General discussion

IMPACT OF SKIN AGING RESEARCH

From cosmetic relevance towards healthy skin aging

Facial skin aging has a large impact on the general population, as it is directly visible and influences the perception of a person's wellbeing. The cosmetic relevance is clear (as described in **Chapter 1**), but there is much more to it. Increasing life expectancy and the resulting aging of the population have made skin aging a topic that is of growing concern. With older age, prevalence of dry skin rises; it affects 60% of the middle-aged and elderly population[1]. This gives rise to higher risk of skin diseases such as late onset atopic dermatitis and pruritus. A better understanding of skin aging will help to unravel aging in general and can be of importance for promoting healthy aging. The skin is the most feasible organ to investigate using non-invasive approaches, making it an ideal target to understand aging as it may even be seen as a mirror of the internal organs. Skin aging could therefore act as a robust biomarker of aging in general and even of survival[2-4], which makes it valuable in clinical decision-making. Furthermore, global disease prevention strategies might benefit from emphasizing that certain risk factors or protective measures are also linked to facial aging. An example of this is the finding that certain dietary habits are also associated with facial wrinkling[5]. Creating a tailored approach to prevention of (skin) aging and related diseases is an important ambition. In doing so, many different phenotypes can be used. Finding the most suitable and standardized one is important to be able to replicate findings and reach the above-mentioned goals.

A MULTI-FACTORIAL PHENOTYPE

Confirming known and discovering new determinants

As described in the general introduction (**Chapter 1**), facial skin aging is a complex phenotype. There are distinctive characteristics between intrinsic and extrinsic aging, but in practice it is difficult to separate these two in UV-exposed areas such as the face. The combined effects of both intrinsic and extrinsic facial aging result in a wide range of observable physical characteristics, which can be divided into four major phenotypes: wrinkles, pigmented spots (PS), telangiectasia and sagging. Perceived age (PA, the estimated age of a person based on facial appearance) is composed of all these aspects. To date there is no gold standard approach to investigate skin aging; a wide range of assessments are used, mostly regarding skin aging as a compound phenotype, rather than specifying a certain component. In the past, two main phenotypes for photoaging have been described based on photoaging characteristics: hypertrophic aging (leathery inelastic skin with coarse wrinkles) and atrophic aging (telangiectasia, hypertrophic sebaceous glands and the striking absence of wrinkles) as depicted in Figure 1[6,7]. There are indications that individuals with atrophic-type skin aging have an increased risk of keratinocytic skin cancer (KC)[8]. Given the complexity of facial skin aging and the need of homogeneous phenotypes to increase the

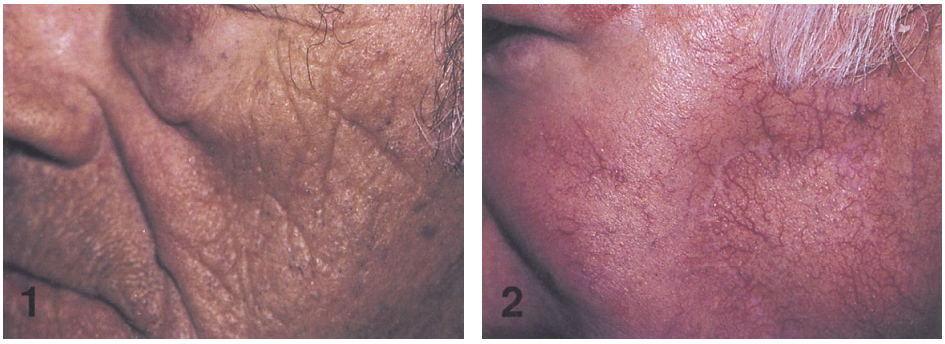

Figure 1. Hypertrophic (**1**) and atrophic (**2**) skin aging, adapted from Calderone et al[6]
1: Coarse wrinkles; inelastic and leathery appearance.
2: Smooth skin without wrinkles; prominent telangiectasia and hypertrophic sebaceous glands.

power to identify (genetic) determinants, in this thesis we studied different skin aging aspects separately, allowing to discover different determinants associated with each one of them. This is shown in several chapters of this thesis: **Chapter 3** and **4** (wrinkles), **Chapter 5** (PS), **Chapter 6** (telangiectasia), and **Chapter 7** (PA). In this thesis we mainly used continuous digital skin aging measures, quantified using image analysis techniques. They correlated well with the different manual grades (**Chapter 2**) and provide an objective, consistent and fast method to produce powerful continuous variables. We have not added sagging as separate feature as it is arguably mainly due to subcutaneous changes in the musculature and bone structure rather than to cutaneous changes.

We replicated known determinants, but also encountered surprising associations. Age was the strongest determinant for wrinkles (**Chapter 3**). It was non-linearly related to wrinkle area; with increasing age, older individuals showed a smaller increase in wrinkle area than younger individuals did. Interestingly, the effect of age was smaller for PS and telangiectasia. As age has more influence on wrinkles than on the other phenotypes, perhaps it is a more suitable proxy for chronological age.

One of the most striking differences in determinants per phenotype was found for skin color. Light skin color was associated with less wrinkles (**Chapter 3**), but with more pigmented spots (**Chapter 5**) and more telangiectasia (**Chapter 6**). The negative association of light skin color with wrinkles seems contradictive at first, because people with Fitzpatrick skin types IV and higher have less wrinkles than those with skin types I-III[9,10]. The possible explanation that very light-skinned individuals are known to avoid the sun because of the ultraviolet (UV) sensitive nature of their skin does not suffice because our model also included UV-exposure related variables which should correct for this effect. Furthermore, pigmented spots and telangiectasia are also influenced by UV-exposure[11-14]. This strengthens the hypothesis that the association between skin color and the skin aging features are independent of UV-exposure and confirms the concept that different skin types age in a different manner. Fair-skinned individuals tend to show more atrophic

signs of aging (e.g. PS and telangiectasia), whereas darker-skinned individuals show more hypertrophic signs of aging (e.g. wrinkles).

We discovered important sex differences for wrinkles, stressing the importance of a sex-tailored approach regarding skin aging management (**Chapter 3**). Men are first at developing wrinkles (<75 years old: wrinkle area is higher in men), but women catch up with them at a higher age (≥75 years old: wrinkle area is higher in women). This difference could be due to hormonal changes in postmenopausal women, as early menopause has been associated with more wrinkles[15]. Women with premature ovarian insufficiency are shown to have an unfavorable cardiovascular risk profile[16], although perhaps the cardiovascular risk is related to the association with their increased free androgen index (FAI; total testosterone in nmol/l divided by sex hormone binding globulin in nmol/l)[17]. In contrast, endogenous estrogen exposure in women was found to be harmful for all-cause mortality[18]. In this thesis, however, we found no association between estradiol levels and wrinkles. Nonetheless, we did find an inverse association of FAI and wrinkles in women. Moreover, female pattern hair loss was also inversely associated with wrinkles. These findings suggest that testosterone could play a role in protecting women against wrinkles. However, this does not coincide with the abovementioned association of an increased FAI with an unfavorable cardiovascular risk profile, when assuming skin aging might reflect internal aging. Surprisingly, we have not found a significant association of testosterone and wrinkles in men. This could be explained by the fact that we have used an inferior measurement; total testosterone is less suitable than bioavailable testosterone in men. Interestingly, the effect sizes of the determinants also differ per sex. The effect sizes for skin color and smoking are larger in women than in men (**Chapter 3**). Furthermore, higher educational level is associated with less wrinkles in women, but not in men.

UV-exposure has well-known negative effects, but it is also essential for vitamin D synthesis in the skin[19], which is vital for our health. In the past decades, vitamin D deficiency has been seen as a marker for health status, as it has been associated with cardiovascular disease as well as with mortality[20-23], although recent studies debate this[24,25]. We found that higher serum 25-hydroxyvitamin D was associated with more skin wrinkling and a higher PA (**Chapter 8**). In order to discover whether these associations were causal, we performed Mendelian Randomization (MR).

Genetically determined 25-hydroxyvitamin D (i.e. the genetic variants that are associated with serum 25-hydroxyvitamin D) was not associated with wrinkling, PS or PA, suggesting that these associations are not likely causal. The observational associations between serum 25-hydroxyvitamin D and the two skin aging phenotypes are most probably due to residual confounding (e.g. UV-exposure) or reverse causality. These findings confirm the need for more MR studies to investigate causality.

Understanding the underlying correlation structure of skin aging

The abovementioned differences between the distinct skin aging features prompted us to investigate the underlying variance of these phenotypes using principal component analysis (PCA)[26], as

described in **Chapter 9**. Besides wrinkles, PS, telangiectasia and PA, we also used data on actinic keratosis (AK) and keratinocytic cancer (KC), the most common skin (pre)malignancies[27,28]. The PCA identified three main components of skin aging that explained 73% of the total variance (Figure 2). It can be argued that principal component 1 (PC1) is related to the hypertrophic skin aging component, while PC2 corresponds to the atrophic component. This analysis also revealed a third component (PC3), which represents a cancerous component. It suggests that hypertrophic aging, atrophic aging, and the cancerous component occur via partly different pathophysiological mechanisms. This PCA confirms what has been described earlier, but is more robust because it is data-driven and hypothesis-free, making it an objective measure (as opposed to the subjective clinical measurement done by clinicians). As expected, the associations between the main lifestyle and physiological determinants and the three components of skin aging differed in magnitude and direction of effects and confirmed the distinctions found in earlier analyses. This highlights the importance of understanding their underlying mechanisms. It also has clinical implications; it can be of use in risk stratification and personalized approach of skin aging prevention strategies. Furthermore, the finding that the cancerous component is separated from the skin aging components stresses the relevance of skin cancer screening even in the absence of obvious signs of skin aging.

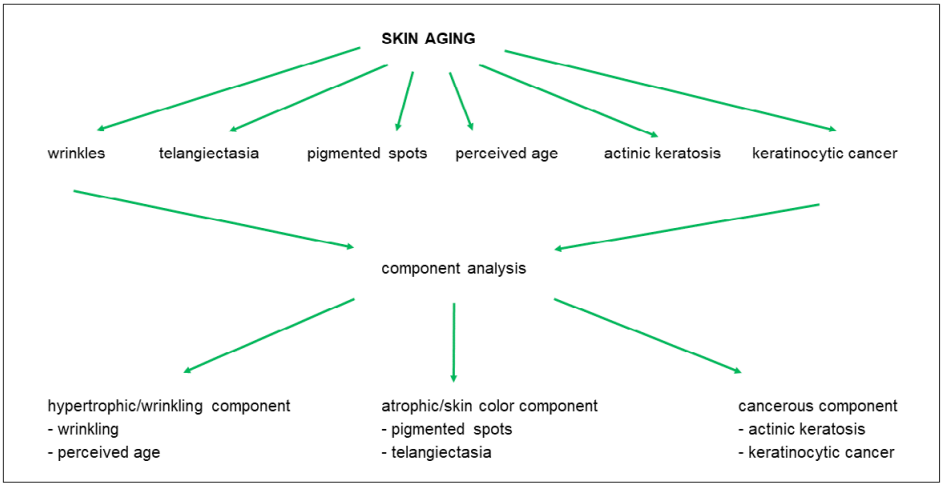

Figure 2. The accordion model. Skin aging is a complex phenotype, consisting of six different features, which can be re-grouped into three main components: hypertrophic, atrophic, and cancerous.

Genetic epidemiology – usual suspects *MC1R* and *IRF4* not related to wrinkles

Results from previous genetic association studies have been conflicting. In the general introduction (**Chapter 1**), we discussed suggestive single-nucleotide polymorphisms (SNPs) from previous skin aging genome-wide association studies (GWAS) and candidate gene studies, of which *MC1R* and *IRF4* have the strongest effects. In this thesis, we found that these and other pigmentation

variants were associated with the phenotypes PS and PA. For PS we found associations at four genetic loci (**Chapter 5**): *IRF4* (rs12203592, $P=1.8\times10^{-27}$), *MC1R* (compound heterozygosity score, $P=2.3\times10^{-24}$), *RALY/ASIP* (rs6059655, $P=1.9\times10^{-9}$), and *BNC2* (rs62543565, $P=2.3\times10^{-8}$). These four genes are associated with skin color variation and skin cancer risk, but remained highly significant ($P<2\times10^{-8}$) after adjustment for skin color. Therefore, these genes seem to contribute to PS through pathways independent of the basal melanin production as they may have pleiotropic effects.

The *MC1R* gene is also associated with PA ($P=2.7\times10^{-12}$, **Chapter 7**). Individuals carrying the homozygote *MC1R* risk haplotype looked on average up to 2 years older than non-carriers did. A diminished *MC1R* activity can cause a weaker UV shielding capacity and cause more photodamage[29]. The effect was, however, found to be independent of UV variables. This is in line with findings that *MC1R* has pleiotropic effects, perhaps influencing aging via different pathways, for example via inflammation[30]. Of importance, the association of the *MC1R* gene with PA was only found when PA was corrected for wrinkles. Therefore, it can be hypothesized that this corrected PA represents the "atrophic" perceived age, including PS and telangiectasia, as well as sagging, which is not quantified in this thesis but also plays a role in PA.

In line with this, *MC1R* was not associated with wrinkles (**Chapter 4**). We only found a suggestive hit for the SNP rs10476781 ($P=2.2\times10^{-8}$), an intergenic variant 628 KB downstream of the Neuromedin U Receptor gene. In contrast to the other phenotypes, the classical skin pigmentation genes did not play a role in global wrinkling. This is somehow unexpected since we found a clear association between skin color and wrinkles. Perhaps other yet to be discovered skin color genes influence wrinkles. The remarkable differences between wrinkles and PS/PA imply different genetic backgrounds for these skin aging components. The genetic architecture of wrinkles seems more complex than that of PS and PA.

Missing heritability

The estimated heritability using all SNP data[31] was highest for wrinkles, namely 46%. For PS it was 28% and for PA 33% (**Chapter 9**). However, in our GWAS the top hit accounted for <1% of wrinkling variation (**Chapter 4**). The remaining genetic variation for wrinkles remains unknown. There are several hypotheses for these gaps[32]. A large part of missing heritability is likely explained by variants with effects too small to be detected. Most probably, there are many different genes affecting wrinkles, but all with small effect sizes. There seems to be too much genotypic and phenotypic heterogeneity. In other words, the well-known but unfortunate power problem for genetic studies. Increasing the sample size can solve this problem. In addition, non-tagged SNPs could be of importance. Previously, GWAS on skin aging mainly took into account common variants with a frequency of >0.01. However, also rare variants may have substantial effects[33]. Focusing on rare SNPs can offer important additional information. Finally, a part of the missing heritability could simply be due to artificial factors, such as imprecise phenotypic measurement, genotyping or imputation errors.

FUTURE PERSPECTIVES – FILLING GAPS OF MISSING HERITABILITY AND BEYOND

In order to discover the beforementioned missing heritability, higher sample sizes are required, for which collaborations are important. With future collaborations in mind, it is important to be able to use a standardized phenotype. Which phenotype is most suitable is under discussion, but several factors are important: it should be easy to retrieve, non-invasive, objective and of high quality. The use of photographs seems the most feasible, as these can be stored for later use and can be replicated by several graders or digital methods. In this thesis, perceived age was assessed by human grading. This was however quite time-consuming; this could possibly be replaced by artificial intelligence in the future, as this is a fast evolving field[34]. Nevertheless, perhaps there is no such thing as "the perfect phenotype" for research in skin aging. It may be more appropriate to select the phenotype based on the underlying question. Combining different assessments for skin aging may obscure identification of genes if variants have different effects on the phenotypes (as shown for wrinkles and PS). On the other hand, PCA-like approaches may help to understand the outcomes from previous studies or be of use for clinical advice. Furthermore, the phenotype PA could perhaps help to identify parameters for healthy aging.

In this thesis, we showed that for certain phenotypes such as wrinkles, large samples will be needed to identify new genes associated with facial skin aging. In GWAS, both common DNA variants and rare DNA variants can be targeted, depending on the underlying genetic architecture of the phenotype. For both, large sample sizes are needed. In future studies, there should be special attention for rare DNA variants. GWAS based on whole-genome sequencing permit the full frequency spectrum of variants to be studied, including rare variants that are difficult to capture using SNP arrays and imputation. This could help solve the missing heritability problem. Performing GWAS will hopefully allow to identify new pathways for skin aging. Another approach is to look into already identified pathways or determinants to reduce the burden of multiple testing associated with testing millions of markers. For example, by using MR we can confirm associations that have been seen in epidemiological studies. In **Chapter 3,** we showed that body mass index (BMI) was negatively associated with wrinkles. It is unclear if this is simply due to the "filling effect" or if there is more to it. In preliminary analyses, we performed MR to understand whether this association was causal. The results showed that some BMI-SNPs were associated with wrinkle variation (data unpublished).

Other major factors are gene-gene and gene-environment interactions. In this thesis, we only evaluated SNP associations one at a time (main effects). However, it is very likely that there are gene-gene interactions and gene-environment interactions for skin aging features, e.g. polymorphisms in repair genes that affect skin aging features via UV-exposure. Therefore, in the future it is important to also take these interactions into account (Figure 3). However, several other challenges are then likely to arise[35] and the importance of environmental exposures is not always straightforward. Also, it requires large sample sizes, only reachable by generating consortia.

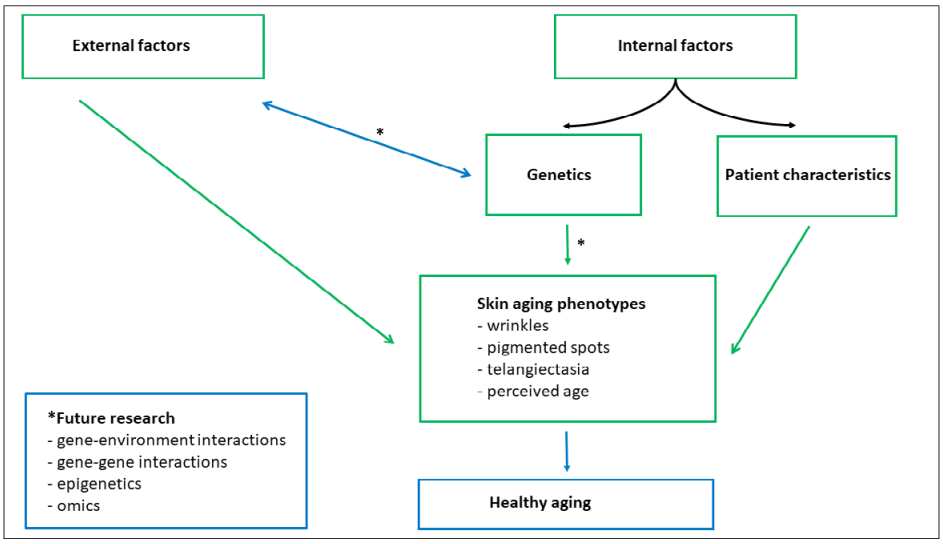

Figure 3. An overview of associations investigated in this thesis (depicted in green) and future possibilities (depicted in blue). In this thesis, we have investigated external factors (lifestyle and environmental exposures) and internal factors (patient characteristics and genetics) related to the skin aging phenotypes. This has offered valuable information, although much is still unexplained. This can perhaps be solved by focusing future research on gene-environment interactions, gene-gene interactions, epigenetics and omics. Furthermore, the interesting link towards healthy aging can be made.

Epigenetics is described by modifications that regulate gene activity and/or expression by methylation, but do not directly change the DNA sequence[36]. Epigenetic modifications are highly dynamic and display great tissue specificity. To what extent epigenetic modifications contribute to skin aging is unknown. Examples of epigenetic studies are next-generation sequencing (especially to search in detail for rare/unknown variation in a specific genetic area) and epigenome-wide association studies (EWAS) researching DNA-methylation profiles. The concept of epigenetic drift describes changes in methylation that occur with age across various tissues, including skin. Several studies show contradictory results of either hypo- or hypermethylation related to skin aging[37,38]. The strong effects of smoking in methylation make it likely that smoking may play an important role in skin aging via epigenetics.

"Omics", high-throughput analyses including genomics, epigenomics, transcriptomics, proteomics and metabolomics, are widely applied in aging research[39]. For skin aging, however, this field has not been extensively researched. Most proteomics research is done on photodamaged skin[40], but there are also examples of comparisons between UV-exposed and UV-unexposed sites, revealing differences in relative protein abundance[41,42]. At the UV-exposed sites, age-altered proteins were associated with conferring structure, energy and metabolism. At the UV-unexposed site, proteins associated with gene expression, free-radical scavenging, protein synthesis and protein degradation were most frequently altered[41], giving rise to inferences on intrinsic and extrinsic aging.

Needless to say, it is first and foremost important that our GWAS findings are replicated in independent cohorts. The relevance of these gene variants can then be confirmed by functional follow-up studies, e.g. through gene expression.

Further missing links may be found in other research fields, as the microbiome. Investigation of dietary habits has revealed interesting findings[43-49]. Most recently discovered, a red meat and snack-dominant dietary pattern was associated with more facial wrinkles in women, whereas a fruit-dominant dietary pattern was associated with fewer wrinkles[5]. Although not much is known on microbiome and skin aging, it could be an interesting new field to discover.

CONCLUDING REMARKS

In this thesis, I discovered lifestyle and genetic associations for different skin aging features, using validated digital measurements. I identified valuable determinants and gene variants for several features and created robust evidence for the hypertrophic and atrophic components. However, this is only the start of understanding skin aging, as it seems much more complex; the relationships are not simply linear, but involve multiple complicated pathways, still to be discovered. This and future research on associations with other aging organ systems will contribute to a better understanding of (healthy) aging.

REFERENCES

1. Mekic S, Jacobs LC, Gunn DA, Mayes AE, Ikram MA, Pardo LM, et al. Prevalence and determinants for xerosis cutis in the middle-aged and elderly population: A cross-sectional study. *J Am Acad Dermatol*. 2019;81(4):963-9 e2.
2. Christensen K, Thinggaard M, McGue M, Rexbye H, Hjelmborg JV, Aviv A, et al. Perceived age as clinically useful biomarker of ageing: cohort study. *BMJ*. 2009;339:b5262.
3. Borkan GA, Norris AH. Assessment of biological age using a profile of physical parameters. *J Gerontol*. 1980;35(2):177-84.
4. Christensen K, Iachina M, Rexbye H, Tomassini C, Frederiksen H, McGue M, et al. "Looking old for your age": genetics and mortality. *Epidemiology*. 2004;15(2):251-2.
5. Mekic S, Jacobs LC, Hamer MA, Ikram MA, Schoufour JD, Gunn DA, et al. A healthy diet in women is associated with less facial wrinkles in a large Dutch population-based cohort. *J Am Acad Dermatol*. 2019;80(5):1358-63 e2.
6. Calderone DC, Fenske NA. The clinical spectrum of actinic elastosis. *J Am Acad Dermatol*. 1995;32(6):1016-24.
7. Gilchrest BA. Photoaging. *J Invest Dermatol*. 2013;133(E1):E2-6.
8. Brooke RC, Newbold SA, Telfer NR, Griffiths CE. Discordance between facial wrinkling and the presence of basal cell carcinoma. *Arch Dermatol*. 2001;137(6):751-4.
9. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol*. 1988;124(6):869-71.
10. Vashi NA, de Castro Maymone MB, Kundu RV. Aging Differences in Ethnic Skin. *J Clin Aesthet Dermatol*. 2016;9(1):31-8.
11. Bastiaens M, Hoefnagel J, Westendorp R, Vermeer BJ, Bouwes Bavinck JN. Solar lentigines are strongly related to sun exposure in contrast to ephelides. *Pigment Cell Res*. 2004;17(3):225-9.
12. Ezzedine K, Mauger E, Latreille J, Jdid R, Malvy D, Gruber F, et al. Freckles and solar lentigines have different risk factors in Caucasian women. *J Eur Acad Dermatol Venereol*. 2013;27(3):e345-56.
13. Monestier S, Gaudy C, Gouvernet J, Richard MA, Grob JJ. Multiple senile lentigos of the face, a skin ageing pattern resulting from a life excess of intermittent sun exposure in dark-skinned caucasians: a case-control study. *Br J Dermatol*. 2006;154(3):438-44.
14. Green AC, Hughes MC, McBride P, Fourtanier A. Factors associated with premature skin aging (photoaging) before the age of 55: a population-based study. *Dermatology*. 2011;222(1):74-80.
15. Youn CS, Kwon OS, Won CH, Hwang EJ, Park BJ, Eun HC, et al. Effect of pregnancy and menopause on facial wrinkling in women. *Acta Derm Venereol*. 2003;83(6):419-24.
16. Daan NM, Muka T, Koster MP, Roeters van Lennep JE, Lambalk CB, Laven JS, et al. Cardiovascular Risk in Women With Premature Ovarian Insufficiency Compared to Premenopausal Women at Middle Age. *J Clin Endocrinol Metab*. 2016;101(9):3306-15.
17. Daan NM, Jaspers L, Koster MP, Broekmans FJ, de Rijke YB, Franco OH, et al. Androgen levels in women with various forms of ovarian dysfunction: associations with cardiometabolic features. *Hum Reprod*. 2015;30(10):2376-86.
18. Jaspers L, Kavousi M, Erler NS, Hofman A, Laven JS, Franco OH. Fertile lifespan characteristics and all-cause and cause-specific mortality among postmenopausal women: the Rotterdam Study. *Fertil Steril*. 2017;107(2):448-56 e1.
19. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266-81.

20. Andrukhova O, Slavic S, Zeitz U, Riesen SC, Heppelmann MS, Ambrisko TD, et al. Vitamin D is a regulator of endothelial nitric oxide synthase and arterial stiffness in mice. *Mol Endocrinol*. 2014;28(1):53-64.
21. Chowdhury R, Kunutsor S, Vitezova A, Oliver-Williams C, Chowdhury S, Kiefte-de-Jong JC, et al. Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. *BMJ*. 2014;348:g1903.
22. Lee JH, Gadi R, Spertus JA, Tang F, O'Keefe JH. Prevalence of vitamin D deficiency in patients with acute myocardial infarction. *Am J Cardiol*. 2011;107(11):1636-8.
23. El Hilali J, de Koning EJ, van Ballegooijen AJ, Lips P, Sohl E, van Marwijk HWJ, et al. Vitamin D, PTH and the risk of overall and disease-specific mortality: Results of the Longitudinal Aging Study Amsterdam. *J Steroid Biochem Mol Biol*. 2016;164:386-94.
24. Zittermann A, Pilz S. Vitamin D and Cardiovascular Disease: An Update. *Anticancer Res*. 2019;39(9):4627-35.
25. Pilz S, Verheyen N, Grubler MR, Tomaschitz A, Marz W. Vitamin D and cardiovascular disease prevention. *Nat Rev Cardiol*. 2016;13(7):404-17.
26. Everitt B HT. Principal component analysis. In: *An Introduction to Multivariate Analysis with R*. New York: Springer-Verlag; 2011.
27. Flohil SC, Seubring I, van Rossum MM, Coebergh JW, de Vries E, Nijsten T. Trends in Basal cell carcinoma incidence rates: a 37-year Dutch observational study. *J Invest Dermatol*. 2013;133(4):913-8.
28. Flohil SC, van der Leest RJ, Dowlatshahi EA, Hofman A, de Vries E, Nijsten T. Prevalence of actinic keratosis and its risk factors in the general population: the Rotterdam Study. *J Invest Dermatol*. 2013;133(8):1971-8.
29. Dessinioti C, Antoniou C, Katsambas A, Stratigos AJ. Melanocortin 1 receptor variants: functional role and pigmentary associations. *Photochem Photobiol*. 2011;87(5):978-87.
30. Muffley LA, Zhu KQ, Engrav LH, Gibran NS, Hocking AM. Spatial and temporal localization of the melanocortin 1 receptor and its ligand alpha-melanocyte-stimulating hormone during cutaneous wound repair. *J Histochem Cytochem*. 2011;59(3):278-88.
31. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, et al. Common SNPs explain a large proportion of the heritability for human height. *Nat Genet*. 2010;42(7):565-9.
32. Bourrat P, Lu Q, Jablonka E. Why the missing heritability might not be in the DNA. *Bioessays*. 2017;39(7).
33. Yang J, Bakshi A, Zhu Z, Hemani G, Vinkhuyzen AA, Lee SH, et al. Genetic variance estimation with imputed variants finds negligible missing heritability for human height and body mass index. *Nat Genet*. 2015;47(10):1114-20.
34. Patcas R, Bernini DAJ, Volokitin A, Agustsson E, Rothe R, Timofte R. Applying artificial intelligence to assess the impact of orthognathic treatment on facial attractiveness and estimated age. *Int J Oral Maxillofac Surg*. 2019;48(1):77-83.
35. Sandholt CH, Hansen T, Pedersen O. Beyond the fourth wave of genome-wide obesity association studies. *Nutr Diabetes*. 2012;2:e37.
36. Bird A. Perceptions of epigenetics. *Nature*. 2007;447(7143):396-8.
37. Vandiver AR, Irizarry RA, Hansen KD, Garza LA, Runarsson A, Li X, et al. Age and sun exposure-related widespread genomic blocks of hypomethylation in nonmalignant skin. *Genome Biol*. 2015;16:80.
38. Gronniger E, Weber B, Heil O, Peters N, Stab F, Wenck H, et al. Aging and chronic sun exposure cause distinct epigenetic changes in human skin. *PLoS Genet*. 2010;6(5):e1000971.
39. Zierer J, Menni C, Kastenmuller G, Spector TD. Integration of 'omics' data in aging research: from biomarkers to systems biology. *Aging Cell*. 2015;14(6):933-44.

40. Adachi H, Murakami Y, Tanaka H, Nakata S. Increase of stratifin triggered by ultraviolet irradiation is possibly related to premature aging of human skin. *Exp Dermatol*. 2014;23 Suppl 1:32-6.
41. Newton VL, Riba-Garcia I, Griffiths CEM, Rawlings AV, Voegeli R, Unwin RD, et al. Mass spectrometry-based proteomics reveals the distinct nature of the skin proteomes of photoaged compared to intrinsically aged skin. *Int J Cosmet Sci*. 2019;41(2):118-31.
42. Voegeli R, Monneuse JM, Schoop R, Summers B, Rawlings AV. The effect of photodamage on the female Caucasian facial stratum corneum corneome using mass spectrometry-based proteomics. *Int J Cosmet Sci*. 2017;39(6):637-52.
43. Cho S. The Role of Functional Foods in Cutaneous Anti-aging. *J Lifestyle Med*. 2014;4(1):8-16.
44. Yoon HS, Kim JR, Park GY, Kim JE, Lee DH, Lee KW, et al. Cocoa Flavanol Supplementation Influences Skin Conditions of Photo-Aged Women: A 24-Week Double-Blind, Randomized, Controlled Trial. *J Nutr*. 2016;146(1):46-50.
45. Schwartz S, Frank E, Gierhart D, Simpson P, Frumento R. Zeaxanthin-based dietary supplement and topical serum improve hydration and reduce wrinkle count in female subjects. *J Cosmet Dermatol*. 2016;15(4):e13-e20.
46. Zmitek K, Pogacnik T, Mervic L, Zmitek J, Pravst I. The effect of dietary intake of coenzyme Q10 on skin parameters and condition: Results of a randomised, placebo-controlled, double-blind study. *Biofactors*. 2017;43(1):132-40.
47. Nagata C, Nakamura K, Wada K, Oba S, Hayashi M, Takeda N, et al. Association of dietary fat, vegetables and antioxidant micronutrients with skin ageing in Japanese women. *Br J Nutr*. 2010;103(10):1493-8.
48. Cosgrove MC, Franco OH, Granger SP, Murray PG, Mayes AE. Dietary nutrient intakes and skin-aging appearance among middle-aged American women. *Am J Clin Nutr*. 2007;86(4):1225-31.
49. Purba MB, Kouris-Blazos A, Wattanapenpaiboon N, Lukito W, Rothenberg EM, Steen BC, et al. Skin wrinkling: can food make a difference? *J Am Coll Nutr*. 2001;20(1):71-80.