Neonatal ichthyosis–sclerosing cholangitis syndrome: report of a novel mutation and a review of the literature

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List of key features

Sclerosing cholangitis
Ichthyosis
CLDN1
Cholestasis

Introduction

Neonatal ichthyosis–sclerosing cholangitis syndrome (NISCH), also known as ichthyosis, leukocyte vacuoles, alopecia, and sclerosing cholangitis (ILVASC), is caused by homozygous mutations in the *claudin 1* gene (*CLDN1*; MIM 603718) (Oji *et al*., 2010). NISCH syndrome was first described by Baala *et al*. (2002). *CLDN1* gene encodes claudin-1 which is a component of tight junction proteins (Hadj-Rabia *et al*., 2004). Dysfunction of this protein leads to various clinical manifestations including sclerosing cholangitis, ichthyosis, alopecia, scalp hypotrichosis, and abnormalities of hair and teeth. Defects on claudin-1 expression disturb the barrier function of epidermal tight junctions, impair the keratinization of epidermis and increase the paracellular permeability in polarized hepatic cell lines (Morita *et al*., 2011; Grosse *et al*., 2012). Zorn-Kruppa *et al*. (2018) showed that claudin-1 protein has also an important role in the barrier function of the hair follicles and hair growth. We present a 3-year-old boy who had a homozygous novel mutation in *CLDN1* gene (c.89G>A; p.Trp30Ter) along with a brief literature review. Literature search was performed on Pubmed using the terms 'NISCH syndrome', 'ILVASC syndrome', '*CLDN1* mutation' and 22 cases published between 2002 and 2022 were collected. One case report written in French was excluded from the collection. The remaining 21 cases were discussed and compared with our own case (Table 1).

Clinical report

A 26-month-old boy was referred to our hospital with complaints of skin peeling, hypotrichosis, and itching. He was born at term with a birth weight of 2850 g after an uneventful pregnancy to his first cousin parents. Conjugated hyperbilirubinemia was detected on investigation of complaints including dark colored urine and dry skin with erythematous rash during the neonatal period. Ursodeoxycholic acid was initiated with 15 mg/kg/day dosage when he was 10-day-old. Cytomegalovirus IgM and cytomegalovirus PCR were found to be positive at 2 months of age. But, our patient had no signs of congenital cytomegalovirus infection rather than cholestasis. In order to exclude hepatic involvement of congenital cytomegalovirus infection, liver biopsy was performed. Histologic examination of the liver biopsy was consistent with cholangitis without cytomegalovirus inclusion bodies. Other laboratory tests of the patient are presented in Table 2. On follow-up, conjugated hyperbilirubinemia gradually decreased during infancy down to the normal range at the age of 5 months; however, pruritus and ichthyosis that started around 6 months persisted, and was initially considered as features of *Ichthyosis vulgaris*. Ursodeoxycholic acid treatment was discontinued at 9 months of age. Family medical history did not reveal any specific health issue.

On admission his weight was 12.5 kg (–0.18 SD), length 86.5 cm (–1.2 SD), BMI 17 (0.83 SD), and weight for length 0.63 SD. Generalized ichthyotic dry skin, frontoparietal cicatricial alopecia, hypotrichosis, sparse eyebrows, and eyelashes were noticed on physical examination. Also, hypodontia and enamel hypoplasia were detected where as hepatosplenomegaly or icterus were not present.

Routine laboratory tests including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, total protein, bilirubin, gamma-glutamyl transferase, L-lactate dehydrogenase, and prothrombin time were within normal limits and remained normal

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Table 1 Clinical and histopathological findings of the patient compared to the previously reported patients

	Literature	Present case
Sex (male/female)	10/11	Male
Parental consanguinity	17/21	+
Clinical features		
Neonatal jaundice	17/21	+
Hepatomegaly	9/18	–
Ichthyosis	21/21	+
Alopecia/sparse hair	20/21	+
Abnormal eyebrow and/or eyelash morphology	15/15	+
Abnormality of the dentition	9/14	+
Biochemical cholestasis/liver failure	15/18	–
Histologic findings		
Cholestasis	9/9	+
Portal fibrosis	4/9	–
Ductular proliferation	4/9	–
Ductopenia	2/9	+
Treatment and clinical course		
Liver transplantation	3/21	–
Improvement of cholestasis with Ursodeoxycholic acid	8/13	+
Cirrhosis	4/10	–
Portal hypertension	4/10	–

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throughout the follow-up until now. Abdominal ultrasonography showed normal liver size and parenchyma without any defect in intrahepatic and extrahepatic bile ducts. Spleen size and portal venous pressure were also within normal range. Whole exome sequencing was planned to elucidate the etiology of ichthyosis and transient cholestasis.

Methods

Genomic DNA was extracted from peripheral blood of the affected individual and unaffected parents after the receipt of the informed consent. The study protocol was approved by Hacettepe University Local Ethics Committee (GO 15-530/25). Exome library was prepared by using Ion AmpliSeqTM Exome RDY Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to the manufacturer's instructions. Emulsion PCR was performed on Ion OneTouchTM 2 instrument using the Ion PI Hi-Q OT2 200 Kit and enrichment of Ion Sphere Particles (ISPs) was performed using the Ion One TouchTM ES module. ISPs were loaded on an Ion PI chip and sequenced with Ion Proton semiconductor sequencer (Thermo Fisher Scientific).

Variant filtering steps were performed as outlined previously (Utine *et al.*, 2017). Torrent Mapping Alignment Program was used for aligning reads to the hg19/GRCh37 human reference genome. Raw sequence file was processed using the Torrent Suite software (version 5.16) for generating mapped reads, analyzing coverage data, and variant calling. Variants with coverage less than 5X for single-nucleotide polymorphisms (SNPs) and 10X for INDELs were excluded for further analysis. Phred Quality Scores were specified as Q15 for SNP variants and Q20 for INDELs. In order to identify the runs of homozygosity in the index case, HomSI software was used (Gormez *et al.*, 2014).

The verification and segregation analysis of the disease-causing variant was done by using sanger sequencing. The BigDye Terminator v.3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) was used for chain termination method and reaction products were applied onto ABI 3500 genetic analyzer (Thermo Fisher Scientific).

Results

In data analysis, totally 52 289 variants were identified, coverage depth was 74.25 and 90% of the mapped reads were aligned over a target region. We filtered out common variants (minor allele frequency >0.01). We identified 11 homozygous regions in 10 different chromosomes. In these regions, 12 variants remained which were not observed in homozygous state in gnomAD. A clinical interpretation of the variants directed the analysis, and the overall process revealed a homozygous nonsense causative variant: c.89G>A (p.Trp30Ter) in exon 1 of *CLDN1* (NM_021101), a disease-causing gene previously implicated in 'Ichthyosis, leukocyte vacuoles, alopecia, and sclerosing cholangitis' (MIM 607626). This variant was validated in the index case and then studied in the parents, revealing that the both were heterozygous carriers.

Discussion

NISCH syndrome is a rare genetic disorder with a heterogeneous clinical spectrum. Data on the clinical course are insufficient due to the small number of the reported cases. Hair and skin anomalies are common in NISCH syndrome. Characteristic dermatological findings include scalp hypotrichosis, dystrophic hair, cicatricial frontoparietal alopecia, sparse eyelashes and eyebrows, and ichthyosis with diffuse white scales not involving the skin folds (Baala *et al.*, 2002). Alopecia, hypotrichosis, and ichthyosis have been reported in all of the published cases like in our case and these signs seem to be the hallmarks of NISCH syndrome (Feldmeyer *et al.*, 2006; Nagtzaam *et al.*, 2010, 2018; Shah and Bhatnagar, 2010; Paganelli *et al.*, 2011; Kirchmeier *et al.*, 2014; Youssefian *et al.*, 2017; Izurieta Pacheco *et al.*, 2020; Salik *et al.*, 2022). Our patient had alopecia at birth and hair growth began when he was 2-years old. On admission, he presented with hypotrichosis predominantly on frontoparietal regions. Anomalies of eyebrows and eyelashes have been reported in only 15 of 21 previous cases whereas, the remaining case reports did not mention this sign. Our patient also had sparse eyebrows and eyelashes, so this sign can be speculated as a common sign in NISCH syndrome (Baala *et al.*, 2002; Hadj-Rabia *et al.*, 2004; Shah and Bhatnagar, 2010; Paganelli *et al.*, 2011; Kirchmeier *et al.*, 2014; Youssefian *et al.*, 2017; Nagtzaam *et al.*, 2018; Izurieta Pacheco *et al.*, 2020; Salik *et al.*, 2022). Histopathological findings of skin identified in NISCH syndrome include follicular keratosis, orthokeratosis, acanthosis, parakeratosis, papillomatosis,

Table 2 Clinical, laboratory, radiological, and histopathological assessments of the patient during follow-up

Table 2	Neonatal	2 months	5 months	3 years
Total bilirubin	11.2 mg/dL	11.16 mg/dL	1.68 mg/dL	0.32 mg/dL
Direct bilirubin	4.5 mg/dL	5.97 mg/dL	0.88 mg/dL	0.08 mg/dL
Alanine aminotransferase	42 U/L	437 U/L	105 U/L	33 U/L
Aspartate aminotransferase	44 U/L	447 U/L	152 U/L	38 U/L
Gamma-glutamyl transferase	71 U/L	63 U/L	33 U/L	23 U/L
Alkaline phosphatase	923 U/L	923 U/L	407 U/L	189 U/L
Albumin	3.1 g/dL	3.64 g/dL	4.2 g/dL	4.2 g/dL
Urinary and blood amino acid patterns		Normal		
Carnitine and acylcarnitine profile		Normal		
Organic acids urine test profile		Normal		
Very long chain fatty acids profile		Normal		
Creatine kinase		67 U/L		242 U/L
International normalized ratio		1.18	1.02	1.03
Prothrombin time		13.7 s	11.8 s	11.6 s
Anti-toxoplasma IgM		Negative		
Anti-cytomegalovirus IgM/IgG		Positive/positive		
Anti-rubella IgM		Negative		
Anti-Ebstein Barr IgM		Negative		
Cytomegalovirus PCR		605 copy/mL – negative without treatment		
Ferritin		784 ng/mL		
Ceruloplasmin		Normal		
Serum alpha1-antitrypsin		Normal		
Antinuclear antibody		Normal		
Anti-smooth muscle antibody		Normal		
Liver/kidney microsome type 1 antibody		Normal		
Hepatitis B surface antigen		Negative		
Hepatitis C virus antibody		Negative		
Echocardiography		Secundum atrial septal defect		
Hepatobiliary and doppler ultrasound			Normal	
Detailed eye examination		Normal		
Liver biopsy		Ductopenia, reactive cholangiocytes proliferation, giant cell hepatocytes formation and multifocal leukocytes and eosinophils infiltration in portal area		

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intracytoplasmic vacuoles in keratinocytes, and granular layer hyperplasia (Baala *et al.*, 2002; Paganelli *et al.*, 2011). White scaly ichthyosis was evident in our patient after birth and he had been also suffering from accompanying pruritus, while skin biopsy was not evaluated. Additionally, improvement of cutaneous findings after liver transplantation has been described (Baala *et al.*, 2002). Our patient had dental anomalies such as hypodontia and enamel hypoplasia which appear to be a common problem in NISCH syndrome, present in nine of the reported cases (Baala *et al.*, 2002; Hadj-Rabia *et al.*, 2004; Feldmeyer *et al.*, 2006; Shah and Bhatnagar, 2010; Paganelli *et al.*, 2011; Youssefian *et al.*, 2017; Salik *et al.*, 2022).

Interestingly, liver involvement in NISCH syndrome presents heterogeneously. Three patients were reported, who did not show any clinically evident liver abnormalities, yet was diagnosed with NISCH syndrome (Nagtzaam *et al.*, 2018; Salik D *et al.*, 2022). Neither jaundice nor hepatosplenomegaly were noticed in our patient on admission and follow-up; however, he had a history of neonatal cholestasis. Therefore, medical records in neonatal period should be examined carefully for detection of early transient liver involvement. In the rest of the 17 cases reported, the presence of cholestasis was a heterogeneous feature. Liver transplantation was performed in three cases, whereas two cirrhotic patients were followed-up without liver transplantation (Baala *et al.*, 2002; Hadj-Rabia *et al.*, 2004; Nagtzaam *et al.*, 2010; Paganelli *et al.*, 2011). Follow-up findings of four cases who had liver involvement at the time of diagnosis were not available (Nagtzaam *et al.*, 2010; Shah and Bhatnagar, 2010; Youssefian *et al.*, 2017). Last eight cases showed improvement on cholestasis after infantile period and their liver function tests returned to normal (Baala *et al.*, 2002; Hadj-Rabia *et al.*, 2004; Feldmeyer *et al.*, 2006; Nagtzaam *et al.*, 2010; Paganelli *et al.*, 2011; Kirchmeier *et al.*, 2014; Youssefian *et al.*, 2017). Ursodeoxycholic acid was found useful in reducing bilirubin levels in some cases (Paganelli *et al.*, 2011). After ursodeoxycholic acid prescription, our patient's liver test abnormalities improved, consistent with the literature but further controlled studies are needed (Paganelli *et al.*, 2011). Liver biopsies were performed only in 9 of 21 cases. Hepatocellular cholestasis were reported in all previous biopsies, which was also detected in our patient (Baala *et al.*, 2002; Hadj-Rabia *et al.*, 2004; Feldmeyer *et al.*, 2006; Nagtzaam *et*

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al., 2010; Shah and Bhatnagar, 2010; Youssefian *et al.*, 2017). Ductular proliferation was reported in four cases whereas, two of them also had ductopenia (Baala *et al.*, 2002; Hadj-Rabia *et al.*, 2004; Nagtzaam *et al.*, 2010). Our patient's biopsy showed ductopenia, reactive cholangiocytes proliferation, giant cell hepatocytes formation and multifocal leukocytes and eosinophilic infiltration in the portal area.

Nagtzaam *et al.* (2010) presented three brothers who had congenital ductopenia and giant cell hepatocyte formation without obvious inflammation in the portal area. This study suggested that congenital paucity of bile ducts could be a manifestation of NISCH syndrome which was inconsistent with our patient who had obvious portal inflammation and ductopenia. Further studies are needed to clarify the course of liver involvement in patients with NISCH syndrome.

CLDN1 encodes one of several claudin proteins, which are found associated with tight junctions. This gene is expressed in many tissue types, and is specifically abundant in liver and skin. To date, there are three different point mutations in *CLDN1* reported as 'pathogenic' in the ClinVar database (two nonsense and one frameshift variants). A homozygous 2 bp deletion (p.Val66_Phe67insTer) in exon 1 of the *CLDN1* was previously identified (Baala *et al.*, 2002; Hadj-Rabia *et al.*, 2004). In one of these studies, this variant caused complete 'loss-of-function' based on the fact that CLDN1 expression was not detected in skin and liver samples of patients (Hadj-Rabia *et al.*, 2004). Feldmeyer *et al.* (2006) identified a homozygous 1 bp deletion (p.Val120SerfsTer15) in exon 2 of the *CLDN1* resulting in a premature stop codon and no protein expression detected in the skin sample of the patient. No protein expression analysis was performed in the current study in order to analyze CLDN1 expression; however, we consider that the nonsense variant identified in this study represent a loss-of-function variant.

The differential diagnosis includes other genetic conditions associated with ichthyosis and liver involvement, such as Chanarin–Dorfman syndrome, mental retardation, enteropathy, deafness, peripheral neuropathy, ichthyosis, and keratoderma (MEDNIK), and arthrogryposis, renal dysfunction, and cholestasis (ARC) syndrome (Youssefian *et al.*, 2017). Also, NISCH syndrome should be considered in patients with cutaneous manifestations even if there are no evident liver abnormalities, since the severity of hepatic involvement is variable (Nagtzaam *et al.*, 2018). Furthermore, no obvious genotype–phenotype correlation could be established, while hypomorphic mutations have been proposed to cause milder phenotypes (Hadj-Rabia *et al.*, 2004).

In conclusion, NISCH syndrome should be contemplated in patients presented with ichthyosis and cholestasis, considering clinical heterogeneity. In two different reports including three patients from Turkey, the same nonsense mutation in *CLDN1* (c.181C>T, p.Gln61Ter) has been demonstrated, suggesting a founder effect (Kirchmeier *et al.*, 2014; Nagtzaam *et al.*, 2018). However, we report on an additional Turkish patient harboring a novel nonsense variant.

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Conflicts of interest

There are no conflicts of interest.

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