

Gene expression changes in peripheral blood mononuclear cells in occupational exposure to nickel

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Abstract: Allergic contact dermatitis is preceded by a clinically silent phase of sensitisation. In this study, we investigated whether the expression levels of six genes were related to nickel exposure and/or nickel sensitisation, and whether they could predict allergic manifestations to nickel. The mRNA expression level of six genes involved in cell growth (PIM1 and ETS2), metabolism/synthesis (HSD11B1 and PRDX4), apoptosis (CASP8) and signal transduction (CISH) was investigated by means of quantitative real-time RT-PCR in a cohort of 110 subjects, including healthy controls ($n = 51$), nickel-exposed workers

($n = 23$) and patients allergic to nickel ($n = 36$). Our findings show that the expression levels of the analysed genes did not differ between allergic patients and healthy controls, while higher expression levels of ETS2 and CASP8 were detected in the nickel-exposed workers. Changes in ETS2 and CASP8 expression are likely to be related to nickel exposure rather than to allergy.

Key words: CASP8 – ETS2 – exposure allergy – nickel – real-time RT-PCR

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Background

Contact dermatitis is the most important cause of occupational skin disease (1). Among chemical agents, nickel is the most frequent cause of allergic contact dermatitis (ACD) in Europe and North America (1). Contact sensitisations to nickel, cobalt and chromate are influenced by age, gender, geographical and historical factors (2,3).

Recently, several studies have been carried out to understand the molecular mechanisms underlying ACD (4–7). These studies have identified genes differently expressed for chemical-induced skin sensitisation in skin (5) and peripheral blood mononuclear cells (PBMC) (4).

Question addressed

By using quantitative real-time RT-PCR, we investigated the messenger RNA (mRNA) expression level of six genes involved in cell growth/maintenance (PIM1 and ETS2), metabolism/synthesis (HSD11B1 and PRDX4), apoptosis (CASP8) and cell communication/signal transduction (CISH) in PBMCs, with no previous *in vitro* exposure to contact allergens.

The aim of this study was to investigate whether the gene expression profiles of the aforementioned molecular markers are related to nickel exposure and/or nickel sensitisation and whether they could predict allergic manifestations to nickel.

Experimental design

A total of 110 blood samples from 51 healthy volunteers with no history of contact dermatitis, 36 patients allergic to nickel and 23 workers daily exposed to nickel compounds were investigated in this study. Eligibility criteria for the allergic patients were positivity to patch test to nickel sulphate and history of cutaneous symptoms after contact (See Supporting information for detail).

Samples of peripheral blood were obtained from each participant and were processed to isolate RNA from PBMCs (Supporting information). Complementary DNA was submitted to quantitative real-time PCR for CISH, CASP8, ETS2, PRDX4, PIM1, HSD11B1 and GAPDH as described in the Supporting information.

Results

The cohort of 110 subjects of this case study included 28 men and 82 women (Table 1). There was no significant difference in age among groups, ($P = 0.13$). Women were prevalent in both the groups of allergics (92%) and nickel-exposed workers (65%) ($P = 0.016$) (Supporting information).

A significant difference between groups was detected for ETS2 ($P = 0.002$) and CASP8 ($P = 0.008$, Fig. 1). The relative expres-

Abbreviations: ACD, allergic contact dermatitis; CASP8, caspase 8; CISH, cytokine inducible SH2-containing protein; ETS2, v-ets erythroblastosis virus E26 oncogene homologue 2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HSD11B1, hydroxysteroid (11-beta) dehydrogenase 1; PBMC, peripheral blood mononuclear cells; PIM1, PIM 1 oncogene; PRDX4, peroxiredoxin 4; RT-PCR, reverse transcription-polymerase chain reaction.

Table 1. Characteristics of the case study by age and gender at the blood collection time

Groups	Age (years)	Gender*	
		Females	Males
Controls ($n = 51$)	47.5 (33–71)	34 (66.7)	17 (33.3)
Allergics to nickel ($n = 36$)	42.5 (21–63)	33 (91.7)	3 (8.3)
Nickel-exposed workers ($n = 23$)	43.0 (26–56)	15 (65.2)	8 (34.8)
Total ($n = 110$)	43.0 (21–71)	82 (74.5)	28 (25.5)

Chi-squared statistic: * $P = 0.016$ (females vs males). Data are given as medians (range) for age and as numbers (%) for gender.

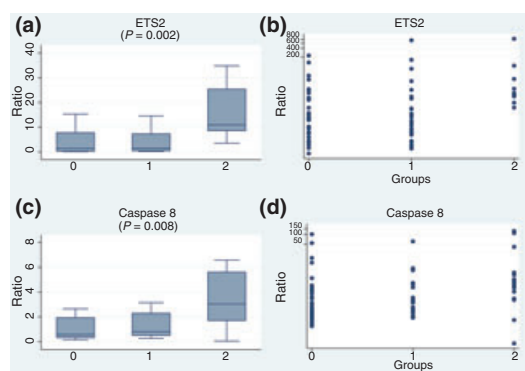


Figure 1. Relative expression level of ETS2 and CASP8 in the case study. 0, controls; 1, patients allergic to nickel; 2, nickel-exposed workers. Summary box plot (a, c) and individual data on a logarithmic scale (b, d) are shown.

sion level of ETS2 and CASP8 in healthy controls and allergic subjects was significantly lower than in nickel-exposed workers, (ETS2: $P = 0.0003$ and $P = 0.0007$; CASP8: $P = 0.001$ and $P = 0.008$). No significant difference was found in the expression level of ETS2 and CASP8 between healthy subjects and allergic patients ($P = 0.38$ and $P = 0.22$, respectively). Gene expression for ETS2 and CASP8 were not related to age or gender.

For the other genes, PIM1, HSD11B1, PRDX4 and CISH, no significant differences were detected in the expression levels between groups ($P = 0.54$, $P = 0.17$, $P = 0.82$ and $P = 0.25$, respectively; Figures S1 and S2).

Conclusion

Nickel is a widely distributed metal and also a common allergen. Nickel sensitisation is influenced by non-occupational exposure and by sociocultural factors (2). Previously, Hansen et al. (4) reported on gene expression changes in allergen-activated PBMC, suggesting that these changes were valid also for nickel allergies.

In this study, we investigated the role of six genes in sensitisation and allergy in a sample of 110 subjects. The main differences in our study in comparison to that of Hansen (4) are as follows: (i) the choice of subjects without any allergic manifestation at the time of PBMC collection; (ii) the analysis of the selected genes in non-stimulated PBMC; and (iii) the sample size of the study (110 subjects in this study versus 11 cases in the aforementioned study).

The allergic group of this study was mainly represented by women (92%). This is consistent with a previous report on the prevalence of nickel sensitisation among women (2).

Our findings showed that the expression levels of the analysed genes did not differ between allergic patients and healthy controls, while a significant higher expression level of ETS2 and CASP8 was

detected in nickel-exposed workers. We recognise that this finding might be because of methodological issues, as we did not stimulate PBMCs with any allergen. Our results suggest that the expression levels of these genes are not predictive of allergic manifestations to nickel in subjects not occupationally exposed to the metal.

ETS2 belongs to ETS transcriptions factors, which seems to be involved in different biological pathways (8–10). Hansen et al. (4) reported a significant higher expression level of ETS2 only in allergic patients who showed a high response in the proliferation test. In our study, we found that the increment in ETS2 expression is likely because of occupational exposure rather than to allergy. As suggested by others (4), the higher ETS2 expression could more likely be related to an increased lymphocyte turnover, rather than to its oncogenic mediating activity.

Our findings on CASP8 are not consistent with the study by Hansen (4). In our study, the nickel-exposed workers showed a significant higher CASP8 expression level. Differences in sample sizes together with the methodological differences may explain the discrepancy between the two studies. An increased activation of Caspase-8 and Caspase-3 following nickel treatment in primary endothelial cells or keratinocytes has been reported (11), but no data are available for PBMCs. The balance between proliferation and death is important in the control of cell number in physiological conditions. Thus, if an increased lymphocyte turnover is related to nickel exposure, the higher Caspase-8 level may be related to cell apoptosis to maintain the physiological range.

The increase in mRNA of ETS2 and CASP8 associated with occupational exposure to nickel may be interpreted as a sign of immunological reaction to the metal. This finding may be explained as a sort of “protection-vaccination effect” preventing or inhibiting nickel sensitisation. This hypothesis seems to be consistent with a recent study in which immune response to the yellow fever vaccine was preceded by coordinated induction of transcription factors, such as ETS2 (12).

In conclusion, our findings suggest that changes in ETS2 and CASP8 genes are likely to be related to nickel exposure rather than to allergy. These genes may represent a possible background to investigate the sensitisation phase towards nickel and represent useful potential biomarkers for nickel occupational exposure.

Acknowledgement

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Conflicts of interests

The authors have declared that they have no conflict of interest.

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Supporting Information

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

Data S1. Methods.

Data S2. Results.

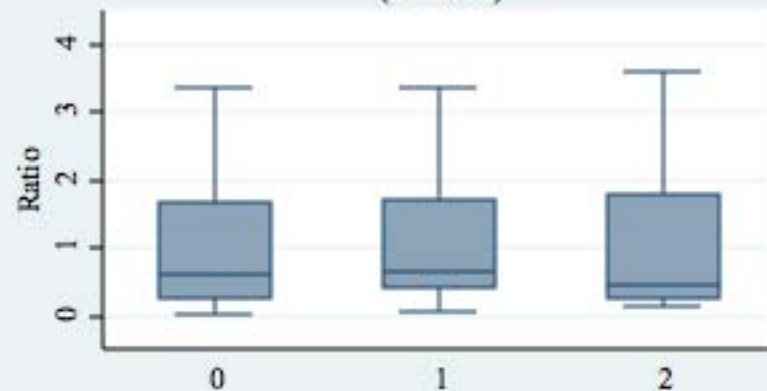
Figure S1. Relative expression levels of PIM1 and CISH in the case study.

Figure S2. Relative expression levels of HSD11B1 and PRDX4 in the case study.

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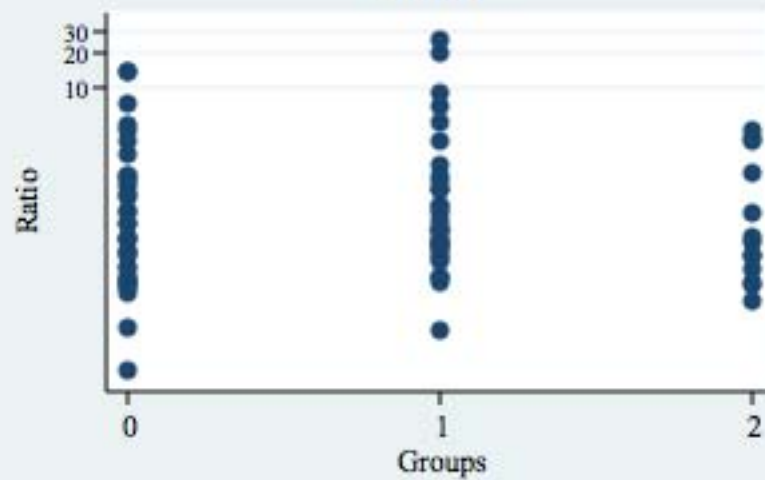
A)

PIM1
($P=0.54$)



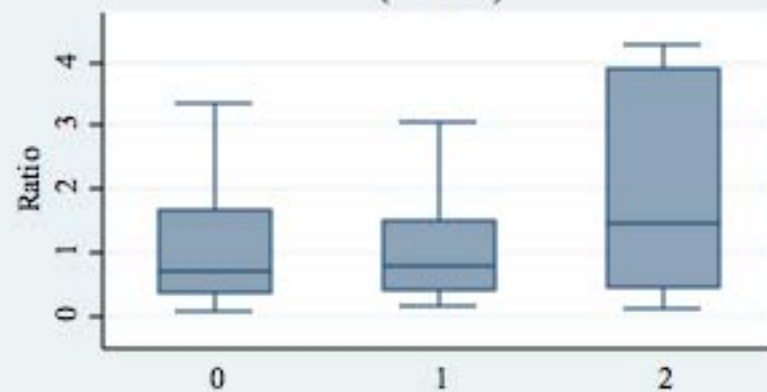
B)

PIM1



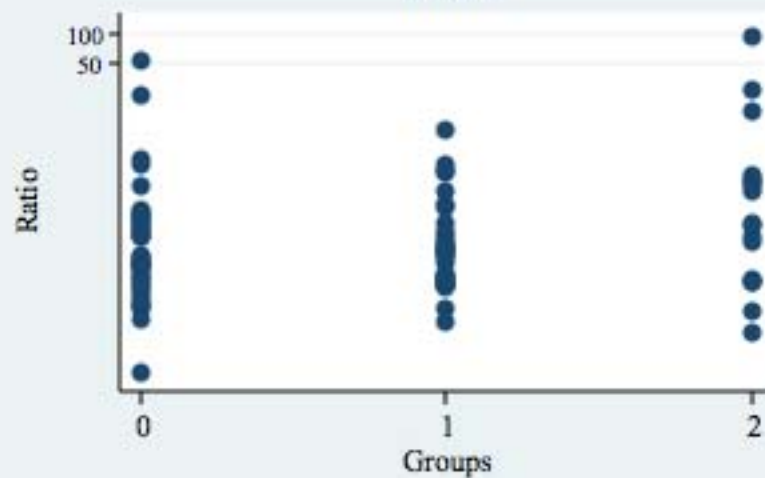
C)

CISH
($P=0.25$)

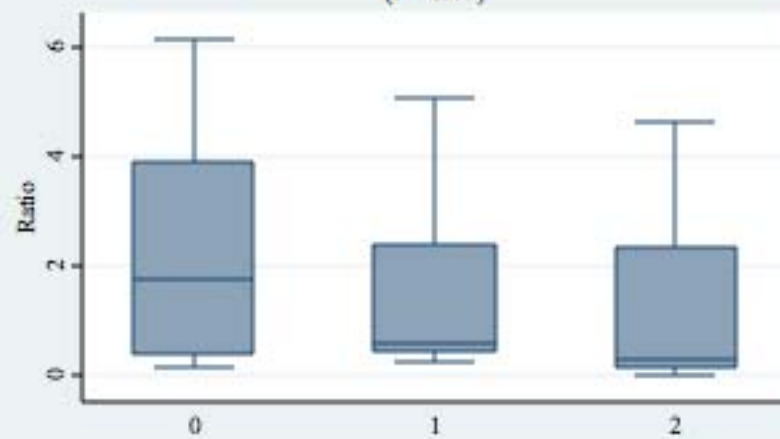


D)

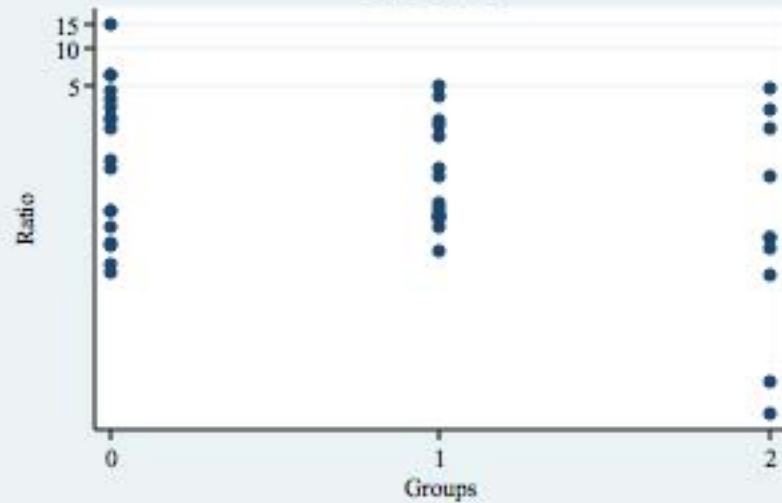
CISH



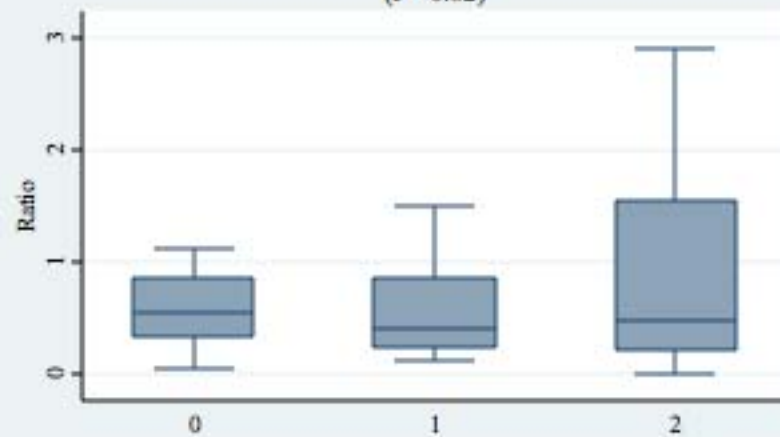
HSD11B1
(P= 0.17)



HSD11B1



PRDX4
(P= 0.82)



PRDX4

