Journal of Academia Vol.7, Issue 2 (2019) 67-75

Copy Number Variation of *FCGR3B* on Negrito-Mendriq

Mohd Helmy Bin Yusof[1*], Umi Shakina Haridan[2] and Muhamad Rahimi Che Hassan[2]

[1]*School of Biology, Faculty of Applied Sciences*
University Teknologi MARA, 40450 Shah Alam, Selangor,
[2]*Center of Foundation Studies*
Universiti Teknologi MARA Cawangan Selangor, Kampus Dengkil, 43800, Dengkil, Selangor, Malaysia

[*]*Corresponding author: mohdhelmy_87@uitm.edu.my*

Abstract

Fc Gamma Receptor 3B (FcγRIIIB, encoded by the gene *FCGR3B*) plays a crucial role in immunity response activated by cellular effectors and regulatory functions. Copy number variation (CNV) of this gene has been previously reported to affect susceptibility to several diseases such as autoimmune diseases and chronic inflammatory response. One of the rarest and smallest tribes of Orang Asli which is Negrito-Mendriq sample was used as a subject in this study. Twenty-two (22) Lymphoblastoid cell lines (LCLs) of Negrito-Mendriq was obtained from the deposited cell archive, Institute Medical Molecular Biotechnology, IMMB (UiTM Sg Buloh, Selangor, Malaysia) and the LCLs were revived. The DNA was extracted from the LCL and finally PRT – REDVR assay was carried for FCGR3B copy number genotyping. Analysis revealed that copy number 2 demonstrated the highest distribution for FCGR3B (90.9%); whilst no copy number loss was found for FCGR3B. For high copy number (>2), 9.1% was obtained. Copy number greater than 3 (>3) was not found in this study. Finally, it was concluded that no significant difference of CNV of *FCGR3B* for Orang Asli Negrito-Mendriq when compared with the reported studies. CNV of *FCGR3B* genes of Negrito-Mendriq was found normal in this study, where the pattern of copy number distribution was almost similar with previously reported studies.

Keywords: *FCGR3B*; Copy number variation; Lymphoblastoid cell line; Orang asli; Negrito-mendriq

Article history:- Received: 18 April 2019; Accepted: 10 October 2019; Published: 16 December 2019

Introduction

In 1990, the beginning of the Human Genome Project (HGP) made massive contribution because it used enormous information in DNA to develop new methods to identify, treat, cure, or even prevent the thousands countless of diseases that befall humanity. The potential for using genes of DNA in molecular medicine can improve diagnosis of disease, earlier exposure of disease, drug design ratio, gene therapy, discovery of noble drugs. This rapidly emergent field holds great potential for treating or even curing genetic diseases. Since that day, genetics study had been vastly carried out including the study on genetic variation. Every person is unique on their own and part of what makes an individual unique is the genes itself. Genes are the instructions inside each of the cells that control genetically and phenotypically. Generally, a person has two copies of each gene, one from the mother and one from the father. Start from here, the slightest changes on their genes for each individual undeniably unique on their own and create a genetic variation.

One of the interesting genes that can be studied is on *FCGR3B* variation. Copy number variation (CNV) is a large segment of DNA (typically >1 kb) that exist a variable number of copies when compared with a reference genome sequence (Redon et al., 2006). These variations involve structural changes of the genome such as insertions , deletions, duplications, translocations and inversions of the DNA segments (Zarrei, MacDonald, Merico, & Scherer, 2015). CNVs can be found in humans genome (Sudmant et al., 2015). Several studies have reported that CNVs may influence disease susceptibility. The changes of CNV in particular gene for the individual are associated with several diseases, including cancer, infectious disease, autism, neurological disorders and autoimmune disease (Sismani, Koufaris, & Voskarides, 2015).

There are three major Fc receptors, comprising Fc-alpha receptors (*FcαR*), Fc-gamma receptors (*FcγR*) and Fc-epsilon receptors (*FcεR*) (Sondermann & Szymkowski, 2016). *FCGR* gene cluster consists of FCGR 1, 2 and 3. *FCGR3B* which encodes the FcγRIIIB protein is located on chromosome 1q23.3. It is a paralogue of the neighbouring *FCGR3A* gene. The attachment to the cell surface and their expression pattern is differ for *FCGR3A* and *FCGR3B.* FcγRIIIA has a transmembrane segment and found on natural killer cells, whereas FcγRIIIB is shortened by a mutation changing on arginine codon to a stop codon. It is attached to the cell membrane by a glycophosphoinositol anchor and is expressed mainly on neutrophils (Heineke & van Egmond, 2017). CNV has been reported to be present both on *FCGR3A* and *FCGR3B* (Franke et al., 2016; Lee et al., 2015; Wang et al., 2016). The CNV on *FCGR3B* is reported to be connected with susceptibility with several number of autoimmune diseases including systemic lupus erythematosus (SLE) and rheumatoid arthritis (Lee et al., 2015; Wang et al., 2016; Yuan et al., 2015).

Point to note, variation of genetic is an interesting topic, but study has been lacking, particularly on specific genetic variation on rare population. Because of this matter, we came up with the idea to study the variation of this gene on the indigenous people, particularly on Negrito-Mendriq tribe. In Peninsular Malaysia, indigenous people are one of the isolated ethnic groups, locally known as *Orang Asli*. According to Department of Statistics Malaysia in 2010, *Orang Asli* represents only 0.5 % of the total Malaysian population. The *Orang Asli* is categorized into three major tribes, namely, Negrito, Senoi and Proto Malay. Negritos are the smallest and oldest population inhabit in peninsular Malaysia which contribute only 0.15% of the total population (Abdullah & Ahmad, 2019; Subramaniam & Nicholas, 2018; The HUGO Pan-Asian SNP Consortium, 2009). The Negritos are further divided into six sub-tribes based on their socio-cultural practices, geographical region, and specific linguistics. The six sub-tribes are Bateq, Mendriq, Jehai, Kensiu, Lanoh and Kintak (Abdullah & Ahmad, 2019).

The smallest *Orang Asli* Negrito is Negrito-Mendriq and we believed that they may have unique variation on their gene (i.e. *FCGR3B*). They had adjusted and adapted to the massive environmental changes in the tropical rainforest environment for a long period and dealing with limited food and resources. Due to their lengthy period of isolation, they have suffered from several specific health problems besides malnutrition and hygiene issues including malaria, tuberculosis, leprosy, upper respiratory and skin infections (Khor et al., 2018; Liu et al., 2015; Loong et al., 2018; Phua, 2015). Although several small scales of biomedical studies were carried out on these people, the majority of the studies focused on helminth and protozoan infections, as well as nutritional status of the communities (Ngui et al., 2015; C. Y. Wong et al., 2015; W. K. Wong et al., 2016). We believed that characterizing the genetic variation of the isolated populations, specifically on copy number variation of *FCGR3B* can offer valuable information to the gene mapping of complex diseases in order to better information how genetic variation contributes to the health of human being. In addition, due to the unique population and migration history of Negrito, we came up with a hypothesized that the CNV of *FCGR3B* Negrito may vary. In addition to that, the findings will reveal further knowledge of genetic variation of *FCGR3B* for Negrito-Mendriq.

Methods

Sample recruitment and DNA Extraction from LCLs

The sample was recruited from established lymphoblastoid cell lines (LCLs) from our previously reported study (Yusof et al. 2016). A total of twenty-two (22) LCLs of Negrito-Mendriq was obtained from the deposited cell archive, Institute Medical Molecular Biotechnology, IMMB (UiTM Sg Buloh, Selangor, Malaysia). LCLs were revived from cryopreserved and were cultured in 75-cm2 T-flasks consist of RPMI 1640 (Gibco) added with 10% fetal bovine serum (FBS). The culture medium was replaced twice or thrice a week with replacement by fresh medium depend on condition of culture. Upon approving proliferation of the cells that achieve desired culture number, the LCLs were harvested and were used for the following procedure.

By using 10 ml serological pipette, the LCLs in 75-cm2 T-flasks were evenly dispersed by pipetting up and down. One ml (1 ml) of LCLs form 75-cm2 T-flask was transferred into 15 ml Falcon tube. The LCL was washed with 12 ml PBS (twice) and centrifuged for 300 g for 10 min at room temperature. The supernatant was discarded and the pellet containing of LCLs sediment was re-suspended with 200 µl of PBS and transferred into 1.5 ml micro centrifuge tube. The micro centrifuge tube was stored at -20°C not more than 6 h before proceeding with the DNA extraction. DNA was extracted from LCLs using the Qiagen Mini Blood Extraction Kit (Qiagen, Germany) by following the manufacture's protocol.

Characterization of FCGR3 CNV using PRT – REDVR assay

Paralogue Ratio Test (PRT)

The PRT-REDVR assay was performed as described by Hollox et al. (2009). The 10 ng/10 µl of DNA was amplified with 0.5 µM FAM- or HEX labelled forward primer and 0.5 µM FAM- or HEX-labelled reverse primer, in a reaction buffer. The primer sequences used were as following;

*FCGR3B*_Forward (HEX)	5'-ATGATCTGGCCCTGAAACTC-3'
*FCGR3B*_Forward (FAM)	5'-ATGATCTGGCCCTGAAACTC-3'
*FCGR3B*_Reverse	5'-TGAGTTCAAGAAAGCAGTTG-3'

The two amplifications were performed with FAM- or HEX- labelled primer, to sanction detection of the amplicons on the capillary electrophoresis for internal calibration. The products were augmented using 30 cycles of: 95˚C for 30 s, 56˚C for 30 s and 70˚C for 30 s followed by single chase of 56˚C for 1 min then 70˚C for 20 min to reduce levels of single-stranded DNA products. After the PCR cycle, each PCR product was added with 10 µl deionized formamide, and analysed by electrophoresis on an ABI 3100 Genetic Analyzer (Applied Biosystems, Warrington, UK), with an injection time of 30 s.

PCR amplicons for size 72 base pairs (bps) corresponding to chromosome 18, and sized 67 bps corresponding to chromosome 1, were recorded for both FAM- and HEX- labelled products. The ratio of the areas under 72 bps and 67 bps peak was compared, and the results were accepted if the coefficient of variation was <0.15. The coefficient is measure as standard deviation divided by the mean. The mean of the FAM and HEX ratio was used in further analysis. Both the mean ratios and 9 reference standards sequence with known copy numbers obtained from Human Random Control DNA (European Collection of Cell Cultures) were used as the calibration for each experiment. The linear regression was evaluated to measure the copy number of *FCGR3* for Negrito-Mendriq samples, as described in Hollox et al. (2009).

Restriction Enzyme Digest Variant Ratio (REDVR)

Two REDVR assays were used in this procedure; first assay was used to differentiate variant from *FCGR3A* and *FCGR3B* and the other to differentiate neutrophil antigens *HNA1a* and *HNA1b* (g.147C>T). The amplifications of these segments were in duplex was carried out using specific primer sequences as following:

38_Forward (FAM)	5'-AAGACTGAGCCACCAAGCAT-3'
38_Reverse	5'- CTCCCTGGCACTTCAGAGTC-3'
234_Forward (HEX)	5'-TTTTGCAGTGGACACAGGAC-3'
234_Reverse	5'-GGGTTGCAAATCCAGAGAAA-3'

2 µ L of PCR product was digested with 10 units of TaqαI restriction enzyme (New England Biolabs)

in 50 mM Tris-Cl (pH 7.9 at 25˚C), 100 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol in a final volume of 10 µl for 4 hours at 65˚C. The products were masured with capillary electrophoresis on an ABI 3100 Genetic Analyzer (Applied Biosystems) and analysed with GeneScan software (Applied Biosystems). Mean ratios of the product along with the reference standard for experimental calibration were applied. The result was recalled to measure the copy number calls. The primer pair 38_ differentiates *FCGR3A* from *FCGR3B* while the primer pair 234 differentiates the *HNA1a* region from *HNA1b*. Both combination PRT analysis and REDVR analysis was performed for copy number call, using likelihood as described previously (Hollox et al., 2009). The total copy number calls for *FCGR3A* and *FCGR3B* genes were computed in PRT analysis, whereas the REDVR analysis compute the copy numbers of *FCGR3A* and *FCGR3B* based on the ratio determined. Analyses were performed using Microsoft Excel except otherwise stated (Hollox et al., 2009). Descriptive analysis of the copy number was done using Microsoft Excel in which the data was expressed as number (n) and percentage.

Result and Discussion

The Institute Medical Molecular Biotechnology LCL Collection

The deposited cell archive, Institute Medical Molecular Biotechnology, IMMB (UiTM Sg Buloh, contains a total of 84 established LCL samples including Malays and Orang Asli samples. Out of 84 LCL samples, a total of 22 samples were from LCL of Negrito-Mendriq. These 22 samples of LCL Negrito-Mendriq were revived from cryopreserved and after achieving the desired cell proliferation, we harvested the cell for DNA extraction. The cryopreserved LCL is in good condition when we revived the samples for harvesting the cell. 100% success rate for the revival of LCLs from the archived collection was achieved. Information on the established LCL was given in Table 1 below.

Table 1: LCL sample form the deposited cell archive, Institute Medical Molecular Biotechnology, IMMB.

LCL samples	Number of LCL samples, n
Malay	25
Orang Asli (Negrito-Mendriq)	22
Another Orang Asli Negrito	37
Total LCL	84

Meanwhile, for the DNA extraction from the LCL, the DNA concentration extracted for each 200 µl of LCL was in range of 75.88 - 140.31 ng/µl and the DNA purity was between 1.84-1.97 when measured by using nanospectrophotometer ND-1000 (Nanodrop). From the result, DNA samples extracted from the LCL fulfilled the quality requirement for genotyping. DNA with purity between 1.8 and 2.0 was acceptable for PRT- REDVR genotyping.

Analysis of FCGR3 CNV

PRT assay produced a copy number call for the sum of the *FCGR3* which included both *FCGR3A* and *FCGR3B* in total; while REDVR produced results in the ratio of *FCGR3A:FCGR3B*. The result was interpreted solely based on the ratio of *FCGR3B* to *FCGR3A*, therefore, copy number call from this assay was in integer (1, 2, 3 etc.). The values of the size of the peak area obtained from fragment analysis of both PRT and REDVR was applied in the equation for the regression during copy number analysis. The copy number likelihood was calculated in an algorithm sheet that contributed by Doctor Edward J Hollox from Leicester University, England. The distribution of CNV for *FCGR3B* was illustrated in the Table 2 and the Figure 1 below.

Table 2: The distribution of CNV for *FCGR3B*

No	Sample of Negrito-Mendriq	*FCGR3B* Copy number
1	K1	2
2	K2	2
3	K4	2
4	K5	2
5	K6	2
6	K7	2
7	K8	2
8	K9	3
9	K10	2
10	K11	2
11	K12	2
12	K16	2
13	K18	2
14	K22	2
15	K23	2
16	K25	2
17	K26	2
18	K28	2
19	K31	2
20	K32	2
21	K36	3
22	K37	2

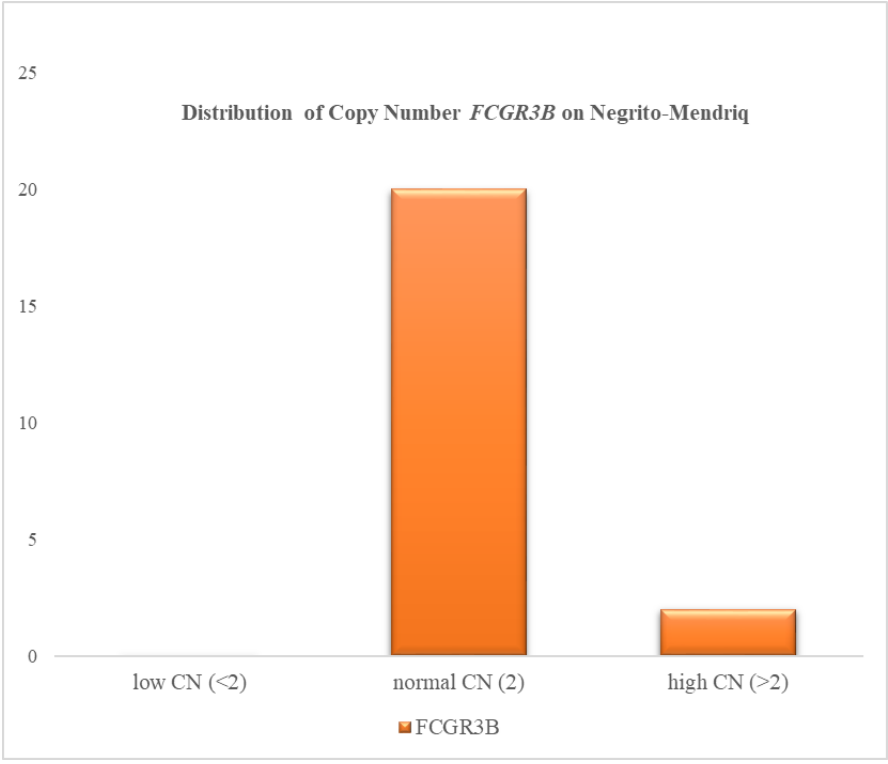

Figure 1: The distribution of copy number for *FCGR3B* based on copy number (low, normal and high)

From the Table 2 and Figure 1 above, the copy number calls obtained from PRT-REDVR were ranged from 2 to 3. Copy number 2 demonstrated the highest distribution for *FCGR3B*; whilst no samples were observed for copy number loss. For the percentage, majority of the samples had two copy numbers (2) for *FCGR3B* which was 90.9%. Then again, high copy number (>2) was observed for *FCGR3B* which is 9.1%. Copy number greater than 3 (>3) was not found in our study.

Previous reports suggested that different ethnic or tribe groups might signify different profiles of CNVs that are scattered in the human population because of their complex demographic histories (Kidd et al., 2008; Redon et al., 2006). Nearly 96% of the current genome-wide association studies were piloted on European ancestry (Bustamante, De La Vega, & Burchard, 2011) and therefore there is an increasing necessity to reveal the variety of human genetic diversity by investigating minority population for example the aboriginal populations in Southeast Asia (SEA) countries. The finding of this study serves to complement the existing information on global CNV map.

Considering CNVs for the entire genome, the CNVs are generally caused by insertions, deletions, and inversions of various sizes of DNA fragments (Redon et al., 2006; Zarrei et al., 2015). CNVs occurred at least 10% of the human genome (Scherer et al., 2007; Sudmant et al., 2015; Zarrei et al., 2015). Some complex medical disorders such as cancer and autoimmune diseases, innate immune system genes, and β-defensin antimicrobial gene clusters related on CNVs (Case et al., 2015; Fanciulli et al., 2007; Green et al., 2016; Sismani et al., 2015; Tansey et al., 2016). For that reason, CNVs give the impression to be a significant source of genetic and phenotypic variation (Sismani et al., 2015).

Therefore, isolated populations such as Negrito-Mendriq, remains an ideal model of study in population genetics, especially in the efforts of locating the signatures of positive selection, and mapping the disease traits. Another point to note is that, reported by JHEOA 2016 (JHEOA, 2016), the Negrito-Mendriq considered the smallest population in Malaysia which is less than 82 people left. Therefore, we considered the study on them is decisive. Detecting CNVs within and between populations is crucial for a better information of the genome and to interpret its possible contribution to disease. This study not only contribute to the continuous sources for genomic of rare ethnic population, but also providing the *FCGR3B* CNV characterization of the indigenous populations in Southeast Asia population, which would be essential in both human evolution and medical implications.

As general, *FCGR3B* gene copy number distribution in this study exhibited the similar patterns of distribution in the study reported by Hollox et al. (2009) and Machado et al. (2013). Both studies had genotyped this gene on a number of populations and found *FCGR3B* was distributed between copy number 1 to 4. They also stated copy number 2 as the highest percentage (60% to 88%) of distribution in every population. This is in line with our finding, of which the frequency of copy number 2 (90.9%) were similar with reported in other populations globally, including Japanese, Chinese, European and African (Hollox, Detering, & Dehnugara, 2009).

On a separate note, none of the samples studied were low copy number. Strikingly, no copy number greater than 3 (>3) was found in our study which was slightly different with the previous study. The Japanese and Chinese populations were reported to have more than 30% of the populations with high copy number (Hollox et al., 2009). This may be attributed to the different evolutionary mechanisms hence leading to the process of natural selection and local adaptation in the immune response against pathogen diversity (Hollox & Hoh, 2014). This postulation is supported by a recent study carried out (Sudmant et al., 2015). However, further investigations should be carried out to address such postulation.

(Almal & Padh, 2015) and (Wang et al., 2016) revealed that multiallelic polymorphism for the copy number of *FCGR3B* was considerably associated with systemic autoimmune disorders. A strong correlation also was found between CNV of *FCGR3B* with systemic lupus erythematosus (SLE) in individuals with low copy number (<2) in UK cohorts and same association was reported in other

populations (Willcocks, Smith, & Clatworthy, 2009). McKinney, (2010) also found a significant result for association of low *FCGR3B* gene copy number (<2) with rheumatoid arthritis which was concurrence with the association study on low copy number of this gene (Almal & Padh, 2015; Y. et al., 2017). Another study documented a significant association between lower copies numbers for *FCGR3B* in the HIV–TB co-infected in the Ethiopian cohort (Lee et al., 2015).

Almost all previous findings were in line with the trend of our study, especially when compare on healthy individual populations globally, including Japanese, Chinese, European and African (Hollox & Hoh, 2014; Qi et al., 2016; Rahbari et al., 2017; Tsang-A-Sjoe et al., 2016). Most studies, if not all, focuses on the low copy number of *FCGR3B,* there is quite lacking on study or disease reported on gain copy number of *FCGR3B* (Yuan et al., 2015). Furthermore, this study may help in the enrichment data information regarding CNV of *FCGR3B* and how this gene plays its role in the human variation, specifically in Southeast Asia population.

Conclusion

In conclusion, due to the unique population and migration history of Negrito, we initialy hypothesized that the CNV of *FCGR3B* among Negrito-Mendriq may vary from the other populations. However, it was found that no significant different of CNV of *FCGR3B* for Orang Asli Negrito-Mendriq when compared with the previous studies. It can be said that *FCGR3B* genes of Negrito-Mendriq was appraised as normal copy number, where the pattern of copy number distribution was almost similar with previous reported study. CNV analysis in big cohorts may offer a better insight to the magnitude to which *FCGR3B* variants outline the risk of the common disease; such inquiries may set early models for consequent hard work to study rare variants through large-scale sequencing. However, further investigation with appropriate population genetics model is required to warrant further postulation. Ultimately, the outcome of this study could serve as a stepping stone to a better understanding of human variation and evolution, and the fundamentals of disease mechanism, hence essential to tomorrow's medical implications.

Acknowledgement

This study was approved by the Research and Ethics Committee of Universiti Teknologi MARA [Ref no: 600-RMI (5/1/6)], and Department of Orang Asli Development (JHEOA) [Ref no: JHEOA.PP.30.052.Jld 5(17)]. This study is supported by the grant MOSTI (100-RMI/BIOTEK 16/6/2B (1/2011)) and 600-IRMI/DANA KCM 5/3/LESTARI (117/2017)

References

Abdullah, J., & Ahmad, C. B. (2019). The Effectiveness of Resettlement of Orang Asli in Peninsular Malaysia. *Environment-Behaviour Proceedings Journal*. https://doi.org/10.21834/e-bpj.v4i10.1636

Almal, S. H., & Padh, H. (2015). Frequency distribution of autoimmunity associated FCGR3B gene copy number in Indian population. *International Journal of Immunogenetics*. https://doi.org/10.1111/iji.12165

Bustamante, C. D., De La Vega, F. M., & Burchard, E. G. (2011). Genomics for the world. *Nature*. https://doi.org/10.1038/475163a

Case, L. K., Wall, E. H., Osmanski, E. E., Dragon, J. A., Saligrama, N., Zachary, J. F., … Teuscher, C. (2015). Copy number variation in Y chromosome multicopy genes is linked to a paternal parent-of-origin effect on CNS autoimmune disease in female offspring. *Genome Biology*. https://doi.org/10.1186/s13059-015-0591-7

Fanciulli, M., Norsworthy, P. J., Petretto, E., Dong, R., Harper, L., Kamesh, L., … Aitman, T. J. (2007). FCGR3B copy number variation is associated with susceptibility to systemic, but not organ-specific, autoimmunity. *Nature Genetics*. https://doi.org/10.1038/ng2046

Franke, L., El Bannoudi, H., Jansen, D. T. S. L., Kok, K., Trynka, G., Diogo, D., … Zhernakova, A. (2016). Association analysis of copy numbers of FC-gamma receptor genes for rheumatoid arthritis and other immune-mediated phenotypes. *European Journal of Human Genetics*. https://doi.org/10.1038/ejhg.2015.95

Green, E. K., Rees, E., Walters, J. T. R., Smith, K. G., Forty, L., Grozeva, D., … Kirov, G. (2016). Copy number

variation in bipolar disorder. *Molecular Psychiatry*. https://doi.org/10.1038/mp.2014.174

Heineke, M. H., & van Egmond, M. (2017). Immunoglobulin A: magic bullet or Trojan horse? *European Journal of Clinical Investigation*. https://doi.org/10.1111/eci.12716

Hollox, E. J., Detering, J.-C., & Dehnugara, T. (2009). An integrated approach for measuring copy number variation at the FCGR3 (CD16) locus. *Human Mutation*, *30*(3), 477–484. https://doi.org/10.1002/humu.20911

Hollox, E. J., & Hoh, B.-P. (2014). Human gene copy number variation and infectious disease. *Human Genetics*. https://doi.org/10.1007/s00439-014-1457-x

Khor, C. S., Mohd-Rahim, N. F., Hassan, H., Chandren, J. R., Nore, S. S., Johari, J., … Abubakar, S. (2018). Seroprevalence of Q Fever among the Indigenous People (Orang Asli) of Peninsular Malaysia. *Vector-Borne and Zoonotic Diseases*. https://doi.org/10.1089/vbz.2017.2153

Kidd, J. M., Cooper, G. M., Donahue, W. F., Hayden, H. S., Sampas, N., Graves, T., … Eichler, E. E. (2008). Mapping and sequencing of structural variation from eight human genomes. *Nature*. https://doi.org/10.1038/nature06862

Lee, Y. H., Bae, S. C., Seo, Y. H., Kim, J. H., Choi, S. J., Ji, J. D., & Song, G. G. (2015). Association between FCGR3B copy number variations and susceptibility to autoimmune diseases: a meta-analysis. *Inflammation Research*. https://doi.org/10.1007/s00011-015-0882-1

Liu, X., Yunus, Y., Lu, D., Aghakhanian, F., Saw, W. Y., Deng, L., … Hoh, B. P. (2015). Differential positive selection of malaria resistance genes in three indigenous populations of Peninsular Malaysia. *Human Genetics*. https://doi.org/10.1007/s00439-014-1525-2

Loong, S. K., Khor, C. S., Chen, H. W., Chao, C. C., Ling, I. S. C., Abdul Rahim, N. F., … Abubakar, S. (2018). Serological evidence of high leptospira exposure among indigenous people (Orang Asli) in peninsular Malaysia using a recombinant antigen-based ELISA. *Tropical Biomedicine*.

Ngui, R., Aziz, S., Chua, K. H., Aidil, R. M., Lee, S. C., Tan, T. K., … Lim, Y. A. L. (2015). Patterns and risk factors of soil-transmitted helminthiasis among orang asli subgroups in peninsular Malaysia. *American Journal of Tropical Medicine and Hygiene*. https://doi.org/10.4269/ajtmh.13-0677

Phua, K. L. (2015). The health of Malaysia's Orang Asli peoples: A review of the outcome, parasite infestation and discussion on implication for clinical practice. *Malaysian Journal of Public Health Medicine*.

Qi, Y. Y., Zhou, X. J., Bu, D. F., Hou, P., Lv, J. C., & Zhang, H. (2016). Comparison of multiple methods for determination of FCGR3A/B genomic copy numbers in hapmap asian populations with two public databases. *Frontiers in Genetics*. https://doi.org/10.3389/fgene.2016.00220

Rahbari, R., Zuccherato, L. W., Tischler, G., Chihota, B., Ozturk, H., Saleem, S., … Hollox, E. J. (2017). Understanding the Genomic Structure of Copy-Number Variation of the Low-Affinity Fcγ Receptor Region Allows Confirmation of the Association of FCGR3B Deletion with Rheumatoid Arthritis. *Human Mutation*. https://doi.org/10.1002/humu.23159

Redon, R., Ishikawa, S., Fitch, K. R., Feuk, L., Perry, G. H., Andrews, T. D., … Hurles, M. E. (2006). Global variation in copy number in the human genome. *Nature*. https://doi.org/10.1038/nature05329

Scherer, S. W., Lee, C., Birney, E., Altshuler, D. M., Eichler, E. E., Carter, N. P., … Feuk, L. (2007). Challenges and standards in integrating surveys of structural variation. *Nature Genetics*. https://doi.org/10.1038/ng2093

Sismani, C., Koufaris, C., & Voskarides, K. (2015). Copy number variation in human health, disease and evolution. In *Genomic Elements in Health, Disease and Evolution: Junk DNA*. https://doi.org/10.1007/978-1-4939-3070-8_6

Sondermann, P., & Szymkowski, D. E. (2016). Harnessing Fc receptor biology in the design of therapeutic antibodies. *Current Opinion in Immunology*. https://doi.org/10.1016/j.coi.2016.03.005

Subramaniam, Y., & Nicholas, C. (2018). The Courts and the Restitution of Indigenous Territories in Malaysia. *Erasmus Law Review*. https://doi.org/10.5553/elr.000096

Sudmant, P. H., Mallick, S., Nelson, B. J., Hormozdiari, F., Krumm, N., Huddleston, J., … Eichler, E. E. (2015). Global diversity, population stratification, and selection of human copy-number variation. *Science*. https://doi.org/10.1126/science.aab3761

Tansey, K. E., Rees, E., Linden, D. E., Ripke, S., Chambert, K. D., Moran, J. L., … O'Donovan, M. C. (2016). Common alleles contribute to schizophrenia in CNV carriers. *Molecular Psychiatry*. https://doi.org/10.1038/mp.2015.143

The HUGO Pan-Asian SNP Consortium. (2009). Mapping Human Genetic Diversity in Asia. *Science*. https://doi.org/10.1126/science.1177074

Tsang-A-Sjoe, M. W. P., Nagelkerke, S. Q., Bultink, I. E. M., Geissler, J., Tanck, M. W. T., Tacke, C. E., … Voskuyl, A. E. (2016). Fc-gamma receptor polymorphisms differentially influence susceptibility to systemic lupus erythematosus and lupus nephritis. *Rheumatology (United Kingdom)*. https://doi.org/10.1093/rheumatology/kev433

Wang, L., Yang, X., Cai, G., Xin, L., Xia, Q., Zhang, X., … Pan, F. (2016). Association study of copy number

variants in FCGR3A and FCGR3B gene with risk of ankylosing spondylitis in a Chinese population. *Rheumatology International*. https://doi.org/10.1007/s00296-015-3384-0

Willcocks, L. C., Smith, K. G. C., & Clatworthy, M. R. (2009). Low-affinity Fcγ receptors, autoimmunity and infection. *Expert Reviews in Molecular Medicine*. https://doi.org/10.1017/S1462399409001161

Wong, C. Y., Zalilah, M. S., Chua, E. Y., Norhasmah, S., Chin, Y. S., & Siti Nur'Asyura, A. (2015). Double-burden of malnutrition among the indigenous peoples (Orang Asli) of Peninsular Malaysia Global health. *BMC Public Health*. https://doi.org/10.1186/s12889-015-2058-x

Wong, W. K., Foo, P. C., Roze, M. N. M., Pim, C. D., Subramaniam, P., & Lim, B. H. (2016). Helminthic Infection and Nutritional Studies among Orang Asli Children in Sekolah Kebangsaan Pos Legap, Perak. *Canadian Journal of Infectious Diseases and Medical Microbiology*. https://doi.org/10.1155/2016/1326085

Y., Q., X., Z., D., B., P., H., J., L., & H., Z. (2017). Low copy numbers of FCGR3A and FCGR3B associated with Chinese patients with SLE and AASV. *Lupus*. https://doi.org/10.1177/0961203317700485

Yuan, J., Zhao, D., Wu, L., Xu, X., Pang, Y., Zhang, J., … Wang, J. (2015). FCGR3B copy number loss rather than gain is a risk factor for systemic lupus erythematous and lupus nephritis: A meta-analysis. *International Journal of Rheumatic Diseases*. https://doi.org/10.1111/1756-185X.12342

Zarrei, M., MacDonald, J. R., Merico, D., & Scherer, S. W. (2015). A copy number variation map of the human genome. *Nature Reviews Genetics*. https://doi.org/10.1038/nrg3871