BJD-2017-0827.R1

Research Letter

A novel *IFIH1* mutation in the pincer domain underlies the clinical features of both Aicardi–Goutières and Singleton–Merten syndromes in a single patient

T. Takeichi[1,*], C. Katayama[2], T. Tanaka[3], Y. Okuno[4,5], N. Murakami[5], M. Kono[1], K. Sugiura[6], Y. Aoyama[2], M. Akiyama[1,*]

Departments of [1]Dermatology and [5]Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Departments of Dermatology and [3]Pediatrics, Kawasaki General Hospital, Kawasaki Medical School, Okayama, Japan; [4]Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, Nagoya, Japan; [6]Department of Dermatology, Fujita Health University School of Medicine, Toyoake, Japan

***Correspondence:** Takuya Takeichi, Department of Dermatology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

Telephone: 81-52-744-2314

E-mail: takeichi@med.nagoya-u.ac.jp

Masashi Akiyama

E-mail: makiyama@med.nagoya-u.ac.jp

Funding sources: This work was supported by funding from Advanced Research and Development Programs for Medical Innovation (AMED-CREST) to M.A. from Japan Agency for Medical Research and Development (AMED). This work was also supported by a Grant-in-Aid for Scientific Research (B) 15H04887 to M.A., and by a Grant-in-Aid for Young Scientists (B) 16K19717 to T.T. from the Japan Society for the Promotion of Science (JSPS).

Conflicts of interest: The authors declare that there are no conflicts of interest.

Short title: Clinical overlap of AGS and SMS by *IFIH1* mutation

Word count: 750/750

References: 8/8

Figures: 1

Tables: 1

Gain-of-function mutations in *IFIH1* encoding interferon-induced helicase C domain-containing protein 1 were identified in a spectrum of human disease phenotypes[1-4] including the overlap between Aicardi–Goutières syndrome (AGS) and Singleton–Merten syndrome (SMS).[5] Here we describe a case with a novel *IFIH1* missense mutation in the pincer domain.

Our case is a 7-year-old Japanese girl, the younger of two siblings born to non-related parents with no family history of any similar disorder. At the age of 6 months, she was noticed as having dry skin with mild erythema. She demonstrated erythematous cheeks and ichthyosis on the extremities (Fig. 1a). Since then, she has shown the following clinical features: low height (-2.8 standard deviation (SD)) and low weight (-1.7 SD), osteopenia (Fig. 1d), poor dentition (Fig. 1e), myopia of the right eye and amblyopia of the left eye, and bilateral calcification of the deep frontal lobes and the globus pallidi on computed tomography (Fig. 1f). She has moderate motor and mental retardation. She can walk with another person's assistance or by using a handrail. At the age of eight years, she was able to run 30 meters. She can feed herself by using dentures. She can almost use the toilet by herself. She is not always spastic, but she does have a febrile seizure every year. She showed low IgA, IgM, and high IgE and IgG levels. Her ichthyotic skin was improved by bathing with baking soda.

Following ethical approval, informed written consent was obtained in compliance with the Declaration of Helsinki guidelines. Whole-exome capture was performed (peripheral blood genomic DNA from the patient and both parents) by in-solution hybridization using SureSelect All Exon 50 Mb Version 5.0 (Agilent, Santa Clara, CA) followed by massively parallel sequencing (HiSeq2500; Illumina, San Diego, CA) with 150-bp paired-end reads. Totally, 24,717 single-nucleotide substitutions were identified in this patient: 10,031 homozygous and 14,686 heterozygous. After filtering, 14 previously unreported heterozygous variants were found as *de novo*. Within these variants, a nonsynonymous heterozygous mutation was identified in *IFIH1* (c.2561T>A; p.Met854Lys), which was confirmed by Sanger sequencing. The mutation had not been described in our in-house database (777 Japanese exome data), nor in the gnomAD Database (http://gnomad.broadinstitute.org/), which includes 123,136 whole-exome and 15,496 whole-genome data. We confirmed paternity/maternity by SNPs on exome data. *In silico* analysis with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and the SIFT (http://sift.jcvi.org/) predict the mutation to be 'damaging'. We did not identify potentially pathogenic mutations in genes implicated in inherited ichthyoses.

A skin biopsy specimen from the right lower leg showed compact hyperkeratosis, parakeratosis with hypogranulosis, a few dyskeratotic cells and mild vacuolar degeneration in the epidermis.

A number of facts support its pathogenicity of the present mutation (p.Met854Lys). First, the mutation was absent in both parents, suggesting a *de novo* condition. Secondly, the mutation affects a residue that is highly conserved among different species (Fig. 1b). The corresponding methionine is a highly conserved amino acid residue that shows homology not only within the *IFIH1*, but also to human *RIG-I* and *LGP2*, which also encode the other antiviral RNA helicase proteins. Thirdly, the mutation lies on the pincer domain, which connects helicase domain 2 (HEL2) and the C-terminal domain (CTD), is composed of two α helices (αP1-αP2) that emerge from the last β-strand of HEL2. p.Met854Lys is located at the first helix, αP1. Although the pincer is neither an ATPase core domain nor a direct RNA-binding site, it transmits information between HEL2 and CTD, regulating the behavior of IFIH1, which is essential for RNA sensing of IFIH1.

Immunohistochemical stainings[6] revealed that p-STAT3 was strongly expressed in the nuclei of keratinocytes in the patients (Fig. 1h), although there were no significant changes in the staining of IFIH1. The interferon-α receptor, the receptor of

type I interferon, activates Janus kinase 1 and tyrosine kinase 2. Phosphorylation of the receptor by these kinases results in the recruitment of STAT proteins, phosphorylation, dimerization and nuclear translocation. The three predominant STAT complexes (STAT1, STAT2 and STAT3) that form in response to type I interferon control distinct gene expression programs. Our observations suggest the possibility that type I interferon signaling is activated in the patient's epidermis.

All 11 previously reported pathogenic mutations in *IFIH1* were located in the Hel1 or Hel2 domains and were associated with upregulated type I interferon signaling (Table 1). The present proband shows characteristic clinical features of AGS (intracranial calcification) and SMS (dental hypoplasia and osteopenia). The heterozygous missense mutation in the pincer domain of IFIH1 might lead to the overlapping clinical phenotypes of type I interferon upregulated signaling.

Acknowledgements

This work was supported by funding from Advanced Research and Development Programs for Medical Innovation (AMED-CREST) to M.A. from Japan Agency for Medical Research and Development (AMED). This work was also supported by a Grant-in-Aid for Scientific Research (B) 15H04887 to M.A., and by a Grant-in-Aid

for Young Scientists (B) 16K19717 to T.T. from the Japan Society for the Promotion of Science (JSPS).

Figure legends

Figure 1. Clinicopathological features and *IFIH1* mutation of the patient, IFIH1 domain structure and immunohistochemical analyses.

(a) At 5 years of age, large whitish scales are seen on the right leg. (b) The corresponding methionine 854 is conserved among diverse species. (c) A schematic of the IFIH1 domain structure. The present mutation in the patient is marked by the black arrow. Domains: CARD, caspase recruitment domain; Hel1, HEL2i and Hel2, helicase domains; P, pincer domain; CTD, C-terminal domain. (d) An X-ray image of her right hand shows carpal hypoplasia (red arrow) and dysplasia of the right thumb's frontal bone (red arrowhead). Radiography of the left foot reveals hypoplasia of the distal phalanxes (red arrows). (e) A pantomogram shows root hypoplasia (red arrows). (f) Head CT demonstrates bilateral calcification of the deep frontal lobes (red arrows) and the globus pallidi (red arrowheads). (g, h) Epidermis from the patient (h) and normal skin from healthy donors (g) were stained with anti-p-STAT3 antibody (p-STAT3, Phospho-Tyr705, Signalway Antibody, College Park, MD). The nuclear localization of p-STAT3 is seen in the epidermal keratinocytes of the patient's epidermis. Scale bars: 50 μm.

Table 1. Summary of reported heterozygous *IFIH1* mutations associated with upregulated type I interferon signalling

No.	Mutation	Phenotype	Exon	Mutation site (functional domain)	Number of pedigrees	Cutaneous features	Refer-ences
1	c.1009A>G, p.Arg337Gly	neuro-regression with spasticity and dystonia	5	Hel1	1	ND	1
2	c.1114C>T, p.Leu372Phe	AGS	6	Hel1	1	ND	2
3	c.1178A>T, p.Asp393Val	neuro-regression	6	Hel1	1	ND	1
4	c.1354G>A, p.Ala452Thr	AGS	7	Hel1	1	ND	2
5	c.1465G>A, p.Ala489Thr	AGS and SMS	7	Hel1	1	chilblains, erythematous cheeks, multiple lentigines on the limbs	5
6	c.1483G>A, p.Gly495Arg	lower-limb spasticity	7	Hel1	1	ND	1
7	c.2159G>A, p.Arg720Gln	AGS	11	Hel2	2	ND	1
8	c.2335C>T, p.Arg779Cys	AGS associated with a severe lupus-like disease	12	Hel2	2	diffuse psoriasiform and ichthyosiform lesions	1, 7
9	c.2336G>A, p.Arg779Hi	AGS, SLE, IgA deficiency	12	Hel2	4	livedo rash, necrotizing	1, 2, 4

	s					cutaneous vasculitis	
10	c.2465G>A, p.Arg822Gln	SMS	12	Hel2	3	psoriasis	3
11	c.2471G>A, p.Arg824Lys	ND	12	Hel2	1	ND	8
12	c.2561T>A, p.Met854Lys	AGS, SMS	12	Pincer	1	erythematous cheeks, ichthyosis, atopic dermatitis	present case

Abbreviations: AGS, Aicardi–Goutières syndrome; SMS, Singleton–Merten syndrome; SLE, systemic lupus erythematosus; Hel1, helicase domain 1; Hel2, helicase domain 2; ND, not described

References

1 Rice GI, del Toro Duany Y, Jenkinson EM *et al.* Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling. *Nat Genet* 2014; **46**: 503-9.

2 Oda H, Nakagawa K, Abe J *et al.* Aicardi-Goutieres syndrome is caused by IFIH1 mutations. *Am J Hum Genet* 2014; **95**: 121-5.

3 Rutsch F, MacDougall M, Lu C *et al.* A specific IFIH1 gain-of-function mutation causes Singleton-Merten syndrome. *Am J Hum Genet* 2015; **96**: 275-82.

4 Van Eyck L, De Somer L, Pombal D *et al.* Brief Report: IFIH1 Mutation Causes Systemic Lupus Erythematosus With Selective IgA Deficiency. *Arthritis Rheumatol* 2015; **67**: 1592-7.

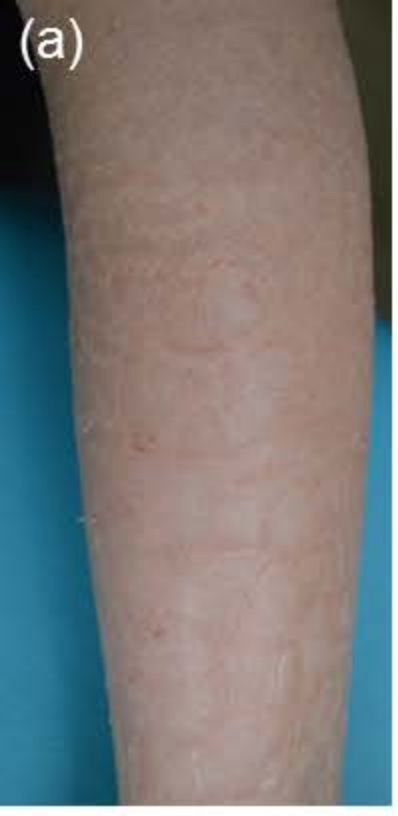
5 Bursztejn AC, Briggs TA, del Toro Duany Y *et al.* Unusual cutaneous features associated with a heterozygous gain-of-function mutation in IFIH1: overlap between Aicardi-Goutieres and Singleton-Merten syndromes. *Br J Dermatol* 2015; **173**: 1505-13.

6 Takeichi T, Watanabe N, Muro Y *et al.* Phosphorylated signal transducer and activator of transcription 3 in the epidermis in adult-onset Still's disease. *J Dermatol* 2017; in press.

7 Marguet F, Laquerrière A, Goldenberg A *et al.* Clinical and pathologic features of Aicardi-Goutières syndrome due to an IFIH1 mutation: A pediatric case report. Am J Med Genet A. 2016; **170A**: 1317-24.

8 Crow YJ, Chase DS, Lowenstein Schmidt J *et al.* Characterization of human disease phenotypes associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR, and IFIH1. Am J Med Genet A. 2015; **167A**: 296-312.

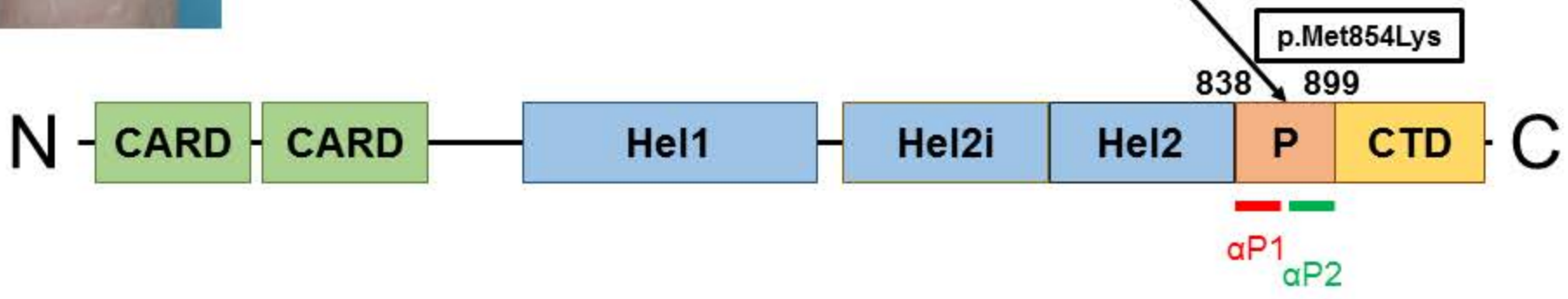
(a)



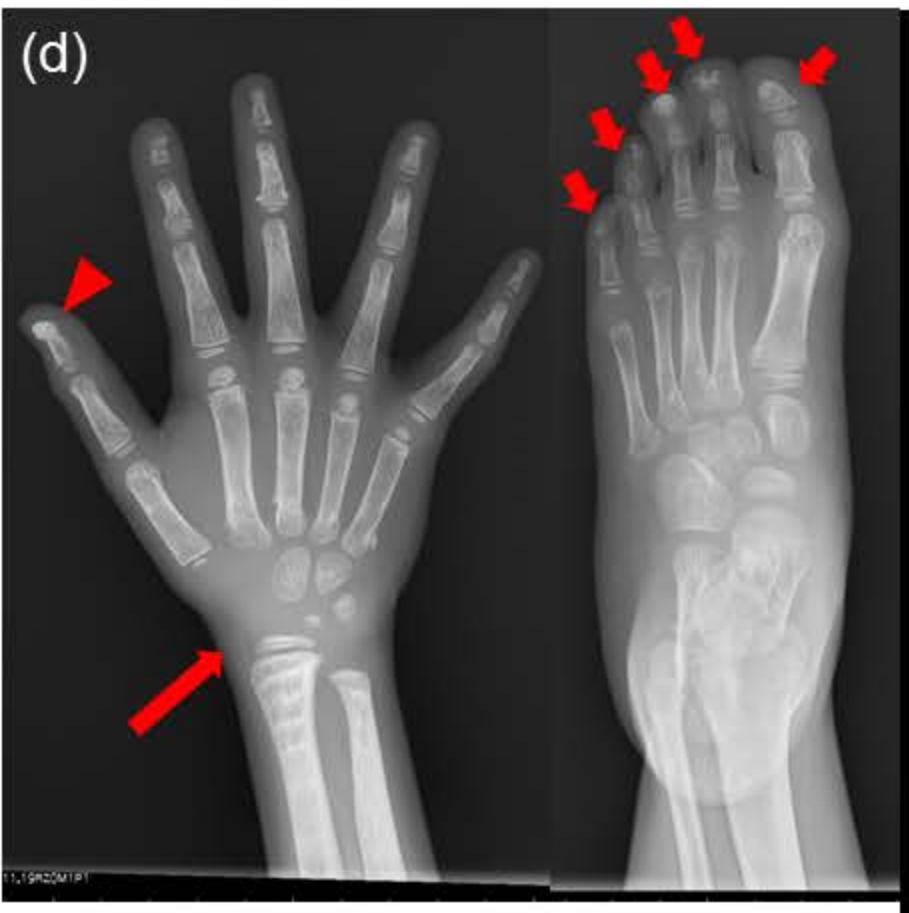
(b)

Species	Alignment (854)
Homo sapiens	HETVNDFREKMMYKAIHCVQNMKP
Felis catus	I VNDFREKMMYKAIDHVQNMN
Macaca mulatta	RETVNDFREKMMYKAIHCVQNMK
Pan troglodytes	RETVNDFREKMMYKAIHCVQNMK
Mus musculus	RE I VNDFREKMMYKAINRVQNMK
Danio rerio	RESVNEYREKMMSKAIAKVCKMN
Caenorhabditis elegans	LMSETISL I QNSP

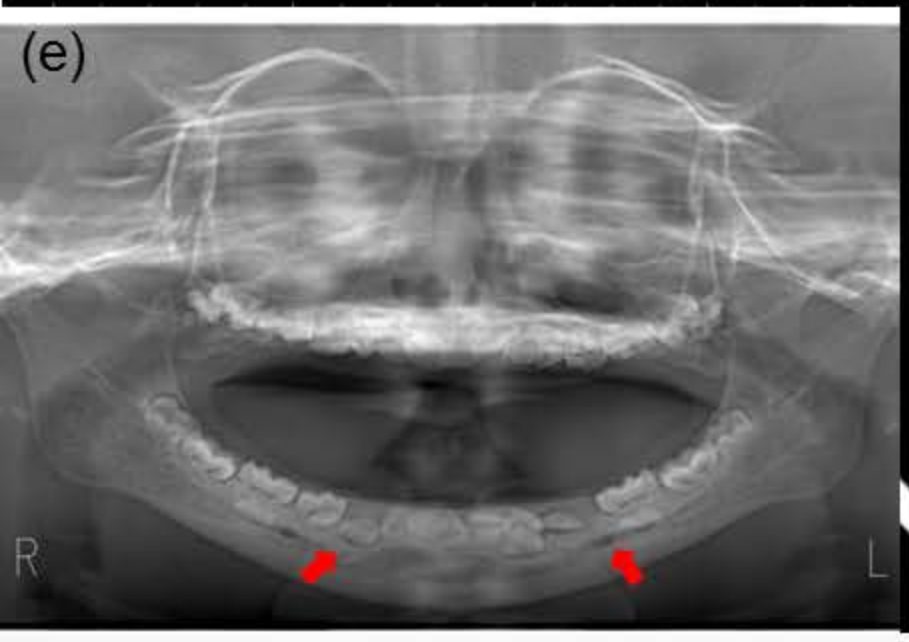
(c)



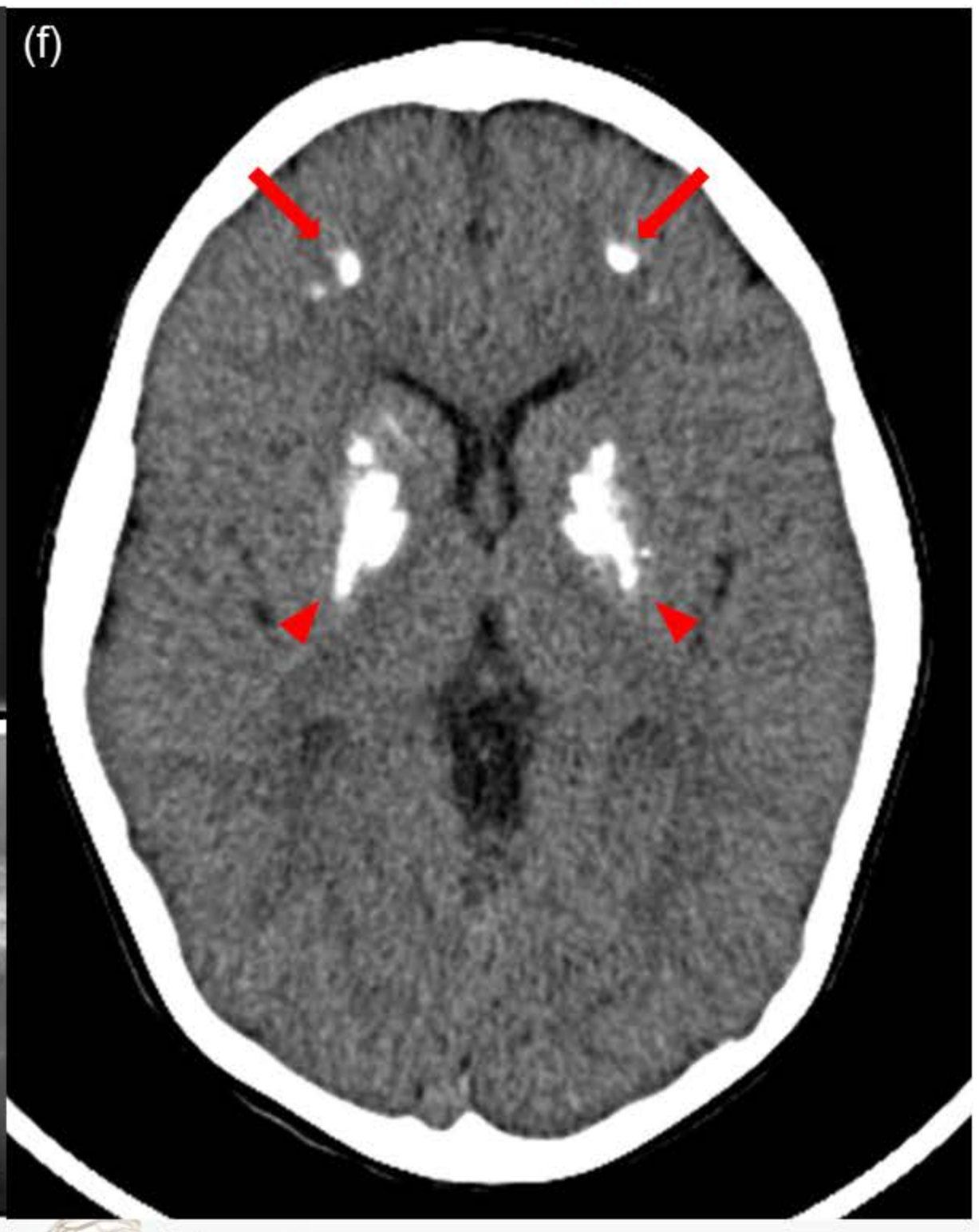
(d)



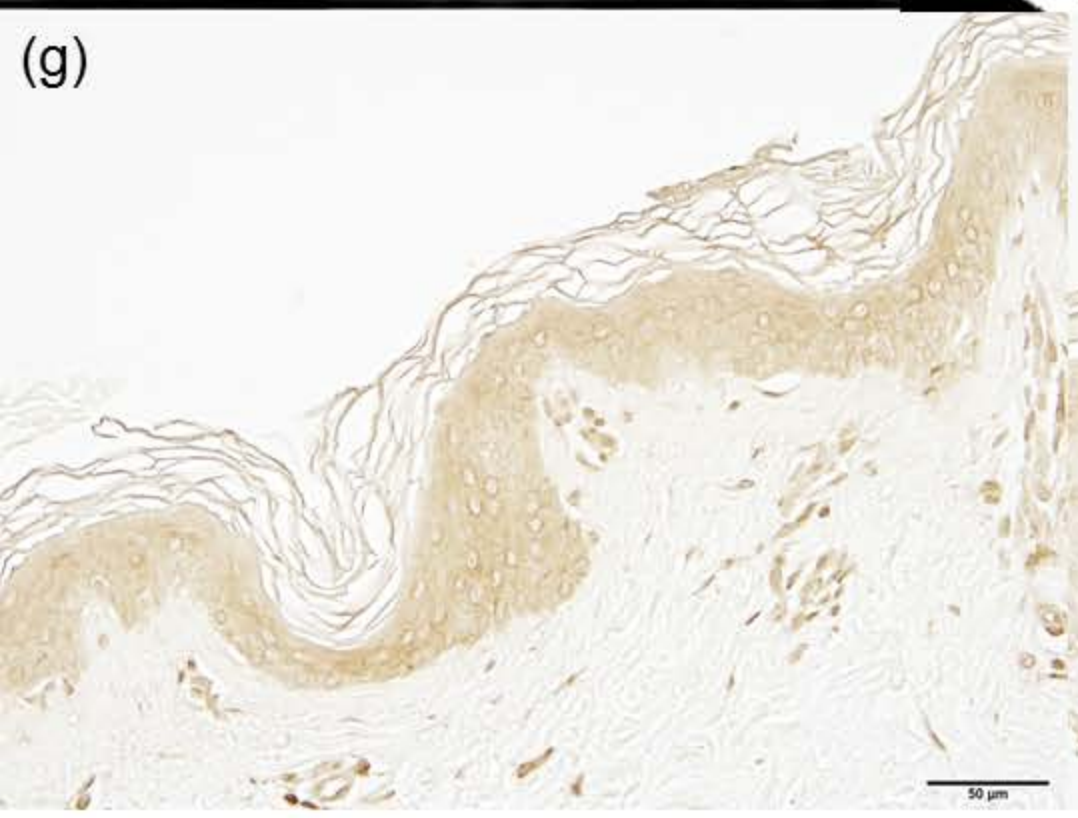
(e)



(f)



(g)



(h)