CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

1. Genetic disorder

A genetic disorder is an illness caused by abnormalities in genes or chromosomes from a discrete mutation in a single base in the DNA to a gross chromosome or set of chromosomes. Most disorders are quite rare and affect one person in every several thousands or millions. There are four different types of genetic disorders (1) single-gene, (2) multifactorial, (3) chromosomal, and (4) mitochondrial.

1.1 Single-gene (also called Mendelian or monogenic)

This type is caused by changes or mutations that occur in the DNA sequence of one gene. Genes code for the proteins for functions and even make up the majority of cellular structures. When a gene is mutated, its protein product can no longer carry out its normal function, causing a disorder. There are more than 6,000 known single-gene disorders, which occur in about 1 out of every 200 births. Single-gene disorders are inherited in recognizable patterns: autosomal dominant (AD), autosomal recessive (AR) and X-linked.

1.1.1 Autosomal dominant

Only one mutated copy of the inherited gene will be necessary for a person to develop a disorder. Each affected person usually has one affected parent. The autosomal dominant diseases have a 50-50 chance of passing the mutant gene and therefore the disorder onto each of their children. Examples of this type of disorder are Huntington's disease, Hyper IgE syndromes, Holt-Oram syndrome, etc.

1.1.2 Autosomal recessive

Two copies of the gene must be mutated for a person to be affected by an autosomal recessive disorder. An affected person usually has unaffected parents who each carry a single copy of the mutated gene (and are referred to as carriers). Two unaffected people who each carry one copy of the mutated gene have a 25% chance with each pregnancy of having a child affected by the disorder. Examples of this type of disorder are cystic fibrosis, sickle cell anemia, glycogen storage disease type II (Pompe disease)

1.1.3 X-linked

X-lined can be classified into 2 classes

1.1.3.1 X-linked dominant

X-linked dominant disorders are caused by mutations in genes on the X chromosome. Only a few disorders have this inheritance pattern. Males are more frequently affected than females, and the chance of passing on an X-linked dominant disorder differs between men and women. The sons of a man with an X-linked dominant disorder will not be affected, and his daughters will all inherit the condition. A woman with an X-linked dominant disorder has a 50% chance of having an affected daughter or son with each pregnancy. Some X-linked dominant conditions, such as Aicardi syndrome, are fatal to boys, therefore only girls have them (and boys with Klinefelter syndrome). Other examples of this type of disorder are X-linked hypophosphatemia (hypophosphatemic rickets, vitamin D-resistant rickets), Aicardi syndrome, and Rett's syndrome.

1.1.3.2 X-linked recessive

X-linked recessive disorders are also caused by mutations in genes on the X chromosome. Males are more frequently affected than females, and the chance of passing on the disorder differs between men and women. The sons of a man with an X-linked recessive disorder will not be affected, and his daughters will carry one copy of the mutated gene. With each pregnancy, a woman who has an X-linked recessive disorder (X^rX^r) has a 50% chance of having sons who are affected and a 50% chance of having daughters who carry one copy of the mutated gene. Examples of this type of disorder include hemophilia A, Duchenne muscular dystrophy, red-green color blindness, and X-linked adrenoleukodystrophy (X-ALD).

1.2 Multifactorial (polygenic or complex disorders)

Genetic disorders may also be complex, multifactorial or polygenic. This means that they are likely associated with the effects of multiple genes in combination with lifestyle and environmental factors. Although complex disorders often cluster in families, they do not have a clear pattern of inheritance. This makes it difficult to determine a person's risk of inheriting or passing on these disorders. Complex disorders are also

difficult to study and treat because the specific factors that cause most of these disorders have not yet been identified. Examples of disorders in this group include heart disease, systemic lupus Erythematosus (SLE), Alzheimer's disease, arthritis and diabetes.

1.3 Chromosomal

Chromosomes are distinct structures made up of DNA and protein, located in the nucleus of each cell. Because chromosomes are carriers of genetic material, such abnormalities in chromosome structure as missing or extra copies or gross breaks and rejoinings (translocations) can result in diseases. Some types of major chromosomal abnormalities can be detected by microscopic examination. Down syndrome is a common disorder that occurs when a person has three copies of chromosome 21.

1.4 Mitochondrial

Mitochondria are small round or rod-like organelles involved in cellular respiration and found in the cytoplasm of plant and animal cells. They can convert the energy of food molecules into the ATP that powers most cell functions. Mitochondrial diseases, rare type of genetic disorders, are caused by mutations in the nonchromosomal DNA of mitochondria. The effects of mitochondrial disease can be quite varied. The mutation that in one person may cause liver disease might in another person cause a brain disorder. Some minor defects cause only "exercise intolerance", with no serious illness or disability. Other defects can more severely affect the operation of the mitochondria and can cause severe body-wide impacts. These diseases that have neuromuscular symptoms are often referred to as a mitochondrial myopathy.

Most genetic disorders are cause by mutations in some genes. Mutation is a permanent change to the nucleotide sequence in the DNA sequence of a gene. It can be caused by copying errors in the genetic material during cell division. Mutations can be subdivided into germ line mutations, which can be passed on to descendants through the reproductive cells, and somatic mutations.

- **Heterozygous mutation** is a mutation of only one allele.
- **Homozygous mutation** is an identical mutation of both the paternal and maternal alleles.

- **Hemizygous mutation is** a mutation on only a single copy of a gene instead of the customary two copies. All of the genes on the single X chromosome in the male are in the hemizygous state
- **Compound heterozygous** mutations comprise two different mutations in the paternal and maternal alleles.

A mutation that is not inherited from either parent is called a *de novo* mutation.

2. Effect of mutation

Mutations can be classified based on their effect into 2 classes.

1. Effect on structure

The sequence of a gene can be altered in a number of ways. Gene mutations have varying effects on health depending on where they occur and whether they alter the function of essential proteins. Structurally, mutations can be classified as:

1.1 Small-scale mutations

It will affect one or a few nucleotides, including

1.1.1 **Point mutations**, often caused by chemicals or malfunction of DNA replication, exchange a single nucleotide for another. These changes are classified as transitions or transversions. Most common is the transition that exchanges a purine for a purine (A ↔ G) or a pyrimidine for a pyrimidine (C ↔ T). Less common is a transversion, which exchanges a purine for a pyrimidine or a pyrimidine for a purine (C/T ↔ A/G). Point mutations that occur within the protein coding region of a gene may be classified into three kinds, depending upon what the erroneous codon codes for :

- Silent mutations: which code the same amino acid.
- Missense mutations: which code a different amino acid.
- Nonsense mutations: which code a stop causing truncation of the protein

1.1.2 **Insertions**, add one or more extra nucleotides into the DNA. They are usually caused by transposable elements, or errors during replication of repeating elements. Insertions in the coding region of

a gene may alter splicing of the mRNA, or cause a shift in the reading frame (frameshift)

1.1.3 **Deletions**, remove one or more nucleotides from the DNA. Like insertions, these mutations can alter the reading frame of the gene.

1.2 Large-scale mutations in chromosomal structure, including

1.2.1 **Amplifications** (or gene duplications) leading to multiple copies of all chromosomal regions, increasing the dosage of the genes located within them.

1.2.2 **Deletions of large chromosomal regions**, leading to loss of the genes within those regions.

1.2.3 **Chromosomal translocations**: interchange of genetic parts from non homologous chromosomes.

1.2.4 **Chromosomal inversions**: reversing the orientation of a chromosomal segment.

1.2.5 **Loss of heterozygosity**: loss of one allele, either by a deletion or recombination event, in an organism that previously had two different alleles

2. Effect on function

Mutations are effect on functions of gene, can classifies as :

2.1 **Loss-of-function mutations** are the result of gene product having less or no function. When the allele has a complete loss of function (null allele), it is often called an amorphic mutation. Phenotypes associated with such mutations are most often recessive. Exceptions are when the organism is haploid, or when the reduced dosage of a normal gene product is not enough for a normal phenotype (this is called haploinsufficiency).

2.2 **Gain-of-function mutations**, change the gene product such that it gains a new and abnormal function. These mutations usually have dominant phenotypes (neomorphic mutation).

2.3 **Dominant negative mutations** (also called antimorphic mutations) have an altered gene product that acts antagonistically to the wild-type allele. These mutations usually result in an altered molecular function (often inactive) and

are characterized by a dominant or semi-dominant phenotype. In humans, Hyper IgE syndromes is an example of a dominant negative mutation occurring in an autosomal dominant disease.

Mutations on codon of DNA sequence of gene that can alter the amino acid sequence of the protein encoded. It can be classified as:

1. **frameshift mutation** is a mutation caused by insertion or deletion of a number of nucleotides that is not evenly divisible by three from a DNA sequence. Due to the triplet nature of gene expression by codons, the insertion or deletion can disrupt the reading frame, or the grouping of the codons, resulting in a completely different translation from the original.
2. **Missense mutations** or *nonsynonymous mutations* are types of point mutations where a single nucleotide is changed to cause substitution of a different amino acid. This in turn can render the resulting protein nonfunctional.
3. **nonsense mutation** is a point mutation in a sequence of DNA that results in a premature stop codon, or a *nonsense codon* in the transcribed mRNA, and possibly a truncated, and often nonfunctional protein product.
4. **Silent mutations** are mutations that do not result in a change to the amino acid sequence of a protein. They may occur in a region that does not code for a protein, or they may occur within a codon that does not alter the final amino acid sequence.

At present, with more than 3,000 genes identified, and over 4,000 diseases caused by genetic disorders, mutation detection has become an increasingly important to detect genetic disorders. Available types of testing can be divided into

Newborn screening: is used just after birth to identify genetic disorders that can be treated early in life of baby.

Diagnostic testing: is used to diagnose or rule out a specific genetic or chromosomal condition. In many cases, genetic testing is used to confirm a

diagnosis. The results of a diagnostic test can influence a person's choices about health care and the management of the disease.

Carrier testing: is used to identify people who carry one copy of a gene mutation. This type of testing is offered to individuals who have a family history of a genetic disorder and to people in some ethnic groups with an increased risk of specific genetic conditions. If both parents are tested, the test can provide information about a couple's risk of having a child with a genetic disorder.

Prenatal testing: is used to detect changes in a fetus's genes or chromosomes before birth. This type of testing is offered to couples with an increased risk of having a baby with a genetic or chromosomal disorder. It cannot identify all possible inherited disorders and birth defects.

Preimplantation genetic diagnosis: Genetic testing procedures that are performed on human embryos prior to the implantation as part of an in vitro fertilization procedure.

Predictive and presymptomatic testing: are used to detect gene mutations associated with disorders that appear after birth, often later in life. These tests can be helpful to people who have a family member with a genetic disorder. It can determine whether a person will develop a genetic disorder. The results of predictive and presymptomatic testing can provide information about a person's risk of developing a specific disorder and help with making decisions about medical care.

Most of the time, practical technique for testing is used to find changes that are associated with inherited disorders. It is most helpful in providing diagnosis, early treatment, or prenatal diagnosis that might help a person make good life decisions. In this time, several hundred genetic tests are currently in use, and more are being developed.

This research will evaluate some practical techniques for mutation detection in patients who were clinically diagnosed with genetic disorders at Pediatric Clinic of the

King Chulalongkorn Memorial Hospital. Selection criteria were based on clinical presentations.

3. X- Linked Adrenoleukodystrophy (X-ALD)

Adrenoleukodystrophy (ALD) is a rare inherited disorder that leads to progressive brain damage, failure of the adrenal glands and eventually death. The prevalence of X-linked adrenoleukodystrophy is approximately 1 in 21,000 males[1]. ALD is one disease in a group of inherited disorders called leukodystrophies. Adrenoleukodystrophy progressively damages the myelin, a complex fatty neural tissue that insulates many nerves of the central and peripheral nervous systems, eventually destroying it. Without myelin, nerves are unable to conduct an impulse, leading to increasing disability as myelin destruction increases and intensifies.

An essential protein, called a transporter protein, is missing in sufferers. This protein is needed to carry very long-chain fatty acids to break down in peroxisomes that found in the normal diet. The lack of this protein can give rise to accumulation of very long-chain fatty acids 24–30 carbon atoms (VLCFA) in the body, which can damage the brain and the adrenal gland. The elevation in VLCFA was originally described by Moser *et al.* in 1981.[2]

Patients with X-linked ALD are all male, but about one in five women carrying the disease develop a milder form in adult life, called *adrenomyeloneuropathy*. There are several different types of the disease which can be inherited, but the most common form is an X-linked condition.

The clinical presentation is largely dependent on the age of onset of the disease. Symptoms normally start between the ages of 4 and 10 and include loss of previously acquired neurologic abilities and die soon after. This severe form of the disease was first described by Ernst Siemerling and Hans Gerhard Creutzfeldt.

The diagnosis is established by clinical findings and the detection of serum very long-chain free fatty acid levels[2], MRI examination which reveals white matter abnormalities, neuro-imaging and mutation analysis in the *ABCD1* gene.

The ALD gene (*ABCD1*), discovered in 1993, is located on chromosome Xq28. The gene consists of 10 exons spanning 20 kb of DNA and encodes a 4.2 kb of RNA.

It coded for a protein of 745 amino acid that was a member of a family of transporter proteins. This gene has 2 domains, as shown in figure 1.

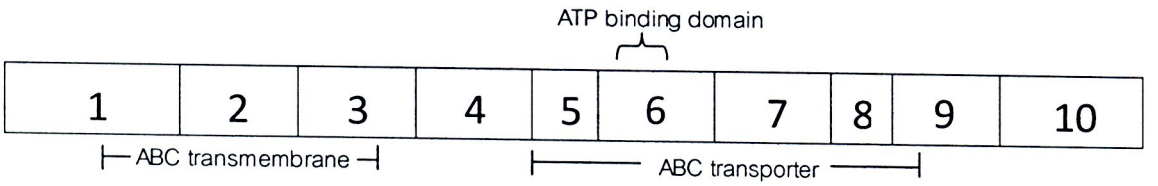

Figure 1. All exons and domains of *ABCD1*

ABCD1 or "ATP-binding cassette, subfamily D, member 1" codes for a protein that transfers fatty acids into peroxisomes, that is the cellular organelles where the fatty acids undergo β-oxidation.[3] A dysfunctional *ABCD1* gene, leads to the accumulation of very long chain fatty acids (VLCFA) because VLCFA cannot be transferred to peroxisomes, which can damage the myelin and neural tissues.

At present, more than 500 mutations in the *ABCD1* gene have been identified. Each mutation was found private and spreaded throughout the *ABCD1* gene.[4]

4. Glycogen Storage Disease Type II (Pompe disease)

Pompe disease is a neuromuscular autosomal recessive metabolic disorder in the family of lysosomal storage diseases caused by a deficiency of the enzyme Acid alpha-glucosidase (EC 3.2.1.20) in lysosomes, which is needed to break down glycogen (a long branched glucose polymer and stored form of sugar used for energy), leads to lysosomal accumulation of glycogen in many different cell types.[5] The disease is named after Johann Pompe, who characterized it in 1932.

The clinical features of Pompe disease have been divided into three forms defined by age of onset and progression of symptoms. Infantile, or early onset, is noticed shortly after birth. Symptoms include severe lack of muscle tone, weakness, and enlarged liver (hepatomegaly) and heart. Mental function is not affected. Development appears normal for the first weeks or months but slowly declines as the disease progresses. Most children die from respiratory or cardiac complications before 2 years of age.[6] Juvenile onset symptoms appear in early to late childhood and include progressive weakness of respiratory muscles in the trunk, diaphragm and lower limbs.

Intelligence is normal. Finally, adult onset symptoms also involve generalized muscle weakness and wasting of respiratory muscles in the trunk, lower limbs, and diaphragm.

Diagnosis and testing: Type II GSD can be diagnosed by determining the activity of the specific enzyme acid alpha glucosidase testing that can be performed on blood samples, muscle biopsy, cultured cells from a skin biopsy, test accumulation of glycogen in lysosomes, and mutation analysis in the *GAA* gene.

The disorder is estimated to occur in about 1 in 40,000-300,000 live births. It has an autosomal recessive inheritance pattern. Children have a 1 in 4 chance of inheriting the disorder when both parents carry one copy the defective gene.

GAA (acid alpha glucosidase) gene is mapped to human chromosome 17q25. This gene consists of 20 exons spanning 18.4 kb of DNA and encodes a 3.8 kb of RNA. It coded for a protein of 952 amino acids. This gene has lysosomal alpha-glucosidase domain, as shown in figure 2.

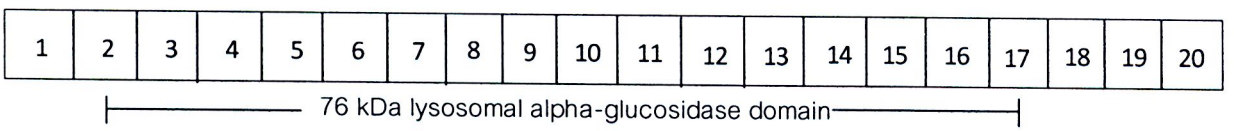

Figure2. All exons and domains of *GAA*

Dysfunctional *GAA* gene, leads to accumulation of glycogen in many different cell types because enzyme acid alpha-glucosidase(GAA) cannot break down glycogen. More than 200 mutations in the *GAA* gene have been identified.

5. Hyper-IgE syndrome (HIES)

HIE or Job's syndrome, is a complex primary immunodeficiency characterized by high levels of serum IgE and recurrent bacterial infection. HIES can be classified into 2 types.[7]

Type 1 HIES (Autosomal Dominant type)

This group of HIES represents the most common and typical form and includes sporadic and familial autosomal dominant type.[8] Its clinical presentations include recurrent skin and pulmonary infections, atopic dermatitis, elevated serum IgE levels. By age 16 years, all patients show distinctive facial appearance: skeletal and dental

abnormalities, asymmetric facial appearance, deep-set eyes, and parenchymal lung abnormality.[9] Recent studies have showed mutations in the signal transducer and activator of transcription-3 (*STAT3*) gene as major causes of AD and sporadic HIES.[10,11]

Type 2 HIES (Autosomal Recessive type)

The patients showed no apparent abnormalities in their skeletal and dental systems but suffered from recurrent and severe infections with *S. aureus*, *S. pneumonia*, or *H. influenzae*, as observed in type 1 HIES.[12] Most of the type 2 HIES patients also suffered from severe viral infections without pneumatoceles, which were not observed in type 1 HIES. In 2006, Minegishi et al. have recently identified a homozygous mutation of the tyrosine kinease-2 gene (*TYK2*) in a patient with AR-HIES.[13] The classification of HIES can be summarized in Table 1

Table 1. A new classification of Hyper-IgE syndrome

Type	Inheritance	Distinguished clinical finding	Genes
Type 1 (multisystem)	Sporadic (most cases), familial with AD inheritance (rare)	skeletal and dental abnormalities, parenchymal lung abnormality	*STAT3*
Type 2 (nonskeletal)	Familial with AR inheritance	severe viral infections, possible CNS involvement	*TYK2* (JAK)

Diagnosis of HIES can be made clinically by eye examination which may reveal signs of dry eye syndrome. A physical exam may show signs of osteomyelitis, and recurrent sinus infections. A chest x-ray may reveal lung abscesses. Tests used to confirm a diagnosis include absolute eosinophil count, complete blood count, serum globulin electrophoresis to look for high blood IgE levels and genetic testing for a mutation in the *STAT3* or/and *TYK2* genes by direct sequencing.

The cytokine can regulate cells by JAK (TYK2) and STAT signaling pathways as shown in figure 3.

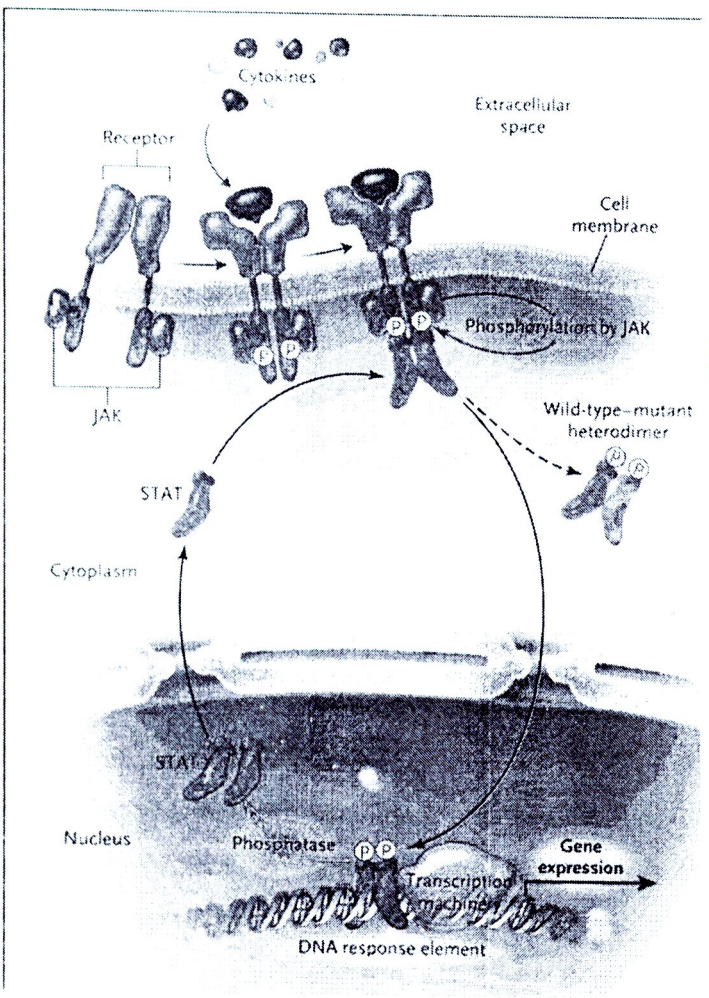

Figure 3. JAK-STAT signaling pathway[14]

From figure 3, when cytokines bind with receptors, receptors will sent the signal to JAK protein. The JAK protein will be phosphorylated and aggregate to the binding site for STAT. The STAT protein will be phosphorylated by JAK and form dimers and accumulate in the nucleus. The STAT dimers will bind to the promoters of the target genes in nucleus and activate transcription. If JAK or STAT does not function properly, it can have an affect on the regulation of cytokines causing HIES.[14]

In this study, the patient had clinical features consistent with type 1 HIES. Therefore, mutation analysis in the *STAT3* gene was performed. *STAT3* is mapped to human chromosome 17q21. This gene consists of 24 exons spanning 30 kb of DNA and encodes a 4.7 kb of RNA. Its protein contains 770 amino acids. This gene has 6 domains as shown in figure 4.

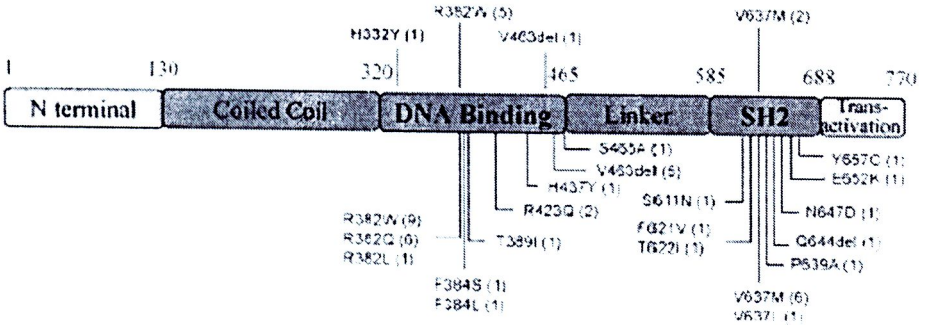

Figure4. Mutations detected in the *STAT3* gene.[15]

From the figure 4, all mutations described recently were localized to regions encoding the DNA binding domain and the SH2 domain.[10] We therefore could select the regions for primer design to detect mutations in the *STAT3* gene.

6. Holt-Oram syndrome (HOS)

Holt-Oram syndrome or heart-hand syndrome has an autosomal dominant inheritance pattern. The disorder is estimated to occur in about 1 in 100,000 live births.[16] HOS was first reported in 1960 by Holt, M. and Oram, S. They found patients with atrial septal defect and upper limb abnormality in 4 generations.[17] In 1997, Bosson et al. found the T-box transcription factor 5 (*TBX5*) gene on chromosome 12q24.1 responsible for this syndrome and 70%of HOS patients harbored mutations in this gene.[18] The *TBX5* gene, a member of T-box gene family, is important transcription factor of heart and upper limb development in embryogenesis. In 2001, Bruneau, B.G et al studied homozygous knockout mice ($Tbx5^{-/-}$) and found that they died early in embryogenesis while heterozygous knockout mice ($Tbx5^{-/+}$) appropriately mimicked Holt-Oram syndrome.[19]

Clinical features of HOS include at least one limb abnormality that affects bones in the wrist, a missing thumb or a thumb that looks like a finger, partial or complete

absence of bones in the forearm, and an underdeveloped bone of the upper arm. In some cases, only one arm and/or hand is affected.

About 75 percent of individuals with Holt-Oram syndrome have congenital heart defects such as atrial septal defects (ASD), or ventricular septal defects (VSD). The most common problem is a defect in the muscular wall, or septum. Sometimes people with Holt-Oram syndrome have cardiac conduction disease, which is caused by abnormalities in the electrical system that coordinates contractions of the heart chambers leading to problems such as a slow heart rate or a rapid and ineffective contraction of the heart muscles.

The diagnosis is established by clinical findings, radiographs of affected bones, echocardiograms to detect congenital heart defects and genetic testing for the analysis of the mutation in *TBX5*.

TBX5 is mapped to human chromosome12q24.1. This gene consists of 9 exons spanning 54 kb of DNA and encodes a 3.9 kb of RNA. It codes for a protein of 518 amino acids. This gene has 2 domains as shown in figure 5

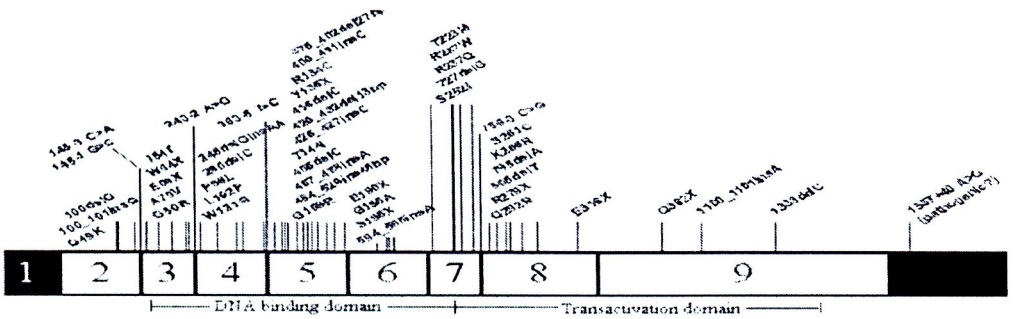

Figure 5. TBX5 Domains and mutations of *TBX5*.[16]

From figure 5, mutations in the *TBX5* gene can be found at exons 2-9 with the most commonly detected is a point mutation.[16] If mutations occur on the DNA binding domain. TBX5 transcription factor cannot bind to the promoter of the target genes. Mutations on transactivation domain lead to a decrease of efficiency of transcription.[20]

A previous cytogenetic report of a de novo pericentric inversion of chromosome 20q13.2, where *SALL4* was located, revealed that it was associated with a clinical presentation of bilateral absence of the thumbs and an atrial septal defect.[21] This phenotype was taken to represent Holt-Oram syndrome and the suggestion was made that there were likely to be other causes of apparent Holt-Oram syndrome but without *TBX5* mutation. From a report in 2003, they suggested that patients with clinical features consistent with Holt-Oram syndrome but without mutations in *TBX5*, *Sall4* might be another candidate gene for analysis.[22]

SALL4 is mapped to human chromosome 20q13.2. This gene consists of 4 exons spanning 18.4 kb of DNA and encodes a 3.4 kb of RNA. It codes for a protein of 1053 amino acids. This gene has a zinc finger domain as shown figure 6.

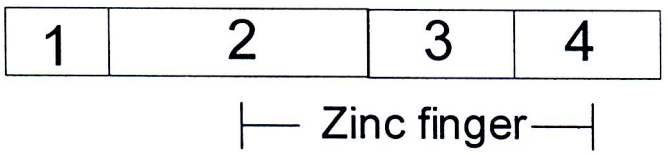

Figure 6. Structure of SALL4

7. Systemic Lupus Erythematosus (SLE)

Systemic lupus erythematosus is a potentially severe autoimmune disease. The abnormalities are caused by defects in apoptosis leading to the accumulation of autoreactive T and B cells resulting in damage to multiple organs.

Clinical features of SLE can be characterized by arthritis, proximal muscle aching or weakness, recurrence of inflammatory rashes, patchy or diffuse loss of hair, oral or nasal ulcerations, pericardial pain, evidence of kidney disease.

A number of tests are required before SLE can be diagnosed definitively. The diagnosis is established by tests for autoantibodies for measuring ANA, antiphospholipid antibodies, antibodies to SR proteins. Other tests include blood tests to measure C3, C4, C1q, and CH50 levels and protein in urine

The exact etiology of SLE has not been elucidated, but it is obvious that genetic factors, gender, and environment are involved in its pathogenesis.[23] It is a polygenic

disease, and as many as 30 susceptibility loci with possible links to its pathogenesis have been identified in mice.[24] In mice, mutations in the prototype proapoptotic molecules *Fas* or *Fas ligand* (*FasL*) lead to the occurrence of an SLE-like syndrome[25], but human SLE patients rarely have mutations in *Fas* or *FasL*.[26] However, it is possible that some molecules in the Fas/FasL pathway are SLE risk factors.

Decoy receptor 3 (DcR3)/TR6 is a secreted protein belonging to the tumor necrosis factor (TNF) receptor family. It binds to Fas ligand (FasL), LIGHT, and TL1A that are all TNF family members. It was noted that soluble or solid phase DcR3-Fc co-stimulated proliferation, lymphokine production and cytotoxicity of mouse and human T cells upon T-cell receptor (TCR) ligation. Recently, the investigators found that the serum level of soluble DcR3 was higher in SLE patients than in healthy control subjects. Taken together, the investigators propose that in autoimmune diseases, SLE activated T cells secrete more DcR3 than non-autoimmune controls, which may in turn costimulate T cells further and cause dysregulated lymphocyte activation. With the aim to establish the possible correlation between DcR3 and autoimmune phenotypes in children, we analyzed the serum DcR3 level and performed mutation analysis in the *DcR3* gene in children with SLE. The genetic analysis on the *DcR3/TR6* gene and circulating DcR3 level will be compared between SLE and non-autoimmune control subjects.

The disease prevalence is ~0.05% in the general population, with 80-90% of patients being women.[27] DcR3 is mapped to human chromosome at 20q13. This gene consists of 3 exons spanning 3.7 kb of DNA and encodes a 1.1 kb of RNA. It codes for a protein of 300 amino acids. This gene has 3 domains as shown in figure 7.

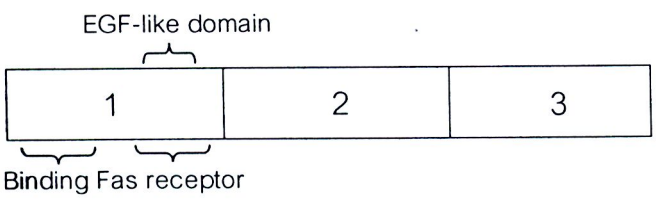

Figure 7. Structure of DcR3

Practical techniques of genetic testing are very helpful in providing additional information for patients with genetic diseases and family members leading to more

accurate diagnosis, effective therapy and prevention. This study presented practical techniques for mutation detection in the *ABCD1*, *GAA*, *STAT3*, *TBX5*, *SALL4*, *DcR3* genes responsible for ALD, Pompe disease, HIE, HOS, and SLE, respectively.