Confirmation of a Rare Carbohydrate Metabolic Disorder through Next Generation Sequencing Technology

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Philosophy in Biotechnology

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In the Name of Allah, the Most Blessed, the Most Merciful.

“If Allah should aid you, no one can overcome you; but if He should forsake you, who is there that can aid you after Him? And upon Allah let the believers rely” [Quran 3:160]

Declaration of Originality

I declare that the work accomplished in this thesis is my research effort carried out in the Medical Genetics Research Laboratory, Department of Biotechnology, Quaid-i-Azam University Islamabad, written and composed by me. This thesis has not been published previously nor contains any material from the published resources that can be considered a violation of international copyright law. I also declare that I know the terms „œopyrights and "plagiarism". I will be responsible for the consequences of any violation of these rules (if any) found in this thesis. The thesis has been checked for plagiarism by Turnitin software.

Signature: _____

Ubaid Ur Rehman

Dated:

Dedication

This dissertation is dedicated specially to my brother **Fawad Khan**

and to my **Mother** and **Father.**

Having you people in my life gives me the wings to fly higher than eagle

and day and night prayers enable me to complete this work.

Thank you for being my best supporters.

Acknowledgment

All praise to Allah, the most Merciful and Sustainer of the worlds, who granted me the ability to acquire knowledge from his unseen infinite treasures. I dedicate this work to our Prophet **Muhammad** (Peace Be Upon Him), the last prophet. Without him, the world would be incomplete, He (S.A.W) had brought the light of knowledge from the darkness of illiteracy.

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I want to extend my gratitude to my brother **Fawad Khan**. For the sacrifices, you have made for me to pursue my MPhil degree. I know no matter, what life throws at me, you will always have my back. You have given me the most precious life lessons, and I owe all that I gained to you. I am fortunate to have a brother with a large heart and lots of love.

Special thanks to my friends who has always been there to share a laugh, smiles, and thoughts, especially cracking jokes when things get too serious. Their unconditional participation and emotional support have proved to be a gift. A special thanks to my beloved father, who always stands by my side and wants to see me shine brighter, and my dear mother, who continually prays for my success. It is due to my parents and my loved ones that I am pursuing my educational career successfully, and I owe their prayers and support for my success in every field of life. May Allah shower his countless blessings on my family.

Ubaid Ur Rehman

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LIST OF ABBREVIATIONS

%	Percentage
µl	Microliter
µg	Microgram
µM	MicroMolar
$^{\circ}$C	Degree Celsius
Bp	Base pair
C	Cytosine
CNS	Central nervous system
DNA	Deoxy ribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
G	Guanine
HSP	Hereditary Spastic Paraplegia
mA	Milliampere
MEDOC	Mendelian disorder of cornification
Mg	Milligram
mM	Millimolar
NaCl	Sodium chloride
Ng	Nano gram
nM	Nano mole
OMIM	Online Mendelian inheritance in man
P	Short arm of chromosome
PNS	Peripheral nervous system
PCR	Polymerase chain reaction
Q	Long arm of chromosome
QAU	Quaid I azam university
RPM	Revolution per minute
RXLI	Recessive X-linked ichthyosis
Sec	Second
SNP	Single nucleotide polymorphism
T	Thymine

Taq	Thermus aquaticus
TBE	Tris borate EDTA
TE	Tris EDTA
FBS	Fanconi Bickel Syndrome
SLC2A2	Solute Carrier Family 2 Member 2
NGS	Next Generation Sequencing
GLUT2	Glucose 2 Transporter
ATP	Adenosine Triphosphate
IMD	Inherited metabolic disorder
PKU	Phenylketonuria
GSD	Glycogen Storage Diseases
NIDDM	Non-insulin-dependent diabetes mellitus
HGMD	Human genome mutation database
G6P	Glucose-6-phosphate
GWAS	Genome-wide association studies
MAF	Minor Allele Frequency
SER	Serine
PRO	Proline

Abstract

Fanconi Bickel Syndrome is a rare carbohydrate metabolic disease which is characterized by hepatomegaly, fasting hypglycemia, excessive proximal tubular dysfunction, rickets, growth retardation and excessive urination (polyuria). Fanconi Bickel Syndrome is also termed as glycogen storage disorder type XI. Abnormal glycogen aggregation in the liver and kidneys, which affects the body use of galactose and glucose and results in proximal renal tubular failure, is the first cause of FBS. The disease is caused by the mutation in the *SLC2A2* gene, which is located on chromosome 3 and contains total 11 exon. The candidate gene encodes for the facultative glucose transporter 2 (GLUT2) and its pattern of inheritance is an autosomal recessive. The encoded protein facilitates glucose transport in both directions and has been known for glucose sensor. The encoded gene is mainly expresses in the liver and kidney. The aim of our study was to diagnose the disorder having an autosomal recessive pattern. Whole exome sequencing was performed to analyze the genomic DNA of the affected individual having disease causing pathogenic mutation. The exome data were then analyzed and different filters were used to find out the variant which are responsible for the disorder. A novel variant c.298T>C (p. Ser100Pro) was detected in the *SLC2A2* gene of the proband in our targeted family. The different insilico tools were applied on the detected variant and these showed that the variant might be pathogenic and likely disease causing, so it might be the exact cause of the metabolic disorder. Moreover, protein expression studies are further recommended to better recognize the expression, structure and functional aspects of the novel variant. This proband family will be counseled on the basis of this result and the instruction will be provided to them for future planning. Furthermore, our study will expand the understanding of mutation spectrum and etiology of this disorder.

Chapter 1

1) INTRODUCTION

1.1) Metabolism

According to this definition, the main focus of biochemistry which is defined as "the discipline of science concerned with the chemical and physico-chemical processes and substances which occur within living beings" is metabolism [1]. As a result, it is obvious how metabolism and biochemistry are related. One theoretical difference is that metabolism refers to the body's interconversion of metabolites as well as the changing of nutrients into metabolites, although metabolite research is the focus of biochemistry. The physical significance is that metabolism is fundamentally the study of molecular change, incorporating energetics and biological chemistry into a setting that is inevitably governed by physiology. According to the definition, metabolism is a process heavily focused on producing energy through catabolism and manufacturing cellular and extracellular components anabolicaly [2]. For the body to grow, develop, and function properly, metabolism is a critical component. It has been divided into two categories, Catabolism and Anabolism, based on how they function. The body's use of the molecules differs significantly between catabolism and anabolism. Utilizing energy, anabolism produces the chemicals that the body needs to function. the body can use the energy that is released through catabolism, which breaks down complex molecules.

Catabolism is the breakdown of substances (for instance, the conversion of glucose to pyruvate through cellular respiration) while anabolism is the synthesis of substances (such as nucleic acid, lipids, carbohydrates, and protiens). Energy is often released by catabolism while being consumed by anabolism [3]. The fundamental interaction between organisms and their environment is represented by metabolism, which allows them to defy the thermodynamic requirement to "feed upon negative entropy."

1.1.1) Catabolism

The term catabolism refer to the collection of pathways which are associated in the breakdown of large molecules into small molecules or also known as monomer.

A different method of obtaining useful energy is the simple breakdown of a small number of these molecules into waste products. Catabolic processes include, among others:

- Cycle of citric acid
- Lipolysis
- Glycolysis
- Breakdown of muscle tissue
- Deamination by oxidation

1.1.2) Anabolism

The series of enzyme catalyzed based processes that make use of nutrients to make complex molecules from simple molecules (monomers) in the living cell. These anabolic processes also known as biosynthesis. The process involves the formation of cellular building blocks such carbohydrates, protiens and lipids, which required energy in the shape of energy-rich molecules called ATP (adenosine triphosphate). These chemicals are created during breakdown processes like catabolism. In growing cells, anabolic mechanisms control catabolic reactions. Both are in equilibrium in cells that are not developing.

1.1.3) Metabolic pathways

Metabolic pathways are used to arrange the chemical processes of metabolism. These enable the basic molecules obtained from food to be changed into a different chemical through a number of processes by a succession of enzymes [4]. Because they enable organisms to carry out favoured energy-demanding reactions, enzymes are essential to metabolism. Alongside those that release energy, there are also responses like these. Enzymes help these processes happen rapidly and effectively by acting as catalysts. Using enzymes, metabolic pathways can also be controlled in response to alterations in the cell's environment or signals from neighbouring cells [5].

The majority of metabolic transformations, including both synthesis and degradation, are carried out by a succession of enzyme-catalyzed reactions that are organised in a certain order. A precursor or beginning chemical is transformed into a specific end product through a chain of reactions known as a metabolic pathway.

The first substrate is frequently changed by an enzyme into a product that serves as a substrate for a different enzyme in a subsequent process. This process continues in a linear chain of reactions until a finished product is generated [6].

To maintain the appropriate operation of the organism as a whole, each cell and tissue's various metabolic pathways must be properly coordinated. Because the availability and demand for a specific molecule fluctuate over time, the flow of metabolites through catabolic or anabolic pathways must be continuously adjusted to fulfil the needs and challenges that a cell or organism must face. An organism's capacity to control metabolic processes and maintain the consistency of the intracellular and extracellular media within specific ranges required to carry out essential tasks is crucial to its ability to survive [7].

1.2) Inherited metabolic disorders

Although they are uncommon on their own, inborn metabolic mistakes are common overall [8]. Garrod first identified in 1900s the inborn disease of metabolism. These disorders were caused by a blockage in a pathway of metabolism brought on by a lack of an enzyme, which in turn directly disrupted cellular metabolism [9, 10]. Inherited metabolic disorders (IMDs) are a complex and diversified class of diseases that manifest clinical symptoms as a result of a genetic mistake that causes an enzyme in a specific intermediate pathway of metabolism to function less or insufficiently [11, 12]. There are over one hundred inborn metabolic abnormalities that might manifest during the neonatal era, but less than twenty of them are treatable. Individually rare but frequently occurring inborn metabolic errors [8].

IMDs have serious clinical repercussions, Consequently, they are a major contributor to morbidity and mortality in clinical practise, particularly in paediatrics. A number of negative effects, for instance, mild to severe cognitive impairment, death and mental retardation result from delayed investigation and treatment of these illnesses. Although each condition is uncommon on its own, the total frequency of these disorders is substantial, between 1 in 1500 and 1 in 5000 live births [13, 14]. IMDs exist in every community, but because they were not acknowledged and documented, particularly in many Asian countries, they are thought to be quite uncommon.

IMD prevalence has often been underestimated, with the exception of areas where extensive population screening has been done. There is a wide range in the prevalence of certain

illnesses across different groups. For instance, phenylketonuria (PKU) has been shown to occur 1 in 5000 times more frequently in Dublin than in Japan (1 in 200,000) [15, 16]. Between illnesses and populations, clinical severity varies greatly. IMDs caused by problems with purines, pyrimidines, organic acids, carbohydrates, or the urea cycle are the ones that are more likely to manifest in newborns and young children [17, 18]. In order to prevent serious morbidity or death, newborn screening is an important technique that identifies infants who appear healthy but actually have substantial hereditary abnormalities, typically of a metabolic nature, that can usually be treated with dietary or pharmaceutical interventions [19]. IMDs are further divided into the three major categories listed below.

1.2.1) Group 1 (Illnesses that lead to intoxication)

This group consist of inherited disorders of intermediate metabolism that result in an severe or accumulation of intoxication caused by the accumulation of dangerous substances nearby the metabolic block. This category encompasses the bulk of organic acidaemias, such as propionic, methylmalonic, and isovaleric acidaemias, as well as aminoacidopathies and sugar intolerances such maple syrup urine sickness and type I tyrosinaemia (galactosaemia, hereditary fructose intolerance). Clinically speaking, all of these illnesses share the same pattern of a period of no symptoms followed by indication of "intoxication," such as nausea, lethargy, coma, liver failure, etc. Amino or organic acid chromatography in plasma and urine are the mainstays of biological diagnostics, which is simple [8, 20].

1.2.2) Group 2 (Disorders affecting the metabolism of energy)

It consists of inherited abnormalities of metabolism with manifestation brought on, at least in parts, by a deficit in the generation or consumption of energy as a result of a problem with the heart, liver, brain or muscle. This category includes conditions that exhibit a predominance of hypoglycemia, such as glycogenosis, gluconeogenesis abnormalities, hyperinsulinism, congenital lactic acidemias and issues with fatty acid oxidation (disorders of the Krebs cycle, mitochondrial respiratory chain, pyruvate carboxylase, and pyruvate dehydrogenase deficits). Failure to thrive, severe hypoglycemia, hyperlactacidaemia, severe generalised hypotonia, myopathy, cardiomyopathy, heart failure, arrhythmias, conduction abnormalities, circulatory collapse, dysmorphia, and anomalies are among the symptoms this population frequently experiences [8, 21].

1.2.3) Group 3 (Complicated molecule-related disorders)

Includes conditions that interfere with the production or breakdown of complex compounds. Permanent, progressive, unrelated to concurrent events, and unrelated to dietary intake are the characteristics of symptoms.This group encompasses all lysosomal abnormalities, peroxisomal conditions, problems with intracellular movement and processing (such a lack of -1-antitrypsin, congenital glycosylation deficiencies (CDG syndrome), and genetic mistakes in cholesterol production). In an emergency, very few of these illnesses can be treated [8, 22].

1.3) Fanconi Bickel Syndrome

Hepatomegaly, postprandial hyperglycemia, severe proximal tubular dysfunction, rickets, and growth retardation are the hallmarks of Fanconi-Bickel syndrome, a disease of carbohydrate metabolism (FBS). Glycogen storage disorder type XI, which was first recognised by Guido Fanconi and Bickel in 1949, is another name for FBS [23]. Abnormal glycogen aggregation in the liver and kidneys, which affects the body's use of galactose and glucose and results in proximal renal tubular failure, is the primary cause of FBS [24]. The *SLC2A2* gene, located on chromosome 3, which codes for the facultative glucose transporter 2 (GLUT2), is autosomal recessively inherited and causes FBS [25]. Hepatocyte plasma membranes and pancreatic b cells also contain GLUT2 protein, in addition to absorptive epithelium cells of the gut and kidney [26, 27]. The encoded protein facilitates glucose transport in both directions and has been suggested as a glucose sensor [28]. Clinical evaluation of the symptoms, biochemical analysis, and confirmation by molecular testing all play a role in the diagnosis [29]. There is no proper treatment, but it include dietary modification to control glucose levels and limit galactose as well as compensating for electrolyte losses from the kidneys through supplementation with vitamin D and phosphorus [30, 31]. Although some patients pass away while still children, the outlook is generally good, and adult survival is conceivable [28]. Although the prevalence of FBS is not yet determined, it is thought to be highly rare given that there have only been less than 200 cases reported globally [32].

1.3.1) Historical aspect

Researcher named horst bickel and guido Fanconi originally reported a patient in 1949 who had a rare symptoms of widespread renal tubulopathy and glycogen buildup in hepatorenal as part of their research into the various clinical disorders linked to hyperaminoaciduria [23]. The clinical signs of this patient have remained into maturity, and they are still present. This sickness can be viewed as a part of the larger category of conditions known as the glycogen storage diseases (GSD). According to the order of their initial descriptions or the discovery of the underlying problem, several authors have assigned numbers to certain members of this group. Hug coined the designation "GSD XI" in 1976 to describe a patient who displayed some clinical symptoms resembling those of Fanconi and Bickel's patient [33]. Hug later concluded that it was plausible that the patient's underlying condition was caused by a congenital phosphoglucomutase deficiency. Many reviewers have historically categorised their works in this way [34].

A 9-year-old child was already documented in 1921 as having FBS symptoms before the first case of Fanconi and bickel syndrome has been officially reported. The first case of any GSD has likely never been reported before [35]. Her clinical features, including her serious hepatomegaly, the glucosuria level up to 45g d-1 per 1, 73 m2, and abnormally little height strongly point to FBS, despite the fact that the liver's glycogen level was not assessed. Gitzelmann reevaluated the initial case of Fanconi and Bickel in 1957 and made a new discovery of enhanced glucagon resistance [36]. Beginning in the 1960s, a number of published case reports have contributed to a clearer definition of the clinical presentation and pathophysiology of the illness. A girl with the age of 4.5 years having chemical and clinical signs of Fanconi bickel syndrome was described by Rotthauwe et al. in 1963 [37] she also demonstrated galactose intolerance, which was a novel finding. In this instance, the amount of liver glycogen per 100 g of wet weight increased to 11 g from the typical 7 g. Two independent cases with identical clinical and chemical findings were documented by Odievre in 1966 [31]. The effects of oral and intravenous galactose loading, he observed, were severe and protracted hypergalactosemia. He came to the conclusion that only the utilisation of glucose and galactose were hindered because, after oral and intravenous fructose doses, this sugar disappeared normally. This result was later validated by Odievre's group in test-tube tests using portions of liver and carbohydrates that were [1-14C]-labeled [38].

When Fellers et al. [39] discovered in 1967 that the removal of renal glucose was equivalent to or highest than the removal of renal inulin in their patient, they surmised that there may have been an unidentified renal transport problem. The phrase "renal glucose losing syndrome" was proposed by Brodehl et al. [40] to emphasise that glucosuria in these people is abnormally severe relative to other tubular functions. They use the word "pseudo-phlorizin diabetes" to emphasise a deficiency of renal glucose reabsorption. Hug [41] however, a follow-up investigation found that a typical FBS case showed normal phosphoglucomutase activity in red cells. This study's findings supported a phosphoglucomutase deficit and classed this shortage as glycogen storage disease type XI [42].

A sodium glucose secondary active transporter, which is in charge of transporting galactose and glucose in several organs, including the kidney and liver, is deficient in Fanconi-Bickel Syndrome (FBS) [24]. The plasma membrane glucose transporter is implicated in glucose sensing, uptake of glucose, and its possible role in non-insulin-dependent diabetes mellitus, according to research done in 1989 using Xenopus oocytes injected with synthetic human liver type glucose transporter mRNA construct [43]. It was discovered in Xenopus oocytes in 1994 that a highly conserved GLUT2 missense mutation (Val197 is swapped for Ile197) in one allele of the gene causes GLUT2 malfunction [44, 45]. This mutation might significantly increase NIDDM's susceptibility to infection (non-insulin dependent diabetes mellitus). Then, in 1997, Santer et al. (1997) discovered for the first time that three FBS-affected families had GLUT2 (*SLC2A2*) gene mutations. These families included the first case described by Fanconi and Bickel in 1949 [25, 46].

1.3.2) *SLC2A2* gene

On chromosome 3, the *SLC2A2* gene, which has 10 introns and 11 exons, is a member of the soluble carrier family 2 (fig.1.1) [47]. The liver, islet beta cells, gut, and kidney epithelium all contain an integral plasma membrane glycoprotein that is encoded by this gene. Facilitated bidirectional glucose transport is made possible by the encoded protein. It has been claimed that it could serve as a glucose sensor because of its low affinity for glucose [48]. Glucose Transporter 2 is another name for the gene (GLUT2). Fanconi-Bickel syndrome and non-insulin-dependent diabetic mellitus susceptibility are two conditions for which this gene is mutated (NIDDM). Multiple transcript variants of this gene are produced by alternative splicing [49, 50].

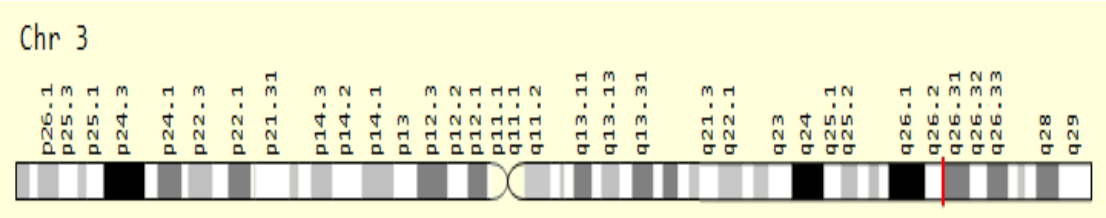

Figure.1.1. Localization of *SLC2A2* gene on chromosome 3 (The figure is taken from Genecard https://www.genecard.org)

1.3.2.1) *SLC2A2* gene expression

he gene *SLC2A2* (solute carrier family 2 member 2) is expressed in several body tissues. The kidney, pancreas, liver, and small intestine all exhibit high levels of the gene's expression (fig.1.2). It expresses itself extensively in the liver because it is the primary organ for storing glucose and because it is essential for maintaining glucose homeostasis. Although not as much as was initially stated, *SLC2A2* is also expressed in the brain, lung, spleen, liver, heart, and smooth muscle [51]. The kidney proximal tubule is where GLUT2 is present and have a main role in the urine glucose reabsorption into the blood circulation. Massive glucosuria and considerable mineral losses (calcium and phosphate), which are necessary for bone growth and strength, are also brought on by the kidney's failure to produce GLUT2 and result in bone abnormalities [52].

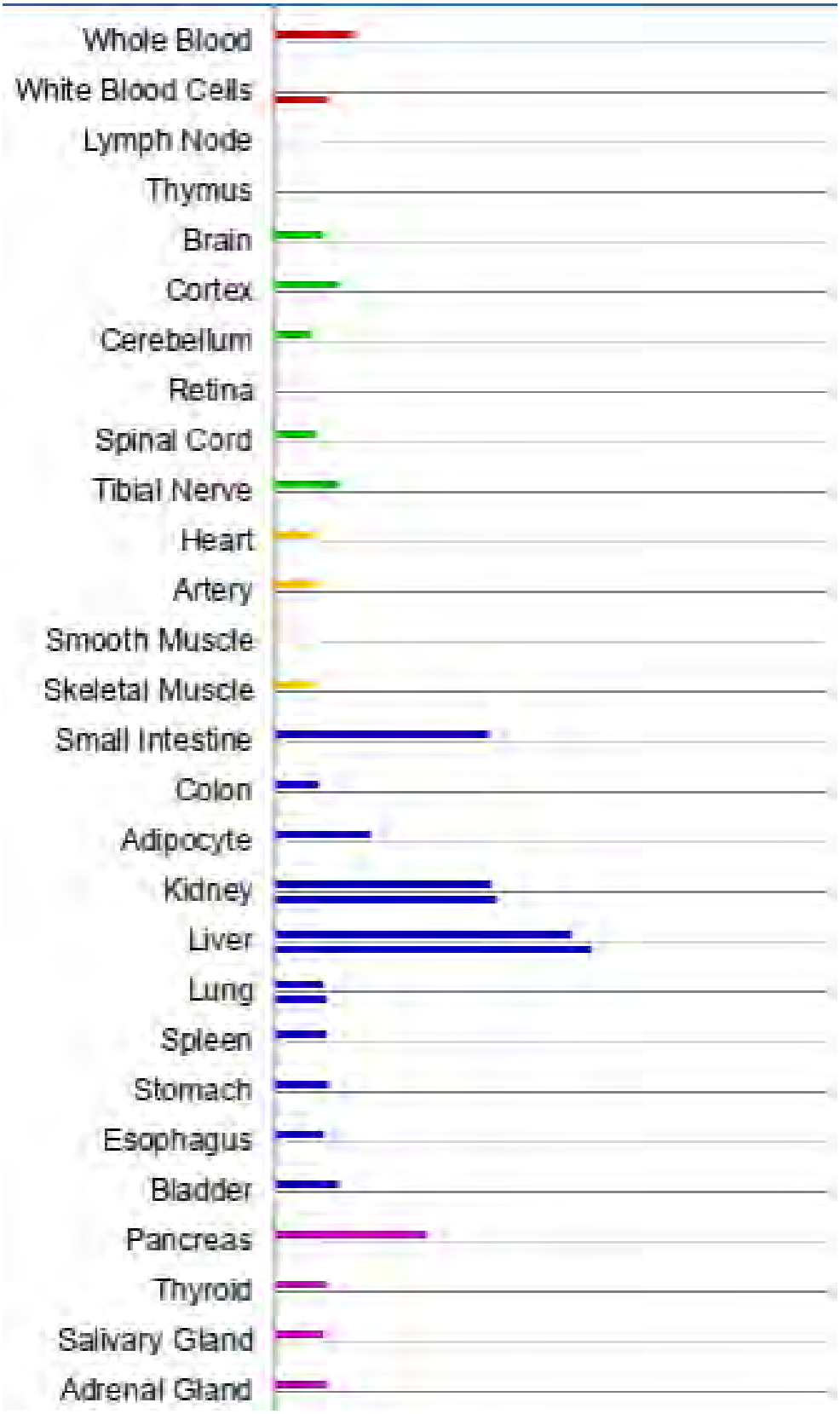

Figure.1.2. Expression of *SLC2A2* gene in different tissues of the body (The figure is taken from Genecard https://www.genecard.org)

1.3.3) Mutations in *SLC2A2*

According to the HGMD, 109 distinct variants have so far been found in the *SLC2A2* gene. There are 109 mutations in all, of which 80 are directly linked to the Fanconi-Bickel syndrome. The remaining different types of mutations are linked to other illnesses, including diabetes, non-insulin-dependent diabetes mellitus, osteogenesis imperfecta, myelomeningocele, and others. Nonsense, missense, in-frame deletion and insertion, frame-shift deletions and insertions, and splice site variations are examples of these. A total of 144 instances with 70 different types of mutations have been documented globally between 1987 and 2020 [46]. Nine of which are compound heterozygous mutations, 19 are missense, 10 are nosense, 21 are indel, and 13 are intronic (fig.1.3).

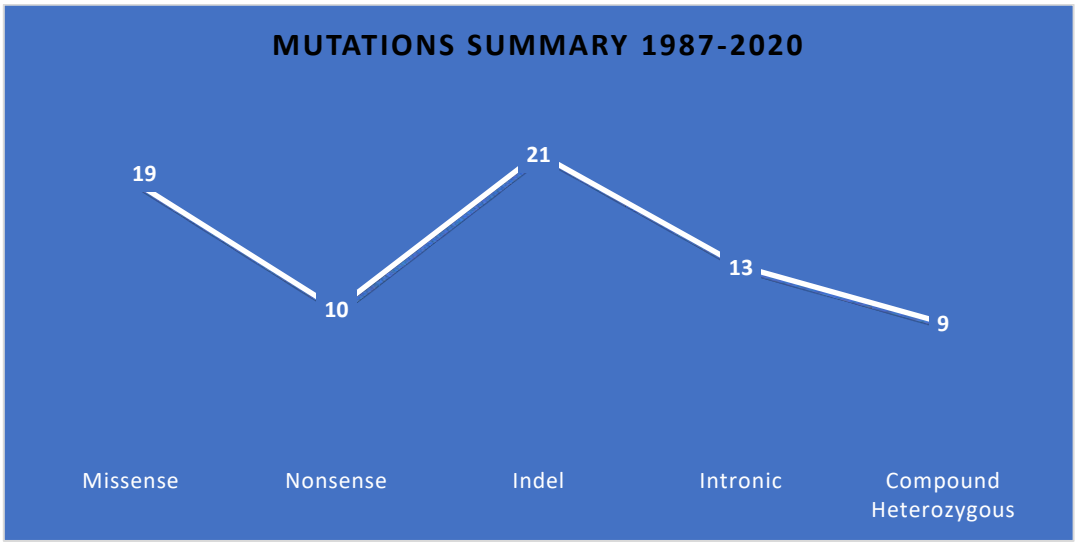

Figure.1.3. Various mutations reported between 1987 to 2020 [46].

As an autosomal recessive condition, an individual who is compound heterozygous for two harmful *SLC2A2* mutations or homozygous for both of them must have FBS [53, 54]. Although GLUT2 variants linked to reported FBS cases have been discovered, the majority of these variants have not been examined for their effects on GLUT2 function. There are only five different types of Fanconi bickel syndrome [55], have been functionally described as of March 2019. Since 2000, numerous additional FBS cases have been discovered from all over the world, exhibiting a variety of unique variations [56-58]. The occurrence of glycemic disturbances (i.e., glycosuria, decreased glucose tolerance) in the carriers of heterozygote FBS variations, that is siblings or parents of Fanconi Bickel Syndrome patients, has been

described [59]. Sakamoto and associates proposed that persons which are heterozygous for Fanconi bickel syndrome GLUT2 missense mutation would experience a dominantly detrimental effect (i.e., haploinsufficiency) from GLUT2 proteins.

In addition to those who are homozygous for uncommon, harmful GLUT2 variations that cause FBS, individuals who have at least one *SLC2A2* allele with a common minor allele frequency > 5% or are less likely to have an allele with a common minor allele frequency between 1% and 5% may be at risk for a number of metabolic traits [60]. Missense mutations are also described, even though shortened proteins are the result of over 70% of FBS-associated mutations. A GLUT2 variants is not always found in patients, despite the presence of classic clinical characteristics [28].

The GLUT2 gene has been the site of many FBS-related alterations over the past ten years. By reporting homozygosity for GLUT2 gene mutations in four patients, Santer [25] stablished the fundamental flaw of this disease. Congenital membrane protein deficiencies caused by these mutations made it easier for glucose transporters to function in numerous organs. 49 individuals with 109 diagnosed FBS patients were analysed, The mutation analysis showed that these people have 23 unique mutations in homozygosity or compound heterozygosity. The people came from 39 families, with members hailing from North America, Turkey, Europe, and the Near East [28].

Two patients in Pakistan were identified in 2019 to have the c.497-2A >T mutation [61]. India additionally discovered three individuals with the c.952G>A mutation in 2018 [62] , and another patient there revealed having the c.1246G>A mutation in the *SLC2A2* gene in 2017 (table.1.1) [63]. Four FBS patients with the identical gene mutation (c.952G>A) were also reported by Govindarajan et al [64]. Researcher has also noted reports of the c.474A > C mutation in Saudi Arabia [65]. Another mutation which are reported in the *SLC2A2* gene are c.474A>C [66], c.482_483insC [67], c.901C>T [68], c.1194T>A [69], c.322A>T [28], nt 1580T>A [70], c.1250C>T [71], c.372A>C [72], 1213 C>T or c.1251C>T [31], c.1478T>C and c.1171C>T [70], p. Trp420* [73]. Missense mutations are also described, even though shortened proteins are the result of over 70% of FBS-associated mutations. However, a GLUT2 mutation is not always found in patients with conventional clinical characteristics [28].

Table.1.1. Various *SLC2A2* gene mutations have been reported in various geographical locations

No. of patients	Mutations	Amino acid change	Origin	References
2	c.497-2A >T	p.(Gly166_Ser169del)	Pakistan	[61]
3	c.952G>A	p.Gly318Arg	Indian	[62]
1	GCCATCCTTCAGTCTT ins CAGAAAA	p.A229 QfsX19	Iran	[74]
1	c.474A>C	p. Arg158Ser	Kuwaiti	[66]
6	c.482_483insC	p. Gly162ArgfsTer17	Turkey	[67]
1	Cys224Del	NA	African	[75]
2	c.901C>T	p. Arg301Ter	Palestine	[68]
1	c.1194T>A	p.Tyr398X	Chinese	[69]
1	c.1250C>T	p. P417L	Egyptian	[72]
1	c.1439C>G and c.1469delA	T480R and L490SfsX24	Caucasus	[76]
1	c.322A>T	K5X	Korea	[28]
1	nt 1580T>A	V423E	Japan	[70]
2	c.372A>C	p. Arg124Ser	Israel	[72]
2	IVS 4-2A>G	p. Gln166AspfsTer4	Dominican	[72]
1	c.1251C>T or 1213 C>T	R301X	Switzerland	[31]

1.3.4) Glucose transport 2 (GLUT2) protein

Researchers first published clones of cDNA from liver of human and cDNA libraries of human kidney in 1988 [26]. These clones encode a protein that shares 55% of its amino acids with the GLUT1 protein. In situ and Somatic cell hybridization have identified chromosome 3q26.1-q26.3 as the location of the gene encoding this protein, which was subsequently given the name GLUT2 and is also known as the *SLC2A2* gene. With a lesser expression in the kidney and gut, [27] identified that the product of gene was concentrated in the human liver. Later studies by [77] and [43] also showed that GLUT2 was expressed in the human

pancrease, but only in beta cells of human pancrease. GLUT2 is also prominent in the plasma cell membrane, Whereas in the flat portions of the membrane, it is more common at microvilli surface facing nearby endocrine cells. Human GLUT2's chromosomal structure was first characterised in 1993. The gene consists of 11 exons, each of which measures about 30 kb [78]. Analysis of variants in patients with FBS has been aided by knowledge of intronic regions close to exons. Various isoforms of GLUT2 are expressed in various types of bodily tissues (table.1.2).

A low-affinity monosaccharide transporter known as GLUT2, D-glucose can be transported by GLUT2 pathway and little amount of D-fructose, D-mannose and D-galactose can also be transported through this pathway. Among the recognised individuals from the family of facilitative glucose transporters, it possesses the highest Km for glucose [79]. With a Km of approximately 42 mM, GLUT2 acts as a glucose sensor, transporting glucose with an essentially linear relationship to concentration when extracellular glucose concentrations are within a healthy range. Consequently, when glucose levels are low, peripheral tissues with specialized transporters are more effective in absorbing glucose than hepatocytes and beta cells that express GLUT2. As will be discussed in more detail below, GLUT2 plays different roles and have various functions in various types of tissues.

1.3.4.1) GLUT2 function in beta Cells

It is still debatable and unclear how GLUT2 functions in human pancreatic -cells. However, GLUT2 has a well-established function in rodent pancreatic -cells and is crucial for the transport of glucose [80]. In contrast to human -cells, rat -cells express glucose transporters principally GLUT1 and GLUT2, whereas human -cells express GLUT1 and GLUT3 [81]. Similar glucokinase gene expression is seen in rat and human pancreatic -cells, however glucose transporter gene expression is different. In human -cells, GLUT1 mRNA and protein expression are quite abundant, and GLUT1 has several properties with other glucose transporters in terms of kinetics [82]. GLUT1 and GLUT2 both proteins are expressed in the foetal islets during the development of the human pancreas [83], approximately 18 weeks of gestation, GLUT1 reaches adult transcriptional levels, while GLUT2 mRNA is detectable as early as 13 weeks of gestation. Though GLUT1 and GLUT2 expression in the -cells is modest throughout the entire human pancreatic development [84]. In addition to its function as a glucose transporter, GLUT2 in rats may also play a part in the pancreatic beta-ability

cell's to sense glucose A GLUT2-GFP fusion protein is shown in one study to move from the cytoplasm to the nucleus at high glucose concentrations when the cells of liver are injected with cDNA encoding the green fluorescent protein (GFP) and the intracellular loop amino acids (aa) 237–301.

Table.1.2. Isoforms of GLUT2 gene and their expression in different tissues

Isoforms of GLUT	Tissues in which they express	Defects	Amino acids	Localization of Gene on chromosome	References
GLUT 1	Fetal tissues, blood vessel endothelial cells (the "blood-brain barrier"), erythrocytes, the placenta, and the retina.	Syndrome of the glucose transporter protein.	492	1p35- 31.3	[85]
GLUT 2	Enterocytes, pancreatic ß cells, renal tubular cells, and hepatocytes.	**Fanconi-Bickel syndrome (FBS).**	524	3q26.1-26.3	[26, 43]
GLUT 3	Brain cells and (many others)	…….	496	12p13	[86]
GLUT 4	Adipocytes, myocardial cells, and muscle cells	…….	509	17p13	[87]
GLUT 5	The basolateral and luminal membrane of enterocytes, as well as the fructose transporter-primarily spermatozoa.	……	501	1p31	
GLUT6	……	……	….	5q(pseudogene)	[88]
GLUT 7	……	Glycogen storage disease's earliest proposed cause.	….	……	[89]
GLUT 8	Blastocyst, and testis (adrenal gland, brain, muscle, liver, kidney).	……	477	9	[34, 90, 91]
GLUT 9	Liver and Kidney	……	540	4p15.3-16	[92]
GLUT10	Pancrease, liver, and (heart, lung, brain, muscle, placenta, kidney)	Around a locus which are associated with Non-insulin dependent diabetes mellitus (NIDDM).	541	20q12-13.1	[93, 94]
GLUT11	Skeletal muscles and Heart muscles.	……	496	22q11.2	[95]

This implies that GLUT2 might be able to transmit a signals of glucose from the membrane of plasma to the cell nucleus by interacting with proteins through its wide loop [96]. In another investigation, transgenic mice that produced the same fusion protein displayed a range of problems in their glucose metabolism [97]. Despite the lower insulin release from the cells, these mice too have exhibited urine which contain higher amount of glucose, it may be essential in keeping the animals from developing hyperglycemia. A glucose facilitative transporter with a low affinity and high KM (15–20 mM), GLUT2 allows the transfer of glucose in accordance to the levels of blood glucose in circulation. This guarantees the liver and pancreas' ability to sense and respond appropriately to maintain normal blood glucose levels [79]. The molecular mechanisms behind insulin production that is induced by glucose are thought to be significantly regulated by GLUT2. This chemical pathway entails glucose transport through glucose phosphorylation by glucokinase, increasing in glycolysis, synthesis of ATP and GLUT2 in beta cells.

1.3.4.2) GLUT2 function in Liver

Depending on whether a person is fasting or eating, liver GLUT2 regulates the absorption and release of glucose, which is crucial for maintaining glucose homeostasis [98, 99]. GLUT2 is known as the main transporter of glucose in the liver of both humans and rats [26, 79, 100]. The basolateral (sinusoidal) plasma membrane region of hepatocyte cells contains GLUT2 [101]. The liver's GLUT2 regulates glucose absorption during the eating state, leading to the creation of liver glycogen, and release of glucose (generated either from the process of glycogenolysis or gluconeogenesis) during the starvation state [102]. Glycogen synthase (GS), a crucial enzyme involved in the production of glycogen, is activated by the allosteric stimulator phosphorylated glucose (glucose-6-phosphate), whereas glycogen synthase kinase-3 (GSK-3) is deactivated by insulin. Glycogenolysis is, however, prevented by the activation of glycogen phosphorylase kinase [103]. As a result of GLUT2 facilitating glucose transport across cell membranes during hyperglycemia, the glycogen production pathway is activated. In hypoglycemia, glycogenolysis is facilitated by the enzyme glycogen phosphorylase (GP), which is activated by either AMP or protein kinase A (PKA).

A great opportunity to learn more about GLUT2's function in the physiology of glucose metabolism is provided by the inactivation of GLUT2 in adult mouse hepatocytes [104]. Due to enhanced glycogen storage, these animals have 40% larger livers, and fasting had no effect on their phenotypic [105]. In these mice with normal hepatic glucose production, the loss of GLUT2 reduces hepatic glucose uptake but has no effect on glucose output, indicating that the release of glucose from hepatocytes may occur through a different pathway without the aid of any facilitative diffusion at the plasma membrane, such as GLUT2 [106, 107]. A few abnormalities in glucose metabolism identified in FBS patients are explained by GLUT2's critical role in the liver (postprandial hyperglycemia, glycogen storage and fasting hypoglycemia).

1.3.4.3) GLUT2 function in Kidney

Three glucose transporters, SGLT1 (sodium glucose contransporter 1), SGLT2 (sodium glucose cotransporter 2), and GLUT2 (glucose transporter 2), are crucial for the reabsorption of glucose from glomerular filtrate in the kidney's proximal tubule. While GLUT2 is located in the basolateral membrane, SGLT2 and SGLT1 are both found in the kidney's apical membrane [108]. The SGLT1 serve as a reserve for the filtration of glucose and that large number of glucose in the filter form is then reabsorbed by the SGLT2. GLUT2 is crucial for completing the glucose reabsorption in the basolateral membrane. The kidney proximal tubules accumulate glycogen in FBS patients as a result of GLUT2 deficiency [109]. The symptoms of GLUT2 deficiency in the proximal renal tubules include glucosuria, phosphaturia, metabolic acidosis, nephropathy, generalised aminoaciduria, bicarbonaturia, hypophosphatemia, and hypercalciuria [34, 110]. Recent research indicates that diabetes increases GLUT2 expression in the renal proximal tubules [111].

1.3.4.4) GLUT2 function in Intestine

How soon glucose enters the bloodstream is determined by how rapidly the stomach empties (gastric emptying) and how quickly glucose is absorbed in the intestine. The small intestine's polarised epithelial cells, known as enterocytes, play crucial roles in the uptake of glucose into the blood vessels and nutrient absorption. The main way that other nutrients and glucose from enter into the bloodstream from the gut is through this mechanism. SGLT1 and (GLUT) is expressed by the enterocytes cells [112]. But the SGLT1 is found at the brush border of the intestinal membrane, GLUT2 transporters are present in the enterocytes basolateral

membrane. SGLT1 transports luminal glucose into the enterocyte and through the basolateral membrane via an active process. Phosphorylation of glucose to glucose-6-phosphates (G6P) occur through hexokinase inside the enterocytes where it reside. In order to transfer glucose to the portal vein, glucose-6-phosphate (G6P) is then dephosphorylated by glucose-6-phospatase. The dephosphorylated glucose from the enterocytes is then passively transported by GLUT2 in the basolateral membrane. Endosomal GLUT2 can migrate quickly to the apical membrane in response to a high glucose load in the gut, improving glucose absorption [102]. Surprisingly, artificial sweeteners can increase glucose absorption from the rat gut due to increased apical GLUT2 expression [113]. The facilitative glucose transporter intestinal GLUT2 may also move galactose, mannose, glucosamine, and fructose [114].

1.3.4.5) GLUT2 function in Brain

Glucose transporters (GLUTs) move glucose across the blood-brain barrier because brain neurons cannot store or manufacture it. The two most significant and dominant transporters in brain cells are GLUT2 and GLUT3. A blood-brain barrier endothelial cell protein called GLUT1 carries glucose from the blood to the brain's extracellular space. GLUT3 is the main glucose transporter from the extracellular environment into neuronal cells, whereas the primary glucose transporter in astrocytes is GLUT2. GLUT2 serves as a sensor of glucose in the brain in addition to delivering glucose to the astrocytes. Studies on GLUT2 knockout mice have demonstrated that the deletion of GLUT2 suppresses brain glucose sensing, which affects feeding behaviour possibly by modulating the pathway that is hypothalamic melanocortin [115]. Through the autonomic nervous system, GLUT2 also connects pancreatic beta-cell bulk and function with brain glucose sensing. Because of decreased -cell bulk and proliferation, nervous system GLUT2 ablation in mice causes the development of late-onset glucose intolerance [116]. Glucose had no effect on the parasympathetic or sympathetic activities, which were both decreased. The establishment of a connection between the endocrine pancreas and the central neurological system depends on GLUT2.

1.3.4.6) GLUT2 genome-wide association studies

According to genome-wide association studies, variations in GLUT2 (specifically the Thr110Ile variant represented by the rs5400 single nucleotide polymorphism) have been linked to type 2 diabetes, impaired fasting glucose, and a higher probability of advancing from impaired fasting glucose to diabetes [117-119]. When GLUT2 SNPs are investigated in

association with other phenotypes, such as exercise intensity, the presence of the major allele predicts that low physical activity increases the risk of developing type 2 diabetes by roughly three times as compared to high physical activity; the minor allele has no predictive value [120]. The GLUT2 locus significantly affects blood cholesterol levels, according to a search for genetic areas linked to plasma lipid profiles that was controlled for food and physical activity[121]. Only the minor risk allele of the gene GLUT2 was significantly linked to an increased risk of cardiovascular illnesses, according to another study that looked at 46 genetic variants [122].

Therefore, these genetic findings imply that GLUT2 participates in regulatory mechanisms that control low fasting glucose, the risk of acquiring type 2 diabetes, the liking for sweet foods, cholesterol levels, and the likelihood of getting cardiovascular disease. Since GLUT2 is only one of the glucose transporters present in beta cells, it is unlikely that slight alterations in expression will have an impact on insulin secretion. Glut2 expression in the neurological system controls glucose-regulated autonomic nerve activity, which may have an impact on the function of the heart, liver, and adipocytes, according to studies on mice. It is tempting to speculate that type 2 diabetes is preceded by GLUT2 dysregulation and that GLUT2 serves a crucial role in the human nervous system's glucose sensing.

1.3.5) Relationship Between GLUT2's Structure and Function in FBS

It has been investigated the structure-function relationships of several naturally occurring FBS *SLC2A2* mutations and several engineered mutations and their effects on kinetic parameters, protein expression, cell differentiation, and insulin secretion using Xenopus oocytes, hepatocytes, and pancreatic cells [123]. Despite positioning the protein on the plasma membrane similarly to wild-type and exhibiting similar or lower levels of protein expression, Loss of transport function was seen in the naturally occurring FBS *SLC2A2* mutations. Despite the transport protein's correct targeting at the plasma membrane, these functional alterations in GLUT2 may be caused by a decreased ability for glucose transport or by a decrease in protein expression. These findings demonstrate how crucial GLUT2's role in glucose transport is. It is found that all of the GLUT2 produced mutants are localised at the plasma membrane and have altered kinetic characteristics and abilities to transport glucose. They also exhibit enhanced -cell differentiation and insulin secretion. The increased number of pancreatic -cells in three designed GLUT2 transporter mutants, which may play a

significant role in the development of pancreatic -cells, is a significant finding. These findings suggested that GLUT2 has a functional role as a key extracellular receptor and glucose transporter that influences the differentiation of pancreatic cells and insulin secretion.

1.3.6) Facilitative glucose transport pathway

Three processes are used to move the hydrophilic monosaccharide glucose across the cell membrane's lipid bilayer. Simple diffusion, which has little physiological significance, sodium-dependent glucose transporter (SGLT) family members' "active" energy dependent transport, and facilitative diffusion (also known as "passive" transport), which is mediated by a GLUT family member, are some of these (fig.1.4).

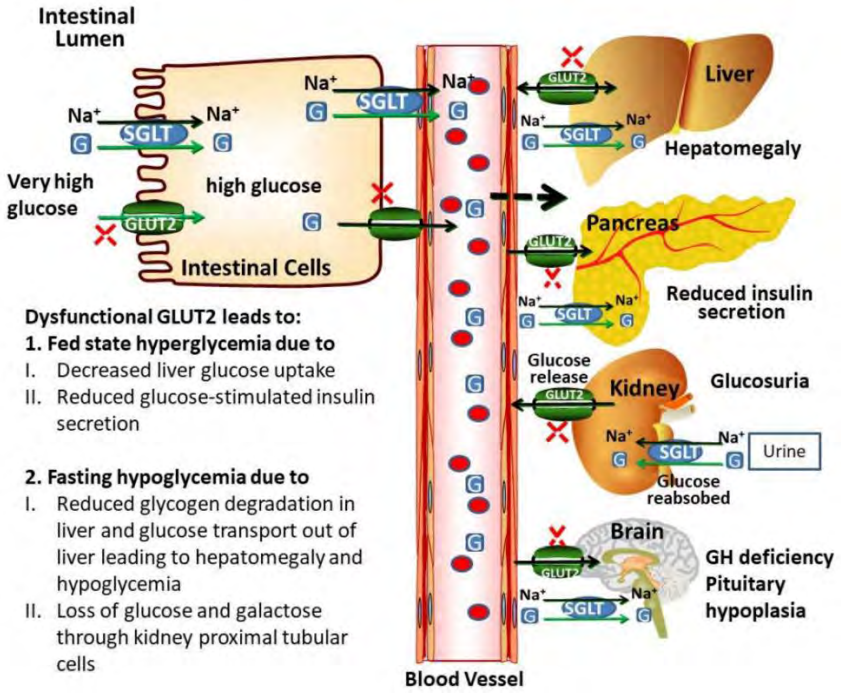

Figure.1.4. Facilitative glucose transport pathway [85].

Almost all mammalian cells include GLUT proteins. These 12 transmembrane domain proteins range from 478 to 541 amino acids in length and from 35 to 58 kDa in molecular weight. The transmembrane domains of the GLUT proteins are thought to make a tunnel which are hydrophilic and that contains the binding site of the glucose, but currently still little knowledge available about the three dimensional structure of GLUT2 [85]. According to the paradigm for alternative conformation, The glucose binding site of GLUT proteins can change conformation to point to either the extracellular or cytoplasmic side of the membrane

[124]. The free amount or bound molecules of glucose is influenced by the transporter's kinetic characteristics, binding site availability, and glucose concentration. In this way, the protein GLUT resembles the enzyme because it produces a saturable reaction that is substrate- and stereo-specific and creates an equilibrium of glucose across the membrane of the cell. Nevertheless, other monosaccharides, ring compounds, or even the channels of waters can be transported by various GLUT proteins in addition to glucose [34]. Therefore, the dissociation constants Km and maximum velocity vmax used to describe their response with various substrates. While net glucose transfer will only happen along a concentration gradient, as opposed to energy-dependent transporters, GLUT protein-mediated transport of glucose is in theory bidirectional.

Due to their inactivity for glucose-6- phosphatase and cannot create free glucose, the majority of cell types only net absorb and metabolise glucose. Hepatocytes, on the other hand, store a lot of glycogen, and while fasting, they use the breakdown of that glycogen and gluconeogenesis to make free glucose. Through energy-dependent transport at their apical membranes, enterocytes and renal tubular cells can store and transport glucose, raising the intracellular glucose content above the plasma level. It has long been believed that facilitative diffusion, which is mediated by GLUT proteins, causes net outflow from all of these cells.

Facilitative glucose transporter isoforms are controlled by several hormones. Regulation may occur at the level of transcription, membrane expression, transporter protein internalisation, or the movement of membrane vesicles transporting transporter proteins from an intracellular storage reservoir. Facilitative glucose transporters distribution and their differences in substrate specificity and kinetics are likewise tissue-specific. In order to maintain glucose homeostasis, transport of monosaccharide is therefore tailored for the particular requirements of the tissue [125, 126].

The complementary DNA cloning sequence determines the numbering of the GLUT (glucose transports) proteins. The first gene of GLUT, GLUT1, which was first cloned and discovered in the 1985 [85]. The protein of GLUT1 is expressed in numerous foetal and adult organs and is extremely prevalent on the erythrocytes surface, making up around 5 percent of the total protein content of the membrane. Although endothelial cells play a critical role in the activity of GLUT1, which is the main glucose transporter in numerous organs. A GLUT1 deficit will lead to inefficient glucose delivery for the central nervous system because endothelial cells

create the tight, glucose-impermeable junctions of the blood-brain barrier at the tiny arteries of the central nervous system. GLUT1 deficiency is a highly sporadic dominant autosomal disorder also known as "glucose transporter protein syndrome" or "De Vivo syndrome.". Sole GLUT1 and GLUT2 have been identified as the only genes that can result in congenital abnormalities of facilitative glucose transport [127]. There are further GLUT gene family members that have been cloned. Although several of them appear to play a crucial function in the transport of monosaccharides in particular tissues, congenital abnormalities for GLUT3 to GLUT10 have not yet been identified.

1.3.7) Pattern of inheritance

Since the first familial instances were originally documented in 1975 [128] FBS has frequently been identified in siblings [31]. Never have successive generations of the disease been noted. Both males and females may be impacted. 50 males and 40 females out of the 90 instances in which gender was reported. The clinical trajectory seems to be the same in both genders. All of these findings are consistent with recessive autosomal inheritance, which were demonstrated by the discovery of compound heterozygous or homozygous mutations in the patient's gene GLUT2, which is located on chromosome number 3 [28].

Any chromosome besides the X or Y chromosomes can contain an autosomal gene (sex chromosomes). Genes typically appear in pairs, just like chromosomes. Recessive means that both copies of the illness-causing gene (pathogenic variant) must carry the disease-causing modification for an individual to develop the condition. However, the outdated term "mutation" is occasionally used to describe a pathogenic variant. A pathogenic variant-containing gene is inherited from each parent by a person with an autosomal recessive condition. Each parent is a carrier because each bears the pathogenic mutation in just one copy of the gene. Most autosomal recessive disease carriers do not exhibit any illness symptoms. A 25% (1 in 4) probability of having a kid with the disease exists when two autosomal recessive disease carriers have children.

1.3.7.1) Prevalence

FBS appears to be a rare condition, however its exact frequency is unknown. FBS is a rare diagnosis, even in hospitals that only treat GSD patients. There have been reports of the disease from every region of Israel, Turkey, Europe the Arab world, Japan, and North

America and Japan. Consanguinity was found in the affected families, while the exact frequency was unknown [31, 34]. Recently, a small number of instances have been identified in neonatal screening programmes using the Guthrie testcards [70, 129, 130] to measure blood galactose, however this frequency is extremely low. The incidence of mutant alleles in most populations is quite low, according to the discovery that 74 percent of Fanconi bickel syndrome patients have GLUT2 homozygous variants and that 71 percent are the result of consanguineous marriages [28].

1.3.7.2) Heterozygous GLUT2 mutation carriers

The topic of whether people with heterozygous GLUT2 mutations exhibit any clinical symptoms arose because of the autosomal-recessive pattern of inheritance. Contrary to FBS, an autosomal-dominant feature is seen in the severe neurologic disease caused by haplo-insufficiency of GLUT1. This condition is brought on by inadequate transport of glucose across the blood brain barrier (BBB) [34, 131].

An example of a woman who had prenatal diabetes and later went on to develop NIDDM and who was identified as having a heterozygous GLUT2 mutation was described prior to the identification of the FBS molecular deficit [45]. Studies on in vitro expression have shown that the GLUT2 T110I mutation impairs glucose transport [132]. Considering that most FBS relatives won't develop diabetes [31] and that there hasn't been any evidence of a relationship between the locus of GLUT2 and type 2 diabetes in the population in general [133-136]. This observation may have been accidental. However, a comprehensive analysis of families of Fanconi Bickel Syndrome looking at secretion of insulin and the onset of diabetes in the heterozygotes is still being completed.

Isolated renal glucosuria has also been noted in GLUT2 heterozygotes [70]. It's interesting to note that a lowered kidney glucose threshold has only been discovered in GLUT2 heterozygous missense mutation carriers, yet not in nonsense mutation carriers. It is believed that the mutated protein expressed in the case of a missense mutation having a dominantly negative effect on GLUT2 transporter polymers composed of varying numbers of mutant and wild-type monomers causes a minor impairment of glucose transport at the basolateral membrane of renal tubular cells.

1.4) Clinical findings in FBS

Fanconi Bickel Syndrome is presently understood the basis of 112 patients. We were aware of 82 patients in 1998, and the review publication lists their clinical information [31]. Hereditary fructose intolerance has since been declared to be the patient's condition in case 61. 28 more cases from personal communications and three more cases from the literature [137, 138] have now been recognised. The diagnosis has since been verified by molecular genetic research with clinical indications in patient thought to be classical for Fanconi Bickel Syndrome as well as in some of the atypical cases. The clinical spectrum can now be more accurately described as a result of this.

Cases of FBS have been documented from every region of Israel, Turkey, Europe, the Near Eastern Arabian countries, and Northern Africa and eastern arabian countries. The majority of cases in North America are Caucasian [28] However, a GLUT2 heterozygous mutation has been identified in an American-African person who had taken part in a study on glucose-stimulated insulin secretion in non-insulin-dependent diabetes mellitus before the discovery of the underlying FBS anomaly. The first FBS cases from Japan have only recently been recorded [28, 70, 139].

Around three to ten months of age, patients frequently make their initial appearance. When all of the typical clinical symptoms of FBS are present, such as hepatomegaly brought on by glycogen accumulation, a severe Fanconi type renal tubulopathy with disproportionately severe glucosuria, glucose and galactose intolerance, and severely stunted growth, the diagnosis of the condition is straightforward. However, at an prompt phase, they could experience vomiting, fever, stunted growth, and persistent diarrhoea. The instances first indicated by [129] It demonstrates that hepatomegaly is not a condition sine quanon when the recognition was confirmed by molecular genetics tools. Fat deposits on the shoulders and abdomen, a moon-shaped face, and a very protruding stomach are common in patients who are in the final stages. Puberty and growth are greatly delayed. Hypophosphatemic rickets symptoms frequently include joint swelling, limb bowing, pathological fractures and dental issues that have a high risk of developing severe caries. Cascade, an often reported hypergalactosemia-related complication, are only occasionally observed.

Most individuals have rather big kidneys [70]. Despite the fact that patients have symptoms of generalised tubular dysfunction, the diagnosis is characterised by an excessive loss of glucose at both high and low plasma glucose conditions (Polyuria, mild tubular proteinuria, hyperuricosuria, hypercalciuria, renal tubular acidosis, hyperphosphaturia, and hyperaminoaciduria). There have been cases of patients whose tubular glucose reabsorption appears to be completely absent. Even when glucose loss is measured using body surface area, age-related increases have been seen [70] daily excretion rates of up to 325 g per 1,73 m2 have been observed [38]. Glomerular function is typically normal, however in one patient with a recently discovered GLUT2 mutation, microalbuminuria, glomerular hyperfiltration, and a progression to glomerular insufficiency have been documented [42].

At birth, the liver's size is normal, and it generally grows larger throughout infancy. By using histologic or electron microscopy techniques, accumulation of glycogen in the liver can be seen, and glycogen concentrations of more than 15 g per gramme of moist tissue were found after the glycogen content was measured [70]. The results of the iodine spectra were consistent with a normal structure for the stored glycogen. The size of the liver does shrink when using treatments like a ketogenic diet, frequent feedings, or slowly absorbed cornflour. However, during adolescence, hepatomegaly recedes even without dietary modifications. Patients with FBS have never experienced the hepatic adenomas or malignancies that have been documented for other kinds of hepatic glycogen disorders.

Plasma glucose homeostasis impairment is another clinical indication of FBS patients. Patients typically experience postprandial hyperglycemia, as seen in the data. A few patients have received insulin treatment after being diagnosed with diabetes mellitus. This could increase the likelihood of developing hypoglycemia when fasting, that is a trait of Fanconi bickel syndrome. There have been many descriptions of fasting hypoglycemia, and in FBS patients, plasma glucose levels as low as 18 mg/dl have been recorded [23]. However, symptomatic hypoglycemia seems to be the exception, maybe as a result of patients' blood having higher levels of alternate brain substrates such lactate and ketone bodies [31].

The prognosis quoad vitam looks to be favourable in the majority of cases. 7 patients have been indicated who have passed away from disease-related difficulty, 2 of them were children who passed away after surgery in acidotic situations [140], and 1 who developed chronic renal problems similar to diabetic glomerulosclerosis and died from glomerular insufficiency

[42]. A little cases, counting the original patient of Fanconi and Bickel, have grown up with stable conditions. The fertility rates for both sexes are unknown, despite the fact that at least one of these individuals is married. The primary subjective issue for the majority of adult patients is their short size; Patients with Fanconi bickel syndrome have adult heights between 131.5 and 158 cm [31]. Numerous of these patients have rickets and osteomalacia, which can also affect their joints.

1.5) Molecular genetic findings in FBS

FBS is caused by a congenital deficiency in the GLUT2 gene, according to a 1997 report by a research team [28]. Four individuals, including the one Fanconi and Bickel first described in 1949, had homozygous or compound heterozygous GLUT2 mutations at that time. Additional mutations have now been indicated [28, 34, 139]. Molecular genetic investigations on 49 total Fanconi bickel syndrome patients from various parts of the globe have recently been summarised by scientists [28]. Molecular genetic techniques have been used to study 63 cases, or more than 50% of documented FBS patients. The GLUT2 gene has been shown to have 34 distinct naturally occurring mutations in total [28]. There are 7 splice site mutations, 10 missense mutations, 10 frame shift mutations, 7 nonsense mutations, and 10 missense mutations among them (fig.1.6). There are numerous mutations throughout the majority of the coding region and surrounding intron segments. Only exon 1 of the GLUT2 gene's eleven exons, which has a modest size of only five codons, has never been discovered to be impacted. According to the global survey, none of the GLUT2 mutations are extremely frequent.

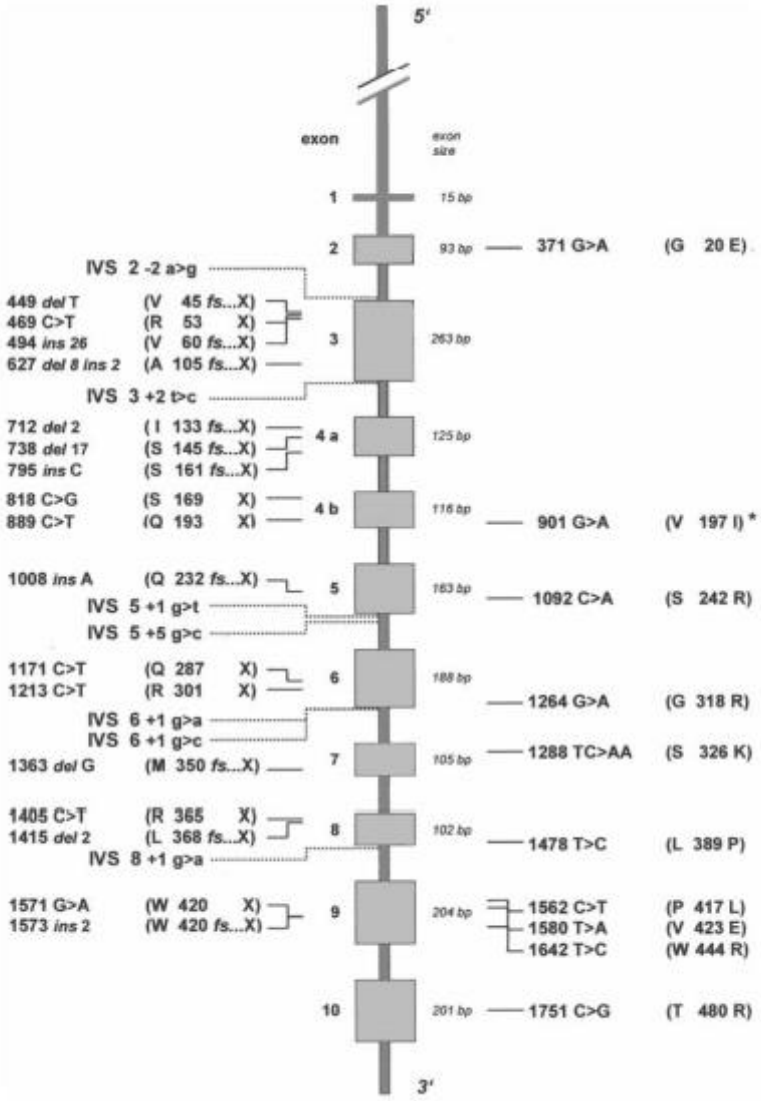

Figure.1.6. 33 GLUT2 mutations were discovered in 63 persons with FBS. Exons 1 through 10 of the GLUT2 gene are depicted in scale. Frame shift, nonsense, and splice site mutations are shown on the left, while missense mutations are shown on the right. A heterozygous GLUT2 mutation is present in a person with gestational diabetes [28].

Similar to this, there isn't just one common mutation in GLUT1 deficiency. None of the GLUT2 mutations match a change in the GLUT1 gene's structure [141]. This shows that the GLUT gene family does not contain mutational hot spots. R301X and R365X, the two most frequently altered GLUT2 alleles, each make up less than 10% of the total. However, there might be regional variations. For instance, In Israel's Arabian population, the R301X mutation appears to be localised in a tiny cluster. This genotype has been identified in five FBS cases (including a recently observed unreported prenatally indicated case) from the three families, none of which can be definitively proven to be connected. It's interesting to note that

the original patient of Fanconi and Bickel [28] and another Italian patient both have the same mutation. It has been feasible to prove that the existence of these two mutations in more than one patient is not the consequence of a founder effect but rather that they have occurred separately in various locations throughout the world since intragenic markers distinguish different haplotypes in these patients. The fact that both mutations affect CpG dinucleotides, which are known to have higher mutation rates, serves as additional evidence for this [28].

When glucose transport is assessed when GLUT2 is expressed in Xenopus laevis oocytes, for example, the physiological significance of the GLUT2 mutation in FBS instances has not been shown. However, the outcomes of various in vitro mutagenesis experiments investigating the characteristics of particular facilitative glucose transporter protein domains can be used to assess the consequences of mutations. The bulk of GLUT2 mutations are expected to result in proteins with shorter C terminals. The alternative conformation model forecasts their inactivity [124]. A loss of more than 24 C terminal amino acids in GLUT1 stabilises the protein's inwardly facing substrate binding site and prevents it from changing [142]. Therefore, we presume that truncating mutations are non-functional even if they have no impact on protein distribution within the cell membrane.

Missense mutations' physiological impact is more challenging to demonstrate. It is unclear if specific missense mutations are linked to any lingering transporter activity and whether this activity could result in less severe symptoms. The discovery of GLUT2 missense mutations, such the P417L variation, in many FBS families but not in controls is one proof that these mutations are harmful. Function may also be impacted by mutations that alter the amino acid sequences in the highly conserved motifs unique to facilitative glucose transporters. The W444R mutation in GLUT2 [28] intriguingly modifies a tryptophan residue in the eleventh transmembrane region of the GLUT2 protein. Numerous in vitro studies have demonstrated the importance of this highly conserved position, which is represented by the amino acid W412 of the GLUT1 protein, for human facilitative glucose transporters and the role of this amino acid in the intracellular binding of glucose [143-145]. The following missense mutations in GLUT2 are other examples: T480R, V423E, S326K and V197I. These novel results influence the less conserved GLUT2 protein domains compared to other GLUT family members. The other three mutations have not been investigated, despite the fact that the V197I mutation has been shown to affect glucose transport in vitro [132]. However, a number of alignment analyses have recently found that these three mutations do exist in regions that

are conserved in class I GLUTs, a category that includes GLUT1 to GLUT4 [95]. The seventh extracellular loop has an STS motif, which is where the S326K mutation is located. It has been proposed that this motif contributes to the transporter's glucose selectivity [146]. There are currently no reports that link the clinical characteristics of FBS patients to the type of mutation, and there does not appear to be a connection between genotype and phenotype.

1.6) Treatment and Management

For patients with FBS, there is no set course of treatment. To counteract renal tubular acidosis, alkali is given, for example, in the form of Shohl's solution or bicarbonate solutions, as well as the restoration of water and electrolytes are therapeutic procedures aimed at alleviating the effects of renal tubulopathy. Phosphate and vitamin D supplements are needed to treat hypophosphatemic rickets.Patients with FBS should be given a diet with enough calories to command the significant losses in kidney glucose. Frequent feedings of slowly digested carbohydrates are advised due to the tendency to develop both hypo and hyperglycemia and hepatic glycogen build up. Uncooked cornflour administration has been shown to have a favourable effect on metabolic control, notably on growth, as is already recognised for other kinds of GSD [89]. Fructose is tolerated when taken orally in contrast to GSD 1, most likely because enterocytes exhibit the fructose-specific transporter GLUT5 at their apical and basolateral membranes [147, 148]. If malabsorption predominates in the clinical picture and there is truly a problem with monosaccharide transfer across enterocytes, then its use might be beneficial. The use of galactose is not clearly defined. Despite postprandial hypergalactosemia due to poor hepatic absorption being confirmed, cataracts have not been seen in Fanconi and Bickel's initial patient, who has consumed vast amounts of milk for decades [31]. It is unclear whether galactose restriction should be advised and whether the levels of galactose in the blood and galactose-1-phosphate in the patient's erythrocytes are useful markers for keeping track of the patient.

Drugs that are difficult to obtain, such as phosphate, vitamin D analogues, or even solutions to restore acid-base balance, should be obtained even at the local level in many underdeveloped nations. Some individuals responded well to treatment, with improvements in their acidity and rickets symptoms, although longer-term monitoring is required to assess their growth and puberty [149]. A better prognosis may result from early detection and

appropriate therapy, but late identification worsens the situation and increases death, mainly as a result of respiratory infections or liver failure [150, 151].

1.7) Aims and objectives of this study

- Detection of the mutational spectrum in hereditary Fanconi Bickle Syndrome in familial and sporadic cases.
- Insilco analysis will confirm the probable pathogenicity of discovered mutations in candidate genes.
- To examine the biomarkers' patterns of expression in relation to the development of Fanconi Bickle Syndrome phenotypes.
- Our findings will be helpful for understanding the genotype to proteome linkages in Fanconi Bickle Syndrome, and further research may assist to identify new therapeutic targets and pathways.
- Contribute to literature, prenatal diagnosis, personalized medicine and genetic counselling.
- To improve diagnostic and therapeutic approaches.

Chapter 2

2) MATERIAL AND METHODS

2.1) Family description

The family was traced in the holy family hospital Rawalpindi, Pakistan and they belong to the province Punjab, Pakistan. The family was investigated having suspected case of Fanconi bickel syndrome. The blood samples were taken from the suspected case. The family agreed to the study in order to carry out the sample molecular analysis. To ascertain the family's pattern of inheritance, a pedigree was created.

2.2) Pedigree construction

During the interview with the family members, all the data essential to create a pedigree was obtained. Pedigree construction is done using Biorender. To represent gender, illness state, and consanguinity in pedigrees, many shapes are utilised. Squares represent men, and circles represent women. Circles and squares with shading stand in for the impacted family members. Crosses on the square and circle signify departed souls. The arrow indicates the study's proband. A double line separating the partners signifies a consanguineous union. Arabic numbers are used to designate each member of the family within the generation, whereas Roman numerals are used to represent generations.

2.3) Sample collection and ethical consent

Blood was drawn from the patient after taking informed consent from the guardian of the family. Blood was drawn through venipuncture using syringes and placed in two 3 ml Ethylene Diamine Tetra Acetate (EDTA) tubes to prevent clotting. After that, the collected blood samples were sent to the Medical Genetics Research Laboratory at the Quaid-i-Azam University in Islamabad's Department of Biotechnology, where they were maintained in a freezer at 4°C.

2.4) Genomic DNA extraction

The organic (Phenol-Chloroform) approach is used to recover genomic DNA from human blood.

2.4.1) Composition of solutions

In (table.2.1), the solution compositions for the phenol-chloroform method of DNA extraction are listed.

Table 2.1. Chemical make-up of DNA extraction solutions

Solutions	**Composition**	**Quantity**	**Function**
Solution A	Sucrose (0.3 M) Tris-HCL 5mM MgCl2 Triton X-10 Distilled Water	54.7g 5ml 2.5ml 5ml 400ml	Makes sample heavier Maintains Ph Lysis buffer Used as a detergent to burst membrane
Solution B	NaCl (400mM) Tris-HCL (10mM) EDTA (2mM) Distilled Water	40ml 5ml 1ml Make total volume of 500ml	Increase buffer capacity Maintains pH Capture Mg ions to protect DNA from action of nuclease
Solution C	Phenol Hydroxyquinoline Tris-HCL	100ml 10mg 10ml	Perform protein denaturation Prevent phenol from oxidation Maintains pH
Solution D	Chloroform Isoamyl Alcohol	24ml 1ml	Involved in protein denaturation Increase the efficiency of chloroform to denature protein

2.4.2) Phenol Chloroform Method

Before starting the DNA extraction process, all blood samples from family members are taken out of the refrigerator and placed at room temperature (37 °C) for an hour. The steps below are used to extract DNA.

- Equi volume, consisting of 1.5 ml of solution A and 750 µl of blood, was added to an Eppendorf microcentrifuge tube, and the tube was repeatedly inverted to mix the two components.

- The tubes were then left at room temperature for ten to fifteen minutes. Centrifugation for one minute at 13000 rpm followed.

- A microcentrifuge tube containing 400 µl of solution A was filled to dissolve the pellet after the supernatant was removed. The tube was then centrifuged for one minute at 13000 rpm.

- The pellet was dissolved by adding 400 µl of solution B, 25 µ of 10% SDS, and 5-8 µl of Proteinase K. The supernatant was once more discarded.

- The pellet was broken up by tapping and overnight incubation at 37 °C since it was stiff.

- On the following day, the microcentrifuge tube that had been incubating received equal amounts of 500 µl of solutions C and D. It was followed by a 10-minute centrifugation period at 13000 rpm.

- The upper aqueous layer was collected using a brand-new, sterile microcentrifuge tube.

- 500 µl of solution D was added to this tube, and it was then centrifuged for 10 minutes at 13000 rpm.

- A fresh, sterile microcentrifuge tube was used to transfer the top water layer.

- A microcentrifuge tube was filled with 500 µl of cold iso-propanol (-20 °C) and 55 µl of sodium acetate in order to precipitate the DNA.
- The mixture was then centrifuged for 10 minutes at 13000 rpm.
- The supernatant and DNA pellet were carefully discarded.
- 200 µl of 70% cooled (-20 °C) ethanol were added to the microcentrifuge tube to wash the DNA pellet. Next, there is a seven-minute period of 13000 rpm centrifugation.
- The tube was kept open after the ethanol was thrown away to let the pellet dry in the concentrator for roughly 10 minutes at 45 °C.
- The final. 80-100 µl of deionized water were added to rehydrate the DNA, which was subsequently incubated at 37 °C overnight.

2.5) Molecular Analysis of extracted DNA

To conduct a molecular analysis on extracted DNA, agarose gel electrophoresis was employed. There were three procedures: loading, setting, and preparation of the gel.

2.5.1) Gel preparation

Genomic DNA is examined using a 1% agarose gel. For the creation of 1% agarose gel, the following components are required.

- Distilled water 45ml
- Agarose 0.5g
- 10X Tris-Borate EDTA (TBE) 5ml
- Ethidium Bromide 5µl

The steps for creating 1% agarose gel are as follows.

- 0.5 grammes of agarose and 5 millilitres of 10x TBE were combined with 45 ml of distilled water.
- After 2 to 3 minutes in the microwave, the agarose is fully dissolved in the liquid, producing a clear solution.
- After that, the solution is let to cool for 5 to 7 minutes at room temperature.
- After the solution had cooled, 5 µl of ethidium bromide was added and well incorporated.

2.5.2) Gel setting

- The gel solution was placed in a casting tray.
- The gel is separated into wells using the appropriate combs. In order to make wells in the gel, I have consequently inserted the comb.
- The solution put into the casting tray required around 20 to 25 minutes at room temperature to solidify.
- After the gel had formed, the casting tray containing the solidified tray was put into the electrophoretic tank with 10X TBE as the electrolyte.

2.5.3) Sample loading

- DNA mobility inside the gel was noticed using bromophenol blue as a loading dye.
- To avoid harming the gel, the extracted DNA was mixed with an equal volume of 3 µl of bromophenol blue before being carefully poured into the wells.
- A Bio-Rad power supply connected to the electrophoretic tank supplied electricity for the electrode.

- It was programmed to run for 25 to 30 minutes at 400 amps of current and 120 volts of voltage.

- We will be able to detect it once the bands have travelled a certain distance. Then we will take off the electrodes and stop the power.

- The gel was carefully removed and placed under a UV trans-illuminator to view the bands and their intensity.

- By adding 60 µl of pcr water to 20 µl of extracted DNA, the isolated DNA was diluted to a final concentration of 40 ng/µl.

2.6) DNA Sequencing

2.6.1) Whole Exome Sequencing

Whole exome sequencing was used to sequence the proband's DNA. The sureselect V5-Post kit from Agilent Technologies in Santa Clara, California, was used to construct the exome libraries. Illumina Hiseq 400 was used for sequencing (illumine san diego, CA, USA). For 150 bp termination readings, the target region's average depth was 142X. Using the Burrows-Wheeler alignment programme (BWA-men; http://bio-bwa.sourceforge.net/bwa.shtml), the resultant reads were mapped to the human reference genome (UCSC, human genome assembly 19). The GATK, or Genome Analysis Toolkit, was used to call variants. Any duplicate reads were removed using Pi-card tools. Variant alleles were annotated in the VCF format using the HaplotypeCaller pipeline and SnpEff (SnpEff v4.1).

In whole exome sequencing, the following steps are taken to find the disease-related variation among the thousands of other variants.

- A number of substitutes, including frameshift, non synonymus/synonymus, and others, were selected.

- Then altering the coding of exons or introns.

- Stop codons are increasing and decreasing.

- Variants from the gnomAD-genome aggregate database were selected with allele frequencies less than 1% (https://gnomad.broadinstitute.org) accessed on 8th August,2022.

- Mutation Taster, SIFT, and Polyphen2 are examples of in silico techniques that are used to identify whether a mutation is harmful or pathogenic.

- Different varieties were discovered in the proband based on filtration.

- Sanger sequencing will be used to confirm a specific disease-causing variation.

- The individuals who are afflicted must be homozygous for the candidate gene (*SLC2A2*).

- Heterozygous peaks ought to be seen in those who carry this variation.

- In exon's coding areas, the variant should exist.

- The mutation must be nonsensical or missense.

- In-silico technologies would be used to determine whether the variations were pathogenic.

- The 1000 Genome Project states that a variant's pathogenicity, or minor allele frequency, should be less than 0.01 (http://www.1000genomes.org).

2.6.2) Primer Designing

The ensembl genome browser (https://asia.ensembl.org/index.html/) was used to identify the mutation [c. 298 T>C (p. Ser100Pro)] on exon 3 after downloading the genomic sequence of the *SLC2A2* gene in FASTA format. Oligo Calc and Sequence Manipulation Suite, PCR Primer Stats (SMS) were used to analyse the specific properties of the primers (table.2.2).The homology and hits of the primers are examined using BLAST at https://asia.ensembl.org/Homosapiens/Tools/Blast/. To determine the product size and whether a virtual PCR using the chosen genomic sequence together with forward and reverse

primers is feasible or not, in-silico PCR was also carried out. It is necessary to acquire the chosen primer pair from MACROGEN Korea.

Table.2.2. List of primers for amplification of exon 3 of *SLC2A2* gene.

Exon No.	**Type F/R**	**Primer sequence 5' to 3'**	**Primer length (bp)**	**T_m (c)**	**Gc content (%)**	**Product size (bp)**
03	F	GGATGACCGAAAAGCTATCAACA	23	59	43%	229
	R	TGCCTACCTTCCAAGTGTGT	20	58	50%	229

Chapter 3

3) RESULTS

3.1) Description of the family

The family was traced in the holy family hospital Rawalpindi, Pakistan and they belong to the province Punjab, Pakistan. The family was investigated having suspected case of Fanconi bickel syndrome. The blood samples were taken from the suspected case. The family agreed to the study in order to carry out the sample molecular analysis. To ascertain the family's pattern of inheritance, a pedigree was created.

3.2) Family pedigree analysis

The family was enlist from holy family hospital Rawalpindi, Punjab,Pakistan. The three generation pedigree was created with one consanguineous marriage in the 2^{nd} generation as shown in (fig 3.1). One member of the family in the 3^{rd} generation had the disease and he was sampled at that time for analysis. The parents of the proband were phenotypically healthy and had no appearance of the disease. The proband, a 5-year-old boy with consanguineous parents, exhibits clinical traits that are very similar to those of a typical features of Fanconi-Bickle disease (FBS).

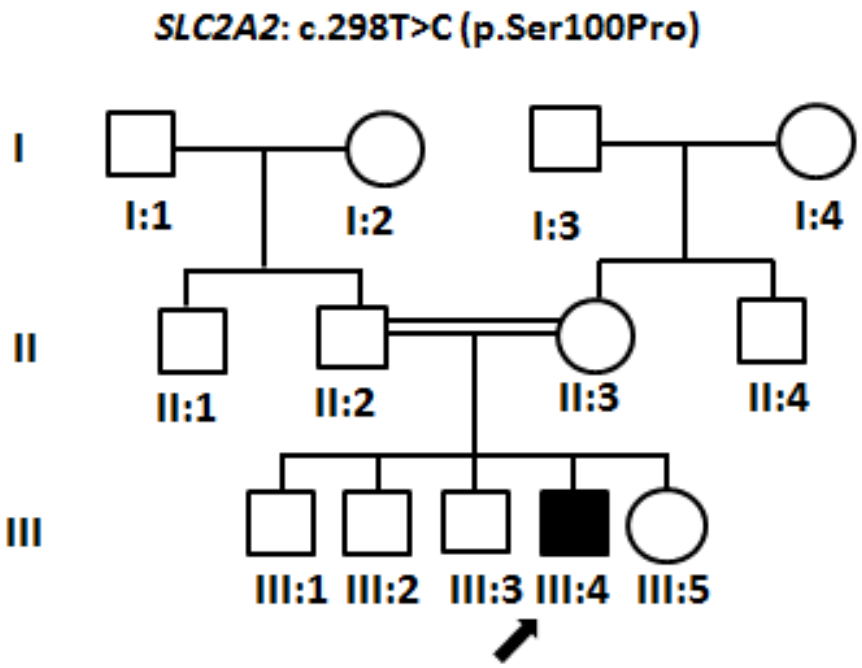

Figure. 3.1. Pedigree of family. Three generation pedigree contain one consanguineous marriage in the 2nd generation. One affected individual with black colour in the third generation. The arrow represent the proband in the family.

3.3) Clinical features of the patient

Numerous characteristics shared by the one patient in this family whose sample was taken for DNA analysis are listed in (table 3.1) below. At the time of the sampling, the patient was five years old. The patient was checked by the doctor, who made a clinical diagnosis of Fanconi Bickel syndrome in the patient.

Table.3.1. Clinical profile of the patient

1.	**Sex**	Male
2.	**Age**	5 years
3.	**Stature**	Short stature
4.	**Hepatomegaly**	Patient have enlarged liver
5.	**Failure to thrive**	Yes
6.	**Polyuria**	Yes
7.	**Abdominal distension**	Yes
8.	**Doll like facies**	Yes
9.	**Rickets**	Yes

The patient was examined by the doctor in detail and all that features that support Fanconi bickel syndrome were present in the patient. At the time of sampling the age of the patient was 5 years. He has short stature as compared to normal, the liver size was also larger than normal as the glycogen storage in the liver increase and the size of liver become larger. Failure to thrive (growing slower than normal), Polyuria (Excessive urination), abdominal distension, doll like facies and rickets (Weakened bones) were all that signs which were present in the patient at the time of examination.

3.4) Gel electrophoresis

The DNA that was isolated from the patient's DNA was examined using gel electrophoresis to determine its quality and concentration. The gel's bands demonstrated a high concentration of usable DNA for PCR and were free of any protein contamination.

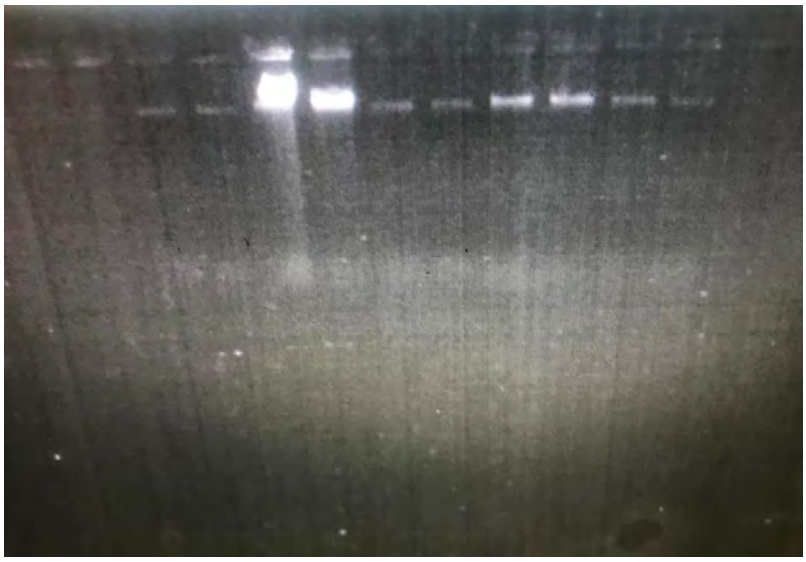

Figure.3.2 Gel electrophoresis. A gel-doc picture of the patient DNA.

3.5) DNA Sequencing

Whole exome sequencing was used to sequence the affected person's DNA, but sanger sequencing has not yet been used to validate allele segregation.

3.5.1) Whole exome sequencing data analysis

The patient entire exome was sequenced, and 50,592 variants were discovered. These variants are subject to various filters.

3.5.1.1) Variants filtration

50,592 variants were found in the patient DNA after whole exome sequencing. By removing variants that were synonymous with or unrelated to the condition of interest, the variants were reduced to the one variant that was most likely to be the cause of the disease as shown in (fig.3.3).

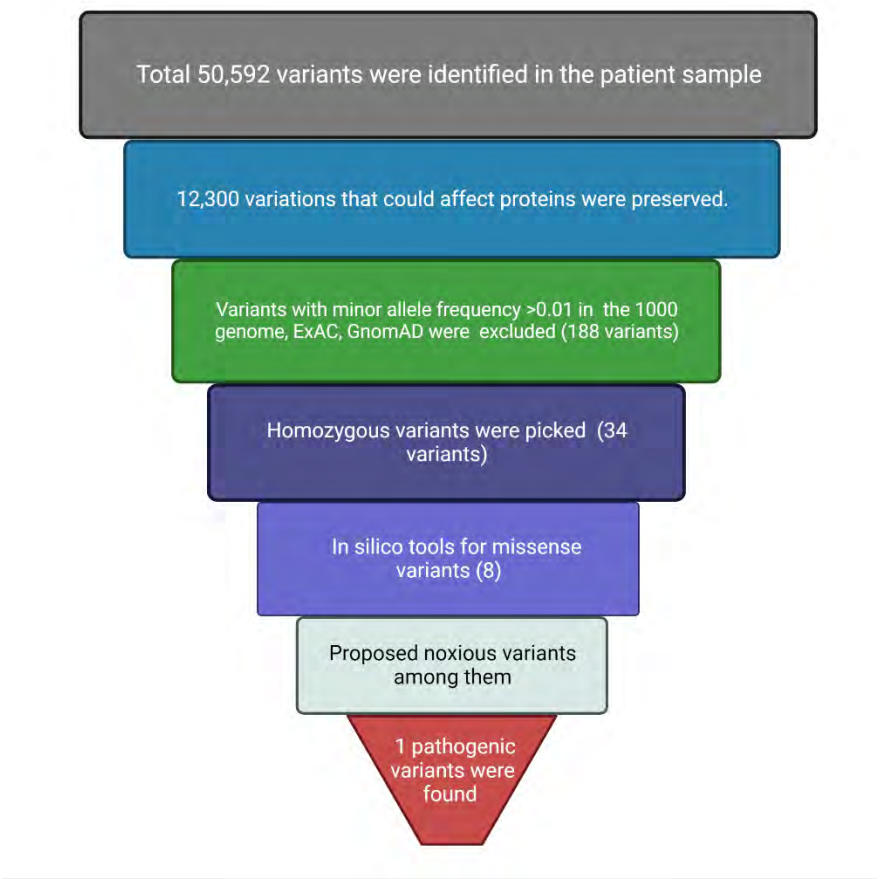

Figure.3.3. Approach for applying different filters for the analysis of exome sequencing data.

Various filters were used to analyzed the data of exome sequencing and to get the specific variant which are the main hallmark of the disease. At first total variants were 50,592 then filter for the regions of exons were applied and all those regions were selected which are protein coding and all others i.e intronic regions, UTR‟s etc were removed, so the total

number of variants reduced to 12,300 variants. Then the next filter were applied for the minor allele frequency (MAF) in different browsers i.e 1000 genome, Exome aggregation consortium (ExAC) and genome aggregation database (gnomAD), so all those variants were excluded which have the minor allele frequency (MAF) greater than 0.01, so 188 total variations were left behind. Then filter were applied for the zygosity and all those variants were selected which are homozygous and all remaining were excluded, the total variants at this stage were 34. All the 34 variants at this stage were missense, so again insilico tools which are specially designed for missense mutation were used and finally one pathogenic variant were detected.

After applying of all filters, it was concluded from the exome sequence analysis that patient had missense mutation c.298T>C and amino acid p. (Ser100Pro) in the *SLC2A2* gene of proband (transcript ID: ENST00000314251.8) (Table.3.2). The variant is present in the exon 3 of *SLC2A2* gene, chromosome 3. This variant could be the hallmark of the disease. The mutation is novel, not reported in the literature and is absent in HGMD, gnomAD and Clinvar. For the verification of the variant various bioinformatics tools were used such as Mutation taster, Provean, SIFT and Polyphene. All these tools predicted that this variant (c.298T>C) might be disease causing and damaging.

Table.3.2. Mutation detected in the family

Exon No.	**Mutation**	**Sequence change**	**Mutation type**	**Amino acid change**
3	c.298T>C	TCT-CCT	Missense	p.(Ser100Pro)

3.5.1.2) Conservation analysis of the *SLC2A2* variant

SLC2A2 variant conservation analysis was done through constraint based multiple alignment tool (COBALT) of National Center for Biotechnology Information. It gives information for multiple protein sequence alignment by utilising local sequence and conserved domain similarities of proteins. Human *SLC2A2* protien amino acid sequence was aligned with the amino acid sequence of eight other organisms that is zebrafish, red jungle fowl, cattle, wild boar, western clot frog, monkey and chimpanzee respectively to examine the homology and sequence conservation as shown in (fig.3.3). The sequence in which a mutation is reported is remarkably conserved across all organisms, as can be seen in the picture below. Consequently, cell lines from all the organisms shown in the picture could be used to conduct functional research on this protein.

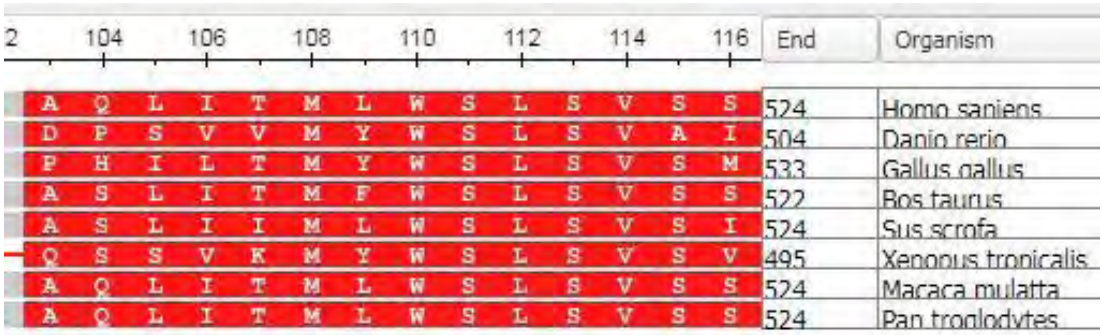

Figure.3.3. *SLC2A2* variant conservation analysis

Chapter 4

4) DISCUSSION

Fanconi-Bickel Syndrome (FBS) is a rare autosomal recessive condition of glucose metabolism. Symptoms secondary to glycogen accumulation, glucose metabolism, and renal tubular dysfunction result from a deficiency in the GLUT 2 receptors in the hepatocytes, pancreas, and renal tubules [152]. Carbohydrate metabolism characterised by hepatomegaly, fasting hypoglycemia, postprandial hyperglycemia, extensive proximal tubular dysfunction, rickets, and growth retardation (FBS). FBS is also known as glycogen storage disorder type XI, which was first identified by Guido Fanconi and Bickel in 1949 [23]. Abnormal glycogen aggregation in the liver and kidneys, which affects the body's use of galactose and glucose and results in proximal renal tubular failure, is the primary cause of FBS [153].

The *SLC2A2* gene, located on chromosome 3, which codes for the facultative glucose transporter 2 (GLUT2), is autosomal recessively inherited and causes FBS [25]. The encoded protein facilitates glucose transport in both directions and has been suggested as a glucose sensor [154]. On chromosome 3, the *SLC2A2* gene, which has 10 introns and 11 exons, is a member of the soluble carrier family 2 [47]. The liver, islet beta cells, gut, and kidney epithelium all contain an integral plasma membrane glycoprotein that is encoded by this gene. Facilitated bidirectional glucose transport is made possible by the encoded protein. Glucose Transporter 2 is another name for the gene (GLUT2). Clinical evaluation of the symptoms, biochemical analysis, and confirmation by molecular testing all play a role in the diagnosis [29].

There is no specific treatment, but it involves dietary changes to control glucose levels and limit galactose as well as compensating for electrolyte losses from the kidneys through supplementation with vitamin D and phosphorus [30, 155]. Although some patients pass away while still children, the outlook is generally good, and adult survival is conceivable [154]. Although the prevalence of FBS is not yet determined, it is thought to be highly rare given that there have only been less than 200 cases reported globally [32].

Researcher named horst bickel and guido Fanconi originally reported a patient in 1949 who had a rare symptoms of widespread renal tubulopathy and glycogen buildup in hepatorenal as

part of their research into the various clinical disorders linked to hyperaminoaciduria. The clinical signs of this patient have remained into maturity, and they are still present. This sickness can be viewed as a part of the larger category of conditions known as the glycogen storage diseases (GSD). According to the order of their initial descriptions or the discovery of the underlying problem, several authors have assigned numbers to certain members of this group. Hug coined the designation "GSD XI" in 1976 to describe a patient who displayed some clinical symptoms resembling those of Fanconi and Bickel's patient [33]. Hug later concluded that it was plausible that the patient's underlying condition was caused by a congenital phosphoglucomutase deficiency. Many reviewers have historically categorised their works in this way [34].

The gene *SLC2A2* is expressed in various human tissues. The kidney, pancreas, liver, and small intestine all exhibit high levels of the gene's expression. It expresses itself extensively in the liver because it is the primary organ for storing glucose and because it is essential for maintaining glucose homeostasis. *SLC2A2* is also expressed in the brain, lung, spleen, liver, heart, and smooth muscle, though not to the extent that was previously claimed [52]. Till date109 distinct mutations have so far been found in the *SLC2A2* gene. There are 109 mutations in all, of which 80 are directly linked to the Fanconi-Bickel syndrome. The remaining different types of mutations are linked to other illnesses, including diabetes, non-insulin-dependent diabetes mellitus, osteogenesis imperfecta, myelomeningocele etc (https://www.hgmd.cf.ac.uk).

In this research, a patient from pakistani family was studied, to recognize the main cause of the clinical indication and to help that family in the diagnosis of the disease. The patient was born to consanguineous parents and was 5 years old at the time of sampling. The proband had symptoms such as rickets (Weakened bones), short stature, hepatomegaly (enlarged liver), failure to thrive (growing slower than normal), abdominal distension, doll like facies and polyuria (excessive urination). The three generation pedigree was designed at that time and in that pedigree there were consagineous marriage in the 2nd generation of the pedigree. The parents and other siblings of the proband were healthy and phenotypically normal. The proband was examined by the doctor at the time of sampling.

Whole exome sequencing of the patient DNA recognized numerous variants in the exome. All these variants were filter out and for this purpose many filter were used i.e (filter for coding region, minor allele frequency (MAF), zygosity, and based on insilico tools) to find out the exact variant which are the main hallmark of the disease.

A novel variant c.298T>C was mark in the exon 3 of *SLC2A2* gene, chromosome 3. The mutation was missense and have effect on protein. The mutation is not present in the Human genome mutation database (HGMD) and Genome aggregation database (gnomAD). The mutation is also not described in clinvar and nor have been reported in the literature till now.

To verify the new variant's pathogenicity, we performed a bioinformatic investigation on it and checked on the online tools i.e MutationTaster, Polyphene, SIFT and provean. The mutation was predicted to cause disease and likely pathogenic as it is missense and very damaging.

The conservation analysis of the *SLC2A2* gene was performed using the constraint based multiple alignment tool (COBALT). The amino acid sequence of the *SLC2A2* protien were aligned with amino acid sequence of the eight other organisms i.e (red jungle fowl, wild boar, monkey, chimpanzee etc) respectively to examine the similarity and sequence conservation of the *SLC2A2.* The sequence in which the new variant is detected is conserved in all the organisms. Consequently, cell line from all those organisms can be used for the further functional based research on this protein.

Conclusion

The finding of our research is that the mutation in the *SLC2A2* gene is the main hallmark of the Fanconi Bickel syndrome. Pedigree and history of the family which are used in this study support the autosomal recessive pattern of inheritance, it will then further be validated by using Sanger sequencing. Our findings will be helpful in the future for genetic counselling, prenatal diagnosis and targeting of new pathways for therapeutic purposes. NGS made it possible to easily diagnose such type of rare and complex disorders.

Whole exome sequencing is a useful method for detecting and confirming the onset of disease. Additionally, a precise diagnosis of the condition and genetic counselling for those who are carriers of the disorder depend on the genetic study of the patient's healthy family members as well as the proband parents. Moreover, to understand the potential impact of the novel variant and the region in which it is present on the protein structure and function, protein expression studies should be conducted. The research may aid in a better understanding of the protein and the condition it is linked to, which will aid in the development of more effective treatment alternatives.

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