Minimal changes in the B- and T-cell compartments of school-aged children with haploinsufficiency of filaggrin: The Generation R Study

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Abstract

Background: Mutations in the filaggrin gene (FLG) affect epidermal barrier function and increase the risk of atopic dermatitis (AD). We hypothesized that FLG mutations affect immune cell composition in a general pediatric population. Therefore, we investigated if school-aged children with and without FLG mutations have differences in immune cell numbers. Methods: This study was embedded in a population-based prospective cohort study, the Generation R Study, and included 523 children of European genetic ancestry aged 10 years. The most common FLG mutations in the European population (R501X, S1085CfsX36, R2447X and S3247X) were genotyped. Additionally, 11-color flow cytometry was performed on peripheral blood samples to determine helper T (Th), regulatory T (Treg) and CD27+ and CD27- memory B cells. Sensitivity analysis was performed in 102 AD cases, assessed by parental questionnaires. Results: FLG mutations were observed in 8.4% of the total population and in 15.7% of the AD cases. Children with any FLG mutation had higher Th22 cell numbers compared to FLG wild-type children. Children with and without FLG mutations had no difference in Th1, Th2, Th17, Treg or memory B cell numbers. Furthermore, in children with AD, FLG mutation carriership was not associated with differences in T- and B-cells or their subsets. Conclusions: School-aged children with FLG mutations have higher Th22 cell, which might suggest an immunological defense mechanism to an altered skin barrier function. No associations between Th or Treg cells and FLG mutations suggests that FLG mutations do not otherwise affect immune composition in a general pediatric population.

TITLE PAGE

Title

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Conflict of Interest Statement:

The Generation R Study is conducted by Erasmus MC University Medical Center Rotterdam in close collaboration with the School of Law and Faculty of Social Sciences of Erasmus University Rotterdam, the Municipal Health Service Rotterdam Metropolitan Area, the Rotterdam Homecare Foundation, and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond. The Dept Immunology of the Erasmus MC funded the immunological measurements. LD received funding from the European Union's Horizon 2020 research and innovation programme (LIFECYCLE, grant agreement No 733206, 2016; EUCAN-Connect grant agreement No 824989; ATHLETE, grant agreement No 874583). MCvZ is supported by the Australian National Health and Medical Research Council (NHMRC, Senior Research Fellowship 1117687). All authors declare that no competing interests exist. The Department of Dermatology of the Erasmus MC University Medical Center Rotterdam received an unrestricted grant from Micreos Human Health, the Netherlands.

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ABSTRACT

Background: Mutations in the filaggrin gene (*FLG*) affect epidermal barrier function and increase the risk of atopic dermatitis (AD). We hypothesized that *FLG* mutations affect immune cell composition in a general pediatric population. Therefore, we investigated if school-aged children with and without *FLG*mutations have differences in immune cell numbers.

Methods: This study was embedded in a population-based prospective cohort study, the Generation R Study, and included 523 children of European genetic ancestry aged 10 years. The most common*FLG* mutations in the European population (R501X, S1085CfsX36, R2447X and S3247X) were genotyped. Additionally, 11-color flow cytometry was performed on peripheral blood samples to determine helper T (Th), regulatory T (Treg) and $CD27^+$ and $CD27^-$ memory B cells. Sensitivity analysis was performed in 102 AD cases, assessed by parental questionnaires.

Results: *FLG* mutations were observed in 8.4% of the total population and in 15.7% of the AD cases. Children with any*FLG* mutation had higher Th22 cell numbers compared to *FLG*wild-type children. Children with and without *FLG* mutations had no difference in Th1, Th2, Th17, Treg or memory B cell numbers. Furthermore, in children with AD, *FLG* mutation carriership was not associated with differences in T- and B-cells or their subsets.

Conclusions: School-aged children with *FLG* mutations have higher Th22 cell, which might suggest an immunological defense mechanism to an altered skin barrier function. No associations between Th or Treg cells and *FLG* mutations suggests that *FLG*mutations do not otherwise affect immune composition in a general pediatric population.

KEYS MESSAGE

This study in a general pediatric population showed that filaggrin haploinsufficiency is associated with higher Th22, but not with other B- and T-cell subsets. *FLG* mutations did not affect immune cell composition in a subset of children with AD. Therefore, FLG mutations pose a risk to allergy development, but do not directly affect or confound immune measurements in children with atopic dermatitis.

INTRODUCTION

Filaggrin is a filament-associated protein that is encoded by the Filaggrin gene (*FLG*),[1] and is an important contributor to the preservation of the skin barrier.[1,2] Approximately 10% of the European population is heterozygote carrier of a disrupting mutation in*FLG* .[3] Both complete loss-of-function and reduced functional activity of filaggrin lead to destruction of the stratum corneum (SC) and to skin barrier dysfunction.[2,4] This barrier dysfunction due to*FLG* mutations is presumed to be caused by lower numbers of tight junctions, reduced density of the protein corneodesmosin and impaired maturation and excretion of lamellar bodies in the epidermis which are important in maintaining cell-to-cell integrity.[1]

Failure in barrier function through mutations in *FLG* results in increased skin permeability for percutaneous transfer of exogenous particles including allergens and pollutants including an increased permeability of the skin for percutaneous protein transfer.[1,2,4] Accordingly, *FLG* mutations are the strongest genetic risk factor for atopic dermatitis (AD), predisposing to a form of AD that starts in early infancy and persists into adulthood.[2,3,5] Previous meta-analysis showed that *FLG* haploinsuffiency results in an odds ratio (OR) of 3.12 for AD.[6] In addition, *FLG* mutations in AD are associated with a higher incidence of skin infections or superinfections such as eczema herpeticum and a higher likelihood of having asthma, inhalant or food allergies.[1,7-9]

The increased permeability of the skin as a result of *FLG*mutations is thought to affect immune responses and maturation of adaptive immune cells. Filaggrin is also expressed in the thymus, the primary lymphoid organ in which T cells are formed.[10]Hence, *FLG* mutations potentially affect the peripheral immune cell compartment through effects in skin and thymus, and previous studies observed higher γδT17, T helper (Th) 17 in mice bi-allelic for*FLG* disruption.[10] A case study reported higher numbers of circulating thymus-emigrated regulatory T (Treg) cells, Th2 in with 6 AD patients with a heterozygote *FLG*mutation.[11] Another study with 2 heterozygous, 2 homozygous and 1 compound heterozygous AD patient showed increased Th17

cells in *FLG* -mutation group.[12] On the other hand, literature on the role of B cell dysregulation in AD is scarce and conflicting.[13-16]. It can be hypothesized that mutations in *FLG* can affect B-cell numbers due to skewing of the Th-cell population.

Because of the function of filaggrin in skin barrier tissue and in thymus, the presence of *FLG* mutations might be a contributor to the shaping of T -and B cell maturation in children. However, no studies on this association have been performed in the general pediatric population and only case studies have been performed in AD patients.[11,12] Therefore, we here studied the associations between common *FLG* mutations in the European population and immune cell numbers, as determined using with 11-color flow cytometry, within a population-based birth cohort study including a subgroup of AD patients.

METHODS

Study design

This study was embedded within the Generation R Study, a prospective birth cohort study located in Rotterdam, the Netherlands. The Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam approved the study (MEC-2012-165).[17] Written informed consent was obtained from parents or legal representatives of all children. We included all children with European genetic ancestry[18] with information on filaggrin mutation (homozygous, compound heterozygous, heterozygous or wild type) and information on at least one of the immune cell outcomes. This resulted in a total number of 523 children (Figure 1).

Our subgroup of children with AD, defined as physician-diagnosed eczema from parental questionnaires obtained at the child's age of 10 years ('Was your child ever diagnosed by a physician with atopic dermatitis', 'yes; no') consisted of 102 subjects.[16]

FLG genotype

DNA samples obtained from umbilical cord blood were genotyped by modified Taqman allelic discrimination assays with the use of primers as described previously.[19] The following 4 *FLG*mutations were identified: R501X (rs61816761), S1085CfsX36 (rs41370446), R2447X (rs138726443), and S3247X (rs150597413).[19,20]Children were classified as having a *FLG* mutation if they were homozygous, compound heterozygous or heterozygous for any of the four mutations. Children without any of the four mutations were classified as wild type.

Immune cell numbers

Peripheral blood samples from children were obtained at the age of 10 years.[16] Absolute counts of $CD3^+$ T cells and $CD19^+$ B cells per μLblood were determined with diagnostic lyse-no-wash protocol and detailed immunophenotyping was performed with 11-color flow cytometry (LSRII Fortessa, BD Biosciences). We determined naive ($CD45RO^-CCR7^+$), effector memory RO-positive T cells (TemRO; $CD45RO^+CCR7^-$) and effector memory RA-positive T cells (TemRA; $CD45RO^-CCR7^-$) within $CD4^+$ and $CD8^+$lineages.[16,21,22] Within Treg cells, the differentiation in naive ($CD45RA^+$) and memory ($CD45RA^-$) was determined.[16] Finally, the following T helper (Th) cell subsets ($CD4^+CD45RA^-$) were determined after exclusion of Treg cells: Th1 ($CCR6^-CXCR3^+CCR4^-$), Th2 ($CCR6^-CXCR3^-CCR4^+$), Th17 ($CCR6^+CXCR3^-CCR4^+CCR10^-$), Th17.1($CCR6^+CXCR3^+CCR4^-$) and Th22 ($CCR6^+CXCR3^-CCR4^+CCR10^+$).s In addition $CD27^+$ and $CD27^-IgG^+$, IgA^+, $IgE^+CD19^+CD38^{dim}IgD^-$memory B cell subsets were defined.[16] Gating strategies for immune cell determination were published previously.[16]

Statistical Analyses

First, characteristics of the study population were determined, stratified for *FLG* mutations . P-values for determining differences between the categorical variables between the two groups were calculated with chi-squared tests. Next, median cell numbers with interquartile range (IQR) were determined. Differences in cell numbers between children with and without *FLG* mutations were determined with the non-parametric Mann-Whitney U tests. Sensitivity analyses on the associations of *FLG* genotype with immune cell numbers were

performed within children who were diagnosed with AD. No adjustment for multiple testing was performed because of strong correlation between the immune cells studied. Statistical analyses were performed with SPSS version 21.0 (IBM Corp.) and R version 3.3.3 (R Foundation for Statistical Computing).

RESULTS

Study population

Characteristics of the study population are presented in Table 1. Within the total group of 523 children with European ancestry, filaggrin mutations were detected in 44 (8.4%) children, including 3 biallelic mutations (2 compound heterozygous and 1 homozygous). The proportion of patients with AD was lower in the wild-type group compared to the group with *FLG* mutations (20% versus 42%; p<0.01). Within the atopic dermatitis population, 15.7% children had a *FLG*mutation, including one biallelic mutation (homozygous). No differences were observed between other atopic diseases, including asthma, inhalant and food allergy and *FLG* mutation status (data not shown).

Higher Th22 cell counts in children with *FLG* mutations

Children with *FLG* mutation had higher Th22 cell numbers compared to children of the wild-type population (Figure 2A, eTable1). The median cell number within the *FLG* mutation group was 5.60/μL (IQR 4.04;8.94) and within the wild-type group 4.54/μL (IQR 2.50;7.38, p =0.03). In contrast, when studying this association in the atopic dermatitis population, no significant associations were observed (eFigure 1A).

No associations between Th1, Th2, Th17 and Treg and *FLG*mutations

No differences in median cell numbers between the *FLG* mutation and wild type group were observed for Th1, Th2, Th17 and Treg (Figure 2A, eTable 1). This was similar in the sensitivity analyses that only included children with atopic dermatitis (eFigure 1A). No differences in median cell numbers between the *FLG* mutation and the wild type group were observed for the effector memory $CD4^+$ and $CD8^+$ T cell subsets: naive, Tcm, TemRA, TemRO (Figure 2B, eFigure 1B).

No associations between memory B cells and *FLG* mutations

No differences in median cell numbers between the *FLG* mutation and the wild type group were observed for total B cells and naive mature B cells (eTable 1). In addition, no associations between *FLG*mutations and the following $CD27^+$ and $CD27^-$ memory B cell subsets were observed: IgA^+, IgE^+, IgG^+, IgM^+ (Figure 3). Similarly, no changes were observed in the sensitivity analyses, which only included children with atopic dermatitis (eFigure 2, eTable 1).

DISCUSSION

In this population-based study among children of European genetic ancestry, we observed a prevalence of 8.4% for *FLG* mutations. In addition, we demonstrated that children with *FLG* mutations had higher Th22 cell numbers than children without *FLG* mutations. In contrast, the Th1, Th2, Th17, Treg and memory B cell numbers were comparable between children with and without *FLG* mutations. In addition, among children with AD, those with or without *FLG*mutations had no differences in B or T cell subsets.

Comparison with literature and interpretation

All previous studies on *FLG* mutations and immune cell numbers have been performed within mice models or smaller numbers of AD patients.[10-12,23] This is the first study that provides insight in the role of *FLG* mutations on immune cell numbers in school-aged children of a general population. The setting of this study within a population-based pediatric cohort study is unique to study the association of *FLG* on immune cell numbers in a general population.

In line with a previous study in mice, we observed higher Th22 cell numbers in children with *FLG* mutations than in to children with wild type *FLG* alleles. [23] Th22 cells are characterized by the production of IL-22 and contributes to skin integrity.[24,25] Moreover, IL-22 is known for its role in the defense of different pathogens

in the skin by the production of antimicrobial proteins.[24,25] In AD, acute skin lesions are associated with an upregulated Th22 immune response.[26,27] We speculate that the observed higher number of Th22 cells is an immunological defense mechanism to the damaged skin barrier. Nevertheless, future studies should confirm this finding. When studying Th22 numbers only in AD children, no differences were observed between both groups. This is could be explained by the fact that children with AD have higher Th22 independent of their *FLG* genotype. [26] In addition, the expression of the protein filaggrin is decreased in AD which can cause Th22 alterations through skin barrier dysfunction independent of *FLG* mutation status.[1,5] Unfortunately, literature on the effect of *FLG* mutations on the numbers of Th22 in AD is lacking.

In contrast with small previous case studies in AD, we did not observe differences in Th2, Th17 and Treg cell numbers between children with and without *FLG* mutations both in the total study population and in the subgroup of patients with AD. [10-12,23] The associations of Th2, Th17 and Treg cell numbers with filaggrin in previous studies were explained by increased TSLP activity due to impaired skin barrier and impaired thymus functioning.[11,28] In the thymus, an altered filaggrin expression affects maturation of T cells and induces dendritic cells to stimulate the differentiation of naive Treg cells into functionally different Treg cells. [10,29] The discrepancies between previous studies and our current study could be explained by differences in investigated populations and species. The mice studies studied *FLG* null mutations, in which no expression of filaggrin is present.[10] This is in contrast with our population, encompassing mainly haploinsufficient children which leads to a 50% reduction in filaggrin expression. [1] In addition, *FLG* knockdown skin equivalents in previous studies could represent a different immunological setting than is present in human skin. [23] Furthermore, it is likely that previous results on immune cell numbers in AD populations are affected by disease severity. Namely, immune cells in active AD skin can induce downregulation of filaggrin protein expression in the skin independent of *FLG* mutations, subsequently affecting immune cell composition. Although we do not have information on disease severity in our AD population, this study included a relatively healthy cohort in which we expect relatively mild AD. A more severe AD phenotype has been linked to homozygous and compound heterozygous *FLG* mutation phenotype.[1] In addition, activated immune cells in more active AD skin can in itself downregulate filaggrin protein expression in the skin independent of *FLG* mutations and thereby affect skin barrier function and subsequently immune cell alteration.[1,5] In accordance, a previous study in AD patients observed that circulating Treg cell numbers were associated with AD activity and not with *FLG* genotype.[11] In addition, we previously showed that children with AD had higher Th17 and Treg memory cell numbers.[16] Therefore, alteration in immune cell numbers is probably not only dependent on *FLG* mutation genotype, but also on AD severity and epigenetic and environmental factors that affect these two, as our data suggest.

In addition, we did not observe differences in memory B cell numbers between children with and without *FLG* mutations, nor any associations between memory B cell numbers and AD in our previous study.[16] No previous studies have investigated B cell subsets in relation to *FLG* mutations. Possibly, T cells are more affected by *FLG* mutations than B cells because of the effects of filaggrin in the thymus.[10]

Methodological considerations

A major strength is that this study investigated the association between *FLG* genotype and a large panel of B and T cells in the general population for the first time. We had detailed and extensive information on immune cell numbers from 11-color flow cytometry and obtained objective information on genetic ancestry. However, the following limitations need to be addressed. First, the AD population for the sensitivity analyses was relatively small which could have limited the power in the statistical analyses. Nevertheless, in comparison to previous studies, only including a maximum of 6 AD patients with *FLG* mutations, this is the largest study on *FLG* mutations in both the general population and AD patients. Second, our AD population was defined by ever having physician diagnosed AD before or at the age of 10 years. Therefore, it is likely that a subset of the children has outgrown AD at the age of 10 and this might affect their immunophenotype. In addition, the use of questionnaires for AD diagnosis could have introduced recall bias. Third, as mentioned previously, our study included the four most common *FLG* mutations in the European population. To prevent misclassification, we selected children with genetic European ancestry for the current study. Although the

choice for including the most common *FLG* mutations in European populations is in line with previous studies [11,12], other less frequent *FLG* mutations could exist in low numbers since up to 113*FLG* mutations resulting in premature protein termination have been described. A recent study including patients with AD and Ichthyosis Vulgaris (IV), showed that screening the entire encoding region of*FLG* for mutations led to an improvement of the diagnostic yield.[30] Furthermore, biallelic genotypes were only present in 3 children of the study population and therefore not studied separately. Since homozygous or compound heterozygous mutations lead to complete absence of filaggrin expression, these mutations might have different effects on immune cell numbers compared to heterozygous*FLG* mutations which lead to 50% reduction in filaggrin expression.[1] Therefore, studies investigating biallelic mutations compared to heterozygous *FLG* mutations and wild type *FLG* are needed. Furthermore, as this is the first study in a general cohort addressing the association between *FLG*mutation and immune cell numbers, future studies are needed for validation of our results.

In conclusion, within the general population, school-aged children with*FLG* mutations have higher Th22 cell numbers. Furthermore, children with and without *FLG* haploinsufficiency did not differ in other B and T cell subsets. This suggests that with age, other factors such as environmental exposure contribute to risk that*FLG* mutations poses to the development of allergy.

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ETHICAL APPROVAL STATEMENT

The study was conducted according to the principles of the Declaration of Helsinki. The Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam approved the study (MEC-2012-165). Written informed consent was obtained from parents or legal representatives of all children.

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TABLES

Table 1. Descriptives of the study population

		Total population	**Total population**	**Total population**	**Total population**	**Sensitivity analyses**
Child characteristics	**Total (n=523)**	**Wildtype population (n=479)**	***FLG* mutation population (n=44)**	**P-value**	**Missing in total study population (N, %)**	**Atopic dermatitis (n=102)**
Sex (N, %)				1.0	0.0	
Female	280 (53.5)	256 (53.4)	24 (54.5)			48 (47.1)
Male	243 (46.5)	223 (46.6)	20 (45.5)			54 (52.9)
FLG mutations (N, %)				–	0.0	
Wildtype	479 (91.6)	479 (100.0)	–			86 (84.3)
1 or more mutations	44 (8.4) +	–	44 (100.0)			16 (15.7)
Type *FLG* mutations (N, %)						
S1085CfsX36 (rs41370446)	22 (4.2)	–	22 (4.2)	–	0.0	7 (6.9)
R2447X (rs138726443)	6 (1.1)	–	6 (1.1)	–	0.2	1 (1.0)
R501X (rs61816761)	18 (3.5)[2]	–	18 (3.5)[2]	–	0.4	9 (9.0) ++
S3247X (rs150597413)	0 (0.0)	–	0 (0.0)	–	0.4	0 (0.0)
Ever physician diagnosed atopic dermatitis (N, %)§	102 (22.2)	86 (20.4)	16 (42.1)	**0.004**	12.0	102 (100.0)

		Total population	**Total population**	**Total population**	**Total population**	**Sensitivity analyses**
Table 1 represents the child and maternal characteristics for the study population stratified for *FLG* mutations. Values are based on the non-imputed dataset and represented as number (%). Chi-squared tests were conducted to examine possible differences in baseline characteristics between the different genotypes. Abbreviations: *FLG*, filaggrin gene; N, number; + Including 3 biallelic mutations (2 compound heterozygous and 1 homozygous) ++ Including one biallelic mutations (homozygous) § Based on parental-reported questionnaires obtained at the child's age of 10 years: ever atopic	Table 1 represents the child and maternal characteristics for the study population stratified for *FLG* mutations. Values are based on the non-imputed dataset and represented as number (%). Chi-squared tests were conducted to examine possible differences in baseline characteristics between the different genotypes. Abbreviations: *FLG*, filaggrin gene; N, number; + Including 3 biallelic mutations (2 compound heterozygous and 1 homozygous) ++ Including one biallelic mutations (homozygous) § Based on parental-reported questionnaires obtained at the child's age of 10 years: ever atopic	Table 1 represents the child and maternal characteristics for the study population stratified for *FLG* mutations. Values are based on the non-imputed dataset and represented as number (%). Chi-squared tests were conducted to examine possible differences in baseline characteristics between the different genotypes. Abbreviations: *FLG*, filaggrin gene; N, number; + Including 3 biallelic mutations (2 compound heterozygous and 1 homozygous) ++ Including one biallelic mutations (homozygous) § Based on parental-reported questionnaires obtained at the child's age of 10 years: ever atopic	Table 1 represents the child and maternal characteristics for the study population stratified for *FLG* mutations. Values are based on the non-imputed dataset and represented as number (%). Chi-squared tests were conducted to examine possible differences in baseline characteristics between the different genotypes. Abbreviations: *FLG*, filaggrin gene; N, number; + Including 3 biallelic mutations (2 compound heterozygous and 1 homozygous) ++ Including one biallelic mutations (homozygous) § Based on parental-reported questionnaires obtained at the child's age of 10 years: ever atopic	Table 1 represents the child and maternal characteristics for the study population stratified for *FLG* mutations. Values are based on the non-imputed dataset and represented as number (%). Chi-squared tests were conducted to examine possible differences in baseline characteristics between the different genotypes. Abbreviations: *FLG*, filaggrin gene; N, number; + Including 3 biallelic mutations (2 compound heterozygous and 1 homozygous) ++ Including one biallelic mutations (homozygous) § Based on parental-reported questionnaires obtained at the child's age of 10 years: ever atopic	Table 1 represents the child and maternal characteristics for the study population stratified for *FLG* mutations. Values are based on the non-imputed dataset and represented as number (%). Chi-squared tests were conducted to examine possible differences in baseline characteristics between the different genotypes. Abbreviations: *FLG*, filaggrin gene; N, number; + Including 3 biallelic mutations (2 compound heterozygous and 1 homozygous) ++ Including one biallelic mutations (homozygous) § Based on parental-reported questionnaires obtained at the child's age of 10 years: ever atopic	Table 1 represents the child and maternal characteristics for the study population stratified for *FLG* mutations. Values are based on the non-imputed dataset and represented as number (%). Chi-squared tests were conducted to examine possible differences in baseline characteristics between the different genotypes. Abbreviations: *FLG*, filaggrin gene; N, number; + Including 3 biallelic mutations (2 compound heterozygous and 1 homozygous) ++ Including one biallelic mutations (homozygous) § Based on parental-reported questionnaires obtained at the child's age of 10 years: ever atopic

	Total population	Total population	Total population	Total population	Sensitivity analyses

FIGURE LEGENDS

Figure 1. Flow chart of participants included in the study.

Abbreviations: Th, helper T cell; Treg, regulatory T cell.; Tcm, central memory T lymphocytes; TemRA, effector memory RA-positive T lymphocytes; TemRO, effector memory RO-positive T lymphocytes

Figure 2. Absolute numbers of blood T cell subsets stratified by *Filaggrin Gene* mutation status

Figure 2A shows the median (IQR) cell count per µLblood for Th and Treg cell numbers stratified for *FLG* mutation. *Figure 2B* shows the median (IQR) cell count per µLblood for $CD4^+$ and $CD8^+$ effector memory T cell numbers stratified for *FLG* mutation. *Abbreviations* : IQR, interquartile range; Tcm, central memory T lymphocytes; TemRA, effector memory RA-positive T lymphocytes; TemRO, effector memory RO-positive T lymphocytes; Th, helper T cell; Treg, regulatory T cell. * denotes a two-sided P-value <0.05. Supplementary Table 1 and 3 show absolute numbers and p-values.

Figure 3. Absolute numbers of blood memory B cell subsets stratified by *Filaggrin Gene* mutation status *Figure 3*shows the median (IQR) cell count per µL blood for B memory cell numbers stratified for *FLG* mutation. Abbreviations: IQR, interquartile range; Ig, Immunoglobulin. Supplementary Table 2 shows absolute numbers and p-values.