

XomeDxSlice – Ichthyosis

Panel Gene List: ABCA12, ABHD5, AGPS, ALDH3A2, ALOX12B, ALOXE3, AP1S1, ARSE, CASP14, CERS3, CLDN1, CYP4F22, EBP, ELOVL4, FLG, GJB2, GJB3, GJB4, GJB6, KRT1, KRT10, KRT2, KRT9, LIPN, LOR, NIPAL4, PEX7, PHGDH, PHYH, PNPLA1, PNPLA2, POMP, PSAT1, SDR9C7, SLC27A4, SNAP29, SPINK5, ST14, STS, TGM1, TGM5, VPS33B, ZMPSTE24

Clinical Features and Genetics:

Congenital ichthyoses are a heterogeneous group of disorders manifesting at birth or infancy with visible scaling and/or thickening of the skin, which may be accompanied by variable degree of redness (erythema), skin fragility or blistering, abnormalities of hair, nails, or mucous membranes. Scaling and/or thickening of the outermost layer of the skin (so-called hyperkeratosis) may be generalized or localized, may involve other organ systems (syndromic ichthyosis) or be limited to skin and skin appendages (non-syndromic ichthyosis). The major types of ichthyoses are: i) Epidermolytic ichthyosis (EI) inherited in an autosomal dominant manner, ii) autosomal recessive (non-syndromic) congenital ichthyoses (ARCI), iii) various syndromic forms of autosomal recessive congenital ichthyosis, iv) X-linked ichthyosis (steroid sulfatase deficiency) and related disorders of cholesterol metabolism and v) various other related disorders of cornification with hyperkeratosis (rather than scaling) of the skin.

i) Epidermolytic ichthyoses (EI)

EI presents at birth with erythroderma, blisters or erosions, and larger areas of denuded skin. While skin fragility decreases with age, severe hyperkeratosis with a verrucous, ridged or cobblestone surface develops over time. Palms and soles may be severely involved or completely spared. There is also a mosaic form of the disease due to somatic variants during embryonic development with skin findings often following the lines of Blaschko. A mild variant of EI with superficially peeling or denuded areas described as ‘molting’ or ‘Mauserung’ is known as superficial epidermolytic ichthyosis (SEI). EI is caused by heterozygous variants in the genes encoding keratin 1 or 10 (KRT1, KRT10), while SEI is due to variants in keratin 2 (KRT2). A variant of EI limited to palms and soles (so-called epidermolytic palmoplantar keratoderma) is caused by variants in the KRT9 gene. This group of disorders is inherited in an autosomal dominant pattern, whereby about 50% of variants occur sporadic (de novo variants).

ii) Autosomal recessive (non-syndromic) congenital ichthyoses (ARCI)

Most neonates with ARCI present as collodion babies with a taut, translucent membrane encasing the entire body that lasts for up to two weeks. In severe cases, ectropion, eclabium, and scarring alopecia of the scalp and eyebrows may be present. After shedding the collodion

membrane, the presentation and severity of ARCI between individuals can vary significantly. At one end of the spectrum is severe 'classic' lamellar ichthyosis (LI), which is characterized by large, dark brown, plate-like scale without underlying erythroderma. At the other end is severe 'classic' CIE, with fine, whitish scale and intense redness (erythroderma) of the skin. The clinical features of CIE can be milder than in LI and demonstrate a greater variability in the intensity of redness, scale, and involvement of palms and soles. However, CIE may cause substantial metabolic stress in young children and growth delay. There are also cases of almost complete resolution of the skin disorder, so-called 'self-improving collodion ichthyosis'. The most severe and life-threatening form of ARCI is Harlequin ichthyosis. Infants are usually born prematurely encased in a thick, hard, armor-like covering with deep fissures that severely restricts movements. The tautness of skin results in ectropium, eclabium, malformation nose and ear cartilage, alopecia, microcephaly, and swellings of hands and feet. The postnatal period is usually complicated by respiratory distress, dehydration, electrolyte imbalance, temperature instability, feeding problems and bacterial infections, often with fatal consequences. Survivors developed clinical features of severe CIE or LI.

Among all patients with ARCI, the majority have autosomal recessive variants in the transglutaminase-1 gene (TGM1), which account for >90% of patients with LI. The remainder of patients have autosomal recessive variants in various genes involved in the fatty acid/triglyceride metabolism or lipid transport of the skin, including ABCA12, ALOX12B, ALOXE3, CERS3, CYP4F22, LIPN, NIPAL4, and PNPLA1.

iii) Syndromic forms of autosomal recessive congenital ichthyoses

The skin manifestations of many syndromic forms of ichthyosis can be indistinguishable from those of non-syndromic forms. Nevertheless, patients develop progressive signs of involvement of the central and peripheral nervous system (Sjogren-Larsson sy., Chanarin-Dorfman sy., Ichthyosis, Spastic quadriplegia, and Mental Retardation sy., Refsum disease, Neu-Laxova sy.), liver (Chanarin-Dorfman sy., MEDNIK sy.), kidneys (Arthrogyrosis-Renal Dysfunction-Cholestasis Sy.), eyes (Sjogren-Larsson sy., Refsum disease, CEDNIK sy., Chanarin-Dorfman sy.), hearing (Chanarin-Dorfman sy.) and/or other organs. In Netherton syndrome, patients have generalized erythema and superficial peeling or scaling associated with hair shaft abnormalities ("bamboo hair"), allergies and highly elevated serum levels of Immunoglobulin E. In severe cases, failure to thrive, growth retardation, and immune defects resulting in serious recurrent infections may complicate NTS. Neonates with Ichthyosis Prematurity Syndrome (IPS) are born prematurely due to polyhydramnios and present at birth with a thick, caseous, desquamating skin. Life-threatening complications such as asphyxia and respiratory complications may occur in the perinatal period, while skin findings later transition to a mild form of generalized ichthyosis with severe pruritus and other signs of atopic diathesis. The vast majority of these autosomal recessive disorders are caused due to deficiency in lipid transport or secretion or other impairments of the normal lipid composition of the epidermis.

- iv) X-linked ichthyosis (steroid sulfatase deficiency) and related disorders of cholesterol metabolism

This X-linked recessive disorder almost exclusively affects males and is caused predominantly by a deletion of the STS gene locus on the short arm of the X-chromosome, resulting in steroid sulfatase deficiency and disturbed cholesterol metabolism. Ichthyosis presents within the first weeks after birth with mild erythroderma and generalized peeling or exfoliation of large, translucent scales, and transitions into large, polygonal, tightly adhering and dark-brown scale symmetrically distributed over extremities, trunk and neck. The neck is almost invariably involved, while palms, soles and face are characteristically spared. Asymptomatic corneal opacities occur in 10%-50% of affected males and in some female carriers, but cryptorchidism and increased risk for developing testicular cancer and hypogonadism are rare. X-linked recessive chondrodysplasia punctata (CDPX1) is characterized abnormal cartilage and bone development, hypoplasia of distal phalanges (brachytelephalangy), stippled epiphyses on X-ray (chondrodysplasia punctata) especially in the hands and feet, hearing loss and short stature. Typical for X-linked dominant chondrodysplasia punctata (CDPX2) are linear or whorl-like hyperkeratosis, atrophy and pigmentary changes of the skin, coarse alopecia, cataracts, and skeletal abnormalities including short stature, rhizomelic shortening of the limbs, epiphyseal stippling, and craniofacial defects. CDPX1 is caused by pathogenic variants in the ARSE gene, while CDPX2 caused by pathogenic variants in the EBP gene, which both encode proteins critical for normal cholesterol metabolism. Chondrodysplasia punctate without ichthyosis is much more common, and inherited in an autosomal recessive manner due to pathogenic variants in several different peroxisomal genes, including AGPS and PEX7.

- v) Autosomal dominant ichthyosis and Ichthyosis vulgaris

Ichthyosis vulgaris (IV) is the most common type of ichthyosis. The disorder initially presents during infancy or early childhood with dry skin and mild to moderate scaling of the extremities with sparing of the groin and flexural areas. In more severe disease, scaling extends to large areas of the trunk, scalp, forehead and cheeks, and there may be itchiness and heat intolerance. IV is frequently associated with keratosis pilaris and features of atopic disease, such as atopic dermatitis, asthma, and hay fever. IV is inherited as an autosomal semi-dominant disorder, with more subtle features in heterozygotes (penetrance up to 90%), and severe disease with complete penetrance in individuals who are homozygous or compound heterozygous for FLG pathogenic variants¹.

- vi) Various other related disorders of cornification with hyperkeratosis (rather than scaling) of the skin.

Autosomal dominant variants in various connexin genes have been observed in patients with localized hyperkeratosis and erythema. Variants in GJB2 (connexin 26) and very rarely GJB6 (connexin 30) are associated with sensorineural hearing loss and a diffuse, honeycomb

hyperkeratosis of palms and soles, with or without development of digital constricting bands, knuckle pads, and leukonychia (white nails). In contrast, variants in the lorcrin gene (LOR), which is a crucial protein during keratinocyte differentiation, lead to similar skin findings without hearing loss. KID syndrome is the most severe connexin disorder with susceptibility to mucocutaneous infections, which can be fatal in the neonatal period, increased risk for squamous cell carcinoma, photophobia and progressive keratitis leading to vision impairment or blindness. Variants in GJB3 and GJB4 (connexin 31 and 30.3) cause erythrokeratoderma variabilis, which presents in infancy with transient, erythematous patches that remain for minutes to hours or longer. In addition, hyperkeratosis develops which can be either generalized or localized and fixed. Both erythema and hyperkeratotic plaques have sharply demarcated edges, and palmoplantar keratoderma is not uncommon.

Test Methods:

Using genomic DNA from the submitted specimen(s), the exonic regions and flanking splice junctions of the genome are captured with RNA baits (Agilent) and sequenced by massively parallel (NextGen) sequencing on an Illumina sequencing system with 100bp or greater paired-end reads. Reads are aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants in the genes selected for this test using a custom-developed analysis tool (Xome Analyzer). Capillary sequencing or another appropriate method is used to confirm all potentially pathogenic variants identified. Sequence alterations are reported according to the Human Genome Variation Society (HGVS) nomenclature guidelines.

Please note that while XomeDxSlice captures and sequences the whole exome, analysis is targeted to the limited and specific phenotype-driven gene list for ichthyosis or related disorders (43 genes). As needed, based on the referring diagnosis and coverage achieved by the XomeDxSlice-Ichthyosis for a given patient, critical exons with a high yield of variants will be filled-in by dideoxy sequencing. For any autosomal recessive gene, if one definitive variant is found by XomeDxSlice sequencing, AND the gene fits the type of ichthyosis reported by the referring physician, capillary sequencing will be used to fill in sequence for exons that are not sufficiently covered (>10X) to find the second variant. If no second variant is found by sequencing, deletion/duplication analysis of that gene can be performed.

Test Sensitivity:

The clinical sensitivity of the XomeDxSlice-Ichthyosis test including 43 genes depends in part on the patient's clinical phenotype. Specific information about the diagnostic yield for each gene in selected populations is summarized in the table below. The technical sensitivity of the sequencing test is estimated to be greater than 99%. All genes have 97-100% coverage with a depth of 10 or more reads. This indicates that most single nucleotide changes and small insertions and deletions greater than or equal to 11 base pairs will be identified by

XomeDxSlice-Ichthyosis. Note that small sections of a few individual genes have inherent sequence properties that yield suboptimal data and variants in those regions may not be identified reliably.

References:

1. Smith et al. (2006) Nature Genetics 38 (3):337-42 (PMID: 16444271)