

Patch test study of 90 patients with tattoo reactions: Negative outcome of allergy patch test to baseline batteries and culprit inks suggests allergen(s) are generated in the skin through haptenization

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Summary

Background. Tattoo reactions, especially in red tattoos, are often suggested as allergic in nature, however, systematic evaluation by patch testing has not performed in the past.

Objective. To report the results of patch testing in 90 patients with non-infectious chronic tattoo reactions.

Materials/methods. From 2009 to 2013 at the 'Tattoo Clinic', Department of Dermatology, Bispebjerg University Hospital, 90 patients were patch tested with batteries of baseline allergens, disperse dyes/textile allergens, and a selection of tattoo ink stock products, which, according to case observations, were problematic, supplemented with individual culprit inks when accessible.

Results. Patients with reactions to the tattoo colour red, the most predominant colour associated with skin reactions, showed negative patch test results with common allergens. Outcomes were also negative in patients who had experienced concomitant reactions in another hitherto tolerated tattoo of the same colour as the problematic tattoo.

Discussion/conclusion. The allergen or allergens responsible for tattoo reactions are not present directly in tattoo ink stock products. This is despite the fact that clinical histories suggest that the vast majority of clinical reactions, especially reactions to red and red nuances, are likely to be allergic events caused by the injected inks. We suggest that the responsible allergen results from a complicated and slow process of haptenization, which may even include photochemical cleavage of red azo pigment.

Key words: allergy; ink; patch testing; pigment; reaction; tattoo; textile.

The worldwide fashion for tattooing is paralleled by an increasing number of tattoo reactions requiring treatment (1). The reactions have a range of clinical manifestations and possible mechanisms, with infection and suspected allergy being prominent. Chronic reactions

are associated with fiery itching and a reduced quality of life, in line with other chronic dermatoses (2). In a recent review, Kluger and Koljonen found no risk of cutaneous malignancy associated with the pigments of tattoos (3). The dominant problem with chronic tattoo reactions is confined to red inks or nuances of red, for example pink, orange, violet, and Bordeaux (1). Azo chemicals are suspected culprit sensitizers. Reactions in red tattoos typically appear after weeks to years, with involvement of the entire area tattooed with the colour, including any other tattoos on the same individual performed with the same red colour (4). Concomitant reactions in older, red-coloured tattoos performed with different ink brands may be triggered. Therefore, clinical

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observations suggest allergy to red tattoo ink chemical(s) as a common pathomechanism responsible for reactions in these tattoos.

Present-day tattoo ink stock products are commercial products of dubious quality composed of particulate pigments suspended in vehicles containing additives and preservatives (5). Impure materials may be used, and the inks are manufactured without appropriate health-related controls. Inks are sold under numerous brand names across national borders, without any meaningful regulation or control. Tattooed individuals are exposed to unidentified and potentially serious allergens and other agents.

Traditionally, tattoo ink pigments were composed of soot and charcoal containing amorphous carbon and insoluble inorganic salts of bright colour, as exemplified by red mercury salts, yellow cadmium salts, and green and blue chromium salts (6, 7). In recent decades, organic pigments such as red azo pigments and blue and yellow phthalocyanins have replaced inorganic compounds (5). Black is typically carbon black produced from the combustion of mineral oil. Modern pigments used in tattoo inks are nanoparticles and microparticles made in bulk for paints, leather, etc. (8). Disperse azo dyes used in textiles are known to be potential contact allergens (9, 10).

To the best of our knowledge, no systematic study of patch testing of patients with tattoo reactions has been performed since the introduction and widespread use of organic pigments by tattooists. Earlier case reports and small series showed allergy to metals, especially mercury, chromium, and cadmium; these metals are no longer used in tattoo ink products, although they may be occasionally identified as impurities or contaminants (11).

In this study, we report the results of patch testing 90 patients with chronic tattoo reactions. Patients were tested with batteries of baseline allergens, disperse dyes/textile allergens, and tattoo ink stock products, supplemented with individual culprit inks when available.

Materials and Methods

The study participants were patients referred to the 'Tattoo Clinic' of the Department of Dermatology, Bispebjerg University Hospital from 2009 to 2013 with non-infectious, chronic reactions in their tattoos, that is, reactions representing a clinical problem for > 3 months. Reactions consisted of itching, swelling, scaling, hyperkeratosis and dermal inflammation confined to one specific colour. Patients were examined clinically, and 3-mm punch biopsies for histology were obtained to verify chronic inflammation in the elements and exclude specific skin diseases such as psoriasis, lichen planus, and lichen simplex chronicus (neurodermatitis).

Table 1. The European baseline series of allergens purchased from Chemotechnique Diagnostics AB (Vellinge, Sweden) as series S-1000 was supplemented with the following 14 substances

	Test substance (all in pet. except where indicated)	Concentration (%, wt/wt)
1	Methylisothiazolinone (aq.)	0.2
2	2-Bromo-2-nitropropane-1,3-diol	0.25
3	Sorbic acid	2
4	Sorbitan sesquiolate	20
5	Diazolidinyl urea	2
6	Imidazolidinyl urea	2
7	<i>Evernia furfuracea</i> (tree moss absolute)	1
8	Ethylenediamine dihydrochloride	1
9	Phenoxyethanol	1
10	Chlorhexidine digluconate	0.5
11	Hydrocortisone-17-butyrate	1
12	DMDM hydantoin	2
13	Textile mix (6.6%)*	6.6
14	Iodopropynyl butylcarbamate	0.2

*Textile mix included Disperse mix (0.3%), Disperse Blue 106 (1%), Disperse Red (1%), Disperse Blue 35 (1%), Disperse Blue 124 (1%), Disperse Orange 3 (1%), Disperse Yellow 3 (1%), Disperse Blue 126 (0.3%), Disperse Red 17 (1%), and Disperse Orange 1 (1%).

Patch testing with the European baseline series (Chemotechnique Diagnostics AB, Vellinge, Sweden; series S-1000) of allergens was performed with 8-mm Finn Chambers® on Scanpor® tape (Epitest Ltd Oy, Tuusula, Finland) (Table S1). All patch tests were applied for 2 days on the backs of the individuals, and readings were performed on D2 and D3 according to ICDRG criteria. The European baseline series of 28 substances was supplemented with 14 substances (Table 1).

In addition to the above, patients were tested with a series of disperse dyes ('Textile colour and finish TF-1000'; Chemotechnique Diagnostics), developed to diagnose contact allergy to textile dyes (Table S2). The series contains 33 test substances, but Disperse Blue 106/124 was, according to the routine of our clinic, included in the extended European baseline series (see above).

Patients were also tested with an empirical selection of problematic tattoo ink stock products, eight in all, which were selected for this study on the basis of our observations and experience in the 'Tattoo Clinic'. Inks of the brand 'Tattoo', produced in Taiwan (no further details are given on the product label), especially red, had caused major local reactions. In 1 case, there was ulceration, and in another case there was necrotizing lymphadenopathy confirmed by histology, which occurred a few weeks after tattooing, suggesting that allergic sensitization had taken place. Red and brown colours of this brand were banned for the purpose of tattooing from the Danish market by the Danish Ministry of the Environment on 21 December

Table 2. Patients tested by number, sex and age, including number of patients with positive results

Type of test	No. of patients tested	Sex [females (F)/males (M)]	Age (years), mean (range)	No. of patients with positive results (%)
European baseline series	89	62 F/27 M	34 (20–61)	26/89 (29)
Disperse dyes/textile allergens	74	55 F/19 M	34 (20–59)	5/74 (5)
Problematic tattoo ink stock products	79	57 F/22 M	34 (20–59)	9/79 (11)
Individual tattoo ink samples	25	20 F/5 M	33 (21–61)	2/25 (8)

2011, owing to the contents of primary aromatic amines (PAAs), aniline and 3,3'-dichlorbenzidine (found in the red ink brand 'Tattoo') and aniline (found in the brown ink brand 'Tattoo') exceeding the requirements of the 2003/2008 guideline of the European Council (12, 13). The 'Tattoo'-branded colours tested were as follows: red, white, blue, yellow, green, black, and purple. The labels on the brand 'Tattoo' range of products gave no information on the chemical specificity of pigments, no colour index number or CAS number, and no details on product composition, sterility, preservation, or durability. No address or precise origin of manufacturer was provided, except for simply 'Taiwan'. The selection of test inks also included 'True Magenta' (Intenze Products, Rochelle Park, NJ, USA) (product label: CI 73900, isopropanol, witch hazel, water, glycerine, proprietary; article ST2009 TRM, Batch #Q6IMX40), which had also caused a strong and delayed reaction in a single case. Ink stock product was applied as the original and undiluted ink product, tested under Finn Chambers® and read as described above.

Finally, identified culprit ink products were tested as individual samples in those cases when the culprit ink could be identified and provided in an original stock bottle or sample from the tattooist. Customers/patients contacted their tattooists, presenting them with a formal letter prepared by the 'Tattoo Clinic' requesting their help, explaining that culprit inks were required exclusively for diagnostic clarification and for study of their tattoo reactions. Samples of identified inks were supplied either in original stock bottles or as samples in laboratory containers provided by our laboratory. The request also explained the explicit need for a precise product name and colour (product label and colour index number). Nevertheless, in most cases, test materials were received without precise information. However, in this study we only included inks that we deemed to be the reliable culprits. Tattooists were generally helpful.

A subgroup of patients who had experienced concomitant reactions in another hitherto tolerated tattoo of the same colour as the problem tattoo are reported and included in the results. Patients with concomitant reactions are index cases of special interest, as the reaction in

a distant anatomical site can only be explained through an immune mechanism. Thus, the outcomes of patch tests in these patients are particularly important for the clarification of the nature of the allergen(s).

Results

In total, 90 patients [63 females and 27 males; mean age 34 years (range 20–61 years)] participated in the study. There were a total of 94 tattoo reactions, with 48 located on the upper extremities, 31 on the lower extremities, 13 on the trunk, and 2 on the head/face. Fifty-six patients experienced reactions to red, 18 to black, 16 to purple, 6 to blue, 3 to green, 3 to pink, 2 to white, 2 to brown, 2 to yellow, and 1 to grey. Three reactions were described as multicoloured. Nineteen patients had reactions to more than one colour. Reactions appeared between 2 weeks and 5 years after tattooing.

According to patient histories, 21 patients had known nickel or metal allergy, 2 were allergic to cobalt, and 1 was allergic to chromium, as documented by their dermatologists. Seven patients reported a past history of atopic dermatitis, 2 of psoriasis, 1 of hand dermatitis, and 2 of rash not diagnosed. No patient had active skin disease at the time of patch testing apart from their tattoo reaction.

Patients tested with the different test series by number, sex and age, including numbers of patients with positive results, are presented in Table 2.

Patch testing with the European baseline series of allergens

Of the 90 patients tested in this study, 89 were tested with the baseline series; the last patient was only tested with an empirical selection of problematic tattoo ink stock products.

The results from 89 patients (62 females and 27 males) tested with this series showed 26 (29%) positive test results (22 females and 4 males) and 59 (66%) with negative outcomes of the test. The results are shown in Table 3.

There were no reactions to *p*-phenylenediamine (PPD). Doubtful reactions were noted in single cases tested with nickel sulfate, epoxy resin, and *Myroxylon pereirae*. Five patients showed irritant reactions to cobalt chloride.

Table 3. Results of patch testing of 89 patients suffering from tattoo reactions, tested with 42 substances included in the European baseline series of allergens; in total, 3738 test applications were performed[†]

	D2					D3					D7					No.*
	1+	2+	3+	IR	?	1+	2+	3+	IR	?	1+	2+	3+	IR	?	
Nickel sulfate	–	1	4	–	–	2	5	9	–	1	3	6	2	–	–	19
Cobalt chloride	–	1	1	2	–	3	1	–	4	–	2	–	–	–	–	5
Fragrance mix I	–	–	–	–	–	2	1	–	–	–	–	1	–	–	–	3
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	2
Textile dye mix	–	–	–	–	–	2	–	–	–	–	–	1	–	–	–	2
Fragrance mix II	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1
Epoxy resin	–	–	–	–	–	–	1	–	–	1	–	–	–	–	1	1
Butylphenol formaldehyde resin	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1
Mercaptobenzothiazole	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1
Imidazolidinyl urea	–	–	–	–	–	–	1	–	–	–	–	1	–	–	–	1
Colophonium	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
<i>Myroxylon pereirae</i>	–	–	–	–	1	–	1	–	–	–	–	–	–	–	–	1
Methyldibromo glutaronitrile	–	–	–	–	–	–	–	1	–	–	–	1	–	–	–	1
Ethylenediamine dihydrochloride	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1

IR, irritant reaction.

*No. indicates number of patients with one or more reactions. IRs and doubtful reactions are not included in the number.

[†]Test substances giving an entirely negative outcome in patch testing are not included in this table; see complete listing of substances in Materials and Methods.**Table 4.** Results of patch testing of 74 patients suffering from tattoo reactions, tested with 32 substances included in a series of disperse dyes/textile allergens; in total, 2368 test applications were performed[†]

	D2					D3					D7					No.*
	1+	2+	3+	IR	?	1+	2+	3+	IR	?	1+	2+	3+	IR	?	
Disperse Yellow 3	1	–	–	–	–	2	–	–	–	–	1	–	–	–	–	2
Disperse Blue 85	–	–	–	–	–	1	–	–	–	1	–	–	–	–	–	1
Disperse Red 17	1	–	–	–	1	–	–	–	–	–	–	–	–	–	–	1
Urea formaldehyde	–	–	–	–	–	–	1	–	–	–	–	1	–	–	–	1
Disperse Orange 1	–	–	–	–	1	–	–	–	–	1	–	–	–	–	–	0
Basic Red 46	–	–	–	–	–	–	–	–	4	–	–	–	–	1	–	0
Reactive Red 123	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	0
Reactive Red 238	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	0
Reactive Red 228	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	0
Disperse Red 1	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	0

IR, irritant reaction.

*No. indicates number of patients with one or more reactions. IRs and doubtful reactions are not included in the number.

[†]Test substances giving an entirely negative outcome in patch testing are not included in this table; see complete listing of substances in Materials and Methods.

Patch testing with a series of disperse dyes/textile allergens

The results from 74 patients (55 females and 19 males) tested with this series showed 5 female patients (7%) with positive test results. Negative results were seen in 64 patients (87%) (Table 4).

Two patients reacted to Disperse Yellow 3, 1 to Disperse Blue 85, 1 to Disperse Red 17, and 1 to urea formaldehyde. Single patients had doubtful reactions to Disperse Blue 85, Disperse Red 17, Disperse Red 1, and Disperse Orange 1.

Four patients were deemed to have irritant reactions to Basic Red 46, and a single patient showed irritant reactions to Reactive Red 123, Reactive Red 238, and Reactive Red 228.

Patch testing with an empirical selection of problematic tattoo ink stock products

The results from 79 patients (57 females and 22 males) tested with this series showed 9 patients (11%) with positive test results. The remaining 69 patients had blank negative reactions (87%).

Table 5. Results of patch testing of 79 patients suffering from tattoo reactions, tested with eight substances included in an empirical selection of problematic tattoo ink stock products; brand name 'Tattoo' (produced in Taiwan, manufacturer not specified)[†]

	D2					D3					D7					No.*
	1+	2+	3+	IR	?	1+	2+	3+	IR	?	1+	2+	3+	IR	?	
Tattoo Red	–	2	–	1	–	1	7	–	–	–	1	3	–	–	–	9
Tattoo Purple	–	–	–	–	–	–	3	–	–	–	–	1	–	–	–	3
Tattoo Green	–	–	–	–	–	–	2	–	–	–	–	1	–	–	–	2
Tattoo Black	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1
Tattoo White	–	–	–	–	–	–	1	–	–	–	–	1	–	–	–	1
Tattoo Yellow	–	–	–	–	–	1	–	–	–	–	1	–	–	–	–	1

^{IR}irritant reaction.

*No. indicates number of patients with one or more reactions. IRs and doubtful reactions are not included in the number.

[†]Test substances giving an entirely negative outcome in patch testing are not included in this table; see complete listing of substances in Materials and Methods.

Table 6. Eight cases with concomitant reactions occurring in a previously tolerated tattoo triggered by a recent tattoo of the same colour; =case information, according to history

Sex	Age (years)	Problem colour	Location of trigger tattoo	Location of prior tattoo	Elicitation period between trigger and cross-reaction
Male	36	Red	Trunk	Leg	9 months
Female	42	Purple	Leg	Arm	2 weeks
Female	61	Purple	Trunk	Arm and leg	8 months
Female	28	Purple	Arm	Arm	2–4 weeks
Female	44	Blue	Leg	Arm	2 months
Male	46	Red	Arm	Leg	6 months
Female	53	Red	Arm	Leg	1–2 weeks
Female	29	Purple	Arm	Arm	2 weeks

Positive readings were as follows: 9 patients reacted to Tattoo Red, 3 to Tattoo Purple, 2 to Tattoo Green, and single patients to Tattoo Black, Tattoo White, and Tattoo Yellow (Table 5). Of 9 patients with positive test results, 2 reacted to four of the tested colours, which may indicate that reactions were elicited not by the pigment alone, but possibly by ingredients of the products as well. No participant reacted to the True Magenta sample.

Only 1 patient had an irritant reaction to Tattoo Red, and no doubtful reactions were observed.

Patch testing with identified or culprit ink products tested as individual samples

Twenty-five patients (20 females and 5 males) were tested with their respective culprit inks obtained from their tattooists; 2 patients (8%) reacted, and 23 (92%) did not. One patient with problems in a red tattoo reacted to red (2+) on the third day, and likewise to purple (2+), and 1 patient with problems in a purple tattoo reacted to purple (1+) on the second day. One patient had full-thickness inflammation of the dermis, necrosis, and ulceration. Another patient initially had ulceration, which was followed by lichenoid

reaction with perivascular inflammation of the outer dermis.

Observations in patients who had experienced concomitant reactions in another hitherto tolerated tattoo of the same colour as the problematic tattoo

From our test group of 90 patients, 8 patients (9%) (6 females and 2 males) experienced concomitant reactions in a previous tattoo performed independently of the trigger tattoo and possibly with another tattoo ink. The prior tattoo was acquired from 2 to 6 years before the trigger tattoo. The colours red, purple and blue were exclusively associated with these distal flare-up reactions, which appeared after an interval of 1–2 weeks to 9 months. Purple ink may be a mix of red and blue pigments, thus, these two colours are implicated as inducers of concomitant reactions (Table 6).

Patch testing showed individual positive reactions to fragrance mix I, hydroxyisohexyl 3-cyclohexene carboxaldehyde, nickel (in 2 cases), and cobalt. The textile dye series tested in 4 cases gave negative results, except for 1 case, which showed irritant reactions to Basic Red 46, Reactive Red 123, Reactive Red 238, and Reactive



Fig. 1. (a–d) Clinical reactions exclusively in red areas of tattoos. Reactions were suggested to be allergic according to history and objective findings. Reactions have a range of manifestations. (a) Ulcerating reaction with necrosis. The tattoo depicted a red heart. (b) Ulcerating reaction with crusting. The tattoo depicted a red 'Superman' logo. (c) Inflammatory reaction with major swelling and elevation. The tattoo depicted a red heart. (d) Inflammatory and hyperkeratotic reaction. The underlying skin may erode. The tattoo depicted the emblem of 'Football Club Barcelona'. Clinical photos: the Tattoo Clinic of the Department of Dermatology Bispebjerg University Hospital, Copenhagen, Denmark, with permission.

Red 228. The tattoo series were performed in 5 cases, and 1 case tested with the culprit ink proved negative.

Discussion

The general outcome of the study showed that patients with clinically significant reactions in their tattoos

presented mainly negative or inconsistent results when patch tested with common allergens, textile dyes, problematic tattoo ink stock products, and even with their individual culprit inks when available for testing. Even patients with concomitant reactions, considered to be convincingly allergic in nature, failed to react. Clearly,

the putative allergen is not present in the tattoo ink stock product, despite the fact that the vast majority of clinical reactions, especially reactions to red and red nuances, behave as allergic reactions (Fig. 1a–d).

With the European baseline series of allergens, 21% patients were patch test-positive, but 23% gave information on verified nickel allergy or reactions to metal prior to tattooing and patch testing. Thus, they appeared not to have been sensitized by their tattoos. Positive nickel patch test reactions in routine patients in our clinic were found in 320 of 2099 (15%) of tested patients between 2010 and 2013. In a European surveillance report, comprising 17 centres in nine European countries, positive reactions to nickel sulfate were reported in 17.3%, with large variations among centres (14). Tattooed persons might be inclined to use piercings containing metal, and thereby be more frequently sensitized to nickel.

All 65 tattoo ink stock products analysed in a recent Danish study contained nickel as a trace element or in higher amounts (11). Therefore, nickel allergy could play a role in already sensitized individuals, and cause elicitation reactions in new tattoos during the first days after tattooing, but with the allergic reaction being obscured by the healing process or misinterpreted as infection. This may occur until nickel is washed out of the skin (15). We identified cases with strongly positive patch test reactions to nickel and a history of severe reactions to tattoos diagnosed as infections. However, allergy to nickel is unlikely to explain chronic tattoo reactions, which commonly develop after a period of months or years. Individuals considering a tattoo should be warned about a possible initial allergic reaction in their newly acquired tattoo if their history or patch test results show allergy.

It is noteworthy that none of our patients had contact allergy to PPD, which is known to be a potent sensitizer. PPD is a PAA that might be present in tattooed skin as a metabolite of azo pigments. Textile allergy studies have suggested that PPD allergy might be associated with reactions to disperse azo dyes, but no statistical significance was found (16). Other PAAs, consisting of aniline, *o*-anisidine, and 3,3-dichlorobenzidine, have been discussed as representing metabolites of azo pigments and eliciting allergy to tattoo inks (13). However, a recent study of these amines by Raman spectroscopy of shave biopsies from tattoo reactions to red pigment failed to confirm their presence in red tattoo reactions (17). Therefore, this study using Raman spectroscopy and patch tests with PPD do not support the 'metabolism theory', which claims that amines produced from the breakdown of azo chemicals are the relevant allergens in tattoo reactions. In textile allergy, the metabolism theory

failed to be confirmed by patch testing with relevant and specific PAAs (10, 18).

With the association between red tattoos and the allergic reactions observed, it should be possible to deduce the allergen from the structure of red pigments, degradation products or contaminants that are found only in red pigments.

Light is known to induce photochemical cleavage of tattoo pigments, in particular azo pigments, after exposure to natural sunlight, ultraviolet light, and laser light *in vitro*, with formation of hazardous chemicals (19–21). The study using Raman spectroscopy indicated that such cleavage might occur in the laboratory even after only 1 day of exposure to natural daylight (17). Clinical studies have indicated that photosensitivity is common in tattoos (22, 23), so it is possible that tattoo pigment *in vivo* absorbs light to a degree that may produce chemical cleavage products. Light exposure may be more important for pigment breakdown *in situ* than enzymatic local metabolism in the dermis. Red-pigmented tattoos absorb wavelengths in the blue and ultraviolet end of the spectrum, which are known to be more potent in eliciting photochemical reactions. Thus, it may be speculated that, in the skin, light produces cleavage of chemicals that form allergens. Future studies might include photo-patch testing and testing with materials pretreated with ultraviolet light.

Disperse dyes are more soluble than tattoo pigments. Allergy to textile disperse dyes has been investigated by Swedish researchers (9, 10). Patients with reactions to red tattoos and azo chemicals would be expected to react or show cross-reactions with textile dyes, which they fail to do. Tattoo pigments are nanoparticles and microparticles, which are poorly soluble in lipophilic media and water (8). In the skin, they become embedded in the connective tissue of the dermis, making them not easily bioavailable. It is unknown whether the tattoo ink stock products used for testing in our study had any potential for skin penetration, although they were tested undiluted and under occlusion to enhance penetration. Testing tattoo inks with needle prick introduction or intradermal needle injection might be considered, but this approach is not realistic, as it would leave a permanent tattoo on the individual. We are aware of experienced tattooists who have performed small 'test tattoos' to predict allergy, but the results cannot be relied on, as the time between tattooing and allergic sensitization and response can be months or years. Testing an individual who already has an allergic reaction in a red tattoo with the problematic ink might also have an unreliable outcome, owing to the complexity of the haptenization process, which may take a long time.

Testing with the empirical selection of problematic tattoo ink stock products in 79 individuals showed positive reactions to red ink in 9 cases, and 1 irritant reaction to red. Three patients reacting to purple, which was possibly a mix of red and blue pigment, also reacted to red but not to blue. There were sporadic reactions to green, yellow, white, and black. Thus, patch testing with red tattoo ink may, in selected cases, have indicated allergy.

Tattoo ink pigments of the azo type are crude industrial products of low purity, making it difficult to identify the precise chemical constituent that is responsible for allergic sensitization (24–28).

This study indicates that the putative allergen(s) causing tattoo reactions are formed inside the dermis through a process of haptenization, which can be slow and develop after weeks, months, or years. It is unlikely that simple chemical cleavage products such as PAAs, which are often found in free form in inks, contribute to sensitization. PPD is excluded as playing any role. Allergen formation may originate from the many constituents of the ink or their interactions, including chemical or metabolic interactions. Allergy to tattoos is more complicated than the presence of primary, secondary and tertiary amines found in free form in tattoo ink stock bottles can explain. This accords with the view that

allergic sensitization often occurs through a complicated process involving pre-haptens and pro-haptens, and through haptenization involving host proteins (28–32). External factors, especially light producing photochemical cleavage of tattoo pigments *in situ* in the skin, may contribute to allergen formation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Patch testing with a European baseline series of allergens was performed. All test substances were purchased from Chemotechnique Diagnostics AB (Vellinge, Sweden) as series S-1000 and applied without further dilution.

Table S2. Patch testing with a baseline series of disperse dyes, the 'Textile colour and finish TF-1000' of Chemotechnique Diagnostics AB.

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