

BRIEF COMMUNICATION

The role of filaggrin mutations during pregnancy and postpartum: atopic dermatitis and genital skin diseases

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Abstract

Mutations in the epidermal filaggrin gene (*FLG*) are associated with skin barrier dysfunction (dry skin, less acidic skin, and fissured skin), and atopic dermatitis (AD) with a severe and persistent course. Because pregnancy and delivery further impairs normal skin barrier functions (immune suppression, mechanical stress), we studied the possible role of *FLG* mutations on the risk of AD flares, genital infections, and postpartum problems related to perineal trauma. *FLG*-genotyping was performed in a population-based sample of 1837 women interviewed in the 12th and 30th weeks of pregnancy and 6 months postpartum as part of the Danish National Birth Cohort study 1996–2002. We found that *FLG* mutations also influence pregnancy-related skin disease; thus, women with *FLG* mutations had an increased risk of AD flares during pregnancy (OR 10.5, 95% CI 3.6–30.5) and of enduring postpartum physical problems linked to perineal trauma during delivery (OR 11.1, 95% CI 1.1–107.7).

Filaggrin proteins are essential to the skin barrier formation and function. Filaggrin gene (*FLG*) mutations reduce or even abolish filaggrin expression and are observed in 8–10% of persons with European descent (1). Clinically, the mutations is a cause for ichthyosis vulgaris, but they also strongly increase the risk of atopic dermatitis (AD) subtypes characterized by early onset, a severe course, persistence into adulthood, and increased risk of superinfections, with *Staphylococcus aureus* and *Herpes simplex* (1–3). Notably, filaggrin proteins are also expressed in epithelia of the vagina and cervix, but the possible impact of filaggrin deficiency at these sites has been insufficiently studied (4).

A developmental bias of T helper (Th) cell 2 is observed during normal pregnancy, which contributes to the prevention of miscarriage and premature labor (5). AD is also characterized by Th2 cell predominance in the acute phase, which may explain why among parous women with AD, half report a deterioration in their skin condition during previous pregnancies (6). Several epidermal and epithelial infections occur more often during pregnancy – these are mainly genital infections such as vaginitis, primary herpes simplex, and genital warts (7). In addition, normal delivery often traumatizes the sensitive skin of the perineum, which

can lead to persistent perineal pain, incontinence, and dyspareunia (8).

We hypothesized that pregnant women with *FLG* mutations have a relatively higher risk of AD flares, genital infections (herpes, warts, and vaginitis), and persistent (postpartum) problems related to perineal trauma sustained at delivery. To study this hypothesis, we took advantage of the rich self-reported information from the Danish National Birth Cohort study (DNBC). Between 1996 and 2002, the DNBC enrolled pregnant women ($n = 91\,769$), who participated in telephone interviews in the 12th and 30th weeks of pregnancy and 6 and 18 months postpartum, and also provided blood samples (see www.dnbc.dk). Our study population consisted of women from a previous case–control study nested in the DNBC (9). In the earlier study (9), women with spontaneous preterm (cases, $n = 1000$, ≤ 36 weeks) and term (controls, $n = 1000$, 40 weeks) delivery of a living singleton after an uncomplicated pregnancy were selected for genome-wide association study (Table S1); (9) 1937 were successfully genotyped. We included in our study all previously genotyped women with remaining DNA ($n = 1907$, 98%) and successfully genotyped 4 relevant *FLG* mutations in 1837 (96%) women (see Table S2). Of these women, 1551 (84%)

had DNBC antenatal interview information (i.e., information on pregnancy events) and 1336 (73%) had postnatal interview information (i.e., information on postpartum outcomes). Antenatal outcomes of interest included skin and genital conditions present before pregnancy (1st antenatal interview) and occurring during pregnancy (1st and 2nd antenatal interviews). For AD, only women diagnosed by physician before pregnancy were considered to have the condition (as DNBC had no information on AD diagnosis performed *during* pregnancy); thus, six women were considered noncases because they only reported AD during pregnancy (none mutation carriers). Information on postpartum outcomes was derived from responses to the postnatal interview questions (6 months postpartum) 'Do you currently have any physical problems that are connected with your pregnancy or delivery?' and 'What kind of problems?'. Answers to the latter question were assigned to one or more prespecified categories, two of which included perineal damage. Associations between *FLG* genotype and outcome prevalences were evaluated by odds ratios (OR) estimated using exact logistic regression with mid-p confidence intervals, in PROC GENMOD in SAS, version 9.4 (SAS Institute, Inc., Cary, NC, USA). When investigating outcome subtypes, each type was compared with the noncase group, thereby approximating multinomial logistic regression by pairwise logistic regression.

Study women were 16–44 years (mean, 30), born in 1954–1984, and 34% were nullipareous at the time of enrollment in the DNBC. *FLG* mutations were observed in 8.2% of women. Table 1 shows that *FLG* mutations were associated with a threefold increased odds of AD overall. However, the strength of this association increased to 10.5 (95% CI 3.6–30.5) for women with AD flares during pregnancy. Otherwise, there were no other significant associations between *FLG* mutations and skin or genital diseases overall (Table 1), although the ORs were increased for the herpes genitalis with onset before pregnancy and untreated vaginitis during pregnancy. As shown in Table 2, women with *FLG* mutations were more likely to have three types of persistent perineal problems significantly related to difficulties during delivery: problems attributable to cuts in the perineum (OR 11.4, 95% CI 1.1–107.7), problems due to perineal rupture or tearing (OR 11.1, 95% CI 1.9–65.5), and involuntary bladder release (OR 11.1, 95% CI 1.1–107.7). The results were not materially different when adjusted for preterm delivery, age at the pregnancy, and AD if not the outcome (see Table S3).

Our study documented that *FLG* mutations confer a strong and significant risk of AD flares during pregnancy. As in most previous studies on AD during pregnancy (6), although not all (10), there was no information about whether the flares were persistent since childhood, or relapsed as a result of pregnancy after years with no symptoms. In either case,

Table 1 Odds ratio (OR) of self-reported skin/genital diseases before and/or during pregnancy by *FLG* genotype among 1551 women interviewed in the 12th and 30th week of pregnancy, Danish National Birth Cohort, 1996–2002

Self-reported history of skin/genital disease	Filaggrin genotype				OR (95% CI)†
	Wild type (reference)		Mutations*		
	Cases	Non Cases (%)	Cases	Non Cases (%)	
Atopic dermatitis (physician-diagnosed)	46 (3.2)	1378	13 (10.4)	114	3.4 (1.7–6.4)
Before pregnancy only	38 (2.7)	1378	6 (4.7)	114	1.9 (0.7–4.4)
Before and during pregnancy	8 (0.6)	1378	7 (5.5)	114	10.5 (3.6–30.5)
<i>Herpes labialis</i>	487 (34.2)	937	40 (31.5)	87	0.9 (0.6–1.3)
Before pregnancy only	328 (23.0)	937	29 (22.8)	87	1.0 (0.6–1.5)
During pregnancy	159 (11.2)	937	11 (8.7)	87	0.7 (0.4–1.4)
<i>Herpes genitalis</i>	64 (4.5)	1360	10 (7.9)	117	1.8 (0.9–3.5)
Before pregnancy only	39 (2.7)	1360	8 (6.4)	117	2.4 (1.0–5.1)
During pregnancy	25 (1.8)	1360	2 (1.7)	117	0.9 (0.1–3.4)
Genital warts	151 (10.6)	1273	16 (12.6)	111	1.2 (0.7–2.1)
Before pregnancy only	139 (9.8)	1273	14 (11.0)	111	1.2 (0.6–2.0)
During pregnancy	12 (0.8)	1273	2 (1.6)	111	1.9 (0.3–7.7)
Vaginitis during pregnancy					
Any	284 (19.9)	1140	29 (22.8)	98	1.2 (0.8–1.8)
Treatment for vaginitis					
None	68 (4.8)	1140	13 (10.2)	98	2.2 (1.1–4.1)
Tablets, or suppositories	112 (7.9)	1140	10 (7.9)	98	1.0 (0.5–2.0)
Other	104 (7.3)	1140	6 (4.7)	98	0.7 (0.3–1.5)

*Defined in Table S2.

†ORs based on diseases that were not mutually exclusive, that is one woman may have reported more than one disease.

Table 2 Odds ratio (OR) for enduring physical problems connected with pregnancy or delivery self-reported at 6 months postpartum by *FLG* genotype among 1336* women interviewed both in the 12th week of pregnancy and 6 months postpartum, Danish National Birth Cohort, 1996–2002

Self-reported physical problems enduring 6 months postpartum	Filaggrin genotype				OR (95% CI)‡
	Wild type (reference)		Mutations†		
	Cases (%)	Non Cases (%)	Cases	Non cases	
Problems related to epidermal or epithelial damage					
Problems related to cuts in perineum	2 (0.2)	1027	2 (1.8)	92	11.4 (1.1–107.7)§
Problems related to perineal rupture/tearing	3 (0.2)	1027	3 (2.8)	92	11.1 (1.9–65.5)§
Urinary incontinence	2 (0.2)	1027	2 (1.8)	92	11.1 (1.1–107.7)§
Fecal/gas incontinence	2 (0.2)	1027	0 (0.0)	92	–
Hemorrhoids	7 (0.6)	1027	1 (0.9)	92	1.6 (0.1–10.5)
Problems related to cesarean scar	14 (1.1)	1027	0 (0.0)	92	–
Psychological distress¶	0 (0.0)	1027	0 (0.0)	92	–
Problems unrelated to epidermal or epithelial damage					
Pelvic girdle pain (PGP)	39 (3.2)	1027	3 (2.8)	92	0.9 (0.2–2.6)
Back/lumbar pain, not PGP	34 (2.8)	1027	3 (2.8)	92	1.0 (0.2–3.0)
Varicose veins	2 (0.2)	1027	0 (0.0)	92	–
Weight gain	2 (0.2)	1027	0 (0.0)	92	–
Weight loss	0 (0.0)	1027	0 (0.0)	92	–
Hip trouble	3 (0.2)	1027	1 (0.9)	92	3.7 (0.1–35.2)
Swollen joints	1 (0.1)	1027	0 (0.0)	92	–
Accumulation of fluid in the body	0 (0.0)	1027	0 (0.0)	92	–
Other problem(s)**					
Nonepithelial location	87 (7.1)	1027	2 (1.8)	92	0.3 (0.0–0.9)
Genital area	2 (0.2)	1027	0 (0.0)	92	–
Location not specified	13 (1.1)	1027	1 (0.9)	92	0.9 (0.0–5.0)

*Four women answered 'Do not know' to the question about any physical enduring problems and were grouped as noncases.

†Defined in Table S2.

‡Self-reported problem categories not mutually exclusive, that is one woman may have multiple problems.

§None of the cases had history of atopic dermatitis. The ORs were based on 13 cases, of whom three reported multiple persistent problems: One reported two problems related to the perineum, one also reported fecal/gas incontinence, and one also reported a problem classified under 'Other'.

¶Although inconsistent with the question, 17 women reported this problem among all women in DNBC interviewed 6 month postpartum, and we therefore chose to present the problem in the table.

**The answer recorded as text, which for the present study was evaluated and categorized as shown.

the strong association we observed emphasizes that this particular genetic predisposition leads to a more troublesome course of AD and therefore represent a distinct clinical phenotype (11). *FLG* mutation status in pregnant women with AD may be valuable in predicting AD flares during pregnancy (positive predictive value of *FLG* mutation for AD flare = 54% [7/13] vs an AD flare prevalence of 17% [8/46] in women without *FLG* mutations).

Our study did not support a strong association between *FLG* mutations and genital infections during pregnancy. In agreement with our hypothesis, we found a strong association between *FLG* mutations and persistent postpartum physical problems connected with perineal trauma during delivery, including urinary incontinence. The mechanism underlying these associations is unclear, but because *FLG* mutations produce dry, fissured, and less acidic (less antimicrobial)

skin, we speculate that the epidermis may heal more slowly after trauma, e.g. due to wound infection and less re-epithelization (3, 12).

The advantages of our study included its population-based setting and the relatively unique information on skin and genital outcomes during pregnancy and postpartum. Disadvantages include the questionnaire-based design and small size. Other studies are needed to confirm our findings. In conclusion, women with *FLG* mutations may have an increased risk of AD flares during pregnancy and of enduring postpartum problems attributable to perineal trauma during delivery.

Author contributions

Drs Bager and Wohlfahrt had full access to all of the data in the study and took responsibility for the integrity of the data

and the accuracy of the data analysis. Drs Bager, Melbye, and Wohlfahrt involved in study concept and design. Drs Bager, Boyd, Wohlfahrt, Thyssen, and Melbye did acquisition, analysis, and interpretation of the data. Dr Bager drafted the manuscript. Drs Bager, Wohlfahrt, Boyd, Thyssen, and Melbye performed the critical revision of the manuscript for important intellectual content. Drs Bager and Wohlfahrt (statistician) did the statistical analysis.

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Conflict of interests

The authors declare that they have no conflicts of interest.

Supporting Information

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

Table S1. Selection criteria in the earlier genetic study of preterm delivery (9)

Table S2. Genotyping results and test for Hardy-Weinberg equilibrium (HWE) for 4 filaggrin gene mutations in 1907 women from the Danish National Birth Cohort

Table S3. Crude and adjusted odds ratio (OR) for important outcomes by *FLG* genotype (any mutation vs wildtype), Danish National Birth Cohort 1996–2002

Table S4. *FLG* mutation carriers according to age at pregnancy and parity, Danish National Birth Cohort 1996–2002

SUPPLEMENTS

Table S1

Selection criteria in the earlier genetic study of preterm delivery.⁽⁹⁾

Cases: 1000 women who delivered preterm (≤ 36 weeks, spontaneous preterm delivery)

Controls: 1000 women who delivered at term (40 weeks)

Selection criteria:

- Child and parents (i.e. the DNBC mother and the baby's father) born in Denmark
- Child's grandparents born in Denmark or northwestern Europe
- Singleton live births
- No placental abruption, placental insufficiency, placenta previa, congenital abnormalities, preeclampsia, induced labor, or Caesarean section

Table S2. Genotyping results and test for Hardy-Weinberg equilibrium (HWE) for 4 filaggrin gene mutations in 1907 women from the Danish National Birth Cohort.

Mutation	OBSERVED GENOTYPES						EXPECTED GENOTYPES			HWE exact p-value
	wt/wt	wt/mt	mt/mt	N	Missing	allele mt	wt/wt	wt/mt	mt/mt	
R501X	1820	67	0	1887	1.0%	1.8%	1820.6	65.8	0.6	1
2282del4	1791	62	13	1866	2.1%	2.4%	1779.0	85.9	1.0	2.74*10 ⁻¹²
S3247X	1887	6	1	1894	0.7%	0.2%	1886.0	8.0	0.0	0.0074
R2447X	1877	12	0	1889	0.9%	0.3%	1877.0	12.0	0.0	1
Study										
Inclusion	1677	146	14	1837	3.7%†					
Exclusion†	70									

wt, wildtype; mt, mutation; HWE, Hardy-Weinberg equilibrium.

† 70 women (3.7% of the study population) were excluded from the study because one or more of the mutations was not called. Consequently, the study's reference group consisted of women who had wildtype genotypes at all 4 markers for the 4 mutations.

Comment

Genotyping was performed by LGC Genomics Ltd (Herts, UK) using KASP™ technology. We defined women with *FLG* mutations as women who were heterozygous (wt/mt) or homozygous (mt/mt) for ≥ 1 *FLG* mutation (146+14=160 women). Wildtype (i.e., ancestral type) women were women homozygous for the wildtype allele (wt/wt) for all 4 markers of the mutations (1677 women); *FLG* genotyping was considered unsuccessful for 70 women.

HWE was tested using an exact test to take into account type I error if the Chi-square test is used.(1) There were no significant deviations from HWE for R501X and R2447X, however, there were deviations for 2282del4 and possibly S3247X.

Consequently, zygosity for 2282del4 and S3247X were uncertain. However, because we did not analyze the effect of zygosity (we analysed only the main effect, i.e. the effect of carrying at least one mutant allele), our results are unlikely to be influenced by this deviation from HWE.

Ethics

The study was approved by the Scientific Ethics Committee of the Capital City Region (Copenhagen) (j.nr. (KF) 01-471/94, j.nr. H-A-2007-0017), the Danish Data Protection Agency (j. nr. 2008-54-0472) and the DNBC Steering Committee (j. nr. 2013-24).

(1) Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. *Am J Hum Genet* 2005 May;76(5):887-93.

Table S3. Crude and adjusted odds ratio (OR) for important outcomes by *FLG* genotype (any mutation vs. wildtype), Danish National Birth Cohort 1996-2002.

Outcome	OR (95% CI)	Adjusted for*			
		Preterm delivery	Age at pregnancy	Atopic dermatitis	Parity
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Atopic dermatitis	3.4 (1.7-6.4)	3.4 (1.7-6.4)	3.4 (1.7-6.4)	-	3.4 (1.7-6.4)
Pregnancy**	10.5 (3.6-30.5)	10.6 (3.6-30.7)	10.5 (3.6-30.3)	-	11.0 (3.7-32.2)
Herpes labialis	0.9 (0.6-1.3)	0.9 (0.6-1.3)	0.9 (0.6-1.3)	0.8 (0.6-1.3)	0.9 (0.6-1.3)
Herpes genitalis	1.8 (0.9-3.5)	1.8 (0.7-3.6)	1.8 (0.9-3.5)	1.8 (0.8-3.5)	1.9 (0.9-3.7)
Genital warts	1.2 (0.7-2.1)	1.2 (0.7-2.1)	1.2 (0.7-2.1)	1.2 (0.7-2.1)	1.2 (0.7-2.0)
Vaginitis	1.2 (0.8-1.8)	1.2 (0.8-1.8)	1.2 (0.8-1.8)	1.1 (0.7-1.8)	1.2 (0.8-1.9)
Postpartum, any [†]	0.9 (0.5-1.6)	0.9 (0.5-1.6)	0.9 (0.5-1.6)	0.9 (0.5-1.53)	0.9 (0.7-1.0)
Perineal ^{††}	9.5 (3.0-30.0)	9.4 (2.9-29.5)	9.5 (2.9-30.0)	10.2 (3.1-32.1)	8.8 (2.7-27.7)

* ORs adjusted separately for preterm delivery (yes/no), age at pregnancy (<30, 30-34, 35+ years), a history of physician-diagnosed atopic dermatitis (never, before pregnancy only, before and during pregnancy) if not the outcome, or parity (nullipara, multipara).

** Includes atopic dermatitis before and during pregnancy (see Table 1).

† Any of 17 the postpartum problems presented in table 2. The prevalence was 16.2%.

†† Includes the only 3 mutation-associated problems among 17 problems (marked † in Table 2). The OR adjusted for both age at pregnancy and parity was 8.8 (95% CI 2.7-28.0).

Table S4. *FLG* mutation carriers according to age at pregnancy and parity, Danish National Birth Cohort 1996-2002.

<i>FLG</i> mutation carriers			
	Table 2 sample N=1336 carriers/n (%)	Table 1 sample N=1551 carriers/n (%)	Entire sample N=1837 carriers/n (%)
Age at pregnancy (years)			
<30	59/734 (8.0)	70/847 (8.3)	86/1004 (8.6)
30-34	37/453 (8.2)	43/535 (8.0)	55/624 (8.8)
35+	13/149 (8.7)	14/169 (11.0)	19/209 (9.1)
	P=0.96	P=0.99	P=0.96
Parity			
Nullipara	62/632 (9.8)	70/716 (9.8)	89/884 (10.1)
Para	47/704 (6.7)	57/835 (6.8)	71/953 (7.5)
	P=0.04	P=0.03	P=0.05