

Letter to the Editor

Antibody-encoding repertoires of bone marrow and peripheral blood—a focus on IgE

To the Editor:

Allergic responses are initiated by the interaction between antigen and IgEs carried on effector cells such as mast cells and basophils. However, the detailed molecular nature of IgE is not amenable to analysis using many of the most commonly used methodologies because B cells/plasma cells that produce them are scarce. Lately, next-generation sequencing (NGS) has allowed us to analyze immunoglobulin-encoding genes at a rate unimaginable in the past.¹ Although such investigations tend to focus on antibody repertoires other than IgE, the technology has already enhanced our understanding of IgE responses in allergic disease (see [Table E1](#) in this article's Online Repository at www.jacionline.org). Many antibody sequencing studies focus on repertoires encoded by cells in peripheral blood (PB), a readily available source of antibody-producing cells. However, studies have defined differences between repertoires of immunoglobulin-encoding genes of PB and those encoded in various immunologically active tissues.² Furthermore, a recent study demonstrated that IgE produced by cells in PB poorly represented circulating IgE.³ In all, because much IgE is produced by cells localized to tissues beyond PB, analysis of this disease-causing isotype is lacking in breadth and relevance.

To address the nature of immunoglobulin repertoires, including IgE, at a site readily available for analysis (PB) and at a major site of antibody production (bone marrow [BM]), we have now examined the immunoglobulin heavy chain repertoire, a major carrier of antibody specificity,⁴ encoded by cells residing at these sites. Samples were collected from 6 allergic subjects in biological duplicates so as to specifically allow analysis of the IgE repertoire in subjects in whom it actually inflicts disease. These subjects (see [Table E2](#) in this article's Online Repository at www.jacionline.org) suffered from allergic rhinitis but had not undergone specific immunotherapy (SIT). Samples were collected out of season of most seasonal allergens, but stimulation with perennial allergens around the time of sample collection cannot be excluded. At the time of sample collection, these subjects, though, had no symptoms of an ongoing allergic response. Sequencing (MiSeq Technology; Illumina, Inc, San Diego, Calif) and analysis were performed as outlined in the [Methods](#) section in the Online Repository at www.jacionline.org (see also [Tables E3 and E4](#) and [Figs E1 and E2](#) in this article's Online Repository at www.jacionline.org).

Sequence analysis revealed a large number of clones derived from a diversity of germline genes. Repertoires of IgA, IgG, and IgM of BM and PB showed very similar composition in terms of IGHV gene, IGHJ gene, and IGHD subgroup usage, and CDRH3 length distributions (see [Tables E5 and E6](#) and [Figs E3-E6](#) in this article's Online Repository at www.jacionline.org). IgA, IgG, and IgM repertoires in BM were more mutated than their corresponding repertoires encoded in PB (see [Figs E7 and E8](#) in

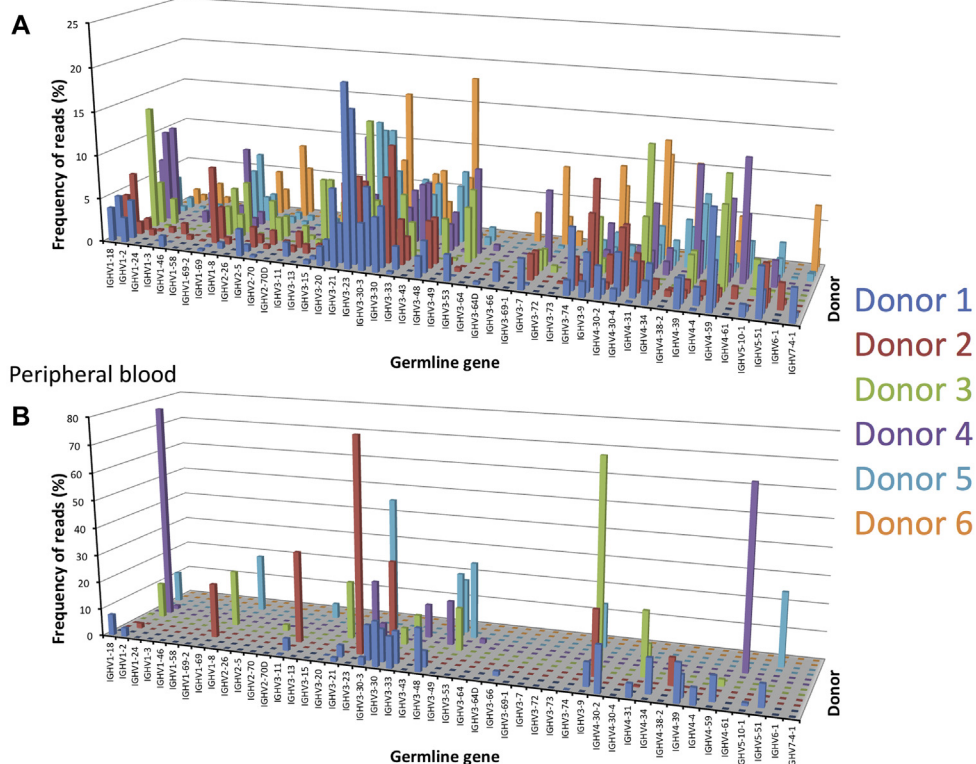
this article's Online Repository at www.jacionline.org). Analysis of duplicated samples of BM and PB demonstrated an appreciable degree of reproducibility in terms of overlap in these samples' description of the IgA, IgG, and IgM repertoires ([Fig 1, C](#); see [Tables E5 and E6](#) and [Figs E3 and E5](#)). Furthermore, a substantial clonal overlap was identified between samples obtained from BM and PB ([Fig 2](#)). Both sample types thus appeared to adequately describe a sizable fraction of the available repertoires. Furthermore, a small but detectable fraction of clones from IgA and IgG repertoires, in particular, were also detected in other isotypes' repertoires (except IgE) ([Fig 2](#)).

In stark contrast to the reproducibility of IgA, IgG, and IgM repertoires of BM and PB, we found that samples of PB of a commonly available volume (10 mL) did not adequately describe the IgE repertoire. Very few, entirely different rearrangements were in general found in biological duplicate samples. The sequences' germline gene origin and the length of their encoded third hypervariable loop differed dramatically, and 1 of 6 donors lacked amplifiable IgE-encoding rearrangements in PB altogether. In contrast, the IgE-encoding repertoires found in duplicate BM samples were larger in size, reproducible in composition, and more diverse in terms of germline gene usage and length of the encoded third hypervariable loop ([Fig 1](#); see [Tables E5 and E6](#) and [Figs E3 and E9](#) in this article's Online Repository at www.jacionline.org). Clonally diversified sequences were identified among these sequences (see [Fig E10](#) in this article's Online Repository at www.jacionline.org). However, irrespective of tissue origin, clustering analysis of transcript populations largely failed to associate IgE-encoding transcript populations to the donor's transcriptomes encoding other isotypes ([Figs 1, C, and 2](#)), suggesting that IgE's clonal origin is to a large degree different from those of other isotypes. In summary, IgE repertoires of BM samples are more diverse than their corresponding PB samples. Individual PB samples of a commonly available size not only poorly describe the IgE-encoding transcriptome but also substantially underestimate the diversity of IgE encoded by transcriptomes in BM, a major site of antibody production. IgE-encoding transcriptomes defined in PB must consequently be used with caution in studies of IgE.

Antigen-specific IgG₄ is an antibody associated with prevention of allergic reactions.⁵ All 6 donors produced IgG₄ (see [Table E2](#)). The sequencing format allowed us to identify IgG₄-encoding transcripts in 5 of 6 donors. IgG₄-encoding sequences in general used a larger number of IGHV genes, a wider distribution of CDRH3 lengths, and mostly represented a larger number of independent clones ([Fig 1, D and E](#); see [Tables E5 and E6](#)) than did the IgE-encoding repertoires of the same samples. The length distribution of IgG₄-encoding CDRH3, IGHV gene usage pattern, and clonal composition of BM were also mostly well replicated between biological duplicate samples (see [Figs E3, E9 and E11](#) in this article's Online Repository at www.jacionline.org), suggesting that each BM sample adequately represented major dominant features of the IgG₄ repertoire. In contrast, such features of the IgG₄ repertoire were not as well reproduced in duplicate samples of PB (see [Figs E3, E9 and E11](#)).

To assess the relationship of the IgE and IgG₄-encoding transcriptomes, both regulated in similar ways,⁶ and transcriptomes

Bone marrow



Peripheral blood

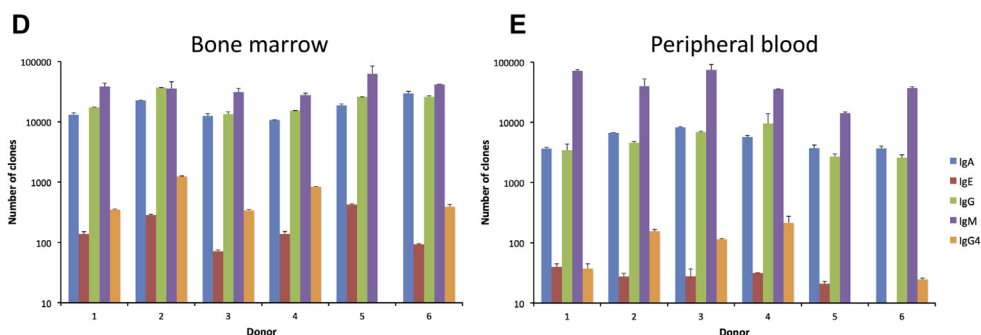
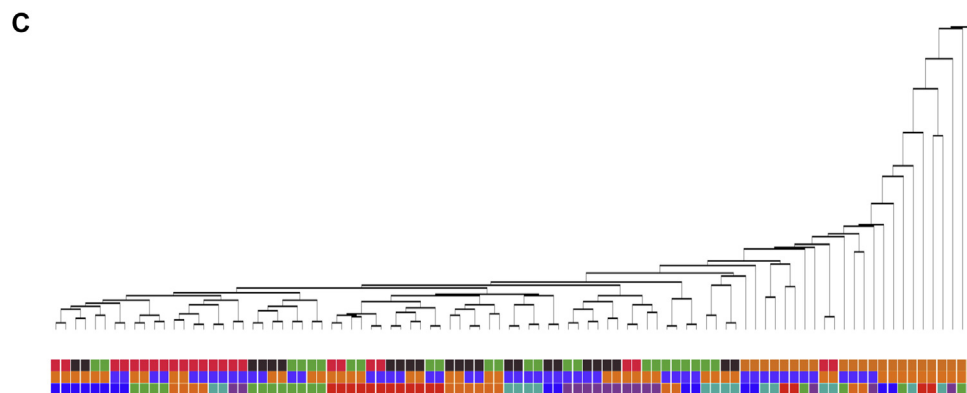
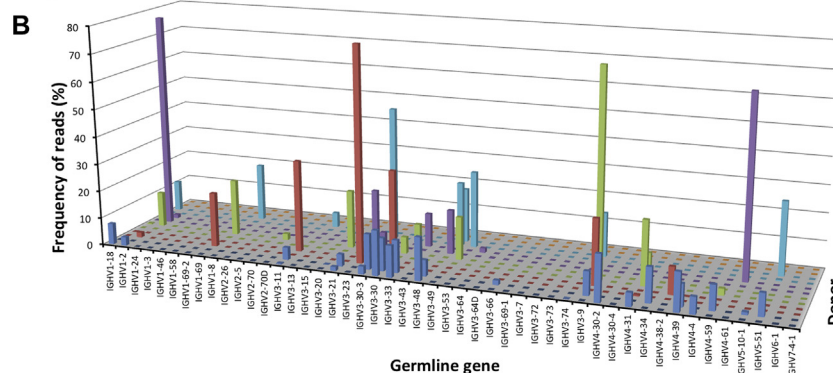


FIG 1. IgE repertoires encoded by cells in BM are more diverse than those encoded by cells in PB. IGHV germline genes origins of IgE-encoding transcripts (present at >0.1%) found in duplicate samples of BM (**A**) and PB (**B**) of 6 donors. Hierarchical clustering (**C**) of V gene usage percentages of 70 genes (Euclidean distances, average linkage) of duplicate samples encoding different isotypes (*top boxes*: IgA, *black*; IgE, *orange*; IgG, *green*; IgM, *red*) in different tissues (*middle boxes*: PB, *orange*; BM, *blue*) as derived from

encoding isotypes other than IgE, we analyzed the clonal overlap between these repertoires. The analysis used the 50 most prominent IgE clones and the entire set of sequences encoding other isotypes. The algorithm used to assess the overlap between different transcriptomes required that matching sequences originated in the same IGHV gene (not considering allelic variation) and had the same CDRH3 length but it allowed for up to 20% diversification of CDRH3 at the nucleotide level, to minimize false negatives caused by somatic hypermutation targeting CDRH3. Despite using these relaxed criteria, a very minor part (<0.2%) of the transcriptomes encoding IgG₄ (as well as those encoding IgA, IgG, and IgM) in BM and PB showed evidence of clonal relatedness to the IgE-encoding population. In fact, in most cases, no related sequences were identified at all (see Fig E12 in this article's Online Repository at www.jacionline.org). Altogether these findings indicate that cells of BM, a major site of antibody production, and PB of these subjects who have not undergone SIT mostly produce IgE with no clonal relatedness to clones that encode other major antibody isotypes.

SIT induces allergen-specific antibodies, often IgG₄,⁵ only some of which (most likely those that target the same epitopes as those bound by IgE) protect against allergen-mediated reactions.⁷ We hypothesize, on the basis of present observations, that immunotherapy that is able to exploit the same clonal rearrangements as those used for production of IgE also for production of protective antibodies (eg, IgG₄) will be particularly successful in alleviating symptoms and in providing cure. We therefore hypothesize that sequence-based analysis demonstrating enhanced clonal overlap between IgE and IgG₄ (or other antibody isotypes/subclasses) may provide objective biomarkers in the analysis of SIT efficacy.

Antibody-encoding gene sequences as such do not *per se* define the specificity of an encoded antibody. Indeed, the vast diversity of the immune repertoire suggests that the response to a complex antigen composed of multiple epitopes, like a protein, will be diverse in terms of sequence. In several instances, though, specific sequence features (stereotyped rearrangements) are highly enriched in a given antigen-specific antibody response.⁸ IgEs specific for grass pollen allergen Phl p 2 often display a 10-residue long CDRH3 with glycine as the fifth residue, and are encoded by genes originating from IGHV4-30-4 or IGHV4-31 germline genes.⁹ When searching for such sequences in the presently investigated transcriptomes, we identified 3 donors of BM (donors 2, 3, 6) and 1 donor of PB (donor 3) whose samples contained large frequencies of such IgE-encoding transcripts (see Fig E13 in this article's Online Repository at www.jacionline.org). Such sequences were very rarely represented in these subjects' primary repertoires (transcripts encoding unmutated IgM), and in IgG (Fig E13). Importantly, these 3 donors, but not donors 1, 4, and 6, were also seropositive for Phl p 2-specific IgE (see Table E7 in this article's Online Repository at www.jacionline.org). In summary, there was a correspondence between the presence of a particular rearrangement and

the presence of allergen-specific, circulating IgE. This finding sets the foundation for using antibody variable domain sequences as objective biomarkers in those instances in which stereotyped rearrangements can be associated with a specific immune response. By greatly expanding our current small set of human allergen-specific IgE,¹⁰ we envisage that we will be able to identify additional stereotyped allergen-specific IgE responses, signatures that will find use as biomarkers in the future.

In conclusion, IgE repertoires of BM samples are more diverse and more reproducible in their composition than those of commonly sized PB samples. Samples from BM should consequently be preferred over those from PB in future studies of IgE and its sequence-based biomarkers. Studies of allergen-specific antibodies of isotypes other than IgE must not *a priori* be used to define the character of IgE because the degree of overlap between these repertoires is very small. Furthermore, we propose that there is a window of opportunity for NGS to assist in the assessment of successful SIT, if such treatment is able to induce a high degree of clonal overlap between IgE and other isotypes, and the IgG₄ subclass in particular. Finally, we envisage that BM-encoded repertoires, through their larger diversity, may also be an excellent source of IgE diversity for the development of human monoclonal IgE through cellular cloning or combinatorial library approaches,¹⁰ antibodies that will enhance our understanding of IgE-allergen interactions and their outcomes.

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different donors (bottom boxes). Duplicate samples of IgA, IgG, and IgM cocluster, and in several cases samples of these isotypes as derived from 1 donor cocluster. In contrast, duplicate samples representing the IgE-encoding repertoire derived from PB, and to some extent BM, do not cluster together. Number of clones encoding different isotypes identified in 10-mL samples of BM (D) and PB (E). Each value represents the mean ± 1 SD of 2 duplicate biological samples collected simultaneously. PB samples from donor 6 did not contain IgE-encoding transcripts and IgG₄-encoding transcripts were not identified in samples from donor 5.

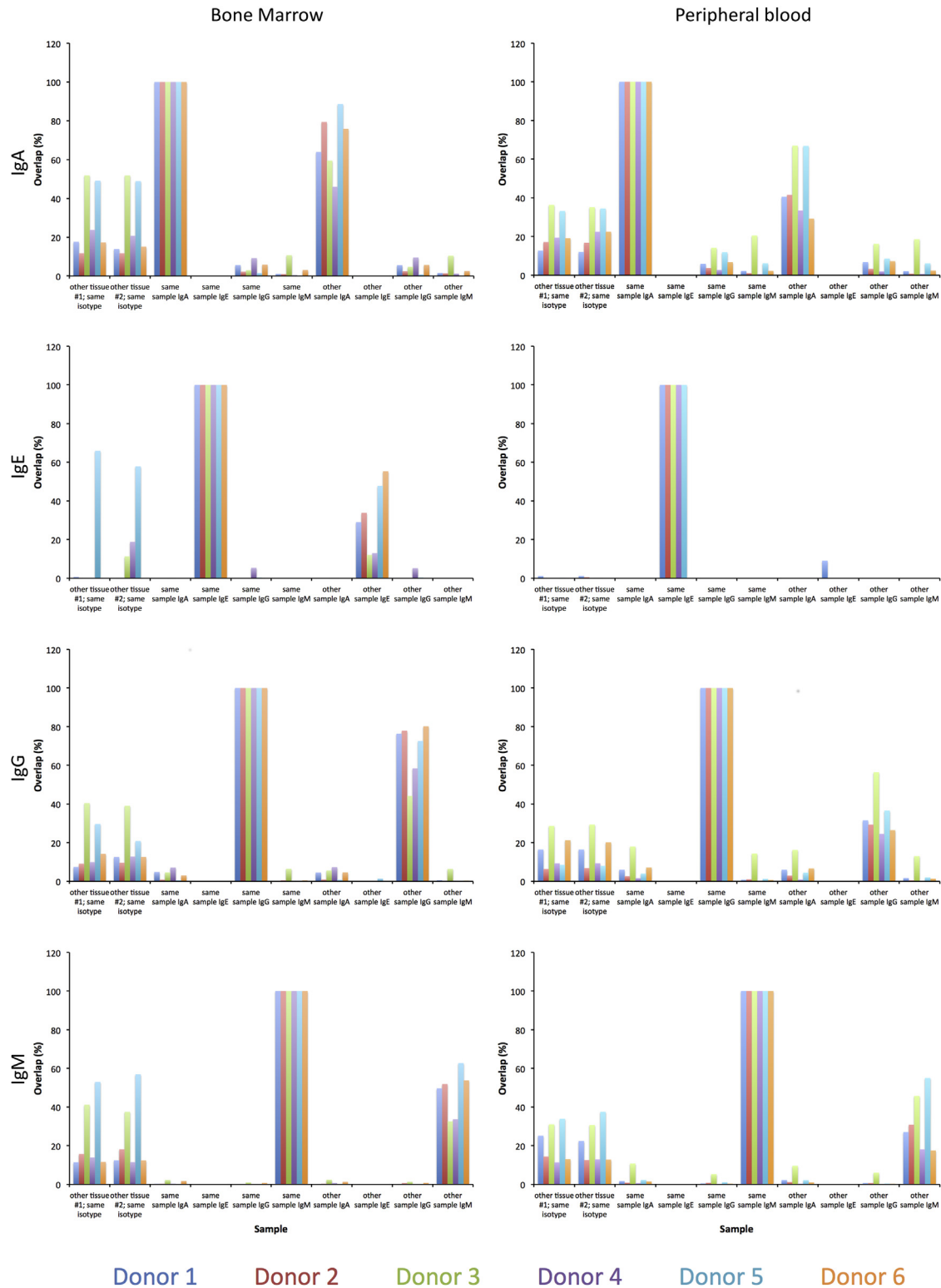


FIG 2. Frequency of transcripts of IgA, IgE, IgG, and IgM encoded by BM and PB and found as related members in duplicate samples of the other tissue (PB and BM, respectively) and in other isotypes of the same tissue type. The analysis included up to 1000 of the commonest transcript variants of each sample (a *variant* was defined as sequence originating from the same germline gene irrespective of allelic variant and with identical CDRH3-encoding nucleotide sequences; each analyzed sequence set must be larger than 0.01% of the largest sequence set in the sample, and it must be represented by at least 2 sequence reads).

om Läkarutbildning och Forskning)), serves as a member on the board from Nares, holds a patent with Nares, receives royalties from Nares, and has stocks from Nares. The rest of the authors declare that they have no relevant conflicts of interest.

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Supplementary information

**Antibody-encoding repertoires of bone marrow and peripheral blood
– a focus on IgE**

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Supplementary Materials and Methods

Patient samples

Samples were collected at Skåne University Hospital, Lund, Sweden, after approval by the regional ethical review board at Lund University. Peripheral blood (PB) (20 mL) and bone marrow (BM) (20 mL aspirated from the iliac crest bone into a heparin rinsed aspiration syringe) samples were obtained from 6 donors diagnosed with allergic rhinitis based on disease history and positive skin prick tests (Supplementary Table EII). Sampling was carried out during the fall, i.e. out of season of most seasonal allergens, but it is recognized that these subjects might have been recently exposed to perennial allergens. Concentrations of IgE and IgG4 were determined at the Skåne University Hospital Laboratory for Clinical Immunology and Transfusion Medicine (Lund, Sweden).

cDNA preparations

Mononuclear cells were isolated from blood (PBMC) and BM (BMC) through density centrifugation using Lymphoprep (Axis-Shield, Oslo, Norway) immediately after sampling. Isolated mononuclear cells were divided into biological duplicates. RNA was purified from PBMC ($6-10 \times 10^6$ cells/sample) using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and from BMC ($11-105 \times 10^6$ cells/sample) using the RNeasy Maxi Kit (Qiagen). Following RNA purification, cDNA was produced from 1 μ g of RNA per samples using the Quantitect Reverse Transcription Kit (Qiagen) and oligo-dT primers.

Library construction and NGS

Transcripts encoding immunoglobulin heavy chain variable domains were amplified using PCR from cDNA corresponding to 200 ng of total RNA. A total of 24 barcoded libraries carrying sequencing adapters optimized for MiSeq sequencing (Illumina, San Diego, CA) were produced. Libraries were produced in two separate rounds of PCR in which 8-nucleotide barcodes and MiSeq sequencing adapters were introduced, respectively. Initial PCR was carried out using isotype-specific primers (Supplementary Table EIII) targeting the region encoding the first constant domain of each isotype in combination with a mix of six 5' primers annealing to sequences encoding the first framework region to allow for

simultaneous amplification of transcripts with origin in all seven IGHV germline gene families. Each isotype was amplified individually, to minimize the risk of PCR crossover errors involving sequences encoding different antibody isotypes, to achieve good amounts of product. All amplifications but two (amplification of the IgE repertoire from donor 6 PB (both samples)) resulted in proper PCR products. The number of cycles needed for PCR amplification varied as amplification of the IgE repertoire in particular required a larger number (approximately 10) of PCR cycles (Supplementary Table EIII) to yield an appropriate, visible product upon agarose gel electrophoresis. The 8-nucleotide barcodes were introduced to allow for separation into donor and sample origin, while 3'-primer usage and finally amplified (non-primer encoded) parts of constant domain encoding transcripts were used for isotype separation. PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA) and quantitated using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Libraries representing transcripts encoding each isotype of donors 1-3 (n=12) and 4-6 (n=12) were finally pooled. Paired end sequencing (2 lanes) was performed using MiSeq technology (Illumina) (MCS 2.4.1.3/RTA 1.18.54) at the National Genomics Infrastructure (SciLifeLab, Stockholm) with a 2x301 setup in High Output mode. Bcl to Fastq conversion was performed using bcl2Fastq v1.8.3 from the CASAVA software suite. Average quality scores (Sanger / phred33 / Illumina 1.8+) for the individual samples (pooled sequences encoding IgA, IgE, IgG, and IgM derived from the 24 single sample) were 29.49-30.88. A total of 38.1 million read pairs (Supplementary Table EIV) were obtained through this process.

Sequence processing and filtering

Sequence FASTQ file processing and filtering were performed using pRESTO version 0.4.4 (1). Sequence quality filtering and trimming were conducted with the FilterSeq.py (q=20) and MaskPrimers.py scripts, respectively. Sequences were paired and assembled using the PairSeq.py and AssemblePairs.py scripts, and assigned to Ig isotype using the SplitSeq.py script. Finally, the isotype-specific sequence FASTA files generated were filtered to remove obvious contaminants by an in-house Java program that discards sequences that do not contain expected signature sequences. The amplified isotype-specific sequences that were expected to be present in sequences were: IgA: CCGACCAGCCCCAAGG; IgE:

ACACAGAGCCCATCCG; IgG: CCACCAAGGGCCCATC; and finally IgM sequences were required to end with GGGA. IgG4-encoding sequences were identified in the set of IgG-encoding sequences by the presence of the CTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAG sequence defining IGHG4*01 (very few reads matching to IGHG4*04, the other allele with a known sequence in this part of the gene as defined by the IMGT database, were seen in the samples). The generated FASTA files were used for further analyses. The number of reads that remained in each data set during this processes is summarized in Supplementary Table EIV.

Immunoglobulin sequence analysis

All sequence files were submitted to HighV-QUEST (program version 3.3.2; reference directory release 201511-4 or 201515-3) (2) for assignment of V genes and CDR3 region. Statistics were generated from the output summary files using an in-house program after removal of sequences that displayed no detectable rearrangement, were out-of-frame, or carried stop codons within the sequence. Separate file of IgM-encoding sequences of each sample lacking evidence of insertions or deletions and with mutation frequencies <2%, as scored by HighV-QUEST, and of IgG4-encoding transcripts were similarly prepared. The number of reads that remained in each data set during these processes is summarized in Supplementary Table EIV. Each donor was found to use a unique combination of 40-45 germline genes that each encoded at least 0.1% of the reads (Supplementary Figure E1 in the Online Repository). Overall features of these germline gene repertoires largely matched well with those observed in an unrelated study (3) (Supplementary Figure E2 in the Online Repository), suggesting that the procedure was comparable to those used by others. For further sequence comparisons the HighV-QUEST output files were processed to collapse reads with the same V gene assignment and identical CDR3. In a second step the entries were further collapsed, to avoid effects of sequencing artifacts and to merge clones into clonotypes, so that reads with the same V gene assigned, and with the same CDR3 length, were collapsed into the entry with most reads if they differed with 3 or less nucleotides. The FASTA files generated after these processing steps contained, for each entry, the number of reads, V gene and the sequence of the CDR3, The entries that existed with at least

two reads were used to estimate the number of clonotypes in each sample. An in-house java program was then used for further overlap comparisons. Unless otherwise stated, overlap was calculated by comparing the 1000 most common sequences in a sample with the 1000 most common in another, and the percentage of entries in one sample that also existed in the other data set was calculated. A minimum of two identical reads was needed to include any sequence in the comparisons. Furthermore, entries were not considered to overlap if the read ratio between the two samples was larger than 10000.

Hierarchical clustering of V gene usage percentages of 70 genes (Euclidean distances, average linkage) of duplicate samples encoding different isotypes was generated using the Amberbio iPhone app (<http://www.amberbio.com>).

Clone lineage analysis was performed by first sequences from the High-VQuest output files with matching CDRH3-length and no more than 20% nucleotide substitutions in the CDRH3 sequence compared to the target CDRH3 sequence. The extracted sequences were required to have the same V-gene assignment with V-genes with minor differences considered the same (for example all genes starting with IGHV3-23 were considered the same). To avoid sequencing artefacts, sequences were extracted for CDR1, FR2, CDR2, FR3, CDR3, and FR4 from IMGT HighV-QUEST output files. The extracted sequences were dereplicated and the number of occurrences of each sequence counted using USEARCH (4) before performing multiple sequence alignments.

Supplementary Figure Legends

Supplementary Figure E1. Frequency of germline gene usage in the six donors' IgM-encoding repertoire having <2% nucleotide mutations in the IGHV gene. Each value represent the average of the duplicate BM and PBMC samples with error bars displaying the SD.

Supplementary Figure E2. Germline gene frequency usage in the present investigation of unmutated (<2%) IgM-encoding transcriptomes (blue) and in a past study of genomic rearrangements (3) (red). Error bar defines the SEM. Only genes that were identified in all subjects of both studies are included.

Supplementary Figure E3. Distribution of H chain CDR3 length in IgA, IgE, IgG, IgG4, and IgM-encoding transcriptomes in individual samples derived from PB and BM (all transcripts and the subset of transcripts with <2% difference from the closest germline gene). Duplicate samples of each donor are shown. No samples of IgE-encoding transcripts from PB of donor 6 were available. As very few transcripts encoding IgG4 were detected in samples derived from donor 5, these were not analyzed (<0.02% of all IgG-encoding transcripts).

Supplementary Figure E4. Distribution of IGHV subgroups in IgA (blue), IgE (red), IgG (green), and IgM (violet)-encoding transcriptomes. Data represents a combined analysis of repertoires of PB and BM. No samples of IgE-encoding transcripts from PBMC of donor 6 were available. Error bars indicate the SD observed between samples of each set.

Supplementary Figure E5. Relative germline gene usage of the IgA, IgG and IgM-encoding transcriptomes in BM and PB. Duplicate BM samples are depicted in light and dark blue, respectively while duplicate PB samples are depicted in light and dark orange, respectively.

Supplementary Figure E6. Frequency of IGHJ (A) and IGHD (B) germline genes among IgA, IgG and IgM-encoding transcripts found in BM and PB of six donors. Each value was

determined as the average of duplicate samples. The coefficients of variation between duplicate samples were in the range 0.005-18 % (average: 2.1 %).

Supplementary Figure E7. Frequency of unmutated (<2% difference from the closest germline gene in the IMGT database) transcripts encoding IgA, IgE, IgG, and IgM found in 6 donors' BMC and PBMC. Error bars show the SD. As the IgE-encoding populations were very different in duplicate samples, the corresponding SD were comparably large (range: 0.01-17.1%) (not shown). No samples of IgE-encoding transcripts from PBMC of donor 6 were available.

Supplementary Figure E8. Frequency of mutations in the mutated (>2% difference from the closest germline gene in the IMGT database) population of 6 donors' transcripts encoding IgA, IgE, IgG, and IgM found in BM and PB. SD between duplicate samples was <0.12% for all samples except for IgE transcriptomes for which SD <0.18 and 2.6% were recorded for samples derived from BM and PB, respectively.

Supplementary Figure E9. Germline gene usage frequency in the IgE and IgG4-encoding transcriptomes of the six lymphocyte donors. Duplicate BM samples are depicted in light and dark blue, respectively, while duplicate PB samples are depicted in light and dark orange, respectively. PB samples from donor 6 did not contain IgE-encoding transcripts and IgG4-encoding transcripts were not identified in samples from donor 5.

Supplementary Figure E10. Examples of seven (A-G) clonally related sets of IgE-heavy chain variable domain-encoding sequences found in the two BM samples obtained from donor 1. Sequence names include donor number, encoded isotype, sample number, clone number, and the number of reads representing this sequence identified in the sample. Only sequences found at least 10 times in either sample are shown, minimizing the representation of sequencing errors and sequence variants created by PCR errors. Some sequences are represented in both samples while other sequences, although clonally related, are unique to one of the duplicate samples. Bases found in >50% of the different sequences are highlighted by a dark background.

Supplementary Figure E11. Overlap of clonal origin of sequence reads between duplicate samples of IgG4-encoding transcriptomes of BM and PB. The transcriptomes of donor 5 contained no definable IgG4-encoding transcripts. The analysis included up to 1000 of the commonest transcript variants of each sample (a variant is defined as sequence originating from the same germline gene (irrespective of allelic variant) and with identical CDRH3-encoding nucleotide sequences; each analyzed sequence set must be larger than 0.01% of the largest sequence set in the sample, and it must be represented by at least 2 sequence reads).

Supplementary Figure E12. Frequency of overlap of transcriptomes encoding isotypes other than IgE with IgE-encoding transcripts in samples of BM and PB using an algorithm that allows for diversification of the sequence encoding CDRH3. The analysis included up to 50 of the commonest transcript variants of IgE (a variant is defined as sequence originating from the same germline gene (irrespective of allelic variant) and with identical CDRH3-encoding nucleotide sequences) found in one of the duplicate samples. These sequences were compared with all sequences of the same donor and sample type that were derived from the same germline IGHV gene (irrespective of allelic variant) encoding other isotypes. Up to 20% nucleotide differences (but no insertions or deletions) in CDRH3 were allowed. Donor 1: blue; donor 2: red; donor 3: green; donor 4 violet; donor 5: cyan; donor 6: orange. Donor 5 had no identifiable IgG4-encoding transcripts in his amplified transcriptomes, and donor 6 had no detectable IgE-encoding transcripts in his amplified transcriptome from peripheral blood.

Supplementary Figure E13. Frequency of transcripts displaying the motif frequently found in Phl p 2-specific IgE (12) in different repertoires of the six donors of this study. Three of these donors (donor 2, 3, and 6), donors who displayed high levels of IgE-encoding transcripts fulfilling the motif criteria, also had plasma IgE specific for Phl p 2 (Supplementary Table EVII).

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262 *Design & Selection* 2008;21:485-93.
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264 numbering for immunoglobulin and T cell receptor variable domains and Ig
265 superfamily V-like domains. *Dev Comp Immunol* 2003;27:55-77.

Supplementary Table EI. Examples of studies employing NGS to investigate the nature of human IgE repertoires.

Title	Year of Publication	Supplementary information reference
Human immunoglobulin classes and subclasses show variability in VDJ gene mutation levels.	2014	(5)
IgE-associated IGHV genes from venom and peanut allergic individuals lack mutational evidence of antigen selection	2014	(6)
Influence of seasonal exposure to grass pollen on local and peripheral blood IgE repertoires in patients with allergic rhinitis	2014	(7)
Peanut oral immunotherapy transiently expands circulating Ara h 2-specific B cells with a homologous repertoire in unrelated subjects.	2015	(8)
Human B-cell isotype switching origins of IgE	2016	(9)
Single B-cell deconvolution of peanut-specific antibody responses in allergic patients	2016	(10)
Persistence and evolution of allergen-specific IgE repertoires during subcutaneous specific immunotherapy	2016	(11)

Supplementary Table EII. Donor characteristics.

Donor ID	Age	Sex	Positive skin prick test ¹	Plasma IgE level (kU/L)	Plasma IgG4 level (g/L)
1	25	M	BP, GP, HDM, CD	148	0.22
2	37	M	BP, GP	101	0.40
3	28	M	GP, CD, DD	127	0.58
4	25	M	BP, GP, MP, HDM, CD	143	1.16
5	26	M	GP, MP, HDM	251	0.73
6	26	M	GP, HDM	23	0.21

¹ BP, birch pollen; CD, cat dander; DD, dog dander; GP, grass pollen; HDM, house dust mite; MP, mugwort pollen

Supplementary Table EIII. Primers used for preparation of MiSeq sequencing libraries, with 8-nucleotide barcodes in bold, and the total number of PCR cycles run to achieve equally good amounts product after the 2nd PCR for each antibody isotype.

	Name	Sequence	Number of PCR cycles in isotype-specific PCR
First PCR	VH1_FR_1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNGGCCTCAGTGAAGGTCTCCTGCAAG	
	VH2_FR_1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNGTCTGGTCCTACGCTGGTGAAACCC	
	VH3_FR_1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNCTGGGGGGTCCCTGAGACTCTCCTG	
	VH4_FR_1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNCTTCGGAGACCCTGTCCCTCACCTG	
	VH5_FR_1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNCGGGGAGTCTCTGAAGATCTCCTGT	
	VH6_FR_1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNTCGCAGACCCTCTCACTCACCTGTG	
	IgA	AGACGTGTGCTCTTCCGATCTAGCCCTGGACCAGGCA	32-34
	IgE	AGACGTGTGCTCTTCCGATCTGGAGGTGGCATTGGAGGGAATGTT	42-44
	IgG	AGACGTGTGCTCTTCCGATCTCAGGCAGCCCAGGGC	32-34
	IgM	AGACGTGTGCTCTTCCGATCTGGGTTGGGGCGGATGCAC	32-34
Second PCR	N501_fw	AATGATACGGCGACCACCGAGATCTACACTAGATCGCACACTCTTTCCCTACACGACG	
	N502_fw	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCTTTCCCTACACGACG	
	N701_rev	CAAGCAGAAGACGGCATAACGAGATTGCGCTTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	
	N702_rev	CAAGCAGAAGACGGCATAACGAGATCTAGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	
	N704_rev	CAAGCAGAAGACGGCATAACGAGATGCTCAGGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	
	N707_rev	CAAGCAGAAGACGGCATAACGAGATGTAGAGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	
	N708_rev	CAAGCAGAAGACGGCATAACGAGATCCTCTCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	
	N709_rev	CAAGCAGAAGACGGCATAACGAGATAGCGTAGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	

Supplementary Table EIV. Summary of number of sequences during the different stages of data analysis.

Donor	Tissue	Number of paired reads	Isotype	Number of reads after PRESTO pipeline	Number of reads after confirmation of isotype-sequence in amplicon	Number of functional reads after HighV-QUEST analysis	Number of unmutated (<2%) reads of IgM	Number of reads representing IgG4*01
1	BM	1.34×10^6	IgA	171837	168975	166441		
			IgE	288838	285977	282889		
			IgG	386877	382786	377445		3536
			IgM	261967	259580	254483	121181	
	BM	1.67×10^6	IgA	265099	260614	256905		
			IgE	335428	332116	328373		
			IgG	418031	413817	408406		4008
			IgM	389986	386671	379003	181681	
	PB	1.90×10^6	IgA	302268	299201	294536		
			IgE	498938	493526	487090		
			IgG	275945	273656	269152		1534
			IgM	527232	523089	510205	305584	
	PB	1.45×10^6	IgA	201916	199670	196722		
			IgE	384827	379835	375228		
			IgG	124551	123393	121217		892
			IgM	540818	535334	522553	323581	
2	BM	1.40×10^6	IgA	286529	282739	278806		
			IgE	311779	306528	302519		
			IgG	390805	388039	382999		9893
			IgM	197949	195163	190721	103762	
	BM	1.67×10^6	IgA	303994	299667	295550		
			IgE	411263	406673	401626		
			IgG	386223	383516	378672		9556
			IgM	333387	328788	321270	174996	
	PB	$0.70 \times$	IgA	133441	132118	130097		

		10^6	IgE	105140	94290	87793		
			IgG	137207	136064	133589		3641
			IgM	204810	202544	197001	118264	
	PB	1.31×10^6	IgA	255218	252598	248708		
			IgE	317359	296227	283702		
			IgG	167747	162955	159959		4836
			IgM	343418	339035	329486	195629	
	BM	1.35×10^6	IgA	273356	269388	265655		
			IgE	330855	325544	320708		
			IgG	249108	246924	243707		4995
			IgM	281711	279241	274197	101969	
3	BM	2.54×10^6	IgA	560448	550559	542730		
			IgE	614456	603812	597781		
			IgG	522759	517410	510729		10665
			IgM	439169	432862	425126	159474	
	PB	2.34×10^6	IgA	427524	423204	416669		
			IgE	311773	264355	218546		
			IgG	326436	324043	318841		2488
			IgM	818475	810205	792631	342840	
	PB	1.97×10^6	IgA	503599	497920	490245		
			IgE	359285	350235	343120		
			IgG	340061	337517	332087		3069
			IgM	467047	461623	451548	198275	
4	BM	2.04×10^6	IgA	326294	321247	316947		
			IgE	649376	638888	622031		
			IgG	453658	449147	444007		18907
			IgM	345021	340589	333979	119034	
	BM	1.59×10^6	IgA	330348	324586	320402		
			IgE	411883	405835	398503		
			IgG	375329	372228	367901		15495
			IgM	294677	291240	285592	100624	

	PB	0.88×10^6	IgA	164625	162800	160368		
			IgE	233660	180968	93948		
			IgG	76359	74460	72679		3162
			IgM	216128	214470	208029	135285	
	PB	1.17×10^6	IgA	149257	147681	145444		
			IgE	406326	386228	348839		
			IgG	226098	223945	218711		10024
			IgM	240047	238280	231053	148671	
	BM	1.48×10^6	IgA	294241	291295	287788		
			IgE	365867	363201	359596		
			IgG	340048	337664	333823		6
			IgM	324269	319332	312204	164023	
5	BM	2.23×10^6	IgA	381089	377016	372412		
			IgE	455103	450465	445911		
			IgG	418751	413343	408732		12
			IgM	734189	725385	709198	371395	
	PB	0.98×10^6	IgA	227516	225606	222720		
			IgE	194033	188156	184471		
			IgG	192048	190647	188395		5
			IgM	261977	259493	255157	75887	
	PB	1.44×10^6	IgA	417461	413076	407972		
			IgE	355947	349740	344823		
			IgG	267239	264804	261557		6
			IgM	249165	247776	243448	72047	
6	BM	2.48×10^6	IgA	741359	730983	722274		
			IgE	436393	420626	416644		
			IgG	570354	564338	558073		6413
			IgM	412689	407879	400172	173196	
	BM	2.29×10^6	IgA	443442	437814	432757		
			IgE	272312	258006	255857		10491
			IgG	872651	859115	849924		

	PB	1.06×10^6	IgM	378378	373889	366840	162072	
			IgA	286298	283701	279817		
			IgE	No product was obtained by PCR				
			IgG	291422	288818	285273		2317
	PB	0.86×10^6	IgM	363306	359433	352261	183611	
			IgA	216169	213999	211053		
			IgE	No product was obtained by PCR				
			IgG	212351	208755	206085		1509
			IgM	329727	326626	319979	162070	
Average number of reads $\pm$ 1SD/ 10^5	BM		IgA	3.6 $\pm$ 1.5	3.6 $\pm$ 1.4	3.5 $\pm$ 1.4		
			IgE	4.1 $\pm$ 1.2	4.0 $\pm$ 1.1	3.9 $\pm$ 1.1		
			IgG	4.5 $\pm$ 1.5	4.4 $\pm$ 1.5	4.4 $\pm$ 1.5		
			IgM	3.7 $\pm$ 1.3	3.6 $\pm$ 1.3	3.5 $\pm$ 1.2		
	PB		IgA	2.7 $\pm$ 1.1	2.7 $\pm$ 1.1	2.7 $\pm$ 1.1		
			IgE	3.2 $\pm$ 1.1	3.0 $\pm$ 1.1	2.8 $\pm$ 1.2		
			IgG	2.2 $\pm$ 0.8	2.2 $\pm$ 0.8	2.1 $\pm$ 0.8		
			IgM	3.8 $\pm$ 1.7	3.8 $\pm$ 1.7	3.7 $\pm$ 1.7		

Supplementary Table EV. Number of germline genes that each encoded >0.1 % of the transcriptome of each isotype.

Donor	BMC										PBMC									
	IgA		IgE		IgG		IgG4		IgM		IgA		IgE		IgG		IgG4		IgM	
1	46	46	29	28	43	43	39	37	45	44	43	44	12	13	44	45	29	25	45	46
2	42	42	34	33	41	41	39	40	41	40	42	41	4	4	38	40	26	27	39	39
3	43	43	26	22	42	43	32	34	42	41	40	40	3	10	43	41	28	26	41	41
4	42	42	24	23	39	40	28	31	38	37	40	40	4	5	39	38	21	22	40	40
5	47	46	35	36	46	46	*	*	45	44	44	41	4	6	43	40	*	*	40	40
6	42	42	21	19	44	44	39	40	42	42	41	43	**	**	42	42	9	15	41	43

* <0.02% IgG-encoding sequences encoded IgG4 in this sample. These sequences were not analyzed.

** No PCR product was obtained when amplifying the IgE transcriptome from this sample.

Supplementary Table EVI. Number of lengths of CDRH3 between 1-30 codons that were used by >0.1 % of the transcriptome encoding the given isotype.

Donor	BMC										PBMC									
	IgA		IgE		IgG		IgG4		IgM		IgA		IgE		IgG		IgG4		IgM	
1	23	22	15	16	24	25	19	19	22	22	22	22	10	11	24	23	13	16	22	23
2	24	24	18	17	24	24	21	23	24	24	21	22	5	4	24	23	20	21	23	22
3	25	24	14	17	24	23	17	19	21	21	23	21	3	8	23	24	15	16	22	22
4	23	23	17	14	25	23	22	20	24	22	24	24	4	6	24	23	19	17	23	23
5	23	22	19	21	23	23	*	*	24	25	22	22	4	4	22	22	*	*	21	21
6	22	23	13	13	23	24	22	19	24	24	22	23	**	**	22	22	11	9	21	22

* <0.02% IgG-encoding sequences encoded IgG4 in this sample. These sequences were not analyzed.

** No PCR product was obtained when amplifying the IgE transcriptome from this sample.

Supplementary Table EVII. Plasma concentration of allergen-specific IgE and IgG4, and the frequency of transcripts carrying the Phl p 2-related sequence motif (12) in different immunoglobulin-encoding transcriptomes.

Donor	Allergen-specific antibodies				Frequency of motif-carrying sequences in the BM transcriptome (%)			
	IgE (kU/L)			IgG4 (mg/L)				
	Phl p 1	Phl p 2	Phl p 5	Phl p 2	Unmutated IgM	IgE	IgG	IgG4
1	3.4	<0.1	11.0	0.00	0.01	<0.01	0.02	0.8
2	9.2	6.4	14.9	0.02	0.02	3.0	<0.01	<0.1
3	6.8	7.9	<0.1	0.02	0.01	7.5	<0.01	<0.1
4	<0.1	<0.1	<0.1	0.00	0.02	<0.01	0.02	<0.1
5	25.5	<0.1	32.7	0.00	0.01	<0.01	0.04	*
6	2.9	1.7	<0.1	0.00	0.01	12.2	0.02	<0.1

* Bioinformatic analysis could not identify the IgG4-encoding transcript population in samples derived from this donor.

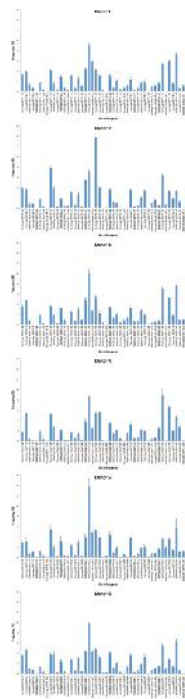


Fig E1.

Mattias Levin, Fredrik Levander, Robert Palmason, Lennart Greiff, Mats Ohlin

Antibody-encoding repertoires of bone marrow and peripheral blood—a focus on IgE

Journal of Allergy and Clinical Immunology, 2016, Available online 9 August 2016

<http://dx.doi.org/10.1016/j.jaci.2016.06.040>

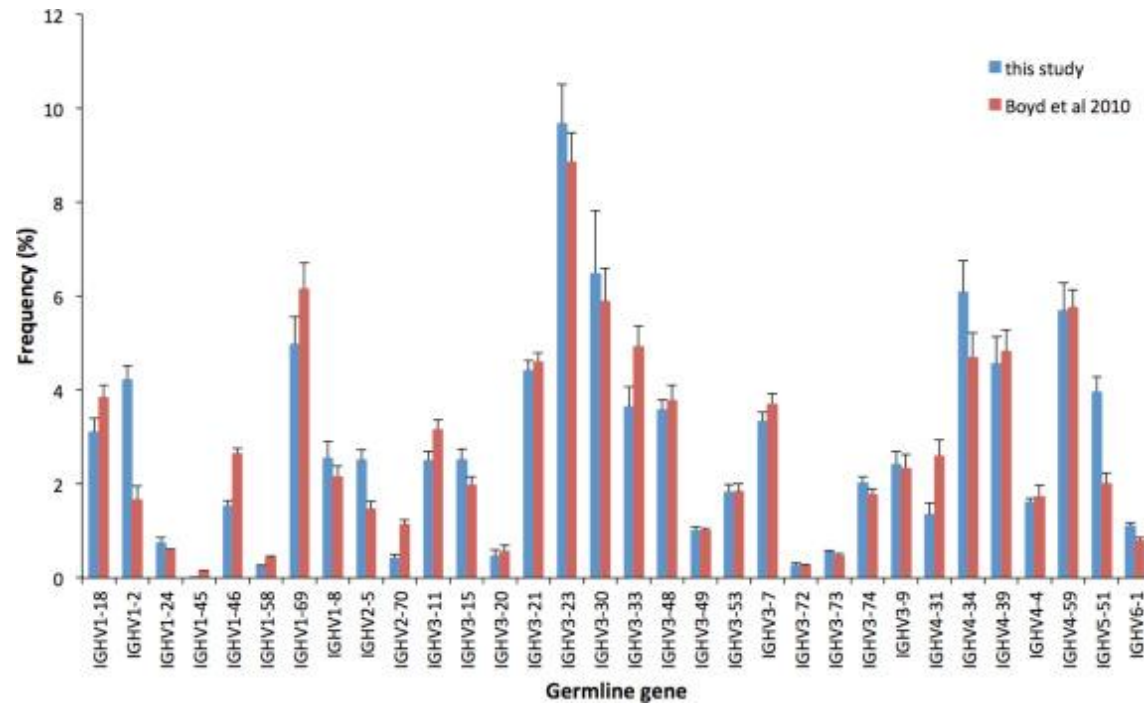


Fig E2.

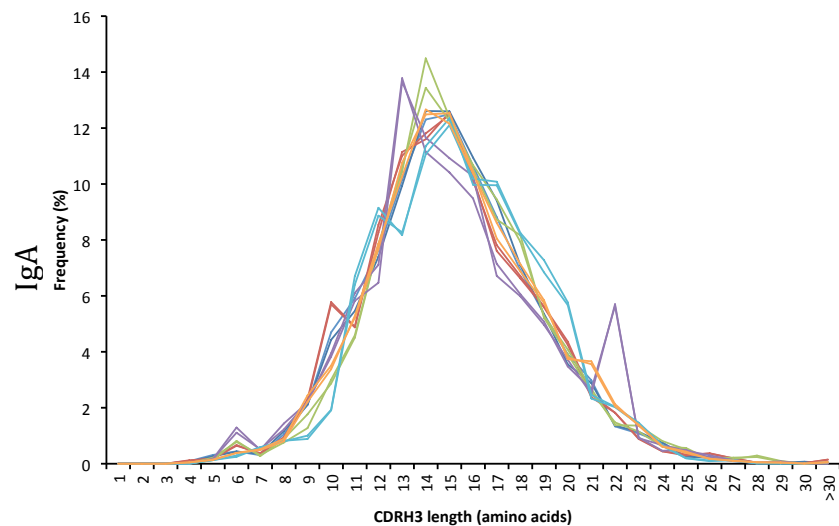
Mattias Levin, Fredrik Levander, Robert Palmason, Lennart Greiff, Mats Ohlin

Antibody-encoding repertoires of bone marrow and peripheral blood—a focus on IgE

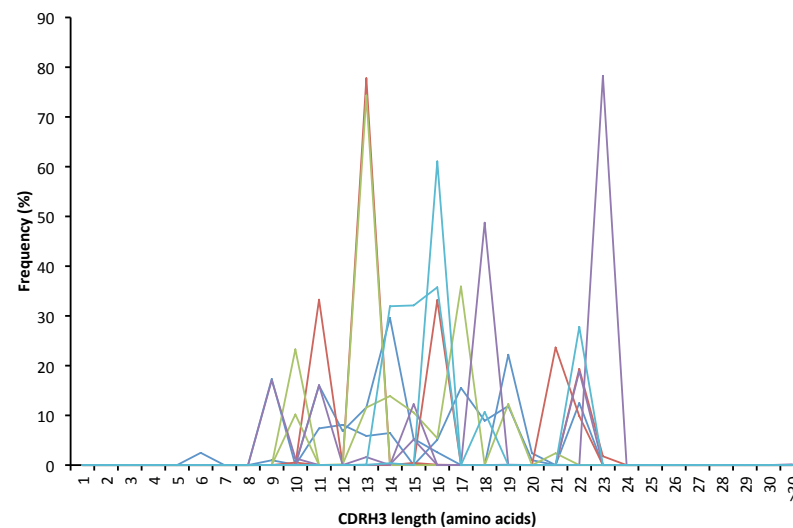
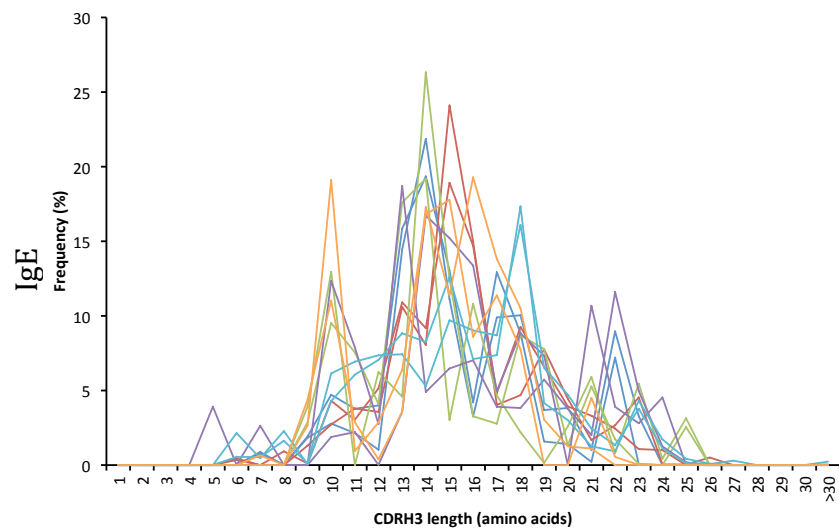
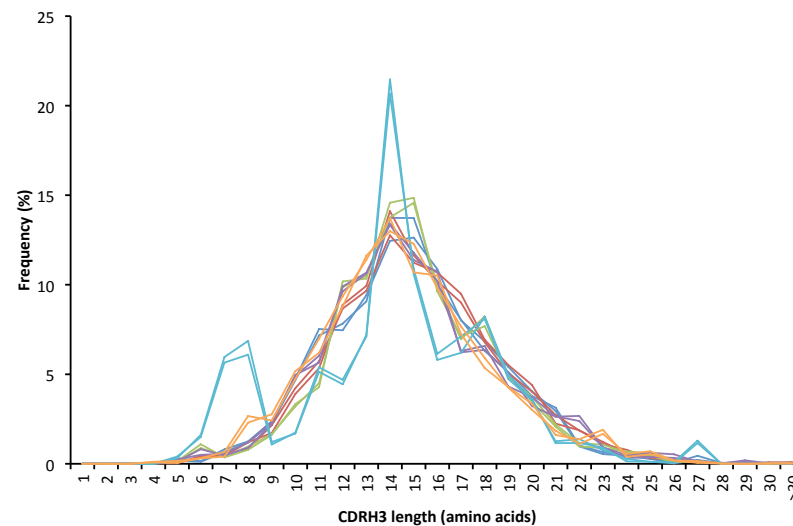
Journal of Allergy and Clinical Immunology, 2016, Available online 9 August 2016

<http://dx.doi.org/10.1016/j.jaci.2016.06.040>

Bone marrow



Peripheral blood



Donor 1

Donor 2

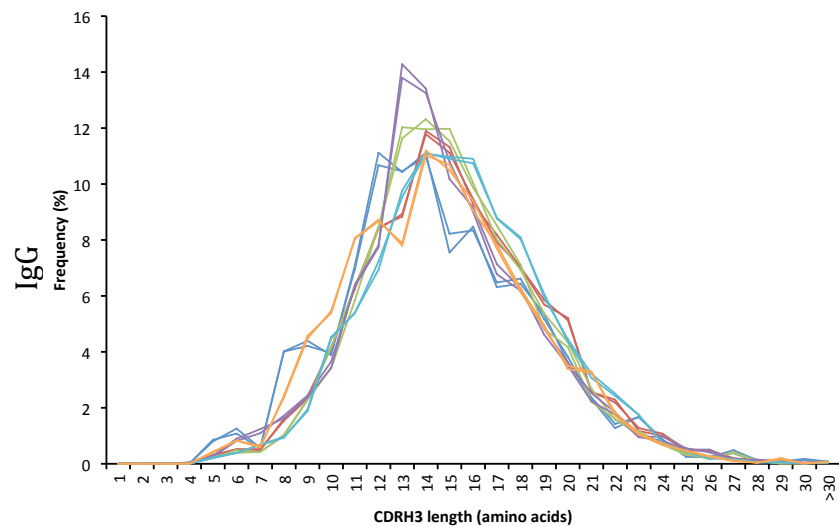
Donor 3

Donor 4

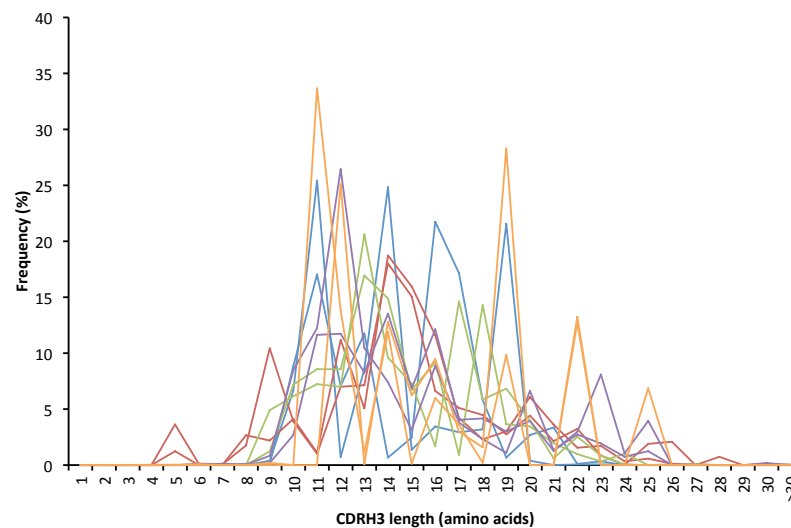
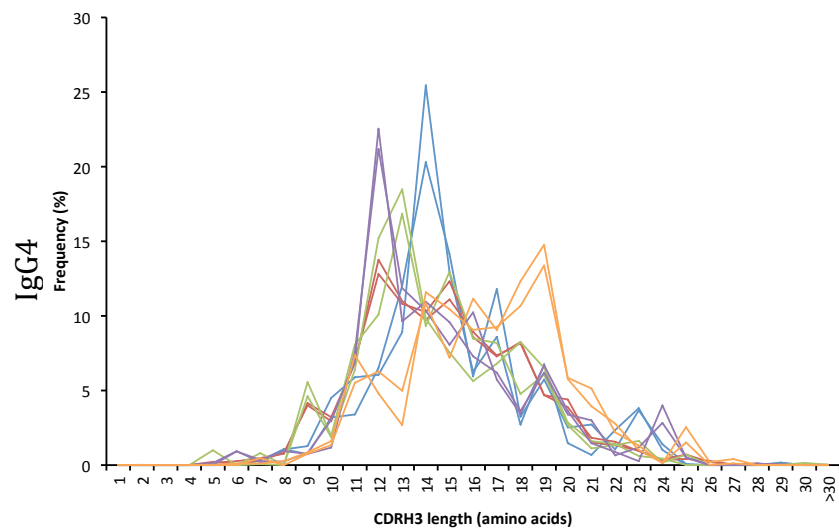
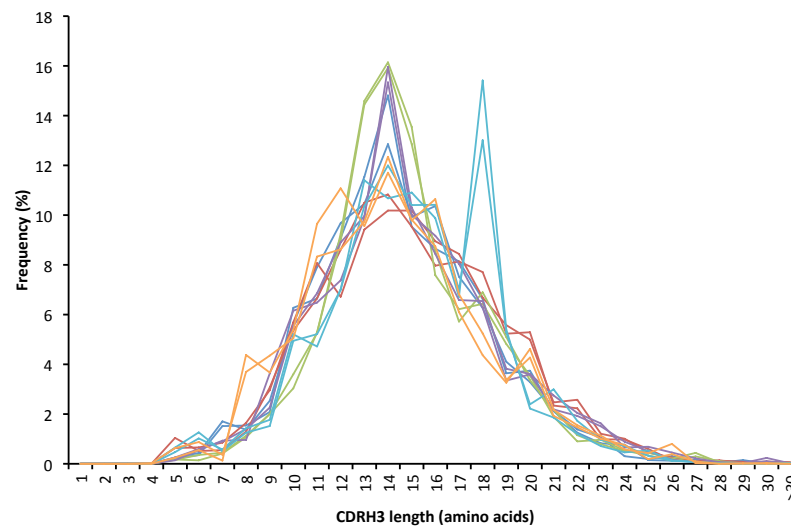
Donor 5

Donor 6

Bone marrow



Peripheral blood



Donor 1

Donor 2

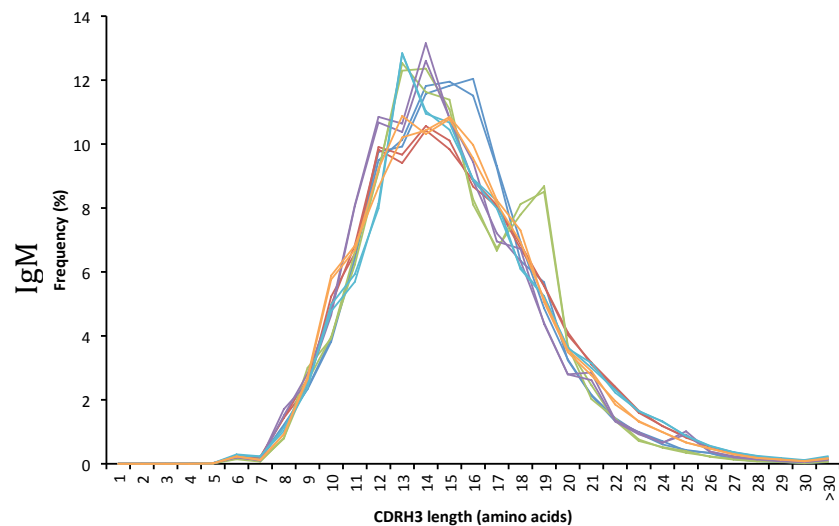
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Donor 4

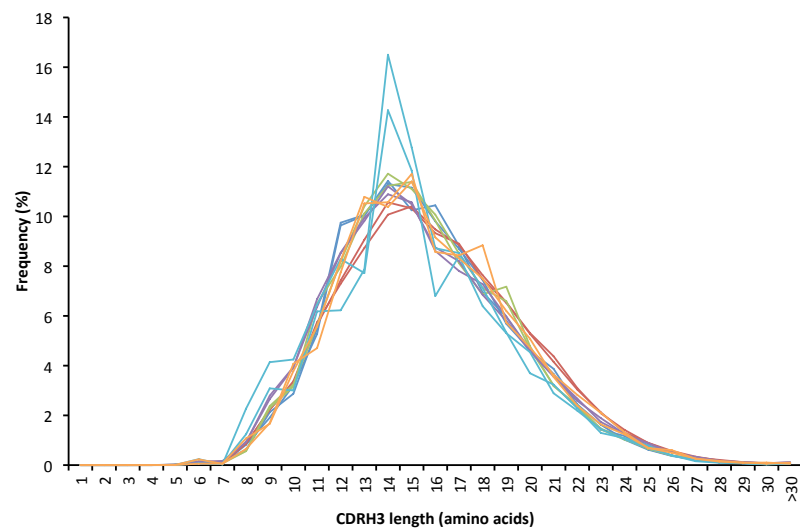
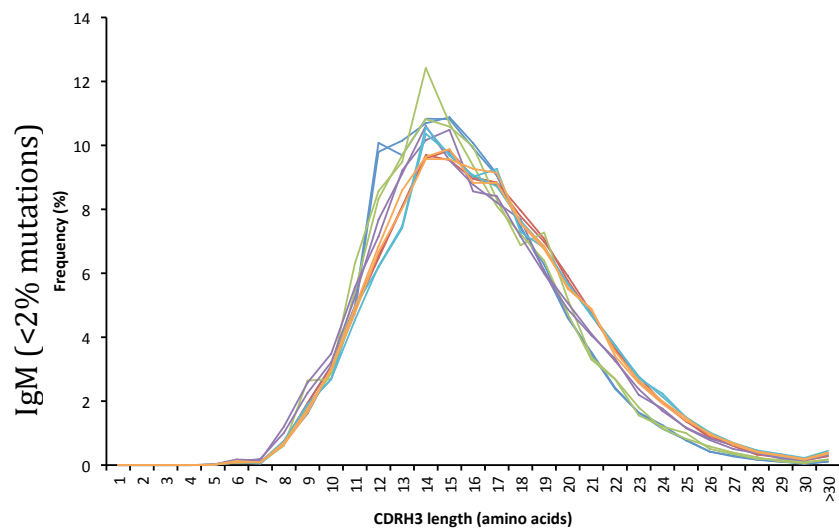
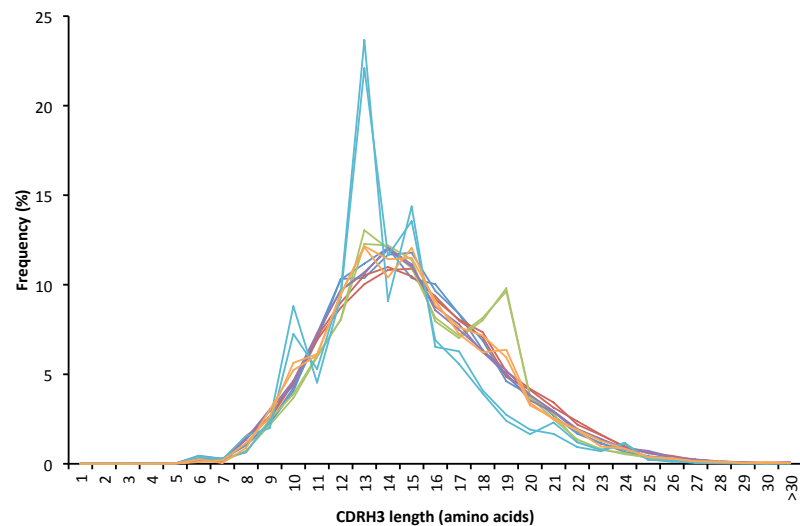
Donor 5

Donor 6

Bone marrow



Peripheral blood



Donor 1

Donor 2

Donor 3

Donor 4

Donor 5

Donor 6

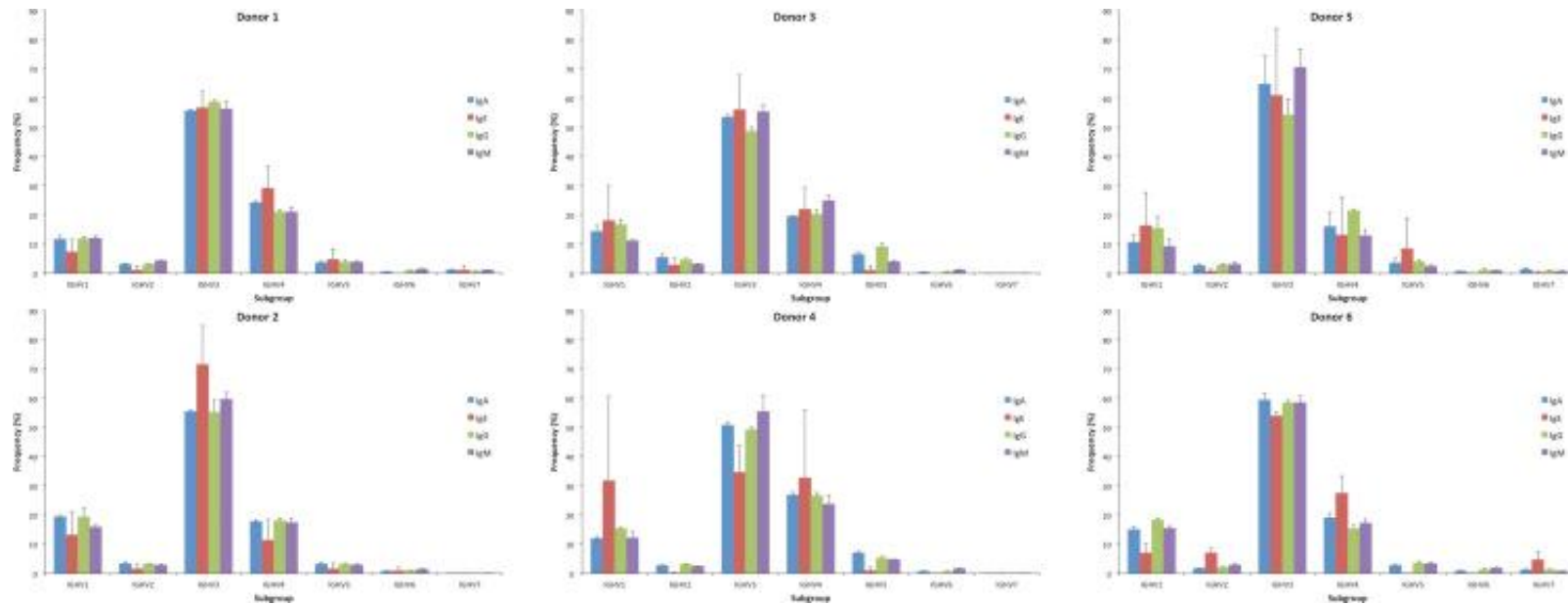


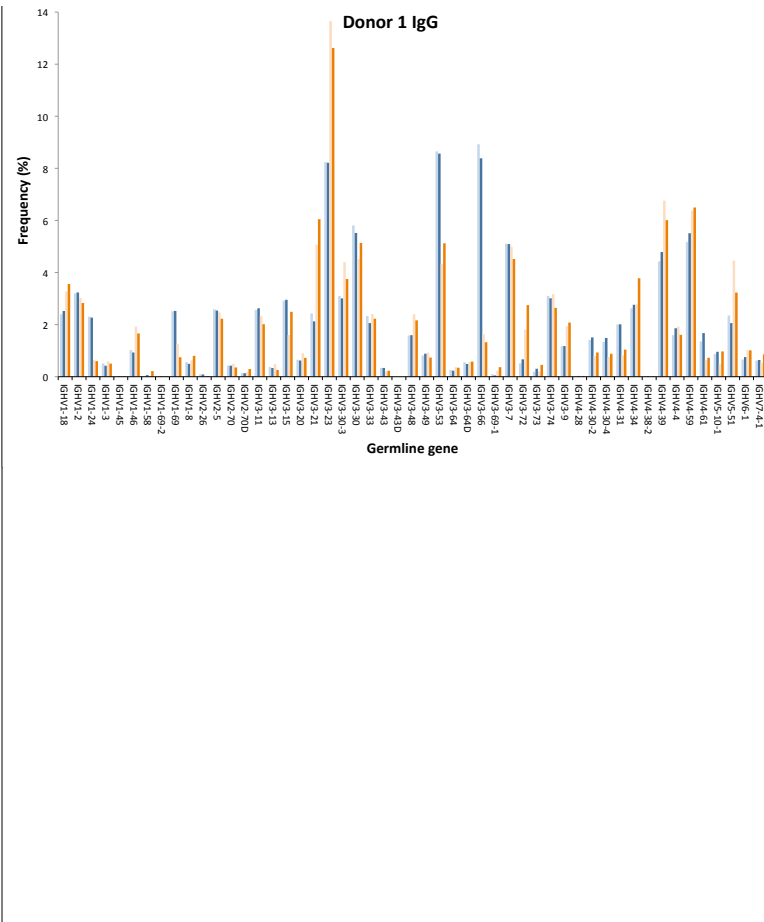
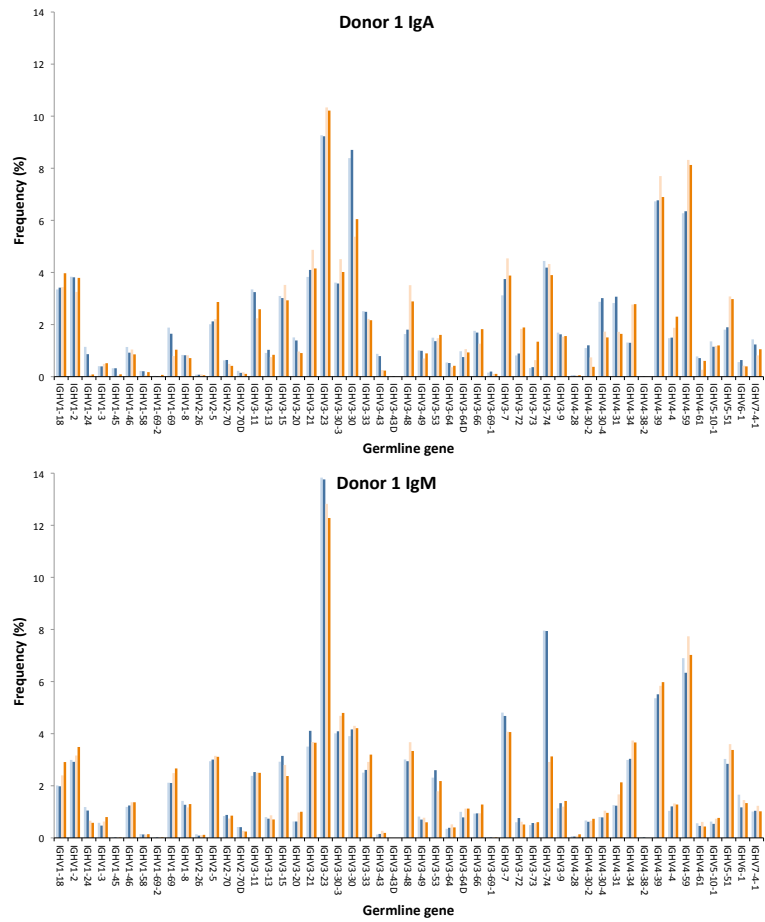
Fig E4.

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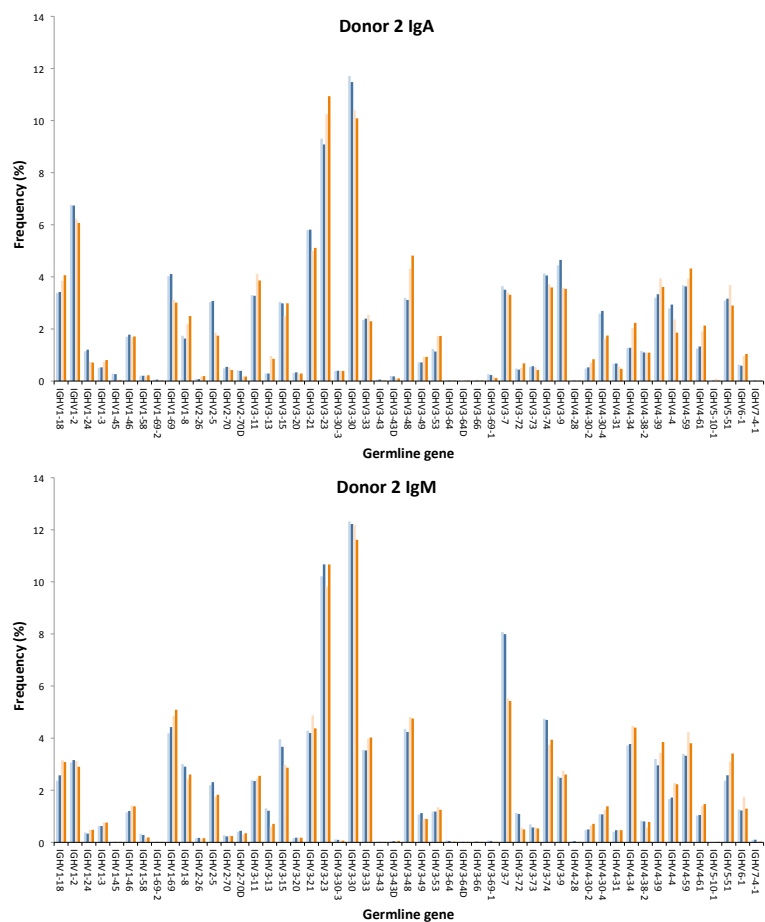


BM sample 1

BM sample 2

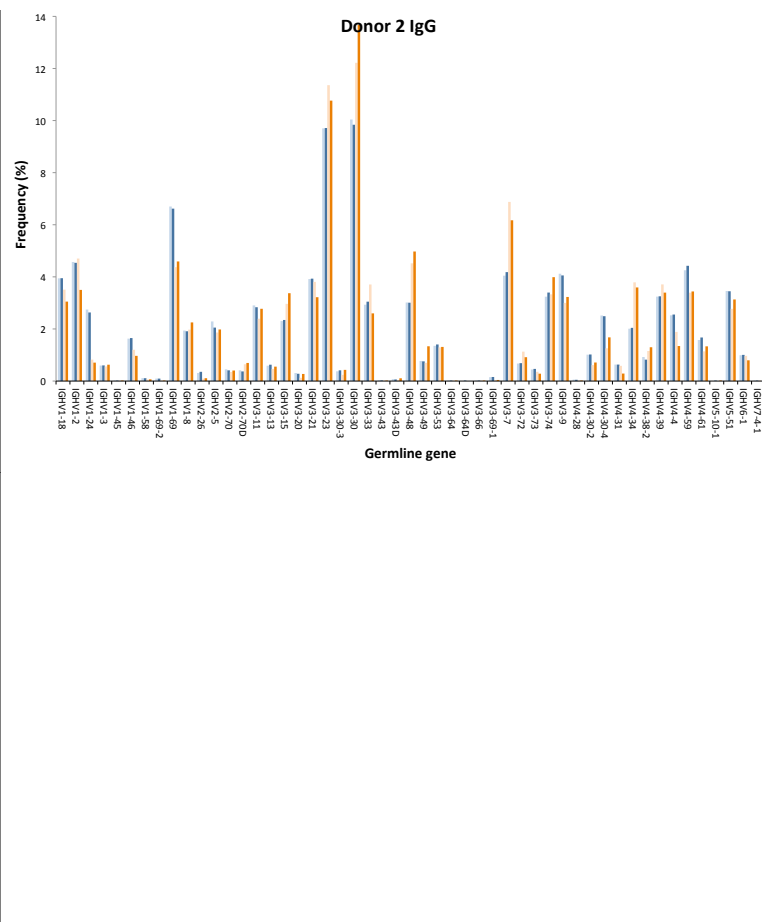
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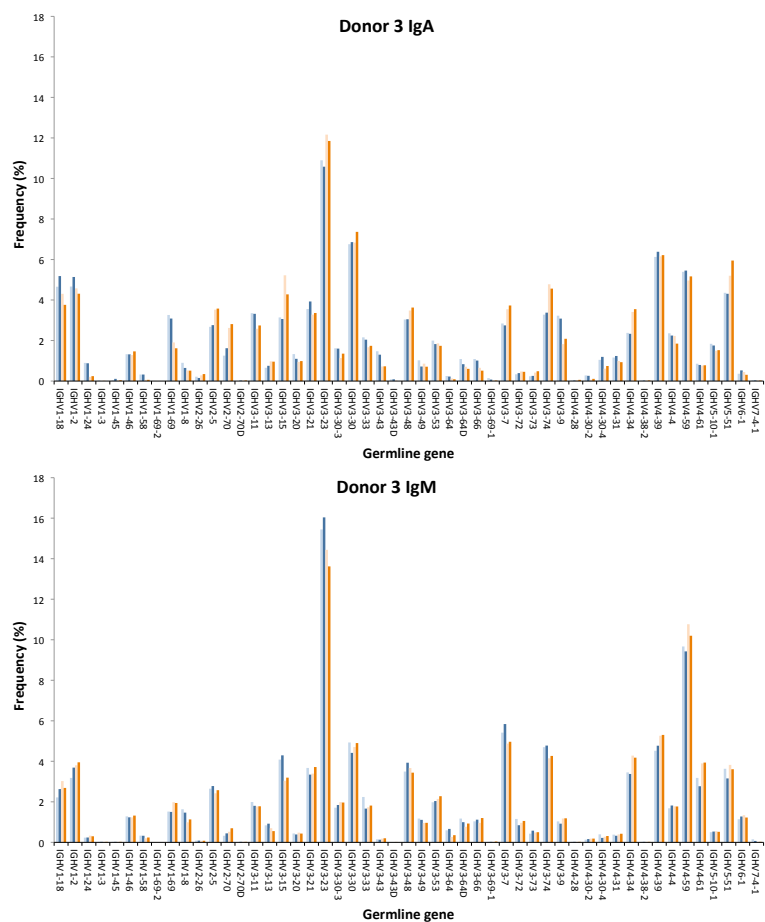
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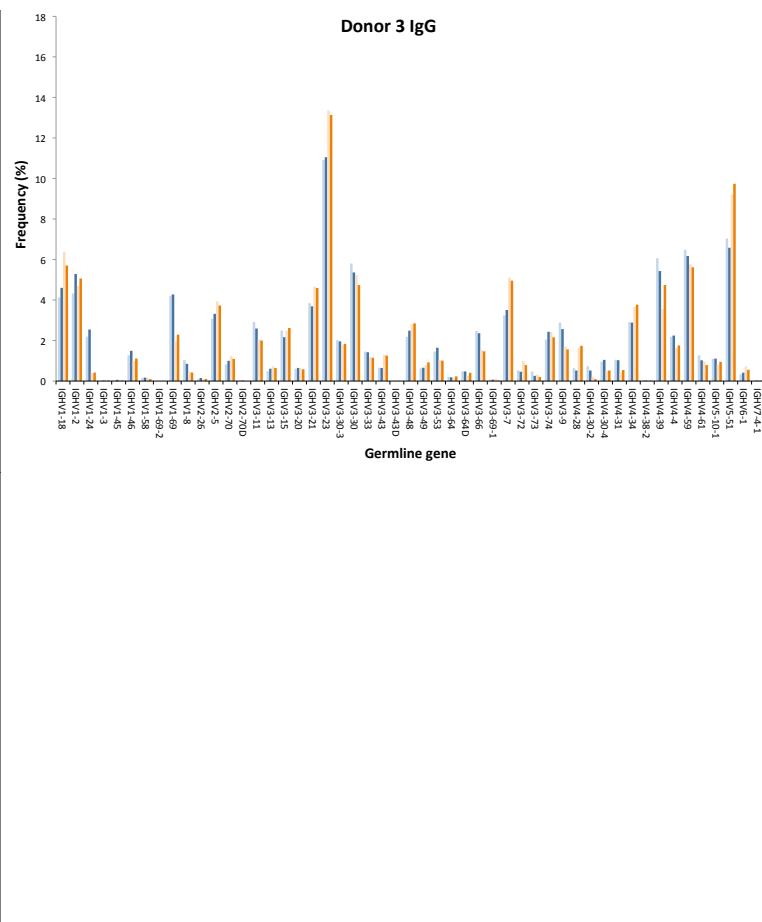
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PB sample 2



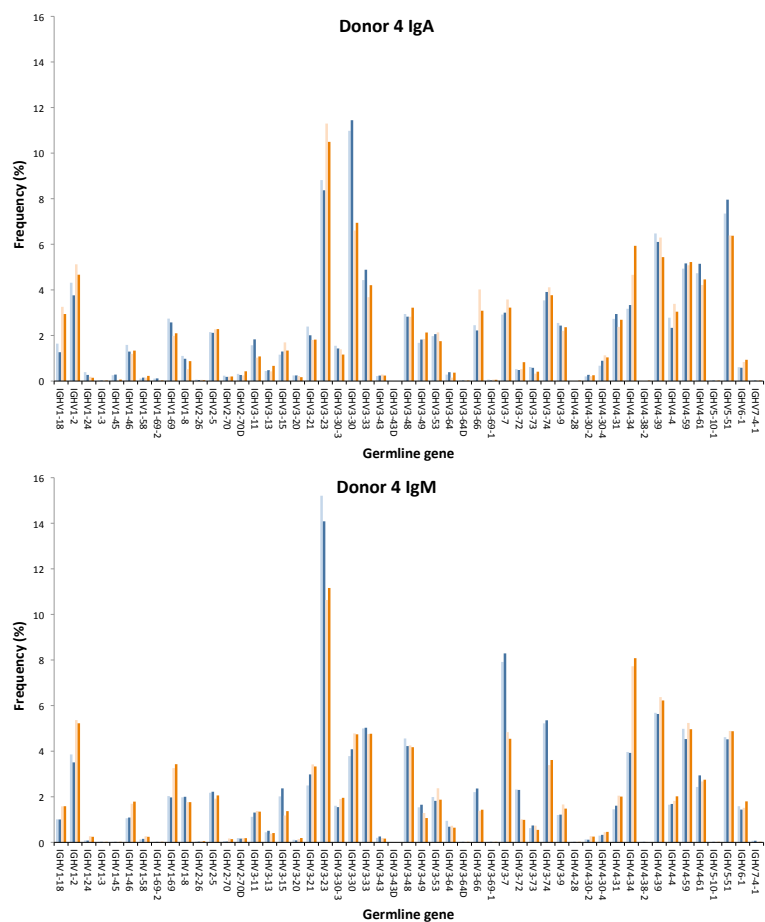
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BM sample 2



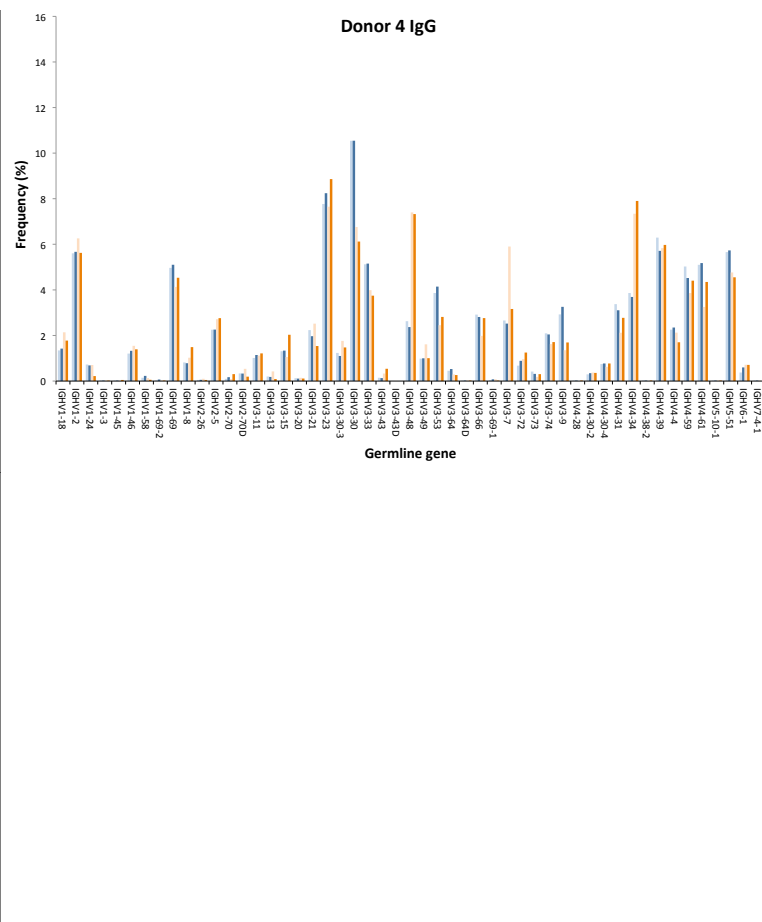
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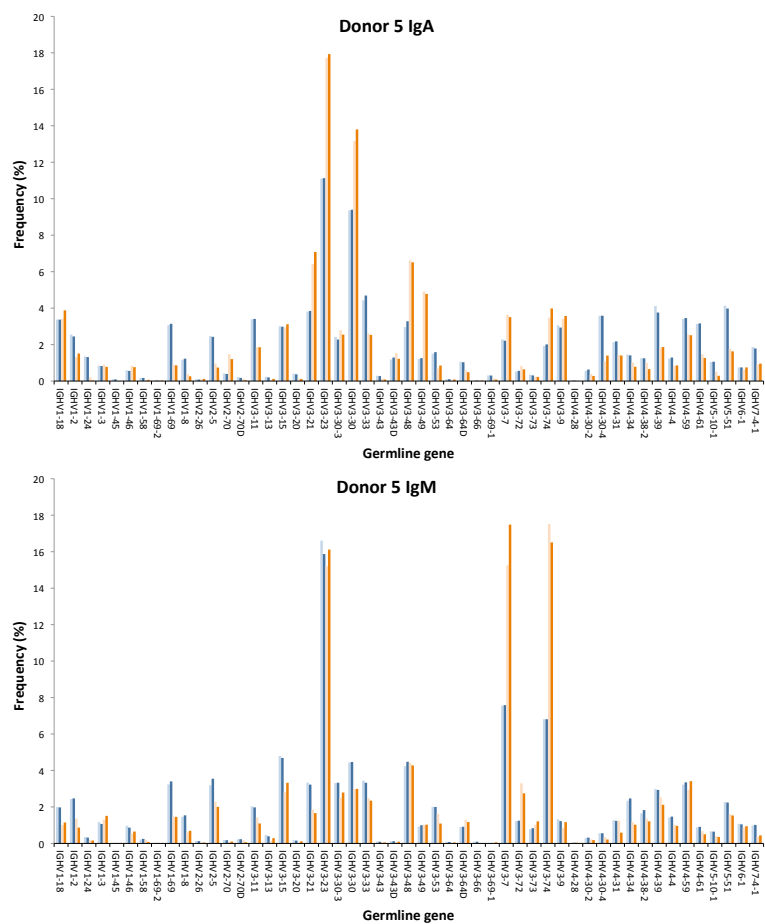
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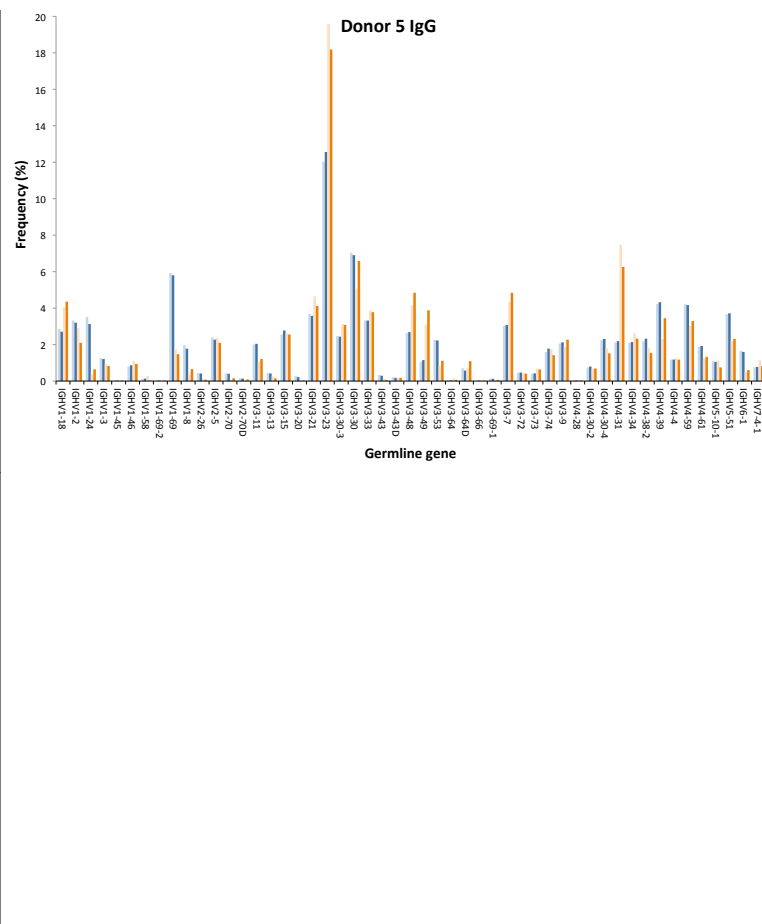
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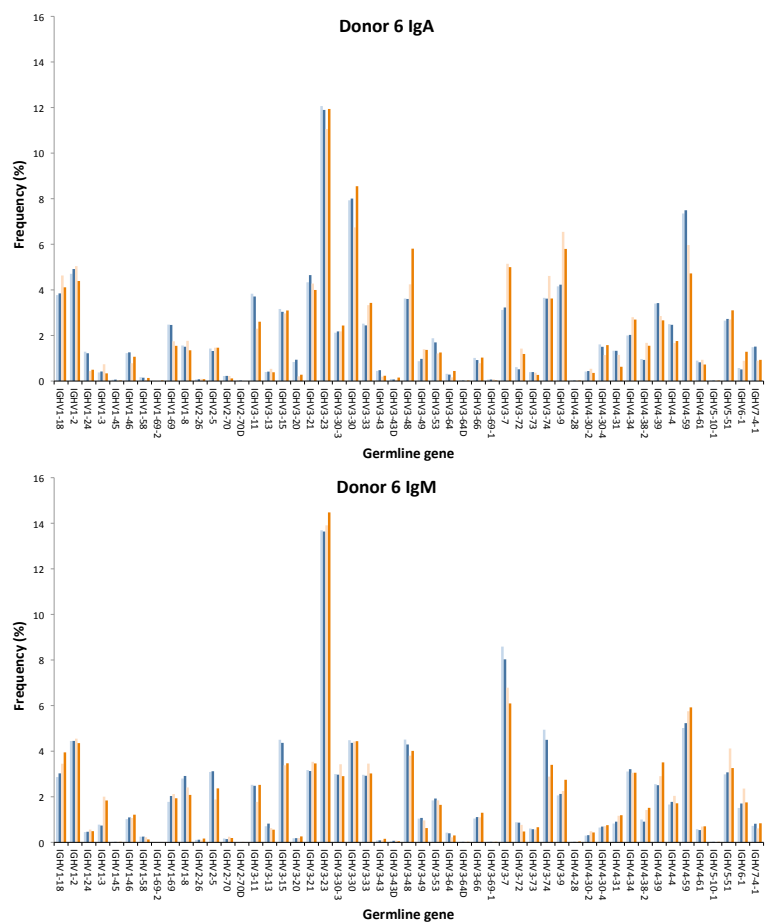
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BM sample 2



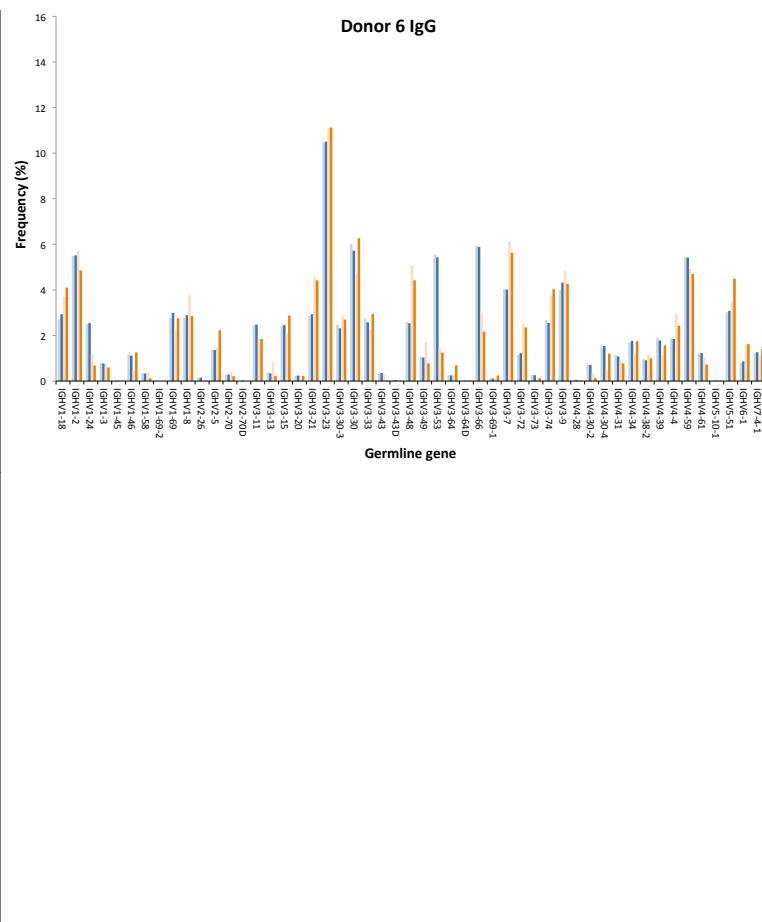
PB sample 1

PB sample 2



BM sample 1

BM sample 2



PB sample 1

PB sample 2

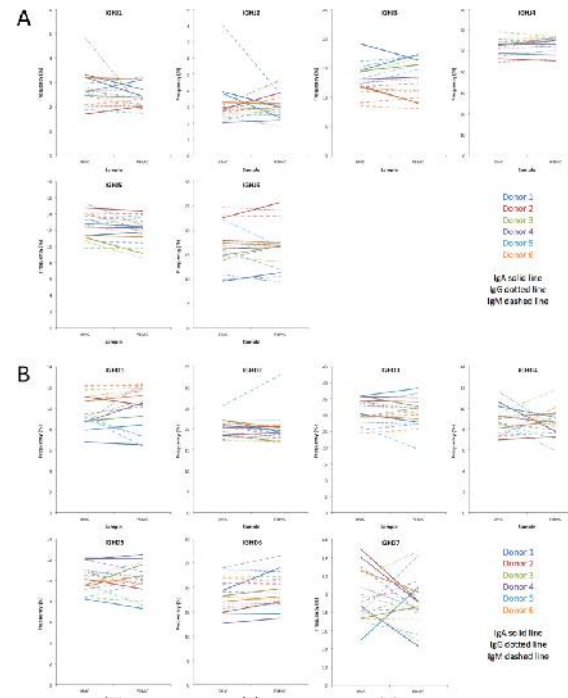


Fig E6.

Mattias Levin, Fredrik Levander, Robert Palmason, Lennart Greiff, Mats Ohlin

Antibody-encoding repertoires of bone marrow and peripheral blood—a focus on IgE

Journal of Allergy and Clinical Immunology, 2016, Available online 9 August 2016

<http://dx.doi.org/10.1016/j.jaci.2016.06.040>

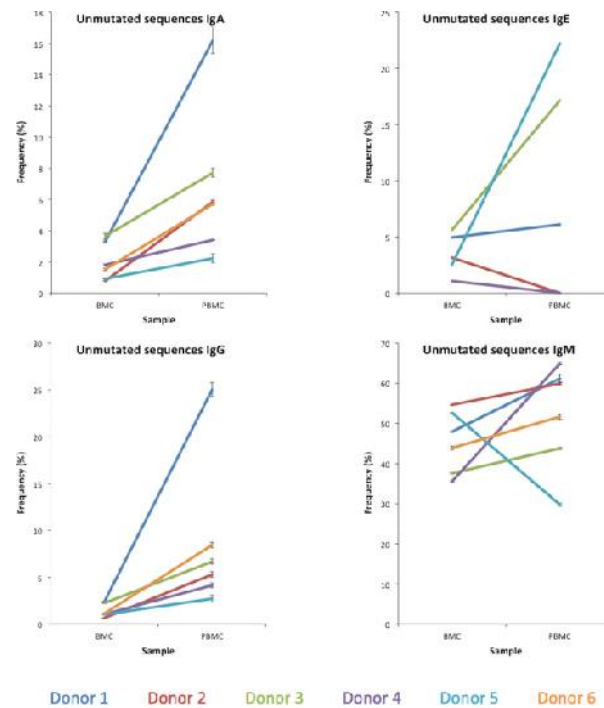


Fig E7.

Mattias Levin, Fredrik Levander, Robert Palmason, Lennart Greiff, Mats Ohlin

Antibody-encoding repertoires of bone marrow and peripheral blood—a focus on IgE

Journal of Allergy and Clinical Immunology, 2016, Available online 9 August 2016

<http://dx.doi.org/10.1016/j.jaci.2016.06.040>

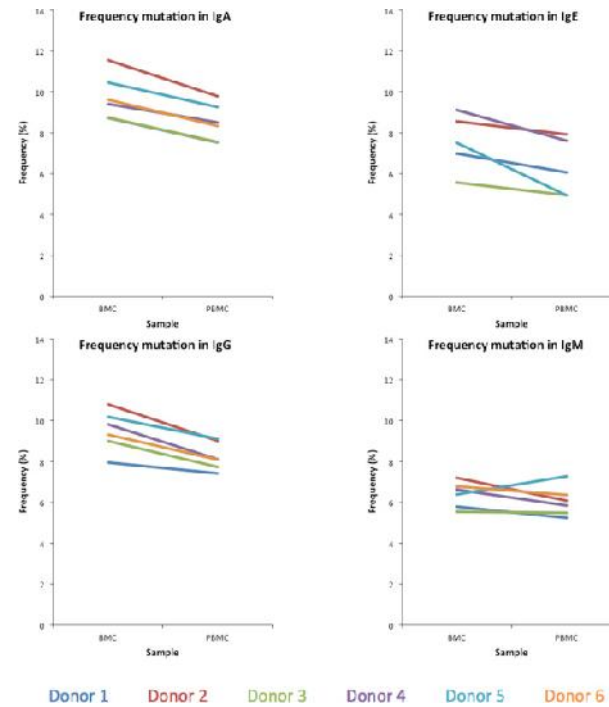


Fig E8.

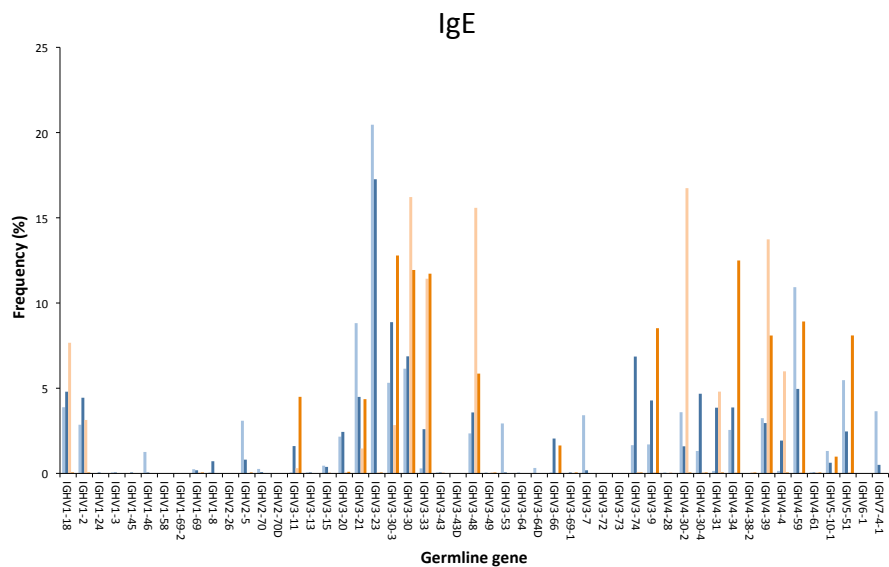
Mattias Levin, Fredrik Levander, Robert Palmason, Lennart Greiff, Mats Ohlin

Antibody-encoding repertoires of bone marrow and peripheral blood—a focus on IgE

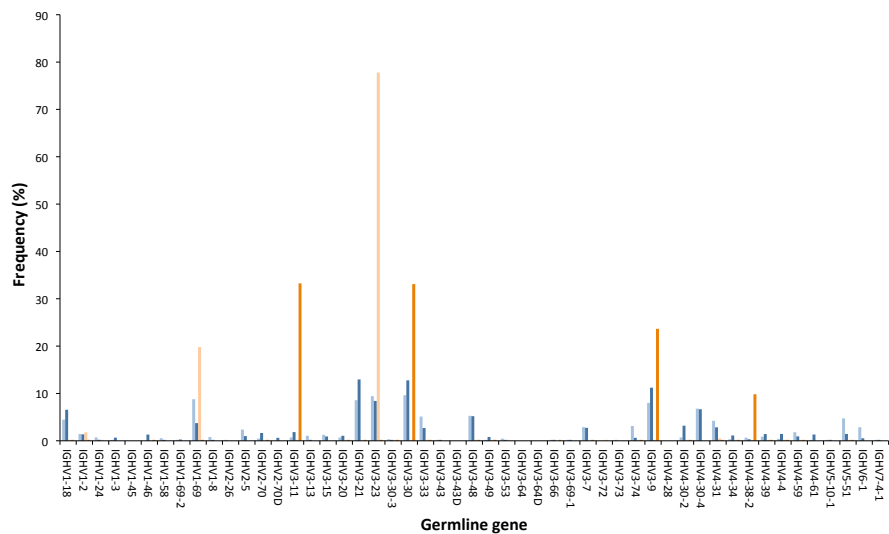
Journal of Allergy and Clinical Immunology, 2016, Available online 9 August 2016

<http://dx.doi.org/10.1016/j.jaci.2016.06.040>

Donor 1



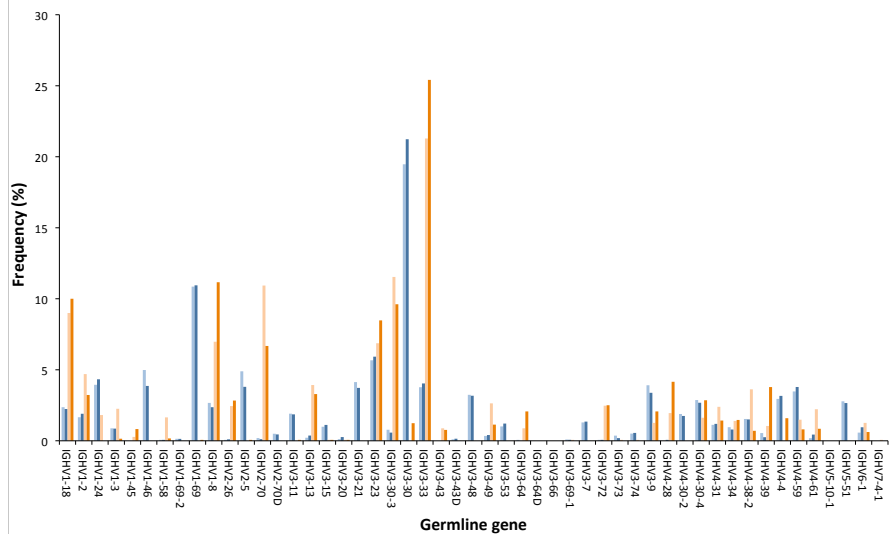
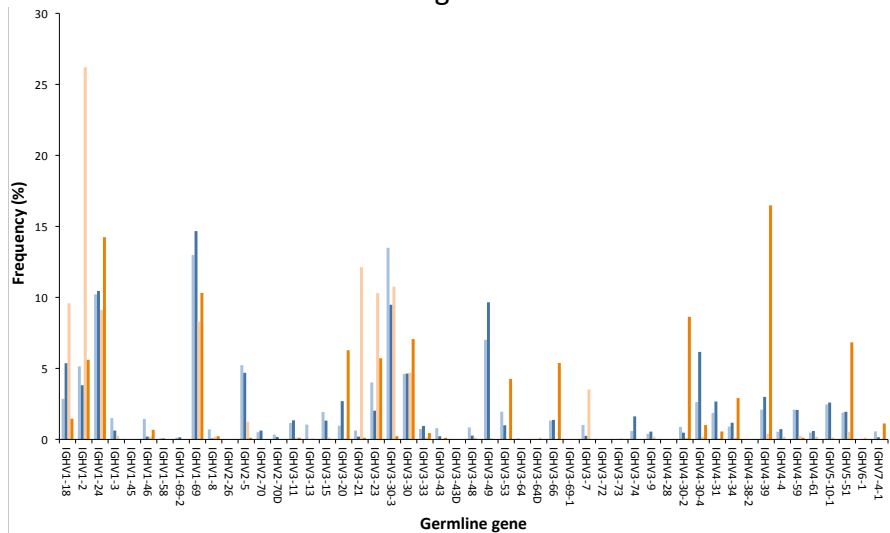
Donor 2



BM sample 1

BM sample 2

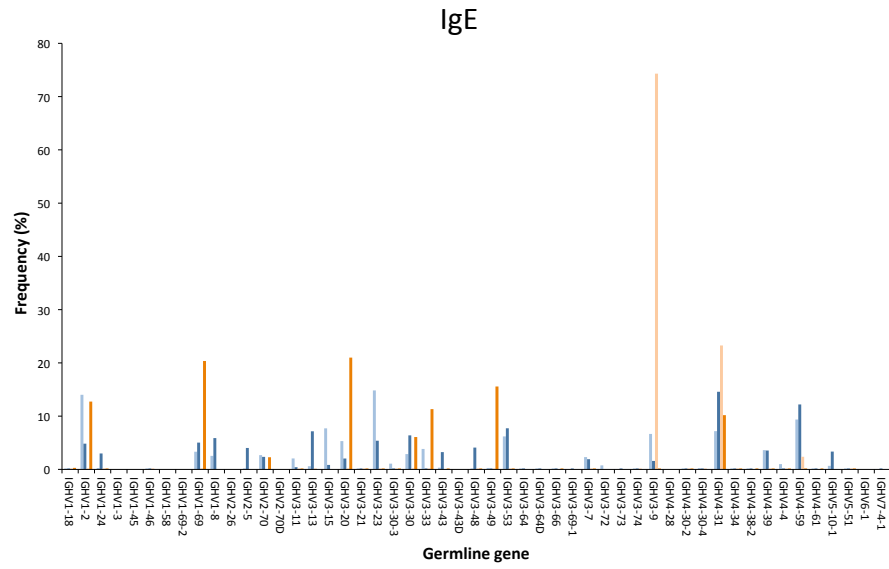
IgG4



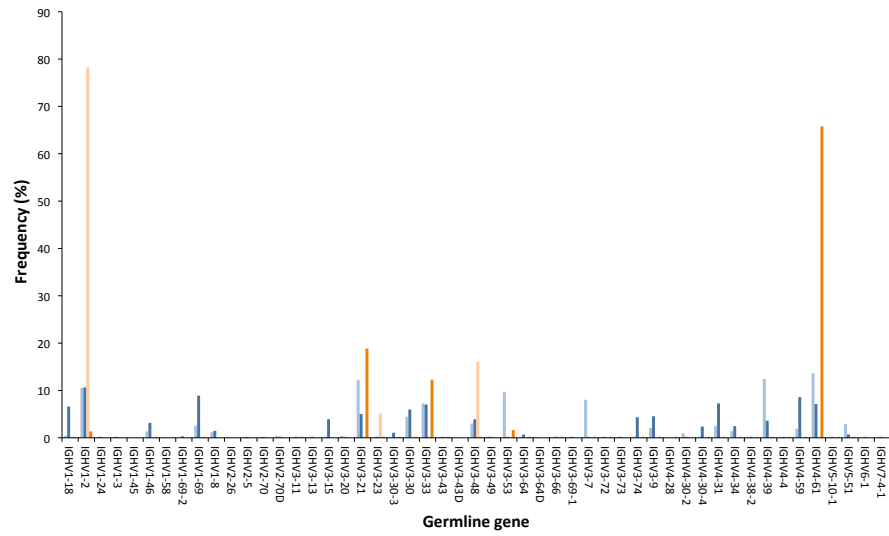
PB sample 1

PB sample 2

Donor 3



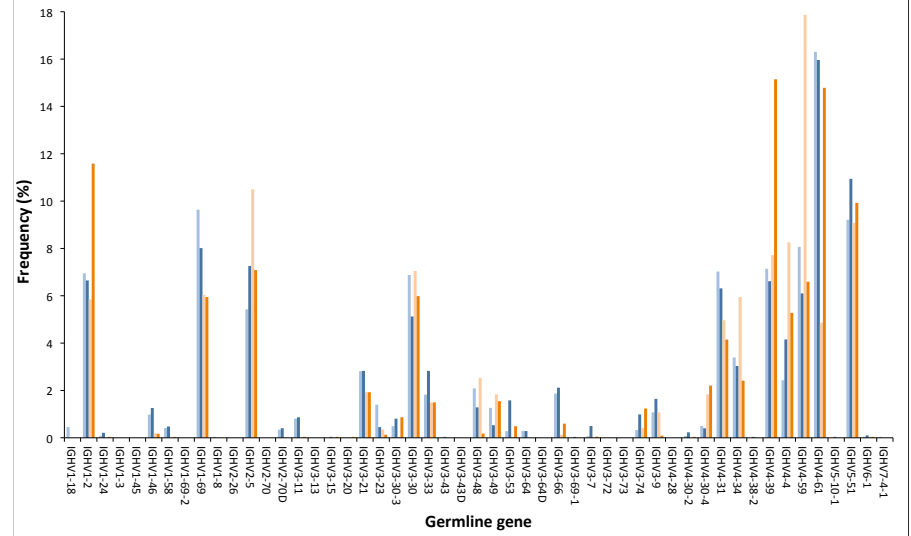
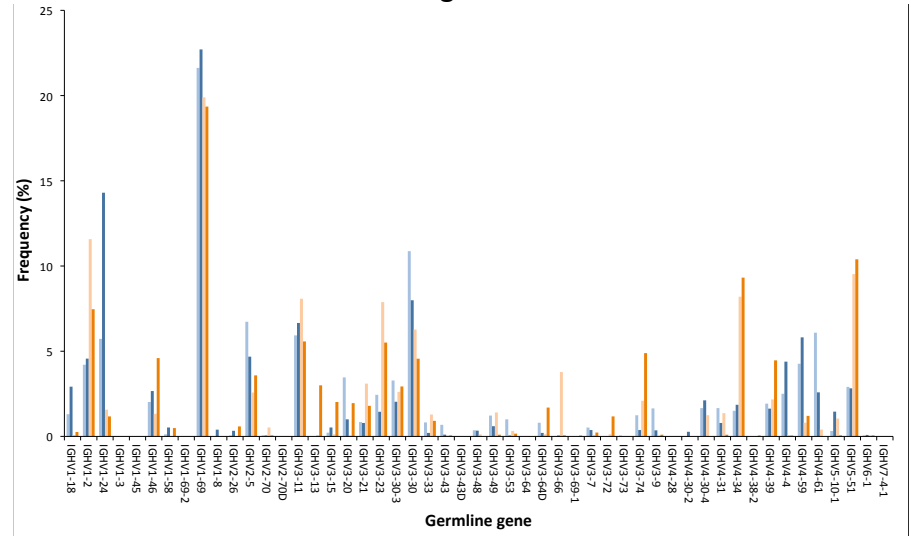
Donor 4



BM sample 1

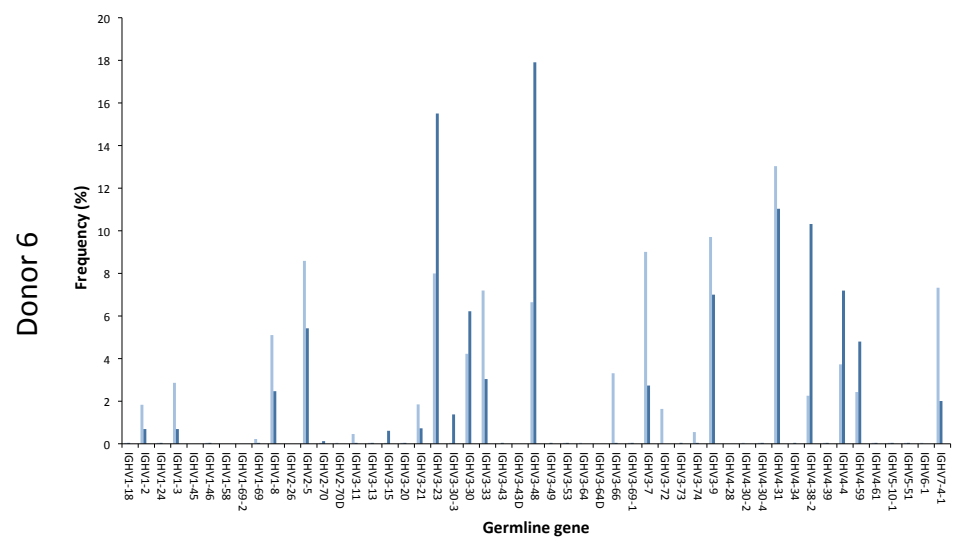
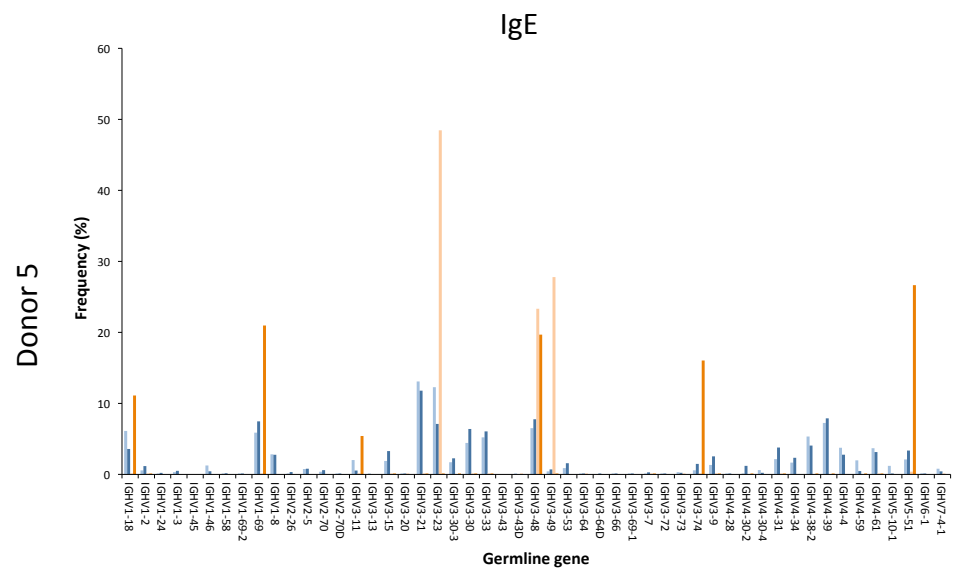
BM sample 2

IgG4



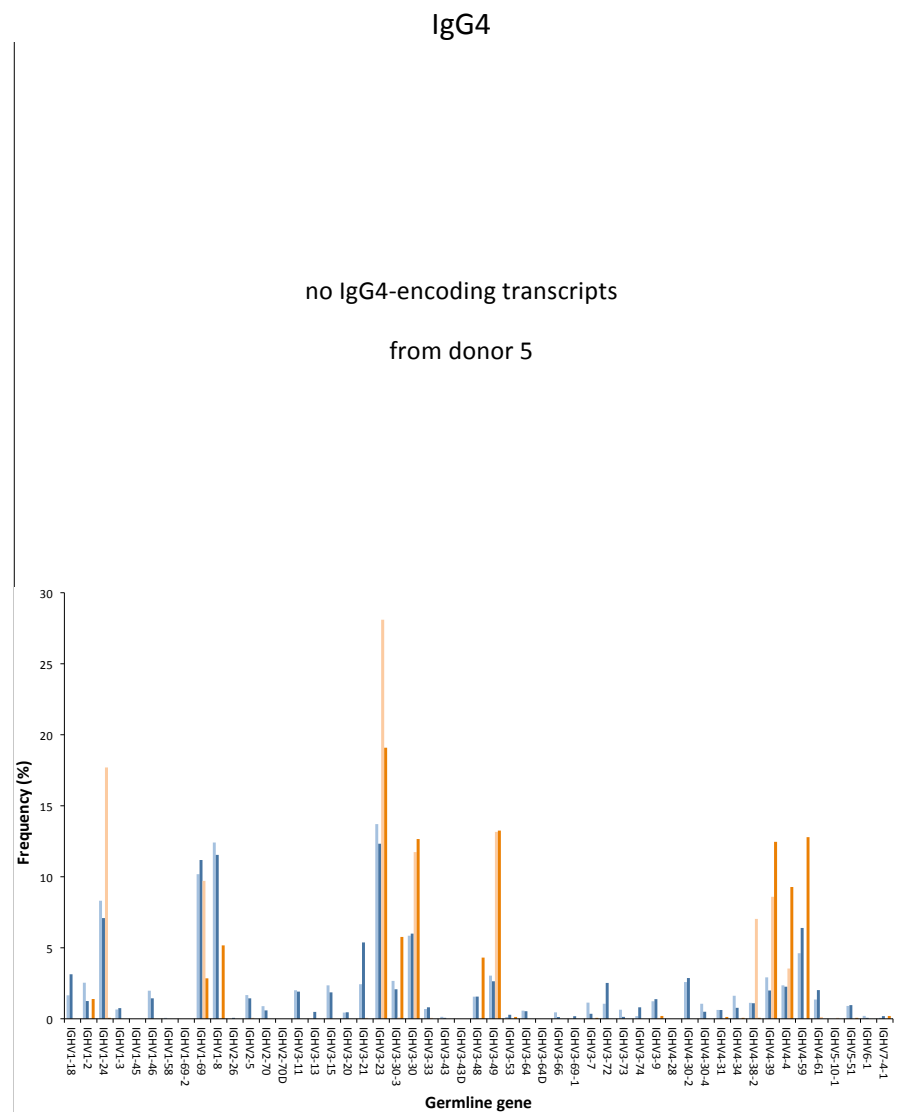
PB sample 1

PB sample 2



BM sample 1

BM sample 2



PB sample 1

PB sample 2

A

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IGHV3-23*01
Donor1_NG_Sample1 80561912E=10462
Donor1_NG_Sample1 79481912E=+1
Donor1_NG_Sample1 79481912E=+19
Donor1_NG_Sample1 82691912E=+16
Donor1_NG_Sample1 84031912E=+16
Donor1_NG_Sample1 60431912E=+12
Donor1_NG_Sample1 84111912E=+11
Donor1_NG_Sample1 17261912E=+47
Donor1_NG_Sample2 17261912E=+188
IGHV3*02
GGAATTCACCTTTAGTCGTTATGGCATGAGCTGGGTCGCCAGAGCTCCAGGGAAGGGGTGGAATGGGCTCAGCTTATAGTGGTATGTTGGTATGACACAT
IGHV3-23*01
Donor1_NG_Sample1 80561912E=10462
Donor1_NG_Sample1 79481912E=+1
Donor1_NG_Sample1 79481912E=+19
Donor1_NG_Sample1 82691912E=+16
Donor1_NG_Sample1 84031912E=+16
Donor1_NG_Sample1 60431912E=+12
Donor1_NG_Sample1 17261912E=+47
Donor1_NG_Sample2 17261912E=+188
IGHV3*02
GGAATTCACCTTTAGTCGTTATGGCATGAGCTGGGTCGCCAGAGCTCCAGGGAAGGGGTGGAATGGGCTCAGCTTATAGTGGTATGTTGGTATGACACAT
IGHV3-23*01
Donor1_NG_Sample1 80561912E=10462
Donor1_NG_Sample1 79481912E=+1
Donor1_NG_Sample1 79481912E=+19
Donor1_NG_Sample1 82691912E=+16
Donor1_NG_Sample1 84031912E=+16
Donor1_NG_Sample1 60431912E=+12
Donor1_NG_Sample1 17261912E=+47
Donor1_NG_Sample2 17261912E=+188
IGHV3*02
GGAATTCACCTTTAGTCGTTATGGCATGAGCTGGGTCGCCAGAGCTCCAGGGAAGGGGTGGAATGGGCTCAGCTTATAGTGGTATGTTGGTATGACACAT
```

B

```
IGHV3-23*01
Donor1_NG_Sample1 11081912E=+7620
Donor1_NG_Sample1 113011912E=+43
Donor1_NG_Sample1 106031912E=+17
Donor1_NG_Sample1 122171912E=+17
Donor1_NG_Sample1 11131912E=+16
Donor1_NG_Sample1 111791912E=+13
Donor1_NG_Sample1 111791912E=+12
Donor1_NG_Sample1 111791912E=+11
Donor1_NG_Sample1 106011912E=+10
Donor1_NG_Sample1 104901912E=+10
Donor1_NG_Sample1 10991912E=+1991
Donor1_NG_Sample1 80481912E=+51
Donor1_NG_Sample1 82691912E=+50
Donor1_NG_Sample1 84471912E=+36
Donor1_NG_Sample1 82691912E=+17
Donor1_NG_Sample1 79481912E=+17
Donor1_NG_Sample1 802251912E=+14
Donor1_NG_Sample1 77481912E=+13
Donor1_NG_Sample1 81841912E=+10
Donor1_NG_Sample1 80481912E=+10
Donor1_NG_Sample2 84601912E=+10
IGHV3-23*01
Donor1_NG_Sample1 11081912E=+7620
Donor1_NG_Sample1 113011912E=+43
Donor1_NG_Sample1 106031912E=+17
Donor1_NG_Sample1 122171912E=+17
Donor1_NG_Sample1 11131912E=+16
Donor1_NG_Sample1 111791912E=+13
Donor1_NG_Sample1 111791912E=+12
Donor1_NG_Sample1 111791912E=+11
Donor1_NG_Sample1 106011912E=+10
Donor1_NG_Sample1 104901912E=+10
Donor1_NG_Sample1 10991912E=+1991
Donor1_NG_Sample1 80481912E=+51
Donor1_NG_Sample1 82691912E=+50
Donor1_NG_Sample1 84471912E=+36
Donor1_NG_Sample1 82691912E=+17
Donor1_NG_Sample1 79481912E=+17
Donor1_NG_Sample1 802251912E=+14
Donor1_NG_Sample1 77481912E=+13
Donor1_NG_Sample1 81841912E=+10
Donor1_NG_Sample1 80481912E=+10
Donor1_NG_Sample2 84601912E=+10
IGHV3-23*01
Donor1_NG_Sample1 11081912E=+7620
Donor1_NG_Sample1 113011912E=+43
Donor1_NG_Sample1 106031912E=+17
Donor1_NG_Sample1 122171912E=+17
Donor1_NG_Sample1 11131912E=+16
Donor1_NG_Sample1 111791912E=+13
Donor1_NG_Sample1 111791912E=+12
Donor1_NG_Sample1 111791912E=+11
Donor1_NG_Sample1 106011912E=+10
Donor1_NG_Sample1 104901912E=+10
Donor1_NG_Sample1 10991912E=+1991
Donor1_NG_Sample1 80481912E=+51
Donor1_NG_Sample1 82691912E=+50
Donor1_NG_Sample1 84471912E=+36
Donor1_NG_Sample1 82691912E=+17
Donor1_NG_Sample1 79481912E=+17
Donor1_NG_Sample1 802251912E=+14
Donor1_NG_Sample1 77481912E=+13
Donor1_NG_Sample1 81841912E=+10
Donor1_NG_Sample1 80481912E=+10
Donor1_NG_Sample2 84601912E=+10
```

C

```
IGHV3-23*01
Donor1_NG_Sample1 110401912E=+1150
Donor1_NG_Sample1 116071912E=+10
Donor1_NG_Sample2 106091912E=+148
IGHV3-23*01
Donor1_NG_Sample1 110401912E=+1150
Donor1_NG_Sample1 116071912E=+10
Donor1_NG_Sample2 106091912E=+148
IGHV3-23*01
Donor1_NG_Sample1 110401912E=+1150
Donor1_NG_Sample1 116071912E=+10
Donor1_NG_Sample2 106091912E=+148
IGHV3-23*01
Donor1_NG_Sample1 110401912E=+1150
Donor1_NG_Sample1 116071912E=+10
Donor1_NG_Sample2 106091912E=+148
```

D

```
IGHV3-23*01
Donor1_NG_Sample1 110501912E=+1398
Donor1_NG_Sample1 106401912E=+10
Donor1_NG_Sample1 116031912E=+617
Donor1_NG_Sample2 80701912E=+261
IGHV3-23*01
Donor1_NG_Sample1 110501912E=+1398
Donor1_NG_Sample1 106401912E=+10
Donor1_NG_Sample1 116031912E=+617
Donor1_NG_Sample2 80701912E=+261
IGHV3-23*01
Donor1_NG_Sample1 110501912E=+1398
Donor1_NG_Sample1 106401912E=+10
Donor1_NG_Sample1 116031912E=+617
Donor1_NG_Sample2 80701912E=+261
```

Clonally related variants in duplicate samples 1 and 2 share multiple mutations in relation to the IGHV3-23 germline gene sequence. One sequence variant is present in both samples. One further mutated sequence is only present in one sample (sample 2). In addition rare reads representing sequence variants, potentially caused by PCR errors, were found.

Three clonally related variants (two found in sample 1 and one found in sample 2). Two mutations are shared between two of the major sequence variants (represented by clone number 11058 of sample 1 and clone number 8055 of sample 2) but no mutations in relation to the IGHV3-23 germline gene sequence are shared by all three variants. In addition rare reads representing sequence variants, potentially caused by PCR errors, were found.

Identical sequences of one clone were detected in duplicate samples 1 and 2. They have seven mutations in relation to the IGHV3-23 germline gene sequence. In addition a few reads representing a sequence variant, potentially caused by PCR errors, were found.

Two clonally related variants (two found in sample 1 and one found in sample 2) were identified. They all share six mutations in relation to the IGHV3-23 germline gene sequence. In addition rare reads representing a sequence variant, potentially caused by PCR errors, were found.

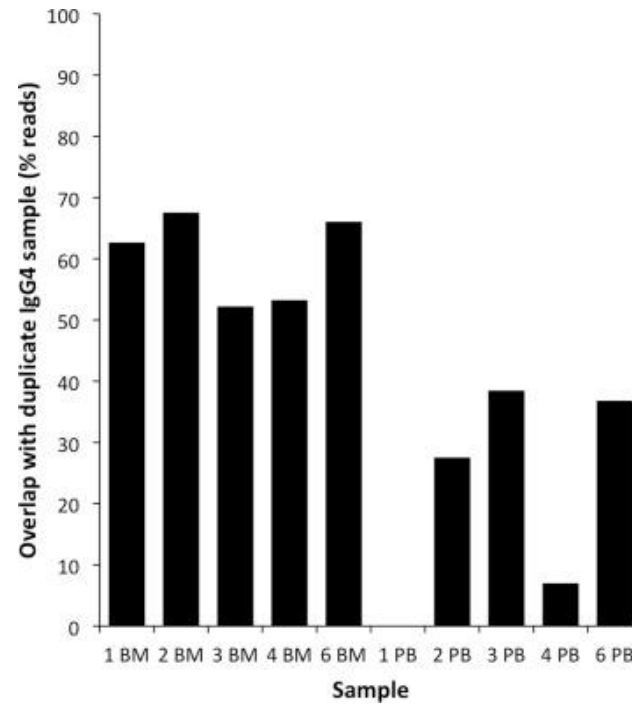


Fig E11.

Mattias Levin, Fredrik Levander, Robert Palmason, Lennart Greiff, Mats Ohlin

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Journal of Allergy and Clinical Immunology, 2016, Available online 9 August 2016

<http://dx.doi.org/10.1016/j.jaci.2016.06.040>

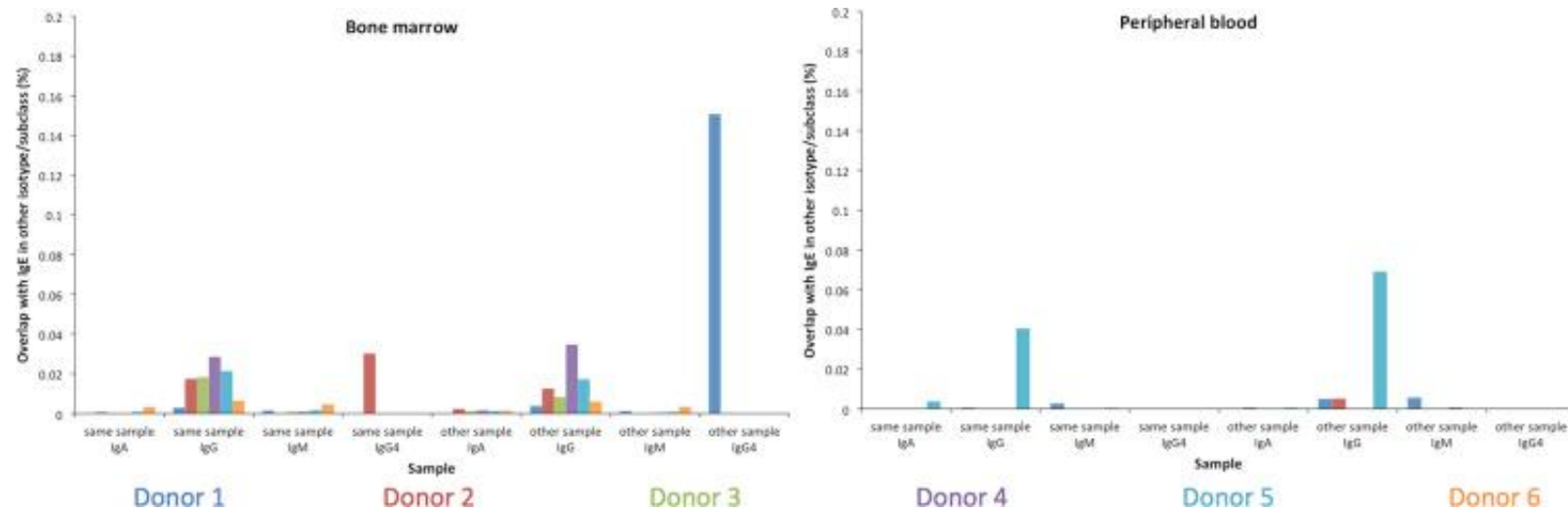


Fig E12.

Mattias Levin, Fredrik Levander, Robert Palmason, Lennart Greiff, Mats Ohlin

Antibody-encoding repertoires of bone marrow and peripheral blood—a focus on IgE

Journal of Allergy and Clinical Immunology, 2016, Available online 9 August 2016

<http://dx.doi.org/10.1016/j.jaci.2016.06.040>

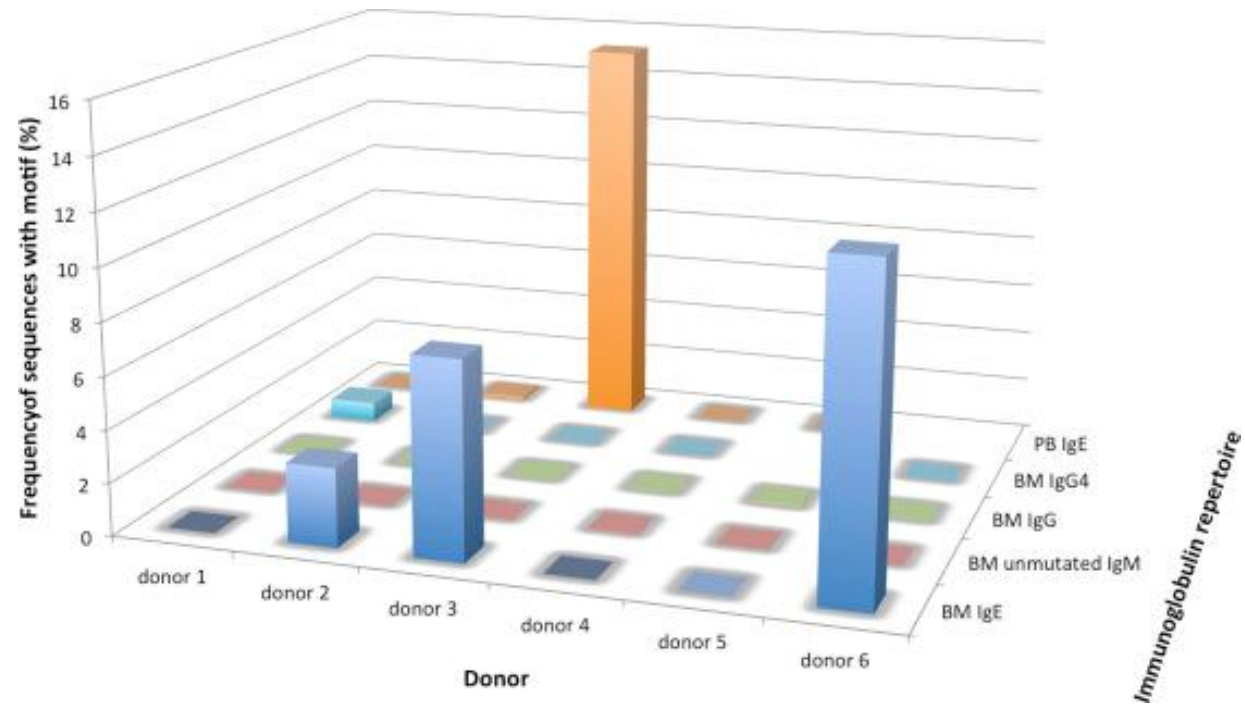


Fig E13.

Mattias Levin, Fredrik Levander, Robert Palmason, Lennart Greiff, Mats Ohlin

Antibody-encoding repertoires of bone marrow and peripheral blood—a focus on IgE

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<http://dx.doi.org/10.1016/j.jaci.2016.06.040>