Immunohistochemical staining indicated that MBTPS2 is mainly expressed in the upper granular layer in normal skin, as previously shown (Aten *et al.*, 2010); however, in OS skin, MBTPS2 was expressed throughout the epidermis (Figure 2c). There was no apparent difference in MBTPS2 localization in the skin of a KFSD patient with the p.N508S mutation (Aten *et al.*, 2010). It is unclear why this is but it may be because of differences in processing of the mutants in the two diseases.

In summary, we demonstrate a novel association between an *MBTPS2* mutation and an X-linked form of OS. This expands the number of disorders linked to *MBTPS2* mutations and reveals clinical heterogeneity associated with different *MBTPS2* mutations.

Written, informed consent was obtained from all family members or their legal guardians. This study was approved by the South East NHS Research Ethics Committee and was performed according to the Declaration of Helsinki Principles.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Aten E, Brasz LC, Bornholdt D *et al.* (2010) Keratosis follicularis spinulosa decalvans is caused by mutations in MBTPS2. *Hum Mut* 31:1125–33

Bergonse FN, Rabello SM, Barreto RL *et al.* (2003) Olmsted syndrome: the clinical spectrum of mutilating palmoplantar keratoderma. *Pediatr Dermatol* 20:323–6

Cambiaghi S, Tadini G, Barbareschi M *et al.* (1995) Olmsted syndrome in twins. *Arch Dermatol* 131:738–9

Ding YG, Wang JY, Qiao JJ *et al.* (2010) A novel mutation in MBTPS2 causes ichthyosis follicularis, alopecia and photophobia (IFAP) syndrome in a Chinese family. *Br J Dermatol* 163:886–9

Larregue M, Callot V, Kanitakis J *et al.* (2000) Olmsted syndrome: report of two new cases and literature review. *J Dermatol* 27:557–68

Lin Z, Chen Q, Lee M *et al.* (2012) Exome sequencing reveals mutations in TRPV3 as a cause of Olmsted syndrome. *Am J Hum Genet* 90:558–64

Mevorah B, Goldberg I, Sprecher E *et al.* (2005) Olmsted syndrome: mutilating palmoplantar keratoderma with periorificial keratotic plaques. *J Am Acad Dermatol* 53: S266–72

Naiki M, Mizuno S, Yamada K *et al.* (2012) MBTPS2 mutation causes BRESEK/BRESHECK syndrome. *Am J Med Genet A* 158A:97–102

Oeffner F, Fischer G, Happle R *et al.* (2009) IFAP syndrome is caused by deficiency in MBTPS2, an intramembrane zinc metalloprotease essential for cholesterol homeostasis and ER stress response. *Am J Hum Genet* 84:459–67

Olmsted HC (1927) Keratodermia palmaris et plantaris congenitalis. *Am J Dis Child* 33:757–64

Sakai J, Nohturfft A, Goldstein JL *et al.* (1998) Cleavage of sterol regulatory element-binding proteins (SREBPs) at site-1 requires interaction with SREBP cleavage-activating protein. Evidence from *in vivo* competition studies. *J Biol Chem* 273:5785–93

Tang L, Liang J, Wang W *et al.* (2011) A novel mutation in MBTPS2 causes a broad phenotypic spectrum of ichthyosis follicularis, atrichia, and photophobia syndrome in a large Chinese family. *J Am Acad Dermatol* 64:716–22

Tang L, Zhang L, Ding H *et al.* (2012) Olmsted syndrome: a new case complicated with easily broken hair and treated with oral retinoid. *J Dermatol* 39:1–2

Yaghoobi R, Omidian M, Sina N *et al.* (2007) Olmsted syndrome in an Iranian family: report of two new cases. *Arch Iran Med* 10:246–9

Ye J, Rawson RB, Komuro R *et al.* (2000) ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell* 6:1355–64

Zelenski NG, Rawson RB, Brown MS *et al.* (1999) Membrane topology of S2P, a protein required for intramembranous cleavage of sterol regulatory element-binding proteins. *J Biol Chem* 274:21973–80

Targeted Sequence Capture and High-Throughput Sequencing in the Molecular Diagnosis of Ichthyosis and Other Skin Diseases

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TO THE EDITOR

The inherited ichthyoses are a large and genetically heterogeneous group of Mendelian disorders of cornification, characterized by scaling and/or hyperkeratosis over the majority of the integument (Oji *et al.*, 2010). The two main nonsyndromic ichthyosis groups are autosomal recessive congenital ichthyosis (ARCI), encompassing lamellar ichthyosis (LI), congenital ichthyosiform erythroderma (CIE), and harlequin ichthyosis (HI), in which patients are

Abbreviations: ARCI, autosomal recessive congenital ichthyosis; CIE, congenital ichthyosiform erythroderma; HI, harlequin ichthyosis; KPI, keratinopathic ichthyosis; LI, lamellar ichthyosis; NGS, next-generation sequencing; SPPK, striate palmoplantar keratoderma; SNP, single-nucleotide polymorphism

usually born as collodion babies; and keratinopathic ichthyoses (KPI), the two main groups of which are epidermolytic ichthyosis (EI) and superficial epidermolytic ichthyosis (SEI) (Oji *et al.*, 2010). KPI are linked to mutations in keratin genes. Mutations in *ABCA12* are associated with HI, the most devastating and frequently lethal form of ichthyosis (Akiyama *et al.*, 2005; Kelsell *et al.*, 2005). However, mutations in multiple genes (Table 1), including *ABCA12*, are linked to LI and CIE, which display clinical overlap (Akiyama *et al.*, 2003).

As mutations in multiple genes are linked to nonsyndromic and syndromic ichthyosis, undertaking genetic screening by PCR and Sanger sequencing in a gene-by-gene approach is labor-intensive and expensive. Screening known ichthyosis genes simultaneously in a patient (or multiple patients) could enable a swifter identification of gene mutations in patients and facilitate prenatal diagnosis and genetic counseling.

Next-generation sequencing (NGS) has proven effective in the discovery of mutations in novel disease-associated genes, for example, by exome sequencing (Ng *et al.*, 2009) or by targeted sequence capture of chromosomal regions previously linked to a condition (Blaydon *et al.*, 2011, 2012). For conditions associated with mutations in multiple known genes, targeted gene capture coupled with NGS has shown promise in the molecular diagnosis of disease (Berg *et al.*, 2011; Artuso *et al.*, 2012). Here, we designed a custom NimbleGen microarray for sequence capture and NGS of the *ABCA12* gene locus, as well as the exons of 23 other genes linked to nonsyndromic and syndromic forms of ichthyosis, acral peeling skin syndrome/peeling (APSS) skin syndrome (PSS) or striate palmoplantar keratoderma (SPPK; Table 1), on pooled DNA consisting of 14 genomic DNA samples (Supplementary Information and Supplementary Table S1 online).

We discovered 12 sequence variants, which, to our knowledge, have not been reported previously (three deletions leading to frameshifts, seven missense, one nonsense, and one splice site), and four known disease-associated mutations (Table 2). The heterozygous whole-exon 8 deletion previously discovered by multiplex and oligonucleotide array analysis (Thomas *et al.*, 2006) in Sample 11 was not detected, indicating that currently NGS may not be sensitive enough to detect all complex or large insertion/deletion mutations. However, a predicted intronic splice site mutation was found, and this is likely to be the second disease-causing mutation in Sample 11. In contrast, a homozygous large 101 bp deletion (p.I1128EfsX38) within exon 24 of *ABCA12* was detected by this capture method. Known whole-exon deletions have been undetectable in other sequence capture investigations (Berg *et al.*, 2011). Other non-microarray

Table 1. Genes implicated in ichthyosis and other skin diseases on the custom capture microarray

Gene	OMIM	Cytogenetic location (hg19)	Disease(s) with skin phenotype
Exons captured			
ABHD5	*604780	3p21.33	CDS
AP1S1	*603531	7q22.1	MEDNIK
ALOXE3	*607206	17p13.1	ARCI (CIE)
ALOX12B	*603741	17p13.1	ARCI (CIE)
CDSN	*602593	6p21.33	Hypotrichosis, PSS
CSTA	*184600	3q21.1	AREI
CYP4F22	*611495	19p13.12	ARCI (LI)
DSG1	*125670	18q12.1	SPPK1
DSP	*125647	6p24.3	DCWHK, LAEB, SFWHS, SPPK2
GJB2	*121011	13q12.11	BPS, HID, KID, PPK with deafness
GJB3	+603324	1p34.3	EKV
GJB4	*605425	1p34.3	EKV
KRT1	*139350	12q13.13	AEI, EI, EPPK, ICM, NEPPK, SPPK3
KRT2	*600194	12q13.13	SEI
LOR	*152445	1q21.3	LK
NIPAL4	*609383	5q33.3	ARCI (CIE)
POMP	*613386	13q12.3	KLICK
SLC27A4	*604194	9q34.11	IPS
SLURP1	*606119	8q24.3	MDM
SPINK5	*605010	5q32	Atopy, NS
STS	#308100	Xp22.31	XLI
TGM1	*190195	14q12	ARCI (CIE, LI)
TGM5	*603805	15q15.2	APSS
Exons, introns			
ABCA12	*607800	2q35	ARCI (CIE, LI, HI)

Abbreviations: AEI, annular epidermolytic ichthyosis; APSS, acral peeling skin syndrome; ARCI, autosomal recessive congenital ichthyosis; AREI, autosomal recessive exfoliative ichthyosis; BPS, Bart-Pumphrey syndrome; CDS, Chanarin-Dorfman syndrome; CIE, congenital ichthyosiform erythroderma; DCWHK, dilated cardiomyopathy with woolly hair and keratoderma; EI, epidermolytic ichthyosis; EKV, erythrokeratoderma variabilis; EPPK, epidermolytic palmoplantar keratoderma; HI, harlequin ichthyosis; HID, hystrix-like ichthyosis with deafness; ICM, ichthyosis histrix, Curth-Macklin type; IPS, ichthyosis prematurity syndrome; KID, keratitis–ichthyosis–deafness syndrome; KLICK, keratosis linearis–ichthyosis congenital–keratoderma; LAEB, lethal acantholytic epidermolysis bullosa; LI, lamellar ichthyosis; LK, loricrin keratoderma; MDM, mal de Meleda; MEDNIK, mental retardation, enteropathy, deafness, peripheral neuropathy, ichthyosis, and keratodermia; NEPPK, nonepidermolytic palmoplantar keratoderma; NS, Netherton syndrome; PPK, palmoplantar keratoderma; PSS, peeling skin syndrome; SEI, superficial epidermolytic ichthyosis; SFWHS, skin fragility–woolly hair syndrome; SPPK, striate palmoplantar keratoderma; XLI, X-linked recessive ichthyosis.