

Analysis of Deep-sequenced Trio-genomes of the Peninsular Malaysia Orang Asli and North Borneo Populations

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Abstract

Background Recent advances in genomic technologies have facilitated genome-wide investigation of human genetic variations. However, most efforts have focused on the major populations, yet trio genomes of indigenous populations from Southeast Asia have been under-investigated. **Results** We analyzed the whole-genome deep sequencing data ($\sim 30\times$) of five native trios from Malaysia, and discovered approximately 6.9 million single nucleotide variants (SNVs), 1.2 million small insertions and deletions (indels), and 9,000 copy number variants (CNVs) in the 15 samples. We found 3.9% SNVs, 4.7% indels and 22% CNVs were novel, implying the insufficient coverage of population diversity in existing databases. We identified a higher proportion of novel variants in the Orang Asli (OA) samples, i.e., the indigenous people from Peninsular Malaysia, than that of the North Bornean (NB) samples, likely due to more complex demographic history and long-time isolation of the OA groups. We used the pedigree information to identify de novo variants and estimated the mutation rates to be 0.81×10^{-8} – 1.33×10^{-8} , 1.0×10^{-9} – 2.9×10^{-9} , and ~ 0.001 per site per generation for SNVs, indels, and CNVs, respectively. The trio-genomes also allowed for accurate haplotype phasing with high accuracy, which serves as references to the future genomic studies of OA and NB populations. In addition, high-frequency inherited CNVs specific to OA or NB were identified. One example was a 50-kb duplication in DEFA1B detected only in the Negrito trios, implying plausible effects on host defense against the exposure of diverse microbial in tropical rainforest environment of these hunter-gatherers. The CNVs shared between OA and NB groups were much fewer than those specific to each group. Nevertheless, we identified a 142-kb duplication in AMY1A in all the 15 samples, and this gene is associated with the high-starch diet. Moreover, novel insertions shared with archaic hominids were identified in our samples. **Conclusion** Our study presents a full catalogue of the genome variants of the native Malaysian populations, which is a complement of the genome diversity in Southeast Asians. It implies specific population history of the native inhabitants, and demonstrated the necessity of more genome sequencing efforts on the multi-ethnic native groups of Malaysia and Southeast Asia.

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Results

We analyzed the whole-genome deep sequencing data ($\sim 30\times$) of five native trios from Peninsular Malaysia and North Borneo, and characterized the genomic variants, including single nucleotide variants (SNVs), small insertions and deletions (indels) and copy number variants (CNVs). We discovered approximately 6.9 million SNVs, 1.2 million indels, and 9,000 CNVs in the 15 samples, of which 3.9%

SNVs, 4.7% indels and 22% CNVs were novel, implying the insufficient coverage of population diversity in existing databases. We identified a higher proportion of novel variants in the Orang Asli (OA) samples, i.e., the indigenous people from Peninsular Malaysia, than that of the North Bornean (NB) samples, likely due to more complex demographic history and long-time isolation of the OA groups. We used the pedigree information to identify *de novo* variants and estimated the mutation rates to be $0.81 \times 10^{-8} - 1.33 \times 10^{-8}$, $1.0 \times 10^{-9} - 2.9 \times 10^{-9}$, and ~ 0.001 per site per generation for SNVs, indels, and CNVs, respectively. The trio-genomes also allowed for accurate haplotype phasing with high accuracy, which serves as references to the future genomic studies of OA and NB populations. In addition, high-frequency inherited CNVs specific to OA or NB were identified. One example was a 50-kb duplication in *DEFA1B* detected only in the Negrito trios, implying plausible effects on host defense against the exposure of diverse microbial in tropical rainforest environment of these hunter-gatherers. The CNVs shared between OA and NB groups were much fewer than those specific to each group. Nevertheless, we identified a 142-kb duplication in *AMY1A* in all the 15 samples, and this gene is associated with the high-starch diet. Moreover, novel insertions shared with archaic hominids were identified in our samples.

Conclusion

Our study presents a full catalogue of the genome variants of the native Malaysian populations, which is a complement of the genome diversity in Southeast Asians. It implies specific population history of the native inhabitants, and demonstrated the necessity of more genome sequencing efforts on the multi-ethnic native groups of Malaysia and Southeast Asia.

Background

The rapid development of genome sequencing technology and analysis capabilities has spawned large scale human genome sequencing projects in recent years, for instance, the 1000 Genomes Project, the Simons Genome Diversity Project, the Estonian Biocentre Human Genome Diversity Project, UK10K Project, [1–4] the All of Us Research Program (<https://allofus.nih.gov/>), and others. A major undertaking of these projects is to conduct a comprehensive inventory of all detectable variations of global modern human populations, which is important for characterizing the human genetic diversity as well as identifying disease risk variants. The fine-scale analyses of the human genome require accurate identification of variants, imputation and phasing of genotypes, which may be greatly facilitated by increasing the sequencing depth and using pedigree information, especially for genomic regions containing large and complex variations like structural variants (SVs) and insertions and deletions (indels) [5]. In addition, the trio information allows verification of the detected variants using Mendel's law of inheritance and detecting *de novo* mutations. Understanding the rates and patterns of *de novo* mutations is important for analyzing the population relationship [6,7], detecting natural selection [8,9], and mapping genes underlying complex traits [10]. To date, most trio-based sequencing studies are disease-related [11–13]. Whole-genome sequencing studies of healthy trios are less biased than those of the disease-based ones, but publications on these are rather limited, except for the one Vietnamese trio and 10 Danish trios that were sequenced to high coverage in recent years [14,15].

Located at the crossroads of Southeast Asia, Malaysia is rich with human population diversity, including native Malays and Orang Asli (OA, a collective term of indigenous populations) occupying the Peninsular Malaysia, and over 40 native ethnic groups categorized based on linguistic and socio-economy practices in North Borneo [16]. However, these native populations are largely underrepresented in the whole-genome sequencing projects. The genomic architecture of these populations were characterized by a handful of SNP-array-based genome-wide studies [17–22]. Recently, using the whole genome sequencing data of 12 unrelated individuals, we have also revealed the population structure and divergence between native populations from Peninsular Malaysia and North Borneo [23].

In this study, we present the variant catalogue of five native trios (father-mother-offspring) from Peninsular Malaysia (OA, including Bateq, Mendriq and Semai) and North Borneo (NB, including Dusun and Murut) by whole-genome sequencing to a mean depth of 30×. Our data revealed a large number of novel genomic variants, including the single nucleotide variants (SNVs), indels and copy number variants (CNVs), in the native Malaysian trios, particularly in OA. The rates of *de novo* genomic variants were estimated. In addition, the inherited novel insertions were identified from the unmapped reads of these samples, some of which could have been shared with archaic hominins.

Results

Discovery of SNVs and indels

The five native Malaysian trios were sequenced at coverage of 28-38× (~30× on average). One Mendriq (MDQ) sample had the lowest sequence coverage at 28.3× (Table 1). On average 97.5% (Phred Score ≥ 10) of the reads were mapped to the reference genome. As shown in Table 1, more than 6.9 million SNVs (3.4 million per genome) and approximately 1.2 million bi-allelic indels (< 100 bp, 0.6 million per genome) were discovered in the fifteen genomes. The average Ti/Tv ratio was similar across all the native Malaysian populations (2.1 per genome), which was consistent with published reports [14,38,39]. The individual genome heterozygosity ranged between 51.6-56.7% for SNVs and 59.5-66.8% for indels, lower than other global populations (Table 1; Figure 1a), suggesting that the native Malaysian populations are genetically more homogenous.

We further examined whether there were genomic regions enriched with variation. Hotspots of variants were determined by selecting the top 1% non-overlapping windows across the genome, each spanning 1 Mb, with top counts of mutations that passed the quality control (GQ ≥ 50 ; DP = 10-120; allele balance = 0.3-0.7). SNVs and indels were treated independently. Regions adjacent to 1 Mb from the telomeres and centromeres were excluded. As expected, the region Chr6:29-33 Mb harboured the largest number of both SNVs and indels, followed by Chr8:3-4 Mb (Table S2-S3). These two regions encompass immunity-related protein-coding genes (the MHC Class II genes, *ANGPT2*, *DEFA*, and *DEFB* on chromosome 6; *CSMD1* on chromosome 8) [40–42], and have been reported previously as SNV hotspots in the Singapore Malays [38]. Particularly noteworthy is *CSMD1*, which is highly expressed in the brain [43], and could play a role in the susceptibility of malarial infection [41,42]. The region Chr22:49-50 Mb was another hotspot of SNVs

and indels, spanning two immune related genes *FAM19A5* and *C22orf34*. Protein-coding genes underlying the mutation hotspots regions were significantly enriched in olfaction, immunity and hemoglobin among others. (Table S4), suggesting that genomic regions which are 'sensitive' towards environmental responses tend to be more variable.

We applied SnpEff version 4.3T [36] to classify the variants according to their functional effects, and summarized the number of SNVs and indels of each category in each population in Table S5-S6. We found 98.5% of the SNVs and 99% of the indels were non-coding variants; while the remains included possibly harmful variants with low (1.1% SNVs and 0.08% indels) and moderate (0.4% SNVs and 0.15% indels) impact, and disruptive variants with high impact (0.03% SNVs and 0.07% indels, e.g., exon-loss, frameshift, splice-acceptor, splice-donor, start-lost, stop-gained, stop-lost, and transcript-ablation variants). Each genome carried 290 loss-of-function (LOF) SNVs on average (Table S7), consistent with previously reported number of LOF variants (200-800) in each healthy human genome [44]. Although fewer samples were sequenced, the number of LOF-SNVs in our data was comparable with that reported in the 1000 Genomes Project data (Table S5), which represents a larger sample size with low sequencing depth. When comparing across the five native Malaysian populations, the number of LOF-SNVs per genome between OA and NB were similar (291 vs. 289 per sample) (Figure 1c; Table S7).

On average, 486 high-impact and 320 LOF-indels were identified in each sample, similar with other global populations (Figure 1d; Table S6; Table S7) [45]. Of these, 354 were homozygous deletions in at least one sample, and 555 indels presented in more than one sample. Frameshift indels (FS-indels) are generally thought to be pathogenic and may confer significant phenotypic consequences [45]. We observed 644 FS-indels in the 15 samples (on average 327 each), of which 171 were homozygous deletion in at least one sample, and 580 FS-indels presented in more than one sample. One example of high-frequency FS-indels in the 15 samples include an 11-bp mutation affecting *MICA* (frequency = 0.87). *MICA* has been attributed to autoimmune diseases and viral infection [46,47]. Details of the FS-indels identified are tabulated in Table S8. Protein-coding genes affected by LOF-indels showed significant enrichment in Ca²⁺ dependent cell adhesion and olfactory transduction (Table S4). A similar functional enrichment pattern was observed on genes overlapping with FS-indels.

Identification of novel SNVs and indels

We observed a total of 271,562 SNVs (3.9%) and 55,399 indels (4.7%) not reported in dbSNP150. The overall novelty rate across autosomal chromosomes was similar, ranging from 3.44% (chromosome 21) to 4.19% (chromosome 2) for SNVs, and from 4.2% (chromosome 4) to 5.1% (chromosome 9) for indels. Genomic regions emerged with higher densities of novel SNVs or indels are listed in Table S9-S10. The variant-enriched region Chr8:3-6 Mb, again, harbored the largest number of novel SNVs; Chr7:4-7 Mb showed a substantial excess of novel indels than other regions.

Comparing across the native Malaysian populations, we found that OA populations harbored more novel variants than NB populations did on both population (1.0-1.5% of SNVs and 2.8-3.0% of indels in OA;

0.6% of SNVs and 2.7% of indels in NB) and individual (1.0-1.6% of SNVs and 2.9-3.2% of indels in OA; 0.6-0.7% of SNVs and 2.7-2.8% of indels in NB) levels (Table 1; Figure 1b; Table S5-S6). Notably, the two Negrito populations, especially the Bateq (BTQ) trio, harbored the highest proportion of putative novel SNVs and indels (novelty rates are 1.5-1.6% for the SNVs and 3.1-3.2% for the indels in each BTQ sample) (Table S5-S6). OA and NB populations shared a smaller number of SNVs (5,786, making up 3.0% and 7.1% of the novel SNVs in OA and NB, respectively), but more indels (24,778, making up 53.7% and 72.8% of the novel SNVs in OA and NB, respectively) in common.

de novo mutations and estimating mutation rate

We further identified *de novo* mutations in the offspring of each trio. We applied stringent control for genotyping quality and have filtered out the mutations with allele balance ≤ 0.3 or ≥ 0.7 . Therefore, the *de novo* mutations identified could be considered in the germline (see Methods). The number of *de novo* SNVs ranged in 37-62 for each offspring (listed in Table S11). Correspondingly, the germline *de novo* mutation rate was estimated to be 0.81×10^{-8} - 1.33×10^{-8} per site per generation for SNVs (Table 2), which falls within the expected range [15,48]. As listed in Table S11, there were a total of 121 *de novo* SNVs in the five offspring, affecting 137 genes, of which 108 were protein-coding genes. These genes showed significant functional enrichment in epidermal growth factor (8 of the 108 genes, Table S4). All the *de novo* SNVs were individual-specific, but we found two mutations in MDQ (Chr2:141,474,240) and Dusun (DSN) (Chr2:141,657,309) which fell in the same gene, *LRP1B*, and encodes for a member of the low-density lipoprotein receptor family. These two *de novo* mutations were also novel SNVs in our data. In addition, *CACNA1C* and *SLC43A2* were affected by multiple *de novo* SNVs in MRT. Two adjacent intronic allele substitutions (at positions 2,605,335 and 2,605,336, respectively; both were novel mutations) occurred in *CACNA1C*. This gene encodes a subunit of voltage-dependent calcium channel, and plays important roles in a wide range of biological functions, e.g. muscle contraction, hormone or neurotransmitter release, gene expression, cell motility, cell division and cell death, and might be attributed with cardiovascular diseases. Other interesting *de novo* SNVs include a 'modifier' C > T substitution at rs72668090 in *EGLN3* and a T > C mutation at position 84,692,399 in *NRG3* in the MDQ offspring. Both genes were reported to function in cardiovascular diseases [49,50].

Compared with SNVs, the *de novo* mutation events for indels occurred less frequently. The mutation rate was estimated to be 1.0×10^{-9} - 2.9×10^{-9} per site per generation according to the 5-10 *de novo* indels identified in the five offspring (Table 2; Table S12), in accordance with previous reports [14,54]. We did not observe any direct physical or functional attribution between the *de novo* indels and *de novo* SNVs in each sample – they were located distant from each other (> 1 Mb) and in different genes. A candidate gene of interest affected by a *de novo* indel was *CDH13* in the Murut (MRT) offspring. *CDH13* is a member of GPI-anchored member of the cadherin superfamily, which encodes for the protein T-cadherin that is prominently expressed in heart. It is associated with blood pressure regulation, atherosclerosis protection and regulation of adiponectin level [53–55]. Interestingly, this gene was also reported to be associated with malaria susceptibility [56], and consistently exerted as a signature of positive selection in the Negrito populations from Peninsular Malaysia [17,18].

Analysis of copy number variants

To minimize potential false positive calls, we utilized both ERDS and CNVnator to identify CNVs on the individual level (see Methods). Consequently, 9,152 CNVs over 100 bp in size were detected in the 15 samples, including 7,470 deletions and 1,682 duplications. Each sample carries 551-777 CNVs (610 on average) (Table S13). The number of CNVs identified in each genome was similar (~1,700), except that the MDQ trio was observed to carry a higher number of CNVs (2,172). The size distribution of CNVs is shown in Figure 2a. Deletions were enriched in the length of 461 bp (43 deletions), and duplications were enriched in the length of 1 kb (458 duplications). The largest CNV was a duplication found in the MRT trio, spanning 529 kb at 18q11.2. It encompassed *RBBP8*, which encodes for protein that regulates cell cycles and proliferation [57]. Using the 50% reciprocal overlap criteria to compare with the Database for Genome Variants (DGV), a substantial amount of the CNVs identified (~22.1%; 742 deletions and 1,276 duplications) are previously unreported, of which 1,214 (13%) were recurrent (observed in at least 2 out of the 15 genomes studied). These novel CNVs were enriched in size range <1 kb for deletions and in 1-10 kb for duplications. In the total of 9,152 CNVs, 42% (3,832) were genic variants, disrupting 694 genes (i.e., CNV breakpoints fell within the exons; average 139 genes per genome). We observed a large number of duplications (copy number (CN) > 2) in this study, which suggests that the duplication events may have been under-reported in previous array-based platforms, likely due to the limitation of the nature of the technology. We observed 12 *de novo* CNVs in this study, which converts to a mutation rate of ~0.001, consistent with the range of the reported rate (Table 2; Table S14) [48]. All the 12 *de novo* CNVs were deletions ranging in 281-2,778 bp. Two candidate genes of interest affected by the *de novo* CNVs were *LMF1* and *CLDN14* identified in MDQ and DSN, respectively. *LMF1* encodes for protein lipase maturation factor, which involves in maturation and transport of lipase. *CLDN14* encodes an integral membrane protein and a component for tight junction strands regulating the cell-cell adhesion in epithelial or endothelial cell sheets.

We then investigated the CNV sharing among the native Malaysian trios, and grouped them as Orang Asli CNVs (OA-CNVs; shared by BTQ, MDQ and Semai (SMI)), Negrito CNVs (NGO-CNVs; shared by BTQ and MDQ), North Bornean CNVs (NB-CNVs; shared by DSN and MRT), and Malaysian CNVs (MLS-CNVs; shared by OA and NB populations). As expected, populations that are historically closer tended to share more CNVs. For instance, we observed more CNV regions shared within OA population (302 OA-CNVs) and within NB population (386 NB-CNVs), than those shared between these two groups (227 MLS-CNVs) (Figure 2b; Table S15). Candidate genes affected by the OA-CNVs were enriched in the synapse-related ion transduction (Table S4).

We further investigated the inheritance of several candidate genes of interest that were known to either lie on the segmental duplication region, or carry multiple allelic CNVs. Numerous studies have reported the attributions and roles of CNVs underlying these genes in a wide range of disease traits. Genes affected by those reported CNVs are listed in Table S16, including: *CCL3L1*, *DEFA/B*, *FCGR2/3*, *AMY1/2*, *GSTT/GSTM*, *LPA*, and *CYP2D6* [58–67]. The copy number of these candidate genes were surprisingly lower than average as previously reported [59,60,62,68]. All five trios showed duplication (CN = 3) in *AMY* and

DEFB103A (except MRT) but a deletion (CN = 1) in gene *DEFB130*. BTQ and MDQ showed duplication for the *DEFA1B* gene (CN > 2) but not the rest of the trios. The most variable gene among all trio members were *LPA*, ranging in 4 (DSN) -10 (MDQ). Some of the CN of these candidate genes of interest were not called, probably due to the stringent quality control criteria during the SV call, which had filtered out the 'noisy' calls. Validation is recommended for identifying these CN harbouring the complex and segmental duplicated regions [59,69–71].

Novel insertions shared with archaic hominids

Novel insertions (NIs) (fragment size in 47 – 19,045 bp) to the human reference genome have been identified in the native Malaysian genomes. To avoid possible artefacts in sequencing and variants calling, we only focused on the inherited NIs that was present in the offspring and at least one of his/her parent. The number of inherited autosomal NIs in OA (5,984-6,145 in each trio) was slightly more than that in NB (5,979-5,991 in each trio), probably due to the ancient origin and long-term isolation of the OA hunter-gatherers. A full list of inherited NIs can be found in Table S17. We found 547 of these NIs were unique to the OA, and 205 were unique to NB. Interestingly, 45.2-46.4% of the NIs in each sample could also be observed in the Neanderthal or Denisovan genomes (Table 3). Consistent with the lower proportion of Denisovan-like genomic segments in the native Malaysian populations [23], only around 50 of the archaic-like NIs in each sample could be specifically observed in the Denisovan genome; while the Neanderthal-like segments constituted a large proportion of the archaic-like NIs. Enrichment analysis revealed that genes underlying Neanderthal-like segments are enriched for synapse-related cell junction, immunity, ion channel, etc. (Table S4).

Discussion

This study presents for the first time, a comprehensive catalogue of genomic variants of the native trio inhabitants from Peninsular Malaysia and North Borneo. Although whole-genome sequencing has been commonly applied in population genomic studies, very few publications have reported on 'healthy' trios [14,15,51], and of particular interest are the lack of data on deep-sequenced trios of native inhabitants such as the Orang Asli and North Borneans. Identification of variants and the frequencies of these populations could provide better insights to population-specific disease susceptibility and serve as an important stepping-stone for advancing clinical and public health genomic study [72]. Studying trios allows us to confirm rare and putatively population specific variants that are often of clinical importance but under-represented, since they are being transmitted, therefore of high confidence.

On a separate note, sequencing of trios remains the most straightforward strategy to estimate *de novo* mutation rate [15]. This is crucial to date the evolutionary events and to investigate the underlying causes for genetic diseases [10]. We caution that *de novo* mutations may be affected by paternal age [73,74], which was not available in our data. However, the estimated rate of *de novo* mutations observed in this study is in line with other recent studies [15,48]. *De novo* mutations are often thought to undergo a different nature of selective pressure and are more deleterious than the inherited variants, thus, are more

likely to be the causative factor for many diseases [27,48]. The *de novo* SNVs in the genomes studied here (Table 2) were enriched in genes related to EGF related pathways. Earlier study have shown that EGF receptor pathway may show adaptive changes against micro-environmental forces specifically hypoxia, acidosis and reactive oxygen species, thus able to induce genetic instability [75].

Haplotype phasing on trios using identity by descent (IBD) essentially is thought to be more informative and accurate than the phasing of unrelated individuals based on the haplotype frequency information [76,77]. When both parents are genotyped, variants that are not triply heterozygous in the parents and offspring could be phased. The IBD-based phasing using the trios of this study, along with the population haplotype frequency models, could be used as a reference and applied to additional populations, especially the Southeast Asians. It works particularly for imputing variants with low frequency. The task of phasing in isolated populations is somewhat a special case, as individuals from such populations exhibit much higher levels of relatedness, and tend to share much longer stretches of IBD-sequences than a pair of unrelated individuals from a non-isolated population.

Higher discovery rate of novel variants was observed in the trio genomes from Peninsular Malaysia and North Borneo compared to the global populations (Table 2) possibly implies an insufficient coverage of population diversity in the attempt of unveiling the genome architecture. Therefore, more sequencing attempts on the indigenous and more diversified populations (in particular the Southeast Asia region) should be carried out in the near future.

In line with the genetic relationship among the native Malaysian populations, more CNVs were shared among OA populations and among NB populations than across the two groups. Our functional enrichment analyses suggested that the genes underlying CNVs in OA differed from those in NB. The OA-specific CNVs were enriched in genes involved in immunity; whereas the NB-specific CNVs were enriched in protein secretory related pathways, suggesting possibility of different population history between these two different groups. In addition, we observed putatively NIs shared with the archaic hominid in the OA and NB populations, and they are significantly enriched in post-synapse membrane and Pleckstrin homology-like domain. Given the potential roles of the underlying candidate genes, collectively these enrichments imply that archaic hominin introgression may have helped to shape the specificity of the native Malaysian populations, and could possibly affect the regulation of immune response of these populations. Further investigations are warranted in order to provide further insights to the evolutionary process of immune systems of anatomical modern humans.

Several limitations were acknowledged in this study. First, recruitment of trios from the natives like OA and NB has been challenging due to the small size of these populations, and identification of the biological matched trio members had been rather difficult. This limitation indeed hindered population-based analyses of this study. Second, there is a gap to precisely identify breakpoints of the CNVs called, due to the limitation of technology and power of the analysis tools.

Conclusions

In this paper, we analyzed the deep sequenced genomes of five native trios from Peninsular Malaysia and North Borneo, and presents a full catalogue of the genome variants. It has several important implications to regional human population genetics. First, the higher discovery rate of novel variants in our genomes, although with a small sample size, compared to global populations suggested insufficient coverage of population diversity in the existing map of genomic variations, hence emphasizing the necessity of conducting further genomic studies on 'minor' populations of the world, such as the native inhabitants from Malaysia. Second, the estimated mutation rate and accurate phasing of the trio haplotypes could potentially be used as a reference to genomic studies of similar populations. Third, the differentiation of OA and NB genomes imply discrepant demographic history of these two populations, in accordance with previous studies [20]. Last, the inherited novel insertions with shared with archaic hominids identified in our samples may imply unique population history of the native inhabitants in Malaysia.

Methods

Sample collection and genome sequencing

Fifteen peripheral blood samples consisting of five sets of trios (father-mother-offspring) were collected from Malaysia under the approval of the Research and Ethics Committee of Universiti Teknologi MARA [Ref no: 600-RMI (5/1/6)], the Department of Orang Asli Development (Jabatan Kemajuan Orang Asli Malaysia, JAKOA) [JHEOA.PP.30.052.Jld 5(17)], and the Universiti Malaysia Sabah Medical Research Ethics Committee [code: JKEtika 4/10(3)], as well as the district offices, village chief, and the chairperson of the Committee of Village Development and Security. Informed written consent was obtained from the volunteers aged 18 years and above. Their family history, pedigree, and self-reported ethnicity were recorded via an interview using local dialect. These five trios were from five native groups (one for each). Three of them were from Peninsular Malaysia, including Bateq (BTQ), Mendriq (MDQ) (both are the Negrito tribes) and the Semai (SMI) (Senoi) groups. They are collectively known as Orang Asli (OA). The other two were from North Borneo (abbreviated as NB population), including Dusun (DSN) and Murut (MRT) populations. The information of the trios is listed in Table S1. The sampling procedure, as well as the protocol for genome sequencing were described in detail in Yew et al (2018)[23]. Briefly, genomic DNA was isolated from peripheral samples using DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany), and the integrity met the whole genome sequencing requirement (OD 260/280 reading ranging from 1.8-2.2). These samples were sequenced using Illumina HiSeq 2000, with a mean coverage of 30× (range 28 – 38×), targeted for 100 bp paired-end reads, with insert sizes of 300–400 bp according to the manufacturer's instruction.

Reads mapping and variants calling

Briefly, the pair-end reads in fastQ files were mapped to human reference genome GRCh37 with BWA 0.7.5a [24], and were subsequently merged and sorted to BAM format using samtools 0.1.16 [25]. Low quality reads and potential duplicates produced by the polymerase chain reaction in the library construction were removed. We then realigned the reads mapped around potential small insertion or

deletions using GATK 2.2-3 [26], and recalibrated the base quality scores. Reads with mapping quality (MQ) larger than 30 were kept for trio-aware variants calling with unified genotyper module in GATK2. Details of variants calling can be found in Yew et al (2018)[23].

Identification of de novo SNVs and indels

A variant in the offspring was defined as a *de novo* if it met the following criteria: i) the locus did not fall in the regions with poor mappability, low complexity or with enriched aberrant SNPs (as reported by Mallick et al.) [2]; ii) the read depth (DP) at this locus should be between 10-120, and 30-70% of the reads should support the alternative allele (allele balance in 0.3-0.7), as suggested by Kong et al. [27] and Neale et al. [28], to remove putative CNV regions in the offspring where the reads from highly similar regions are often mixed together; iii) the Genotype Quality (GQ) of the variant should be ≥ 50 in both offspring and parents; iv) no read supporting the alternative allele in each parent; and v) the alternative allele should be absent from both parents but present in the offspring.

Identification of copy number variants (CNVs)

We defined autosomal CNVs in this study as deleted or duplicated DNA segments larger than 100 bp to distinguish it from the indels (< 100 bp). CNVs were identified using CNVnator [29] and ERDS [30] in combination as recommended by Trost et al. (2018) [31]. A filtered set of CNVs was then generated by removing those variants that were: i) identified by only one method (<50% overlap); ii) overlapped with any repetitive and low-complexity regions; or iii) with $\geq 50\%$ overlap with gaps and segmental duplications.

Identification of novel and de novo CNVs

A novel CNV was defined if it had <50% overlap with a CNV region reported in the Database for Genome Variants (DGV) (<http://dgv.tcag.ca/dgv/app/home>, GRCh37 [32]). We defined a *de novo* CNV as a deletion or duplication that was only present in the offspring but absent in both parents. The following steps of stringent criteria were used to identify *de novo* CNVs: (i) we compared whether the putative CNV in the offspring was present in his/her parents with consistent type (i.e copy number (CN) gain or CN loss) under the reciprocal overlapping threshold of 50%; (ii) CNV that was not found in step (i) were genotyped by CNVnator in the trios, and we then filtered out the variants of which the genotyping results were consistent in the trios; (iii) we subsequently manually checked all the remaining CNVs in the last step to confirm if the variant was indeed true *de novo*.

Estimating the de novo mutation rate

We calculated *de novo* mutation rate for SNVs and indels in each trio based on a callability-based method in which the probability of each site that can be called as a *de novo* mutation is considered in denominator rather than simple counts of sites [15]. For a site s with an actual *de novo* mutation, the callability $C_k(s)$ is defined by the probability of calling s as a *de novo* mutation in the family k . The *de novo* mutation rate of a family k is (due to technical limitations see Supplementary Files for equation)

For CNVs, the *de novo* mutation rate was calculated as the number of *de novo* CNVs divided by the total CNVs in the offspring and averaged across trios.

Analysis of novel insertions

We used Poplns [33] with default settings to assemble the human reference genome (GRCh37) unmapped reads from the five trios into contigs together with those unmapped reads from the archaic hominin genome sequences (e.g., Neanderthal and Denisovan, obtained from <http://cdna.eva.mpg.de/neandertal/altai/>) [34,35]. These assembled contigs were candidate novel sequences which were absent from the human reference genome. We further filtered out the novel sequences in the trios if they violated Mendelian inheritance as they are more likely to be false positives.

Variants annotation and functional enrichment analysis

Annotation of the variants (SNVs, indels and CNVs) were performed using SnpEff version 4.3T [36], which provide estimated biological effects for each variant. Functional enrichment analysis of each set of gene was conducted using the database for annotation, visualization and integrated discovery (DAVID) v6.8 [37]. Enrichment score > 1.3, as proposed by the authors [37], and Benjamini-FDR-corrected p value < 0.05 were considered as thresholds of significance.

Haplotype Phasing

Parent-trio haplotype phasing was carried out using SHAPEIT for the five trios [5]. SHAPEIT allows for the inference of haplotypes using identity-by-descent (IBD) at any sized pedigrees. Prior to the haplotype phasing, we removed 39,429 SNPs, 31,657 indels, and 77 CNVs with missing rate >10% in the fifteen samples or exhibiting Mendelian error in two or more trios. For each trio, SNVs, indels and CNVs were phased in a combined dataset.

Abbreviations

BTQ: Bateq; CN: copy number; CNV: copy number variant; DAVID: Database for Annotation, Visualization and Integrated Discovery; DGV: Database for Genome Variants; DP: read depth; DSN: Dusun; FS: frameshift; GQ: genotype quality; IBD: identity by descent; indel: insertions and deletions; LOF: loss-of-function; MDQ: Mendriq; MQ: mapping quality; MRT: Murut; NB: North Bornean; NI: novel insertion; OA: Orang Asli; SMI: Semai; SNV: single nucleotide variant; SV: structural variant

Declarations

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Availability of data and materials

The genome sequence of the samples has been submitted to the National Omics Data Encyclopedia (NODE) (<http://www.biosino.org>) with the assigned accession number: NODEP00371760. SVs detected in the samples have been deposited to dbVar (<https://www.ncbi.nlm.nih.gov/dbvar/>).

Author's contributions

SX, BPH, SVK and YYT conceptualised the study; LD, BPH, SX and SVK drafted the manuscript; DL, LPW, CWY, RTHO performed the variant calling analyses; LD, HL, XZ, CL, BX, WX performed the SNV and indel analyses; HL, TB, CRM, SWS performed the CNV analyses; MZH, CWY, AF, ART, BJ, MEP, SVK and BPH contributed to the sample collections. All authors have read and approved the manuscript.

Ethics approval and consent to participate

This study was approved by the Research and Ethics Committee of Universiti Teknologi MARA [Ref no: 600-RMI (5/1/6)], the Department of Orang Asli Development (Jabatan Kemajuan Orang Asli Malaysia, JAKOA) [JHEOA.PP.30.052.Jld 5(17)], and the Universiti Malaysia Sabah Medical Research Ethics Committee [code: JKEtika 4/10(3)], as well as the district offices, village chief, and the chairperson of the Committee of Village Development and Security. It was also approved by the Biomedical Research Ethics

Committee of Shanghai Institutes for Biological Sciences (ER-SIBS-261903). Informed written consent was obtained from the volunteers aged 18 years and above. Their family history, pedigree, and self-reported ethnicity were recorded via an interview using local dialect.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables, Figures And Equations

Due to technical limitations, tables, figures and equations are only available as a download in the supplemental files section.

Additional Files

Additional file 1: This file contains Table S1-S3, Table S5-S7, Table S9-S14, Table S16.

Additional file 2: This file contains Table S4.

Additional file 3: This file contains Table S8.

Additional file 4: This file contains Table S15.

Additional file 5: This file contains Table S17.

Figures

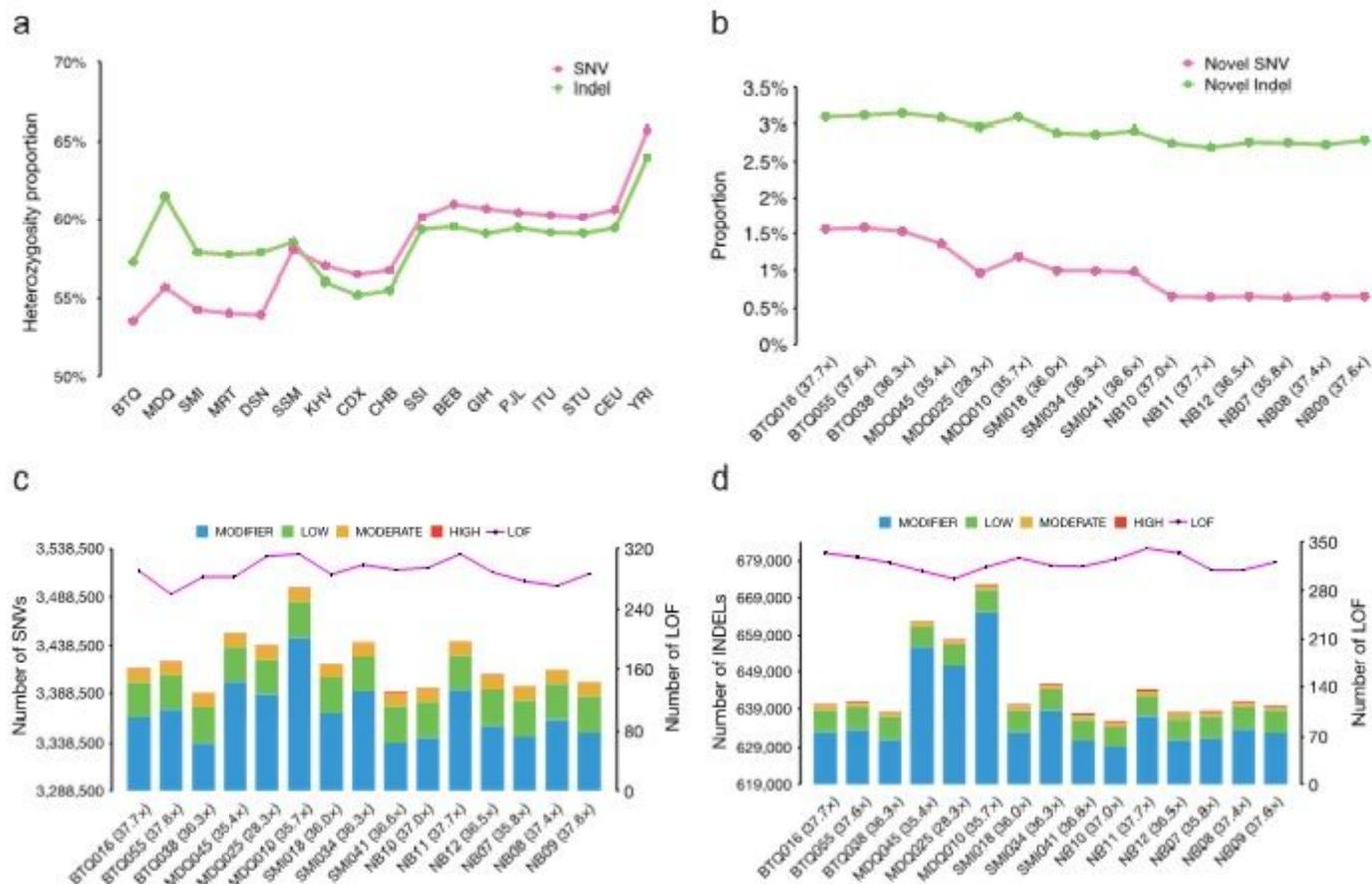


Figure 1. Characterization of SNVs and indels identified in the 15 genomes. (a) Heterozygosity proportion of SNVs (green) and indels (pink) identified from 15 individuals. The number of homozygous alternative variants and heterozygous variants for each population were calculated separately. The heterozygosity proportion was calculated as the number of heterozygous variants divided by the sum of the number of homozygous alternative variants and heterozygous variants. **(b)** Proportion of novel SNVs (green) and novel indels (pink) in all 15 individuals with the mean mapped depth. **(c)** Number of SNVs with different impacts per sample. **(d)** Number of indels with different impacts per sample.

Figure 1

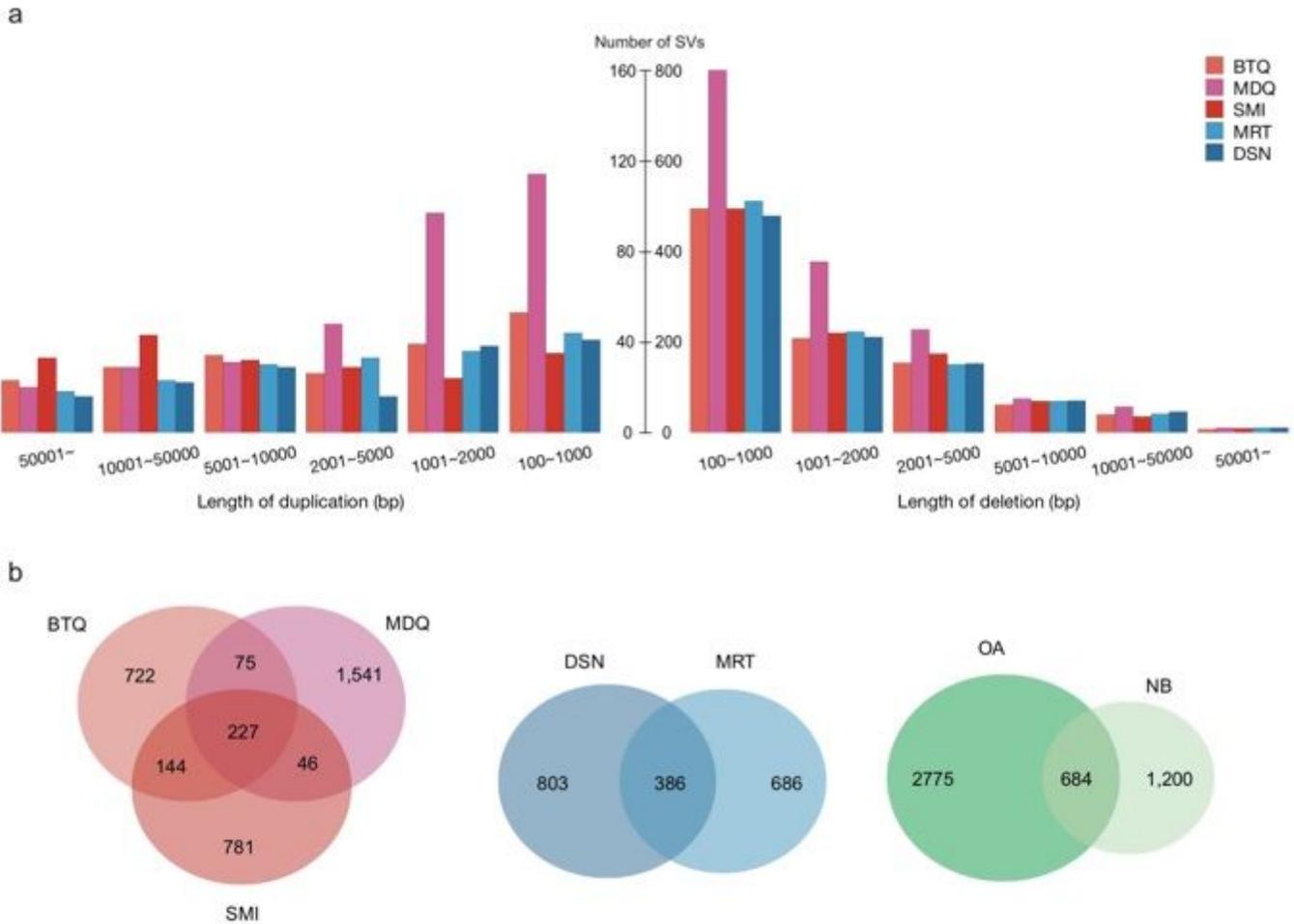


Figure 2. Structural variants identified in the 5 trios. (a) The number and length distribution of the duplications (left) and deletions (right) in each trio. (b) Venn diagrams represented the number of shared and unique SVs among three OA trios (left), within two NB trios (medium) and between these two groups (right).

Figure 2

Supplementary Files

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