Genetic risk factors for colorectal cancer in multiethnic Indonesians

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Colorectal cancer is a common cancer in Indonesia, yet it has been understudied in this resource-constrained setting. We conducted a genome-wide association study focused on evaluation and preliminary discovery of colorectal cancer risk factors in Indonesians. We administered detailed questionnaires and collecting blood samples from 162 colorectal cancer cases throughout Makassar, Indonesia. We also established a control set of 193 healthy individuals frequency matched by age, sex, and ethnicity. A genome-wide association analysis was performed on 84 cases and 89 controls passing quality control. We evaluated known colorectal cancer genetic variants using logistic regression and established a genome-wide polygenic risk model using a Bayesian variable selection technique. We replicate associations for rs9497673, rs6936461 and rs7758229 on chromosome 6; rs11255841 on chromosome 10; and rs4779584, rs11632715, and rs73376930 on chromosome 15. Polygenic modeling identified 10 SNP associated with colorectal cancer risk. This work helps characterize the relationship between variants in the SCL22A3, SCG5, GREM1, and STXBP5-AS1 genes and colorectal cancer in a diverse Indonesian population. With further biobanking and international research collaborations, variants specific to colorectal cancer risk in Indonesians will be identified.

Colorectal cancer is one of the most common cancers in the world and a leading cause of cancer-related deaths1,2. There is growing evidence that colorectal cancer rates are changing in Asian countries, but the causes are still under investigation3,4. Colorectal cancer is now one of the top three cancers in many Asian countries5. Currently, Asia contributes to 48% of the total number of new colorectal cancer cases in the world, of which the majority are found in Eastern Asia6. Specifically in Indonesia, the age-standardized incidence for males and females has been reported as 15.9 and 10.1 per 100,000 respectively6.

The heritability of colorectal cancer is estimated to be between 12 and 35%. However, germline mutations that are highly penetrant contribute less than 5% to colorectal cancer7. Nonetheless, increasing evidence is finding that heritability plays a potential, crucial role in colorectal cancer pathogenesis. Currently, mutations in 14 genes are suspected to underlie different subtypes of colorectal cancer, including mutations in the APC that increases predisposition to familial adenomatous polyposis (FAP) and defects in mismatch repair genes associated with Lynch Syndrome7. Recent genome-wide association studies have identified common genetic variants linked to colorectal cancer predisposition, highlighting a greater association between heritable risk and the disease. Thus far, over 40 genetic variants have been identified, within several well-known biological pathways that have been shown to be highly relevant to oncogenesis, including the TGF-beta/BMP pathway and the mitogen-activated protein kinases (MAPK) pathway7.

However, many of these colorectal cancer genetic associations were discovered in European-ancestry populations but do not replicate well in other ancestry groups, demonstrating the need for studies in diverse populations worldwide8. The Asia Colorectal Cancer Consortium was initiated in 2009 among East Asian nations and has successfully identified novel relevant, genetic regions9,10. However, colorectal cancer cases from South East Asian cohorts have been under represented.

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Given the changes in colorectal cancer rates in Asia and the differences in risk factors present in ethnically diverse South East Asia, we present results of the first genomic association study of colorectal cancer in Indonesia. We present results from the initial phase of this study, focused on cases from South Sulawesi, Indonesia.

**Results**

**Characteristics of study sample.** The characteristics of the colorectal cancer cases and controls are summarized in Table 1. The mean age of the colorectal cancer cases was 54 years. The majority of cases were male (57%). Among ethnicities, most cases were self-reported Bugis (44%) or Makassar ethnicity (27%). Controls appeared to be adequately frequency matched to cases by age, sex, and ethnicity ($p > 0.05$). Colorectal cancer cases had lower average body mass index (BMI) and were more likely to be smokers than controls ($p < 0.01$). Estimated genetically, the majority of both cases and controls were of East Asian ancestry. 82% of the cases had late stage cancer (III or IV) which unfortunately is consistent with recent reports in Indonesia. As seen in other studies, the most common colorectal cancer site was rectum (43%).

**Genome-wide association analysis.** As expected given the sample size, no SNPs met the historical cut-off set for genome-wide significance (Supplementary Figs. 6 and 7). The summaries for all variants with a marginal p-value < 5E-5 are included in the "Supplementary materials" (Table 4). These include two intergenic SNPs and two SNPs in the MRO gene on chromosome 18.

Results for previously reported colorectal cancer SNPs are presented in Fig. 1 and Supplementary Table 3. There is evidence of replication for the following genetic variants: rs9497673, rs6936461 and rs7758229 on chromosome 6; rs4779584, rs11632715, and rs73376930 on chromosome 15. The regions are characterized in Figs. 2, 3, 4, and 5. The pattern of associations is rather diffuse in the STXBP5-AS1 (STXBP5 Antisense RNA 1) and SLC22A3 genes of chromosome 6, representing the correlation among

### Table 1. Characteristics of South Sulawesi colorectal cancer cases and controls.

|                      | Cases N = 89 | Controls N = 84 | P     |
|----------------------|--------------|----------------|-------|
| **Age**              | 53.8 (13.2)  | 50.5 (14.5)    | 0.12  |
| **Gender**           |              |                | > 0.99|
| Female               | 38 (42.7%)   | 36 (42.9%)     |       |
| Male                 | 51 (57.3%)   | 48 (57.1%)     |       |
| **Ethnicity**        |              |                | 0.68  |
| Bugis                | 39 (43.8%)   | 45 (53.6%)     |       |
| Makassar             | 24 (27.0%)   | 23 (27.4%)     |       |
| Mandar               | 2 (3.3%)     | 1 (1.2%)       |       |
| Toraja               | 10 (11.2%)   | 8 (9.5%)       |       |
| Non South Sulawesi   | 9 (10.1%)    | 4 (4.8%)       |       |
| Non Sulawesi         | 5 (5.6%)     | 3 (3.6%)       |       |
| **BMI**              | 21.2 (3.1)   | 24.5 (3.6)     | < 0.01|
| **Smoking status**   |              |                | < 0.01|
| Smoker               | 39 (43.8%)   | 15 (17.9%)     |       |
| Non smoker           | 50 (56.2%)   | 69 (82.1%)     |       |
| **Ancestry (estimated)** |          |                |       |
| East Asian (EAS)     | 0.92         | 0.94           | 0.02  |
| South Asian (SAS)    | 0.07         | 0.05           | 0.15  |
| African (AFR)        | < 0.01       | < 0.01         | 0.02  |
| European (EUR)       | 0.01         | 0.01           | 0.36  |
| **Cancer site**      |              |                |       |
| Right colon          | 15 (16.9%)   | –              |       |
| Transversum          | 9 (10.1%)    | –              |       |
| Left colon           | 1 (1.2%)     | –              |       |
| Sigmoid              | 26 (29.2%)   | –              |       |
| Rectum               | 38 (42.7%)   | –              |       |
| **Staging**          |              |                |       |
| I                    | 3 (3.4%)     | –              |       |
| II                   | 9 (10.1%)    | –              |       |
| III                  | 62 (69.7%)   | –              |       |
| IV                   | 11 (12.4%)   | –              |       |
the variants in these regions (Figs. 2 and 3). Similarly, the association pattern tapers along chromosome 10. The strongest association pattern can be found on chromosome 15. This region has a more defined peak than the other regions with associations spanning two genes: SCG5 (secretogranin V) and GREM1 (gremlin 1, DAN family BMP antagonist).

The polygenic analysis identified 10 SNPs which appear to have a relatively strong association (i.e., large effect size) with the risk of developing colorectal cancer as can be seen in Table 2. These variants have marginal
Figure 4. Association plot for 100 kb region flanking rs11255841 on chromosome 10.

Figure 5. Association plot for 100 kb flanking rs11632715 on chromosome 15. The top associated SNP in the region was rs10083612.

| Description | Chr | Position | Gene      | Ref | MaF | Estimate |
|-------------|-----|----------|-----------|-----|-----|----------|
| Intercept   | 0   | 0.90     |           | 0.00| 0.00| 0.00     |
| Gender      | 0   | 0.00     |           | 0.00| 0.00| 0.00     |
| Age         | -1  | -3.75    |           | -1  | -1  | -1       |
| BMI         | 0   | 0.00     |           | 0.00| 0.00| 0.00     |
| Smoking     | 1   | 1.32     |           | 1.32| 1.32| 1.32     |
| rs11919079  | 3   | 57086348 | Intron:ARHGEF3 | G  | 0.07| 2.40     |
| rs4888186   | 16  | 81947156 | Intron:PLCG2 | C  | 0.08| 0.85     |
| rs11016111  | 10  | 129963848| Intergenic  | C  | 0.34| -1.32    |
| rs76757157  | 5   | 98125016 | Intron:RGMB | G  | 0.05| 1.95     |
| rs72066763  | 5   | 164113078| Intergenic  | T  | 0.12| 1.65     |
| rs2446103   | 6   | 77328692 | Intergenic  | A  | 0.04| 1.22     |
| rs219420    | 17  | 45800299 | Intergenic  | T  | 0.36| 1.32     |
| rs78165118  | 3   | 12816282 | Intergenic  | A  | 0.03| 2.13     |

Table 2. Polygenic risk model learned from colorectal cancer data. Presented results include the chromosome (Chr) and position of the significant genetic variants, the gene they lie on (Gene), reference allele (Ref), minor allele frequency (MaF), and estimated effect (Estimate).
cases23,24. With the risk allele of T, this variant had an odds ratio of 2.2 in our study, while previous reports had an odds ratio of 1.1–1.2.

lncRNAs are involved in all stages of colorectal cancer. In colorectal cancer pathogenesis, lncRNAs are implicated DNA, protein, microRNA and mRNA. The different expression of lncRNAs in colorectal cancer indicate that linked to the level of patient responsiveness towards cancer treatments20; in particular, platin-based cytotoxic (p = 0.010). Interestingly, the smallest p-value in the region was rs10083612 within an intron of SCG5 replicated colorectal cancer associations for rs4779584 (p = 0.018), rs11632715 (p = 0.004), and rs73376930

Using bioinformatics tools, it is predicted that changes from T to A in rs6936461 and A to G in rs9497673, has the STXBP5-AS1 on chromosome 6. see Supplementary Table 3). These SNPs are located in the intronic regions of SLC22A3 and colorectal cancer is needed. SLC22A3 encodes for the protein OCT3, which is an organic cationic transporter. While OCT3/SLC22A3 is well characterized within neurochemistry, it has been found to play a role within oncology as well. The upregulation of SLC22A3 in head and neck squamous cell carcinoma is associated with improved prognosis while the downregulation of SLC22A3 leads to enhanced metastasis and invasion of the tumor18. SLC22A3 has also been implicated in the pathogenesis of prostate cancer and its expression is elevated in these neoplastic tissues19. The level of OCT3/SLC22A3 expression has also been linked to the level of patient responsiveness towards cancer treatments20; in particular, platin-based cytotoxic cancer treatments in colorectal cancer21 patients, as well as head and neck squamous cell carcinoma patients20.

Intergenic variant rs11255841 on chromosome 10 was identified in an colorectal cancer GWAS of European ancestry individuals22 and has replicated in a Japanese study and a large meta-analysis with nearly 37,000 metastasis and invasion of the tumor18. SLC22A3 has also been implicated in the pathogenesis of prostate cancer and its expression is elevated in these neoplastic tissues19. The level of OCT3/SLC22A3 expression has also been linked to the level of patient responsiveness towards cancer treatments20; in particular, platin-based cytotoxic cancer treatments in colorectal cancer21 patients, as well as head and neck squamous cell carcinoma patients20.

Several previously identified colorectal cancer associated SNPs replicated in this population. And we can begin characterizing these regions by examining neighboring variants. The rs7758229 variant within SLC22A3 on chromosome 6 was originally identified and subsequently replicated in large case-control study of a Japanese population (OR of 1.3)16. Interestingly, in a subsequent study in a Chinese population, this SNP was not associated with colorectal cancer (OR of 0.95)17. However, in S. Sulawesi, we detect a statistically significant association with colorectal cancer (p = 0.009, OR of 2.2). Given these differences among East Asians, further work to understand variation in SLC22A3 and colorectal cancer is needed. SLC22A3 encodes for the protein OCT3, which is an organic cationic transporter. While OCT3/SLC22A3 is well characterized within neurochemistry, it has been found to play a role within oncology as well. The upregulation of SLC22A3 in head and neck squamous cell carcinoma is associated with improved prognosis while the downregulation of SLC22A3 leads to enhanced metastasis and invasion of the tumor18. SLC22A3 has also been implicated in the pathogenesis of prostate cancer and its expression is elevated in these neoplastic tissues19. The level of OCT3/SLC22A3 expression has also been linked to the level of patient responsiveness towards cancer treatments20; in particular, platin-based cytotoxic cancer treatments in colorectal cancer21 patients, as well as head and neck squamous cell carcinoma patients20.

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The region on chromosome 15 nearby SCG5 and GREM1 have been flagged in multiple GWAS, e.g.,. We replicated colorectal cancer associations for rs4779584 (p = 0.018), rs11632715 (p = 0.004), and rs73376930 (p = 0.010). Interestingly, the smallest p-value in the region was rs10083612 within an intron of SCG5 (p = 1.61e−5, see Fig. 5). The role of SCG5 in colorectal cancer has not been well characterized, while much is known about its neighbor GREM1’s role in colorectal cancer. GREM1, which is one of the antagonists of the bone morphogenetic proteins (BMPs) found within the TGF-beta signaling pathway, has been found to be important for the survival and proliferation of several types of cancers20. In particular, modulated expression of GREM1 is found in cancer-associated stromal cells. GREM1 is also found to be a proangiogenic factor, suggesting a role in cancer development when it is upregulated27. SCG5 and GREM1 genes have been found to be associated with polyposis syndromes that are associated with colorectal cancer28. A duplication that spans the 3’end of SCG5 and the immediate, adjacent upstream region of GREM1 is associated with hereditary mixed polyposis syndrome (HMPs) as well as tumorigenesis in juvenile polyposis. This duplication results in a 40-kb extra segment that leads to the upregulation of GREM1 expression. The duplication is the basis for an autosomal dominant HMPs condition that is prevalent among the Ashkenazi Jewish population and is a recommended biomarker/genetic test to detect CRC in this population. Aberrant expression of GREM1 has also been shown to underlie oncogenesis within the large intestines and colon29.

Two of the previously identified colorectal cancer markers replicate in this study (rs6936461 and rs9497673; see Supplementary Table 3). These SNPs are located in the intronic regions of STXBP5-AS1 on chromosome 6. Using bioinformatics tools, it is predicted that changes from T to A in rs6936461 and A to G in rs9497673, has the potential to alter the splicing of the gene. STXBP5-AS1 is an long non-coding (IncRNA) gene. IncRNAs drive many important cancer phenotypes through their interactions with other cellular macromolecules including DNA, protein, microRNA and mRNA. The different expression of IncRNAs in colorectal cancer indicate that IncRNAs are involved in all stages of colorectal cancer. In colorectal cancer pathogenesis, IncRNAs are implicated...
in a variety of signaling pathways including the Wnt/-catenin signaling pathway, epidermal growth factor receptor (EGFR)/insulin-like growth factor type I receptor (IGF-IR) signaling pathway, KRAS and phosphatidylinositol-3-kinase (PI3K) pathways, transforming growth factor-beta (TGF-) signaling pathway, p53 signaling pathway, and the epithelial-mesenchymal transition (EMT) pathway. While it is still unclear how STXBPS-AS1 contributes to colorectal carcinogenesis, it plays a role in predicting the prognostic survival with good sensitivity and specificity. The IncRNAs may act as competing endogenous RNAs (ceRNAs) and interfere in the binding of miR-190b to certain targets such as ERG, STK38L, and FND3A and thus contribute to breast cancer pathogenesis. STXBPS-AS1 may act similarly in colorectal cancer; it may hinder the binding of microRNAs to their target genes and subsequently modulate colorectal cancer tumorigenesis. Interestingly, STXBPS-AS1 was identified among genes that are methylated in buccal samples in a genome-wide screen for cigarette smoke exposure, indicating its possible role in smoking-related diseases. Since there is a significant difference in smoking status between cases and controls in our cohort, it is plausible that genetic variants associated with tobacco smoke are also associated with the presence of colorectal cancer in our study population.

The polygenic model represents a strategy for jointly modeling SNP effects in a GWAS and development of risk prediction models in a specific population. These models can be used to estimate an individual’s risk of colorectal cancer based on easily obtainable genotypes. While most of the variants flagged in the polygenic model are novel, the gene ARHGFI3 has been implicated in promoting nasopharyngeal carcinoma in Asians. RGMB has been shown to promote colorectal cancer growth. Additional samples will enable us to refine and validate a polygenic colorectal cancer risk model in Indonesians.

Methods

Study participants. Indonesia is an archipelago consisting of more than 14,000 islands. There are five major islands, and one of them is Sulawesi. Makassar is located in the southern part of Sulawesi. It is the largest city in eastern Indonesia. 162 colorectal cancer cases were recruited from seven hospitals throughout Makassar between 2014 and 2016. The hospitals were Wahidin Sudirohusodo Hospital, Hasanuddin University Hospital, Ibn Sina Hospital, Akademis Hospital, Grestelina Hospital, Stella Maris Hospital, and Hikmah Hospital. 193 controls were frequency matched to cases on age category, sex, and ethnicity. Informed consents were obtained from all subjects, and all methods were carried out in accordance with the relevant guidelines and regulations as determined by ethical review approved by the Hasanuddin University Ethical Committee (registration number: UH 15040389).

Data and DNA sample collection. Questionnaires and medical records were recorded into study data collection forms and entered into a study database. The case forms contained 382 questions and the control forms contained 319 questions. The forms included information on demographics, cancer history in the family, smoking behavior, alcohol use, and detailed dietary history. For colorectal cancer cases, the forms collected information on cancer symptoms, staging (post operation), tumor, location, histopathology, and type of surgery. The questionnaire is included as a “Supplementary file.” The database was managed by the Bioinformatics and Data Science Research Center (BDSRC) at Bina Nusantara University (Jakarta, Indonesia). A blood sample was collected from the basilic/cephalic vein on all participants for genotyping. These blood samples were stored in Data Science Research Center (BDSRC) at Bina Nusantara University (Jakarta, Indonesia). A blood sample was allocated for this preliminary study and filled based on the DNA quality. Extracted DNA were sent to RUCDR Infinite Biologics for genotyping (Piscataway, NJ, USA) under Material Transfer Agreement (MTA) approved by the Indonesian Health Ministry (registration number: LB.02.01/I/12749/2016).

Genototyping and imputation. DNA samples were collected at the hospital where surgery was performed (Wahidin Hospital). DNA was extracted from samples at Mochar Radyi Institute for Nanotechnology (MRIN) Laboratory https://www.overleaf.com/project/5efa1240b367400001bf3549 (Tangerang, Indonesia). Genomic DNA was extracted from 200 μL of whole blood sample using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. DNA concentration was determined using NanoDrop ND-1000 spectrophotometer, version 3.3 (Thermo Fisher Scientific, Wilmington, DE, USA) and adjusted to a concentration of 20 ng/μL. The quality of DNA extracted was verified by purity index of OD260/OD280 (1.8–2.0) and OD260/OD230 (> 1.5). The DNA was inspected through Gel Electrophoresis using 1% molecular biology grade Agarose (Biorad, Hercules, CA, USA). Two plates of samples (92 cases and 92 controls) were allocated for this preliminary study and filled based on the DNA quality. Extracted DNA were sent to RUCDR Infinite Biologics for genotyping (Piscataway, NJ, USA) under Material Transfer Agreement (MTA) approved by the Indonesian Health Ministry (registration number: LB.02.01/I/12749/2016).

DNA samples from study cases and controls were genome-wide genotyped on the Smokescreen Genotyping Array. Using 200 ng of genomic DNA, array plates were prepared using the Axiom 2.0 Reagent Kits and then processed on the GeneTitan MC instrument (Thermo Fisher Scientific, Wilmington, DE, USA). Analysis of the raw data was performed using Affymetrix Power tools (AFT) v-1.16 according to the Affymetrix best practices workflow. 183 samples remained after completing these steps. Additional steps were performed using SNPolisher to identify and select best performing probe sets and high quality SNPs for downstream analysis. 524,765 SNPs remained after QC filtering. Additional sample quality control included verifying concordance of study replicates, checking for unintentional duplicates and unexpected relatives, and verifying genetic versus reported gender. After filtering samples with missing covariates, 173 samples (84 cases and 89 controls) remained for statistical analysis.

Genome-wide imputation was performed on the Michigan Imputation Server v1.0.2. Briefly, quality controlled study genotypes were reported on the forward strand and uploaded in vcf format. 1000 Genomes Phase 3 was selected as a reference panel, phasing was performed using Eagle v2.3, and allele frequencies were estimated using the IMPUTE program.
compared against the 1000 Genomes East Asian (EAS) populations. The server automatically excludes variants with alleles other than (A, C, T, G), variants with duplicate positions, indels, monomorphic sites, and allele mismatches with the reference panel.

**Statistical analysis.**  Ancestry analysis.  Ancestry categories were estimated from 5515 ancestry informative markers contained on the Smokescreen Genotyping Array using fastStructure 1.0. Combining study and reference data from the 1000 Genomes Project Phase 3, we estimated the ancestry proportions of East Asian (EAS), South Asian (SAS), European (EUR), and African (AFR).

Genome-wide association analysis. We filtered out variants with poor imputation quality (< 0.3) and rare variants (minor allele < 1%). We then performed a marginal analysis of the remaining SNP genotype dosage fitting logistic regression models, with sex, age, body mass index, smoking status and estimated ancestries proportions (i.e., SAS, EUR, AFR) as covariates. The threshold for statistical significance in the discovery scan was set at the historical traditional genome-wide value of $5 \cdot 10^{-8}$. This association model was implemented using glm in R.

We queried the scan results for markers previously reported to be associated with colorectal cancer. These variants were identified through previous genotyping in an independent sample of South Sulawesi colorectal cancer cases (R. Kusuma, I. Suriapranata, personal communication) and a recent catalog of colorectal cancer SNPs for a genome-wide association scan in Hispanics. The source and annotation for these variants are provided in Supplementary Table 3. Variants with evidence of replication (p-value < 0.05) were flagged for further investigation. Regional association plots were generated in LocusZoom.

We also developed a polygenic model considering the joint effect of multiple genetic variants on colorectal cancer. We included a screening step as a practical way to keep the number of variants under consideration in the polygenic model close to the total sample size. In this screening step the top 200 genetic associations were selected, based on Bayes factors, as candidate predictors in this joint model. Bayes factors were computed for the marginal versus the null models for each SNP while controlling for gender, age, BMI, and smoking status. To jointly model these variants, we use a Bayesian variable selection technique. In particular, we fit a logistic regression model utilizing shrinkage priors for each of the explanatory variables; i.e., the covariates listed above as well as the remaining candidate SNPs. In this analysis, the generalized double Pareto shrinkage prior was specified and the parameters of the joint model were estimated via a maximum a posteriori (MAP) estimator which was obtained via an expectation-maximization (EM) algorithm. The MAP estimator under these specifications simultaneously completes parameter estimation and variable selection by obtaining a sparse estimator, i.e., some of the regression coefficients are estimated to be identically equal to zero thus removing the effect of the corresponding explanatory variable. The EM algorithm was developed following the techniques illustrated by Armagan et al. and Polson et al. and the regularization parameters were selected via the Bayesian information criterion. These algorithms were implemented in R and completed within 90 s on an Intel based laptop, see Joyner et al. for details including the source code.

**Conclusions**

We demonstrate replication of several colorectal cancer genetic risk factors in an Indonesian population. This study overcame the many challenges of genomic research in resource-constrained settings and provides rational for additional data collection in this population to characterize these regions more precisely and identify genetic risk factors unique to this diverse population. The primary focus of this study was replicating associations of known colorectal cancer risk variants in an Indonesian population. A secondary focus was computing genome-wide summary statistics for contributions to international colorectal cancer consortia. With additional data collections in Indonesia, we may examine and report on environmental factors (e.g., dietary factors) as well as gene–environment interactions.

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The authors declare no competing interests.

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