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Evidence That Loss-of-Function *Filaggrin* Gene Mutations Evolved in Northern Europeans to Favor Intracutaneous Vitamin D3 Production

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Abstract

Skin pigmentation lightened progressively to a variable extent, as modern humans emigrated out of Africa, but extreme lightening occurred only in northern Europeans. Yet, loss of pigmentation alone cannot suffice to sustain cutaneous vitamin D3 (VD3) formation at the high latitudes of northern Europe. We hypothesized that loss-of-function mutations in the epidermal structural protein, filaggrin (FLG), could have evolved to sustain adequate VD3 status. Loss of FLG results in reduced generation of trans-urocanic acid, the principal endogenous ultraviolet-B (UV-B) filter in lightly-pigmented individuals. Accordingly, we identified a higher prevalence of *FLG* mutations in northern European populations when compared to more southern European, Asian and African populations that correlates significantly with differences in circulating 25-OH-VD3 levels in these same populations. By allowing additional UV-B penetration and intracutaneous VD3 formation, the latitude-dependent gradient in *FLG* mutations, likely together with other concurrent mutations in VD3 metabolic pathways, provide a non-pigment-based mechanism that sustains higher levels of circulating VD3 in northern Europeans. At the time that *FLG* mutations evolved, xerosis due to FLG deficiency was a lesser price to pay for enhanced VD3 production. Yet, the increase in *FLG* mutations has inadvertently contributed to an epidemic of atopic diseases that has emerged in recent decades.

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Keywords

Filaggrin; Human evolution; Pigmentation; Skin color; Vitamin D3

Introduction

Ultraviolet-B (UV-B) exposure of the skin generates the majority of circulating vitamin D3 (VD3) in humans (Bikle 2010; Holick 2011). VD3 production begins with UV-B-induced photolysis of 7-dehydrocholesterol (7DHC) into pre-VD3, followed by thermal isomerization of 7DHC into VD3, its subsequent transport from the skin first to the liver, and then the kidneys, where the active hormone, 1,25-dihydroxy vitamin D (1,25(OH)₂D), is formed. Because VD3 is required not only for bone and mineral metabolism, but also for other innate immune/anti-inflammatory processes (Bikle 2010; Holick 2011), VD3 deficiency could negatively impact survival. UV-B exposure declines progressively with distance from the Equator, creating a gradient that reduces intracutaneous VD3 production. Yet paradoxically, northern-dwelling Europeans display higher than normal circulating 25-(OH)D3 levels, a finding usually ascribed to differing cultural attitudes towards sun exposure (van Schoor and Lips 2011), but the lengthy, sun-deprived seasons of the far North, coupled with the relatively short half-life of 1,25(OH)₂D (Jones 2008), argue against a crucial behavioral effect alone as an explanation for these higher 25-(OH)D3 levels (Rhodes et al. 2010). A higher intake of seafood likely cannot explain these higher 25-(OH)D3 levels, because in contrast to Inuits and other populations of the far North, modern humans who migrated northward in the late Pleistocene behind retreating glaciers 11 thousand years ago (kya), consumed primarily cold-tolerant fauna of the tundra, such as reindeer, rather than seafood (Bjorn and Wang 2000). Many of these northerners, even when residing near the sea, preferred land-based sources of protein, rather than seafood (Gillie 2012; McPhee 1992), and even today the diets of most Scandinavians is not centered on seafood (Halldorsson et al. 2007). Although VD3 is found in the lichen species, *C. arbuscula* and *C. rangiferina*, which represent important winter feeds for reindeer and caribou, whether this dietary source altered VD3 status in humans is unclear (Snellman et al. 2009; Wang et al. 2001). Alternatively, we hypothesize that evolutionary differences in cutaneous structure and function are more likely to provide an explanation for the observed increase in circulating 25-(OH)D3 levels in northern Europeans. As described below, there is a strong genetic component that links epidermal structure to VD3 status, that further correlates the individual's ability to generate VD3 during summer months' UV exposure.

Potential Impact of Pigment Dilution on Vitamin D Synthesis

Darkly-pigmented modern humans, progressing out of Africa towards northern latitudes, doubtlessly encountered reduced levels of incident UV-B, potentially threatening them with VD3 deficiency. Because these early migrants risked the consequences of VD3 deficiency, natural selection should have favored mechanisms that enhanced the cutaneous bioavailability of this key vitamin. Current dogma holds that it was mutations in genes, such as MC1R, SLC24A5, and SLC45A2 that resulted in a dilution of epidermal pigmentation which facilitated additional cutaneous VD3 production (Chaplin and Jablonski 2009). Recent fossil finds in northern Spain from the Mesolithic era (≈9 kya), however, show that

at least some modern humans retained two genes associated with dark pigmentation in Africans (i.e., SLC24A5 and SLC45A2) (Olalde et al. 2014), suggesting first, they were still quite darkly pigmented; and second, that pigmentation did not progressively lighten as modern humans departed from Africa $\approx 50\text{--}60$ kya. Moreover, there is no south-to-north gradient of mutations in another key regulator of epidermal pigmentation, the melanocortin receptor type 1 (MC1R) (Rana et al. 1999). Likewise, mutations in SLC24A5 show a horizontal latitudinal arc (Basu Mallick et al. 2013), rather than a south-to-north sweep that should have occurred to facilitate VD3 production, and in contrast to the gradient that we show for *filaggrin* (FLG) mutations (see below).

Though pigmentation in northern migrants eventually lightened moderately in a quasi-latitude-dependent fashion throughout Europe and Asia (Loomis 1967), dramatic pigment lightening occurred only in northern Europeans (Norton and Hammer 2008; Norton et al. 2007) (Fig. 1a). In contrast, substantial pigmentation has persisted in Inuits and northern Asians who reside at comparable latitudes of the far north, but who consume a marine seafood-enriched diet.

While population genetic studies in both European and eastern Asian populations show that loss-of-function mutations accrued in several genes that regulate melanin pigment production, the specific mutations varied widely in these two groups (Anno et al. 2008; Norton et al. 2007; Sturm 2009). Though these correlations strongly suggest that natural selection occurred for mutations that resulted in pigment dilution, they do not resolve whether these mutations were ‘driven’ by an increased need for cutaneous VD3 production, or whether they served other purposes. The many shortcomings of the ‘pigment-vitamin D’ hypothesis (Elias and Williams 2013; Robins 2009) include: (1) low prevalence of rickets in late Pleistocene and early Holocene human fossils; (2) erythematous doses of incident UV-B produce comparable elevations in circulating (25-OH)D3 levels in darkly and lightly-pigmented humans (Bogh et al. 2010); (3) the paradoxical; i.e., lower prevalence of osteoporosis in many darkly-pigmented populations (Aloia 2008); (4) lack of preferential pigment dilution in exposed versus unexposed skin sites (facial skin and skin on the backs of the hands), a change that should have occurred to facilitate further UV-B-induced VD3 production; and (5) darkly-pigmented humans display polymorphisms that allow increased bioavailability of 1,25-dihydroxy VD3, despite lower circulating VD3 levels (Powe et al. 2013). Finally, hair follicles, which do not manufacture VD3, lightened in parallel with epidermal pigmentation in northerners.

Because of these concerns with the pigment-VD3 hypothesis, we recently proposed an alternate explanation for pigment dilution in northern Europeans, based upon the universal biologic imperative for metabolic conservation (Elias and Williams 2013). As pigmentation became optional in populations living far from the tropical onslaught of UV-B, the organism no longer needed to expend precious energy resources in the production of large amounts of melanin pigment, a metabolically-expensive protein.

Though the further loss of pigmentation that occurred in northern Europeans likely reflected an attempt to increase cutaneous VD3 synthesis, even the most fair-skinned residents receive insufficient UV-B to sustain VD3 levels at these latitudes (Gillie 2012; Rhodes et al. 2010).

Thus, non-fish-eating hunter-gatherers of the far North needed to evolve alternate or additional mechanisms to generate additional intracutaneous VD3.

Epidermal Filaggrin, Ultraviolet Protection and Vitamin D Synthesis

Non-pigment-based mutations that could have evolved to enhance cutaneous VD3 production fall into two broad categories. First, a genome-wide study in Europeans identified polymorphisms in genes involved in vitamin D metabolism, including cholesterol synthesis, VD3 hydroxylation, and VD3 transport, that correlate with increased circulating VD3 levels (Wang et al. 2010). Mutations in two of these genes could enhance VD3 levels; i.e., loss-of-function 7-dehydrocholesterol reductase (7DHCR) and/or gain-of-function mutations in the VD3 binding protein. Indeed, a very recent study suggests that polymorphisms in the gene encoding 7DHCR could have allowed additional VD3 to be generated as humans emigrated into northern Europe (Kuan et al. 2013). Yet, mutations in 7DHCR can result in the syndromic disorder, Smith-Lemli-Opitz Syndrome, which also appeared in a southward-to-northward trajectory in humans over the last 3–6 kya (Witsch-Baumgartner et al. 2008). Although acquisition of this disease certainly would confer no evolutionary benefits, asymptomatic mutation carriers could have benefitted, analogous to the protection against malaria that accrues to carriers of the sickle cell trait. We provide here data that identifies a second, non-pigment-based, genetic mechanism that likely evolved to facilitate enhanced UV-B-induced, cutaneous VD3 production, thereby improving survival of lightly-pigmented, northern Europeans. Filaggrin is a histidine-rich protein, generated in abundance late in epidermal differentiation, that plays an initial structural role both in the formation of the cornified envelope, and in promoting filament aggregation in corneocytes (Eckert et al. 2004; Presland 2009). Higher in the stratum corneum, FLG detaches from the cornified envelope, followed by its humidity-dependent hydrolysis by caspase 14 and other proteases into its constituent amino acids (Brown and McLean 2012; Harding et al. 2013; Scott et al. 1982). These amino acids then are further deaminated into a series of osmotically active, polycarboxylic acids, including histidine-derived trans-urocanic acid (t-UCA) (suppl. Fig. 1). t-UCA is the major endogenous UV-B filter of the SC, interdicting at least 50 % of incident UV-B in lightly-pigmented skin (Brookman et al. 2002; Thomson 1955). Accordingly, down-regulation of FLG expression in organotypic human keratinocyte cultures yields lower-than-normal levels of t-UCA; while simultaneously enhancing UV-B sensitivity; while conversely, caspase 14 over-expression accelerates the conversion of FLG into t-UCA, providing further photoprotection (Devos et al. 2012; Mildner et al. 2010).

Latitude-Dependent Changes in the Prevalence of *Filaggrin* Mutations

Common loss-of-function mutations in the *FLG* gene cause xerotic skin, characterized by reduced stratum corneum hydration and elevated transcutaneous water-loss rates, resulting in the dominantly-inherited disorder, ichthyosis vulgaris (Thyssen et al. 2013), and a strong propensity for atopic disorders, such as atopic dermatitis, asthma and food allergies (Irvine et al. 2011). Heterozygous *FLG* mutations affect about 10 % of normal Northern Europeans and 5 % of normal Asians. The number of identified loss-of-function mutations in *FLG* now exceeds 40, with prominent, population-specific differences (Table 1; Suppl. Tables 1–3). While the R501X and 2282del4 account for more than 80 % of common mutations in Northern Europeans, eight different mutations account for ≈80 % in Asians (Irvine et al.

2011). Though these prevalence data are correlative, when they are stratified by latitude, a striking north–south gradient is observed within Europe and Africa, with a similar, though less striking pattern in Asian populations (Fig. 1b; Suppl. Tables). Notably, the latitudes of Japan, Korea and northern China largely correspond to those of southern Europe. Accordingly, the highest prevalence of reported *FLG* mutations in Asians were from Shenyang, China (41° latitude, 6.5 %) and Fukui, Japan (36°, 6.5 %), both relatively far to the north in Asia. Most pertinently, population cohorts of Tunisians, Ethiopians (Winge et al. 2011), Italians (Cascella et al. 2011), Bangladeshi (Sinclair et al. 2009), and very recently, a homogenous population of native Xhosa from South Africa (Thawer-Esmail et al. 2014) show a very low prevalence of *FLG* mutations even in atopic populations. The relatively low mutation prevalence of Austrians in comparison to other central-to-northern Europeans likely reflects centuries of downward, transnational migration of more pigmented Slavs from northern Eurasia. Likewise, the somewhat elevated prevalence of *FLG* mutations in today's southern Chinese, Singaporeans and Taiwanese likely also reflects relatively recent, transnational patterns of migration from further North. Although the gradient of *FLG* mutation prevalence at different latitudes will certainly be modified as more population sequencing data become available, it seems highly unlikely that such data will modify the latitude-dependent gradient that we have identified here.

Parallel Gradients for Light Pigmentation, Filaggrin Mutations and 25-(OH)VD3 Blood Levels

Based upon available data, it is now clear that three parallel, latitude-dependent north–south prevalence gradients exist in Europe: (1) Loss-of-pigmentation in Europeans of the far North (Fig. 1a); (2) loss-of-function *FLG* mutations in these same northerners (Fig. 1b); and (3) a corresponding increase in (25-OH)D3 serum levels in these same populations (Fig. 1c).

The relationship between *FLG* mutation prevalence, 25-OH D3 serum levels and latitude shows a high degree of statistical correlation (Fig. 1d). Thus, the Pearson's R correlation coefficients between latitude and, respectively, VD3 and *FLG* mutations are moderate-to-strongly positive ($R^2 = 0.77$ and $R^2 = 0.55$). Notably, the correlation between VD3 and *FLG* mutations also showed a strong correlation, $R^2 = 0.74$. Yet, these significant correlations remain correlative, and do not provide direct evidence for our proposed causal relationship. McLean et al. recently proposed that *FLG* mutations, by compromising skin barrier function, might have evolved to favor transcutaneous vaccinations by external pathogens, thereby increasing survival during epidemics, such as the plague during the Middle Ages (Brown and McLean 2012). However, this interpretation fails to explain the much lower prevalence of *FLG* mutations towards Equatorial climes, where infections are much more common (Mackintosh 2001).

To test our hypothesis further, it would be useful to determine both the prevalence and *FLG* mutation profiles in northerly-residing Inuits, who have always consumed a marine seafood diet. If the prevalence of *FLG* mutations is lower in Inuits than in lightly-pigmented northern Europeans, this observation would lend strong support to the hypothesis that *FLG* mutations likely evolved to increase VD3 synthesis. In contrast, if the prevalence of *FLG* mutations proves comparable in Inuits and northern Europeans, then the *FLG*–VD3 hypothesis

becomes more tenuous. Finally, it would be relatively straight-forward to assess whether *FLG* deficient-humans generate higher circulating 25(OH)D3 levels in response to incident UV-B irradiation than do wild-type individuals with comparable degrees of pigmentation.

The Evolutionary Trade-Off: Further VD3 Production in Exchange for Xerosis and a Propensity for Atopy

Our data support a different evolutionary explanation that connects these three, seemingly interdependent gradients. We suggest that *FLG* mutations became prevalent relatively recently in lightly-pigmented northern Europeans to further optimize cutaneous VD3 synthesis, and therefore survival. The repeatedly-observed, intra-European gradient in circulating (25-OH)D3 levels is better explained by *FLG* mutations than by either loss of pigmentation and/or differences in cultural habits. Our hypothesis is supported not only by the parallel gradients described above, but also by the following additional observations: (1) Serum (25-OH)D3 levels, independent of known confounders, were 10 % higher in $\approx$ 16,000 *FLG* mutation carriers of all ages from five independent population cohorts from Germany and Denmark; and in addition, these blood levels demonstrated an allele dose-dependency, with the highest levels in homozygous carriers (Thyssen et al. 2012); (2) Loss-of-function mutations in *FLG* correlate with corresponding reductions in the levels of the UV-B photoreceptor, t-UCA (Thyssen et al. 2012); (3) A seasonal effect also was found in these populations, with a further enhancement in 25(OH)D3 levels during summer months (Thyssen et al. 2012), despite the potentially-mitigating wide-spread use of tanning beds and sun-soaked holidays among northerners during Winter months; and finally; (4) In $\approx$ 10,000 German children and adolescents with atopic dermatitis, significantly higher serum 25(OH)D3 levels were found in *FLG*-deficient patients in comparison to wild-type controls (Heimbeck et al. 2013). *FLG* mutations result in xerosis, and a possible tendency for increased cutaneous infections, as well as a propensity to develop AD. Pertinently, *FLG* degradation products are acidic and help to maintain a low pH on the skin surface, which likely reduce the growth of pathogenic micro-organisms such as *Staphylococcus aureus*. Yet, patients with single- and double-allele mutations in *FLG*, with ichthyosis vulgaris, but not inflammation, do not exhibit a propensity to develop cutaneous infection. Fortunately, AD was not a problem in the late Paleolithic era, due to a rural-agrarian lifestyle and lack of exposure to dust mite and cockroach antigens, as occurs in contemporary, urban settings. Nonetheless, there would have been a necessary evolutionary trade-off of dry skin in service to the need to increase cutaneous VD3 generation.

In summary, we propose that an increased prevalence of *FLG* mutations evolved relatively recently to insure adequate VD3 status in northern Europeans, who lived at high latitudes, and in cultures where seafood was not extensively consumed. A decrease in *FLG* results in a corresponding reduction in levels of t-UCA, the principal endogenous UV-B filter in lightly pigmented individuals. Loss of pigmentation alone could not suffice, since UV-B exposure is scarce, even in the summer at these high latitudes. Hence, the abrupt latitude-dependent gradient in *FLG* mutations shown here, likely coupled with other mutations that modify VD3 formation, transport or metabolism, provides separate, non-pigment-based explanations for the maintenance of VD3 levels in Europeans of the far North.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

7DHC	7-Dehydrocholesterol
FLG	Filaggrin
KYA	Thousand years ago
t-UCA	Trans-urocanic acid
UV	Ultraviolet
VD3	Vitamin D3

Fig. 1.
a Distribution of human skin pigmentation before 1492. Modified from Loomis, WF. Science, 157: 501–506, 1967, according to T.B. Fitzpatrick: the validity and practicality of sun-reactive skin types I through IV. Arch Dermatol. 1988) [I (lightest)–VI (darkest)]. **b** Latitude-dependent changes in the prevalence of FLG mutations in general populations. **c** Latitude-dependent changes in serum 25-hydroxy vitamin D levels in nmol/L in Europe. **d** Latitude-dependent correlation between FLG mutations prevalence and correlating 25-OH VD3 levels

Table 1

Filaggrin mutation frequencies by regions, countries and latitudes

Countries	Latitude	Article's first author	Identified mutations	Total subjects (mutation carriers) ^a	Mutation carrier prevalence by country (%)	Mutation prevalence with CI > 95 %
Northern Europe	Sweden	Ballardini, Winge	R501X, S2282del4, R2447X, S3247X	2,195 (154)	7.02	8.3 % (8.0-8.6)
	Denmark	Thyssen	R501X, 2282del4	6,565 (516)	7.86	
	UK/Ireland	Barker, Brown, Henderson, Palmer, Rice, Sandilands, Van Limbergen,	R501X, 2283del4, R2447X, S3247X, 3673delC	20,263 (1,878) (C)	9.27	
	Germany	Zhao, Zivab Betz, Cramer, Greisenegger,	R501X, 2282del4, R2447X, S3247X, 3702delG	11,316 (808) (C)	7.14	
Southern Europe	Netherlands	Huffmeier, Marenholz,	R501X, 2282del4, R2447X, S3247X	543 (43)	7.92	
	Poland	Novak, Ohi, Stemmer, Weichenthal, Weidinger DeJongh, Visser Poninska	R501X, 2283del4	510 (24)	4.71	
	Austria	Gruber	R501X, 2282del4	133 (2)	2.73	3.5 % (2.5-4.9)
	France	Militz, Droitcourt	R501X, 2282del4, R2447X	188 (9)	4.79	
Northern Asia	Croatia	Sabolic Pipinic	R501X, 2282del4, R2447X, S3247X	423 (11)	2.60	
	Italy	Cascella	R3638X	201 (9) (C)	4.48	
	Korea	Lee	S2554X, 3321delA	133 (2)	1.50	3.7 %
	Japan	Imoto, Nomura, Nemoto-Hasabe	Q1701X, R501X, S2554X, S2889X, S3298, I695X, 2282del4, 3321delA, 1702delG, Lys4021X	1,922 (107) (C)	5.57	
Southern Asia	China	Chen, Ching, Hu, Li, Ma, Xiong, Zhang	E2422X, K4002X, K4671X, Q1070X, Q1712X, Q1790X, Q2397X, Q2417X, R501X, R826X, R1474X, R2447X, R4306X, R4307X, R1140X, S1302X, S1515X, S2554X, S2706X, S2889X, S3296X, W2583X, 441delA, 478insA, 1249insG, 2231delA, 2282del4, 3321delA, 3222del4, 4026delT, 4271delAA, 5757del4, 6218delAA, 6834del5, 6950del8, 7145del4, 7945delA, 8001 del4	2,014 (78) (C)	3.87	
	Taiwan	Whang	T454A, P478S, E498D, H519N, R3270C, Q3322Q, R501X, 2282del4, S3247X, S3296X, S2554X, S2889X, 3321delA, E1795X, E2422X, Q2417X, P478S	212 (8)	3.77	5.1 %
Southern Asia (cont.)	Singapore	Chen, Common	E2422X, G323X, Q368X, Q1745X, Q2417X, R501X, R4307X, S406X, S1302X, S1515X, S2706X, 441delA, 1249insG, 2284del4, 3321delA,	967 (62) (C)	6.41	

Countries	Latitude	Article's first author	Identified mutations	Total subjects (mutation carriers) ^a	Mutation carrier prevalence by country (%)	Mutation prevalence with CI > 95 %
North America	USA	Gao	4275del12, 6834del5, 6950del8, 6957del8, 7945delA, 8157delC			
			4275del12, 6834del5, 6950del8, 6957del8, 7945delA, 8157delC			
			R501X, 2282del4	329 (3) (AA)	0.91	8.0 % (6.6–9.5)
Africa	Canada	Brown	R501X, 2282del4,			
			R2447X, S3247X	156 (9) (EA)	5.77	
				891 (98) (EC)	11.00	
Australia	Tunisia	Palmer	R501X, 2282del4	124 (0)	0.91	0 % (0–1.6)
			R501X, 2282del4, R2447X, S3247X	103 (0)		
			R501X, 2282del4, R2447X, S3247X, 3702delG	126 (5) (C)	3.97	3.97 %

AA African American, EA European American, EC European Canadian
^a (C) = populations that underwent sequencing of the entire *FLG* gene