SUPPLEMENTARY MATERIAL

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

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Complete Maternal Isodisomy of Chromosome 5 in a Japanese Patient with Netherton Syndrome

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TO THE EDITOR

In genetic counseling of autosomal recessive diseases, parents of patients are normally informed on the basis of the Mendelian principle that both parents are asymptomatic heterozygous carriers and the risk of recurrence is 25% in subsequent pregnancies (Fassihi *et al.*, 2006). However, there are several exceptions, including cases with uniparental disomy (UPD). UPD is a condition in which a pair of chromosomes are inherited from only one parent (Zlotogora, 2004). There are two types of UPD, uniparental isodisomy and uniparental heterodisomy (Zlotogora, 2004). When one chromosome is present in duplicate, the situation is described as isodisomy. When both chromosomes from one parent are present, the situation is heterodisomy (Kotzot, 2001; Siegel and Slavotinek, 2005). Thus, homozygosity for a recessive mutation may result from uniparental isodisomy. This study reports a case of Netherton syndrome (NS, MIM 256500), an autosomal recessive syndromic type of ichthyosis, showing complete maternal isodisomy of chromosome 5 with a pathogenic mutation in *serine protease inhibitor Kazal-type 5* (*SPINK5*; Bitoun *et al.*, 2002; Sun and Linden, 2006).

The patient was a 10-year-old Japanese girl, whose clinical and histopathological details have been described recently (Akagi *et al.*, 2013). In brief, she demonstrated typical symptoms of NS, including ichthyosiform erythroderma, bamboo hairs, and atopic features (Figure 1a and b). She showed moderate disease severity without any additional extracutaneous abnormalities. See Supplementary Data for further clinical information.

All described studies were performed following the guidelines of the medical ethical committee of Kurume University School of Medicine. Written informed consent was obtained from each individual, and the study was conducted according to the Declaration of Helsinki Principles.

Direct nucleotide sequencing of exon 19 of *SPINK5* for the patient's genomic DNA disclosed a recurrent homozygous mutation p.Arg578X (Figure 1c). However, although the mother was a heterozygous carrier of this mutation, the

Abbreviations: CP, crossing point; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NS, Netherton syndrome; SPINK5, serine protease inhibitor Kazal-type 5; UPD, uniparental disomy

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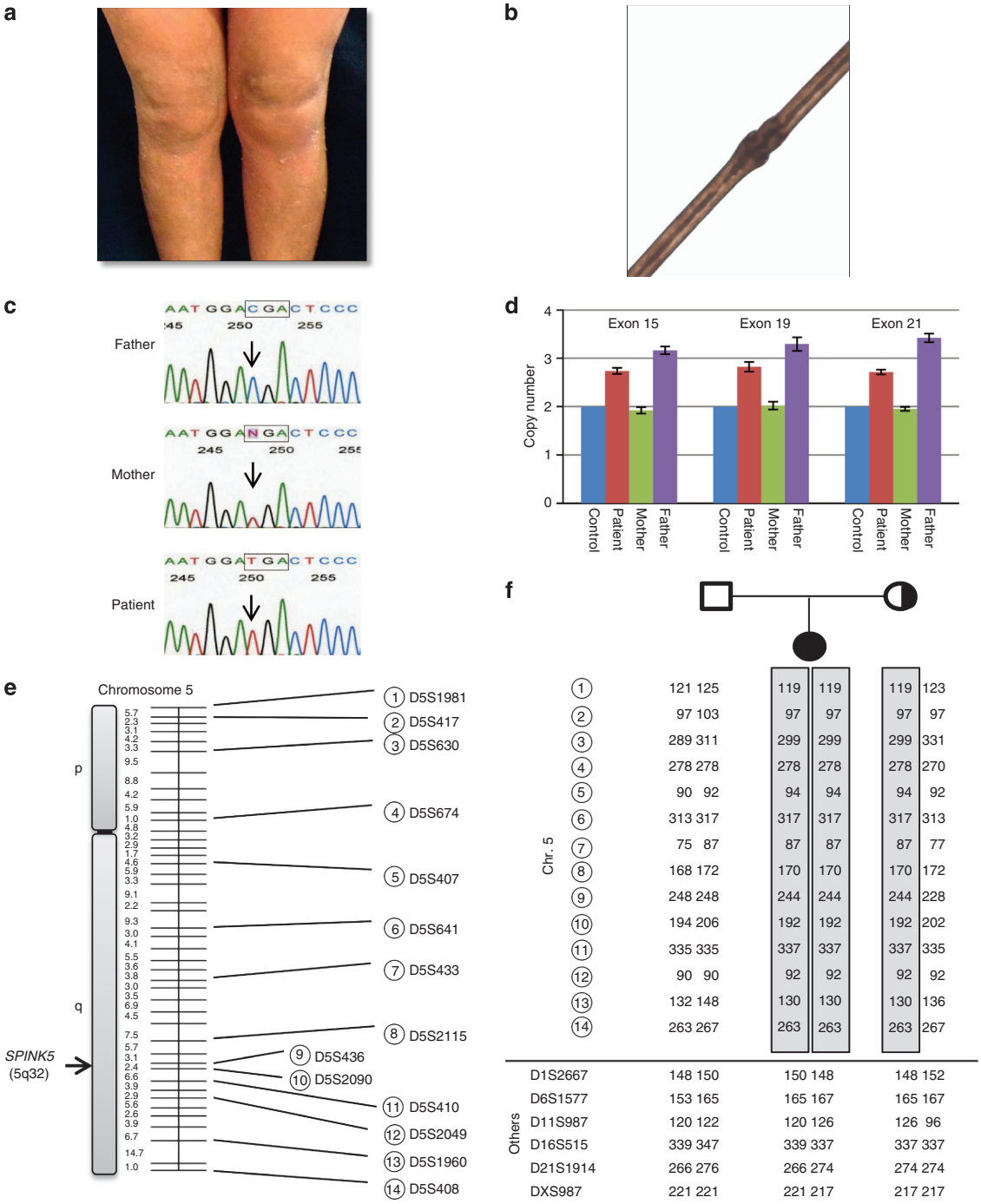

Figure 1. Clinical features and results of genetic analysis of our patient with Netherton syndrome. (**a**) Clinical features of the patient. Whitish thin scales and diffuse erythemas were present on legs at the age of 10 years. (**b**) Light microscopic analysis showed trichorrhexis invaginata (bamboo hair). (**c**) Nucleotide sequencing revealed homozygous mutation p.Arg578X in the patient. (**d**) Genomic copy-number quantification of present family. The patient and the parents did not show deletion of these exons, and no genomic copy-number alteration at *SPINK5* region in the patient was indicated. (**e**) Scheme of chromosome 5 and positions of 14 microsatellite markers. (**f**) Pedigree and results of genotype analysis in chromosome 5 and other six chromosomes of the patient and the parents. The patient has complete maternal isodisomy of chromosome 5.

father showed a wild-type sequence (Figure 1c). Polymorphism analysis of *SPINK5* between intron 2 and intron 27 indicated that all five polymorphisms examined were homozygous, although only three of them were fully informative (Table 1).

To verify whether the patient had a gene deletion in *SPINK5*, we examined the gene copy number of this family using real-time quantitative PCR for exons 15, 19, and 21. Each crossing-point (CP) value was compared with that of *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* by the relative

Table 1. Haplotype analysis of *SPINK5* using five intragenic polymorphisms

Location	Nucleotide position[1]	Major allele	Minor allele	Father	Patient	Mother
Intron 2	82 −31	G	A	G/G	G/G	G/G
Exon 13	1,157	G	A	G/G	A/A	A/A
Intron 18	1,693 −32	T	C	C/C	T/T	T/C
Exon 26	2,475	G	T	G/G	T/T	T/G
Intron 27	2,666 +13	T	A	A/A	A/A	A/A

[1]According to *SPINK5* gene sequence, GenBank accession no. NM_001127698.

quantification system and corrected CP values were used for the evaluation of copy numbers. The patient and her parents did not show deletion of these exons, and no reduced genomic copy number at *SPINK5* region was observed (Figure 1d).

The origin of the second mutant allele in the patient was studied by genotype analysis of 14 microsatellite markers spanning the entire chromosome 5 (ABI Prism Linkage Mapping Set Version 2.5, Applied Biosystems, Warrington, UK; Figure 1e). The patient was homozygous for all 14 markers, nine of which indicated inheritance of two copies of maternal chromosome 5 (Figure 1f). The allele sizes were analyzed using GeneMapper software (Applied Biosystems). In addition, normal segregation seen in six non-chromosome 5 markers (D1S2667, D6S1577, D11S987, D16S515, D21S914, and DXS987) excluded non-paternity (Figure 1f). Finally, karyotyping of peripheral blood from the patient revealed 46, XX. The results of all studies indicated that our NS case was caused by complete maternal isodisomy of chromosome 5.

There are 40 reported cases of various autosomal recessive diseases caused by UPD, including three cases with genes on chromosome 5 (Engel, 2006). The first case of spinal muscular atrophy (MIM 253300) was caused by complete paternal isodisomy of chromosome 5. The second case of child-onset schizophrenia (MIM 181500) was caused by segmental paternal isodisomy of 5q32-qter. The third case of NS was caused by a homozygous missense mutation p.Arg267Glu in *SPINK5*, resulting from a *de novo* mutation in combination with segmental maternal isodisomy (Lin *et al.*, 2007). To our knowledge, complete maternal isodisomy of chromosome 5 with a pathogenic *SPINK5* mutation is previously unreported, and this is the fourth case of UPD in chromosome 5, including one previous NS case (Lin *et al.*, 2007).

Depending on the source of the parental chromosome, UPD gives rise to unique phenotypes, and the phenomenon is known as genomic imprinting. To date, an association of genomic imprinting with intrauterine growth retardation, developmental delay, and reduced stature has been reported (Miozzo and Simoni, 2002; Coan *et al.*, 2005).

UPD is induced by diverse mechanisms, including gamete complementation, trisomy rescue, monosomy rescue, and post-fertilization error (Robinson, 2000). As common processes, trisomy rescue causes heterodisomy, in which both chromosomes from one parent are present, or monosomy rescue causes isodisomy, in which one chromosome is present in duplicate. In monosomy rescue, a nullisomic gamete is fertilized with a haploid gamete. The single chromosome from the other parent is duplicated and produces isodisomy of the chromosome. Because most monosomies are lethal and result in spontaneous abortion in the early stage of pregnancy, monosomy rescue is considered to occur early after fertilization (Fassihi *et al.*, 2006). The most likely cause of UPD in our patient is fertilization of a normal egg by a nullisomic sperm with subsequent salvage of a monosomy by post-fertilization duplication of the maternal chromosome 5, resulting in homozygosity for the *SPINK5* locus containing the mutation p.Arg578X (Kotzot and Utermann, 2005). Although UPD is a rare cause of autosomal recessive disorder, it is important. Therefore, it is important that DNA samples are obtained from both the patient and the parents in mutation analysis for autosomal recessive conditions.

In summary, to our knowledge, complete maternal isodisomy in chromosome 5 was previously unreported and was the fourth case of UPD in chromosome 5. As the patient did not have any symptoms other than those seen in NS, we speculate that genes on chromosome 5 are not imprinted. However, a careful follow-up for possible late-onset symptoms should be necessary. Our findings should provide important implications into mutation screening and genetic counseling in NS.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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IL-17A RNA Aptamer: Possible Therapeutic Potential in Some Cells, More than We Bargained for in Others?

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TO THE EDITOR

IL-17A is one of the main pathogenic factors orchestrating inflammation in diseases such as psoriasis and rheumatoid arthritis (Martin *et al.*, 2012). Although effective therapies already exist for these diseases, biologics are restricted to severely affected patients due to the considerable costs of current antibody-based therapies and potential systemic immunosuppressive and drug-induced side effects. New topical strategies are therefore required for chronic inflammation.

Aptamers are considerably cheaper to produce and furthermore are non-immunogenic. The use of stable RNA aptamers for therapy is based on their ability to fold into unique 3-dimensional structures that bind to the target with high specificity and affinity. The first aptamer-based therapeutic agent, which targets vascular endothelial growth factor (Huang *et al.*, 2001), was FDA-approved in 2004 for treatment of macular degeneration and several other aptamers are currently in clinical trials (Zhou *et al.*, 2012).

Here, we have sought to show the functional efficacy of a previously described aptamer, termed Apt21-2, which blocks IL-17A binding to its receptor (Ishiguro *et al.*, 2011). So far, the functional properties of this aptamer have not been investigated in human pathophysiological settings. To overcome this lack of information we analyzed the efficacy of Apt21-2 in a co-culture system, which mimics psoriatic inflammation, using T cells isolated from healthy controls and psoriatic patients. All human samples were taken in accordance with the Declaration of Helsinki Principles, and participants gave their written informed consent (ethics approval for this study is covered by IRAS REC number: 11/YH/0368). This *in-vitro* model, using patient-derived and healthy cells, is based on our own previous work (Muhr *et al.*, 2010) with blood-derived IL-17 producers (CD4+ CCR6+ T cells) activated with TCR stimulatory antibodies (antiCD3 and antiCD28; Biolegend, Hatfield, UK). These cells were co-cultured with healthy human primary fibroblasts. The protocols used for the culture of primary human skin cells are also based on previous work (Wittmann *et al.*, 2012). To measure the functional response, a bead-based ELISA assay (FlowCytomix; eBioscience, Hatfield, UK) in addition to an IL-6 and IL-8 ELISA (DuoSet human ELISA kit; R&D Systems, Abingdon, UK) was used. IL-17A (Biolegend)-mediated production of IL-6 was determined to establish the efficacy of the aptamer, as this has been employed in the previously published work (Ishiguro *et al.*, 2011) and we have extended this to include IL-8. It is well known that both keratinocytes and fibroblasts produce IL-6 and IL-8.

Our results confirmed that IL-6 release in recombinant IL-17A-treated fibroblasts can be significantly abrogated in a dose-dependent fashion by addition of Apt21-2, and this effect was not seen when a control aptamer (synthesized to the polymerase from foot-and-mouth disease virus, 47tr; Ellingham *et al.*, 2006) was used (Figure 1a). The effect was also not present if recombinant tumor necrosis factor-α (Immunotools, Friesoythe, Germany) was used as a stimulus (Figure 1b). The same effect was confirmed for fibroblasts isolated from psoriatic patients (data not shown). Higher doses of IL-17A were used and an efficient inhibitory effect of IL-6 production could be seen in doses up to $20\,ng\,ml^{-1}$. However, the aptamer failed to consistently neutralize the effect of $40\,ng\,ml^{-1}$ of rIL-17A on IL-6 production in fibroblasts. In a co-culture system of healthy fibroblasts and healthy or psoriatic CD4+CCR6+ T cells, a neutralizing capacity of Apt21-2 was

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