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Research paper

The quantification of antibody elements and receptors subunit expression using qPCR: The design of VH, VL, CH, CL, FcR subunits primers for a more holistic view of the immune system

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ABSTRACT

The expression levels of immunoglobulin elements and their receptors are important markers for health and disease. Within the immunoglobulin locus, the constant regions and the variable region families are associated with certain pathologies, yet a holistic view of the interaction between the expressions of the multiple genes remain to be fully characterized. There is thus an important need to quantify antibody elements, their receptors and the receptor subunits in blood (PBMC cDNA) for both screening and detailed studies of such associations. Leveraging on qPCR, we designed primers for all Vκ1-6, VH1-7, Vλ1-11, nine CH isotypes, Cκ, Cκ, Cλ1 &3, FcεRI α,β, and γ subunits, all three FcγR and their subunits, and FcαR. Validating this on a volunteer PBMC cDNA, we report a qPCR primer set repertoire that can quantify the relative expression of all the above genes to the GAPDH housekeeping gene, with implications and uses in both clinical monitoring and research.

1. Introduction

There are large numbers of immune genes in the adaptive immune response and their expressions levels have been associated to various states of health and disease (Lindström, 1970; McKelvey and Fahey, 1965). In healthy individuals, the ratio of expression of these immune genes (e.g. antibodies) can be associated with pre-disease states. Biases in Immunoglobulin (Ig) variable heavy chains (VH chains) 1, 3, and 4 families were found to be more prominent in asthma (Snow et al., 1995), with VH1 reported to be dominant in patients suffering from peanut allergy (Janezic et al., 1998). High levels of certain antibody isotypes were also found to be associated with specific diseases e.g. IgE for allergy (Stanworth, 1993), IgA in IgA nephropathy (Suzuki et al., 2011), and IgM in hyper-IgM syndrome (Lougaris et al., 2005) amongst an inexhaustive list. The constant region (forming the isotype) was also able to influence the ability of V-regions to bind antigens (Lua et al., 2018) and the variable light chain (VL) family frameworks were also found to impact the interaction with antigens (Su et al., 2017), antibody production (Ling et al., 2018), and the interaction with Ig receptors (FcRs, see Ling et al., 2018), often in conjunction with VH frameworks (Su et al., 2018; Lua et al., 2019). Given the effects of the various antibody parts and their association with certain pathologies, the relative quantification of specific antibody elements and genes together with the antibody receptors and their subunits are important markers in health screening and to provide a more holistic investigation to disease associations. Since antibodies often work in tandem with effector immune cells via the FcR, quantifying FcR expression levels in PMBC provide an insight to the responses available to the various antibody isotypes. To address this, we aim to develop a set of primers for qPCR to study the spectrum of immune genes in PBMCs. The method developed here aims to aid the diagnostic and further study of immunological conditions and disease.

Our primer repertoire targets the six kappa variable light chain families (Vκ1-6), eleven lambda variable light chain families (Vλ1-11), seven variable heavy chain families (VH1-7), one kappa constant light chain (Cκ), seven lambda constant light chains (Cλ1-7), and nine constant heavy chains (IgG1-4, IgA1&2, IgE, IgM and IgD), along with

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the respective Fc receptor subunits: FcγRIα, IIα, IIβ, IIc, IIIα, IIIβ, FcαR, FcεRIα, FcεRIβ, FcεRIγ & FcεRII. We quantified the target messenger ribonucleic acid (mRNA) through complementary deoxyribonucleic acid (cDNA) synthesis using quantitative real-time polymerase chain reaction (qPCR) and showed their levels in human PBMC cDNA.

2. Material and methods

2.1. Designing qPCR primers

Full receptor DNA sequences were obtained from GenBank ® (https://www.ncbi.nlm.nih.gov/genbank, Benson et al., 2013), and immunoglobulin DNA sequences were obtained from IMGT (http://www.imgt.org, Lefranc et al., 1999), with the following accession numbers shown in Table A.1. DNA sequences of target genes were analysed using the Primer3plus program (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi, Untergasser et al., 2007) for qPCR. Primer output were subjected to UCSC In-Silico PCR program (https://genome.ucsc.edu/cgi-bin/hgPcr, Kuhn et al., 2013) for verification of specificity on the target gene along with melting curves analysis for those with only a single peak.

2.2. Real-time polymerase chain reaction (qPCR)

The quantification of gene expression was obtained from Applied Biosystem StepOnePlus™. PowerUp™ SYBR® Green Master Mix (2X) reagents were used according to the manufacturer's recommendations. Each gene target quantification reaction was performed separately with the respective primer sets. For simultaneous operation, the amplification and melt curve reaction settings utilized a constant annealing temperature despite the different theoretical melting temperatures of each primer set. The settings are as follows: Stage 1 (1 cycle) at 50°C, for 120 seconds, followed by 95°C for 2 seconds; Stage 2 (40 cycles) at 95°C for 3 seconds, followed by 60°C for 30 seconds; and melt curve at 95°C for 15 seconds followed by 60°C for 60 seconds, and finally 95°C for 15 seconds. Optimization and validation of primer pairs consisted of triplicate independent runs with technical triplicates including "No Template Controls" (NTC) and samples for each run.

2.3. Gene expression levels

Gene expression results were analysed using Applied Biosystem software, StepOnePlus™ Version 2.3. Threshold Cycle (CT) values were auto-populated with all parameters set on default with Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene as the endogenous control. The GAPDH gene was used as baseline to deduct each gene expression CT mean value from triplicate independent runs (including technical triplicates), further divided by GAPDH gene CT mean value and converted to percentage using the following formula: $\frac{GAPDH\ CT\ mean - Gene\ \Delta\ CT\ mean}{GAPDH\ CT\ mean} \times 100\%$

3. Results and discussion

3.1. Primer design

Since antibodies are formed by V(D)J recombination rather than by the artificial VH and VL frameworks (discussed in Phua et al., 2019), many antibody target genes have several different germline sequences classified within the same VH or VL family. It is thus difficult to design primers for every individual germline sequence. To overcome this issue, we selected the representatives of each target gene (of the VH and VL families) through sequence alignment of all other germlines to determine the most conserved regions. The resulting list of qPCR primers was generated based on Vκ families (Table 1), VH families (Table 2), Vλ families (Table 3), CH, and both CLs: Cκ and Cλ (Table 4) alongside the immunoglobulin receptor subunits and GAPDH (Table 5) using the Primer3plus program (refer to 2.1)

For the primers to amplify the correct gene targets in the pool of cDNA, the specificity is important. To assess the specificity of our primers, we ensured that only a single melting curve was present for the target genes (Figure S1-8, Appendix B). However, highly related gene targets such as Cλ 2, 4, 5, 6 and 7 complicated the design of specific primers, and were thus excluded.

Primers were chosen to keep the melting temperature (Tm) close to 60 °C and GC contents around 50% with amplicon product sizes between 70 to 150 base pairs (bp).

Since FcαμR (with dual specificity to antibody isotype IgA and IgM) is only expressed and found in niche populations of IgD+/CD38+ mature B cells in tonsil tissue (Kikuno et al., 2007) and activated macrophages (Feng et al., 2010), it was also excluded (Table 5) given the purpose towards blood PBMC cDNA quantification.

Table 1
List of qPCR forward and reverse primers presented in 5′ to 3′ fashion generated using Primer3plus program against Vκ families as gene targets with respective primer lengths, melting temperatures and GC contents listed.

Primer name	Sequence (5′ to 3′)	Length (bp)	Tm (°C)	GC content (%)
Vκ1F	AAAGTGGGGTCCCATCAAG	19	59.8	52.60
Vκ1R	AGGCTGCTGATGGTGAGAG	19	59.1	57.90
Vκ2F	TGGAGTGCCAGATAGGTTCAG	21	60.3	52.40
Vκ2R	TAAACCCCAACATCCTCAGC	20	59.9	50.00
Vκ3F	ATCCCAGCCAGGTTCAGTG	19	61.1	57.90
Vκ3R	GCAAAATCTTCAGGCTCTAGG	21	58.2	47.60
Vκ4F	CTGGGACAGATTTCACTCTCAC	22	58.8	50.00
Vκ4R	AATAAACTGCCACATCTTCAGC	22	58.4	40.90
Vκ5F	ATCCCACCTCGATTCAGTG	19	58.4	52.60
Vκ5R	GAAGTAATATGCAGCATCCTCAG	23	58.1	43.50
Vκ6F	GTTCAGTGGCAGTGGATCTG	20	59.3	55.00
Vκ6R	TGCAGCATCTTCAGCTTCC	20	61.6	50.00

Table 2
List of qPCR forward and reverse primers presented in 5′ to 3′ fashion generated using Primer3plus program against VH families as gene targets with respective primer lengths, melting temperatures and GC contents listed.

Primer name	Sequence (5′ to 3′)	Length (bp)	Tm (°C)	GC content (%)
VH1F	ACAGAAGTTCCAGGGCAGAG	20	59.5	55.00
VH1R	AGCTCCATGTAGGCTGTGC	19	59.0	57.90
VH2F	ACAGCACATCTCTGAAGACCAG	22	59.5	50.00
VH2R	AGGGTCCATGTTGGTCATTG	20	60.6	50.00
VH3F	AAGGGCCGATTCACCATC	18	60.8	55.60
VH3R	GCTCTCAGGCTGTTCATTTG	20	58.6	50.00
VH4F	ACTACAACCCGTCCCTCAAG	20	59.1	55.00
VH4R	TCAGCTTCAGGGAGAACTGG	20	60.5	55.00
VH5F	TACAGCCCGTCCTTCCAAG	19	61.2	57.90
VH5R	TTCAGGCTGCTCCACTGC	18	61.3	61.10
VH6F	GAAAAGTCGAATAACCATCAACC	23	58.9	39.10
VH6R	GTGTCCTCGGGAGTCACAG	19	59.2	63.20
VH7F	ACGGTTTGTCTTCTCCTTGG	20	59.2	50.00
VH7R	TAATACACGGCAGTGTCCTCAG	22	60.2	50.00

Table 3
List of qPCR forward and reverse primers presented in 5′ to 3′ fashion generated using Primer3plus program against Vλ families as gene targets with respective primer lengths, melting temperatures and GC contents listed.

Primer name	Sequence (5′ to 3′)	Length (bp)	Tm (°C)	GC content (%)
Vλ1F	CGATTCTCTGGCTCCAAGTC	20	60.0	55.00
Vλ1R	CAGTAATAATCAGCCTCATCCTC	23	57.1	43.50
Vλ2F	CAGGGGTTTCTAATCGCTTC	20	58.8	50.00
Vλ2R	GCAGTAATAATCAGCCTCGTCC	22	60.1	50.00
Vλ3F	ATCCCTGAGCGATTCTCTG	19	57.9	52.60
Vλ3R	ACAGTAATAGTCAGCCTCATCC	22	55.1	45.50
Vλ4F	GAGTTCCTGATCGCTTCTCAG	21	59.2	52.40
Vλ4R	CAGTAATAATCAGCCTCATCCTC	23	57.1	43.50
Vλ5F	CAGCCGCTTCTCTGGATC	18	59.6	61.10
Vλ5R	CCTCATCCTCAGACTGGAGC	20	59.9	60.00
Vλ6F	ATCGGTTCTCTGGCTCCATC	20	61.5	55.00
Vλ6R	TAGTCAGCCTCGTCCTCAGTC	21	59.6	57.10
Vλ7F	TCTCAGGCTCCCTCCTTG	18	59.0	61.10
Vλ7R	GTAATACTCAGCCTCATCCTCAG	23	57.2	47.80
Vλ8F	GCTCTTCTGGGGTCCCTG	18	60.8	66.70
Vλ8R	TTCATCATCTGCCTGGGC	18	60.7	55.60
Vλ9F	ATCCCTGATCGCTTCTCAGTC	21	60.8	52.40
Vλ9R	CTTCCTGGATGTTCTTGATGG	21	59.5	47.60
Vλ10F	TGCATCCAGGTCAGGAAAC	19	59.6	52.60
Vλ10R	TAATAGTCAGCCTCGTCCTCAG	22	58.6	50.00
Vλ11F	TCCAAGGAGACCTCAAGTAACAC	23	59.7	47.80
VL11R	TAATAATCGGCCTCGTCCTC	20	59.1	50.00

Table 4
List of qPCR forward and reverse primers presented in 5′ to 3′ fashion generated using Primer3plus program against CHs, Cκ and Cλs as gene targets with respective primer lengths, melting temperatures and GC contents listed.

Primer name	Sequence (5′ to 3′)	Length (bp)	Tm (°C)	GC content (%)
IgG1F	CTGCAACGTGAATCACAAGC	20	60.5	50.00
IgG1R	GTGGGCATGTGTGAGTTTTG	20	60.0	50.00
IgG2F	GCAAATGTTGTGTCGAGTGC	20	60.3	50.00
IgG2R	AGGGTGTCCTTGGGTTTTG	19	59.8	52.60
IgG3F	CCTGCAACGTGAATCACAAG	20	60.3	50.00
IgG3R	TCACAAGATTTGGGCTCTGG	20	61.2	50.00
IgG4F	TTCAACTGGTACGTGGATGG	20	59.4	50.00
IgG4R	TTGTACTCCTTGCCGTTCAG	20	58.9	50.00
IgA1F	ATGGGAAGACCTTCACTTGC	20	59.1	50.00
IgA1R	TGTTTCCGGATTTTGAGAGG	20	60.0	45.00
IgA2F	AACCATGGGGAGACCTTCAC	20	61.2	55.00
IgA2R	CCGGATTTTGTGATGTTGG	19	59.8	47.40
IgEF	ACTATGCCACCATCAGCTTG	20	58.8	50.00
IgER	GTTTTGTTGTCGACCCAGTC	20	58.0	50.00
IgMF	TGACCTTCCAGCAGAATGC	19	59.9	52.60
IgMR	GAAGATGCTGGCAAAGGATG	20	60.7	50.00
IgDF	CTGGCATGCTTGATAACTGG	20	59.3	50.00
IgDR	CGTCTTTGTATCTCAGGGAAGG	22	60.1	50.00
IgκF	ACGCTGAGCAAAGCAGACTAC	21	59.8	52.40
IgκR	CCCTGTTGAAGCTCTTTGTG	20	58.5	50.00
Igλ1F	ACCAAACCCTCCAAACAGAG	20	59.0	50.00
Igλ1R	GCTGTAGCTTCTGTGGGACTTC	22	60.4	54.50
Igλ3F	GTTGCCTGGAAGGCAGATAG	20	59.8	55.00
Igλ3R	TGGCAGCTGTAGCTTTTGTG	20	60.2	50.00
Igλ7F	AGGAGCTTCAAGCCAACAAG	20	59.6	50.00
Igλ7R	AGGAGCTTCAAGCCAACAAG	20	61.8	50.00

Table 5
List of qPCR forward and reverse primers presented in 5′ to 3′ fashion generated using Primer3plus program against immunoglobulin receptor subunits and GAPDH as gene targets with respective primer lengths, melting temperatures and GC contents listed.

Primer name	Sequence (5′ to 3′)	Length (bp)	Tm (°C)	GC content (%)
FcγRIαF	CTTTGGGTTCCAGTTGATGG	20	60.3	50.00
FcγRIαR	TGCAAGGTTACGGTTTCCTC	20	60.1	50.00
FcγRIβF	AATGGCACCTACCATTGCTC	20	60.0	50.00
FcγRIβR	TTGGTAACTGGAGGCCTTTC	20	59.2	50.00
FcγRIIαF	ATCTTGGCTCACTGCAAACC	20	60.3	50.00
FcγRIIαR	ATGGCACATGCCTCTAATCC	20	59.9	50.00
FcγRIIβF	AAAGTTGGGGCTGAGAACAC	20	59.2	50.00
FcγRIIβR	CCCAATGCAAGACAATGGAG	20	61.4	50.00
FcγRIIcF	TGCCATCAGAAAGAGACAACC	21	60.2	47.60
FcγRIIcR	ACTGTTGACATGGTCGTTGG	20	59.4	50.00
FcγRIIIαF	CTTTTTGCAGTGGACACAGG	20	59.3	50.00
FcγRIIIαR	GATGGGGGTCATTTGTCTTG	20	60.2	50.00
FcγRIIIβF	TCAATGGTACAGCGTGCTTG	20	60.9	50.00
FcγRIIIβR	TGGCAGCGTCAATGAAGTAG	20	60.0	50.00
FcεRIαF	TCAGCAGCAGGTCACATTTC	20	60.0	50.00
FcεRIαR	TTGGCTTAGGATGTGGGTTC	20	59.9	50.00
FcεRIβF	CCAAGTGCTTTATGGCTTCC	20	59.7	50.00
FcεRIβR	TTTGAGTTCTTCCCCAGCTC	20	59.4	50.00
FcεRIγF	TCTCCAGCCCAAGATGATTC	20	60.2	50.00
FcεRIγR	TGGCATCCAGGATATAGCAG	20	58.7	50.00
FcεRIIF	TTGAACGAGAGGAACGAAGC	20	60.5	50.00
FcεRIIR	ACTTTTCAGGGCACGTGTTG	20	61.1	50.00
FcαRF	TACGGCACTGAACAAGGAAG	20	58.9	50.00
FcαRR	CACTTGGTGTTCGTGCAAAG	20	60.3	50.00
GAPDHF	CAATGACCCCTTCATTGACC	20	60.2	50.00
GAPDHR	ATGACAAGCTTCCCGTTCTC	20	59.3	50.00

3.2. Threshold cycle (Ct) data of gene targets

After designing the primers, we validated them on PBMCs. Three independent runs with three technical replicates, Ct mean values, delta Ct mean and delta Ct standard error (SE) were calculated as shown in Table 6.

The primer sets were evaluated based on the returned Ct mean values generated from the StepOnePlus™ software. As shown in Table 6, all primer sets had acceptable returned values ranging from the lowest Ct value of 16.23 (GAPDH) to the highest Ct value of 30.79 (Vκ5). One exception was the Vλ11 (Ct value of 35.79 that is near the end of total amplification cycles) gene that had either insufficient template or unspecific amplification. The former was more likely since the values were populated from three independent runs with technical triplicates showing low Ct standard error (SE), suggesting that the expression levels of Vλ11 were sufficient for the assay. It should be noted that despite having a return CT mean value, Vλ11 expression levels in the volunteer were measured to be close to 0% (Table 6). Testing the linearity of our qPCR experimental data with selected representative genes (due to limited volunteer samples) from each variable families (Vκ1 from Vκ, VH3 from VH, Vλ1 from Vλ), constant regions (IgE, IgD, Igκ), Fc receptor (FcεRIα) and endogenous control (GAPDH), we found a high linearity in our tested range of 12.5 ng, 1.25 ng, 0.125 ng, 0.0125 ng and 0.00125 ng of cDNA (Figure S9).

Table 6
CT value of various gene targets populated from StepOnePlus™ Version 2.3. Gene target GAPDH were used as endogenous control as well as normalization. CT mean, delta CT mean and CT SE values are obtained from independent triplicate run with each run containing triplicate technical samples.

Gene target	Acronym	Ct mean	ΔCt mean	ΔCt SE
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH	16.23	-	-
Variable heavy 1	VH1	23.14	6.58	0.05
Variable heavy 2	VH2	26.04	9.48	0.07
Variable heavy 3	VH3	17.17	0.61	0.07
Variable heavy 4	VH4	21.78	5.22	0.05
Variable heavy 5	VH5	21.78	5.22	0.05
Variable heavy 6	VH6	28.32	11.76	0.05
Variable heavy 7	VH7	27.09	10.53	0.06
Variable light kappa 1	Vκ1	17.78	1.23	0.06
Variable light kappa 2	Vκ2	22.37	5.81	0.05
Variable light kappa 3	Vκ3	24.24	7.68	0.06
Variable light kappa 4	Vκ4	25.17	8.62	0.05
Variable light kappa 5	Vκ5	30.79	14.24	0.08
Variable light kappa 6	Vκ6	28.53	11.98	0.05
Variable light lambda 1	Vλ1	19.54	2.98	0.05
Variable light lambda 2	Vλ2	24.27	7.69	0.1
Variable light lambda 3	Vλ3	20.10	3.66	0.41
Variable light lambda 4	Vλ4	22.58	5.99	0.12
Variable light lambda 5	Vλ5	28.04	11.45	0.07
Variable light lambda 6	Vλ6	27.4	10.81	0.05
Variable light lambda 7	Vλ7	26.42	9.83	0.04
Variable light lambda 8	Vλ8	25.7	9.12	0.04
Variable light lambda 9	Vλ9	30.33	13.75	0.04
Variable light lambda 10	Vλ10	27.21	10.63	0.07
Variable light lambda 11	Vλ11	35.79	19.16	0.31
Constant heavy gamma 1	IgG1	18.55	1.97	0.04
Constant heavy gamma 2	IgG2	23.06	6.48	0.05
Constant heavy gamma 3	IgG3	28.4	11.81	0.04
Constant heavy gamma 4	IgG4	16.97	0.39	0.12
Constant heavy alpha 1	IgA1	20.83	4.81	0.05
Constant heavy alpha 2	IgA2	21.73	5.71	0.04
Constant heavy delta	IgD	23.89	7.87	0.06
Constant heavy epsilon	IgE	27.31	11.29	0.06
Constant heavy mu	IgM	19.87	3.86	0.06
Constant light kappa	Igκ	19.02	3.09	0.04
Constant light lambda 1	Igλ1	20.14	4.22	0.04
Constant light lambda 3	Igλ3	17.74	1.82	0.11
Fc alpha receptor	FcαR	26.4	10.47	0.03
Fc epsilon receptor 1 alpha	FcεRIα	23.87	7.85	0.06
Fc epsilon receptor 1 beta	FcεRIβ	27.92	11.91	0.06
Fc epsilon receptor 1 gamma	FcεRIγ	21.42	5.49	0.06
Fc epsilon receptor 2	FcεRII	25.43	9.5	0.03
Fc gamma receptor 1 alpha	FcγRIα	27.56	11.55	0.07
Fc gamma receptor 1 beta	FcγRIβ	28.61	12.6	0.05
Fc gamma receptor 2 alpha	FcγRIIα	24.2	8.19	0.1
Fc gamma receptor 2 beta	FcγRIIβ	26.64	10.63	0.04
Fc gamma receptor 2 c	FcγRIIc	26.2	10.18	0.06
Fc gamma receptor 3 alpha	FcγRIIIα	25.8	9.78	0.06
Fc gamma receptor 3 beta	FcγRIIIβ	29.33	13.31	0.04

3.3. Gene expression level of gene targets

For comparing gene targets, the relative comparison method was deployed using GAPDH as a standard (relative percentage, 100%) among all independent runs and technical triplicates. Our results (Fig. 1) showed that the genes exhibited generally expected levels of expression. VH3 and Vκ1 were expectedly more abundant amongst the VH and Vκ families, a finding in agreement with a previous report (Tiller et al., 2013) There was also expected variations within the CLs, although the Cκ and Cλ levels were relatively balanced in our data and not in agreement with previous findings (Haraldsson et al., 1991; Normansell, 1987). Nonetheless, this can be explained by volunteer factors. Other unexpected quantifications include that of IgG4, IgE and IgD levels of the volunteer. While IgG4 should be at around 4% the total IgG (Hashira et al., 2000; Janeway et al., 2001), the volunteer's IgG4 levels exceeded the expression levels of IgG1. Similarly, the volunteer had a 30% IgE expression level to GAPDH (and ~5.3% of the total heavy chain Igs measured here) when the typical IgE levels in healthy individuals should be extremely low at ~ 0.000175% of the total immunoglobulin levels, and certainly lower than that of IgG4. Since the volunteer has a history of severe Type-I hypersensitivity, and that the IgE percentage to GAPDH corresponds reasonably to FcεRIα (55 %), β (27 %) and FcεRII (41 %), this result may thus be reasonable, and in fact the IgE levels supported previous reports that the ratio between IgG4 and IgE are markers for allergy (Caubet et al., 2012; Noh et al., 2007). For IgD, the volunteer had an elevated level at 52% to GAPDH (at ~9.2% to the Ig heavy chains) while it is typically about 0.23% of the total serum immunoglobulin (Janeway et al., 2001). Regardless, much remains elusive when it comes to IgD, although some studies show that IgD can substitue IgM in IgM immunodeficiency (Lutz et al., 1998), which may not explain our findings as the individual did not suffer from IgM immunodeficiency given the volunteer's IgM levels of 76% to GAPDH or ~13.4% of our tested total heavy chain Igs. Nonetheless, the discrepancies demonstrate the need to study the IgM/IgD ratios and that of the various genes, especially at various health and disease states for a more holistic understanding.

4. Conclusion

We set out to design primers that could detect the various V-region families of antibodies, the antibody isotypes and subtypes, as well receptors subunits to quantify their relative expression in PBMCs. Leveraging on the use of qPCR, the quantification could be performed with very little amounts of blood such as those extracted from finger pricks (Poh and Gan, 2014). By combining finger prick blood methods and qPCR primers, screening and diagnosis in the clinical setting can be performed easily. In the research front, a more in-depth and holistic view of the expression ratios can be gathered to shed light on their association with health and disease.

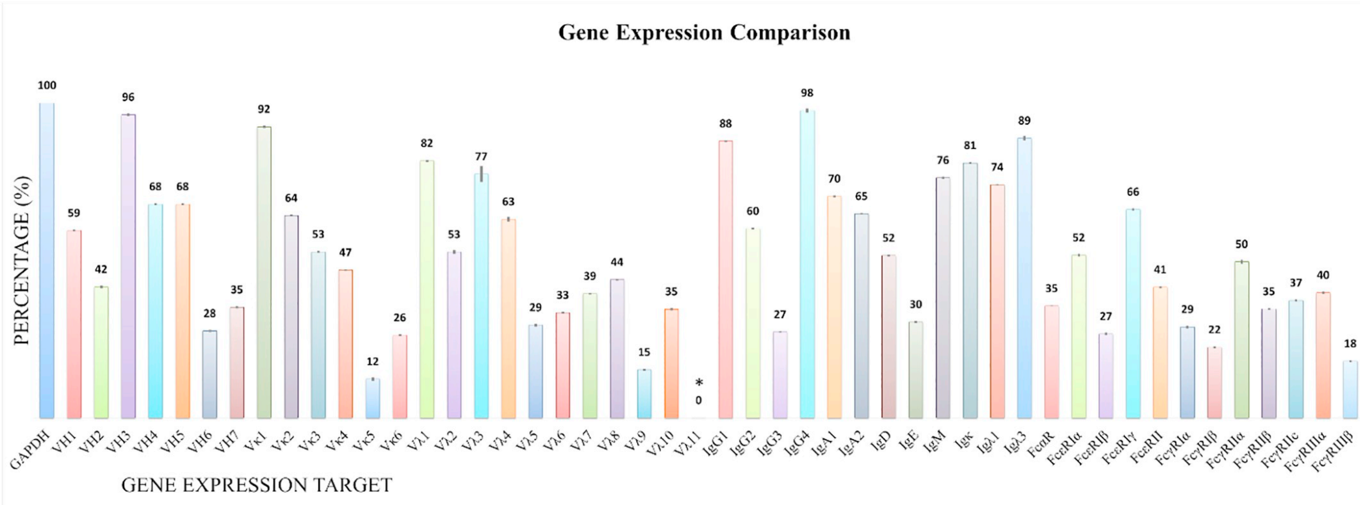

Fig 1. Histogram showing various gene expression percentages. The housekeeping GAPDH was used as an endogenous control for normalization to 100%. Percentages were calculated by subtracting delta CT mean value of each gene from GAPDH CT mean value followed by division of GAPDH CT mean value and multiplied by 100% (refer to 2.1). Asterisk (*) denotes replacement of a percentage value instead of the original value (-18%) due to ΔCT mean reading.

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Author contributions

WWL performed the experiments, analysis and drafted the manuscript. YLN, AW, DPL were involved in the writing and discussions of the manuscript. SKEG conceived and supervised all aspects of the study.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Appendices

Table A.1
List of genes with corresponding identify or reference number used in the designing of qPCR primers.

Gene name	Identification reference / number
Vκ1	IMGT Reference Sequence: **V01577**
Vκ2	IMGT Reference Sequence: **M31952**
Vκ3	IMGT Reference Sequence: **X12686**
Vκ4	IMGT Reference Sequence: **Z00023**
Vκ5	IMGT Reference Sequence: **X02485**
Vκ6	IMGT Reference Sequence: **X63399**
VH1	IMGT Reference Sequence: **Z14296**
VH2	IMGT Reference Sequence: **L21969**
VH3	IMGT Reference Sequence: **M99660**
VH4	IMGT Reference Sequence: **M99684**
VH5	IMGT Reference Sequence: **M996686**
VH6	IMGT Reference Sequence: **J04097**
VH7	IMGT Reference Sequence: **L10057**
Vλ1	IMGT Reference Sequence: **M94116**
Vλ2	IMGT Reference Sequence: **Z73664**
Vλ3	IMGT Reference Sequence: **X97473**
Vλ4	IMGT Reference Sequence: **Z73648**
Vλ5	IMGT Reference Sequence: **Z73670**
Vλ6	IMGT Reference Sequence: **Z73673**
Vλ7	IMGT Reference Sequence: **Z73674**
Vλ8	IMGT Reference Sequence: **Z73650**
Vλ9	IMGT Reference Sequence: **Z73675**
Vλ10	IMGT Reference Sequence: **Z73676**
Vλ11	IMGT Reference Sequence: **D86996**
IgG1	IMGT Reference Sequence: **J00228**
IgG2	IMGT Reference Sequence: **J00230**
IgG3	IMGT Reference Sequence: **D78345**
IgG4	IMGT Reference Sequence: **K01316**
IgA1	IMGT Reference Sequence: **J00220**

Table A.1 (*continued*)

Gene name	Identification reference / number
IgA2	IMGT Reference Sequence: **J00221**
IgE	IMGT Reference Sequence: **J00222**
IgM	IMGT Reference Sequence: **X14940**
IgD	IMGT Reference Sequence: **X57331**
FcγRIα	NCBI Reference Sequence: **NM_000566**
FcγRIβ	NCBI Reference Sequence: **NM_001244910**
FcγRIIα	NCBI Reference Sequence: **NM_021642**
FcγRIIβ	NCBI Reference Sequence: **NM_001190828**
FcγRIIc	NCBI Reference Sequence: **NR_047648**
FcγRIIIα	NCBI Reference Sequence: **NM_000569**
FcγRIIIb	NCBI Reference Sequence: **NM_000570**
FcεRIα	NCBI Reference Sequence: **NM_002001.3**
FcεRIb	NCBI Reference Sequence: **NM_000139**
FcεRIγ	NCBI Reference Sequence: **NM_004106.2**
FcεRII	NCBI Reference Sequence: **NM_001207019**
FcαR	NCBI Reference Sequence: **NM_133269**
GAPDH	NCBI Reference Sequence: **NM_002046**

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jim.2019.112683.

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