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RESEARCH

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Molecular profiling of *BRCA1* and *BRCA2* genes in Turkish patients with early-onset breast cancer

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Abstract

Background Early-onset breast cancer (EOBC) is a specific condition that affects women under the age of 45. *BRCA* pathogenic/likely pathogenic (P/LP) germline variants have been demonstrated to be harbored in a subgroup of EOBC individuals, and *BRCA*-positive genetic result offers an option to ensure more specified therapeutic implications. Establishing comprehensive *BRCA1/2* genetic testing, including both the detection of small-scale mutations and large genomic rearrangements (LGRs), is needed for risk assessment and clinical management. In this study, we described a Turkish EOBC cohort along with their clinico-pathological characteristics and *BRCA1/2* mutational profiles. A total of 67 unrelated patients were enrolled. Both next-generation sequencing (NGS)-based gene panel and multiplex ligation-dependent probe amplification (MLPA) were performed for *BRCA1/2* variant identification. Patients' family medical history and hormone receptor status of the tumors were also recorded.

Results 14 (20.90%) patients were found to carry *BRCA* P/LP germline variants. (Nine were *BRCA2*-positive, and five were *BRCA1*-positive.) Two novel *BRCA2* variants were detected. No significant differences were found between *BRCA* -positive vs. *BRCA*-negative or *BRCA1*-positive vs. *BRCA2*-positive for hormonal status and family history.

Conclusions *BRCA1/2* genes represent a predominant part of the genetic landscape of EOBC. Our results expand the spectrum of *BRCA1/2* variants and provide knowledge of the *BRCA1/2* variant prevalence in our cohort.

Keywords Early-onset breast cancer, *BRCA1*, *BRCA2*, Large genomic rearrangement

Background

Early-onset breast cancer (EOBC) represents a distinct clinical and biological entity affecting women aged 18–45 years, regardless of family history [1]. Defining the threshold age that is considered "early-onset" for getting breast cancer fairly differs in different scientific studies, guidelines, or protocols [2, 3]. Although breast cancer (BC) occurs commonly in elders (50 years old or older) and only 12% of the new diagnoses of breast cancer in the United States are found in females younger than 45 years [4], EOBC has recently been undertaken as a research priority because of several objectives: BC in young women is more likely to be heritable than in older women and is usually more aggressive, harder to treat, and associated with a poor clinical outcome. Furthermore, EOBC patients inevitably experience certain long-term survivorship issues, such as contraception, management of perimenopause and menopause symptoms, and reproductive options (e.g., fertility preservation) [1]. Current evidence indicates that breast cancer is the top cancer-related cause of death in women aged < 45 years [5].

EOBC may be an independent predictor for "Hereditary Breast and/or Ovarian Cancer (HBOC) Syndrome"

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and fulfills the criteria for genetic testing of hereditary cancer-related genes (i.e., *BRCA1/2*) [6]. The probability of detecting a disease-causing variant in HBOC-related genes is maximized in an EOBC patient with a family history of multiple affected individuals on the same side [7, 8]. While the likelihood of identifying HBOC is about 80% when four or more BC patients diagnosed under age 60 are present in the same family [9], it is about 20% when a BC patient has only one more affected relative [10, 11].

BRCA1 and *BRCA2* P/LP variants have been demonstrated to be related to an actionable genetic predisposition to BC. Thus, genetic testing of *BRCA1/2* has got to be taken into consideration in order to ensure personalized breast cancer surveillance, appropriate risk reduction options, and therapeutic indications [12, 13]. In this study, we aim to present the *BRCA1/2* mutational landscape of a Turkish cohort with EOBC and compare the hormonal profiles between *BRCA* P/LP variant carriers and the others.

Methods

Patients

From June 2020 to May 2022, unrelated subjects with EOBC who had applied to the department of medical genetics at Isparta or Eskişehir City Hospitals for genetic testing were recruited. All included individuals were also subjected to a detailed examination, including medical and family history and laboratory assessments, including the hormone receptor status of their tumors.

Genetic analysis

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) according to the standard procedures. Next-generation sequencing (NGS) technology was used to screen for disease-causing variants in *BRCA1/2* genes. For NGS analysis, all coding exons and exon–intron junctions of *BRCA1/2* were sequenced on the MGISEQ-200 platform (BGI, Shenzhen, Guangdong, China) using a custom-designed NGS panel (Twist Biosciences, San Francisco, CA, USA). All detected disease-causing variants were also confirmed by Sanger sequencing.

Patients found to be negative for *BRCA* P/LP variants were further tested for the detection of large genomic rearrangements (LGRs) in *BRCA* genes by the multiplex ligation-dependent probe amplification (MLPA) method. SALSA® MLPA Probemixes (P002 for the *BRCA1* gene, P045 and P090 for the *BRCA2* gene) were used for this purpose following the manufacturer's recommendations (MRC-Holland, Amsterdam, the Netherlands).

Variant interpretation

Each variant was annotated according to the Human Genome Variation Society (HGVS) nomenclature. NM_007300.4 (for *BRCA1*) and NM_000059.4 (for *BRCA2*) were used as the reference sequences.

To predict the effects of novel variants, in silico tools (PolyPhen2 [http://genetics.bwh.harvard.edu/pph2/], SIFT [https://sift.bii.a-star.edu.sg], MutationTaster [https://www.mutationtaster.org], CADD [https://cadd.gs.washington.edu], and MCAP [http://bejerano.stanford.edu/mcap/]) and population databases (1000G [http://www.internationalgenome.org] and gnomAD [https://gnomad.broadinstitute.org/]) were employed, and all identified variants were classified according to the American College of Medical Genetics and Genomics (ACMG) criteria [14].

Statistical analysis

Analyses were performed using IBM® SPSS Statistics version 28.0.1.1 (IBM Corp., Armonk, NY, USA). We used median, minimum and maximum values, mean, and standard deviation (SD) as descriptive statistics. Chi-square and Mann–Whitney U tests were utilized to compare differences between categorical variables. A P value of < 0.05 was considered statistically significant.

Results

Variants in *BRCA1* and *BRCA2* genes

Totally, 67 unrelated individuals with EOBC were included in this study, of whom 53 (79.10%) patients were *BRCA*-negative and 14 (20.90%) patients had *BRCA* P/LP variants. Among the *BRCA*-positive group, nine (64.28%) were *BRCA2*-positive and five (35.72%) were *BRCA1*-positive (Fig. 1).

All variants detected in the *BRCA1* and *BRCA2* genes are described in Table 1. Six *BRCA1* variants were detected, all of which had been previously identified. Two novel *BRCA2* variants, including a missense variant of uncertain significance (VUS) and a large genomic rearrangement (LGR), were identified (Fig. 2), together with 12 variants previously reported.

Clinico-pathological features and mutational profiles

Table 2 summarizes the clinico-pathological findings of all cases. The median age at diagnosis for *BRCA2* P/LP variant carriers was moderately higher than for *BRCA1* P/LP variant carriers (40 vs. 37), although not statistically significant. Patient age groups were similar in *BRCA1*-positive vs. *BRCA2*-positive and *BRCA*-positive vs. *BRCA*-negative ($P = 0.405$ and $P = 0.418$, respectively).

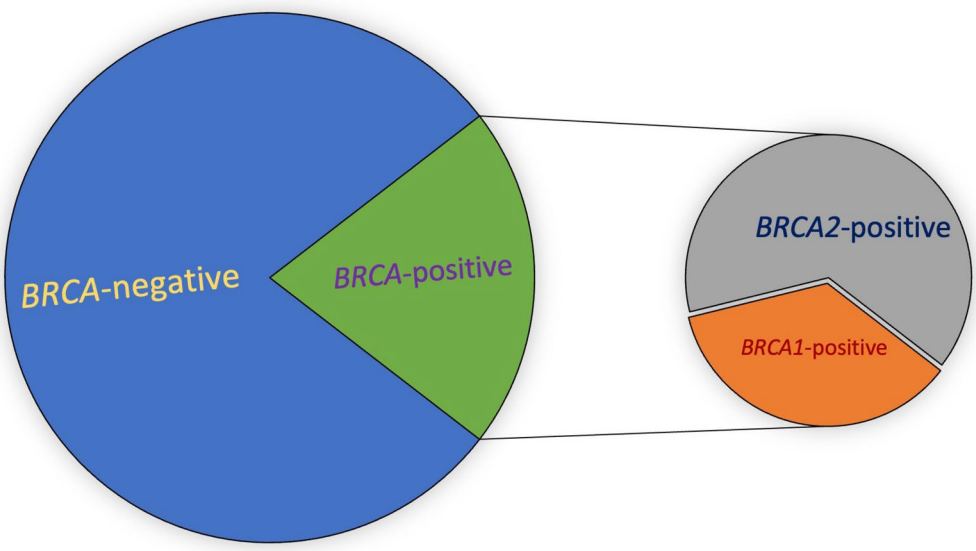

Fig. 1 *BRCA1/2* P/LP variant status of all included patients

Table 1 All detected variants in this study

Gene (Refseq accession number)	cDNA change	Predicted protein product	dbSNP	Number of patient detected	ACMG criteria	Variant Interpretation	Additional Information
BRCA1 (NM_007300.4)	c.981_982del	p.Cys328Ter	rs80357772	1	PVS1, PP5, PM2	Pathogenic	
	c.1059G > A	p.Trp353Ter	rs80356935	1	PVS1, PP5, PM2	Pathogenic	
	c.3607C > T	p.Arg1203Ter	rs62625308	1	PVS1, PP5, PM2	Pathogenic	
	c.3666G > C	p.Glu1222Asp	rs1555587312	1	PM2, BP4	VUS	
	c.5159G > A	p.Arg1720Gln	rs41293459	1	PS3, PP3, PP5, PM1, PM2, PM5	Pathogenic	
	c.5329dup	p.Gln1777ProfsTer74	rs80357906	1	PVS1, PP5, PS3, PM2	Pathogenic	
BRCA2 (NM_000059.4)	c.721A > T	p.Lys241Ter	rs876659100	1	PVS1, PP5, PM2	Pathogenic	
	c.1763_1766del	p.Asn588SerfsTer25	rs80359303	1	PVS1, PP5, PM2	Pathogenic	
	c.2514dup	p.Tyr839IlefsTer42	rs886040433	1	PVS1, PP5, PM2	Pathogenic	
	c.2808_2811del	p.Ala938ProfsTer21	rs80359351	1	PVS1, PP5, PM2	Pathogenic	
	c.2951A > G	p.Glu984Gly	rs767964776	1	PM2, BP4	VUS	
	c.3727G > A	p.Asp1243Asn	rs398122771	1	PP3, PM2	VUS	
	c.4766C > A[a]	p.Pro1589Gln	-	1	PM2, BP4	VUS	
	c.5901G > C[b]	p.Lys1967Asn	rs556893517	1	PM2, BP4	VUS	
	c.6664 T > G	p.Tyr2222Asp	-	1	PM2, PM5, BP4	VUS	Novel
	c.8452G > A[a]	p.Val2818Ile	rs80359094	1	PM2, BP4	VUS	
	c.9018C > G	p.Tyr3006Ter	rs80359154	1	PVS1, PP5, PM2	Pathogenic	
	c.9097dup	p.Thr3033Asnf-sTer11	rs397507419	2	PVS1, PP5, PM2	Pathogenic	
	Exon 6 deletion			1		Pathogenic	Novel
	Exon 12 deletion			1		Pathogenic	

[a] Detected in the same patient

[b] Coinherited with *BRCA1* c.5329dup variant in the same patient

[c] 5329dup variant in the same patient

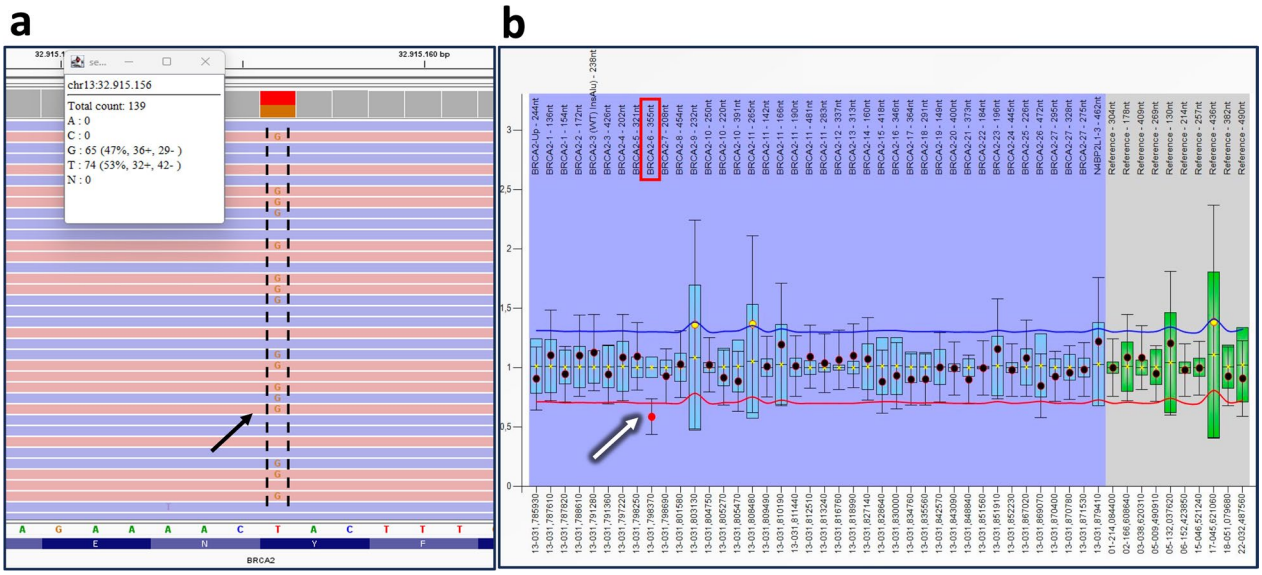

Fig. 2 Images of the novel variants identified in our study. Arrows indicate the site of mutation. **a** *BRCA2*(NM_000059.4): c.6664 T > G (p.Tyr2222Asp) variant in a heterozygous state is demonstrated by the IGV image. **b** Exon 6 heterozygous deletion of *BRCA2* is illustrated by the Coffalyser.Net™ software

There was no statistically significant difference observed between *BRCA*-positive vs. *BRCA*-negative or *BRCA1*-positive vs. *BRCA2*-positive for the family history status ($P=0.075$ and $P=0.648$, respectively).

The percentages of estrogen receptor (ER)-positive and progesterone receptor (PR)-positive patients were 69% (45/65) and 60% (39/65), respectively. Conversely, our cohort was more likely to have human epidermal growth factor receptor 2 (HER2)-negative tumors (62% [36/58]). We did not observe any statistically significant association between hormone receptor status (ER, PR, and HER2), either between *BRCA*-positive vs. *BRCA*-negative or *BRCA1*-positive vs. *BRCA2*-positive. Of 61 subjects with an available hormonal status of breast cancer, 15% (9/61) were triple-negative (TN), and among them, only three patients were found to be *BRCA* P/LP variant carriers.

Discussion

In this study, we identified a Turkish cohort composed of 67 unrelated patients with EOBC, and their clinical, pathological, and genetic characteristics are listed in Additional file 1: Table S1.

The identification of clinically actionable P/LP variants in EOBC patients is especially pivotal given the risk of second primary malignancies, the need for proper surveillance, potential reproductive decision-making, and segregation testing of at-risk relatives that provides early diagnosis and prevention of the disease by determining pre-symptomatic variant carriers. Additionally, uncovering the mutational landscape of breast cancer in this age group may help to optimize therapeutic management; for example, knowledge of *BRCA1/2* status may play a central role in both surgical decision-making and systemic treatment decisions [15].

We observed in our study group that about 21% of EOBC patients have P/LP variants in *BRCA* genes. The diagnostic yield of *BRCA1/2* genetic testing deviates from other similar studies because many other independent factors, such as the size of the study groups, inclusion criteria, or referral/ascertainment bias, influence the results automatically. For example, while Akdeniz Odemis et al. [16] detected a 16.3% diagnostic yield in a total of 1202 EOBC patients, Biancolella et al. [17] calculated their diagnostic yield as 23.52% in a cohort of 51 EOBC women with or without a positive family history from Burkina Faso [16, 17]. Another study from Turkey found that *BRCA1/2* genetic testing provided a diagnostic yield of 17.54% in 171 individuals with EOBC [18].

Out of the 20 variants observed in our study, two (10%) were novel. Missense alterations have been the most detected type of variant in *BRCA2*, and the majority of them are classified as VUS. As of September 2022, 6.188 *BRCA2* VUS variants had been cataloged in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), of which 5.539 (89.51%) were listed as missense variants. Moreover, of the total 6.559 *BRCA2* missense variants, 5.539 (84.44%) were VUS. While vigorous scientific efforts to validate the functional significance of VUS are being made [19–24], a large number of *BRCA2* variants remain unclassified. The rate of receiving a VUS report has also been significantly associated with ethnic origin [25, 26]. Thus,

Table 2 Baseline characteristics and clinico-pathological data of the cohort

	All patients ($n=67$)	*BRCA1*-positive ($n=5$)	*BRCA2*-positive ($n=9$)	*BRCA*-positive ($n=14$)	*BRCA*-negative ($n=53$)	*p* value
Age at diagnosis (years)[a]	40 [32;44] (39.17 ± 3.93)	37 [32;44] (38.60 ± 4.71)	40 [32;44] (38.0 ± 4.83)	38.5 [32;44] (38.21 ± 4.79)	40 [32;44] (39.43 ± 3.63)	0.490 (*BRCA*-positive vs *BRCA*-negative) 0.689 (*BRCA1*-positive vs *BRCA2*-positive
Patient age groups (years)[b]						
≤ 35	16 (24%)	1 (20%)	4 (44.5%)	5 (%36)	11 (%21)	0.418 (*BRCA*-positive vs *BRCA*-negative) 0.405 (*BRCA1*-positive vs *BRCA2*-positive)
36–40	22 (33%)	2 (40%)	1 (11%)	3 (%21)	19 (%36)	
41–44	29 (43%)	2 (40%)	4 (44.5%)	6 (%43)	23 (%43)	
Family history (at least one affected first, second or third degree relative with breast, ovarian, pancreatic, or prostate cancer)[b]						
Positive	44 (%66)	4 (%80)	8 (%89)	12 (%86)	32 (%60)	0.075 (*BRCA*-positive vs *BRCA*-negative) 0.648 (*BRCA1*-positive vs *BRCA2*-positive)
Negative	23 (%34)	1 (%20)	1 (%11)	2 (%14)	21 (%40)	
Estrogen-receptor (ER) status[b]						
Positive	45/65 (%69)	2/5 (%40)	5/9 (%56)	7/14 (%50)	38/51 (%75)	0.078 (*BRCA*-positive vs *BRCA*-negative) 0.576 (*BRCA1*-positive vs *BRCA2*-positive)
Negative	20/65 (%31)	3/5 (%60)	4/9 (%44)	7/14 (%50)	13/51 (%25)	
Missing	2 (%3)	–	–	–	2 (%4)	
Progesterone-receptor (PR) status[b]						
Positive	39/65 (%60)	3/5 (%60)	4/9 (%62)	7/14 (%61)	32/51 (%63)	0.388 (*BRCA*-positive vs *BRCA*-negative) 0.576 (*BRCA1*-positive vs *BRCA2*-positive)
Negative	26/65 (%40)	2/5 (%40)	5/9 (%38)	7/14 (%39)	19/51 (%37)	
Missing	2 (%3)	–	–	–	2 (%4)	
HER2 status[b]						
Positive	22/58 (%38)	2/4 (%50)	5/9 (%56)	7/13 (%54)	15/45 (%33)	0.179 (*BRCA*-positive vs *BRCA*-negative) 0.852 (*BRCA1*-positive vs *BRCA2*-positive)
Negative	36/58 (%62)	2/4 (%50)	4/9 (%44)	6/13 (%46)	30/45 (%67)	
Missing	9 (%13)	1 (%20)	–	1 (%7)	8 (%15)	
Triple-negative (TN)[c] *breast cancer status*[b]						
Yes	9/61 (%15)	2/4 (%50)	1/9 (%11)	3/13 (%23)	6/48 (%12.5)	0.340 (*BRCA*-positive vs *BRCA*-negative) 0.124 (*BRCA1*-positive vs *BRCA2*-positive)
No	52/61 (%85)	2/4 (%50)	8/9 (%89)	10/13 (%77)	42/48 (%87.5)	
Unknown due to lack of data	6 (%9)	1 (%20)	–	1 (%7)	5 (%10)	

[a] Values indicate "median [min;max] (mean ± SD)"

[b] Values indicate "*n* (%)." Patients with missing data were not included in the *p* value calculation. Mann–Whitney *U* test used for continuous variables and chi–square tests for categorical variables, done on patients with complete data

[c] Defined as estrogen-receptor-negative, HER2-negative, and progesterone-receptor-negative

the identification of VUS variants in *BRCA* genes is a significant clinical challenge in terms of risk assessment.

According to the NCCN® Guideline for Breast, Ovarian, and/or Pancreatic Cancer Genetic Assessment (Version 1.2024), VUS variants should not be used to revise the patient's medical management. In this instance, screening and risk reduction strategies should be recommended on the basis of personal and family history. Additionally, testing relatives for a VUS should not be done for clinical reasons unless there are conflicting interpretations of the data.

Specific recurrent variants in *BRCA1/2* genes have been delineated in certain populations, such as the Ashkenazi-Jewish, French-Canadian, Brazilian, Italian, Icelandic, and Polish populations [27–30]. For Turkey, several studies reported that the c.5329dup (also known as c.5266dup in alternate nomenclature) variant in *BRCA1* is the most common detected variant, which is possibly attributed to a founder effect [31–35]. In our study, we also detected the c.5329dup variant in an EOBC patient with a positive family history.

While the majority of germline disease-causing variants in *BRCA1/2* are small-scale, LGRs are also defined in a notable proportion of patients originating from distinct populations [36–40]. A small number of studies from Turkey report different LGRs in *BRCA* genes with a variable frequency [31, 35, 41]. Although the prevalence of rearrangement in the *BRCA1* gene is found to be higher

[42–45], we herein reported two LGRs in *BRCA2,* one of which is novel (deletion of exon 6) detected in a 32-year-old affected individual. It is evident that LGRs in *BRCA* genes comprise a significant proportion of the Turkish population. For this reason, a targeted and affordable genetic testing strategy that also includes the analysis of LGRs should be developed in Turkey for the molecular characterization of high-risk individuals.

Conclusion

In conclusion, enhanced knowledge about the mutational spectrum of druggable genes can aid clinicians in the management of EOBC patients. This study contributes to existing literature by extending the molecular basis of *BRCA1/2* genes.

Abbreviations

EOBC	Early-onset breast cancer
P/LP	Pathogenic/Likely pathogenic
NGS	Next-generation sequencing
MLPA	Multiplex ligation-dependent probe amplification
LGR	Large genomic rearrangement
BC	Breast cancer
HBOC	Hereditary Breast and/or Ovarian Cancer
VUS	Variant of uncertain significance
TN	Triple-negative
ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor receptor 2

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43042-023-00442-w.

Additional file 1. Table S1. Turkish cohort composed of 67 unrelated patients with EOBC, and their clinical, pathological, and genetic characteristics.

Acknowledgements

None.

Author contributions

We declare that we contributed significantly toward the research study; TK designed the study. TK and AK analyzed and interpreted the patients' data. TK was a major contributor in writing the manuscript. TK and AK revised the manuscript. All authors approved the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its Additional files].

Declarations

Ethics approval and consent to participate

This retrospective cohort study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from each subject, after approval of the experimental protocol by the Clinical Research Ethics Committee of Süleyman Demirel University (20/276).

Consent for publication

As participants were completely anonymized, consent for publication is not necessary/applicable.

Competing interests

The authors declare that they have no competing interests.

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