Tobacco smoking upregulates CRLF1 expression in the atherosclerotic lesions in sex- and plaque-type specific manner

T. Lan¹, D.P.V. De Kleijn², H.M.R. Den Ruijter¹, G.P. Pasterkamp¹, M.M. Mokry¹

¹University Medical Center Utrecht, Central Diagnostics Laboratory, Utrecht, Netherlands (The)
²University Medical Center Utrecht, Department of Vascular Surgery, Utrecht, Netherlands (The)

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**Background:** Tobacco smoking is known to impact the development of atherosclerotic disease. Interestingly, females who smoke have a higher risk of cardiovascular disease (CVD) than males. It is established that smoking can lead to changes in DNA methylation and gene expression. We, therefore, hypothesised that smoking influences gene expression in a sex- and plaque-type-specific manner.

**Method:** We executed bulk RNA sequencing of 654 carotid plaques and single sequencing in 46 plaques. We analysed the transcriptomes of 625 carotid plaques from smokers (n=226) and non-smokers (n=399) and performed the differential gene expression in plaques between smokers and non-smokers. The analysis was repeated separately in males and females. The observed gene expression differences were also associated with pathological and molecular plaque characteristics.

**Results:** After correction for multiple testing, CRLF1 was found to be significantly higher expressed in smokers than non-smokers (Log2FC=0.48, FDR =0.012) (Figure 1a). Single-cell RNA-seq analyses revealed specific expression of CRLF1 in the synthetic smooth muscle cell population. The smoking-associated CRLF1 overexpression was larger in females (log2FC=0.93) compared to males (log2FC=0.35) (Figure 1b). Furthermore, the effect was restricted to plaque types with a higher content of smooth muscle cells.

**Conclusion:** Our results show the sex and plaque type-specific effect of smoking on gene expression in carotid plaques. The higher upregulation of the CRLF1 gene in female smokers suggests mechanistic clues to a higher risk of smoking-related CVD in women.

**Figure 1**

(a) current smoking VS previous or non-smoking

(b) CRLF1 gene expression in males and females