

Peer Review File

Sequence variants influencing the regulation of serum IgG subclass levels



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REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Steffanson and colleagues have conducted a GWAS of IgG levels in two human populations. This manuscript identifies novel variants associated with IgG within the immunoglobulin heavy chain locus, as well as in other immune loci elsewhere in the genome. This study provides important insight into the genetic underpinnings of antibody biology, with the potential to inform clinical medicine. In general, the article is quite terse in some places. I would recommend fleshing out several of the results sections to provide additional detail and context for the data used and the analyses performed. Likewise, expansions of the main figures would help improve the readability and comprehension of the results, without the reader needing to flip back and forth between the main text and supplement. I provide specific comments below.

Comments

Line 60: "constant regions" should be changed to "constant region genes" or "constant genes".

Line 61: "IGH" is not italicized. Individual genes within the IGH locus could be italicized depending on Nat Comm. specs.

Line 81: same as comment re line 61.

Line 92: "possibly due to different effects on different IgG subclasses". Would be important to note that the use of microarrays for genotyping in these regions is also potentially a barrier to identifying associations. This will depend on coverage of the array used, as well as the ability of that array to effectively represent the extensive haplotype diversity in the IG loci. This will vary from array to array, and it is likely that no current array will be able to fully represent the diversity in this region. This has been clearly demonstrated for other human IG regions.

Line 111 and Line 125: Some information on the methods used to compile phenotypic datasets (i.e, subclass levels and mRNA expression) should be provided in the results. This can be done concisely, pointing to the methods for more detail, of course, but a minimal level of information should be provided to aid the reader's comprehension.

Line 138: The term "subclass traits" is vague. Do you just simply mean levels? If so, seems more appropriate to say.

Lines 141-144: Seems appropriate to cite the studies relating to the traits identified in the GWAS catalog, so appropriate attribution is given.

Line 155: Regional Manhattan plots for IGH locus section and associations should be provided, along with a map of the locus. This would help readers unfamiliar with the structure of the IGH locus better understand the associations. Within these Manhattan plots, it might also be useful to indicate which SNPs displayed are imputed vs. directly genotyped by the array/sequencing data used.

Line 157: Would be good to provide references for the statement regarding "complexity" so the reader has an understanding of what is meant by this.

Line 164: Again, based on what mRNA data?

Line 206: "IGHG" should be changed to either IGH or "IGHC region". There is no such thing as IGHG locus. There are multiple IGHG genes/loci.

Line 228: It would be useful to expand figure 2 to include a map of the locus and the deletion characterized. Additionally, moving supplemental figures regarding mRNA levels into figure 2 may also be useful.

Line 241: Those variants should be described in a bit more detail. Are these coding variants?

Paragraph starting at line 306: I think this comprehension of these data/results could be greatly improved if the authors expanded their description of these results. There are multiple experimental protocols and objectives here that are tersely presented.

Line 330: This sentence is a bit convoluted. I would consider rewording. Again, this entire paragraph could be expanded further to highlight what analysis is being presented in Figure 3c.

General comment regarding Discussion: Would be ideal if the authors could discuss limitations of their study. Of course, these findings are important and advance our understanding of subclass levels. However, it would be good to discuss why the authors think only a limited amount of variance in these phenotypes is explained by the identified GWAS variants? For one, it would be appropriate for the authors to acknowledge that there is likely more to find with respect to genetic underpinnings. Particularly within IGH, it is likely it is plausible that additional variation in subclass levels will be described as the genetics in these loci are further explored.

Line 360: Important for the authors to note that there are likely additional alleles that have yet to be curated (see <https://pubmed.ncbi.nlm.nih.gov/34864821/> and <https://pubmed.ncbi.nlm.nih.gov/37027017/>). This may change interpretation of allotype and allele frequencies.

Reviewer #2 (Remarks to the Author):

This study offers a compelling analysis of the genetic architectures underpinning IgG subtype levels, providing valuable insights into immune response mechanisms.

The identification of 14 signals surpassing the nominal GWAS significance threshold is noteworthy. Particularly commendable is their integration of pQTL and eQTL data alongside subtype or allotype-level associations of IgG.

I have several points for consideration:

1. Clarification on IgG subclass allotypes:

While the authors delineate IgG subclass allotypes using sequence data, it remains unclear if allotypes were confidently allocated for all subjects. Further elaboration on this aspect would enhance clarity.

2. Improved data presentation:

Table 1's layout could be refined for better comprehension. If "a" row denotes a locus (not a variant) for conditional analyses, clearly indicating this across all rows of the locus would enhance readability and understanding. Additionally, please clarify which signals are known and which are unreported.

3. Exploration of FCGR2B region:

Given IgG4's distinct behavior among the subclasses, it would be valuable to discuss findings from the GWAS paper on IgG4-related disease (DOI: [https://doi.org/10.1016/S2665-9913\(19\)30006-2](https://doi.org/10.1016/S2665-9913(19)30006-2)) to provide a comparative perspective with the present results.

4.Data deposition:

It is strongly advised that the authors deposit their data, including sumstats, to a public repository. This would facilitate transparency and reproducibility within the scientific community.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Steffanson and colleagues have conducted a GWAS of IgG levels in two human populations. This manuscript identifies novel variants associated with IgG within the immunoglobulin heavy chain locus, as well as in other immune loci elsewhere in the genome. This study provides important insight into the genetic underpinnings of antibody biology, with the potential to inform clinical medicine. In general, the article is quite terse in some places. I would recommend fleshing out several of the results sections to provide additional detail and context for the data used and the analyses performed. Likewise, expansions of the main figures would help improve the readability and comprehension of the results, without the reader needing to flip back and forth between the main text and supplement. I provide specific comments below.

We thank the reviewer for the constructive comments. Please see our specific answers below each comment. Text directly from the manuscript is written in *italics* and we have underlined additions made to the manuscript.

Comments

Line 60: “constant regions” should be changed to “constant region genes” or “constant genes”.
This has been changed to “*constant region genes*”

Line 61: “IGH” is not italicized. Individual genes within the IGH locus could be italicized depending on Nat Comm. specs.

This has been corrected and only the individual genes are now italicized.

Line 81: same as comment re line 61.

This has been corrected and throughout the text we have changed that only individual genes are italicized and not when referring to the complex loci of IGH, HLA or FCGR.

Line 92: “possibly due to different effects on different IgG subclasses”. Would be important to note that the use of microarrays for genotyping in these regions is also potentially a barrier to identifying associations. This will depend on coverage of the array used, as well as the ability of that array to effectively represent the extensive haplotype diversity in the IG loci. This will vary from array to array, and it is likely that no current array will be able to fully represent the diversity in this region. This has been clearly demonstrated for other human IG regions.

We thank the reviewer for giving us the chance to further elaborate on this part. First, we want to emphasize that we are not using microarrays but rather whole genome sequenced (WGS) individuals (49,708 Icelanders and 17,408 other individuals of European origin, including 3,704 Swedes) to identify the genomic diversity, including at the IGH locus. The microarray genotyping is then used as a scaffold to impute all the WGS variants into. However, we understand that the phrasing could be more precise, and we have therefore made the following changes to line 93-95: “Moreover, in GWASs on total IgG, IgA, IgM and IgE levels^{9,10}, relatively few sequence variants were found to associate with total IgG, possibly because different sequence variants associate with different IgG subclasses, meaning that individual signals are diluted when studying combined IgG levels. ~~due to different effects on different IgG subclasses.~~”

Line 111 and Line 125: Some information on the methods used to compile phenotypic datasets (i.e, subclass levels and mRNA expression) should be provided in the results. This can be done

concisely, pointing to the methods for more detail, of course, but a minimal level of information should be provided to aid the reader's comprehension.

This is a valid point and we have added the underlined to the descriptions.

Line 114-116: *"We used linear regression to test for association between standardized IgG-subclass levels, as measured with radial immunodiffusion (Iceland) and Optilite® analyser (Sweden), and genotypes (using Illumina technology), in each dataset separately, for about 22 million well-imputed variants with minor allele frequency (MAF) greater than 0.1%."*

Line 129-130: *whereof 4 also affect or are highly correlated with variants affecting mRNA expression (measured by RNA sequencing of whole blood and isolated immune cells) of genes at the same locus in blood (cis-expression quantitative trait loci; cis-eQTLs) (Supplementary Table 3 and 4).*

Line 138: The term "subclass traits" is vague. Do you just simply mean levels? If so, seems more appropriate to say.

We have changed this to "*subclass levels*" line 143 in the updated version of the manuscript.

Lines 141-144: Seems appropriate to cite the studies relating to the traits identified in the GWAS catalog, so appropriate attribution is given.

Thanks to the reviewer for pointing this out. We are already on the upper limit of references and this would require to add 17 references. We therefore chose to give the references only in the supplementary table 10. We have made this clear by adding the underlined to line 149: "*references to the previous publications are found in Supplementary Table 10.*"

Line 155: Regional Manhattan plots for IGH locus section and associations should be provided, along with a map of the locus. This would help readers unfamiliar with the structure of the IGH locus better understand the associations. Within these Manhattan plots, it might also be useful to indicate which SNPs displayed are imputed vs. directly genotyped by the array/sequencing data used.

Regional locus plots are provided in supplementary figure 2 page 3-10, for all of the Ig subclass signals, and we refer to Supplementary Fig 2 in the first sentence of this chapter.

As explained in our answer above and in further elaborated in the methods chapter, all of our association is run on imputed genotypes.

Line 157: Would be good to provide references for the statement regarding "complexity" so the reader has an understanding of what is meant by this.

Thank you to the reviewer for giving us the chance to clarify this. We are referring to the highly homologous sets of genes as we further elaborate on in the discuss chapter. We have added the underlined and a reference to this sentence line 162-165 in the updated version: "*The genetic complexity of the IGH locus with highly homologous sets of genes¹⁷, plus the fact that there were fewer WGS individuals in the Swedish than the Icelandic reference dataset, resulted in lower imputation accuracy for variants at the IGH locus in the Swedish data compared to other regions.*"

Line 164: Again, based on what mRNA data?

We have added the underlined to line 169 in the updated version "*mRNA expression of IGHG1 and IGV4-34 as measured with RNA sequencing of whole blood*"

Line 206: "IGHG" should be changed to either IGH or "IGHC region". There is no such thing as IGHG locus. There are multiple IGHG genes/loci.

This typo has been corrected, and it now says IGH in line 211 in the updated version.

Line 228: It would be useful to expand figure 2 to include a map of the locus and the deletion characterized. Additionally, moving supplemental figures regarding mRNA levels into figure 2 may also be useful.

Supplementary figure 5 is a map of the locus, where we show homozygous and heterozygous carriers of the deletion as well as non-carriers of the deletion. Further, supplementary figure 6 shows the median RNA-sequence coverage of the IGHG1 gene in whole blood stratified by the IGHG1 deletion. Upon request of the reviewer, we have now expanded figure 2 to include 2 additional panels showing a map of the locus (a) and the mRNA coverage (d). Relevant changes to the figure numbering was made in the results chapter: ~~Supplementary Figure 5-2a, Figure 2ab, Figure 2bc, Supplementary Figure 6-2d, Supplementary Figure 7-5, Supplementary Figure 8-6, Supplementary Figure 9 7, Supplementary 10 8.~~

S figure 5

Updated version of Figure 2 can be found below.

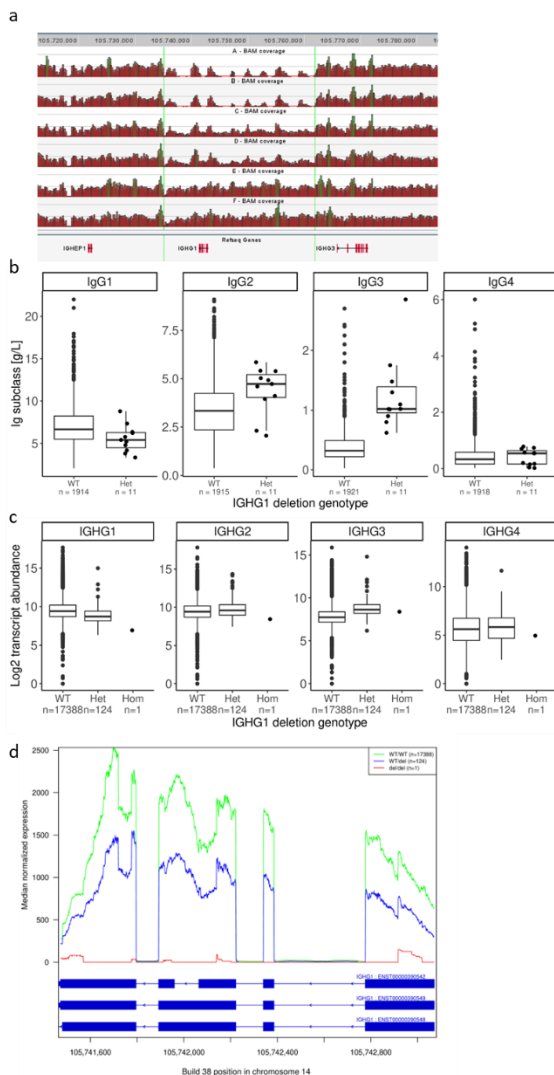


Figure 2. Deletion of the *IGHG1* gene associates with increased IgG3 levels in serum. A rare *cis*-association at the IGH locus with IgG3 levels, where the lead variant, rs587597004, tags a deletion of the *IGHG1* gene. (a) Genomic location of the ~28kb deletion at Hg38-chr14:105734756-105762914. Individuals labelled A and B are homozygotes for the deletion, C and D are heterozygotes and E and F are non-carriers of the deletion. (b) Box plots showing absolute serum levels of each IgG subclass, stratified on *IGHG1* deletion genotype. (c) Normalized expression of each IgG subclass gene mRNA in whole blood, stratified on *IGHG1* deletion genotype, showing effects consistent with the serum

protein levels. (d) A graph showing the median RNA-sequence coverage of the IGHG1 gene region in whole blood, stratified by the IGHG1 deletion alleles. RNA sequencing data was available for one homozygous deletion carrier, showing no *IGHG1* mRNA. In the box plots, the bottom and top of the boxes correspond to the 25th (Q1) and 75th (Q3) percentiles, the line inside the box is the median, and the whiskers are located at $Q1 - 1.5 \text{ IQR}$ and $Q3 + 1.5 \text{ IQR}$ (where IQR is the interquartile range, $Q3 - Q1$).

Line 241: Those variants should be described in a bit more detail. Are these coding variants?

We have added the underlined to further describe those variants in lines 246-251: *“Finally, we identified additional two novel non-coding IGH variants, both of which are common. One is intronic in IGHG3 and associated with increased IgG3 levels (rs142065266 [G>A]), $P = 3.5 \times 10^{-17}$, $\beta = 0.438$, EAF = 5.2% in line with increased IGHG3 mRNA expression detected for this variant in blood (Supplementary Table 4 and Table 1), and the other, located in 3'UTR of IGHG1, associated with decreased IgG4 levels (rs2753545[C>T]), $P = 8.4 \times 10^{-10}$, $\beta = -0.315$, EAF = 8.2%).*

Paragraph starting at line 306: I think this comprehension of these data/results could be greatly improved if the authors expanded their description of these results. There are multiple experimental protocols and objectives here that are tersely presented.

We have added more descriptions of the dataset used in lines 312-326. Additions are underlined. *“The associated region of 17q21.1 spans 160 kb and includes four genes, IKZF3, ZBP2, GSDMB and ORMDL3, all expressed in pre-B cells, B cells, plasma cells and T cells (Figure 3a). We used various datasets to look for transcriptional effects of the IgG4 associated signal and thereby attempted to identify the causal gene. First, we performed eQTL analysis in pre-existing dataset of CD138⁺ immunomagnetic bead-enriched plasma cells isolated from 1,445 multiple myeloma patients³¹. eQTL analysis in plasma cells, ~~the cells that secrete immunoglobulins,~~ showed that the allele that decreased IgG4 associated with increased expression of IKZF3 and ZBP2 but not with the expression of GSDMB or ORMDL3 or other genes in the region (Supplementary Table 17). This was in contrast to eQTL analysis based on RNA sequencing of immunomagnetic bead enriched CD19⁺ B-cells (n=758), CD4⁺ T-cells (n=837), CD8⁺ T-cells (n=807) as well as whole blood (n=17,848) from Icelanders, where the IgG4 decreasing allele also associated with expression of GSDMB and ORMDL3 but in the opposite direction of IKZF3 and ZBP2 (i.e., the IgG4 decreasing allele associated with increased expression of IKZF3 and ZBP2 but decreased expression of GSDMB and ORMDL3; Supplementary Table 4).*

Line 330: This sentence is a bit convoluted. I would consider rewording. Again, this entire paragraph could be expanded further to highlight what analysis is being presented in Figure 3c.

We have added some more explanations to this paragraph, that we hope makes it easier to follow. Lines 341-346:

“In order to understand whether the correlated association signals at 17q21.1 for IgG4, IgM, the cis eQTLs and the trans pQTLs, and the various inflammatory diseases represented the same or distinct signals, we performed a co-localization analyses³⁶. These analyses were not consistent with a one common association signal and rather the signals could be split into two groups (Figure 3c and Supplementary Table 19).”

General comment regarding Discussion: Would be ideal if the authors could discuss limitations of their study. Of course, these findings are important and advance our understanding of subclass levels. However, it would be good to discuss why the authors think only a limited amount of variance in these phenotypes is explained by the identified GWAS variants? For one, it would be appropriate for the authors to acknowledge that there is likely more to find with respect to genetic underpinnings. Particularly within IGH, it is likely it is plausible that additional variation in subclass levels will be described as the genetics in these loci are further explored.

Line 360: Important for the authors to note that there are likely additional alleles that have yet to be curated

(see <https://pubmed.ncbi.nlm.nih.gov/34864821/> and <https://pubmed.ncbi.nlm.nih.gov/37027017/>). This may change interpretation of allotype and allele frequencies.

The reviewer is correct that this was missing, and we thank for the opportunity to add this.

We have added the following paragraph and references suggested by the reviewer in line 421-428: *“Our study has some limitations. First, IgG subclass measurements are performed on limited number of individuals that are all Caucasian origin. Secondly, the highly homologous sets of genes at the IGH locus, results in lower imputation accuracy for variants at this locus than in other regions and recent publications have revealed that we are still lacking comprehensive characterization of polymorphisms within this locus^{39,53} Therefore, further sequencing and imputation of alternate haplotypes in the IGH locus in larger datasets, preferably in different ancestral populations, is needed to better understand polymorphisms at the IGH as well as other loci and their role in antibody mediated immunity.”*

Reviewer #2 (Remarks to the Author):

This study offers a compelling analysis of the genetic architectures underpinning IgG subtype levels, providing valuable insights into immune response mechanisms.

The identification of 14 signals surpassing the nominal GWAS significance threshold is noteworthy. Particularly commendable is their integration of pQTL and eQTL data alongside subtype or allotype-level associations of IgG.

I have several points for consideration:

1. Clarification on IgG subclass allotypes:

While the authors delineate IgG subclass allotypes using sequence data, it remains unclear if allotypes were confidently allocated for all subjects. Further elaboration on this aspect would enhance clarity.

The genetic complexity of the IGH locus is challenging for phasing and imputing genotypes into the samples. For the Icelandic samples the imputation yield for the allotypes (fraction of chromosome with allotypes assigned) was between 93% to 97% depending on gene, and for the Swedish samples the yield was about 93.5%. This is similar imputation yield as for the associated variants at this locus listed in Table 1. For comparison, the imputation yield for the other associated variants in Table 1 is about 99%. We have added the difficulty in imputing at the IGH loci as limitation of the study in the

discussion (line 421, see response to reviewer #1 on limitations of the study) and added this information to the methods section, line 496-502: *"The genetic complexity of the IGH loci is, however, challenging for phasing and imputing genotypes into the samples. For the Icelandic samples the imputation yield for the allotypes (fraction of chromosome with allotypes assigned) was between 93% to 97% depending on gene, and for the Swedish samples the yield was about 93.5%. This is similar imputation yield as for the associated variants at this locus listed in Table 1, but considerable lower than imputation yield for the other associated variants in Table 1 which is about 99%."*

2.Improved data presentation:

Table 1's layout could be refined for better comprehension. If "a" row denotes a locus (not a variant) for conditional analyses, clearly indicating this across all rows of the locus would enhance readability and understanding. Additionally, please clarify which signals are known and which are unreported.

Each row denotes a sequence variant not a locus. We have corrected the labeling of the fourth column from loci to sequence variant. Furthermore, we have added the locus name to each line in the 2nd column instead of labelling it with a repeat symbol (""). Therefore, each line is an independent signal, if there are more than two lines for the same locus that means that conditional analyses revealed more than one independent signal at that locus. We have labelled the 4 previously reported signals with a b in superscript in the table.

3.Exploration of FCGR2B region:

Given IgG4's distinct behavior among the subclasses, it would be valuable to discuss findings from the GWAS paper on IgG4-related disease (DOI: [https://doi.org/10.1016/S2665-9913\(19\)30006-2](https://doi.org/10.1016/S2665-9913(19)30006-2)) to provide a comparative perspective with the present results.

We thank the reviewer for giving us a chance to further elaborate on this. We have added the following to the discussion part line 393-398: *"IgG4-related disease (IgG4-RD) is a chronic, inflammatory disorder that often manifests with tumor-like masses in various organs and often an increased IgG4 levels⁴⁷. Sequence variants at HLA and FCGR2B have been reported to associate with IgG4-RD in a Japanese cohort⁴⁸. Similar to this study we found a significant IgG4 association at the HLA locus although not the same as reported in Japan as expected by the different geographic patterns of HLA frequencies, whereas we found no IgG4 association at the FCGR locus."*

4.Data deposition:

It is strongly advised that the authors deposit their data, including sumstats, to a public repository. This would facilitate transparency and reproducibility within the scientific community.

This has been added in line 756-761: ***"Data availability:*** *The sequence variants from the Icelandic population whole-genome sequence data have been deposited at the European Variant Archive under accession code PRJEB15197. The GWAS summary statistics will be made available at [\[https://www.decode.com/summarydata\]](https://www.decode.com/summarydata) upon publication. The authors declare that the data supporting the findings of this study are available within the article, its Supplementary Information file, and upon reasonable request."*

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have sufficiently addressed my concerns, except for one point, noted below.

Line 92: "possibly due to different effects on different IgG subclasses". Would be important to note that the use of microarrays for genotyping in these regions is also potentially a barrier to identifying associations. This will depend on coverage of the array used, as well as the ability of that array to effectively represent the extensive haplotype diversity in the IG loci. This will vary from array to array, and it is likely that no current array will be able to fully represent the diversity in this region. This has been clearly demonstrated for other human IG regions. We thank the reviewer for giving us the chance to further elaborate on this part. First, we want to emphasize that we are not using microarrays but rather whole genome sequenced (WGS) individuals (49,708 Icelanders and 17,408 other individuals of European origin, including 3,704 Swedes) to identify the genomic diversity, including at the IGH locus. The microarray genotyping is then used as a scaffold to impute all the WGS variants into. However, we understand that the phrasing could be more precise, and we have therefore made the following changes to line 93-95: "Moreover, in GWASs on total IgG, IgA, IgM and IgE levels^{9,10}, relatively few sequence variants were found to associate with total IgG, possibly because different sequence variants associate with different IgG subclasses, meaning that individual signals are diluted when studying combined IgG levels. due to different effects on different IgG subclasses."

– I appreciate the Authors' reply here, but I believe it is still missing the point. First of all, regarding mention of the methods used in the current paper, neither short reads nor microarrays will be able to capture the full extent of variation in the IGHC region. However, specifically with regard to this comment, it was focused on mention of previous GWAS literature. The key here is that associations may have been missed not because subclasses have been grouped, but simply because the genotypes used to represent IGHC were not fully representative of the diversity in the region (and furthermore, could even include erroneous genotypes). This should be clarified.

Reviewer #2 (Remarks to the Author):

The authors addressed my comments in a satisfactory manner.

The authors have sufficiently addressed my concerns, except for one point, noted below.

Line 92: “possibly due to different effects on different IgG subclasses”. Would be important to note that the use of microarrays for genotyping in these regions is also potentially a barrier to identifying associations. This will depend on coverage of the array used, as well as the ability of that array to effectively represent the extensive haplotype diversity in the IG loci. This will vary from array to array, and it is likely that no current array will be able to fully represent the diversity in this region. This has been clearly demonstrated for other human IG regions. We thank the reviewer for giving us the chance to further elaborate on this part. First, we want to emphasize that we are not using microarrays but rather whole genome sequenced (WGS) individuals (49,708 Icelanders and 17,408 other individuals of European origin, including 3,704 Swedes) to identify the genomic diversity, including at the IGH locus. The microarray genotyping is then used as a scaffold to impute all the WGS variants into. However, we understand that the phrasing could be more precise, and we have therefore made the following changes to line 93-95: “Moreover, in GWASs on total IgG, IgA, IgM and IgE levels^{9,10}, relatively few sequence variants were found to associate with total IgG, possibly because different sequence variants associate with different IgG subclasses, meaning that individual signals are diluted when studying combined IgG levels. due to different effects on different IgG subclasses.”

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The reviewer is correct that we misunderstood this comment and we thank for the chance to correct it. We have made the following change to lines: 94-95:

“Moreover, in GWASs on total IgG, IgA, IgM and IgE levels^{9,10}, relatively few sequence variants were found to associate with total IgG, possibly because different sequence variants associate with different IgG subclasses, meaning that individual signals are diluted when studying combined IgG levels. due to different effects on different IgG subclasses **and/or due to limitations of the genotyping methods to represent the extensive diversity at the IGH locus**”