CHAPTER I
INTRODUCTION

A. Introduction to Ectodermal Dysplasia

Background

The ectodermal dysplasias (EDs) comprise a large, heterogeneous group of inherited disorders that share primary defects in the development of 2 or more tissues derived from embryonic ectoderm. The tissues primarily involved are the skin, hair, nails, eccrine glands, and teeth. Current classification of EDs is based on clinical features. Pure EDs are manifested by defects in ectodermal structures alone, while ED syndromes are defined by the combination of ectodermal defects in association with other anomalies.

Pathophysiology

ED results from the abnormal morphogenesis of cutaneous or oral embryonal ectoderm (ie, hair, nails, teeth, eccrine glands).

Hair defects: The scalp hair is absent, sparse, fine, lightly pigmented, or abnormal in texture. The hair may also be fragile and unruly, sticking out in all directions and difficult to comb. The hair is dry because the oil glands are absent or poorly developed.

Some defects of the hair are evident at birth, while others are not noted until later in life. Hair growth is slow and haircuts are not often needed. After puberty hair growth improves in some persons.

The eyebrows, eyelashes, and other body hair may also be absent or sparse, but beard growth in males is usually normal.

A reduction in the number of hair follicles in conjunction with structural hair shaft abnormalities may be seen. Structural hair shaft abnormalities may

result from aberrations in hair bulb formation and include longitudinal grooving, hair shaft torsion, and cuticle ruffling. Hair bulbs may be distorted, bifid, and small.

Eccrine sweat gland defects: The sweat glands are absent, reduced in number, or may not function normally, particularly in patients with hypohidrotic ED. Diminished or absent sweating is a common problem. Reduced sweating may result in very high fevers, because the body regulates its temperature by sweating. Often, the first clue that the sweat glands are absent or are not functioning normally is an elevated temperature.

Elevations in body temperature are often caused by high environmental temperatures, excessive activity, or heavy clothing. When the body temperature is elevated, the skin feels dry, hot and may be flushed or pale.

Other secretory gland defects: Hypoplasia of the salivary and lacrimal glands may occur. In some patients, mucous glands may be absent in the upper respiratory tract and in the bronchi, esophagus, and duodenum.

The generalized underproduction of body fluids leads to several problems. Saliva is sparse, causing problems with chewing, tasting, and swallowing foods. The mucous secretions of the nose are excessively thick, forming a crusty mass. Nasal infections are common. A hoarse, raspy voice is common. Abnormal ear wax production may be noticed in some people with ectodermal dysplasia. The most frequent problem is accumulation of wax in the ear canal. Hearing loss may occur secondary to impacted wax or to nerve degeneration.

Tears are reduced, causing irritation of the eyes, conjunctivitis, and sensitivity to sunlight. There may be cloudy corneas or cataracts.

Dental defects: Abnormal morphogenesis or absence of teeth may occur. The teeth are missing altogether or reduced in number. Teeth that are present are

widely spaced, tapered, or malformed. In persons with some types of ectodermal dysplasia, the enamel (outer layer of the teeth) is defective and there may be an excessive number of cavities. When teeth are missing the jawbones in which they are ordinarily embedded do not develop well, leading to a typical aged appearance in the face.

Nail dystrophy: Most people with ectodermal dysplasia do not have nail abnormalities, although the nails are frequently dry and rough. A distinctive finding in one of the forms of ectodermal dysplasia is a short nail that fails to grow to the end of the finger. In others, the nails may be thin and fragile, thick and distorted, or brittle and slow- growing. Nails with any of the listed abnormalities may be prone to infection.

There are many different types of ectodermal dysplasia. Multiple genes have been discovered that cause ectodermal dysplasias. The most common form of ectodermal dysplasia is linked to the X chromosome and usually affects men. Another form of the disease affects men and women equally.

X-linked hypohidrotic ectodermal dysplasia belongs to the group of diseases known as ectodermal dysplasias. Alternative names for the disorder are anhidrotic ectodermal dysplasia (EDA I) and Christ-Siemens-Touraine syndrome.

X-linked recessive hypohidrotic ED is caused by mutations in *EDA*, which encodes the ectodysplasin protein, a soluble ligand that activates the NF-kappa B and JNK/c-fos/c-jun signaling pathways. Ectodysplasin is important in promoting cell survival, growth, and differentiation. Female carriers may display a blaschkoid distribution of hypohidrosis as a result of lyonization and somatic mosaicism for the abnormal X chromosome. Autosomal recessive and autosomal dominant forms of hypohidrotic ED have been reported but are rare. Intelligence is normal.

Symptoms

An individual with hypohidrotic ectodermal dysplasia either has no sweat glands or their function is reduced. As a result, the natural heat regulation which is needed to lower the body temperature in warm environments or in cases of fever is unsatisfactory. The inability to sweat may cause abnormally elevated body temperatures, especially in children, and even mild infections may induce a high fever. It is important to treat the fever to avoid the risk of complications. Adults with the syndrome may also have problems in the summer, in hot indoor environments and during physical exercise, and children can have difficulties participating fully in physical education or in team sports. Signs of overheating include headache, irritability, sleepiness, fainting and, in severe cases, cramps. Overheating (heatstroke) is a serious condition that may even be life-threatening. It is caused for example by heat wave weather or severe infections. In addition to being heat sensitive, some individuals with the disorder also have reduced development of subcutaneous fat, which means that they are sensitive to cold weather as well.

A common feature is that some or even all of the teeth are missing in both the primary and the permanent dentition, and that the first primary teeth erupt six to twelve months later than normal. The teeth are often unusually small and the front teeth can have an aberrant crown form (peg shape). There are almost always more teeth present in the upper jaw, and in some cases there are no teeth at all in the lower jaw. Tooth agenesis (the absence of tooth formation) also results in decreased jawbone growth in the edentulous areas, and in individuals with few teeth the palate is often flat and the face height low.

The skin is thin, fair and smooth. It is also dry, owing to the absence of sebaceous glands and the reduced number of sweat glands. Individuals with the syndrome often have atopic eczema. The skin around the eyes often looks darker owing to increased keratinization, and the nails may be thin and brittle.

Hair on the head is sparse, and it is often fair, thin, and coarse in structure. The hair tends to grow very slowly, and sometimes boys develop no hair at all on their heads in childhood, although some may come during puberty. There may be no hair at all on arms and legs, while the growth of beard is normal. The hair is often dry owing to the absence of sebaceous glands in the scalp, and dandruff is common. Most patients have fine, sparse, lusterless, fair hair; therefore, little pigmentation in the hair shaft is observed microscopically and the medulla is often discontinuous. When medullation is present, a "bar code" appearance is often seen. Onychodystrophy is common. Extensive scaling of the skin and unexplained pyrexia secondary to anhidrosis may occur in the neonatal period. The development of a chronic eczematous dermatitis is common.

Some individuals have dry eyes, owing to the reduced function of the tear glands or narrow tear ducts. This may result in inflammations of the eyes or hypersensitivity to light, and there is a risk of damage to the cornea.

The ear wax can be thick and sticky and ear wax build-up or clogged Eustachian tubes in auricular infections may cause hearing problems. There is also an increased risk of middleear infections.

A reduction of mucous and saliva-producing glands causes the mucous membranes in the nose and throat to dry out, which increases the risk of nose and throat infections. Foul-smelling dry scabs in the nose can also develop, and the ability to smell and taste may be impaired. When the throat is dry the voice becomes hoarse and it may be painful to speak. The mouth may also be dry and the reduced salivary function makes it difficult to eat, as it is difficult to soften and swallow food. Mouth dryness can also affect speech, and it increases the risk of caries.

Individuals with the full expression of the syndrome have characteristic facial features: a high forehead, a low nasal bridge, protruding lips and low facial height.

Some individuals suffer from constipation, owing to a low number of mucous-producing glands in the intestine. The nipples may be underdeveloped (hypoplastic) or absent.

The risk of overheating when the infant has a temperature or the environment is too warm is particularly great during the first year in life, and early diagnosis is important in order to prevent irreversible complications. However, at birth the facial appearance does not distinguish a child with hypohidrotic ectodermal dysplasia from other newborn babies, even if they have thin, very dry skin and little or no hair on the head. Therefore, in most cases children are not diagnosed until twelve to eighteen months of age when they still have no teeth, or when the first tooth erupts and has an aberrant, conical shape.

Mortality/Morbidity

Morbidity and mortality is related to the absence or dysfunction of eccrine and mucous glands. Beyond early childhood, life expectancy ranges from normal to slightly reduce.

Intermittent hyperpyrexia may occur in infants with decreased sweating. The mortality rate approaches 30%. Recurrent high fever may also lead to seizures and neurological sequelae.

Pharyngitis, rhinitis, cheilitis, and dysphagia may result from reduced numbers of functional mucous glands in the respiratory and gastrointestinal tracts.

Growth failure is common.

Severe inflammatory scalp dermatitis with erosions may result in frequent infections and cause scarring alopecia in patients with AEC (Hay-Wells) syndrome and Rapp-Hodgkin syndrome.

Sex

HED is usually inherited as an X-linked recessive genetic trait; in such cases, the disorder is fully expressed in males only. However, females who carry a single copy of the disease gene (heterozygote carreirs) may exhibit some of the symptoms and findings associated with the disorder. These may include absence and/or malformation of certain teeth, sparse hair, and/or reduced sweating. Researchers also have reported cases in which HED appears to be inherited as an autosomal recessive genetic trait. In such cases, the disorder is fully expressed in both males and females.

History

Individuals affected by ED have abnormalities in different ectodermal structures. Some ED types are mild, while others are devastating. Obvious manifestations of the disorders are not clinically apparent in most newborns. Dental, hair, and nail anomalies usually become evident during infancy or childhood. A family history of similar clinical features is helpful.

The typical facies, which is often not recognized until infancy, is characterized by frontal bossing; sunken cheeks; saddle nose; thick, everted lips; wrinkled, hyperpigmented periorbital skin; and large, low-set ears. Dental manifestations include conical or pegged teeth, hypodontia or complete anodontia, and delayed eruption of permanent teeth.

Complications:

Patients with ectodermal dysplasia may have absent or decreased sweating because of a lack of sweat glands. Children with the

disease may have difficulty controlling fevers. Mild illness may produce extremely high fevers, because their skin can't sweat and control temperature properly.

Affected adults are unable to tolerate a warm environment and require special measures to maintain a normal body temperature.

Patients may have chronic nasal infections with foul-smelling discharge and increased lung infections.

The skin is thin with light coloring. Hair may be absent or very thin. Teeth develop abnormally, and many teeth are missing.

EDA gene

Official Symbol: *EDA* and Name: ectodysplasin A

Gene type: protein coding

Gene name: EDA

Gene description: ectodysplasin A

Gene aliases: ED1; HED; EDA1; EDA2; XHED; XLHED; ED1-A1; ED1-A2

Summary: The protein encoded by this gene is a type II membrane protein that can be cleaved by furin to produce a secreted form. The encoded protein, which belongs to the tumor necrosis factor family, acts as a homotrimer and may be involved in cell-cell signaling during the development of ectodermal organs. Defects in this gene are a cause of ectodermal dysplasia, anhidrotic, which is also known as X-linked hypohidrotic ectodermal dysplasia. Several transcript variants encoding many different isoforms have been found for this gene.

The *EDA* gene responsible for the ectodermal dysplasia was originally isolated by positional cloning. Additional exons of the *EDA* gene have recently been identified, bringing the total number of exons in the gene to twelve. Mutations in affected individuals have been characterized, and interruption of

the orthologous gene in mouse leads to the Tabby phenotype. The Tabby mice have a mutation in the X chromosome region that is the mouse equivalent of the human *EDA* gene location. The mice are afflicted with some of the same symptoms that appear in humans, including missing sweat glands and abnormally shaped and/or missing teeth. Because affected individuals have sparse hair, rudimentary teeth, and no sweat glands, and Tabby mice show similar defects, the gene is believed to function at an early stage in ectodermal development, possibly at a branch point.

Some hints as to the function of the EDA protein have been gained by findings that it associates with the cell membrane and may participate in the regulation of cell-cell or cell-matrix interactions. Consistent with a role in such interactions, exons of the gene encode collagen-like repeat motifs that have been shown to form collagenous trimers in the extracellular domain of the EDA protein. Studies of *EDA* gene expression and protein function have been complicated by the fact that the EDA transcript undergoes alternative splicing and is capable of forming eight distinct isoforms, many of which can be detected by reverse transcriptase-polymerase chain reaction (PCR) in a variety of tissues. In addition, *in situ* hybridization and immunohistochemical analysis of various human embryonic, fetal, and adult tissues have demonstrated that the *EDA* gene and protein are expressed at low levels in several tissues unaffected in *EDA* as well as in the ectodermal tissues that develop abnormally.

A single 858-bp cDNA, representing a full-length transcript composed of two exons, was identified from an adult–sweat gland cDNA library. *In situ* analysis showed that the *EDA* gene is expressed in hair follicles and in the epidermis of adult skin. The putative gene product is a 135–amino-acid protein (isoform I) that has no clear homology to other proteins. The protein is

predicted to contain a single transmembrane domain, and fractionation studies of transfected cell lines showed that the protein product is localized to the plasma membrane.

B. Introduction to Mental Retardation

1. Background and rationale

Mental retardation (MR) is one of the biggest unsolved problems in Medical Genetics. While the etiology of mild or moderate MR is mostly complex, involving a wide variety of environmental and genetic factors which are frequently additive, single genetic factors play a predominant role in severe forms (IQ below 50) including chromosomal abnormalities and single gene defects. There is reason to believe that this is also true for the large majority of sporadic and familial cases which, in the absence of a defined molecular diagnosis, is currently referred to as "idiopathic" mental retardation. It has long been known that mental retardation is more common in males than in females. This skewed sex ratio is not only apparent in institutions for the mentally retarded but also in the percentage of male and female pupils in schools for children with learning deficits. The most straightforward explanation for these data is that this sex difference is due to the involvement of X-chromosomal genes, and it is currently estimated that X-linked defects account for 20-50% of all cases [1]. X-linked mental retardation (XLMR) represents a large group of disorders where mental retardation is a feature of the phenotype and the corresponding gene is on the X chromosome. A wide variety of so-called syndromic forms of X-linked mental retardation (XMR) have been found where mental retardation is associated with a specific, recognizable pattern of other symptoms. For numerous of these disorders, the fundamental defect is already known, including Fragile X syndrome. Individually, and as a group, these syndromic forms of XMR are rare

compared to the larger group of non-syndromic forms (MRX) which cannot be distinguished on the basis of their clinical phenotypes. The situation with MRX is complex mainly because of the genetic heterogeneity underlying an otherwise clinically homogenous phenotype [2]. The prevalence of MRX based on a study from British Columbia was calculated to be 1.83 per 1000 males [3]. However the true prevalence remains uncertain owing to the mild nature of the phenotype segregating in some families. Owing to the genetic heterogeneity and often large non-overlapping linkage intervals, the positional cloning or positional candidate gene approaches can be difficult to carry out [4-5]. A suitable alternative is to identify candidate MRX genes from X chromosomal rearrangements (translocations, inversions, deletions) associated with a MR phenotype and to test these candidate genes in MRX families mapping across the rearranged region [6-10]. Breakpoint cloning in patients with disease-associated balanced chromosome rearrangements is a very powerful strategy for identifying genes that play a role in these genetic disorders [11-13].

We have identified an inverted X chromosome with a pericentric inversion 46,Y,inv(X)(p22.2q13.1) (Fig. 1) in a 6-year-old Thai male patient presented with mild mental retardation (IQ 66-73) and clinical signs of hypohidrotic ectodermal dysplasia (HED). Hypohidrotic (anhidrotic) ectodermal dysplasia (also known as Christ-Siemens-Touraine syndrome) is a group of genetic disorders defined by congenital birth defects of two or more structures derived from ectoderm. HED is usually inherited as an X-linked recessive trait. Gene responsible for X-linked HED, *EDA* gene, has been mapped in the proximal area of the long arm of the X chromosome band Xq12-q13.1 [14]. A decreased expression of the epidermal growth factor receptor has been proposed as playing a causal role in this condition's

phenotype [15-17]. The symptoms and signs of HED include decreased number of teeth, peg-like teeth, delayed or absent tooth formation, inability to sweat, absent tears (occasional), thin skin, decreased pigment, foul-smelling nasal discharge, poor temperature regulation, heat intolerance, scanty hair or absent hair, abnormal nails, low nasal bridge. Hair is sparse, very fine, lightly pigmented, and abnormal texture (scalp, eyebrows, and eyelashes). The intelligence of HED individuals is usually normal. Only 2 HED cases have been reported to be associated with MR and both of them had X-autosome translocation. Cytogenetic study of his mother was also shown to carry this inversion on one X allele (Fig. 2). Because the *EDA* gene is located at Xq13.1, which is one breakpoint of the inv(X), and because its defects are rarely associated with mental retardation, the mental deficits of this patient were deemed likely to be associated with the opposite breakpoint at Xp22.2. This study aims to characterize both breakpoints on X chromosome. We speculate that the breakpoint at Xp22.2 disrupts a novel unidentified gene responsible for the patient's mild mental retardation.

2. Research Questions

Major research question: Does the inversion breakpoint disrupt a novel unidentified gene on Xp22.2?

Minor research question: How does the genotype, chromosomal inversion, cause the phenotype, hypohidrotic ectodermal dysplasia?

3. Objectives

1) To characterize both X inversion breakpoints of this patient.

2) To explain whether the Xp breakpoint can cause mental retardation in this patient or not.

3) To explain how the genotype, chromosomal inversion, causes the phenotype hypohidrotic ectodermal dysplasia.

4. Hypothesis

Breakpoints rearrangement in this patient's inv(X)(p22.2q13) might have disrupted not only the *EDA* gene but also additional unidentified gene that could account for his mental retardation

5. Conceptual Framework

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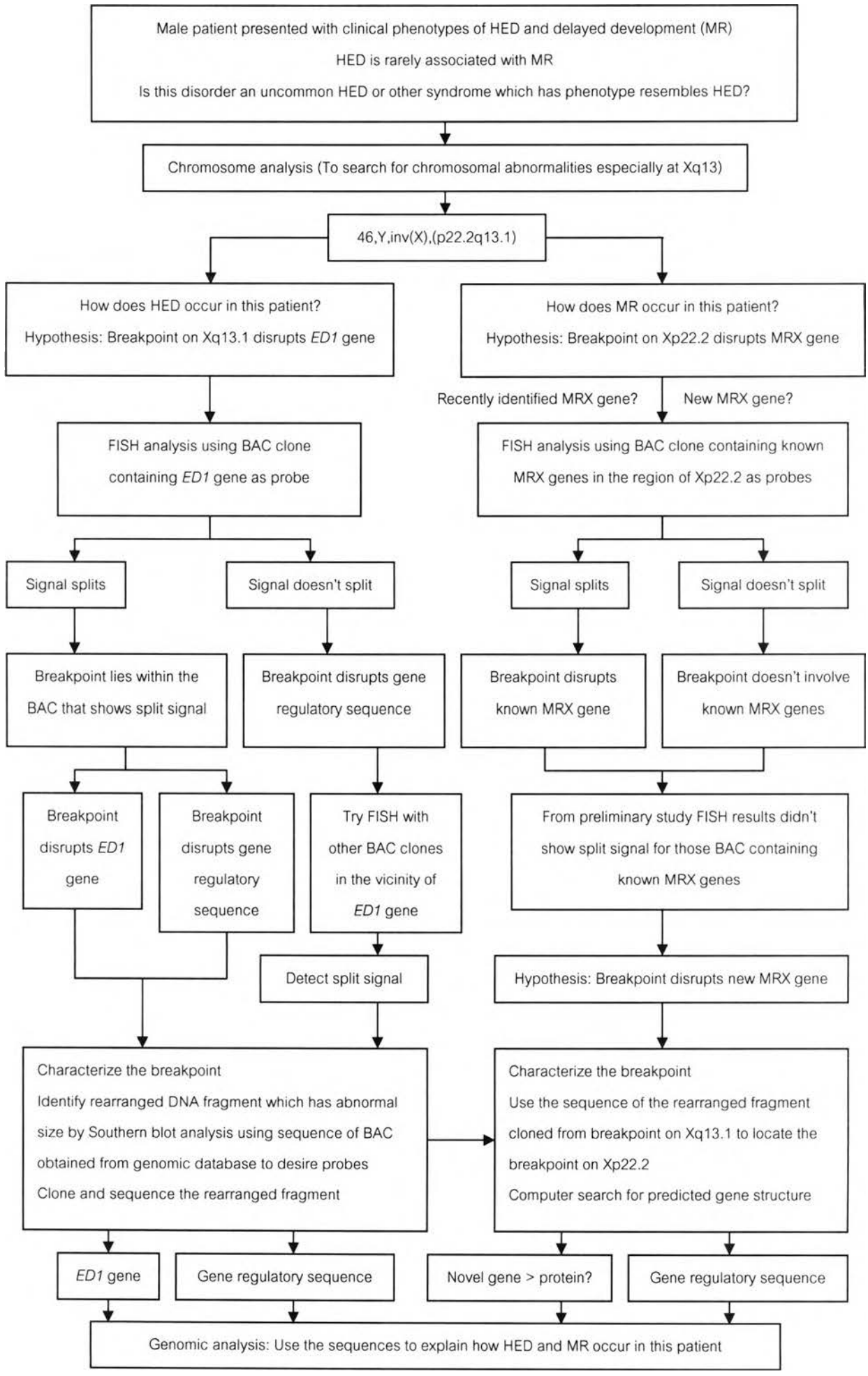
Male patient presented with clinical phenotypes of HED and delayed development (MR)
HED is rarely associated with MR
Is this disorder an uncommon HED or other syndrome which has phenotype resembles HED?
Chromosome analysis (To search for chromosomal abnormalities especially at Xq13)
46,Y,inv(X),(p22.2q13.1)
How does HED occur in this patient?
Hypothesis: Breakpoint on Xq13.1 disrupts ED1 gene
How does MR occur in this patient?
Hypothesis: Breakpoint on Xp22.2 disrupts MRX gene
Recently identified MRX gene?
New MRX gene?
FISH analysis using BAC clone containing ED1 gene as probe
FISH analysis using BAC clone containing known MRX genes in the region of Xp22.2 as probes
Signal splits
Signal doesn't split
Signal splits
Signal doesn't split
Breakpoint lies within the BAC that shows split signal
Breakpoint disrupts gene regulatory sequence
Breakpoint disrupts known MRX gene
Breakpoint doesn't involve known MRX genes
Breakpoint disrupts ED1 gene
Breakpoint disrupts gene regulatory sequence
Try FISH with other BAC clones in the vicinity of ED1 gene
From preliminary study FISH results didn't show split signal for those BAC containing known MRX genes
Detect split signal
Hypothesis: Breakpoint disrupts new MRX gene
Characterize the breakpoint
Identify rearranged DNA fragment which has abnormal size by Southern blot analysis using sequence of BAC obtained from genomic database to desire probes
Clone and sequence the rearranged fragment
Characterize the breakpoint
Use the sequence of the rearranged fragment cloned from breakpoint on Xq13.1 to locate the breakpoint on Xp22.2
Computer search for predicted gene structure
ED1 gene
Gene regulatory sequence
Novel gene > protein?
Gene regulatory sequence
Genomic analysis: Use the sequences to explain how HED and MR occur in this patient

6. Key Words

Hypohidrotic ectodermel dysplsia, X inversion, Mental retardation

7. Operational Definitions

HED: Hypohidrotic ectodermel dysplsia

XLMR: X-linked mental retardation

XMRs: Syndromic forms of X-linked mental retardation where mental retardation is associated with a specific, recognizable pattern of other symptoms

MRX: Non-syndromic X-linked mental retardation defined by the absence of specific phenotypic characteristics except mental retardation

FISH: Fluorescence in situ hybridization

BAC: Bacterial artificial chromosome

PCR: Polymerase chain reaction

Real-time RT-PCR: Real-time polymerase chain reaction

RT-PCR: Reverse transcription polymerase chain reaction

8. Benefit of the Study

Breakpoint cloning in patients with disease-associated balanced chromosome rearrangements is a very powerful strategy for identifying genes that play a role in genetic disorders. More recently, the same strategy has been used by MRX Consortium members and others to identify other genes that play a role in unspecific XMR. So we will employ this strategy to search for a novel gene in a mentally retarded male with a 46,Y,inv(X)(p22.2q13).

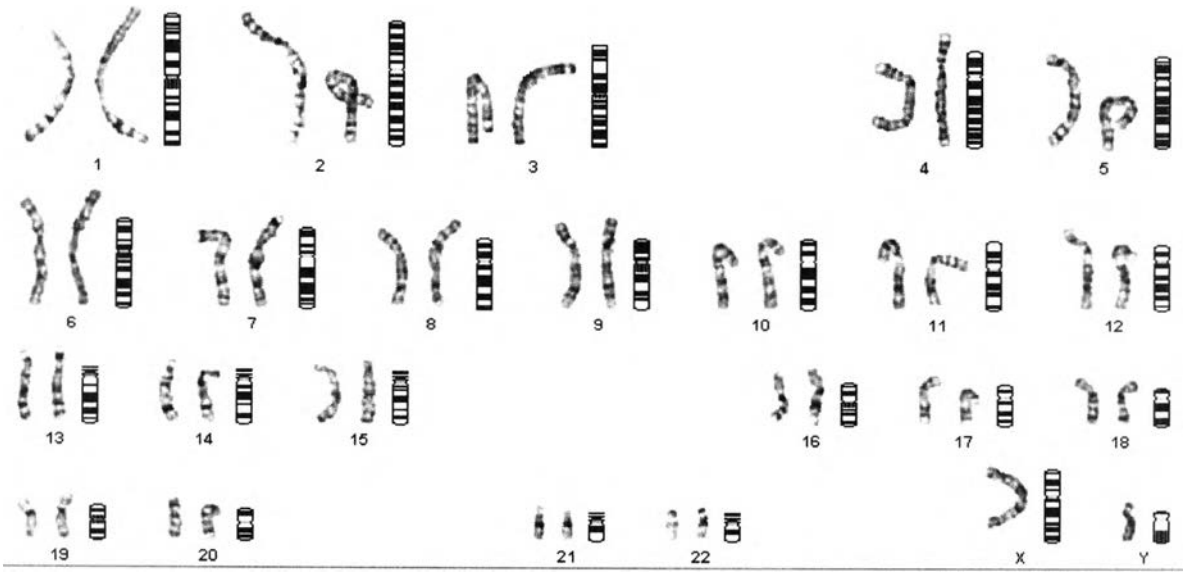

Figure 1. The G banding karyotype of the patient.

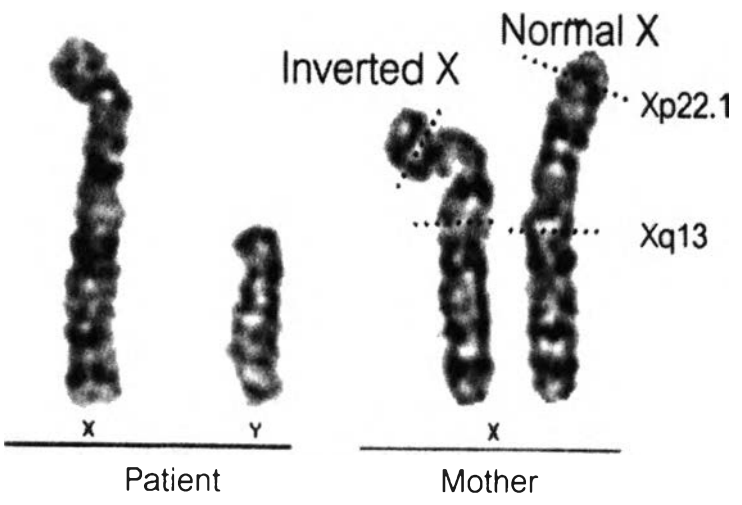

Figure 2. The inversion X chromosome of the patient and his mother.