Chapter

APC and *MSH2* mRNA Quantitative Gene Expression and Bayesian Analysis of Proband in Hereditary Colorectal Carcinoma

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Abstract

Heterozygote relatives have approximately 80% lifetime colorectal cancer (CRC) risk. mRNA gene expression and Bayesian theorem can calculate CRC's family risk through the initial pedigree proportion appended with conditional information. The study is the first to report such an application. The present cross-sectional and translational investigation tracked CRC patients' tissue and blood measurement of adenomatous polyposis coli (*APC*) and MutS homolog (*MSH*)*2 mRNA* quantitative gene expressions, control matching, and ancestral analysis by pedigree and Bayesian theorem. Among 40 CRC patients, mean tissue level and hereditary cutoff of APC are 13,261 (670) fold-change (fc) and 12,195 fc, while 12,219 (756) fc and 11,059 fc for MSH2. A quarter of the CRC patients had a history of familial CRC. Meanwhile, four CRC patients and 10 probands were evaluated for recurrence risk via pedigree, quantitative PCR, and Bayesian analysis. We determined a cutoff point for hereditary mRNA quantitative expression. *APC* and *MSH2* levels in the CRC subjects were significantly lower than controls. The Bayesian analysis builds ways to calculate relative risk in CRC patients' family members and application in clinical practice.

Keywords: hereditary CRC, APC gene, MSH gene, Bayesian analysis

1. Introduction

The continuing morbidity and lethality of colorectal cancer (CRC) do not always stop at the diseased person. In fact CRC holds the top place in familial inherited case prevalence [1]. Hereditary CRC with clear-cut forms overall can be divided into lynch

syndrome (LS) or hereditary non-polyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP), which are inherited by autosomal dominant pattern [2]. Those who related to parents or grandparents with an autosomal dominant trait has at least an 80% chance for lifetime risk of CRC incidence [3].

Screening for cancers is dependent on every individual. Those without any familial cancer history can start colonoscopy for CRC screening at 50 years old. Nevertheless, the age is smaller by a magnitude if you have a CRC first-degree relative. A CRC individual will raise your risk by two to three times more than normal; however, more relatives with the disease may equal to an exponential risk increase [4].

Familial characteristics such as age, disease onset, size, and health history often pose precarious conditions to the internist and gastroenterologist who did CRC hereditary screening by the Amsterdam and Bethesda criteria. These were illustrated in the low guidelines' performances from both Revised Bethesda Guideline and Amsterdam II Criteria against molecular tumor analysis with 50 and 25% sensitivity and 7 and 38% positive predictive values [5]. We accordingly need a more swift and stable method to screen for hereditary CRC, such as with the implementation of family history, molecular expression, and Mendel inheritance concept [6, 7].

The Mendel hereditary concept is well-performed in screening or determining autosomal and gonadal patterns risks, as it can compute recurrence probability; however, it cannot be quickly adjusted for mutation, external factors, and coverage changes, since it focused more on empirical recurrences. Yet the application of only such analysis is questionable, as most traits are not generalizable. Hence, family members' recurrence risk should be calculated with prior Mendelian risk and geared with personal genotyping and environmental conditional probability [8–11].

Genetic studies are becoming more present recently with research around genetic matters like DNA sequencing or polymorphism [12]. They open up a new horizon for disease susceptibility and inheritance analysis, including malignancy. However, RNA study was still rare as mistakes in the nucleotide base or elsewhere will be quickly dealt by the proofreading and the mismatch repair (MMR) genes [13].

Adenomatous polyposis coli (*APC*) gene exhibited a unique causal relationship to the incidence of hereditary FAP from mutation on the fifth chromosome's second region and first band. LS conversely rises from mutations in the second, third, and seventh chromosome of several different genes, including human MutL homolog 1, human MutS homolog 2 and 6, as well as human post-meiotic segregation 1 and 2 (*hMLH1*, *hMSH2*, *hMSH6*, *hPMS1*, and *hPMS2*) [14].

In commencing the current study, research operators or the authors need to be more aware of their surroundings. This time, huge complex calculations and uncommon Bayesian prior and posterior analysis were implemented. There is no former report on the *APC* and *MSH2* genomic RNA expressions to CRC risk with modified Bayesian estimation per the authors' knowledge [15–17]. We hoped the current study was able to officialize an adequate hereditary measure through gene expression and the families able to incorporate Bayesian into their risk of CRC.

2. Materials and methods

2.1 Study design

The current translational study adopted a cross-sectional design in assessing 71 subjects from May 2018 to December 2019. Medical Ethics Committee of Hasanuddin University

ensured the research commencement had followed Helsinki declaration and institutional review board (IRB) standards with certification of 884/H4.8.45.31/PP31-Komite/2018. Every subject had understood and agreed to participate as shown by the signed informed consent form. The author priorly measured the minimum sample size by 5% alpha and 80% power.

2.2 Subject enrollment

Subjects were consecutively gathered from Tarakan General Hospital in Jakarta and Siloam Hospitals Lippo Village in Tangerang. The case group broadly enrolled all 41 CRC patients who had undergone a biopsy in either hospital. Gastroenterologists and oncologists made the CRC diagnosis based on the clinical symptoms, physical examinations, and supporting investigations (i.e., endoscopy and histopathological findings). We contrarily enlisted normal patients or CRC patients' relatives who had been matched by age, sex, and body mass index to the control group.

Exclusion of patients from either group may happen at any time of the study if they had: (1) presence or history of other malignancies or inflammatory bowel disease, (2) ever done chemotherapy or radiotherapy, (3) illnesses that inhibit communication, and (4) refuse to participate.

2.3 Data and sample collection

The current study investigated *APC* and *MSH2* quantitative genotypic expressions as well as hereditary possibilities. 0.3 ml of blood samples were taken from all 71 subjects using one cc syringes, yet only CRC subjects did colonoscopy biopsy. Each of the blood and tissue samples was then laced into separated sample tubes containing L6 buffer preservative, specifically created by Hasanuddin University from a slightly modified version of the buffer in the Boom RNA extraction method. **Figure 1** showed the complete RNA extraction techniques by the NucleoSpin technique (#740200.50) [18]. The isolated extraction results were subsequently amplified using a real-time PCR

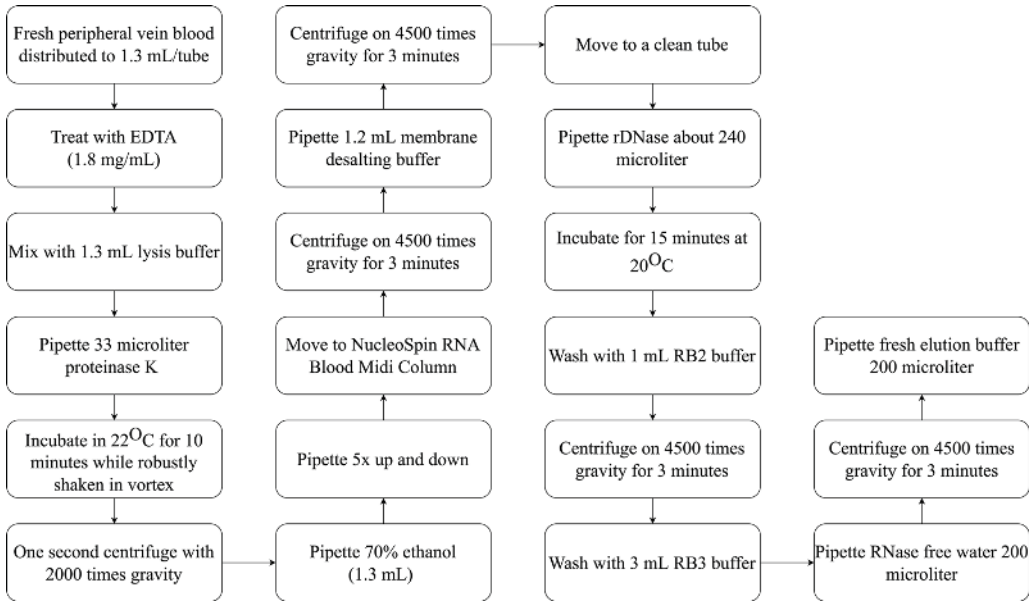

Figure 1.
RNA extraction technique. Step by step pathway of the RNA extraction with NucleoSpin technique (#740200.50) [18].

Gene	Orientation	Sequence	Primer [19, 22]
Specific primer target[a]			
MSH2	Forward	5′ to 3′	CATCCAGGCATGCTTGTGTTGA
	Reverse		GCAGTCCACAATGGACACTTC
APC	Forward		TGTCCCTCCGTTCTTATGGAA
	Reverse		TCTTGGAAATGAACCCATAGGAA
Internal control genes[a]			
ß-actin	Forward	5′ to 3′	ACAGAGCCTCGCCTTTGCCGAT
	Reverse		CTTGCACATGCCGGAGCCGTT
GAPDH	Forward		CGCTCTCTGCTCCTCCTGTT
	Reverse		CCATGGTGTCTGAGCGATGT

[a]*Designed by Macrogen (Seoul, Korea).*

Table 1.
Genetic primers.

(RT-PCR) and then measured with a Bio-Rad CFX Manager 3.1 (Bio-Rad Laboratories, Hercules, USA) [19–21]. After that, we also applied the Bayesian probability analysis on the probands' age, *APC*, and *MSH2* data to yield CRC risk estimations.

2.4 Polymerase chain reaction (PCR)

The present study used RT-PCR to detect the mRNA expression of *MSH2* and *APC* genes with the following primers (**Table 1**). First, they entered the initial denaturing phase with 94°C for 3 minutes. Then the process continued with 38 cycles of annealing stage in 54°C for 30 s and extension stage in 72°C for 30–40 s [19–21]. Note that each gene has its unique amplicon length. For example, *APC* is 89 bp long, 81 bp for *GAPDH*, 215 bp for *MSH2*, and 109 bp for ß-actin [23].

We procured the RT-PCR materials from Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, USA). Additionally, we used CFX Connect real-time PCR system from Bio-Rad Laboratories for the measurement [22, 23].

2.5 Bayesian probability

The current investigation quantified the CRC risk among family members through Bayesian analysis of the Mendelian hereditary, genetic, and direct mutations data [4, 16]. We derived a posterior probability equation (Eq. (1)) to estimate the family CRC risk from the coupling of the conditional and prior probability theorems.

$$\mathrm{p}(\mathrm{A}\,|\,\mathrm{B}) = \frac{\mathrm{p}(\mathrm{B}\,|\,\mathrm{A})\mathrm{p}(\mathrm{A})}{\mathrm{p}(\mathrm{B})} \text{ and } E(\beta) = \int \beta\, p(\beta|\gamma|,\vartheta)\, d\beta$$

$$p(\beta|\gamma|,n) = \frac{p(\gamma,\beta,|\vartheta)}{p(\gamma|\vartheta)} = \frac{p(\gamma,\beta|\vartheta)}{\int p(\gamma,u|\vartheta)\,du} = \frac{f(\gamma|\beta)\varpi(\beta|\vartheta)}{\int f(\gamma|u)\varpi(u|\vartheta)\,du} \quad (1)$$

2.6 Statistical analysis

Statisticians used descriptive statistics to univariately portray the subjects' demographic characteristics and histological findings, as opposed to Shapiro-Wilk for determining the numeric data normality. Further bivariate analysis of the parametric numeric variables used *t*-test, while non-parametric used Mann-Whitney. Meanwhile, they tested categorical variables by either x^2 or Fisher exact. Bayesian analysis was employed last for adjusted estimation of the CRC risk in the probands (i.e., relatives of the hereditary CRC patients).

3. Results

Forty CRC patients and 31 healthy controls had a 100% participation rate within the research period. Those with CRC on average live 5.19 years longer with 1.21 kg/m^2 lower body mass index (BMI) than the counterpart. The sex difference was also apparent with a 1.11:1 vs. 0.72:1 male to female ratio among the case and control groups, respectively. We also determined the cellular differentiation levels among the biopsied CRC subjects, with results in **Table 2**.

There was a significant difference in blood *APC* levels between CRC and control subjects. There was a lower mean value of *MSH2* in CRC but no substantial difference between CRC and control subjects because of the outlier (**Table 3**).

Hereditary screening of the CRC subjects came next. Analyzing the CRC risk with Bayesian Analysis is futile if the disease is not hereditary in the first place. There are however no prior validated data on cutoff amounts for CRC hereditary trait from *APC* and *MSH2* gene expression. Hence, we established the required cutoff values through the fifth percentile technique.

Variable	Subject group		p
	CRC (*n* = 40)	Control (*n* = 31)	
Age (year)[a]	56.80 (8.40)	51.61 (13.44)	
Body mass index (kg/m^2)[a]	22.41 (3.29)	23.62 (3.41)	
Sex[b]			>0.05
• Male	21 (52.5)	13 (41.9)	
• Female	19 (47.5)	18 (58.1)	
Cellular differentiation[b]		—	—
• Adenocarcinoma			
• Well	26 (65.0)		—
Fair	6 (15.0)		
Poor	7 (17.5)		
Neuroendocrine carcinoma	1 (2.5)		

[a]*Mean (standard deviation).*
[b]*n (%).*

Table 2.
Baseline characteristics.

Gene	Subject group (fold-change)		p
	CRC (*n* = 40)	Control (*n* = 31)	
Blood sample			
APC			
• Median (range)	12,156.5 (5848–15,035)	13,260 (12,080–14,376)	0.014
• Mean (SD)	11,578.68 (2638.23)	13,261.74 (670.56)	0.014
MSH2			
• Median (range)	12,554.5 (4230–14,559)	12,146 (11,029–13,633)	0.116
• Mean (SD)	11,411.05 (2912.45)	12,219.87 (756.87)	0.465
Tissue sample			
APC		—	—
• Median (range)	8337.0 (5060–13,087)		
• Mean (SD)	8147.78 (1875.12)		
MSH2		—	—
• Median (range)	7485.0 (4174–14,218)		
• Mean (SD)	7475.20 (1946.24)		

Table 3.
***APC** and* MSH2 *gene expression between groups.*

Table 4 showed the percentiles distribution of both *APC* and *MSH2* quantitative expressions among the control group. The fifth percentile of both genes adequately fits to be hereditary cutoff values since it had no significant difference to the first and third percentile. Henceforth, hereditary CRC was very likely in those with over 12,195.80 fc *APC* or 11,059.60 fc *MSH2*.

The CRC subjects were then distributed nicely into either the hereditary or sporadic category with the determined cutoff. Gene expressions equal to and above the cutoff positively correspond to a hereditary status. A majority proportion (52.5%) of the 40 people with colorectal cancer had hereditary nature based on both *APC* and *MSH2* cutoffs. Nonetheless, the hereditary rate decreased by 2.5% and 20.0% if only using cutoff from either one (**Table 5**).

Complete pedigree analysis of the family age, health, gender, and family history of diseases is essential for the estimation of CRC risk. There were merely eight subjects with positive CRC in the family and even then, half were dropped because of vague recollection or retracted permission. We consequently extracted only 10 probands from the four CRC families for Bayesian analysis (**Figure 2A–D**).

Percentile	Gene expression (*n* = 31)	
	APC (fc)	*MSH2* (fc)
First	12,080.00	11,029.00
Third	12,080.00	11,029.00
Fifth	12,195.80	11,059.60

Table 4.
Gene expression percentile distribution.

Cutoff gene	Hereditary (*n* (%))	Sporadic (*n* (%))
APC	20 (50.0)	20 (50.0)
MSH2	13 (32.5)	27 (67.5)
APC and *MSH2*	21 (52.5)	19 (47.5)

Table 5.
CRC subjects' hereditary distribution.

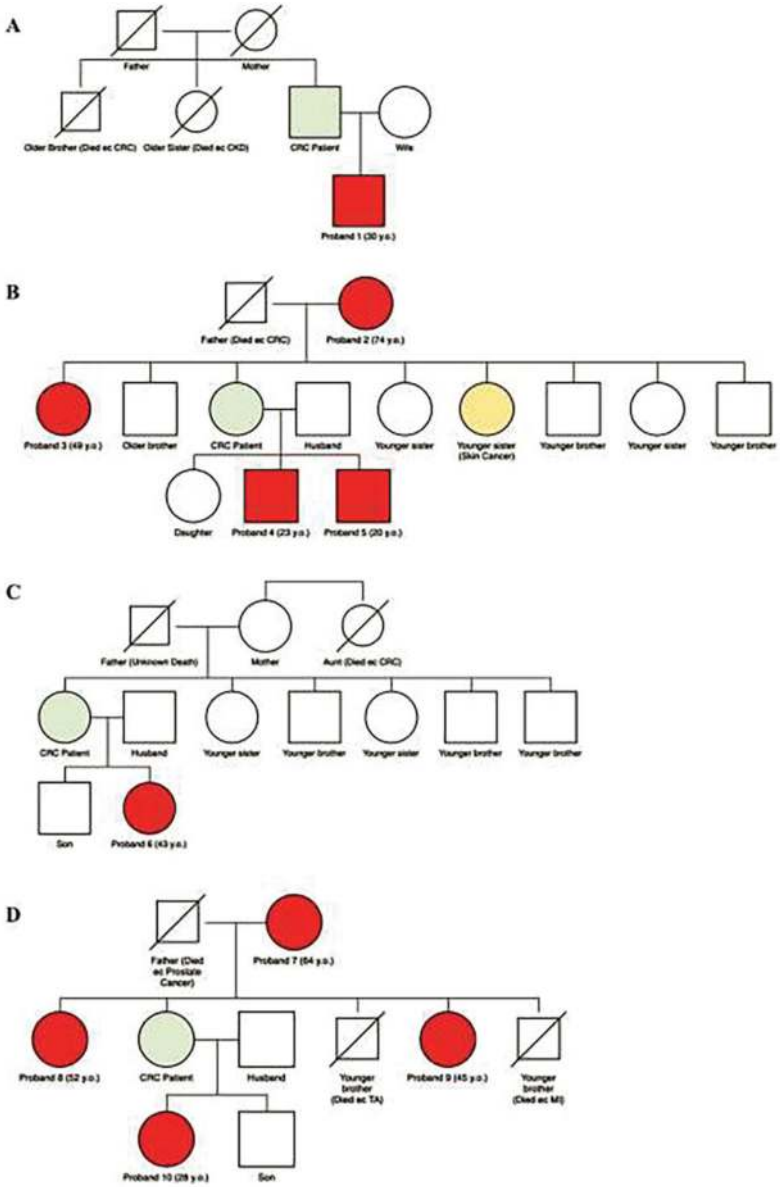

Figure 2.
Hereditary CRC subjects' family pedigree. The pedigrees symbols correspond to the standardized human pedigree nomenclature [17], where a circle denoted a woman, a square for a man, a straight line for a relationship, and a diagonal strikethrough line for death. Colors also exhibited a similar trend. Red represented the proband, yellow for malignancy other than CRC, and light green for CRC subjects. A: Pedigree of 67-year-old man CRC, B: Pedigree of 44-years-old woman with early-onset CRC, C: Pedigree of elderly 62-years-old woman with CRC, D: Pedigree of 47-years-old woman with CRC.

Proband	Prior	Age (year)	*APC* (fc)	*MSH2* (fc)	*Y*	phi
1	0.5	30	7290	9753	1	1.000
2	0.0	74	13,832	14,209	0	0.932
3	0.5	49	8727	9567	1	0.504
4	0.5	23	9757	10,320	1	0.505
5	0.5	20	14,524	13,073	0	0.500
6	0.5	43	11,676	10,673	1	0.500
7	0.0	64	14,020	13,653	0	0.500
8	0.5	52	6884	7073	1	0.500
9	0.5	45	14,341	13,295	0	0.500
10	0.5	28	14,609	13,426	0	0.500

Table 6.
Proband Bayesian CRC risk estimation.

Table 6 described the process of CRC risk estimation among the probands. Bayesian analysis conditionally tweaked each proband's initial risk ('prior' column) with his or her age and gene expressions to yield adjusted odds ('phi' column) in developing or carrying CRC ('Y' column).

The combination of the pedigree in **Figure 2** and Bayesian estimation in **Table 6** gave rise to the complete story of CRC risks in 10 selected probands relative to the four subjects with CRC. A 67-year-old man with CRC of late-onset had a son with almost 100% CRC risk carrier or development (**Figure 2A**). Meanwhile, a 44-year-old woman with early-onset CRC and paternal death due to CRC had a mother with 93% unlikely, a son with 50.51% likely, and another son with 50% unlikely carrier or incidence of CRC (**Figure 2B**). An elderly woman with paternal death of unknown origin and CRC death of the aunt had an adult daughter with 50% of the CRC risk (**Figure 2C**). On the other side, a 47-year-old CRC diseased woman with a huge family and paternal death due to prostate cancer had a 50% likely risk on her sister, but three 50% unlikely probability on her mother, younger sister, and female offspring (**Figure 2D**).

4. Discussion

Several autosomal dominant diseases may appear to be majorly asymptomatic until adulthood or beyond puberty. Given the notion, sophisticated comprehension of the hereditary risk of neoplasm is critical. Direct genomic examination and molecular diagnosis of nucleic acids from the blood, tissue, or other bodily fluids have become more prominent as a screening and investigation standard [4].

DNA is the building block of every living cell in the world. In doing its job, DNA is often destringed into a single-stranded DNA (ssDNA) for DNA replication or mRNA transcription. The first case goes by attachment of complementary nucleic acid base pairs to the corresponding one in both the leading and lagging strand of the partly unzipped ssDNA by DNA polymerase. This created an exact copy of the source DNA. While for the latter case, the RNA polymerase enzyme works separately on the sense and antisense part of the ssDNA. Each strand of ssDNA produced a single mRNA, thus there will be two mRNAs for every transcription of a DNA. These mRNA then moved to the ribosome for translocations into amino acids and eventually proteins.

Cancerous cell arises due to mutations or faulty repair of the nucleic acid bases. Even one deletion, addition, or translocation of the bases drastically changes the transcribed mRNA, codon, and hence the protein. Commonly, uncontrolled proliferation of cells happened if the mistakes occurred on oncogenes or tumor suppressor genes. Constantly activated oncogenes or inhibited tumor suppressor genes direct the cell cycle to bypass checkpoints and not return to the resting phase.

Familial adenomatous polyposis and Lynch syndrome are the top two subtypes of hereditary colorectal cancer with the most incidence count. FAP is almost solely generated because of mistakes in the tumor suppressor gene of *APC*. Whilst a lot of MMR genes can be responsible for LS or HNPCC (e.g., *MLH1*, *MSH2*, *MSH6*, *PMS2*, and epithelial cell adhesion molecule) [24]. The present study accordingly chose APC and one of the repair genes to accommodate both hereditary subtypes of CRC. We inevitably selected *MSH2* among the other mismatch repair (MMR) genes due to the prevalence and missenses amount. Kim et al. stated that approximately 90% of the mutations in the MMR occurred in either *MLH1*, *MSH2*, or both genes [25]. Furthermore, a 13% increase of missenses (i.e., a type of gene mutation which renders genotypic reading and interpretation to be considerably harder) was measured on *MLH1* as opposed to *MSH2* [26]. *MLH* is also more often found in sporadic colon cancer [14]. Next, a three countries assessment on lynch syndrome also gathered that *MSH2* had a substantially higher 10-year-risk of severe adenoma (Δ = 10.1%) and tumor pathogenic variants (11.4 vs. 11.3%) over *MLH1* [27].

Observation among the CRC versus the control group displayed a lesser mean mRNA level of *APC* gene expression than the control group (Δ = 1683.06 fc, p = 0.014). However, the reverse is true for the *MSH2* gene expression (Δ = 808.82 fc, p = 0.465). The minute discrepancy can be because of many gene mutations also somatically involved *APC*. Engel et al. confirmed that from *MSH2*, *MSH6*, and *MLH1* tumor variants, somatic mutations of *APC* happened in 75, 100, and 11% cases [27].

The current study and its prior version in the Indonesian Medical Journal [28], swiftly acted on a novel proposition to determine *APC* and *MSH2* gene expressions cutoff for hereditary cancer classification. Looking over the percentile distribution of healthy controls, the measure of the first to fifth percentiles only had negligible insignificant differences. Following the wrapped Cauchy distribution of circular data techniques with M, D, and A statistics [29], we officialize the fifth percentile mark as the hereditary cutoff (*APC* = 12,195.80 fc and *MSH2* = 11,059.60 fc).

Interesting hereditary proportions had been exhibited by the 40 CRC subjects. Hereditary using only *APC* gene enlisted 50%, while *MSH2* gene gathered 32.5%, and both genes combination enticed 52.5% of the subjects. Only the one with *MSH2* cutoff showed akin prevalence to the 20–30% global familial CRC [30].

The brief, simple, yet informative presentation of the family medical history can be conveniently reflected through a visual pedigree. Taking accurate information on family history should be standard medical practice. The pedigree will subtly enhance oncology prevention, diagnosis, and treatment together with recent genomics advancements. Family history can substantially alter not only genetic testing results but also oncology prevention, including digestive cancer [16]. For instance, a study found that cancer occurrence in an individual relative to a bowel cancer was dependent on familial cancer prevalence, duration of onset, and closeness to the diseased [14]. The current study employs a family pedigree to analyze the health status of CRC patients' relatives. The diagram clearly outlines familial relationships; thus, it will be easier for recognition and interpretation of the inheritance patterns [16, 17].

Individualized medical care has been on the rise of attention. Instead of general medicine for a certain disease, therapeutic care needs to start keenly observing the patient and prescribing medicine that has been tailored for that particular individual [31]. One of the ways in achieving such goals is through personal genetic and biological factors consideration. Particularly, extra attention should be imposed on hereditary disorders like malignancy.

Relatives of a confirmed cancer patient may not experience any cancer-predisposing syndrome from a clinical viewpoint although having an increased risk of developing one. Decreased penetrance and onset age must be factored into consideration especially for autosomal dominant hereditary disorders like colorectal cancer [4, 7, 8, 32].

Investigation on the hereditary risk of diseases in relatives and families accordingly requires a lot of extra information and tests. The natural history of the disease firstly should be made clear (i.e., what is the diagnosis, how does it spread, how does it inherit, and what about its epidemiology). Next, focus on medical examinations or observations that include genetic data (i.e., marker, expression, mutation, and then clinical data). Lastly, we can examine the family pedigree and make objective evidence-based inferences for analysis.

Bayesian analysis of the pedigree from the familial and genetic factors displayed an interesting finding. It was obvious that the majority of probands on the next generation of the current CRC patients had a higher likely probability of CRC incidence or carrier. Probands below the current CRC patients' generation (i.e., the first, fourth, fifth, sixth, and tenth probands) on average had a 60.1% likelihood of CRC risk. Those in the same generation had 50.1% risk, while those above (i.e., the second and seventh probands) had 28.4% risk. These observations were moderately suitable with the autosomal dominant disease hereditary pattern.

The current study bridged the knowledge gaps of CRC hereditary cutoff, gene expressions risk of CRC, and Bayesian pedigree analysis. Nevertheless, some limitations are still presented. First, a small sample size increased the chance for a biased result. The sample selection in the study is also confined to a segmental niche of Indonesian urban citizens, hence the result may not be generalizable to rural or foreign populations. There were also no immunohistochemical or DNA sequencing mutation tests for objective comparisons. Lastly, the method of selecting cutoff from a fifth percentile has not broadly checked for its credibility yet.

5. Conclusion

The current study explored the relationship between *APC* and *MSH2* gene expressions to colorectal cancer risk assessment. Bayesian analysis computed that downregulation of the mRNA gene expression will induce a higher risk of developing or exacerbating CRCs. Yet only *APC* had significance while *MSH2* did not. Therefore, the study establishes the foundation of utilizing *APC* and *MSH2* gene expressions for CRCs risk indicators. Future novel or multiplicity studies should consider family pedigree as a part of CRC prevention strategy among the patient's relatives with expanded cohorts and sample pool, including more profound Bayesian analysis and application with other essential hereditary genes.

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Conflict of interest

The authors declare no conflict of interest. The authors acknowledge that although Vincent Tedjasaputra is currently an American Association for the Advancement of Science (AAAS) Science and Technology Policy Fellow; yet, the current paper is not affiliated with AAAS nor is the product of his position at the National Science Foundation.

Declarations

Please note that an earlier version of this manuscript has been published in the Indonesian Biomedical Journal volume 12 on 2020 with a digital object identifier (DOI) of 10.18585/inabj.v12i4.1329 [28].

Abbreviation summary

APC	adenomatous polyposis coli
BMI	body mass index
bp	base pair
CKD	chronic kidney disease
CRC	colorectal cancer
DNA	deoxyribose nucleic acid
EDTA	ethylenediaminetetraacetic acid
EPCAM	epithelial cell adhesion molecule
FAP	familial adenomatous polyposis
fc	fold-change
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
HNPCC	hereditary non-polyposis colon cancer
IRB	Institutional Review Board
LS	Lynch syndrome
MI	myocardial infarct
MLH	MutL homolog
MMR	mismatch repair
MSH	MutS homolog
PMS	post-meiotic segregation
RNA	ribose nucleic acid
RT-PCR	real-time-polymerase chain reaction
SD	standard deviation
ss	single-stranded
TA	traffic accident
TACSTD	tumor-associated calcium signal transducer
y.o.	years old

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References

[1] Jasperson KW, Tuohy TM, Neklason DW, Burt RW. Hereditary and familial colon cancer. Gastroenterology. 2010;**138**(6):2044-2058. DOI: 10.1053/j.gastro.2010.01.054

[2] Giardiello FM. Hereditary colorectal cancer and polyp syndromes. In: Gearhart SL, Ahuja N, editors. Early Diagnosis and Treatment of Cancer Series: Colorectal Cancer. Philadelphia: Elsevier; 2010. pp. 21-30. DOI: 10.1016/B978-1-4160-4686-8.50008-7

[3] Nussbaum R, McInnes R, Willard H. Cancer genetics and genomic. In: Thompson & Thompson Genetics in Medicine. 8th ed. Philadelphia: Elsevier; 2015. pp. 309-332

[4] Nussbaum R, McInnes R, Willard H. Risk assessment and genetic counselling. In: Thompson & Thompson Genetics in Medicine. 8th ed. Philadelphia: Elsevier; 2015. pp. 333-348

[5] Tranø G, Sjursen W, Wasmuth HH, Hofsli E, Vatten LJ. Performance of clinical guidelines compared with molecular tumour screening methods in identifying possible Lynch syndrome among colorectal cancer patients: A Norwegian population-based study. British Journal of Cancer. 2010;**102**(3):482-488. DOI: 10.1038/sj.bjc.6605509

[6] Turnpenny P, Ellard S, Cleaver R. Patterns of inheritance. In: Emery's Elements of Medical Genetics and Genomics. 16th ed. Philadelphia: Elsevier; 2020. pp. 66-82

[7] Turnpenny P, Ellard S, Cleaver R. Risk calculation. In: Emery's Elements of Medical Genetics and Genomics. 16th ed. Philadelphia: Elsevier; 2020. pp. 94-101

[8] Nussbaum R, McInnes R, Willard H. Patterns of single-gene inheritance. In: Thompson & Thompson Genetics in Medicine. 8th ed. Philadelphia: Elsevier; 2015. pp. 107-132

[9] Turnpenny P, Ellard S, Cleaver R. The cellular and molecular basis of inheritance. In: Emery's Elements of Medical Genetics and Genomics. 16th ed. Philadelphia: Elsevier; 2020. pp. 9-23

[10] Buckingham L. Molecular basis of inherited disease chromosomal abnormality. In: Buckingham L, Flaws ML, editors. Molecular diagnostics. Fundamentals, Methods, & Clinical Applications. Philadelphia: F. A. Davis Company; 2007. pp. 311-326

[11] Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. Science (80). 2013;**339**(6127):1546-1558. DOI: 10.1126/science.1235122

[12] Teama S. DNA polymorphisms: DNA-based molecular markers and their application in medicine. In: Yamin Liu, editor. Genetic Diversity and Disease Susceptibility. London: Intech; 2018. DOI: 10.5772/intechopen.79517

[13] Li M, Wang IX, Li Y, Bruzel A, Richards AL, Toung JM, et al. Widespread RNA and DNA sequence differences in the human transcriptome. Science (80). 2011;**333**(6038):53-58. DOI: 10.1126/science.1207018

[14] Turnpenny P, Ellard S, Cleaver R. The genetics of cancer and cancer genetics. In: Emery's Elements of Medical Genetics and Genomics. 16th ed. Philadelphia: Elsevier; 2020. pp. 117-199

[15] Guttmacher AE, Collins FS, Carmona RH. The family history—More

important than ever. The New England Journal of Medicine. 2004;**351**(22): 2333-2336. DOI: 10.1056/NEJMsb042979

[16] Brock J-AK, Allen VM, Kieser K, Langlois S. Family history screening: Use of the three generation pedigree in clinical practice. Journal of Obstetrics and Gynaecology Canada. 2010;**32**(7):663-672. DOI: 10.1016/S1701-2163(16)34570-4

[17] Bennett RL, French KS, Resta RG, Doyle DL. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. Journal of Genetic Counseling. 2008;**17**(5):424-433. DOI: 10.1007/s10897-008-9169-9

[18] Takara Bio USA Inc. Mini & midi spin kits for isolation of total RNA from blood—NucleoSpin RNA Blood & NucleoSpin RNA Blood Midi [Internet]. United States of America; 2017

[19] Zhou Z, Liu H, Wang C, Lu Q, Huang Q, Zheng C, et al. Long non-coding RNAs as novel expression signatures modulate DNA damage and repair in cadmium toxicology. Scientific Reports. 2015;**5**(1):15293. DOI: 10.1038/srep15293

[20] Hatta M, Surachmanto EE, Islam AA, Wahid S. Expression of mRNA IL-17F and sIL-17F in atopic asthma patients. BMC Research Notes. 2017;**10**(1):202. DOI: 10.1186/s13104-017-2517-9

[21] Sirait R, Hatta M, Ramli M, Islam A, Arief S. Systemic lidocaine inhibits high-mobility group box 1 messenger ribonucleic acid expression and protein in BALB/c mice after closed fracture musculoskeletal injury. Saudi Journal of Anaesthesia. 2018;**12**(3):395. DOI: 10.4103/sja.SJA_685_17

[22] Tambaip T, Br Karo M, Hatta M, Dwiyanti R, Natzir R, Nasrum Mas M, et al. Immunomodulatory effect of orally red fruit (*Pandanus conoideus*) extract on the expression of CC chemokine receptor 5 mRNA in HIV patients with antiretroviral therapy. Research Journal of Immunology. 2018;**11**(1):15-21. DOI: 10.3923/rji.2018.15.21

[23] University of Twente. PCR Primer Information. 2017. Available from: https://www.utwente.nl/en/tnw/dbe/research/pcp-primers/ [Cited: 29 December 2019]

[24] Rashid MU, Naeemi H, Muhammad N, Loya A, Lubiński J, Jakubowska A, et al. Prevalence and spectrum of MLH1, MSH2, and MSH6 pathogenic germline variants in Pakistani colorectal cancer patients. Hered Cancer Clinical Practices. 2019;**17**(1):29. DOI: 10.1186/s13053-019-0128-2

[25] Kim Y-M, Choe C-G, KimCho S-M, Jung I-H, Chang W-Y, Cho M-J. Three novel germline mutations in MLH1 and MSH2 in families with Lynch syndrome living on Jeju Island, Korea. BMB Reports. 2010;**43**(10):693-697. DOI: 10.5483/BMBRep.2010.43.10.693

[26] Lynch HT, de la Chapelle A. Hereditary colorectal cancer. The New England Journal of Medicine. 2003;**348**(10):919-932. DOI: 10.1056/NEJMra012242

[27] Engel C, Ahadova A, Seppälä TT, Aretz S, Bigirwamungu-Bargeman M, Bläker H, et al. Associations of pathogenic variants in MLH1, MSH2, and MSH6 with risk of colorectal adenomas and tumors and with somatic mutations in patients with lynch syndrome. Gastroenterology. 2020;**158**(5):1326-1333. DOI: 10.1053/j.gastro.2019.12.032

[28] Tedjasaputra TR, Hatta M, Massi MN, Natzir R, Patellongi I, Simadibrata M, et al. Risk assessment

in hereditary colorectal cancer family by using APC and MSH2 mRNA gene expression and Bayesian analysis. Indonesia Biomedical Journal. 2020;**12**(4):368-375. DOI: 10.18585/inabj.v12i4.1329

[29] Abuzaid AH, El-hanjouri MM, Kulab MM. On discordance tests for the wrapped cauchy distribution. Open Journal of Statistics. 2015;**5**(4):245-253. DOI: 10.4236/ojs.2015.54026

[30] Brosens LAA, Offerhaus GJA, Giardiello FM. Hereditary colorectal cancer. The Surgical Clinics of North America. 2015;**95**(5):1067-1080. DOI: 10.1016/j.suc.2015.05.004

[31] Meiliana A, Dewi NM, Wijaya A. Personalized medicine: The future of health care. Indonesia Biomedical Journal. 2016;**8**(3):127. DOI: 10.18585/inabj.v8i3.271

[32] Chin L, Andersen JN, Futreal PA. Cancer genomics: From discovery science to personalized medicine. Nature Medicine. 2011;**17**(3):297-303. DOI: 10.1038/nm.2323