



ORIGINAL ARTICLE

The expression pattern of N-acetyltransferase 1 in healthy human skin

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Abstract

Background: N-acetyltransferase 1 (NAT1) is an enzyme expressed among others in keratinocytes in human skin. NAT1 is important in the biotransformation of aromatic amines, an important example being *p*-phenylenediamine (PPD), a hair dye molecule. Unoxidized PPD penetrates the skin and is N-acetylated by NAT1.

Objectives: To investigate in detail the expression pattern of NAT1 in human skin.

Materials and Methods: Cryosections obtained from healthy human skin were stained for NAT1 and expression patterns were observed. NAT1 double stainings were performed with antibodies against different cellular organelles to determine expression patterns.

Result: A speckled, granular expression of NAT1 was seen predominantly in the stratum basale. NAT1 was expressed in a cytoplasmic pattern, perinuclear, and in the nucleus. No co-localisation was seen with the selected cellular organelles. Local differences in NAT1 expression patterns were observed between donors and between different biopsies obtained from the same donor.

Conclusions: NAT1 is expressed predominantly in the stratum basale and can be found in the cytoplasm, nucleus, and perinuclear in human skin. Further studies should be performed to investigate expression of NAT1 in a larger sample size.

KEYWORDS

contact allergy, human skin, immunofluorescence, N-acetyltransferase 1, P-phenylenediamine

1 | INTRODUCTION

Arylamine N-acetyltransferases (NATs) are a group of enzymes involved in the biotransformation of aromatic amines. NAT is widely expressed among various species of animals and bacteria.¹ In humans, two isoforms of NATs are recognized, NAT1 and NAT2, encoded by the *NAT1* and *NAT2* genes. Studies have found that NAT1 is expressed in all investigated fetal² and adult tissues,³⁻⁸ among others, in keratinocytes in human skin.⁹ NAT2 is predominantly expressed in

the liver and gastrointestinal tract.¹⁰ N-acetylation of aromatic amines in the skin is an important metabolic process that changes the chemical to immunologically inactive metabolites. A well-known example of an aromatic amine is the hair dye component *p*-phenylenediamine (PPD, CAS no. 106-50-3). Its low molecular weight and strong protein-binding potential make PPD a strong sensitizer, with the potency to cause severe contact allergic reactions.¹¹ In the hair dying process, most of the PPD oxidizes by air oxygen or via auto-oxidation into its oxidation products, including *p*-benzoquinonediimine,

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TABLE 1 Overview of the six donors, of which two donors (nos. 5 and 6) provided two biopsies

Donor no.	Sex (F/M)	Age (years)	Biopsy	Location biopsy
1	F	54	a	Wrist
2	M	42	b	Axilla
3	M	41	c	Glutes
4	F	17	d	Mamma
5 ^a	F	34	e	Axilla
		35	f	Axilla
6 ^b	F	54	g	Mamma
			h	Mamma

^aBiopsies were obtained 1 year apart.

^bBiopsies were obtained on the same day.

benzoquinone, or Bandrowski's base.¹² The majority of unoxidized PPD that penetrates the skin is N-acetylated by NAT1 into mono-acetyl PPD (MAPPD) and di-acetyl PPD (DAPPD).^{9,13} There is one study in which two highly PPD sensitive cases were reported who showed weak positive reactions to MAPPD and DAPPD.¹⁴ This is rarely seen since these acetylated products are considered as non-sensitizers in local lymph node assay.¹² N-acetylation of PPD can, therefore, be considered as an important step for detoxification of this agent.

Until now, NAT1 has been mainly studied in the human reconstructed epidermis, human primary keratinocytes, HaCaT cells (immortalized human keratinocyte cell line), and neonatal human skin.^{9,15} The aim of this study is to investigate NAT1 expression in healthy human skin.

2 | METHODS AND MATERIALS

2.1 | Skin tissue

Eight skin biopsies of 4 mm were obtained from six healthy human volunteers (four females, six males, age 17–54 years), snap frozen in liquid nitrogen, and stored at -80°C until use. Skin biopsies were obtained from different body locations (Table 1). For immunofluorescence, microscopy cryosections of healthy skin biopsies of 4 μm thickness were mounted on poly-L-lisine coated glass slides and air-dried for 30 minutes under a cold fan. All volunteers gave informed consent to the donation of their skin. The study was performed in accordance with the Declaration of Helsinki and received approval from the ethics committee (University Medical Centre Groningen).

2.2 | Immunofluorescence analysis

After drying, cryosections were circled with a hydrophobic pen (Dako, Glostrup, Denmark), blocked with 1% ovalbumin (Serva, Germany) and incubated with a monoclonal rabbit antibody against

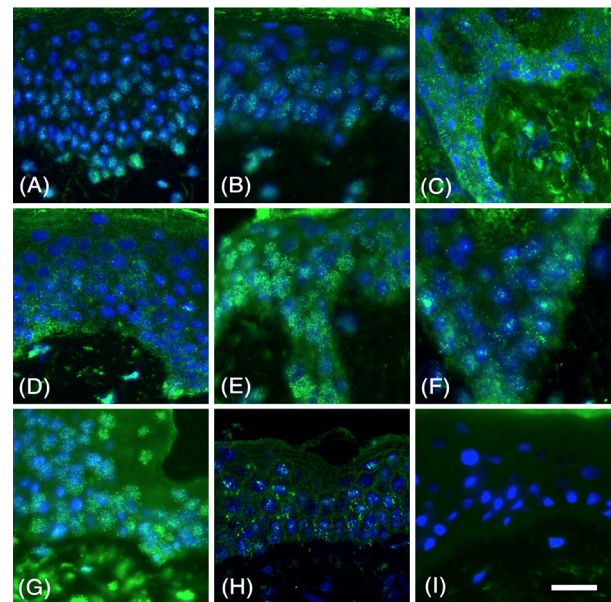


FIGURE 1 N-acetyltransferase 1 is expressed in different epidermal layers in healthy human skin. (A–E, G) Skin biopsies of six different donors were included (see Table 1). Two donors provided two biopsies each: donor 5 (E, F) and donor 6 (G, H). (A–G) Biopsies were incubated with monoclonal rabbit antibody EPR3221 (2) (green). (H) Biopsy included a control staining incubated with polyclonal rabbit antibody ES-195 (green). (I) Tissue section presents a negative control (omission of first antibody) from biopsy (G). In all skin tissues, a speckled/granular expression located primarily in the stratum basale and, to a lesser extent, in the upper epidermis were observed. In all skin biopsies expression was found in the nuclei (blue). The levels of expression in the cytoplasm varied between donors. White bar is 25 μm

human N-acetyltransferase 1 (1:100, EPR3221 [2]), Genetex; Irvine, CA, USA). This was followed by fluorescein isothiocyanate-labeled donkey anti-rabbit IgG (Invitrogen; Bleiswijk, the Netherlands). A control staining was added with a second NAT1 antibody; a polyclonal rabbit anti-human NAT1 (1:100, NAT1 antibody ES-195, a gift from Dr. Sim, Kingston University, London).¹⁶ A negative control staining (Rabbit anti-Human collagen 14, COL14, MyBioSource, San Diego, CA, USA) and in addition omission of the first antibody was performed. For accessing organellar co-localization, double staining was performed with the EPR3221 (2) or ES-195 together with Mouse antibodies against early endosomes (Clone 14/EEA1, BD transduction laboratories, San Jose, CA, USA), lysosomes (H4A3, BioLegend, San Diego, CA, USA), the Golgi apparatus (Golgin97, Invitrogen, Carlsbad, CA, USA) and mitochondria (MTC02, Abcam, Cambridge, UK). In addition, double staining with keratinocyte membrane marker desmocollin 3 (U114, Progen, Heidelberg, Germany) or desmoglein 1 (P23, Progen, Heidelberg, Germany) was performed to investigate the cytosolic location of NAT1. Depending on the antibody employed, secondary antibodies used were chicken anti-Rabbit IgG conjugated with Alexa 488 and Alexa 568 labeled Goat-anti-Mouse IgG (Thermo Fischer, Waltham, MA, USA). Nuclei were made visible with Hoechst 33342. The mounting medium was SlowFade Gold (Thermo

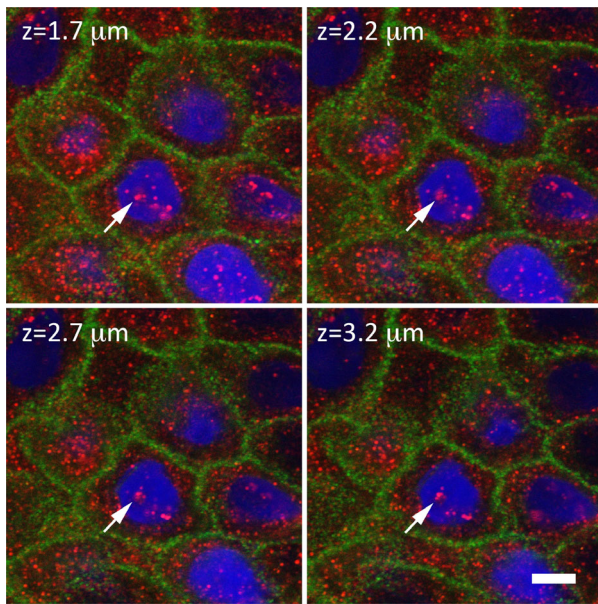


FIGURE 2 N-acetyltransferase 1 (NAT1) is expressed in the cytoplasm. Double staining for NAT1 (red) and desmoglein 1 (green). Tissue sections were taken from biopsy E (see Table 1). Single focal plane scans at the height of z-stack (μm) from the glass slide shows NAT1 is present in the cytosol as it does not localize with membrane bound desmoglein 1. NAT1 is present perinuclear and nuclear (arrows) as well. White bar is $5 \mu\text{m}$

Fischer, Waltham, CA, USA) or for confocal imaging Prolong Diamond Antifade (ThermoFisher, Waltham, MA, USA).

Analysis of the sections were performed using a Leica DMRA fluorescence microscope and images were acquired by Leica DFC350 FX digital camera (Leica, Wetzlar Germany) and recorded using Leica Application Software (Leica). The double stainings were imaged with the Leica SP8X confocal microscope using a white light laser. Lifetime gating was used to eradicate the auto fluorescence background signal.

3 | RESULTS

3.1 | NAT1 expression pattern

NAT1 expression in healthy human skin is shown in Figure 1. Stained cryosections with EPR3221 (2) showed a clear granular or speckled expression pattern, predominantly in the stratum basale and, to a lesser extent, in the upper epidermis. In all skin biopsies, expression was found in the nuclei. The levels of expression in the cytoplasm varied between donors. A control staining with a second NAT1 antibody, ES-195 (Figure 1H), showed a more granular expression pattern, predominantly in the stratum basale as well. The pattern was specific, as the negative control (omission of first antibody) did not show any specific staining pattern (Figure 1I).

Figure 2 shows the results from double staining for NAT1 with the keratinocyte membrane marker, desmoglein 1, to investigate the

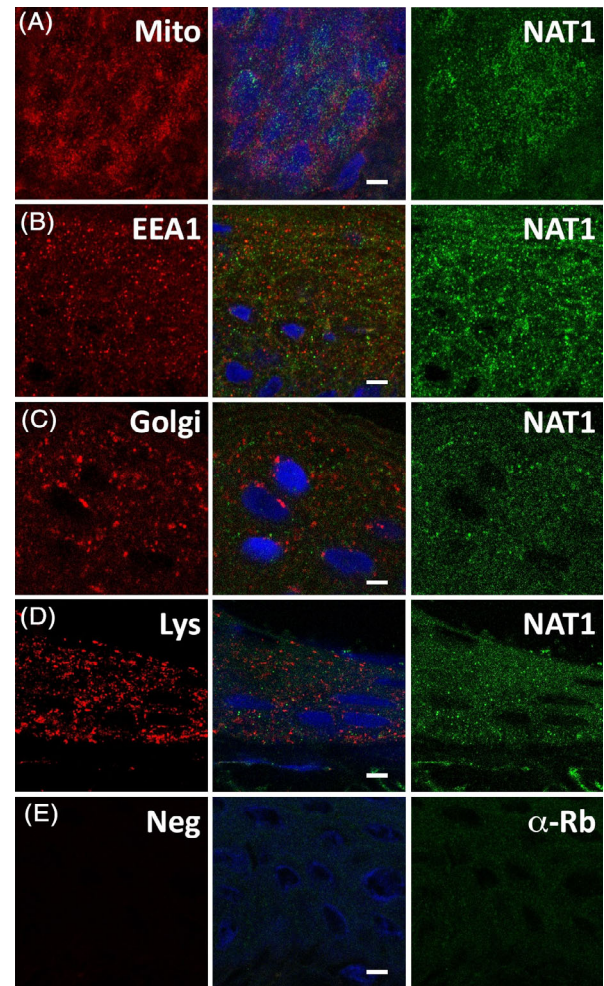


FIGURE 3 No co-localization of N-acetyltransferase (NAT1) and different organelle proteins. (A) mitochondria (Mito; MTCO2) are visible both perinuclear as well as free in the cytoplasm. (B) endosomes (EEA1; clone 14), (C) golgi apparatus (Golgin97) and (D) lysosomes (Lys; H4A3) have a cytoplasmic speckled pattern while (E) negative controls show no fluorescence in the presence of a non-sense 1st Ab (COL14). NAT1 (green, EPR3221 [2]) does not co-localize with either of these four organelle proteins (red, A-D). White bar = $0.5 \mu\text{m}$

cytosolic location of NAT1. High resolution, confocal sequential Z-stack images clearly show that NAT1 is present free in the cytoplasm, and it does not co-localize with the membrane bound protein. In addition, NAT1 is clearly present perinuclear and in the nucleus as well. Another keratinocyte membrane marker, desmocollin 3, showed the same staining pattern (data not shown).

3.2 | Double staining with selected organelles

On the primary single staining in Figure 1, a granular or speckled NAT1 expression pattern was seen, which could also be interpreted as vesicular. The observed expression pattern was suggestive of possible co-localization and, therefore, double staining for different cellular

organelles were performed (Figure 3). The expression of the different organelles are seen both perinuclear and throughout the cytoplasm for mitochondria (Mito), in a cytoplasmic speckled pattern for early endosomes (EEA1) and lysosomes (Lys). For golgi apparatus (Golgi), expression is predominantly observed perinuclear. No obvious co-localization of NAT1 with any of these organelles was seen. The negative control (replacing the anti-NAT1 antibody with a non-epidermal Rabbit antibody) did not show any specific staining pattern (Figure 3E).

4 | DISCUSSION

In the current study we investigated the expression pattern of NAT1 in healthy human skin. We demonstrated that NAT1 was expressed in all six donors, predominantly in the stratum basale and, to a lesser extent, in the upper epidermis.

N-acetylation is an important pathway in the metabolism of PPD. In the hair dyeing process, the majority of unoxidized PPD that penetrates the skin will be converted by NAT1 into its detoxification products MAPPD and DAPPD.^{17,18} Pot et al investigated real-time detection of PPD penetration into human skin in two donors in vivo using raman spectroscopy.¹⁹ PPD penetration into the skin was fast and was detected in a high concentration in the stratum corneum and reached up to the viable epidermis in decreasing concentrations. The detection of PPD up to the viable epidermis supports our outcomes of the localization of NAT1 expression, which was predominantly in the stratum basale, where it is present to N-acetylate the PPD. Pot et al, however, did not detect MAPPD and DAPPD in the viable epidermis. A possible explanation why these PPD derivatives could not be detected is that the formations did not occur in concentrations above the limit of detection of the experimental design. A study by Lichter et al investigated the presence and localization of NAT1 in human reconstructed epidermis and in one tissue section from neonatal skin. NAT1 was expressed throughout the whole epidermis, without indicating a difference between layers and without a specific pattern.¹⁵ In addition, active PPD acetylation in normal human primary keratinocytes (NHEK) in four donors was seen in early, intermediate, and late states of keratinocyte differentiation. The same author reported a high NAT1 activity, particularly in proliferating cells in HaCat cells and primary keratinocytes obtained from single donor cells.²⁰ Although the methods of detection of NAT1 expression and activity from the above mentioned studies differ from the current study, the reported results partly support our outcomes, as we found NAT1 expression predominantly in the stratum basale.

In all skin biopsies expression was found in the nuclei. The levels of expression in the cytoplasm varied between donors. The observed variation in expression pattern was also observed between different biopsies obtained from the same donor. We could not find a clear explanation for this, since the applied methods for staining was equal for all tissue sections. In donor no. 5, sampling of the skin biopsies occurred on separate days. It is known that NATs are unstable and are subject to a high turnover. This can be explained by their regulation with regard to their

function – at least this applies to the circadian control of serotonin-N-acetyltransferase.²¹ The high turnover might explain the differences in expression pattern between the two skin biopsies from donor no. 5. In addition, although all skin biopsies were taken from healthy human skin and sampling procedures were equal for all biopsies, the biopsies were taken from different body locations. Future research is necessary to study whether this could affect the expression pattern. In order to confirm our results an additional control staining with another Rabbit anti-Human antibody (ES-195) was performed and showed NAT1 expression as well. NAT1 expression patterns were similar between staining with EPR3221 (2) and ES-195, allowing marginal differences in expression pattern. Since EPR3221 (2) is a monoclonal rabbit antibody and ES-195 a polyclonal rabbit antibody this could possibly contribute to the difference. In addition, ES-195 was originally made and used to visualize NAT2 expression during normal murine embryonic development.²² In a study validating NAT1 and NAT2 specific antibodies using the In-Cell Western method, ES-195 was two times more specific towards NAT1 than NAT2.²³

Several studies have shown that NAT1 activity is detectable in the cytosol in different tissues in humans and animals.^{9,16,24,25} In this study, we reconfirmed that NAT1 can indeed be considered as a cytosolic enzyme, as we showed NAT1 to be expressed in human skin cytosol. Kawakubo et al investigated the N-acetylation capacities of PPD for MAPPD and DAPPD in human cytosol and compared these findings in cultured human epidermal keratinocytes. PPD N-acetylation activity in human cytosol was slightly lower than n-acetylation activity in cultured keratinocytes. Their presented data suggests that interindividual variation can be observed in human skin and cultured keratinocytes and suggest that a larger study is necessary to confirm these findings.⁹ In the current study, NAT1 expression was not only seen in the cytosol, but also perinuclear and in the nucleus itself. In the context of the results from Kawakubo et al., it could be hypothesized that N-acetylation capacity not only occurs in the cytosol, but can be found in other organelles as well. This is in line with earlier findings where NAT1 expression was found in muscular cytosol and nuclei,²⁶ and is in agreement with Lichter et al who suggested that cellular changes may influence the course of substrate availability and conversion.¹⁵ In addition, Butcher et al concluded that NAT1 is subject to substrate-dependent down-regulation that is due to a loss of NAT1 protein. They suggest that further investigations are necessary to gain insight into the role of NAT1 in both xenobiotic activation and cellular metabolism.^{20,27} We found no previous studies in the literature investigating NAT1 co-localization with organelles in human skin. In the current study, we found a granular or speckled NAT1 expression pattern on the primary single staining, therefore, double stainings with different organelles were performed to study the possible presence of co-localization. No co-localization of NAT1 with early endosomes, lysosomes, mitochondria, and the trans-Golgi network was seen, indicating that NAT1 is not functionally present in any of these organelles. The absence of co-localization with early endosomes, lysosomes or, the trans-Golgi network could indicate that NAT1 is

possibly present in other organelles, (e.g. intracellular storage vesicles, or shedding vesicles). The absence of co-localization with mitochondria was an interesting observation, considering acetyltransferases are assumed to have important functions in mitochondria, as NAT1 is involved in the folate catabolism and mitochondria contain as much as 40% of the cellular folate.²⁸

5 | LIMITATIONS

The interindividual variations and variations in the same donor of NAT1 expression are not completely clear and could be explained by small-study effects. Larger studies including a higher number of biopsies obtained from the same donor, from same donor site, and from multiple donors are needed.

6 | CONCLUSION

NAT1 expression in healthy human skin is seen predominantly in the stratum basale and can be found in the cytoplasm, in the nucleus, and perinuclear. No co-localization with mitochondria, early endosomes, trans-Golgi network, or lysosomes was found. Further research should focus on the expression patterns of NAT1 in a larger sample size and should explore the possibility of a relation between differences in NAT1 expression patterns and NAT1 genotype, to gain a better understanding of NAT1 detoxification capacities in the development of contact allergy.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Cynthia van Amerongen: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing-original draft; writing-review & editing. **Duco Kramer:** Data curation; formal analysis; investigation; methodology; project administration; software; supervision; visualization; writing-review & editing. **Hendri Pas:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; supervision; visualization; writing-review & editing. **Marie Schuttelaar:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing-original draft; writing-review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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