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Overview of germline variants in the BRCA2 gene in cohort of Brazilian women with a high risk of hereditary breast cancer

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ABSTRACT

Introduction: Malignant breast cancer is the second most common type of cancer among women in the world, leaving behind nonmelanoma skin cancer. The aim of this study was to identify germline variants in the *BRCA1* and *BRCA2* genes in women diagnosed with breast cancer in the southeastern region of Brazil. **Methods:** This study is part of a retrospective study, performed from a hospital-based cohort, consisting of 522 women. 92 patients were excluded from the study because they had carcinoma in situ and did not present clinical information, totaling 430 patients. Of these, we performed molecular investigation in 46 patients. *BRCA2* variants were detected in 10/46 (22%) women. From 7 missense variants identified, 5 and 2 showed benign and uncertain significance, respectively. Two synonymous variants not previously reported were considered of uncertain significance (c.2622T>A; c.2721G>A), and one nonsense variant showed pathogenic clinical significance (c.2847T>A). **Results:** The results showed that gene sequencing in individuals with a high risk of hereditary cancer is necessary, as it may reveal new variants, or initially described with uncertain significance. **Conclusion:** Although this study was conducted with a small cohort of selected breast cancer patients, it reinforces the importance of investigating the Brazilian population due to the finding of the pathogenic variant and genetic counseling.

KEYWORDS: breast cancer; BRCA2 gene; hereditary breast and ovarian cancer syndrome; Cohort study.

INTRODUCTION

Malignant breast cancer is the second most common type of cancer among women in the world, leaving behind nonmelanoma skin cancer[1,2], and it has a multifactorial etiology associated with environmental and genetic factors[3]. In Brazil, 66,280 new cases of breast cancer are identified each year, corresponding to an estimated risk of 62 new cases per 100,000 women[1].

It is known that the risk factors for the development of breast cancer are those related to a woman's reproductive life. For example, early age at menarche, late menopause, never having been pregnant or giving birth, first pregnancy after 30 years of age, and use of oral contraceptives and hormone replacement therapies in menopause can contribute to carcinogenesis[3]. In addition to hormonal factors, studies also indicate lifestyle-related risk factors, which include alcohol intake, smoking, physical inactivity, and exposure to ionizing radiation[4].

However, hereditary predisposition is considered an important etiological factor. Approximately 5–25% of cancers are due to hereditary factors related to the multiple stages of carcinogenesis and may involve numerous genes, through gene mutations, chromosomal instabilities, gene amplifications, and epigenetic mechanisms. Among the main tumor suppressor genes involved in this process are the *BRCA1* and *BRCA2* genes[5,6].

The identification of genes related to the development of hereditary cancer provides a better understanding of the disease and contributes to the management of control and earlier diagnosis[7].

Some mutations in *BRCA1* and *BRCA2* are more prevalent in individuals from specific ethnic or geographical groups such as

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Caucasians and Ashkenazi Jews. This is due to the presence of initiating mutations in this population, which probably appeared several generations ago[8-10]. There is evidence that the founding mutations – which are strongly related to hereditary breast and ovarian cancers (HBOC) and are identified in high penetration genes, such as *BRCA1*, *BRCA2*, and others – are the most prevalent pathogenic genetic alterations in the Brazilian population, due to the immigration events of European peoples to our country[11-13].

The state of Minas Gerais, located in the southeastern region of Brazil and initially inhabited by South American Amerindians, has an estimated population of 21,292,666 inhabitants[14]. Its history is determined by the exploration of gold. Consequently, with the great mineral wealth, the state attracted residents from neighboring states, such as Rio de Janeiro and São Paulo, in addition to immigrants, mainly from Portugal, and African slaves who were brought to Brazil. According to Pena et al.[15], European ancestry is prevalent in all Brazilian regions.

In Brazil, in the public health system, the genetic counseling services are principally located in university hospitals. They are carried out based on the investigation of clinical and family history in order to estimate the risk of hereditary cancer and the probability of pathogenic variants in predisposing genes. Genetic testing is offered to patients and families who meet some National Comprehensive Cancer Network (NCCN) eligibility criteria for hereditary breast cancer[7].

The aim of this study was to screen and verify the prevalence of variants in the *BRCA1* and *BRCA2* genes by Sanger DNA sequencing of blood samples of 46 selected and unrelated women, with clinical evidence of HBOC in the state of Minas Gerais[15]. The comprehensive interpretation of the identified *BRCA2* variants was challenging for the genetic counseling support team.

METHODS

Patients

This study is part of a retrospective study, performed from a hospital-based cohort, consisting of 522 women diagnosed with breast cancer between 2014 and 2016, and treated at an oncology referral center in the Zona da Mata of Minas Gerais, in the southeastern region of Brazil[16]. Through the criteria used to assess hereditary breast cancer risk, recommended by the NCCN[17], women were classified into two categories: increased and usual risk for hereditary breast cancer. The group with an increased risk for hereditary breast cancer considered the presence of at least one of the clinical criteria for HBOC Syndrome, such as age at diagnosis ≤45 years; triple-negative subtype diagnosed in women aged ≤60 years; diagnosis of breast cancer between ages of 46 and 50 years, with at least one first- or second-degree relative with malignant neoplasm in the breast or ovary; and a personal history of breast cancer with the presence of secondary malignant tumor in the same organ.

The study excluded women with in situ breast cancer (n=42) and those without information about at least one of the biomarkers of the tumor for estrogen, progesterone, and HER-2 (n=50). Among the 430 women diagnosed with invasive breast cancer who composed our study population, 127 (29.5%) were classified as at increased risk for HBOC Syndrome[16], according to the criteria recommended by the NCCN[17] and 36.2% of women were users of the public health service.

Of the 522 women, 23 (4.41%) died and 2 (1.57%) were part of the increased risk group for HBOC.

The molecular investigations of *BRCA1* and *BRCA2* genes were performed in 46 of the 127 women diagnosed and were classified into the category of increased risk for hereditary breast cancer.

Clinical and pathological information was extracted from medical records, while the complementary information was obtained from contact with patients and the analysis of laboratory results, pathological anatomy[18], and immunohistochemistry.

All procedures followed ethical recommendations and the study was approved by the Ethics Committee in Research of the Federal University of Juiz de Fora (protocol number 5342919.0.0000.5147). All subjects provided written consent for *BRCA* testing.

DNA isolation

Genomic DNA was extracted from buccal epithelial cells using organic solvents, according to Aidar and Line (2007). DNA concentration, purity, and integrity were assessed by spectrophotometry (Nanodrop 2000 – Thermo Fisher Scientific®, Waltham, MA)[19].

Point mutation screening

The entire coding sequence and exon-intron boundaries of the *BRCA1* (NM_007294.3) and *BRCA2* (NM_000059.3) genes were evaluated and detected by polymerase chain reaction (PCR). PCR conditions and primer sequences are available (Supplementary Material). All PCR products were purified using Exo-SAP (Affymetrix®) and sequenced by the Sanger method with the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher®), in ABI 3730 XL genetic analyzer. Copy number variations were not analyzed.

Classification of variants

The identified variants were consulted in reference databases (gnomAD, ExAC, BRCA Exchange, dbSNP, ClinVar, LOVD, and ABraOM – a Brazilian database). The new variants were registered in the LOVD (Leiden Open Variation Database). For the biological significance of all variants, the Mutation Taster software was used, and the variants were classified using the IARC-LOVD[20].

RESULTS

Germline variants

Of the 46 samples evaluated for the presence of *BRCA* mutations, 10 genetic variants were identified as heterozygous in the

Table 1. Variants identified in the *BRCA2* gene for the study population. Minas Gerais State, Brazil, 2014–2016.

Proband	cDNA	Genomic localization (GRCh38)	Alteration	Mutation type	Clinical significance	dbSNP	ClinVar	GnomAD	ExAC	ALFA Project	ABRAOM
BC7	c.2622T>A	13: 32911114	p.Tre874Tre	Synonym	VUS	NF	NF	NF	NF	NF	NF
BC15	c.5744C>T	13: 32340099	p.Thr1915Met	Missense	Benign	rs4987117	T=0.008*	T=0.020*	T=0.018*	T=0.027*	T=0.017*
BC20	c.2847T>A	13: 32337202	p.Tyr949Ter	Nonsense	Pathogenic	rs886040449	NF	NF	NF	NF	NF
BC21	c.2721G>A	13: 32911213	p.Lys907Lys	Synonym	VUS	NF	NF	NF	NF	NF	NF
BC22	c.2813C>A c.2971A>G	13: 32337168 13: 32337326	p.Ala938Glu p.Asn991Asp	Missense	Benign	rs55773834 rs1799944	NF G=0.080	A=0.000 G=0.037*	A=0.000 G=0.053	A=0.000 G=0.038*	NF G=0.045*
BC25	c.2680G>A c.2971A>G	13: 32337035 13: 32337326	p.Val894Ile p.Asn991Asp	Missense	Benign	rs28897715 rs1799944	NF G=0.080	A=0.000* G=0.037*	A=0.000* G=0.053	A=0.000* G=0.038*	NF G=0.045*
BC28	c.2649C>A	13: 32911141	p.Phe883Leu	Missense	VUS	NF	NF	NF	NF	NF	NF
BC28	c.2641G>A	13: 32336996	p.Glu881Lys	Missense	VUS	NF	NF	NF	NF	NF	NF
BC41	c.3055C>G	13: 32337410	p.Leu1019Val	Missense	Benign	rs55638633	G=0.000*	G=0.000*	G=0.000*	G=0.000*	NF
BC45	c.2971A>G	13: 32337326	p.Asn991Asp	Missense	Benign	rs1799944	G=0.080	G=0.037*	G=0.053	G=0.038*	G=0.045*

cDNA: Complementary DNA; GRCh38: Genome Reference Consortium Human Build 38; dbSNP: contains records of allele frequencies for specific population samples that are defined by each submitter and used in validating submitted variations. (O link para o site encontra-se no tópico Websites); ClinVar: ClinVar aggregates information about genomic variation and its relationship to human health. GnomAD: Genome Aggregation Database; ExAC: Exome Aggregation Consortium; ABRAOM: Arquivo brasileiro online de mutações; BC: Breast câncer; NF: not found; VUS: variants of uncertain significance. *MAF: minor allele frequency (<1%).The gray shadings represent the new variants identified in the study population.

BRCA2 gene (Table 1) in nine patients. The variant was considered benign, as the change generated in the nucleotide sequence did not impact the function of the protein or influence the phenotype (missense). However, some missense alterations of conflicting interpretation or unclassified variants and of the synonym type were considered "variants of uncertain significance" (VUS), that is, the variant is detected, but its effect on the function of the gene is unknown; and the variant that generated a premature stop codon (nonsense) was classified as pathogenic, since the alteration interrupts the function of the gene and, therefore, is highly likely to have clinical consequences[21].

In this study, five missense variants identified as benign clinical impact; two missenses as VUS; two synonymous variants not previously reported with clinical impact of VUS; and a nonsense variant, with pathogenic clinical significance associated with HBOC were found.

All detected variants were investigated in the available databases (gnomAD, ExAC, BRCA Exchange, dbSNP, ClinVar, LOVD, and ABraOM). The identified VUS was classified in accordance with the American College of Medical Genetics and Genomics criteria[21], and submitted to the LOVD database. The minor allele frequency (MAF) of the altered allele, shown in the databases in the South Latin American population, is listed in Table 1. Rare variants were defined as MAF <1% and common variants as MAF >5%[22].

Clinicopathological characterization

Of the 46 Brazilian women analyzed, 9 patients had variants in the *BRCA2* gene, and the average age of breast cancer diagnosis was 47.3 years (35–75 years), among self-reported white and non-white ethnoracial groups, users of the public health system (SUS) or private health system. Only three patients reported a positive family history of breast cancer (CM7, CM15, and CMCM28). We also assessed the overall survival of each woman, from the period in which the diagnosis was made until 2019 (Table 2). All of the abovementioned information on 46 women is summarized in the Supplementary Material.

Pathogenic variant

The CM20 proband, with a molecular finding of pathogenic implication (Figure 1), a self-reported non-white user of the private health service, was diagnosed at 45 years old in 2016 when identifying a palpable retroareolar lesion on the left breast, confirmed by mammographic screening images. During anamnesis, she did not have comorbidities or use hormone replacement therapy. The clinical TNM estimate was at stage IIIB, which is considered an advanced stage in this study. In an interview with a geneticist, she reported having a positive family history of cancer, with limited information about her parents and relatives. The patient was the first case of breast carcinoma in the family. This information is illustrated in Figure 2.

The biopsy result indicated invasive ductal carcinoma of histological grade 3, tumor size ≤2 cm with areas of carcinoma in situ and invasive component, solid patterns and comedonecrosis, and the presence of committed lymph nodes and left axilla with carcinoma macrometastasis in one isolated lymph node. Furthermore, the biopsied material from the periareolar lesion of the left breast showed changes in columnar cells without atypia and ectasia, apocrine metaplasia, intraductal papillomas, and florid ductal hyperplasia with the pathological TNM staging pT1c.

The immunohistochemistry analysis demonstrated the positivity of estrogen and progesterone receptors, negative HER2 expression, positive p53 marker, and Ki-67 of 15%. Additionally, the tumor has been classified as luminal subtype B.

Table 2. Clinical characteristics of patients and histopathological findings of breast carcinomas.

Proband	Age at diagnosis	Self-reported ethnoracial group	Health system	Tumor laterality	Tumor size	Lymph nodes committed	GH	Immunophenotype	Ki-67 (%)	pTNM	FH of breast cancer	HRT
CM7	43	White	Public	R	≥2 cm	No	3	Luminal B ER+, PR−, Her2−	≥25	T2N1M0	Yes	No
CM15	75	White	Private	L	≤2 cm	No	2	Triple-negative	≥25	T1N0M0	Yes PH: hysterectomy at 30 years old	NR
CM20	45	Non-white	Private	L	≤2 cm	Yes	3	Luminal B ER+, PR+, Her2−	<25	T4N1M0	No	No
CM21	44	White	Private	R	≤2 cm	No	2	Luminal A ER+, PR+, Her2−	≤25	T1N0M0	No	NR
CM22	41	White	Private	L	≥2 cm	No	2	Luminal B ER+, PR+, Her2−	≥25	T1N0M0	No PH: fibroadenoma	No
CM25	44	Non-white	Public	R	≤2 cm	No	2	Luminal B ER+, PR+, Her2-	≥25	T1N0M0	No	No
CM28	61	Non-white	Private	L	≥2 cm	No	3	Overexpression Her2	≥25	T0N0M0	Yes PH: bilateral oophorectomy, hysterectomy and salpingectomy.	Yes
CM41	35	White	Public	L	≥2 cm	No	3	Triple-negative	≥25	T2N0M0	No	No
CM45	38	Non-white	Public	L	≥2 cm	Yes	3	Luminal B ER+, PR+, Her2−	<25	T2N1M0	No	No

GH: histological grade (provided by the Nottingham classification system); pTNM: pathological TNM[18]: tumor, linfonodo, metástase; FH: familial history; PH: personal history; HRT: hormone replacement therapy; NR: not reported. Ki: Ki67 is a nuclear antigen that is an excellent marker of active cell proliferation in the normal and tumor cell populations; ER: estrogen receptors; PR: Progesterone receptor. Her2: human epidermal growth factor receptor 2.

There was no systemic metastasis at diagnosis as well as no locoregional recurrence or distant metastasis during the course of treatment or follow-up. Regarding the therapeutic approach, a radical mastectomy of the affected breast (left) was performed, followed by adjuvant chemotherapy and radiotherapy, along with hormone therapy, which was prescribed for 10 years.

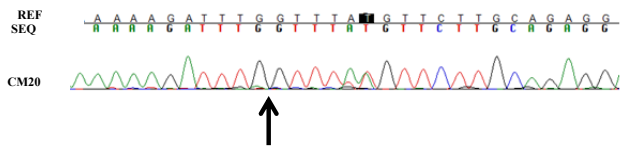


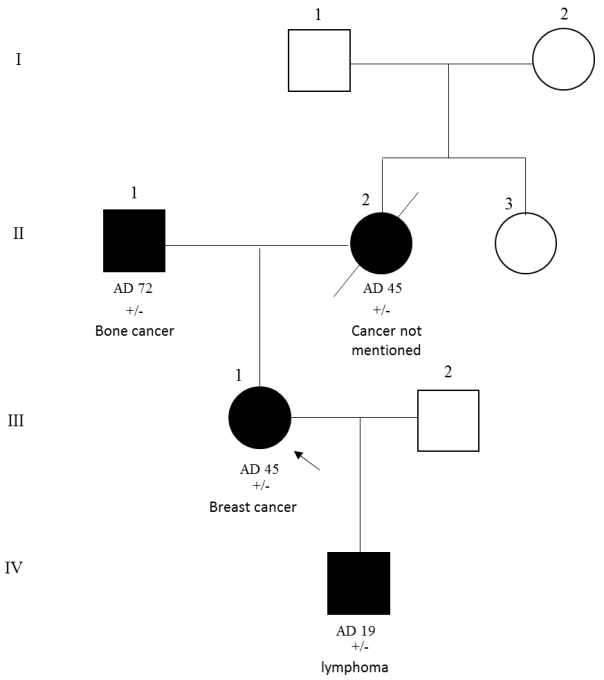
Figure 1. Sequencing of the *Breast Cancer 2* pathological variant c.2847T>A.

DISCUSSION

The use of genomic sequencing techniques has been a fundamental tool in the establishment of genetic diseases, particularly in those where multiple genes can be affected[23]. In this sense, the cause of hereditary predisposition to cancer can be elucidated and help to develop new applications for both the clinic and scientific research[24]. The *BRCA2* gene, a tumor suppressor located on chromosome 13, encodes a protein of 3,428 amino acids and is responsible for repairing the breaks in the double strand of DNA, together with the RAD51 protein[25,26].

Approximately, 1 in 800 women carry *BRCA2* mutations. Similar to the *BRCA1* gene, *BRCA2* is related to 10–15% of hereditary cancers; moreover, the *BRCA2* mutation confers up to 85 and 27% of the cumulative risk of developing breast and ovarian cancers, respectively, throughout life[27,28].

There are some management options that seek to reduce the risk in patients with mutations in known genes that confer high and moderate risk of HBOC, including bilateral risk-reducing mastectomy, salpingo-oophorectomy, chemoprevention, and intensive surveillance with annual breast magnetic resonance imaging[29]. Studies seek to screen the most prevalent mutations



**AD: Age of diagnosis (years)

Figure 2. Heredogram of breast cancer 20 proband.

in *BRCA* in order to reduce costs through a method that is faster and more efficient in detecting mutations in *BRCA1* and *BRCA2*. This strategy would make it possible to include a greater number of investigated patients and a more accurate treatment, offering greater benefits to them[30].

After identifying carriers of *BRCA* mutations, genetic counseling and testing for individuals at increased risk results in control and allows the use of risk-reducing strategies, which often lead to the prevention of primary or secondary tumors and an increased survival rate of the carriers[7]. Regarding these benefits, a study by Palmero et al.[7] warns of the limited genetic testing in Brazil, caused by the reduced supply, since medical genetics services are predominantly located in university hospitals. Furthermore, genetic testing is only offered to those families that fulfill the NCCN criteria for a hereditary breast cancer syndrome through local, national, and/or international collaborative research studies, once genetic testing is not covered by the Brazilian Public Health System.

In this study, we did not find any genetic variants in the *BRCA1* gene. However, in the *BRCA2* gene, we identified 9 single-nucleotide variants in 10 women diagnosed with breast cancer, with an average age of 47.2 years (SD=12.71). Two missense variants, rs4987117 and rs1799944, have already been identified in two other Brazilian studies. The latter was present in three women with the luminal subtype B tumor[31,32]. The variant rs4987117 was identified in 4 of 30 (13.3%) probands with triple-negative breast cancer, corroborating our finding[33]; it was less frequent in a cohort of 117 cases with sporadic breast cancer (positive estrogen receptor), in Poland (OR=0.39; 95%CI 0.19–0.82; p=0.013)[34]. Therefore, Meyer et al.[33] classified the variant as a "probable risk" for triple-negative breast cancer.

The missense variants rs28897715 and rs55638633, also with a benign clinical effect, were not detected in any other Brazilian study. The study by Balia et al. (2011) [35] describes the rs55638633 variant in a 39-year-old metastatic case (4 compromised lymph nodes out of 18 analyzed) with invasive ductal breast carcinoma (luminal subtype B), and histological grade 3. In the referred work, this variant is reported in the BIC (http://research.nhgri.nih.gov/bic/) 22 times. In our study, a 35-year-old patient presented the same variant with breast cancer, a triple-negative subtype, without any family history of cancer[35].

Another missense variant rs55773834 is referred to as probably benign (1) and VUS (8) in ClinVar, but not reported in other Brazilian studies. In general, VUSs are missense substitutions that result in changes to a single nucleotide, but they may also include small deletions, insertions, or other effects that may be unknown[25]. Therefore, the VUSs and its variants with conflicting interpretations represent a challenge for genetic counseling, because more genetic information is necessary to elucidate the clinical impacts in relation to the predisposition to cancer[36].

Four newly identified variants were found, two being missense and two being synonymous changes. It is known that synonymous substitutions can alter the splicing site, creating or destroying a donor or receptor site, which can modify the protein translation, the mRNA structure, and the protein folding[29].

The nonsense variant rs886040449 with a pathogenic clinical effect, mentioned in ClinVar, has no previous identification references in Brazilian studies – not even in the largest multicenter Brazilian study, conducted by Palmero et al. to track mutations in *BRCA*[29]. The study by Li et al. identified a family in which the proband had breast cancer at the age of 21 years and a recurrence at the age of 36 years, with a family history of an older sister diagnosed with breast cancer at the age of 60 years. However, this reference is from a single nucleotide (delT) deletion in amino acid 949 of exon 11 *BRCA2* gene[37]. Our finding is related to a single nucleotide substitution in the same amino acid. Pathogenic variants in the *BRCA1/BRCA2* genes are significantly associated with an increased risk of breast, ovarian, pancreatic, and prostate cancer[38]. Thus, carriers of mutations can become eligible for and, therefore, beneficiaries of treatments with polyADP-ribose-polymerase inhibitors in advanced and recurrent ovarian, breast, pancreatic, and prostate carcinomas.

According to the Brazilian Society of Medical Genetics and some studies on care in the field of genetics carried out in Brazil, there are few genetic professionals for the territorial dimension of our country, the concentration of services is in large urban centers, and there are difficulties in accessing specialized services in the public health service. We know the benefits of counseling and genetic testing in risk management. To minimize limitations on access to specialized services, Achatz et al.[6] recommended a series of strategies that can overcome barriers to adequate early diagnosis and management of identified cases of HBOC in Brazil.

The VUSs, which are routinely identified in genetic testing, are reclassified as benign in 90–95% of cases[21]. The VUS investigation of the Brazilian population, such as the ones described here, is essential for us to know the genetic variability of our population and, thus, for us to have more appropriate data to evaluate the phenotypes and genotypes of individuals.

CONCLUSION

Although this study was conducted with a small cohort of selected breast cancer patients, it reinforces the importance of investigating the Brazilian population due to the finding of the pathogenic variant, not yet reported in the country as well as the VUS. In patients in whom no pathogenic variant was identified, the screening of other hereditary breast cancer genes should be implemented in the future. Therefore, our study provides relevant information for the genetic counseling of hereditary Brazilian breast cancer patients.

Websites

GnoAD: https://gnomad.broadinstitute.org
ClinVar: https://www.ncbi.nlm.nih.gov/clinvar
dbSNP: https://www.ncbi.nlm.nih.gov/snp
LOVD: https://databases.lovd.nl/shared/genes/BRCA2
ABraOM: http://abraom.ib.usp.br
EXAC: http://exac.broadinstitute.org
BRCA EXCHANGE: https://brcaexchange.org
VARSOME: https://varsome.com/variant

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AUTHORS' CONTRIBUTIONS

RMF: Conceptualization, Data Curation, Formal Analysis, Methodology, And Writing – Original Draft. GA: Project Administration, Visualization, Writing – Original Draft. MRG: Supervision, Writing – Review & Editing. AALC: Data Curation, Validation. LD: Investigation, Methodology, Writing – Original Draft. PH: Data Curation; Validation. OM: Data Curation; Validation. RRE: Methodology, Writing – Original Draft. JRDC: Supervision, Validation, Writing – Review & Editing. MTBT: Supervision, Writing – Review & Editing.

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