Mechanism of ABCA12 gene Mutation & its severity with improved management & Treatment

Author* Md. Aktar Hossain

Abstract—ABCA12 is a member of the ATP-binding cassette transporter family, and members of the ABCA subfamily are known to have closely related functions as lipid transporters. ABCA3 is involved in lipid secretion via LGs from alveolar type II cells, and missense mutations in *ABCA12* have been reported to cause lamellar ichthyosis type 2, a milder form of ichthyosis. Therefore, we hypothesized that HI might be caused by mutations that lead to serious ABCA12 defects. We identify 5 distinct *ABCA12* mutations, either in a compound heterozygous or homozygous state, in patients from 4 HI families. Harlequin ichthyosis (HI) is a devastating skin disorder with an unknown underlying cause. Abnormal keratinocyte lamellar granules (LGs) are a hallmark of HI skin. All the mutations resulted in truncation or deletion of highly conserved regions of ABCA12. Immunoelectron microscopy revealed that ABCA12 localized to LGs in normal epidermal keratinocytes. We confirmed that ABCA12 defects cause congested lipid secretion in cultured HI keratinocytes and succeeded in obtaining the recovery of LG lipid secretion after corrective gene transfer of *ABCA12*. We concluded that ABCA12 works as an epidermal keratinocyte lipid transporter and that defective ABCA12 results in a loss of the skin lipid barrier, leading to HI. Our findings not only allow DNA-based early prenatal diagnosis but also suggest the possibility of gene therapy for HI.

Index Terms—ABC, ATP-binding cassette; CT, threshold cycle; HI, harlequin ichthyosis; LG, lamellar granule; MIM, Mendelian Inheritance of Man.

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1 INTRODUCTION

ATP-binding cassette sub-family A member 12 also known as ATP-binding cassette transporter 12 is a protein that in humans is encoded by the ABCA12 gene [1] ABCA12 belongs to a group of genes called the ATP-binding cassette family, which makes proteins that transport molecules across cell membranes. The ABCA12 gene is active in some types of skin cells and in several other tissues, such as testis, placenta, lung, stomach, and fetal brain and liver. This protein appears to be essential for normal development of the skin, which provides a barrier between the body and its surrounding environment. It transports epidermoside, a glucosylceramide, out of the keratinocytes of the stratum corneum of the epidermis [2] The ABCA12 gene is located on the long (q) arm of chromosome 2 between positions 34 and 35, from base pair 215,621,772 to base pair 215,828,656.

Related conditions: Several mutations in the ABCA12 gene are known to cause harlequin-type ichthyosis . Most of these mutations are predicted to lead to an absence of ABCA12 protein or the production of an extremely small version of the protein that cannot transport lipids properly. A loss of functional ABCA12 protein causes numerous problems with the development of the epidermis before and after birth. Abnormalities in lipid transport prevent the skin from forming an effective barrier and result in the hard, thick scales characteristic of harlequin ichthyosis. Mutations in the ABCA12 gene also cause another severe skin disorder, lamellar ichthyosis type 2. People with this disorder have red, scaly, plate-like skin covering most of their bodies. The ABCA12 mutations that cause this disorder substitute one amino acid (a building block of proteins) for another amino acid in the ABCA12 protein. These mutations almost always occur in an important functional region of the protein (the region that binds to ATP, a molecule that supplies energy for chemical reactions). Changes in the structure of the ABCA12 protein likely disrupt its ability to transport lipids, which affects the development of skin before and after birth.[3]

1.1 Normal Function:

The ABCA12 gene provides instructions for making a protein known as an ATP-binding cassette (ABC) transporter. ABC transporter proteins carry many types of molecules across cell membranes. In particular, the ABCA12 protein plays a major role in transporting fats (lipids) in cells that make up the outermost layer of skin (the epidermis). This lipid transport appears to be essential for normal development of the skin. The ABCA12 protein is also found in several other tissues, including the testes, placenta, lung, stomach, and fetal brain and liver.[4]

Aim of the work:

The life expectancy of a baby born with **Harlequin ichthyosis**

was generally given as just a few days. Often, this was due to the severe dehydration the babies were often plagued with or the result of a superimposed infection from the cracking and peeling of the skin. When large amounts of skin come off, the body struggles to regulate the temperature, which can cause the baby to go into shock. Also, with the thick plates of skin covering most of the body surface, the baby can overheat as the skin can't breathe.
Still there is no cure of this disease but this disease can be managed more recently by following aspects:

- Management of Harlequin Ichthyosis.
- Improved management and patient's care of Harlequin Ichthyosis.
- Improved Otologic management.
- Improved Ocular management.
- Improved Patient's skin care.
- Improved control of pain.
- Retinoid therapy.
- Improved knowledge of superstitions.

More recently there have been several advances in the **treatment** of Harlequin ichthyosis. Unfortunately, there is no known cure, but ongoing treatments and management can help to prolong the life of a Harlequin sufferer. The most notable change to the treatment regime is the addition of drugs such as **retinoids.**

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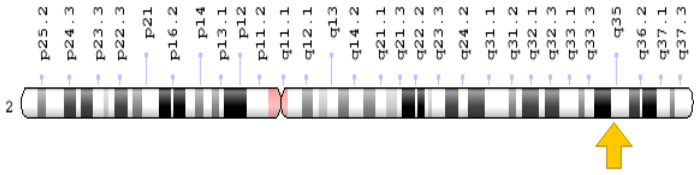
1.2 <u>Health Conditions Related to Genetic Changes:</u>

- Harlequin ichthyosis
- Lamellar ichthyosis

1.3 Chromosomal Location:
Cytogenetic Location: 2q35, which is the long (q) arm of chromosome 2 at position 35 Molecular Location: base pairs 214,931,542 to 215,138,591 on chromosome 2 (Homo sapiens Annotation Release 108, GRCh38.p7) (NCBI)



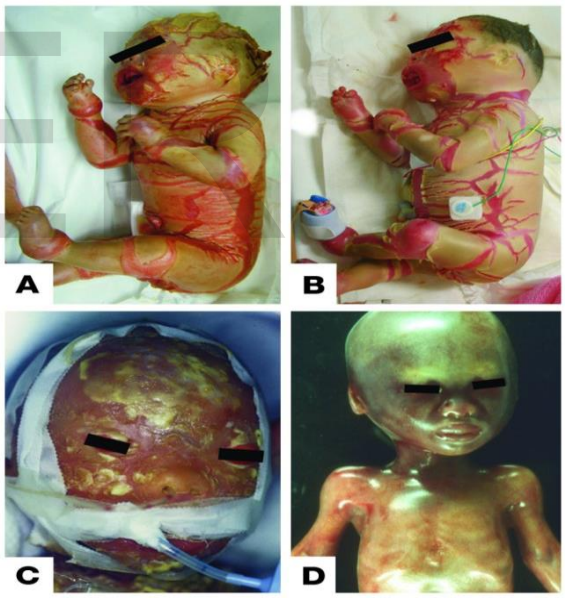
1.3 Figure-1: Chromosomal Location **[**Credit: Genome Decoration Page/NCBI**]**

1.4 Other Names for This Gene:

- ABCAC_HUMAN
- ATP-binding cassette 12
- ATP-binding cassette transporter 12
- ATP-binding cassette, sub-family A (ABC1), member 12
- ATP-binding cassette, sub-family A, member 12
- ICR2B

1.5 Mutations in lipid transporter ABCA12 in harlequin ichthyosis and functional recovery by corrective gene transfer:

During the evolutionary process, when our ancestors left the safety of the aquatic environment, they developed a robust, protective mechanism or process in the skin that allowed adjustment to the new, dry environment; this is now known as keratinization. In humans, congenital defects involving skin keratinization cause a unique Geno dermatosis, ichthyosis which was named after the Greek word ichthys, meaning fish. Among the variety of types of ichthyosis, harlequin ichthyosis (HI) (Mendelian Inheritance of Man [MIM] 242500) is the most serious subtype (Figure 1); it is also the most severe congenital skin disorder of unknown etiology.



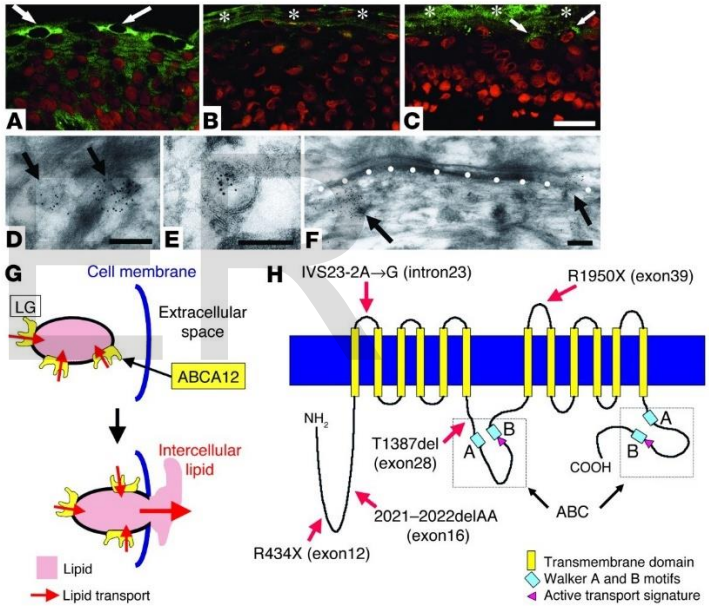
1.5 Figure 2: Effect of HI

Clinical features of HI patients. (A) Patient 1 from family A harboring a homozygous mutation IVS23-2A→G in ABCA12. (B) Patient 2 from family B with compound heterozygous ABCA12 mutations, IVS23-2A→G and 5848C→T (R1950X). (C) Patient 3 (family C) carrying compound heterozygous ABCA12 mutations, 2021_2022del AA and 4158_4160delTAC (T1387del). (D) An affected fetus from family C aborted at 23 weeks' gestation showed no serious symptoms, although some abnormal keratinization was observed mainly on the cheeks and the perioral area. ABCA12 belongs to a large superfamily of ATP-binding

cassette (ABC) transporters, which aid in the transport of various biomolecules across the cell membrane. The ABCA subfamily is thought to be important in lipid transport. ABCA12 is phylogenetically related to ABCA3, which is essential for alveolar surfactant lipid transport/secretion by lamellar granules (LGs) in type II alveolar lung cells. In skin, LGs are the most common secretory granules present in upper epidermal keratinocytes. Abnormal LGs are the most obvious characteristic findings in HI lesional epidermis. Furthermore, relatively minor missense mutations in ABCA12 have been reported to underlie type 2 lamellar ichthyosis (MIM 601277), a milder form of ichthyosis Thus, we hypothesized that ABCA12 mutations leading to serious ABCA12 protein defects might underlie HI. In the present study, we demonstrate that ABCA12 works as an epidermal keratinocyte lipid transporter and that defective ABCA12 results in a loss of the skin lipid barrier, leading to HI. We have found 5 distinct truncation, deletion, or splice-site mutations in 4 independent HI families. These mutations were present on both alleles of our HI patients, either in compound heterozygous or homozygous state. We demonstrate that ABCA12 mRNA is expressed in normal human keratinocytes and that this expression is upregulated during keratinization. Our immunoelectron microscopic findings showed that ABCA12 protein localizes to LGs in the upper epidermal keratinocytes of human skin. Ultrastructural and immunofluorescent examination of human skin and cultured epidermal keratinocytes from HI patients who harbor ABCA12 mutations revealed defective lipid secretion of LG lipid contents. In addition, using ABCA12 corrective gene transfer in cultured HI keratinocytes, we have succeeded in restoring the normal ability of HI cells to secrete LG lipid.

Results:

ABCA12 expression and localization in epidermal keratinocytes. To confirm the expression of ABCA12 mRNA in human epidermal keratinocytes, we performed semiquantitative RT-PCR assays and demonstrated strong expression of ABCA12 transcripts in cultured normal epidermal keratinocytes. To investigate the upregulation of ABCA12 during keratinization under high-Ca2+ conditions, we evaluated the ABCA12 transcript population against the GAPDH transcript using real-time RT-PCR. Calculation of the expression ratios showed that ABCA12 transcription was upregulated 4-fold after 1 week in high-Ca2+ culture. Furthermore, to confirm the expression of ABCA12 protein in the epidermis, we raised a polyclonal anti-ABCA12 antibody that recognizes an epitope near the C terminus of the ABCA12 polypeptide (residues 2567–2580) and performed immunostaining for ABCA12. ABCA12 was positive in the upper epidermal layers, mainly the granular layers, of normal human skin (Figure 2A); in contrast, there was an absence of immunolabeling in epidermal keratinocytes from HI patient 4 (Figure 2B), who harbors the homozygous truncation mutation R434X (see below). This mutation resulted in truncation of the protein, leading to loss of the epitope for the anti-ABCA12 antibody. These findings confirmed specificity of the anti-ABCA12 antibody. In the epidermis of patient 1, who harbors a homozygous splice acceptor site mutation IVS23-2A→G (see below), weak ABCA12 immunostaining was seen in the granular layer keratinocytes (Figure 2C). Immunoelectron microscopy revealed that ABCA12 protein was restricted to the LGs in the cytoplasm of epidermal keratinocytes (Figure 2, D and E). ABCA12-positive LGs were abundant close to the cell membrane and were observed fusing with the cell membrane to secrete their lipid content to the extracellular space of the stratum corneum (Figure 2F). These results indicate that ABCA12 is expressed in keratinocytes during keratinization and is likely to be involved in lipid transport into the extracellular space via LGs to form the stratum corneum lipid barrier (Figure 2G)

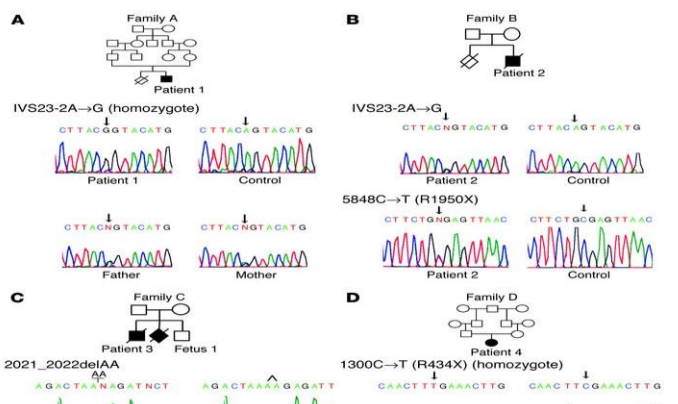


1.5 Figure 3: Mutation of ABCA12 gene.

Localization and structure of ABCA12 protein and the sites of HI mutations. (**A**) ABCA12 protein (green) was localized in the cytoplasm of the upper epidermal keratinocytes (arrows) in healthy skin. (**B**) No ABCA12 immunolabeling was seen in the epidermis of patient 4. (**C**) Weak ABCA12 staining (arrows) was observed in the epidermis of patient 1. Asterisks indicate nonspecific staining in the stratum corneum. Red, nuclear counterstain. Scale bar: 10 μm. (**D–F**) By immunoelectron microscopy,

ABCA12 protein (5 nm gold particles) was restricted to LGs (arrows) in the upper epidermal cell (**D**). Lamellar structures were apparent in some ABCA12-positive LGs (**E**). ABCA12-positive LGs (arrows) were observed close to the keratinocyte-cell membrane (white circles) and they fused with it to secrete their contents into the intercellular space (**F**). Scale bars: 0.2 μm. (**G**) Model of ABCA12 function in the skin. ABCA12 transports lipid into the LG, and ABCA12-positive LGs fuse with the cell membrane to secrete lipid into extracellular space to form the intercellular lipid layer. (**H**) Structure of ABCA12 protein and the 5 mutation sites (red arrows) in HI families. Dark-blue area, cell membrane; bottom of dark-blue area, cytoplasmic surface.

ABCA12 mutations in HI families. Full-length ABCA12 protein comprises 2595 amino acids and includes 2 ABCs containing 3 characteristic, highly conserved motifs (Walker A, Walker B, and active transport signature). In addition, there are 2 transmembrane domains, each consisting of 6 hydrophobic membrane-spanning helices. Mutational analysis of the 53 exons, including the exon-intron boundaries of the entire *ABCA12* gene, revealed 5 novel, distinct mutations in both alleles, either in compound heterozygous or homozygous state, in all patients from 4 HI families (Figure 2H). The mutations in patients were homozygous in the 2 consanguineous families and compound heterozygous in the 2 nonconsanguineous families (Figure 3). The mutations were verified in the heterozygous parents. Each of the three mutations, 1300C→T (R434X), 2021_2022delAA, and 5848C→T (R1950X), resulted in truncation of a highly conserved region of the ABCA12 protein. The deletion mutation 4158_4160delTAC led to an in-frame deletion of a highly conserved threonine residue at codon 1387 (T1387del) within the first ATP-binding domain of the ABCA12 protein (Figure 4D). A splice acceptor site mutation, IVS23-2A→G, was verified by RT-PCR in mRNA from the patient's cultured keratinocytes (Figure 4, A–C). RT-PCR products from the patient showed 2 splice pattern variants different from the normal splicing variant in which 1 mutant transcript loses a 9-bp sequence from exon 24, which results in a 3–amino acid deletion (Y1099_K1101del). These 3 amino acids are located between the transmembrane domains and are highly conserved (Figure 4D). The other mutant transcript lost a 170-bp sequence from exon 24, which led to a frameshift. All these mutations are thought to seriously affect either the function or specific critical structures of the ABCA12 protein.



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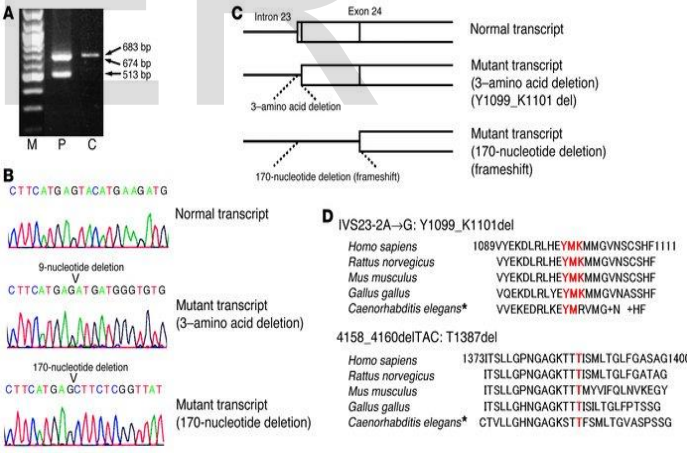
1.5 Figure 4: ABCA12 Gene mutation

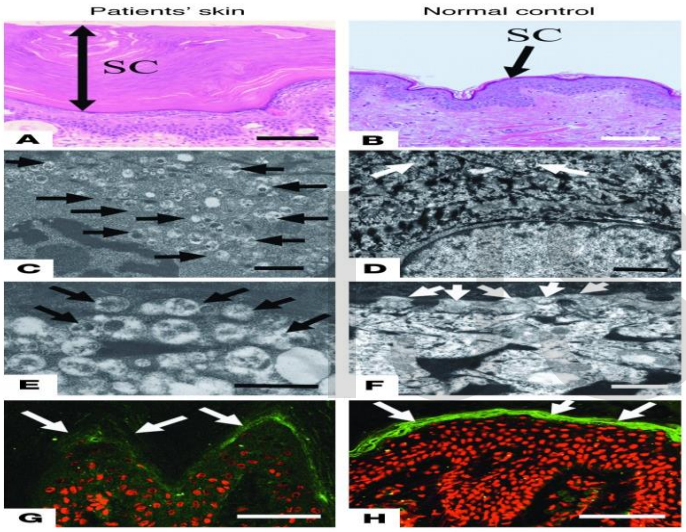
Families with HI and *ABCA12* mutations. (**A**) Patient 1 from family A was a homozygote for the mutation IVS23-2A→G, and both his parents were heterozygous carriers. (**B**) Patient 2 from family B was a compound heterozygote for the mutations IVS23-2A→G and 5848C→T (R1950X). (**C**) Patient 3 from family C was a compound heterozygote for the mutations 2021_2022delAA and 4158_4160delTAC (T1387del). (**D**) Patient 4 from family D was a homozygote for the mutation 1300C→T (R434X), and her 2 parents were heterozygous carriers.



1.5 Figure-5: Transcription of ABCA12 Gene

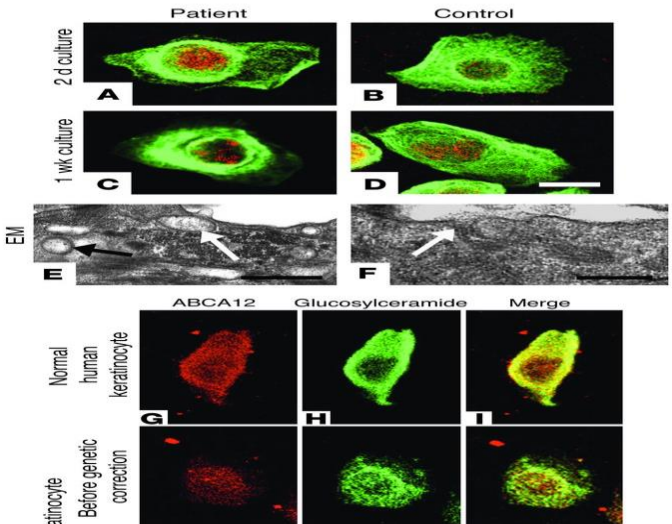
Verification of splice-site mutation IVS23-2A→G and conservation of residues deleted by mutations IVS23-2A→G and 4158_4160delTAC (T1387del). (A) RT-PCR analysis of mRNA fragments around the exon 23–24 boundary indicated that keratinocytes from patient 1 (lane P) showed 2 different mutant transcripts, 674 bp and 513 bp, which were shorter than the control transcript (683 bp) from healthy human keratinocytes (lane C). Lane M, markers. (B) Sequencing of the mutant transcripts and the control transcript revealed that 9 nucleotides and 170

nucleotides were deleted in mutant transcripts. (C) A 9-nucleotide deletion resulted in the loss of 3 amino acids from the N terminal sequence of exon 24 (Y1099_K1101del), and the 170-nucleotide deletion led to a frameshift. (D) ABCA12 amino acid sequence alignment shows the level of conservation in diverse species of the amino acids, Y1099_K1101 and T1387 (red characters), which were deleted by mutations in HI families. Asterisks indicate ABC (abt-4). Disturbed lipid secretion in epidermis of HI patients. Morphological observations revealed extraordinarily thick stratum corneum (Figure 5A) and abnormal LG secretion in keratinocytes of the epidermis of HI patients (Figure 5, C and E). Ultra-structurally, the cytoplasm in the stratum corneum was congested with abnormal lipid-containing droplets and vacuoles that resembled immature LG-like vesicles. In the keratinocyte cytoplasm of the granular layer, no normal LGs were apparent.



1.5 Figure- 6: Difference between normal and HI patient's skin

Extremely thick stratum corneum and severe disruption of the secretion of LGs in the ABCA12-deficient skin of the present series of HI patients. (**A**) Strikingly thick stratum corneum (SC; double arrow) in the patient's skin. (**B**) Control epidermis showing normal, stratum corneum (arrow). (**C**) By electron microscopy, LG secretion was disturbed, and many abnormal immature (lacking proper lamellar structures) LGs (arrows) were observed in the keratinocytes. (**D**) In control skin, LGs (arrows) were distributed in a gradually increasing pattern toward the plasma membrane. (**E**) Abnormal HI LGs (arrows) was localized close to the cell membrane, but not secreted. (**F**) LGs were secreted into the extracellular space (arrows). (**G**) Patient's epidermis including stratum corneum (arrows) showed diffuse staining for glucosylceramide (green), a lipid component of LGs. (**H**) Glucosylceramide staining (green) was restricted and intense in the stratum corneum (arrows) of normal skin. Red, nuclear stain. Scale bars: 50 mm (**A**, **B**, **G**, **H**); 1 mm (**C**, **D**); 0.5 mm (**E**, **F**). Immunofluorescent staining showed that glucosylceramide, a major lipid component of LG and an essential component of the epidermal permeability barrier was diffusely distributed throughout the epidermis of HI patients (Figure 5G); this contrasts with the restricted, intense distribution in the stratum corneum of healthy skin (Figure 5H). Abnormal lipid secretion in keratinocytes of HI patients and recovery of ABCA12 function by corrective gene transfer. Keratinocytes from patient 1, cultured under high-Ca2+ conditions (2.0 mM), expressed only a small amount of mutated ABCA12 protein (Figure 6J). Culture of HI keratinocytes under high-Ca2+ conditions (2.0 mM) induced a large number of cells to exhibit intense glucosylceramide staining around the nuclei, and this glucosylceramide failed to localize to the periphery of the keratinocyte cytoplasm (congested pattern) (Figure 6, A and C). Conversely, culture of healthy keratinocytes in high-Ca2+ conditions resulted in a large proportion of cells with diffuse lucosylceramide staining throughout the cytoplasm (widely-distributed pattern) (Figure 6, B and D). This difference was most pronounced after 1 week in culture (Figure 6, C and D). Electron microscopic observation of HI keratinocytes cultured for 1 week in high-Ca2+ conditions revealed that LGs formed, although proper secretion of their contents was not observed (Figure 6E). This finding suggests defects in LG lipid transport. Double immunostaining for ABCA12 protein and glucosylceramide clearly demonstrated that, before genetic correction of ABCA12, keratinocytes from patient 1 with a low expression of mutated ABCA12 protein showed a congested glucosylceramide distribution pattern (Figure 6, J–L). After genetic correction, however, the HI patient's keratinocytes, now expressing normal ABCA12, showed a normal, widely-distributed pattern of glucosylceramide staining (Figure 6, M–O). Corrective ABCA12 gene transfer into cultured HI keratinocytes resulted in a significant increase in number of cells exhibiting the widely-distributed pattern of glucosylceramide staining from 6.98% ± 3.33% (control non-transfected patients' cells) to 16.70% ± 2.14 % (transfected patients' cells) (Student's t test, $P < 0.02$) (Figure 6P). These results clearly indicate that an ABCA12 deficiency leads to defective LG lipid transport into the intercellular space in HI patients, both in the epidermis and in cultured keratinocytes.



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1.5 Figure-7: Genetic correction of ABCA12 gene

Cultured HI keratinocytes carrying ABCA12 mutations showed abnormal congestion of lipid, and this phenotype was recovered by corrective ABCA12 gene transfer. (A–D) HI keratinocytes cultured in high Ca2+ conditions showed that glucosylceramide, a major component of LG lipid, was distributed densely around the nuclei (in a congested pattern) (green, FITC). Control normal human keratinocytes showed a widely distributed, diffuse glucosylceramide staining pattern. (E) Electron microscopic (EM) observation revealed, in cultured HI keratinocytes, that apparently amorphous, electron lucent LG-like structures (arrows) formed, but were not secreted. (F) Normal secretion of LG contents (arrow) in a control keratinocyte. (G–O) Before genetic correction, an HI patient cell showed weak ABCA12 immunostaining (red, TRITC) and a congested pattern of glucosylceramide staining (green, FITC) (J–L). After genetic correction, an HI patient cell demonstrated stronger ABCA12 labeling (red) and a normal distribution pattern of glucosylceramide (green) (M–O), similar to those of a normal human keratinocyte (G–I). (P) Corrective gene transfer resulted in a statistically significant increase in the number of cells with completely normal, widely distributed glucosylceramide patterns. *P < 0.02, Student's t test. Scale bars: 10 mm (A–D, G–O); 0.5 mm (E, F).

Discussion:

An abnormal synthesis or metabolism of the LG lipid contents was previously suspected as being a possible pathogenetic mechanism underlying HI. Here, we demonstrate that a severe ABCA12 deficiency causes defective lipid transport via LG in keratinizing epidermal cells, resulting in the HI phenotype.

The ABC transporter superfamily is one of the largest gene families, encoding a highly conserved group of proteins involved in energy-dependent active transport of a variety of substrates across membranes, including ions, amino acids, peptides, carbohydrates, and lipids. ABC transporters have nucleotide-binding folds located in the cytoplasm and utilize energy from ATP to transport substrates across the cell membrane. ABC genes are widely dispersed throughout the eukaryotic genome and are highly conserved between species The ABCA subfamily, of which the *ABCA12* gene is a member, comprises 12 full transporter proteins and 1 pseudogene (ABCA11). The ABCA subclass has received considerable attention because mutations in these genes have been implicated in several human genetic diseases.

The ABCA1 protein is mutated in the following recessive disorders involving cholesterol and phospholipid transport: Tangier disease (MIM 205400), familial hypoalphalipoproteinemia (MIM 604091), and premature atherosclerosis, depending on the site of the mutations in the protein The ABCA4 protein is mutated in Stargardt disease (MIM 248200) as well as in some forms of autosomal recessive retinitis pigmentosa (MIM 601718) and in the majority of cases of autosomal recessive cone-rod dystrophy (MIM 604116), depending on the mutation site or the combination of mutation types. Heterozygous mutations in *ABCA4* have also been implicated in some cases of macular degeneration (MIM 153800) In a relatively mild type of congenital ichthyosis (lamellar ichthyosis type 2), 5 ABCA12 mutations were reported in 9 families, and all 5 of these mutations were missense mutations that resulted in only 1 amino acid alteration. In the present series of HI patients, no ABCA12 missense mutations were identified, and most of the defects led to severe truncation of the ABCA12 peptide, affecting important nucleotide-binding fold domains and/or transmembrane domains. The other, nontruncation mutations in HI were deletion mutations affecting highly conserved ABCA12 sequences (Figure 4D). Thus, it is thought that only truncation or conserved region deletion mutations that seriously affect the function of the ABCA12 transporter protein lead to the HI phenotype. This is in contrast with diseases caused by mutations in other members of the ABCA family. Of ABCA4 mutations causing Stargardt disease, 80% were missense, and many of these occurred in conserved domains of ABCA4. Of ABCA1 mutations resulting in Tangier disease, 60% were missense, located within the conserved domains of ABCA1). In this context, ABCA12 mutations underlying HI are unique in that these mutations are restricted to truncation or deletion mutations. Patient 1 was homozygous for the splice acceptor site mutation IVS23-2A→G. This splice-site mutation was shown to lead to comparable amounts of 2 predicted transcripts, an inframe deletion of 3 amino acids, and a transcript with a 170-nucleotide deletion (frameshift) (Figure 4,

A–C). Thus, it is still possible that patient 1 expresses some mutated ABCA12 protein. Indeed, expression of a small amount of ABCA12 protein, although mutated, was detected in the granular cells of the patient's epidermis and cultured keratinocytes by immunofluorescent staining (Figures 2C and 6J). This might have some relevance to the fact that patient 1 survived infancy and is still alive. ABCA1 and ABCA4 are suspected transmembrane transporters for intracellular cholesterol/phospholipids and protonated N-retinylidenephosphatidylethanolamines respectively. ABCA2, ABCA3, and ABCA7 mRNA levels have been reported to be upregulated by sustained cholesterol influx mediated by modified low-density lipoprotein suggesting that ABCA transporters are involved in transmembrane transport of endogenous lipids Keratinocyte LGs are known lipid-transporting granules, and LG contents are secreted into the intercellular space, forming an intercellular lipid layer between the granular layer cells and keratinized cells in the stratum corneum, which is essential for the proper barrier function of human skin. Our results indicate that ABCA12 functions in the transport of endogenous lipid across the keratinocyte cell membrane into the stratum corneum intercellular space via LGs. Immunohistological and immunoelectron microscopic observations have indicated that ABCA3 is involved in lipid secretion of pulmonary surfactant in human lung alveolar type II cells ABCA3 and ABCA12 are very closely related in the ABCA subfamily phylogenetic tree. It is interesting that the functions of both ABCA3 and ABCA12 are involved in alveolar surfactant and stratum corneum lipid secretion, respectively. This suggests that these 2 transporter systems are evolutionary adaptations to aid the respiratory system and the integument in a dry environment, developed as vertebrates left the aquatic environment and began terrestrial lives. HI skin that harbors serious defects in ABCA12 highlights the important role(s) of epidermal lipid transport in adapting human skin to a terrestrial, dry environment.

HI is one of the most severe of all genodermatoses and has a very poor prognosis. Thus, parents' request for prenatal diagnosis is to be taken seriously and with care. However, to our knowledge, the causative gene was not identified until now. For the last 20 years, prenatal diagnoses have only been performed by electron microscopic observation of fetal skin biopsy at a late stage of pregnancy (19–23 weeks estimated gestational age) In this report, we have identified the causative gene in HI, which now makes possible DNA-based prenatal diagnosis using chorionic villus or amniotic fluid sampling at an earlier stage of pregnancy, with a lower procedural risk and a reduced burden on prospective mothers, similar to that of screening for other severe genetic disorders, including keratinization disorders. Furthermore, we have performed corrective gene transfer in HI keratinocytes and succeeded in obtaining phenotypic rescue of a patient's cultured keratinocytes. These data provide significant clues that establish a strategic approach to HI gene therapy treatment. We believe that the genetic information presented in this study will be highly beneficial to our understanding of HI pathogenesis and in optimizing HI patient diagnosis, genetic counseling, care, and treatment.

Methods:

Patients and families. Four HI patients, patients 1–4, and 1 affected fetus, fetus 1 (4 males, 1 female), from 4 independent families, families A–D, showed a serious collapse in the keratinized skin barrier (Figure 1). Family A and family D were consanguineous (marriage of first cousins). All the patients showed severe hyperkeratosis at birth and presented with generalized, thick scales over their entire body surface with the presence of marked fissuring. Severe ectropion, eclabium, and malformed pinnae were apparent in all cases. Patient 2 from family B died 3 days after birth. Patient 3 from family C died 15 days after birth, and an affected fetus from family C was terminated at the parents' request after a positive prenatal diagnostic skin test. After written, informed consent was obtained, blood samples were collected from each participating family member, and skin biopsy or autopsy specimens were obtained from the patients and the affected fetus. The study was given appropriate ethical approval by the Ethics Committee at Hokkaido University Graduate School of Medicine. Mutation screening. Mutation analysis was performed in patients, an affected fetus, and other family members in the 4 families, as far as DNA samples would allow. Briefly, genomic DNA isolated from peripheral blood was subjected to PCR amplification, followed by direct automated sequencing using an ABI PRISM 3100 genetic analyzer (Applied Biosystems). Oligonucleotide primers and PCR conditions used for amplification of exons 1–53 of ABCA12 were originally derived from the report by Lefèvre et al. and were partially modified for the present study. The entire coding region, including the exon/intron boundaries for both forward and reverse strands from all patients, family members, and controls, were sequenced. No mutations were found in 100 control alleles from the Japanese population. Verification of the splice acceptor site mutation. In order to verify the splice acceptor site mutation IVS23-2A→G, RT-PCR amplification of mRNA spanning the exon 23–24 boundary was performed using mRNA samples from cultured HI keratinocytes (see below). After RT-PCR amplification, direct sequencing of the products was performed. Establishment of HI keratinocyte cell culture. A skin sample from patient 1 was processed for primary keratinocyte culture, and cells were grown according to standard procedures in defined keratinocyte serum-free medium (Invitrogen Corp.). Cultures were grown for several passages in low-Ca2+ (0.09 mM)

conditions and then switched to high-Ca2+ (2.0 mM) conditions. RT-PCR of ABCA12 mRNA. RT-PCR of ABCA12 mRNA was performed with Superscript 2 (Invitrogen Corp.) following the manufacturer's instructions. Specific primers for PCR amplification were as follows: forward 5′-GAATTGCAAACTGGAAGGAACTCCC-3′ and reverse 5′-GAGTCAGCTAGGATTAGACAGC-3′. These primers were used for amplification of a 683-bp fragment around exon 23–24 boundary of normal cDNA. Real time-PCR. For quantitative analysis of ABCA12 expression levels, total RNA extracted was subjected to real-time RT-PCR using the ABI PRISM 7000 sequence detection system (Applied Biosystems). Specific primers/probes for human ABCA12, TaqMan Gene Expression Assay (HS00292421; Applied Biosystems) were used. Differences between the mean CT values of ABCA12 and those of GAPDH were calculated as ΔCTsample = CTABCA12 – CTGAPDH and those of the ΔCT for the cultured keratinocytes in the low Ca2+ condition as (ΔCTcalibrator = CTABCA12 – CTGAPDH). Final results, the sample-calibrator ratio, expressed as n-fold differences in ABCA12 expression were determined by 2–(ΔCTsample – ΔCTcalibrator). Cloning of ABCA12 and corrective gene transfer of HI keratinocytes. Using human keratinocyte cDNA as a template, overlapping clones of human ABCA12 cDNA were amplified by PCR. A composite full-length cDNA was constructed, subjected to nucleotide sequencing, and subcloned into a pCMV-tag4B vector (Stratagene). The expression plasmid pCMV-tag4B-ABCA12 was transfected into HI keratinocytes using Lipofectamine reagent (Invitrogen Corp.) according to the manufacturer's recommendation. As a control, pCMV-tag4B was transfected into the cells. The transfected cells and control cells from patient 1 were

stained with anti-glucosylceramide antibody, and the number of keratinocytes showing the 3 distinct types of glucosylceramide distribution patterns — congested, intermediate, and widely distributed — were assessed and calculated by 1 observer after viewing under a confocal laser scanning microscope, the Olympus Fluoview, FV300 confocal microscope (Olympus). Morphological observation. Skin biopsy samples or cultured keratinocytes were fixed in 2% glutaraldehyde solution, postfixed in 1% OsO4, dehydrated, and processed for conventional electron microscopic observation. Antibodies. Polyclonal anti-ABCA12 antibody was raised in rabbits using a 14–amino acid sequence synthetic peptide (residues 2567-2580) derived from the ABCA12 sequence (NM 173076) as the immunogen. The other primary antibody was mouse monoclonal anti-ceramide antibody (Alexis Biochemicals). Immunofluorescent labeling. Immunofluorescent labeling was performed on frozen tissue sections and keratinocyte cultures as previously described Fluorescent labeling was performed with FITC-conjugated secondary antibodies, followed by 10 μg/ml propidium iodide (Sigma-Aldrich) to counterstain nuclei. For double labeling, fluorescent labeling was performed with a TRITC-conjugated secondary antibody for the anti-ABCA12 antibody. The stained samples were observed under a confocal laser scanning microscope. Postembedding immunogold electron microscopy. Normal human skin samples were obtained from skin surgical operations under fully informed consent and were processed for postembedding immunoelectron microscopy as previously described. Cyrofixed, cryosubstituted samples were embedded in Lowicryl K11M resin (Electron Microscopy Sciences). Ultrathin sections were cut and incubated with anti-ABCA12 antibody, a secondary linker antibody, and a 5-nm gold-conjugated antibody for immunogold labeling.[5]

3.1 Epidemiology:

Frequency: Approximately 200 cases of harlequin ichthyosis have been reported. The incidence is calculated to be around 1 case in 300,000 births.

Race: No racial predilection is known for harlequin ichthyosis. A higher incidence may be encountered in cultures where parental consanguinity is common.

Sex: Sex distribution appears to be equal.

3.2 PATHOPHYSIOLOGY:

HI is caused by mutations in the lipid transporter adenosine triphosphate binding cassette A12 (ABCA12). ABCA12 is a member of the subfamily of adenosine triphosphate–binding cassette protein transporters. Its function in the epidermis is to facilitate the delivery of lipid glucosylceramides into lamellar granules, which then deliver them into the extracellular space. Electron microscopy of lamellar granules in HI are abnormally shaped, reduced in number, and in some cases absent. [11]

3.3 Etiology of harlequin ichthyosis:

In 2005 the cause of harlequin ichthyosis was traced to a mutation in the ABCA12 gene. This gene is believed to encode a transporter protein involved in the transport of fats across cell membranes. Parents usually have no signs or symptoms of the condition, but each carry a copy of the mutated gene in their cells. Identification of the gene has made prenatal DNA testing for the condition possible. Mutations in the ABCA12 gene cause the disease. The ABCA12 protein plays a major role in transporting fats in cells that make up the outermost layer of skin. Severe mutations in the gene lead to absence or partial production of the ABCA12 protein. This results in lack of lipid transport and as a result the skin development is affected by varying degrees according to the severity of the mutation.

3.4 SEVERITY OF HARLEQUIN ICHTHYOSIS:

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3.4 Figure 8: Body Of harlequin ichthyosis patients

3.4 FIG 9: FACES OF HARLEQUIN ICHTHYOSIS PATIENTS

3.4 FIGURE-11: NEW BORN HI BABY

hypoventilation and respiratory failure. Patients are often dehydrated, as their plated skin is not well suited to retaining water.

3.6 Signs of harlequin ichthyosis in newborns include:

- ✓ Thick skin plates that crack and split
- ✓ Distorted facial features.
- ✓ Tight skin around eyes and mouth (may force eyelids and lips to turn inside out and affect ability to feed)
- ✓ Restricted breathing (when chest or abdomen is affected)
- ✓ Hands and feet that are small, swollen and partially flexed
- ✓ Deformed ears or ears fused to the head (may appear to be missing)
- ✓ High blood sodium levels.[12]

3.7 Harlequin ichthyosis outlook:

IT WAS ONCE RARE FOR CHILDREN AFFECTED BY HARLEQUIN ICHTHYOSIS TO SURVIVE THE NEWBORN STAGE. NOW, IMPROVED TREATMENTS GIVE SOME A BETTER CHANCE OF LIVING INTO CHILDHOOD AND ADULTHOOD. UNFORTUNATELY, DUE TO THE SEVERITY OF THEIR CONDITION, SOME INFANTS DO NOT SURVIVE DESPITE THE BEST OF CARE. SURVIVING CHILDREN MAY HAVE:

- ✓ DRY, RED SKIN
- ✓ LARGE, THICK SKIN SCALES
- ✓ LIMITED MOVEMENT DUE TO TIGHT, INFLEXIBLE SKIN
- ✓ SPARSE HAIR DUE TO BLOCKED HAIR FOLLICLES
- ✓ DELAYED PHYSICAL DEVELOPMENT.
- ✓ OVERHEATING DUE TO REDUCED ABILITY TO SWEAT
- ✓ IMPAIRED HEARING OR EYESIGHT, DUE TO SKIN BUILD-UP OVER EARS AND EYES. [13]

3.8 CLINICAL CHARACTERISTICS:

Affected neonates are often born prematurely. 15 The thickened armorlike skin can cause pseudocontractures that restrict movement, and impaired perfusion can result in digital necrosis. Skin of surviving infantsgradually adapts from the aqueous intrauterine to the extrauterine environment, with skin phenotype evolving to generalized erythema and scaling, often with associated palmoplantar keratoderma. 15, 17HI can usually bedifferentiated from the less severe collodion baby phenotype (CBP) based on clinical appearance. HI features generalized armorlike yellow scale, whereas the classic skin changes of CBP are more translucent and
waxy. Ectropion and eclabium are often present in patients with CBP but are generally far less severe. [14].

3.5 Signs and symptoms:

Newborns with harlequin-type ichthyosis present with thick, fissured armor-plate hyperkeratosis. Sufferers feature severe cranial and facial deformities. The ears may be very poorly developed or absent entirely, as may the nose. The eyelids may be everted (ectropion), which leaves the eyes and the area around them very susceptible to infection. Babies with this condition often bleed during birth. The lips are pulled back by the dry skin (eclabium). Joints are sometimes lacking in movement and may be below the normal size. Hypoplasia is sometimes found in the fingers. Polydactyly has also been found on occasion. In addition, the fish mouth appearance, mouth breathing, and xerostomia place affected individuals at extremely high risk for developing rampant dental decay. Patients with this condition are extremely sensitive to changes in temperature due to their hard cracked skin, which prevents normal heat loss. Respiration is also restricted by the skin, which impedes the chest wall from expanding and drawing in enough air. This can lead to

4. Harlequin ichthyosis:

Harlequin ichthyosis is a severe genetic disorder that mainly affects the skin. Infants with this condition are born with very hard, thick skin covering most of their bodies. The skin forms large, diamond-shaped plates that are separated by deep cracks

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(fissures). These skin abnormalities affect the shape of the eyelids, nose, mouth, and ears, and limit movement of the arms and legs. Restricted movement of the chest can lead to breathing difficulties and respiratory failure. The skin normally forms a protective barrier between the body and its surrounding environment. The skin abnormalities associated with harlequin ichthyosis disrupt this barrier, making it more difficult for affected infants to control water loss, regulate their body temperature, and fight infections. Infants with harlequin ichthyosis often experience an excessive loss of fluids (dehydration) and develop life-threatening infections in the first few weeks of life. It used to be very rare for affected infants to survive the newborn period. However, with intensive medical support and improved treatment, people with this disorder now have a better chance of living into childhood and adolescence.

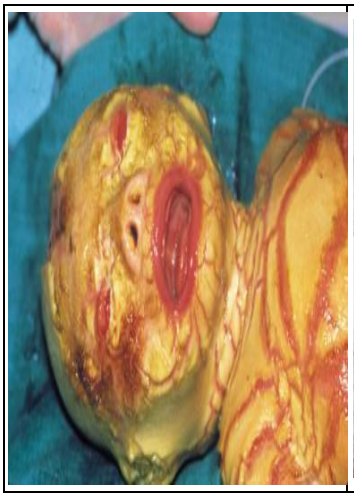
Harlequin-type ichthyosis

Harlequin-type ichthyosis is a very rare severe genetic disease, which causes thickening of the skin. At birth, the child's whole body is encased in an 'armor' of thick white plates of skin, separated by deep cracks. In addition, the eyes, ears, penis, and limbs may be abnormally contracted. Because of resultant cracked skin in locations where normal skin would fold, it is easily pregnable by bacteria and other contaminants, which can result in serious risk of fatal infection. It is an autosomal recessive congenital ichthyosis, which is a group of nonsyndromic disorders of keratinization. It is associated with a mutation in the gene for the protein ABCA12. The disease can be diagnosed in the uterus by way of fetal skin biopsy or by analysis of amniotic fluid cells obtained by amniocentesis. Common features of the disease can be recognized through ultrasound, and follow up with 3D ultrasound to diagnose the condition. Ultrasound can reveal abnormal facial features with ectropion, eclabium, short foot length, incurved toes, clenched fists, poor delineation of nostrils, and polyhydramnios. Constant care is required to moisturize and protect the skin. The overall rate of harlequin ichthyosis is 1 in 300,000 births. The harlequin-type designation comes from the diamond shape of the scales at birth (resembling the costume of Arlecchino).[6]

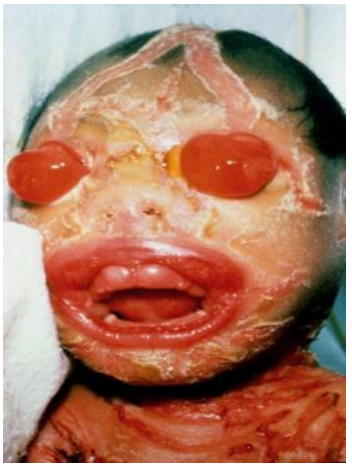
4.1 A Brief History of Harlequin Ichthyosis:

Background:

Harlequin ichthyosis is the most severe form of autosomal recessive congenital ichthyosis. Harlequin ichthyosis is characterized by a profound thickening of the keratin layer in fetal skin. The affected neonate is born with a massive, horny shell of dense, platelike scale and contraction abnormalities of the eyes, ears, mouth, and appendages, as is shown in the images below.



4.1 Figure:13:HI affected baby. [Courtesy of Dr Bernice Krafchik]



4.1 Figure-14: Harlequin ichthyosis. [Courtesy of Jason K Rivers, MD,FRCPC, and Dr Lawler.]

The term harlequin derives from the facial appearance and the triangular and diamond-shaped pattern of hyperkeratosis. The newborn's mouth is pulled wide open, mimicking a clown's smile. Marked eclabium and ectropion are present secondary to the taut, unyielding skin. The ears may be absent or poorly developed. The arms, feet, and digits have flexion contractures and may be hypoplastic. The skin barrier is severely compromised, leading to excessive water loss, electrolyte abnormalities, temperature dysregulation, and an increased risk of life-threatening infection. The tight, armorlike scale can restrict respiration. Poor feeding and impaired intestinal absorption are common. This disease primarily affects the skin. Other systems may be significantly compromised by the hyperkeratosis and concomitant deformities. Neonates are often born prematurely. The underlying genetic abnormality in harlequin ichthyosis is a mutation in the lipid-transporter gene *ABCA12* on chromosome 2. Immunohistocytochemical examination of the skin reveals characteristic abnormalities in the structure of lamellar granules and in the expression of epidermal keratin. In the past, harlequin ichthyosis was uniformly fatal. Improved survival has been achieved with intense supportive care and systemic retinoid therapy in the neonatal period. Patients who survive manifest a debilitating; persistent ichthyosis like severe congenital ichthyosiform erythroderma. Other Medscape articles on ichthyosis include Hereditary and AcquiredIchthyosis Vulgaris, Lamellar Ichthyosis, X-Linked Ichthyosis, and Ichthyosis (ophthalmology focus).[7]

4.2 GENETIC CHANGES:

Mutations in the ABCA12 gene cause harlequin ichthyosis. The ABCA12 gene provides instructions for making a protein that

is essential for the normal development of skin cells. This protein plays a major role in the transport of fats (lipids) in the outermost layer of skin (the epidermis). Some mutations in the ABCA12 gene prevent the cell from making any ABCA12 protein. Other mutations lead to the production of an abnormally small version of the protein that cannot transport lipids properly. A loss of functional ABCA12 protein disrupts the normal development of the epidermis, resulting in the hard, thick scales characteristic of harlequin ichthyosis.[8]

INHERITANCE PATTERN:

This condition is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. The parents of an individual with an autosomal recessive condition each carry one copy of the mutated gene, but they typically do not show signs and symptoms of the condition.[10]

4.3 OTHER NAMES FOR THIS CONDITION:

- Harlequin baby syndrome
- HI
- Ichthyosis Congenita, Harlequin Fetus Type [9]

5. Diagnosis:

The diagnosis of harlequin-type ichthyosis relies on both physical examination and certain laboratory tests. Physical assessment at birth is vital for the initial diagnosis of harlequin ichthyosis. Physical examination reveals characteristic symptoms of the condition especially the abnormalities in the skin surface of newborns. Abnormal findings in physical assessments usually result in employing other diagnostic tests to ascertain the diagnosis. Genetic testing is the most specific diagnostic test for harlequin ichthyosis. This test reveals a loss of function mutation on the ABCA12 gene. This gene is important in the regulation of protein synthesis for the development of the skin layer. Mutations in the gene may cause impaired transport of lipids in the skin layer and may also lead to shrunken versions of the proteins responsible for skin development. Less severe mutations result in a collodion membrane and congenital ichthyosiform erythroderma-like presentation. ABCA12 is an ATP binding cassette (ABC) transporter and is a member of a large family of proteins that hydrolyze ATP to transport cargo across membranes. ABCA12 is thought to be a lipid transporter in keratinocytes necessary for lipid transport into lamellar granules during the formation of the lipid barrier. Biopsy of skin may be done to assess the histologic characteristics of the cells. Histological findings usually reveal hyperkeratotic skin cells, which leads to a thick, white and hard skin layer.

5.1 PRENATAL DIAGNOSIS:

Advances in fetal DNA analysis and ultrasound technology have replaced the more invasive techniques of fetal skin biopsy. 1 Fetal DNA analysis can be offered to parents who had a previous child with HI. Fetal genomic DNA is obtained from amniotic fluid via amniocentesis or chorionic villus sampling. New research has shown that messenger RNA analysis using hair samples can also more easily and less invasively be used to identify ABCA12 mutations. 14In some cases, prenatal ultrasonography may allow detection of signs suggestive of HI, including eclabium, ectropion, rudimentary ears, contractures, and dense floating particles in amniotic fluid ("snowflake sign"). The application of three-dimensional ultrasound theoretically offers a
greatly improved analysis of facial morphology and may aid in prenatal diagnosis; however, detection of these unusual features requires tertiary expertise, and they are not detectable until the second trimester, excluding the option of early termination. [15]

5.2 Laboratory Methods:

Genetic testing for mutations in the *ABCA12* gene is available. Complete sequence analysis of the coding region of this gene is performed to identify specific mutations. Peripheral blood cells or cells from a buccal smear from affected individuals are required. Extensive information regarding genetic testing for harlequin ichthyosis is available from GeneDx. Carrier testing is available for relatives after the proband's mutation is identified. Prenatal diagnosis is available for fetuses with suspected harlequin ichthyosis who may or may not have a family history of the disorder. The following laboratory investigations may be helpful in the newborn period to identify complications of harlequin ichthyosis:

- Check the WBC count and skin and blood cultures for signs of infection.
- Closely monitor serum electrolyte levels, which may be abnormal secondary to dehydration.
- Monitor serum calcium and glucose, as hypocalcemia and hypoglycemia may occur.
- Check BUN and creatinine levels for signs of renal failure.
- Monitor hemoglobin levels because severe anemia is reported.

5.3 Imaging Methods:

Prenatal ultrasonography, particularly 3-dimensional ultrasonography, may show features suggestive of harlequin ichthyosis. This has been particularly helpful in antenatal diagnosis of infants with no family history of harlequin ichthyosis. Characteristic features include a large and gaping mouth, aplasia of

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the nose, abnormal limbs, bulging eyes, rudimentary ears, flexion contractures, and floating particles in the amniotic fluid. Growth restriction and polyhydramnios are also described. Two-dimensional ultrasonography can also demonstrate features of harlequin ichthyosis but not until late in the second trimester, when enough keratin buildup is present to be sonographically detectable. Short feet may be an early marker for harlequin ichthyosis. This may be detectable in the early second trimester before other signs of harlequin ichthyosis are noticeable. Chest radiography may be indicated if respiratory distress is present postnatally. Renal ultrasonography may be indicated if renal failure or poor urine output is evident. Renal dysplasia has been described in harlequin ichthyosis. Further investigations should be based on the history and findings from physical examination.

5.4 Histopathologic Method:

The stratum corneum is thick and compact. Hyperkeratosis may be more marked around hair follicles compared with the interfollicular epidermis. The histopathologic hallmark is an extraordinary thickened and compact orthokeratotic stratum corneum, although in some cases parakeratosis has been observed. Cells within the stratum corneum are abnormally keratinized. Granular, spinous, and basal cell layers appear unremarkable. Inflammatory cells may infiltrate the papillary dermis. Hair follicles show marked, concentric accumulation of keratotic material around hair shafts, which is considered a diagnostic feature of harlequin ichthyosis and has been used to establish the diagnosis prenatally. [16]

6. Treatment and prognosis:

Constant care is required to moisturize and protect the skin.

The hard outer layer eventually peels off, leaving the vulnerable inner layers of the dermis exposed. Early complications result from infection due to fissuring of the hyperkeratotic plates and respiratory distress due to physical restriction of chest wall expansion. Management includes supportive care and treatment of hyperkeratosis and skin barrier dysfunction. A humidified incubator is generally used. Intubation is often required until nares are patent. Nutritional support with tube feeds is essential until eclabium resolves and infants can begin nursing. Ophthalmology consultation is useful for the early management of ectropion, which is initially pronounced and resolves as scale is shed. Liberal application of petrolatum is needed multiple times a day. In addition, careful debridement of constrictive bands of hyperkeratosis should be performed to avoid digital ischemia. Cases of digital autoamputation or necrosis have been reported due to cutaneous constriction bands. Relaxation incisions have been used to prevent this morbid complication.

In the past, the disorder was nearly always fatal, whether.

due to dehydration, infection (sepsis), restricted breathing due to the plating, or other related causes. The most common cause of death was systemic infection and sufferers rarely survived for more than a few days. However, improved neonatal intensive care and early treatment with oral retinoids, such as the drug Isotretinoin (Isotrex), may improve survival. Early oral retinoid therapy has been shown to soften scales and encourage desquamation. After as little as two weeks of daily oral isotretinoin, fissures in the skin can heal, and plate-like scales can nearly resolve. Improvement in the eclabium and ectropion can also be seen in a matter of weeks. Children who survive the neonatal period usually evolve to a less severe phenotype, resembling a severe congenital ichthyosiform erythroderma. Patients continue to suffer from temperature dysregulation and may have heat and cold intolerance. Patients can also have generalized poor hair growth, scarring alopecia, contractures of digits, arthralgias, failure to thrive, hypothyroidism, and short stature. Some patients develop a rheumatoid factor-positive polyarthritis. Survivors can also develop fish-like scales and retention of a waxy, yellowish material in seborrheic areas, with ear adhered to the scalp. The oldest known survivor is Nusrit "Nelly" Shaheen, who was born in 1984 and is in relatively good health as of April 2016. Lifespan limitations have not yet been determined with the new treatments. A study published in 2011 in the Archives of Dermatology concluded, "Harlequin ichthyosis should be regarded as a severe chronic disease that is not invariably fatal. With improved neonatal care and probably the early introduction of oral retinoids, the number of survivors is increasing." [17]

6.1 Treatment for harlequin ichthyosis includes:

Babies born with harlequin ichthyosis need immediate, individual, nursing care in a neonatal intensive care unit. They are less able to maintain a safe body temperature and more prone to fluid loss, dehydration and life threatening infections in the first weeks of life. Measures may include:

- ✓ Intravenous tubes to deliver fluids and nutrition
- ✓ Monitoring of electrolytes and sodium
- ✓ Lubrication and protection of the eyes, if eyelids are forced open.
- ✓ Heated, high humidity incubator to maintain body temperature and prevent skin cracks.
- ✓ Antibiotics may be prescribed to prevent infection.
- ✓ Retinoids may be prescribed to accelerate shedding of the skin scales.[17]

6.2 Complications:

Complications in the neonatal period include the following:

- Sepsis

- Respiratory compromise
- Dehydration, hypernatremia, hypocalcemia, hypoglycemia
- Hyperthermia
- Feeding difficulty
- Nasal obstruction
- Conjunctivitis, keratitis
- Limb or digital constriction, ischemia

Infants who survive the newborn period have a lifelong, severe ichthyosiform erythroderma. Recurrent skin infections may continue after the newborn period. Contractures and painful fissuring of the hands and the feet may occur. Rajpopat et al reported palmoplantar keratoderma in 52% of survivors, causing pain and delay in walking. Pruritus was reported in 44% of patients, heat and cold intolerance was found in 36%, reduced sweating was found in 28%, and photosensitivity and pigmented macules were found in one patient each. Poor hair growth and nail deformities were common. Hearing impairment may result from obstruction of the ear canals by skin debris. Developmental delay and normal intellectual development are described. Rajpopat et al reported that most school-aged survivors were attending mainstream schools, although many needed additional help. Growth must be closely monitored. Short stature is common and weight below average. Nutritional rickets due to vitamin D deficiency is reported. This is likely due to defective vitamin D synthesis in the abnormal skin, calcium loss, and reduced exposure to sunlight. Inflammatory arthritis and permanent contractures may occur. Hypothyroidism and juvenile idiopathic arthritis have been reported in a patient with harlequin ichthyosis. [18]

6.3 Can it be cured?

There is no cure for the disorder, but it can be managed with treatment. In the past the disease was considered fatal. But with advanced technology improved survival rate has been achieved with intense neonatal care. [18]

7. MANAGEMENT:

- Management of HI During the Neonatal Period
- Admission to a NICU.
- Placement in a humidify ed isolate at 50%–70% humidify cation.
- Monitoring of body temperature to prevent TEWL and associated temperature dysregulation.
- Monitoring of daily body weight and fluid status; daily monitoring of electrolytes to prevent
- hypernatremic dehydration during the first week and thereafter based on patient's condition.
- Meeting high caloric demands and nutrition supplementation.
- Skin care with daily bathing (± antiseptics) followed by application of bland emollient every 4–6 h.
- Vigilant monitoring for respiratory compromise with a low threshold for intubation.
- Vigilant monitoring for infection with surveillance cultures from selected skin sites and folds with a low
- threshold for antibiotics based on cultured microbes.
- Eye care with application of bland lubricant every 6–12 h; reserve topical antibiotics for conjunctivitis or corneal abscesses.
- Monitoring of limb and digit perfusion. Consider surgical intervention, splinting, and physical therapy as needed.
- Controlling pain with acetaminophen, nonsteroidal anti-inflammatory agents and/or narcotics as needed.[19]

7.1 Medical Care:

Ensure that the patient's airway, breathing, and circulation are stable after delivery. Early intubation may be required. Babies require intravenous access. Peripheral access may be difficult and umbilical cannulation may be necessary. Place infants in a humidified incubator. Monitor temperature, respiratory rate, heart rate, and oxygen saturation. Once stabilized, transfer newborn with harlequin ichthyosis to a NICU. Exposure keratitis results from ectropion of the eyelids. Apply ophthalmic lubricants frequently to protect the conjunctivae. Bathe infants twice daily and use frequent wet sodium chloride compresses followed by application of bland lubricants to soften hard skin. Dilute bleach baths may reduce the risk of skin infection. Topical keratolytic (eg, salicylic acid) are not recommended in newborns because of potential systemic toxicity. According to Rajpopat et al, early retinoid treatment (by day 7) may require prompt consideration, as these medications can take some days to obtain. See Medication. Tazarotene, a topical retinoid, has been reported to be beneficial. Intravenous fluids are almost always required. Consider excess cutaneous water losses in daily fluid requirement calculations. Monitor serum electrolyte levels. A risk of hypernatremic dehydration exists. Neonates with harlequin ichthyosis initially do not feed well and may require tube feeding. Maintain a sterile environment to avoid infection. Take frequent cultures of the skin. Growth of pathogenic organisms (eg, *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella*) indicates risk of sepsis. Draw blood cultures because sepsis can occur quickly in affected infants. [19]

7.2 Further inpatient care:

Continue careful attention to skin care and use of emollients during retinoid therapy. Infants with harlequin ichthyosis can be successfully breastfed or bottle-fed as the eclabium improves. Involving occupational therapy to aid in feeding strate-

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gies is advised. Carefully monitor weight gain and intake. Affected infants are at risk of failure to thrive. Physical bonding between the parents and the baby should be encouraged.[19]

7.3 Palliative Care:

Palliative care is treatment of the discomfort, symptoms, and stress of serious illness. It provides relief from distressing symptoms including.

- Pain
- Shortness of breath
- Fatigue
- Constipation
- Nausea
- Loss of appetite
- Problems with sleep
- It can also help you deal with the side effects of the medical treatments you're receiving.
- Hospice care, care at the end of life, always includes palliative care. But you may receive palliative care at any stage of an illness. The goal is to make you comfortable and improve your quality of life.[19]

7.4 Surgical Care:

Hyperkeratosis causing constriction of limbs, digits, or nasal obstruction may need to be treated surgically. [19]

7.5 Long-Term Monitoring:

Infants are discharged from the hospital when their cutaneous symptoms are improving, feeding and weight gain are established, and they are free of infection. Social and psychological support should be provided for the parents/caregivers. The primary care physician should closely monitor the infants for growth, development, social issues, and skin surveillance. A dermatologist should monitor affected infants for ongoing assessment and for monitoring of retinoid therapy. Adverse effects of retinoid therapy (eg, mucocutaneous dryness, aberrant liver function tests, hypertriglyceridemia, benign intracranial hypertension) should be noted. Serum AST, ALT, total cholesterol, and triglyceride levels should initially be obtained monthly initially. The clinician should be cognizant of the musculoskeletal abnormalities that can occur with long-term retinoid therapy if treatment is continued. Follow-up with an ophthalmologist is required. Recurrent exposure keratitis can be a problem because of persistent ectropion. [19]

8. Future directions for management of HI:

The thick plate-like skin will gradually split and peel off. Antibiotic treatment may be necessary to prevent infection at this time. Administration of oral etretinate (1 mg./kg. body weight) may accelerate shedding of the thick scales. Most harlequin infants will need one-on-one nursing care for the first several weeks of life. In the past, these infants rarely survived the first few days of life. However, with recent advances in neonatal care and perhaps with the administration of etretinate, 1 mg./kg. body weight, harlequin infants can survive. Several surviving children are now in their teenage years, with several in their twenties. The surviving children display dry, reddened skin, which may be covered by large thin scales, and sparse hair. Physical development may be delayed by the enormous calorie needs their skin function demands, but mental and intellectual developments are expected to be normal. Harlequin ichthyosis demands a meticulous skin care regimen to keep the skin moisturized and pliable and to prevent cracking and fissuring that may lead to infection. [1] It takes a week or two for etretinate to work loosening the scales. Because most of the fatalities from this condition occur in the first few days of life, many of the successes attributed to etretinate use in the medical literature may be equally due to the high quality of care in the immediate newborn period and to a less severely affected newborn. Some newborns with harlequin ichthyosis will not survive, even with the best of care, because of the severity of their condition.

8.1 NEONATAL MANAGEMENT:

Admission to a tertiary care center with a level III NICU is desirable. Intensive care management is largely supportive and involves a multidisciplinary team, including neonatology, dermatology, genetics, ophthalmology, otolaryngology, orthopedic and plastic surgery, nutrition, physical therapy, and nursing. Because of the significant morbidity associated with HI, the high risk of respiratory failure requiring intubation, and potential risk of neonatal demise, discussion regarding aggressivity of support should be held with parents and documented early in the course (see Ethical Considerations section below). Beyond complications of prematurity, minimizing TEWL, preventing electrolyte imbalance, temperature dysregulation, respiratory distress, malnutrition, and infection are key to survival and best performed in the NICU (Table 2).Neonates should be maintained in an incubator with added humidity, as individualized for the patient. 19 Serum electrolytes, urine output, daily weights, prealbumin, and kidney and liver function should be monitored closely (Table 3). Prevention of infection is particularly important. Deep fissuring of the thick scale can penetrate the epidermis and become a source of pain. It is important to provide adequate pain control (see section below). Many neonates will initially require narcotics. Infants with milder phenotypes may have adequate pain control with acetaminophen or nonsteroidal anti-inflammatory agents. [19]

8.2 Securing Lines:

Initial placement of a central venous umbilical line is standard

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for high-risk neonates and useful in patients with HI for hydration, parenteral nutrition, and laboratory sampling. Alternative access is via peripheral scalp vein or a peripherally inserted central catheter line. A protocol to secure lines in infants with fragile skin has been developed.[19]

8.3 Skin Care:

Skin barrier dysfunction in neonates is especially problematic, given the large body surface-to-weight ratio. Skin care should include once to twice daily cleansing to hydrate and promote shedding of the stratum corneum. Some suggest daily buffered dilute hypochlorite baths. 11This can be accomplished by dampening roll gauze with warmed 0.125% sodium hypochlorite mixed 1:10 with warmed sterile water. The optimal pH is 8 to 8.5. The gauze can be applied as a wet wrap, occluded with a plastic wrap layer for 10 to 20 minutes. A bland emollient should be applied immediately after wet wrap removal. Products, such as petrolatum jelly, extra virgin coconut oil, and sunflower seed oil, are considered safe and may even possess antimicrobial properties. The authors recommend handling infants with sterile, latex-free gloves and using single use packets of emollient to minimize colonization with pathogenic microbes. Application of keratolytic-containing emollients should be avoided in the neonatal period due to the risk of percutaneous toxicity. Respiratory distress has been reported after use of topical salicylic acid. Reports on the use of topical ceramide-containing emollients have been conflicting. Although ceramides can stimulate ABCA12 expression through the peroxisome proliferator-activated receptor PPAR δ topical application has not been shown to improve barrier function.Digital necrosis is a common complication in HI, related to a compartmentlike syndrome from epithelial constriction. Surgical intervention can be digit or limb saving . Several fasciotomy techniques have been described, including a linear band incision technique. application of a topical retinoid (eg, 0.1% tazarotene cream) and soft splinting of the hands and feet may be an alternative to or augment surgical intervention.[19]

8.4 Pain Control:

Deep fissures and skin sloughing can be a source of pain in neonates. Assessing the severity of pain in neonates with HI, however, can be challenging. Newborn pain scales include facial expression as a parameter; most also include extremity tone and respiratory rate. Interpreting each of these can be problematic in infants with HI. The characteristic eclabium results in a fixed facial appearance as if in pain. The respiratory rate can be increased due to restricted tidal volumes. The fingers and toes may remain fixed due to encasement. As such, other parameters, such as heart rate, blood pressure, crying, and state of arousal, become relatively more useful. It is imperative that pain is well managed,
and that parents are reassured that this issue has been addressed. Adequate pain control might require the use of narcotics, even if such treatment contributes to a requirement for assisted ventilation. The severity of pain dissipates after the surface layer has been shed, and the underlying skin epithelialized. and exposure keratitis. 1Lubricant ophthalmic ointment should be applied to lid margins a minimum of every 6 to 12 hours. Surgical correction of ectropion has been reported with full-thickness autografts from the thigh and posterior auricular skin as well as from engineered human skin. There is no evidence that early surgery results in less ectropion at 6 to 12 months of age than would occur as part of the natural history of HI. Because retinoids promote stratum corneum desquamation, both oral37and topical retinoids 38may be effective in reducing ectropion. Early ophthalmology evaluation is recommended.[19]

8.5 **Ocular Management**:

The thick stratum corneum on the eyelids results in bilateral ectropion, placing infants at high risk for conjunctivitis, squinting, and exposure keratitis. 1Lubricant ophthalmic ointment should be applied to lid margins a minimum of every 6 to 12 hours. Surgical correction of ectropion has been reported with full-thickness autografts from the thigh and posterior auricular skin as well as from engineered human skin. There is no evidence that early surgery results in less ectropion at 6
to 12 months of age than would occur as part of the natural history of HI. Because retinoids promote stratum corneum desquamation, both oral 37 and topical retinoids may be effective in reducing ectropion. Early ophthalmology evaluation is recommended.[19]

8.6 Otologic Management:

Reports on the otologic manifestations of HI have noted that patients have signs or symptoms of ear discomfort. Possible causes include excessive ear canal debris, dysbiosis, or secondary contact dermatitis. Vigorous ear debridement and skin manipulation should be avoided to minimize the risks of infection. Professional microsuctioning to remove skin debris may help prevent early conductive hearing loss. Ear drops have also been used to soften keratin plugs in the ear canal. Options include 0.25% acetic acid or 2% aluminum acetate. Avoid the potential percutaneous toxicity of salicylic acid and the risk of Malasseziaovergrowth associated with olive oil. Early otolaryngology26and audiology intervention is recommended.[19]

8.7 RETINOID THERAPY**:**

The use of systemic retinoids has become standard-of-care in the management of HI, following a reported 83% survival among 25 treated infants compared with 24% survival of 21 infants who did not receive an oral retinoid. However, these results must be interpreted with some caution because half of the untreated infants died within 3 days after birth, which is earlier

than when retinoid therapy is usually available for administration. Another study documented a 92% survival rate among 12 infants treated with retinoids compared with 50% among those not treated. The efficacy of oral retinoids is not well understood. Some authors suggest that the reported benefit may be an overall improvement in intensive care in addition to oral retinoid therapy. Several oral retinoids have been used in the management of HI as well as other congenital ichthyoses, including etretinate, isotretinoin, and acitretin. The first successful neonatal use of acitretin in HI was reported in 2001 at a dose of 1 mg/kg per day, started on day 10 of life. Acitretin administration has been the retinoid most often used by the authors. Additionally, compounding isotretinoin rapidly isomerizes the 13-cis molecule to all-trans retinoic acid, which may have greater toxicity than the aromatic acitretin. Treatment initiation within the first 7 days of life is recommended for all infants who can tolerate the medication. However, neonatal acitretin administration has been hindered by a lack of commercially available liquid formulations in the United States. The authors have found that capsular acitretin is not water soluble, so preparing a liquid requires compounding expertise. Acitretin can be compounded by extracting the powder from 10 mg capsules. The powder is weighed, and the appropriate dose is administered in a small aliquot of warm milk. At Maimonides Medical Center (Brooklyn, NY) acitretin is prepared in an Ora-Plus solution an aqueous-based vehicle with a slightly acidic pH to help reduce oxidative degradation. The current literature suggests that the acitretin dose be between 0.5 and 1 mg/kg per day.

A 2-day half-life suggests that once daily dosing is sufficient. Surveillance laboratory data includes a complete blood count, comprehensive metabolic panel, and lipids at baseline and monthly. Acitretin should be titrated to the lowest dose possible for individual patients based on clinical improvement with regular skin examinations and monitoring of side effects. Retinoids are typically able to be discontinued by 6 months of age. The early introduction of retinoid therapy is likely related to hastened desquamation

(Fig 1B). Retinoids may be especially useful for improving digital and thoracic constrictions, thus improving functional movement, and breathing in HI neonates.

The benefits of oral retinoid therapy outside of the neonatal period are unclear, because there may be spontaneous skin improvement in HI.

As genetic testing expands, more information on genotype/phenotype correlations may provide additional information on which neonates will respond best to retinoid therapy. In cases where oral retinoid therapy cannot be tolerated, the use of topical retinoids has proved beneficial. A 2014 report of an infant with HI demonstrated improvement in skin.

with application of 0.1% tazarotene cream to the face, scalp, hands, and feet. One report documented application of a topical retinoid as an effective alternative to systemic therapy with improvement in limb contractures 50and another for ectropion management.

Topical retinoid therapy has also been used successfully in older infants as oral retinoid therapy was tapered.[19]

8.8 FAMILY COUNSELING:

The birth of a neonate with HI poses a great challenge for the family. During the first several days, parents will need to cope with the initial shock of an affected child, grieve the loss of the anticipated child, and come to an understanding of the long-term medical issues that their child will face. As in other situations in which dysmorphology is so strongly manifested, the appearance of the neonate may lead parents to harbor feelings of guilt or resentment and avoid seeing the baby after birth. Bond formation between mother and infant has been shown to be delayed in the NICU setting when a neonate's appearance was not compatible with a mother's expectation.

There are approaches that can foster bonding between the family and infant. Touch should be encouraged. Sharing photographs of survivors to family members has been a beneficial intervention. It is also important for healthcare teams to educate and prepare families.

8.9 Genetic Counseling:

Genetic counseling provides information and support to people who have, or may be at risk for, genetic disorders. A genetic counselor meets with you to discuss genetic risks. The counseling may be for yourself or a family member. Or you may get it when you are planning or expecting a baby. You may follow up with genetic testing.

There are many reasons to seek genetic counseling. You may consider it if you.

- Have a personal or family history of a genetic condition or birth defect.
- Are pregnant or planning to be pregnant after age 35.
- Already have a child with a genetic disorder or birth defect
- Have had two or more pregnancy losses or a baby who died.
- Have had ultrasound or screening tests that suggest a possible problem.[19]

8.10 Pregnancy Management:

Affected mothers are at no specific disease-related risks during pregnancy. Anecdotal improvement of the ichthyosis with return to baseline after delivery has been reported.

Babies born with harlequin ichthyosis and, less common, collodion membrane have an increased incidence of premature birth with concomitant perinatal morbidity and mortality.

8.11 BEYOND THE NEONATAL PERIOD**:**

The severely abnormal keratotic epithelium marking HI at birth gradually transitions over 4 to 6 weeks to a severe ichthyosiform erythroderma secondary to the dryness of a post uterine environment. This condition requires treatment with frequent. application of bland emollients and active skin care techniques. Children remain prone to infection, although less so than during the neonatal period. Persistent ectropion requires frequent eye lubrication and protection. As discussed previously, topical retinoids applied to the eyelids may be useful. Additionally, patients can have heat and cold intolerance, pruritus, and hair and nail abnormalities. Physical and occupational therapy is key to optimizing range of motion in infancy and childhood as the hyperkeratotic skin can lead to encasement and constriction of limbs and digits, affecting fine and gross motor skills.
Some infants and children may display impaired cognitive and social functioning, making speech and language therapy necessary. [19]
It is important to note that some children with HI can function well and attend regular schools. Children with HI require long-term coordinated, multispecialty care. Enhanced long-term survival may permit recognition of other associated comorbidities, such as synovitis. The mental health of patients and their families should be addressed, including issues of socialization, self-confidence, and quality of life. [19]

8.12 ETHICAL CONSIDERATIONS:

The diagnosis of HI can present ethical challenges for parents and healthcare providers. The striking physical appearance of the newborn with HI may lead to perceptions of decreased amenability to treatment and greater physical pain than are actually the case. Such perceptions can influence decisions regarding aggressivity of care. In a survey exploring the parental perspectives regarding end-of life care in the PICU, 31% of parents described the way their child looked as being a "very important" consideration. The influence of appearance on ethical decision-making also extends to health care providers. Specialists knowledgeable in HI should thus make effort to ameliorate the potentially disproportionate influence of the physical appearance. It is important that the family and the health care team are helped to understand that the initial appearance is transient, that pain can be controlled, and that the underlying skin disorder can be treated. At the same time, the long-term problems facing children with HI must be given consideration. These have been outlined previously, and include life-long dermatologic problems, potential for chronic pain, increased risk for neurodevelopmental delay, increased frequency of hospitalization, and possible need for surgical interventions. 1It is questionable, however, whether the severity
of these issues rises to the level that foregoing early intensive care management and retinoid therapy should be considered. A patient with HI is not expected to have the severe neurocognitive disabilities that have been used in other situations to justify nonintervention during an early "window," in which survival depends on intensive care management.[19]

8.13 Patient Education:

Advise parents and caregivers that the baby's appearance will improve after the neonatal period. Emphasize the need for attention to skin lubrication and for compliance with systemic therapy. Teach them to recognize signs of infection.

Congenital ichthyoses can have devastating medical and social consequences. Parents may wish to communicate with other families who have been similarly affected. Patient organizations (eg, The Foundation for Ichthyosis and Related Skin Types [FIRST]) are available in several countries to provide support to families.

8.14 Consultations:

Early formation of a multidisciplinary team is recommended and may include the following:

- Neonatologist
- Dermatologist
- Medical geneticist
- Ophthalmologist
- Ear-nose-throat specialist
- Plastic surgeon
- Dietician
- Social worker
- Occupational therapist
- Physical therapist [20]

Prevention of Secondary Complications:

The following measures are appropriate:

- Prevention of infection in the newborn (pivotal to outcome)
- Prevention of dehydration
- Maintenance of body temperature
- Prevention of corneal drying
- Release of collodion membrane on digits, when necessary, to prevent reduced circulation leading to loss of digits.
- Prevention of chest constriction resulting from tautness of membrane to assure adequate respiration.

9. Conclusion:

HI is a rare form of congenital ichthyosis that can present many

challenges throughout a lifetime, but especially during the neonatal period. An understanding of the ABCA12 mutation and skin barrier disruption provides a basis for therapy. Aggressive and supportive care from an interdisciplinary team is required for effective management; additionally, in the absence of data to the contrary, the authors believe it is advisable to institute early retinoid therapy. The life expectancy of a baby born with **Harlequin ichthyosis** was generally given as just a few days. Often, this was due to the severe dehydration the babies were often plagued with or the result of a superimposed infection from the cracking and peeling of the skin. When large amounts of skin come off, the body struggles to regulate the temperature, which can cause the baby to go into shock. Also, with the thick plates of skin covering most of the body surface, the baby can overheat as the skin can't breathe.

More recently there have been several advances in the **treatment** of Harlequin ichthyosis. Unfortunately, there is no known cure, but ongoing treatments and management can help to prolong the life of a Harlequin sufferer. The most notable change to the treatment regime is the addition of drugs such as retinoids**.**

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