Received: 31 August 2019 | Revised: 9 December 2019 | Accepted: 16 December 2019

DOI: 10.1002/jcla.23201

RESEARCH ARTICLE

WILEY

X-linked ichthyosis: Molecular findings in four pedigrees with inconspicuous clinical manifestations

Min Zhang | Hailong Huang | Na Lin | Shuqiong He | Gang An | Yan Wang | Meihuan Chen | Lingji Chen | Yuan Lin | Liangpu Xu

Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China

Correspondence
Liangpu Xu, Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, No. 18 Daoshan Road, Gulou District, Fuzhou City, Fujian Province, 350001, China.
Email: xiliangpu@fjmu.edu.cn

Funding information
This study was supported by the Key Special Projects of Fujian Provincial Department of Science and Technology (grant no. 2013YZ0002-1); the Key Clinical Specialty Discipline Construction Program of Fujian (grant no. 20121589); and the Fujian Provincial Natural Science Foundation (grant no. 2017J01238).

Abstract

Background: X-linked ichthyosis (XLI) is the second most common type of ichthyosis, which is characterized by wide and symmetric distribution of adherent, dry, and polygonal scales on the skin. Steroid sulfatase (*STS*) gene, which is located at chromosome Xp22.31, has been identified as the pathogenic gene of XLI.

Methods: In this study, chromosome karyotype analysis, bacterial artificial chromosomes-on-Beads™ (BoBs) assay, fluorescence in situ hybridization (FISH), and single nucleotide polymorphism array (SNP-array) were employed for the prenatal diagnoses in three pregnant women with high-risk serological screening results and a pregnant woman with mental retardation.

Results: *STS* deletion was identified at chromosome Xp22.31 in all four fetuses. Postnatal follow-up confirmed the diagnosis of ichthyosis in two male fetuses and revealed normal dermatological manifestations in other two female fetuses carrying ichthyosis.

Conclusion: The results of the present study demonstrate that a combination of karyotypying, prenatal BoBs, FISH, and SNP-array may avoid the missed detection of common microdeletions and ensure the accuracy of the detection results, which provides a feasible tool for the reliable etiological diagnosis and better genetic counseling of XLI.

KEYWORDS
epidermal barrier function, prenatal diagnosis, steroid sulfatase deficiency, *STS* gene, X-linked ichthyosis

1 | INTRODUCTION

X-linked ichthyosis (XLI) is an inherited skin disorder characterized by wide and symmetric distribution of adherent, dry, and polygonal scales on the skin. The scales vary in color, with 70% appearing dark brown and 30% showing light gray. This disorder predominantly occurs in the preauricular area, neck, axillae, anterior abdomen, and extension zone of the limbs.[1,2] It is estimated that the incidence of this rare disease is 1/1500-1/6000.[3,4] Steroid sulfatase (*STS*) gene, which is located at chromosome Xp22.31, has been proved to be a

pathogenic gene of XLI. Approximately 90% of XLI patients harbor complete deletions of the *STS* gene, and the others carry partial deletions or point mutations in the *STS* gene.[5] In this study, prenatal diagnosis was performed in three pregnant women with high-risk serological screening results and a woman with mental retardation, including 3 pedigrees with unknown family history of ichthyosis and one pedigree with known family history.

2 | SUBJECTS AND METHODS

2.1 | Subjects

Pedigree 1 was a 31-year-old G2P1 woman at gestational age of 24 weeks. She was admitted to our hospital due to high-risk screening results for trisomy 21 (1/195) in the second trimester. Three-dimensional color Doppler ultrasound displayed no apparent fetal abnormality at gestational age of 23 weeks. The pregnant woman denied family history of ichthyosis and had no complications of pregnancy. Pedigree analysis results revealed skin scales in three members of her pedigree, and all were male. The proband was the woman's 3-year-old nephew. He had white scales on both arms 1 week after birth, and scales were found to gradually extend to the limbs and trunk with age, which manifested adherent, light gray scales on the skin. The pregnant woman's maternal grandfather had died, and had adherent, light brown scales on the neck, trunk, and limbs, with rough skin seen prior to death. The pregnant woman's male cousin was 35 years old and had similar dermatological manifestations like her maternal grandfather's. Apart from skin scales, no involvement of other systems was seen in the pedigree members. The woman's husband was healthy.

Pedigree 2 was a 33-year-old G2P1 woman at gestational age of 24 weeks. She was admitted to our hospital due to family history of ichthyosis and high-risk screening results for trisomy 18 (1/67) in the second trimester. Three-dimensional color Doppler ultrasound displayed no apparent fetal abnormality at gestational age of 23 weeks. There were three members of her pedigree with ichthyosis, and all were male. The proband was the woman's 6-year-old eldest son. He had widely distributed, adherent, and white scales on the abdomen 10 days after birth, and light brown scales gradually developed on the abdomen and bilateral lower limbs with age (Figure 1A-C), which aggravated in winter and at onset and alleviated in summer. In addition, light brown and rough scales were found to adhere to the skin of the trunk and limbs in the pregnant woman's 39-year-old male cousin and 30-year-old younger brother. Except skin scales, no involvement of other systems was detected in the pedigree members. The woman's husband was healthy.

Pedigree 3 was a 34-year-old G3P0 woman at gestational age of 18 weeks. She was admitted to our hospital due to high-risk screening results for trisomy 21 (1/183) in the first trimester. The pregnant woman denied family history of ichthyosis and had no complications of pregnancy. The woman's husband was healthy.

Pedigree 4 was a 27-year-old G1P0 woman at gestational age of 19 weeks. She was admitted to our hospital for prenatal diagnosis due to mental retardation. The pregnant woman' family members denied family history of ichthyosis, and she had no complications of pregnancy. The woman's husband was healthy.

2.2 | Methods

2.2.1 | Sample collection

All four pregnant women underwent B-mode ultrasonography-guided amniocentesis, and 25-30 mL of amniotic fluid was sampled for cell culture, chromosome karyotype analysis, fluorescence in situ hybridization (FISH), prenatal bacterial artificial chromosomes-on-Beads™ (BoBs), and single nucleotide polymorphism array (SNP-array) analysis. Peripheral blood was sampled from the pregnant woman and her nephew and younger cousin in Pedigree 1, the pregnant woman and her son in Pedigree 2 and the two pregnant women in Pedigrees 3 and 4 for SNP-array analysis, while the other pedigree members were given no advice on genetic testing. This study was approved by the Ethical Review Committee of Fujian Provincial Maternity and Children's Hospital.

2.2.2 | Cell culture and chromosome karyotype analysis

Amniotic fluid cells were routinely cultured and subjected to G-band karyotyping (320-400 bands). The cytogenetic and molecular cytogenetic findings were described according to the International System for Human Cytogenetic Nomenclature 2009 (ICSN 2009).

2.2.3 | BoBs assay

Genomic DNA was extracted from amniotic fluid cells using the QIAamp® DNA Blood Mini Kit (QIAGEN), and the concentration and purity of genomic DNA was measured with a NanoDrop micro-volume UV-Vis spectrophotometer (Thermo Fisher Scientific). Prenatal BoBs test kit was used to detect aneuploidies involving chromosomes 13, 18, 21, X, and Y as well as nine common microdeletion syndromes on a multi-analyte suspension array (PerkinElmer LAS, Inc) following the manufacturer's instructions, including DiGeorge I syndrom, DiGeorge II syndrom, Prader-Willi syndrom/Angelman syndrome, Cri-du-Chat syndrome, Williams-Beuren syndrome, Wolf-Hirschhorn syndrome, Smith-Magenis syndrome, Miller-Dieker syndrome, and Langer-Giedion syndrome.

2.2.4 | Fluorescence in situ hybridization assay

Amniotic fluid cells were cultured, prepared into cell suspensions, and dropped on clean slides. FISH assay was performed with an STS/DXZ1 probe by the Becreative Lab (Beijing) Co., Ltd.

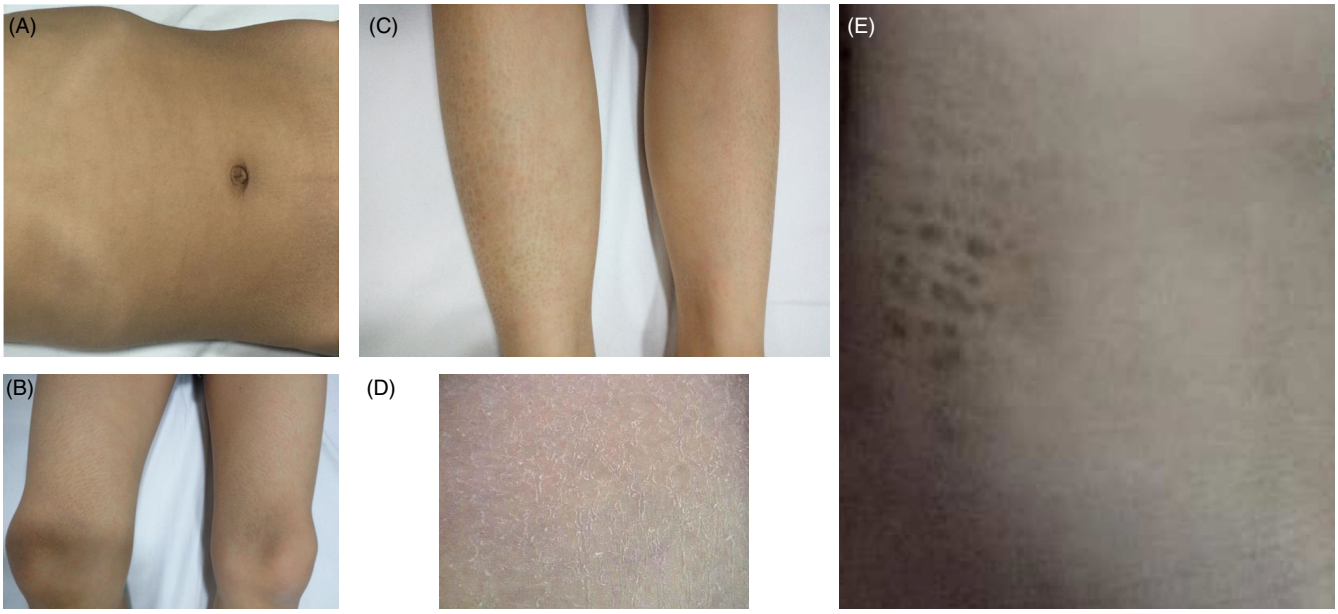


FIGURE 1 Skin presentation of X-linked ichthyosis patients. A, B, and C show the proband in family two, and thin and light brown scales are seen on the anterior abdomen and extension zones of the lower limbs; D, widespread, thin and translucent scales are seen on the anterior abdomen in a one-week-old baby from family one; E, Light brown scales are observed on the ankle in a 1-y-old baby from family two

2.2.5 | SNP-array analysis

Genomic DNA was digested, amplified, purified, fragmented, marked with signals, hybridized on the Affymetrix CytoScan 750K arrays (Affymetrix), and washed, and images were acquired. The original image files were processed with the software Chromosome Analysis Suite (Affymetrix). The reporting threshold was set at ≥200 kb for deletion and ≥500 kb for repetition.

2.3 | Follow-up of pregnancy outcomes

The pregnant outcomes and newborns' dermatological manifestations were collected through review of medical records and telephone follow-up.

3 | RESULTS

3.1 | Genetic testing results

3.1.1 | Pedigree 1

Prenatal BoBs assay revealed a 0.36 sample-to-female reference ratio and 0.5 sample-to-male reference ratio in the fetus of Pedigree 1 using the XC1 probe, suggesting the presence of microdeletions; however, no obvious abnormalities of chromosomes 13, 18, or 21, or the nine common microdeletion syndromes were detected (Figure 2A). SNP-array analysis showed a deletion of 1.68 Mb at chromosome Xp22.31 (arr[hg19] Xp22.31(6645882-8085215)x1) encompassing four OMIM genes in the pregnant woman, including *HDHD1*, *STS*, *VCX*, and *PNPLA4*, and the SNP-array result was arr[hg19] Xp22.31(6455151-8135644)x0 in the fetus (Table 1), suggesting that the deletion was derived from the mother. FISH assay confirmed the deletion of the *STS* gene in the fetus (Figure 3C), and the karyotype was 46,XY. In addition, SNP-array testing revealed the deletion of the *STS* gene in the pregnant woman's younger cousin and nephew, with the deletion size consistent with the pregnant woman's and fetus's.

3.1.2 | Pedigree 2

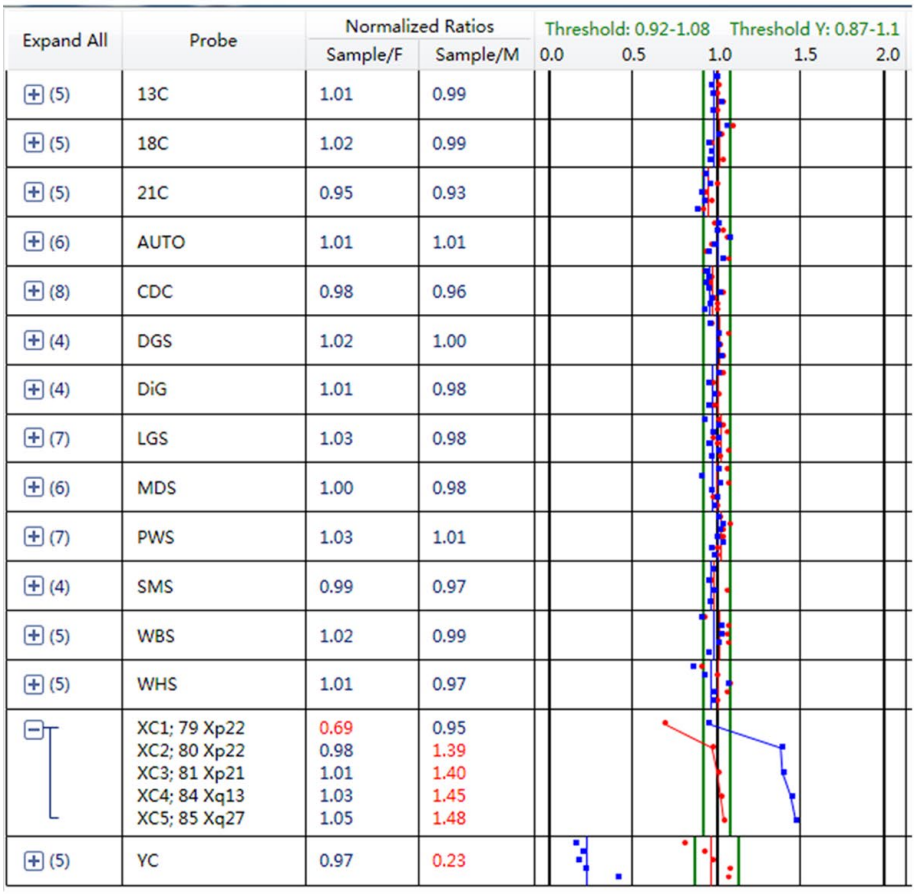
In the Pedigree 2, SNP-array analysis showed a deletion of 1.2 Mb at chromosome Xp22.31 (arr[hg19] Xp22.31(6715163-7918931) in the pregnant woman, the proband and the fetus (Table 1), and the deleted region contained four OMIM genes, including *HDHD1*, *STS*, *VCX*, and *PNPLA4*. FISH assay confirmed the deletion of the *STS* gene in the fetus (Figure 3D), and the karyotype was 46, XY.

3.1.3 | Pedigree 3

In the Pedigree 3, prenatal BoBs assay detected a 0.69 sample-to-female reference ratio and 0.95 sample-to-male reference ratio in the fetus using the XC1 probe, suggesting the presence of microdeletions; however, no obvious abnormalities of chromosomes 13, 18, or 21, or the nine common microdeletion syndromes were detected (Figure 2B). SNP-array analysis showed a deletion of 1.68 Mb at chromosome Xp22.31 (arr[hg19] Xp22.31(6683449-7887990)

Expand All	Probe	Normalized Ratios Sample/F	Sample/M
(5)	13C	1.00	1.00
(5)	18C	1.04	1.01
(5)	21C	0.95	0.93
(6)	AUTO	1.00	1.00
(8)	CDC	0.99	0.98
(4)	DGS	1.01	0.96
(4)	DiG	1.02	1.00
(7)	LGS	1.02	0.99
(6)	MDS	1.04	0.98
(7)	PWS	1.02	1.01
(4)	SMS	0.96	0.95
(5)	WBS	1.01	0.97
(5)	WHS	1.03	0.97
	XC1; 79 Xp22	0.36	0.50
	XC2; 80 Xp22	0.69	0.95
	XC3; 81 Xp21	0.78	1.09
	XC4; 84 Xq13	0.73	0.98
	XC5; 85 Xq27	0.69	0.97
(5)	YC	4.19	1.00

(A)



Expand All	Probe	Normalized Ratios Sample/F	Sample/M
(5)	13C	1.01	0.99
(5)	18C	1.02	0.99
(5)	21C	0.95	0.93
(6)	AUTO	1.01	1.01
(8)	CDC	0.98	0.96
(4)	DGS	1.02	1.00
(4)	DiG	1.01	0.98
(7)	LGS	1.03	0.98
(6)	MDS	1.00	0.98
(7)	PWS	1.03	1.01
(4)	SMS	0.99	0.97
(5)	WBS	1.02	0.99
(5)	WHS	1.01	0.97
	XC1; 79 Xp22	0.69	0.95
	XC2; 80 Xp22	0.98	1.39
	XC3; 81 Xp21	1.01	1.40
	XC4; 84 Xq13	1.03	1.45
	XC5; 85 Xq27	1.05	1.48
(5)	YC	0.97	0.23

(B)

FIGURE 2 Representative bacterial artificial chromosomes-on-Beads™ results of fetus in two families. A, the results of the Xp22 microdeletion of fetus in families one; B, the results of the Xp22 microdeletion of fetus in families two. Sample is defined as a normal disomic if the ratios of the fluorescence intensities for a chromosome region fall within the lower and upper threshold limits, with a ratio of ~1.0. A sample was defined deleted/duplicated at a specific chromosomal locus if the ratios of the fluorescence intensities fall outside the threshold of the mean ± two standard deviations; they typically range between 0.6 and 0.8 (deleted) and between 1.3 and 1.4 (duplicated). Blue dots represent the proportion of tested DNA compared with the male reference DNA. Red dots represent the proportion of tested DNA compared to the female reference DNA. Green lines are the normal range for the signals

TABLE 1 *STS* deletion cases identified through SNP-array

Fetuses	Sex	SNP-array	Deleted OMIM genes
1	Male	1.68 Mb deletion at Xp22.31 (arr[hg19] Xp22.31(6455151-8135644)x0)	*HDHD1*, *STS*, *VCX*, *PNPLA4*
2	Male	1.2 Mb deletion at Xp22.31 (arr[hg19] Xp22.31(6715163-7918931) x0)	*HDHD1*, *STS*, *VCX*, *PNPLA4*
3	Female	1.68 Mb deletion at Xp22.31 (arr[hg19] Xp22.31(6683449-7887990)x1)	*HDHD1*, *STS*, *VCX*,*PNPLA4*
4	Female	1.68 Mb deletion at Xp22.31 (arr[hg19] Xp22.31(6455276-8135568)x1)	*HDHD1*, *STS*, *VCX*, *PNPLA4*

x1) in the pregnant woman, and the deleted region contained four OMIM genes, including *HDHD1*, *STS*, *VCX*, and *PNPLA4*. The SNP-array result was arr[hg19] Xp22.31(6683449-7887990)x0 in the fetus (Table 1), suggesting that the deletion was derived from the mother. FISH assay confirmed the deletion of the *STS* gene in the X chromosome of the fetus (Figure 3E), and the karyotype was 46, XX.

3.1.4 | Pedigree 4

In the Pedigree 4, SNP-array analysis showed a deletion of 1.68 Mb at chromosome Xp22.31 (arr[hg19] Xp22.31(6455276-8135568)x1) in the pregnant woman and the fetus (Table 1), and the deleted region contained four OMIM genes, including *HDHD1*, *STS*, *VCX*, and *PNPLA4*. FISH assay confirmed the deletion of the *STS* gene in the fetus (Figure 3F), and the karyotype was 46, XX.

3.2 | Follow-up of pregnancy outcomes

In the Pedigree 1, the pregnant woman delivered a full-term male baby by cesarean section, and no skin abnormality was seen in the baby at birth. However, white and widespread scales were found to adhere to the abdomen one week after birth, which aggravated in dry air (Figure 1D). The 17-month follow-up revealed slightly rough, light brown scurf in the anterior abdomen and extension zone of the limbs, and no other clinical symptoms were seen. In the Pedigree 2, the pregnant woman delivered a full-term male baby by cesarean section, and no skin abnormality was observed in the baby at birth. However, white and widespread scales were found to adhere to the abdomen 10 days after birth. The 13-month follow-up revealed slightly rough, light brown scurf in the ankle and slightly rough instep skin without scurf, and no other clinical symptoms were found (Figure 1E). In the Pedigrees 3 and 4, full-term babies were delivered, and no abnormalities were seen during the 6- and 3-month follow-up period. The phenotypes between our research and published studies are summarized in Table 2.

4 | DISCUSSION

XLI is an X-linked, recessively inherited disorder of cutaneous keratinization. Since the *STS* gene escapes X chromosome inactivation, female patients with XLI contain the same amounts of STS with healthy males, in whom skin scales are rare.[6,7] However, male patients with XLI often have polygonal, semitransparent, and fine scales on the skin at birth or soon after birth, and the scales gradually become deep dark and rough with age. The skin damages predominantly occur in the trunk and extension side of the limbs. The symptoms of XLI aggravate in winter and dry climates and alleviate in summer.[7,8] In addition, some patients are complicated by extracutaneous manifestations, such as corneal opacity (10%-15%), cryptorchidism, and testicular germ cell cancer (20%) and male hair loss.[9] In 1978, deletion of the *STS* gene was detected in the skin fibroblasts of XLI patients, and *STS* gene was therefore identified as the pathogenic gene of XLI.[10] Approximately 90% of the patients with XLI are reported to harbor complete deletion of the *STS* gene and its flanking sequences, and a minority of patients has partial deletions and point mutations; however, the deletion pattern of the *STS* gene shows no significant correlation with the severity of cutaneous manifestations.[11] It is reported that 8% of the patients with *STS* gene deletion are complicated with the deletions in the neighboring genes, thereby resulting in more complicated phenotypes, such as microsomia, chondrodysplasia punctata, Kallmann syndrome, ocular albinism, epilepsy,

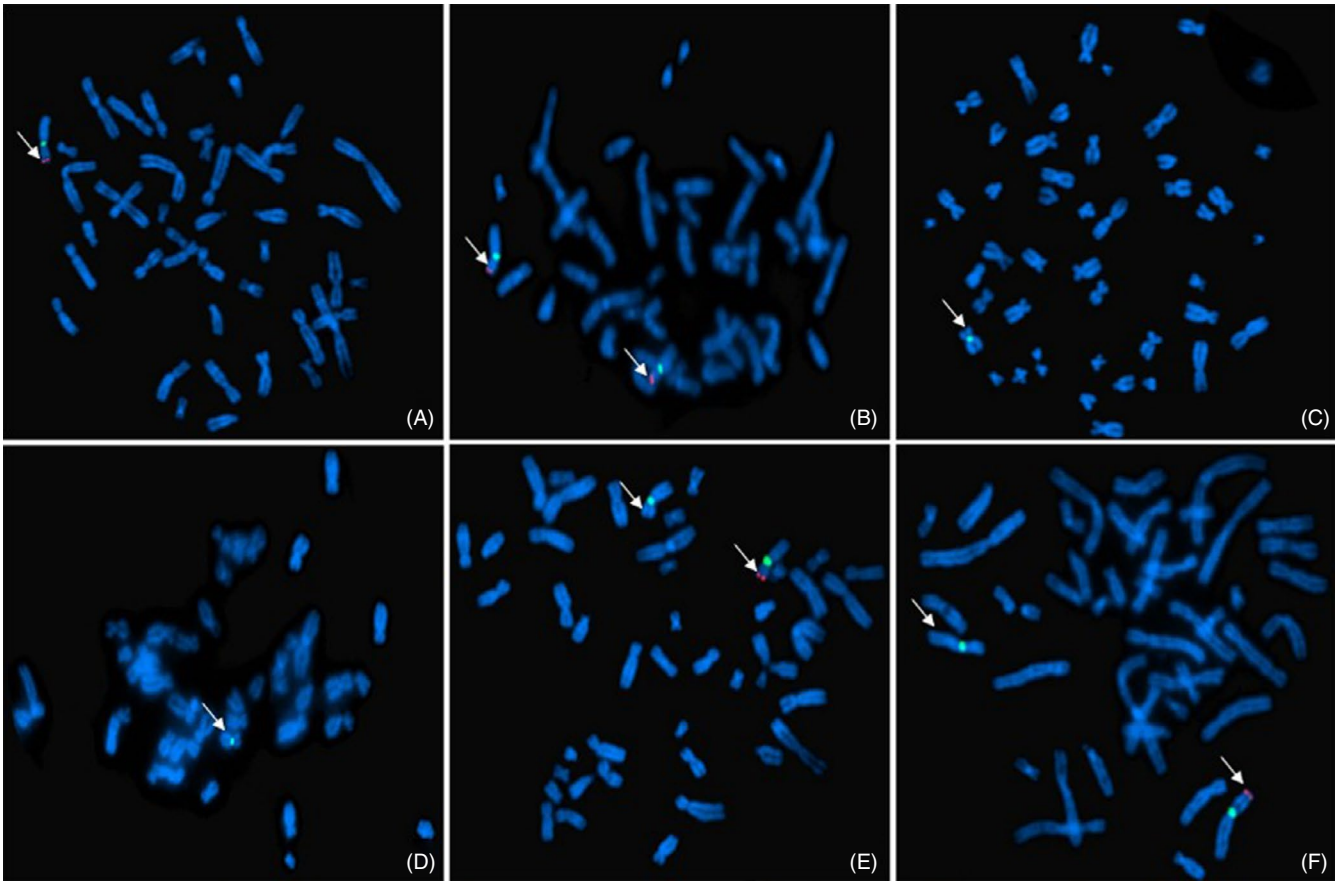


FIGURE 3 Representative images of fluorescence in situ hybridization analysis using a *STS* gene (red)-specific probe and X centromere (green) control probe. A, a normal male with *STS* probe (arrow); B, a normal female with *STS* probe (arrow); C, An XLI affected male fetus with a complete deletion of the *STS* probe (arrow) in family one; D, An XLI affected male fetus with a complete deletion of the STS probe (arrow) in family two; E, An XLI affected female fetus with a complete deletion of the *STS* probe (arrow) in family three; F, An XLI affected female fetus with a complete deletion of the *STS* probe (arrow) in family four

electroencephalography abnormality, mental retardation, hyposmia, attention deficit hyperactivity disorder, autism, and language development disorder.[9-17]

STS, which is located at chromosome Xp22.31, is estimated to be 164 kb in length and contains 10 exons and 2 non-coding regions. The cDNA of the *STS* gene encodes 583 amino acids, which has a relative molecular mass of 62 000. In normal stratum corneum, cholesterol sulfate is decomposed into cholesterol and sulfate by STS, leading to a reduction in the constituent ratio of cholesterol sulfate from approximately 5%-1% in lipids. XLI patients have a deficiency in the *STS* gene, and cholesterol sulfate accumulates in stratum corneum, which consists of 10%-12% of the lipids.[18,19] Excessive accumulation of cholesterol sulfate increases epidermal cell adhesion and stability, resulting in hyperkeratosis and formation of scales. Previous studies have demonstrated a low serum free estriol level in the pregnant woman carrying a fetus with XLI, suggesting that serological test may be indicative of XLI.[3,20-22] In this study, the pregnant women from two pedigrees were admitted due to high-risk screening results in the second trimester, and the serum free estriol had a 0.121 multiple of the median in the pregnant woman from Pedigree 1 and a 0.14 multiple of the median in the pregnant woman from Pedigree 2, both of which were lower than the reference range (0.5-2 multiples of the median). This is consistent with previous reports. The pregnant woman in Pedigree 3 received Down's syndrome screening in the first trimester, and Down's syndrome screening was not performed in the pregnant woman in Pedigree 4. Therefore, free estriol levels are unavailable in these two pedigrees. Further studies to examine the diagnostic value of free estriol in pregnant women carrying XLI-affected female fetuses are warranted.

Previous studies have demonstrated that the dermatological manifestations of XLI are large, jet-black, polygonal, and dry scales. With the continuous development of molecular techniques, more and more XLI patients that have only mild dermatological manifestations are detected, and these patients manifest skin dryness, eczema, and allergic dermatitis.[11] In addition, approximately 30% of XLI patients have adherent, light gray scales, rather than atypical, polygonal, and “dirty” scales, on the skin.[1,23] In this study, the family members and the patient of Pedigree 1 did not know ichthyosis and were not given standard diagnosis or treatment in the department of dermatology. Ichthyosis was mistaken as common skin disorders for the management. In Pedigree 2, the light brown and rough scales were found to adhere to the skin of the trunk and limbs in the male

TABLE 2 Comparative of phenotypes between our research and previous studies

Clinical manifestation	Previous studies	Pedigree 1 of our study	Pedigree 2 of our study	Pedigree 3 of our study	Pedigree 4 of our study
Dermatological manifestations	Main of dermatological manifestations in male patients: the severity of the skin varies, most of manifestations were typical, atypical, polygonal, and "dirty" scales on the skin. A few were mild dry skin, eczema, atopic dermatitis, adherent light gray scales. The skin damages predominantly occur in the preauricular area, neck, axillae, anterior abdomen, and extension zone of the limbs	Male patients: dermatological manifestations were mild, the light brown scales adherent on the neck, trunk, and limbs, with rough skin. The boy was followed up to 17 mo: no skin abnormality was seen in the baby at birth. However, white and widespread scales were found to adhere to the abdomen one week after birth, which aggravated in dry air. The 17-mo follow-up revealed slightly rough, light brown scurf in the anterior abdomen and extension zone of the limbs	Male patients: dermatological manifestations were mild, the light brown and rough scales were found to adhere to the skin of the trunk and limbs. The boy was followed up to 13 mo: no skin abnormality was observed in the baby at birth. But white and widespread scales were found to adhere to the abdomen 10 d after birth. The 13-mo follow-up revealed slightly rough, light brown scurf in the ankle and slightly rough instep skin without scurf	The girl was followed up to 6 mo: normal	The girl was followed up to 3 mo: normal
Extracutaneous manifestations	Main of extracutaneous manifestations in male patients: corneal opacification and cryptorchidism are common accompanying symptoms, but could also accompanied by testicular germ cell cancer, male hair loss, microsomia, chondrodysplasia punctata, epilepsy, electroencephalography abnormality, mental retardation, hyposmia, attention deficit hyperactivity disorder, autism and language development disorder	Male patients: normal. The boy was followed up to 17 mo: normal	Male patients: normal. The boy was followed up to 13 mo: normal	The girl was followed up to 6 mo: normal	The girl was followed up to 3 mo: normal

patients. Only female carriers were found in Pedigree 3 and Pedigree 4, and there were no skin or extra-skin symptoms during follow-up. Because of the characteristics of X-linked recessive inheritance, half of the female offspring generated by male ichthyosis patients and normal women are carriers of ichthyosis, and male offspring are all normal, while half of the female offspring generated by female carriers of ichthyosis and normal men are carriers of ichthyosis and half of the male offspring are ichthyosis patients. Prenatal testing is therefore recommended in the offspring of the patients and carriers of ichthyosis in the four pedigrees.

It has been reported that the patients with deletions of four OMIM genes (*HDHD1*, *STS*, *VCX*, and *PNPLA4*) have diverse manifestations. Puri and colleagues[24] reported a 3-year-old Caucasian male XLI baby with deletions of four OMIM genes including *HDHD1*, *STS*, *VCX*, and *PNPLA4*, and this child had manifestations of skin dryness in the limbs with adherent scales, complicated by pyloric stenosis, epilepsy, polymicrogyria, thin hair, poor dentition, retinitis pigmentosa, malformation of cortical development, and developmental delay. Song and colleagues[25] reported a 12-year-old male XLI patient with deletions of *HDHD1*, *STS*, *VCX*, and *PNPLA4* genes, and the child had

large, thick, dark brown, and polygonal scales, complicated by glomerular sclerosis and microsomia. In addition, Hand and colleagues[11] reported three males with XLI in whom four OMIM genes (*HDHD1*, *STS*, *VCX*, and *PNPLA*) were deleted, and these patients had only invisible scales adherent to the skin, or dryness or eczema, complicating by other clinical manifestations, such as autism, microsomia, neurofibroma, and developmental delay. In the current study, the pregnant women in Pedigrees 1 and 2 manifested adherent light brown scales, and dry and rough skin in the limbs and abdomen, and no other clinical symptoms were observed. Additionally, most female carriers of XLI are reported to have no detectable clinical symptoms, and scales are rarely seen in the skin. It has been reported that neuropsychiatric disorders predominantly occur in male patients with XLI, and there are few reports regarding the development of neuropsychiatric symptoms in female carriers of XLI.[16,26-28] The pregnant woman in Pedigree 4 was a carrier of XLI and had clinical manifestations of risibility, impaired verbal communication, and mental retardation; however, the associations of these clinical symptoms with the deletion of the *STS* gene remained unknown. More clinical studies are required to examine the associations of deletion of the same gene that leads to diverse clinical symptoms with environmental or individual genetic heterogeneity.

5 | CONCLUSION

Currently, conventional karyotyping remains the gold standard for the diagnosis of chromosomal disorders; however, this technique has a limited resolution and may cause a missed detection of deletions of <10 Mb repeat sequences. In this study, karyotyping, prenatal BoBs, FISH assay, and SNP-array analysis were employed and the combination of these tools may avoid the missed detection of common microdeletions and ensure the accuracy of the detection results, which provides a feasible tool for the reliable etiological diagnosis and better genetic counseling of XLI.

ACKNOWLEDGMENTS

We would like to thank the patients and their family members who kindly agreed to participate in this study.

ORCID

Min Zhang https://orcid.org/0000-0003-1027-7196
Hailong Huang https://orcid.org/0000-0001-5775-5082
Meihuan Chen https://orcid.org/0000-0002-7356-8751

REFERENCES

1. Traupe H, Fischer J, Oji V. Nonsyndromic types of ichthyoses - an update. *J Dtsch Dermatol Ges*. 2014;12:109-121.
2. Craig WY, Roberson M, Palomaki GE, et al. Prevalence of steroid sulfatase deficiency in California according to race and ethnicity. *Prenat Diagn*. 2010;30:893-898.
3. Langlois S, Armstrong L, Gall K, et al. Steroid sulfatase deficiency and contiguous gene deletion syndrome amongst pregnant patients with low serum unconjugated estriols. *Prenat Diagn*. 2009;29:966-974.
4. Fernandes NF, Janniger CK, Schwartz RA. X-linked ichthyosis: an oculocutaneous genodermatosis. *J Am Acad Dermatol*. 2010;62:480-485.
5. Takeichi T, Akiyama M. Inherited ichthyosis: non-syndromic forms. *J Dermatol*. 2016;43:242-251.
6. Diociaiuti A, Angioni A, Pisaneschi E, et al. X-linked ichthyosis: Clinical and molecular findings in 35 Italian patients. *Exp Dermatol*. 2019;28:1156-1163.
7. Elias PM, Williams ML, Choi EH, et al. Role of cholesterol sulfate in epidermal structure and function: lessons from X-linked ichthyosis. *Biochim Biophys Acta*. 2014;1841:353-361.
8. Hazan C, Orlow SJ, Schaffer JV. X-linked recessive ichthyosis. *Dermatol Online J*. 2005;11:12.
9. Ohyama A, Nakano H, Imanishi Y, et al. A novel missense mutation of the STS gene in two siblings with X-linked ichthyosis, complicated by short stature, bone density reduction, epilepsy, and cryptorchidism. *Clin Exp Dermatol*. 2019;44:78-79.
10. Koppe G, Marinković-Ilsen A, Rijken Y, De Groot WP, Jöbsis AC. X-linked icthyosis. A sulphatase deficiency. *Arch Dis Child*. 1978;53:803-806.
11. Hand JL, Runke CK, Hodge JC. The phenotype spectrum of X-linked ichthyosis identified by chromosomal microarray. *J Am Acad Dermatol*. 2015;72:617-627.
12. Abasq-Thomas C, Schmitt S, Brenaut E, et al. A case of syndromic X-linked ichthyosis with Léri-Weill dyschondrosteosis. *Acta Derm Venereol*. 2016;96:814-815.
13. Kent L, Emerton J, Bhadravathi V, et al. X-linked ichthyosis (steroid sulfatase deficiency) is associated with increased risk of attention deficit hyperactivity disorder, autism and social communication deficits. *J Med Genet*. 2008;45:519-524.
14. Chatterjee S, Humby T, Davies W. Behavioural and psychiatric phenotypes in men and boys with x-linked ichthyosis: evidence from a worldwide online survey. *PLoS ONE*. 2016;11:e0164417.
15. Huang JW, Tang N, Li WG, et al. Identification of gene mutation and prenatal diagnosis in a family with X-linked ichthyosis. *Chin J Contemp Pediatr*. 2016;18:1136-1140.
16. Malik A, Amer AB, Salama M, et al. X-linked ichthyosis associated with psychosis and behavioral abnormalities: a case report. *J Med Case Rep*. 2017;11:267-267.
17. Shi H, Qi X, Liu T, et al. In vivo confocal microscopy of pre-Descemet corneal dystrophy associated with X-linked ichthyosis: a case report. *BMC Ophthalmol*. 2017;17:29.
18. Elias PM, Williams ML, Maloney ME, et al. Stratum corneum lipids in disorders of cornification. Steroid sulfatase and cholesterol sulfate in normal desquamation and the pathogenesis of recessive X-linked ichthyosis. *J Clin Invest*. 1984;74:1414-1421.
19. Williams ML, Elias PM. Stratum corneum lipids in disorders of cornification: increased cholesterol sulfate content of stratum corneum in recessive x-linked ichthyosis. *J Clin Invest*. 1981;68:1404-1410.
20. Craig WY, Palomaki G, Roberson M, et al. Further insights into implications of undetectable or very low unconjugated estriol in maternal serum during the second trimester. *Prenat Diagn*. 2011;31:616-618.
21. Liaugaudienė O, Benušienė E, Domarkienė I, et al. X-linked ichthyosis: differential diagnosis of low maternal oestriol level. *J Obstet Gynaecol*. 2014;34:737-739.
22. Zalel Y, Kedar I, Tepper R, et al. Differential diagnosis and management of very low second trimester maternal serum unconjugated estriol levels, with special emphasis on the diagnosis of X-linked ichthyosis. *Obstet Gynecol Surv*. 1996;51:200-203.
23. van Steensel MA, Vreeburg M, Engelen J, et al. Contiguous gene syndrome due to a maternally inherited 8.41 Mb distal deletion of chromosome band Xp22.3 in a boy with short stature, ichthyosis, epilepsy, mental retardation, cerebral cortical heterotopias and Dandy-Walker malformation. *Am J Med Genet A*. 2008;146A:2944-2949.

24. Puri PK, Reddi DM, Spencer-Manzon M, et al. Banding pattern on polarized hair microscopic examination and unilateral polymicrogyria in a patient with steroid sulfatase deficiency. *Arch Dermatol*. 2012;148:73-78.
25. Song Y, Chen J, Yi Z, et al. Genetic analysis of a 12-year-old boy with X-linked ichthyosis in association with sclerosing glomerulonephritis. *Mol Med Rep*. 2013;8:1183-1187.
26. Baek WS, Aypar U. Neurological manifestations of X-linked ichthyosis: case report and review of the literature. *Case Rep Genet*. 2017;2017:1-5.
27. Rodrigonicolas B, Buenomartinez E, Martinsantiago A, et al. Evidence of the high prevalence of neurological disorders in non-syndromic X-linked recessive ichthyosis: a retrospective case series. *Br J Dermatol*. 2018;179:933-939.
28. Cavenagh A, Chatterjee S, Davies W, et al. Behavioural and psychiatric phenotypes in female carriers of genetic mutations associated with X-linked ichthyosis. *PLoS ONE*. 2019;14:e0212330.

How to cite this article: Zhang M, Huang H, Lin N, et al. X-linked ichthyosis: Molecular findings in four pedigrees with inconspicuous clinical manifestations. *J Clin Lab Anal*. 2020;34:e23201. https://doi.org/10.1002/jcla.23201