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Acne and telomere length. A new spectrum between senescence and apoptosis pathways.

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Short title: Telomere length, p53 and acne susceptibility

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Acne is a multi-factorial disease with many factors thought to play a role including the skin microflora and nutrition as well as hormonal influences and stress (Suh and Kwon, 2015). Acne patients have increased sebum secretion, and both acne and activity of the sebaceous glands are under significant genetic control (Mourelatos et al., 2007; Bataille et al., 2002). Recent Genome Wide Association Studies (GWAS) have identified several

variants linked to acne susceptibility (Wang et al., 2015; He et al., 2014; Navarini et al., 2014; Zhang et al., 2014).

It has long been noticed by dermatologists that acne patients have reduced skin ageing, often observed many years after the acne has recovered. Signs of ageing, such as wrinkling and skin thinning appear later in acne patients compared to non-affected individuals. This was speculated to be due to increased sebum secretion during a lifetime but other factors are likely to be involved (Downing et al., 1986).

In this study, we investigated leucocyte telomere length (LTL) in subjects with acne compared to controls using data from the TwinsUK registry (http://www.twinsuk.ac.uk/).

Telomeres are repeat TTAGGG sequences at the end of linear chromosomes guarding against loss of genetic material during cellular replication. Repeated cell cycles eventually lead to a critically shortened LTL signalling cellular senescence and triggering apoptosis. Hence, LTL has been shown to be predictive of biological ageing (Hewitt at al., 2012).

Volunteers in the TwinsUK cohort were not recruited on the basis of any specific trait or disease and have been shown to have diseases and lifestyle characteristics similar to the general population (Andrew et al., 2001). Guy's and St Thomas' Hospital NHS Trust Research Ethics Committee approved the study, and all twins provided informed written consent. For historical reasons the TwinsUK registry involves mainly females and males were therefore excluded from this study. The history of acne was self-reported during a nurse-administered questionnaire and the female twins were asked if they had ever suffered from acne and whether their acne was self treated, treated by a GP or by a dermatologist (Bataille et al., 2002). 293 out of 1,205 twin volunteers (24.3%) had experienced acne in their lifetime.

LTL was measured using Southern Blot and was available for all the subjects included in this study (Valdes et al., 2007; see Supplementary Material for details). Mean LTL in the 1,205 subjects was 7.08 kb (median=7.06 kb, range: [5.70-8.67] kb). Mean weight was 67 kg (median: 65 kg; range: [40-115] kg) and mean height was 162 cm (median: 162 cm; range: [144-182] cm). The mean age of the twins at the time of DNA extraction was 48±12 years.

Linear regression (see Supplementary Material for details) showed that acne cases had longer LTL (mean 7.17±0.64 kb) compared to controls (mean 6.92±0.02 kb) after adjustment for age, twin relatedness, weight and height (beta=0.11; se=0.05; p=0.01). Using score tests under a logistic regression model, we further investigated the association between acne and a set of SNPs previously reported to be associated with LTL in a sample of 1,893 cases of severe acne and 5,132 population controls from the UK Acne Genetic study (Codd et al., 2013; Mangino et al., 2015; Navarini et al., 2014). No LTL SNPs were significantly associated with acne after correction for multiple testing. However, rs3027234 in the CTS Telomere Maintenance Complex Component 1 gene (*CTC1)*, was nominally significant (Supplementary Table 1). Finally, we performed a mixed effect logistic regression analysis in whole genome data from healthy skin samples comparing acne cases and controls. The expression data was measured in 705 female individuals from the TwinsUK registry (http://www.muther.ac.uk/) of whom 346 had data on acne history. We selected 195 controls twins who were perfectly age-matched to 39 twins with acne and gene expression data (see Supplementary Material for details)**.** Only the *ZNF420* gene (probe ILMN_1720431) was significantly associated with acne history at FDR 5%, showing a higher expression in controls ($p=7.73x10^{-7}$; Table 1 and Figure 1), Interestingly, *ZNF420* is one of the 30% most expressed genes in skin.

This study investigates reduced skin ageing observed in acne by assessing telomere length in circulating white cells and gene expression in the skin. Acne cases showed longer telomeres after adjusting for age, height and twin relatedness suggesting that the delayed skin ageing may be due to reduced senescence. Only one SNP predicting LTL was found to be associated with acne at nominal significance. This SNP is located within the *CTC1* gene which is a component of the CST complex and plays an important role in protecting telomeres against degradation (Sarek et al., 2015). The reduced expression of the gene *ZNF420* in normal skin in acne cases which encodes the protein Apak (ATM and p53-associated KRAB-type zinc-finger protein) suggests that p53 is up-regulated in acne cases as the *ZNF420* gene is a negative regulator of p53-mediated apoptosis.

Considering longer telomeres and the up-regulation of the p53 pathway in acne cases, it could be speculated that acne susceptibility may be linked to the biology of cancer. A recent study from the Nurses' Health study II, a large USA cohort involving more than 99,000 female nurses (Zhang et al., 2015) found an increased risk of cancer in acne cases (Zhang et al., 2015). Additionally, recent GWASs have reported significant associations between acne and genes involved in cancer susceptibility, including the *MYC* gene and genes linked to the TGFβ cell signalling pathway (Zhang et al., 2014; Navarini et al., 2014). Further work is needed to investigate the associations between cell senescence, acne, and cancer susceptibility but this work shed new light on this very common and often debilitating skin disease.

The authors state no conflict of interest

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TABLES AND FIGURES

Table 1. Whole genome differential expression between acne cases and controls using a mixed linear model to control for intra-pair phenotypic correlation. The table reports the top 20 hits from the mixed effect logistic regression analysis. P values (P), Storey's Q value (Q), effect sizes (Beta) and standard errors (SE) are reported. Only the *ZNF420* gene remained significant after multiple testing at 5% false discovery rate (Q < 0.05).

Gene	Beta	SE	P	Q
ZNF420	-36.52	7.39	7.73×10^{-7}	0.02
KRTAP13-1	16.03	4.24	1.54×10^{-4}	0.85
TP53INP1	-4.11	1.24	9.01×10^{-4}	0.85
PTPN9	8.32	2.53	1.01×10^{-3}	0.85
GOLGA4	-4.70	1.44	1.14×10^{-3}	0.85
BCLAF1	-2.87	0.89	1.17×10^{-3}	0.85
ZC3H10	4.88	1.51	1.23×10^{-3}	0.85
VGLL1	-6.35	1.98	1.35×10^{-3}	0.85
GOLGA5	-3.68	1.15	1.42×10^{-3}	0.85
C20orf106	6.88	2.16	1.45×10^{-3}	0.85
FAM3A	3.22	1.02	1.66×10^{-3}	0.85
ALDH3A1	2.03	0.65	1.78×10^{-3}	0.85
SUV420H1	-8.68	2.80	1.93×10^{-3}	0.85
TNFAIP3	-2.91	0.95	2.10×10^{-3}	0.85
PREB	4.27	1.39	2.18×10^{-3}	0.85
HCFC1R1	2.74	0.90	2.22×10^{-3}	0.85
ING1	-3.88	1.27	2.22×10^{-3}	0.85
WDR17	-8.95	2.93	2.22×10^{-3}	0.85
CXorf1	-7.07	2.31	2.22×10^{-3}	0.85
RAPH1	-2.96	0.97	2.37×10^{-3}	0.85

Figure 1: Gene expression levels for *ZNF420* (probe ILMN_1720431) in acne cases and controls. Gene expression levels are adjusted by age and twin relatedness and have been scaled between 0 and 1. Main plot**:** *ZNF420* expression levels in controls (N=195) and cases (N=39). Inset plot: *ZNF420* expression levels in moderate acne cases, which were treated only by the GP (N=31) and severe acne cases which were treated by a dermatologist (N=8). Reported p values are from logistic regression.