |
|---|---|---|
| | | |

**Interviewers Name:** **Sign**: **Date**:

## APPENDIX IV: Sample of a filter paper



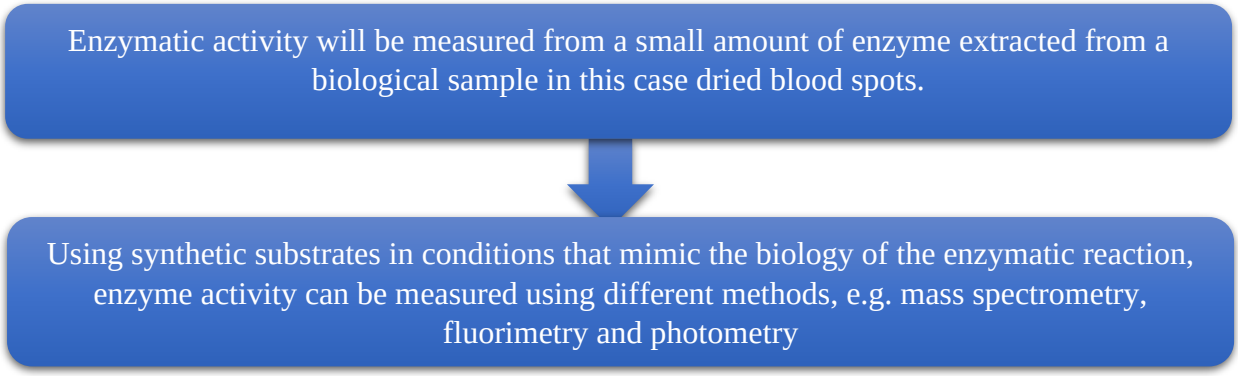



**APPENDIX V: Blood sample collection onto a filter paper**

1. Enter unique coded participant's particulars and clinical information onto the requisition form attached to the filter paper.
2. Disinfect the participant's finger.
3. Prick the chosen finger using a sterile needle, draw about 1ml of the participant's blood, into a sterile container.
4. Allow 1 drop of blood onto the circle. Ensure the entire circle is uniformly saturated.
5. Continue the procedure until full saturation of the 10 required blood spots. After that air-dry the card for at least two hours. The samples should be sent to CENTOGENE within two weeks.
6. Once the sample is spotted on the filter card and it is dried, it is mandatory to store the dry filter paper sealed in the plastic bag at room temperature (+18°C to +28°C). Accepted ranges: -10°C to +30°C; no direct sunlight. Out of these ranges, the enzymes may be damaged.

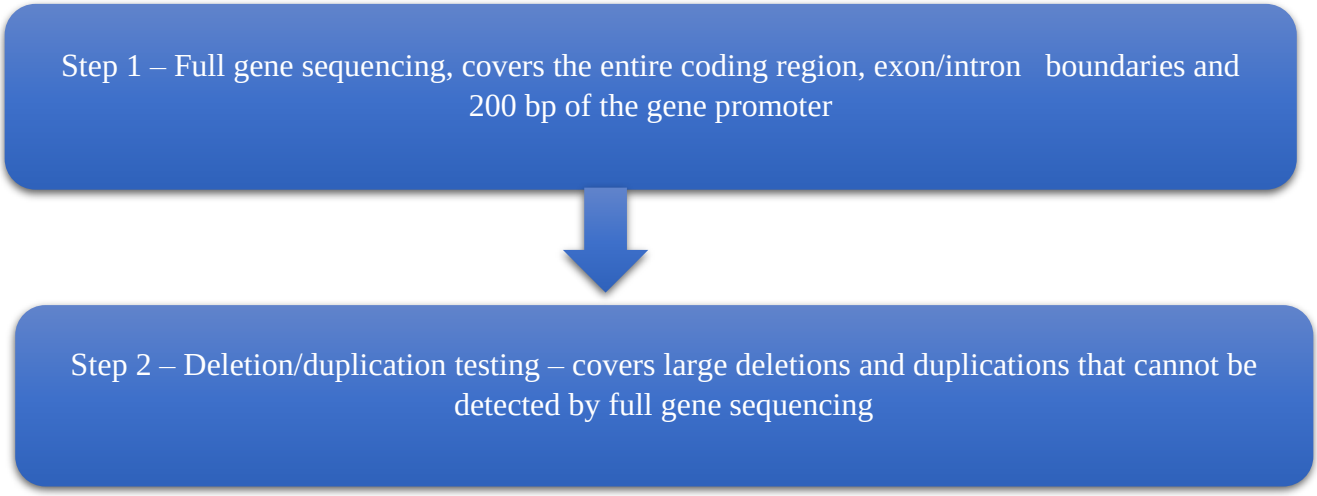
**Appendix V: Testing algorithms for IEM**

**Enzymatic activity testing method:**




**Genetic testing**

Genetic testing is completed by fully sequencing the specific gene associated with IEM, followed by deletion/duplication testing and analysis.




- Based on the screening outcome, Principal Investigator will be issued with one of the following reports:
  −Normal; a biochemistry report
  −Pathogenic; a biochemistry and genetic analysis report
- CENTOGENE will keep all information generated and the unused sample for at least twenty years.
- Medical confidentiality will be observed in the participant's generated results and the unused samples.

- At any time, the participant can request a stoppage of processing of results and destruction of samples without victimization.

## APPENDIX VII: CERTIFICATE OF ACCREDITATION 1

CENTERS FOR MEDICARE & MEDICAID SERVICES
CLINICAL LABORATORY IMPROVEMENT AMENDMENTS
*CERTIFICATE OF ACCREDITATION*

**LABORATORY NAME AND ADDRESS**
CENTOGENE GMBH ROSTOCK
AG AM STRANDE 7
ROSTOCK 18055
GERMANY

**CLIA ID NUMBER**
99D2049715

**EFFECTIVE DATE**
04/03/2021

**LABORATORY DIRECTOR**
PETER K BAUER M.D.

**EXPIRATION DATE**
04/02/2023

Pursuant to Section 353 of the Public Health Services Act (42 U.S.C. 263a) as revised by the Clinical Laboratory Improvement Amendments (CLIA), the above named laboratory located at the address shown hereon (and other approved locations) may accept human specimens for the purposes of performing laboratory examinations or procedures.
This certificate shall be valid until the expiration date above, but is subject to revocation, suspension, limitation, or other sanctions for violation of the Act or the regulations promulgated thereunder.

Monique Spruill
Monique Spruill, Director
Division of Clinical Laboratory Improvement & Quality
Quality & Safety Oversight Group
Center for Clinical Standards and Quality

302 certs2_030921

If you currently hold a Certificate of Compliance or Certificate of Accreditation, below is a list of the laboratory specialties/subspecialties you are certified to perform and their effective date:

| LAB CERTIFICATION (CODE) | EFFECTIVE DATE |
|---|---|
| VIROLOGY (140) | 05/18/2020 |
| ROUTINE CHEMISTRY (310) | 04/03/2013 |
| HEMATOLOGY (400) | 04/03/2013 |
| CYTOGENETICS (900) | 04/03/2013 |

| LAB CERTIFICATION (CODE) | EFFECTIVE DATE |
|---|---|

FOR MORE INFORMATION ABOUT CLIA, VISIT OUR WEBSITE AT WWW.CMS.GOV/CLIA
OR CONTACT YOUR LOCAL STATE AGENCY. PLEASE SEE THE REVERSE FOR
YOUR STATE AGENCY'S ADDRESS AND PHONE NUMBER.
PLEASE CONTACT YOUR STATE AGENCY FOR ANY CHANGES TO YOUR CURRENT CERTIFICATE.

## APPENDIX VIII: CERTIFICATE OF ACCREDITATION 2

COLLEGE of AMERICAN PATHOLOGISTS

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

The College of American Pathologists
certifies that the laboratory named below

**Centogene**
**Centogene AG**
**Rostock, Germany**
**Arndt Rolfs, MD, PhD**

CAP Number: 8574447
AU-ID: 2011867

has met all applicable standards for accreditation and is hereby accredited by the College of American Pathologists' Biorepository Accreditation Program. Reinspection should occur prior to December 2, 2021 to maintain accreditation.

Accreditation does not automatically survive a change in director, ownership, or location and assumes that all interim requirements are met.

Chair, Accreditation Committee

President, College of American Pathologists

## APPENDIX IX: CERTIFICATE OF ACCREDITATION

COLLEGE of AMERICAN PATHOLOGISTS

ISO 15189 CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

The College of American Pathologists accredits

**Centogene AG**
**Rostock, Germany**

in accordance with the recognized International Standard ISO15189:2012, Medical Laboratories – Requirements for quality and competence. This accreditation demonstrates competence for a defined scope and the operation of a laboratory quality management system.

Effective August 6, 2018

Expires August 6, 2021

Frank Schneider, MD, FCAP
Chair, CAP 15189 Committee

R. Bruce Williams, MD, FCAP
CAP President

Centogene AG
CAP #8005167

The scope of this accreditation includes Quality Management Systems and the disciplines of Clinical Biochemical Genetics and Molecular Pathology.

ISO 15189 CAP ACCREDITED

**APPENDIX X: BUDGET**

| Item | Unit | Unit Cost | Amount(Kshs) |
|---|---|---|---|
| **Research Assistants**<br>-To be retained for 12 weeks of study | 2 | 10,000 for each assistant | 20,000 |
| **Transport for participants to KNH** | 78 | 1000 | 78,000 |
| **Specimen collection**<br>-Specimen bottles, Syringes, Needles, Surgical spirit, cotton wool | For 78 study participants | Estimate | 3,000 |
| **Shipping of samples to Germany**<br>-For the genetic testing | 5.2 | 3500 | 18, 200<br>-Shipping cost is charged at about 3500 per 1 kg of sample filter papers.<br>-About 15 filter papers add up to 1kg. Thus 78 filter papers for 78 participants make 5.2 units. |
| **Cost of genetic testing** | For 78 study participants | | CENTOGENE laboratory will carry out the genetic tests for free |
| **Statistician** | 1 | 30, 000 | 30,000 |
| **Printing and Photocopying services for**<br>-proposal write up printing and copies<br>-printing questionnaires<br>-printing thesis | | | 20, 000 |
| **ERC Fee** | 1 | 2000 | 2,000 |
| **Airtime for**<br>-calling supervisors, research assistants, study participants | | | 5,000 |
| **Publication/Dissemination of results** | | | 20,000 |

| | | | |
|---|---|---|---|
| **Contingency fund** | | | 30,000 |
| **TOTAL** | | | 226, 200 |

**APPENDIX XI: TIME FRAME FOR STUDY ACTIVITIES**

| **Activity** | **Estimated time** |
|---|---|
| **Development of Proposal and presentation** | January to March 2021 |
| **Proposal Submission for Ethical review, Internal Marking, Corrections, and Approval** | May to October 2021 |
| **Data Collection** | November 2021 to January 2022 |
| **Data Analysis** | February 2022 |
| **Thesis Writing** | March 2022 |
| **Thesis Submission** | April 2022 |

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
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KNH-UON ERC
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Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
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Ref: KNH-ERC/A/339

27th September, 2021

Dr. Mercy Mapenzi Mwakubo
Reg. No.H58/34485/2019
Dept. of Paediatrics and Child Health
School of Medicine
College of Health Sciences
University of Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 27 SEP 2021 QUALITY HEALTH CARE KNH/UoN-ERC P.O. Box 20723 - 00202, NAIROBI

Dear Dr. Mwakubo

**RESEARCH PROPOSAL: MAGNITUDE AND TYPES OF INBORN ERRORS OF METABOLISM AMONG CHILDREN AND ADOLESCENTS WITH AUTISM SPECTRUM DISORDER AT KENYATTA NATIONAL HOSPITAL (P348/05/2021)**

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 27th September 2021 – 26th September 2022.

This approval is subject to compliance with the following requirements:

viii. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
ix. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
x. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
xi. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
xii. Clearance for export of biological specimens must be obtained from KNH- UoNERC for each batch of shipment.
xiii. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (Attach a comprehensive progress report to support the renewal).
xiv. Submission of an executive summary report within 90 days upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

**PROF. M.L CHINDIA**
**SECRETARY, KNH- UoN ERC**

c.c. The Principal, College of Health Sciences, UoN
The Senior Director, CS, KNH
The Chair, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UoN
The Chair, Dept.of Paediatrics and Child Health, UoN
Supervisors: Dr. Lucy Mungai, Dept.of Paediatrics and Child Health, UoN
Dr. Beatrice Mutai, Dept.of Paediatrics and Child Health, UoN
Dr. Josephine Omondi(Consultant Child and Adolescent Psychiatrist), KNH