

- 5 Sidwell RU, Francis N, Bunker CB. Hormonal influence on reticular erythematous mucinosis. *Br J Dermatol* 2001; **144**: 633–634.
- 6 Cinotti E, Merlo V, Kempf W *et al*. Reticular erythematous mucinosis: histopathological and immunohistochemical features of 25 patients compared with 25 cases of lupus erythematosus tumidus. *J Eur Acad Dermatol Venereol* 2015; **29**: 689–697.
- 7 Kucukunal A, Altunay I, Demirci GT, Sarikaya S, Sakiz D. Reticular erythematous mucinosis on the midline of the back. *Cutis* 2014; **93**: 294–296.
- 8 Suárez-Amor O, Pérez-Bustillo A, González-Morán MA, Ramírez-Santos A, Rodríguez-Prieto MA. Reticular erythematous mucinosis: partial response to treatment with topical tacrolimus. *Actas Dermosifiliogr* 2010; **101**: 105–106.
- 9 Fühler M, Ottmann K, Tronnier M. Reticular erythematous mucinosis- (REM syndrome) in twins. *J Dtsch Dermatol Ges* 2009; **7**: 968–970.

DOI: 10.1111/jdv.13585

Results of patch testing in Indonesian shoe factory workers

Editor

Previously, we presented an overview of putative sources of occupational sensitizing allergens in an Indonesian shoe factory.¹ In this letter, we report relevant positive patch test results to the identified allergens.

Employees were interviewed about occupational skin disease and allergen exposure in the workplace and underwent dermatological examination. Workers with suspected occupational contact dermatitis (OCD) were patch tested with allergens from the European baseline series, the shoe series (Chemotechnique Diagnostics, Vellinge, Sweden) and an additional series with allergens specific for shoe factory work, compiled based on our previously reported inventory of potentially sensitizing allergens.¹ We also patch tested a randomly selected sample of 78 workers without skin disease. A diagnosis of occupational allergic contact dermatitis (OACD) was established in cases that met all of the following criteria:^{2,3}

- 1 Diagnosis OCD.²
- 2 Exposure to relevant occupational allergens.
- 3 Confirmed type 4 sensitization to relevant occupational allergens by patch testing.
- 4 Exposure to allergens confirmed as cause or important aggravating factor in the development of OCD.

Only positive patch test results to allergens to which exposure at the workplace was present were deemed relevant, and were thus included.

Five hundred and fourteen workers (497 female and 17 male) were studied. Mean age was 32 years (range: 19–47); mean duration of employment 8.1 years (SD: 5.5 years); and mean working hours 48 h per week (SD: 8 h). Fifty-two workers (10%) had a history of childhood eczema; 63 workers (12%) had a history of

atopic respiratory disease; and 77 workers (15%) had an atopic skin diathesis.⁴ Thirty-nine workers (8%) had OCD. Dermatitis was located on hands, wrist, and forearms ($n = 30$); on the trunk ($n = 14$); and in the face/neck ($n = 7$). Workers in the preparing/upper sole and assembling department handled adhesives barehanded without personal protective equipment. This could explain the predominance of OCD on hands, wrists, and forearms. Patch tests were adequately performed in 33/39 workers (six refused to be tested, manipulated the patches or did not return for reading of the results). Fourteen (3% of all workers) were diagnosed with OACD. We identified relevant positive reactions to 18 allergens (Table 1). The most frequently sensitizing materials were rubber (11 positive reactions) and adhesives (9 positive reactions). This observation is in line with the results of other studies.^{5,6} Sensitization was most commonly seen to dibutylthiourea (9%; 3/33) and methyldibromoglutaronitrile (9%; 3/33). Although all workers in the studied factory were exposed directly to rubber soles, sensitization to dibutylthiourea was seen only in the preparing/upper sole department. This could be due to long duration of exposure in this department. Methyldibromoglutaronitrile is used as an industrial biocide and is added to paints and adhesives.⁷ Sensitization to

Table 1 Positive patch test reactions relevant to shoe factory work in workers with occupational contact dermatitis

Material to which workers are exposed	Allergens relevant for causing OACD	Workers with OCD ($n = 33$) n (%)	Healthy workers ($n = 78$) n (%)
Leather	Preservatives/biocides		
	Methylchloroisothiazolinone	1 (3)	-
	Tanning agents		
	Potassium dichromate	1 (3)	-
	Formaldehyde	1 (3)	-
	Glutaraldehyde	1 (3)	-
Leather and synthetic leather	Finishing process		
	Polyethyl acrylate	1 (3)	-
Rubber materials	N-isopropyl-n'-phenyl-4-phenylenediamine	1 (3)	-
	p-phenylenediamine	2 (6)	1 (1)
	1,3-diphenylguanidine	2 (6)	-
	Dibutylthiourea	3 (9)	-
	Mercapto mix	1 (3)	-
	Thiuram mix	2 (6)	-
Adhesive	p-tert-buthylphenol		
	formaldehyde resin (PTBP-F-R)	2 (6)	-
	Methyldibromoglutaronitrile	3 (9)	1 (1)
	Dodecylmercaptan	2 (6)	1 (1)
	Epoxy resin	1 (3)	-
	Colophony	1 (3)	1 (1)
Dyes	4-aminoazobenzene	2 (6)	3 (4)
Shoe accessories	Nickel sulfate	2 (6)	-

OACD, occupational allergic contact dermatitis; OCD, occupational contact dermatitis.

Note: some workers had more than one positive reaction.

Table 2 Workers (all female) with occupational allergic contact dermatitis

Age (yrs)	Working section	Material causing/aggravating the skin symptoms	Sensitization	Description of material
Preparation				
30	Cutting	Leather	Glutaraldehyde	Leather tanning agent
		Scissor/handle cutting machine	Formaldehyde	Leather tanning agent
			Nickel sulfate	Scissor and cutting machine
Preparing/upper sole				
29	Sewing	Rubber insoles	N-isopropyl-n'-phenyl-4-phenylenediamine	Rubber antioxidant, antidegradant
			Mercapto mix	Rubber accelerators
			Dibutylthiourea	Rubber accelerator and antidegradant
			4-aminoazobenzene	Dye component
35	Sewing	Shoe adhesive	p-tert-buthylphenol formaldehyde resin	Neoprene based adhesive
		Rubber insoles	p-phenylenediamine	Rubber antioxidant
			1,3-diphenylguanidine	Rubber accelerator
36	Sewing	Rubber adhesive	Colophony	Rubber adhesive and tackifier in inner sole
		Rubber insoles	1,3-diphenylguanidine	Rubber accelerator
30	Sewing	Leather	Methylchloroisothiazolinone	Biocide in leather processing
		Oil paint	Potassium dichromate	Leather tanning agent
		Shoe adhesive	4-aminoazobenzene	Dye component
			p-tert-buthylphenol formaldehyde resin	Neoprene based adhesive
32	Sewing preparation	Shoe adhesive	Dodecyl mercaptan	Neoprene based adhesive
41	Sewing	Shoe adhesive	Methyldibromoglutaronitrile	Preservative in shoe adhesives
41	Sewing	Shoe adhesive	Dodecyl mercaptan	Neoprene adhesive
40	Sewing	Rubber insoles	Dibutylthiourea	Rubber accelerator and antidegradant
		Shoe adhesive	Methyldibromoglutaronitrile	Preservative in shoe adhesives
39	Sewing preparation	Rubber sole	p-phenylenediamine	Rubber antioxidant
			Thiuram mix	Rubber accelerators
Assembling				
38		Shoe adhesive	Methyldibromoglutaronitrile	Preservative in shoe adhesives
		Synthetic leather (polyurethane)	Polyethyl acrylate	Used in polyurethane finishing process
36		Adhesive	Epoxy resin	Preservative in shoe adhesives
		Latex covering inside shoe upper	Dibutylthiourea	Rubber accelerator and antidegradant
29		Shoe buckles and inlets	Nickel sulfate	Nickel shoe buckles and inlets
		Oil paint	4-aminoazobenzene	Dye component
Finishing				
49		Rubber sole	p-phenylenediamine	Rubber antioxidant
			Thiuram mix	Rubber accelerators

methyldibromoglutaronitrile is only rarely reported in adhesives; a case report has been published involving a worker with OACD due to contact with paste glue containing methyldibromoglutaronitrile.⁸ In Table 2, individual patients with OACD are presented. Positive allergens are linked to the material to which workers were exposed at their working department.

The positive patch tests of three healthy workers to 4-aminoazobenzene could be explained by previous sensitization while working in another department of this shoe factory or by sensitization to shoes worn in the past.⁹ Another explanation could be that workers had been involved in the batik industry, where exposure and sensitization to azo-dyes occurs.¹⁰

The fact that our study was carried out in only one shoe factory might be considered a limitation. However, according to the Indonesian Footwear Services, the studied factory is a representative shoe manufacturing facility in Indonesia. Our results

add to the existing knowledge on allergens that may cause allergic contact dermatitis in shoe manufacturers as well as shoe wearers.

Acknowledgements

The authors thank the department of Research and Development, Ministry of Industry and Trade, Republic of Indonesia, and Mr. Ferry, representative of Indonesia Footwear Service Centre, for their assistance.

J.A.F. Oosterhaven,¹ S.A. Febriana,² H. Soebono,²
P.J. Coenraads,¹ M.L.A. Schuttelaar^{1,*}

¹Department of Dermatology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, ²Department of Dermatology & Venereology, Gadjah Mada University, Yogyakarta, Indonesia

*Correspondence: M.L.A. Schuttelaar. E-mail: m.l.a.schuttelaar@umcg.nl

References

- 1 Febriana SA, Soebono H, Coenraads PJ. Occupational skin hazards and prevalence of occupational skin diseases in shoe manufacturing workers in Indonesia. *Int Arch Occup Environ Health* 2014; **87**: 185–194.
- 2 Mathias CG. Contact dermatitis and workers' compensation: criteria for establishing occupational causation and aggravation. *J Am Acad Dermatol* 1989; **20**: 842–848.
- 3 Ale IS, Maibach HI. Diagnostic approach in allergic and irritant contact dermatitis. *Expert Rev Clin Immunol* 2010; **6**: 291–310.
- 4 Diepgen TL, Sauerbrei W, Fartasch M. Development and validation of diagnostic scores for atopic dermatitis incorporating criteria of data quality and practical usefulness. *J Clin Epidemiol* 1996; **49**: 1031–1038.
- 5 Mancuso G, Reggiani M, Berdondini RM. Occupational dermatitis in shoemakers. *Contact Dermatitis* 1996; **34**: 17–22.
- 6 Guedes RV, Leite IB, Baptista AM. Contact allergy to shoe components in professionals and nonprofessionals of the shoe industry. *Dermatitis* 2013; **24**: 147–149.
- 7 Yim E, Baquerizo Nole KL, Tosti A. Contact dermatitis caused by preservatives. *Dermatitis* 2014; **25**: 215–231.
- 8 Mathias CG. Contact dermatitis to a new biocide (Tektamer 38) used in a paste glue formulation. *Contact Dermatitis* 1983; **9**: 418.
- 9 Febriana SA, Soebono H, Coenraads PJ, Schuttelaar ML. Contact allergy in Indonesian patients with foot eczema attributed to shoes. *J Eur Acad Dermatol Venereol* 2015; **29**: 1582–1589.
- 10 Soebaryo R. Batik manufacturing workers. In Rustemeyer T, Elsner P, John SM, Maibach HI, eds. *Kanerva's Occupational Dermatology*, 2nd edn. Berlin Heidelberg, Springer-Verlag, 2012: 1289–1295.

DOI: 10.1111/jdv.13586

The novel *EDAR* p.L397H missense mutation causes autosomal dominant hypohidrotic ectodermal dysplasia

Editor

Hypohidrotic/Anhidrotic ectodermal dysplasia (HED) is the most common form of ectodermal dysplasias and exhibits X-linked recessive, autosomal recessive (AR), and autosomal dominant (AD) modes of inheritance. HED is mainly caused by mutational inactivation of either of three genes namely *EDA*, *EDAR*, or *EDARADD* that encode components of a developmental cell signaling cascade responsible for formation of tissues of ectodermal origin.¹

Four suspected HED patients were evaluated following informed consent and approval from CDFD bioethics committee. The study was performed as per guidelines of the Helsinki declaration revised in 1983. The proband of family 1, a male child aged 10 years born to non-consanguineous parents (Fig. 1a), exhibited decreased sweating and recurrent episodes of hyperpyrexia and heat intolerance since two years of age.

Eyebrows were scanty and eyelashes were absent, whereas the scalp hair was thin, coarse and hypopigmented (Fig. 1b). Skin was dry and scaly (Fig. 1b). Of the 27 teeth, caries were evident in almost all; lower central incisors were small and pointed. Proband's father exhibited similar symptoms including sparse hair and dental caries (Fig. 1b); an elder brother (aged 12 years) was also affected (Fig. 1a), but did not present for examination. Given the autosomal dominant (AD) trait, we performed mutation screening for *EDAR* as described before² and identified a novel heterozygous c.1190T>A (p.L397H) mutation (Fig. 1c and Tables 1 and 2) that was confirmed to be absent in 100 normal chromosomes. Multiple sequence alignment showed a strong conservation of residue L397 (Fig. 1d), which is located in the *EDAR* death domain. The Gribkov scores (+15.0 for Leucine and -2.0 for Histidine at amino acid position 397) clearly showed the mutated residue to be disfavored. Predictions from SIFT,³ Poly-Phen II⁴ and HANSA⁵ unanimously classified the missense mutation as pathogenic (data not shown). The *EDAR* death domain homology model⁶ was used to evaluate the neighborhood of L397. The L397 side chain was found to be buried within the hydrophobic core characterized by W374, A378, F381, L383, M391, F398, and I401 (Fig. 1e). Thus, a buried Histidine in the apolar environment is expected to destabilize *EDAR* death domain structure. Taken together, these analyses strongly suggest the *EDAR* p.L397H mutation to be the cause of HED in family 1. This is the first and only AD-HED family reported from India and symptoms in the affected proband and proband's father were clearly less severe when compared to the AR-HED families reported here and earlier,⁶ especially with respect to scalp hair and teeth as suggested previously.^{7–9}

EDAR mutation screening was performed in the other three probands and the p.G382S mutation was detected in all three (Families 2, 3, and 4 in Table 1). Thus, the p.G382S accounts for 20% of all HED families screened in India so far (Table 2), making it the most frequent cause of HED in the Indian population.

One peculiar aspect of *EDAR* mutation positive HED is the presence of both AD and AR traits. Though a rare phenomenon, additional genes are characterized that similarly exhibit a dual trait.¹⁰ In conclusion, this study has added to the repertoire of *EDAR* mutations causing HED in India and validated our previous observation of the *EDAR* p.G382S mutation being the predominant cause of HED in the Indian population. Finally, the discovery of a novel *EDAR* p.L397H mutation has confirmed that missense mutations resulting in AD inheritance are exclusively localized to exon 12 encoding the death domain (Fig. 1f).

Acknowledgements

The authors acknowledge all patients and family members who kindly agreed to participate in this study. The study was funded by a core grant from the Department of Biotechnology, Government of India to the corresponding author's host institution.