A multicenter study of genotype variation/demographic patterns in 2475 individuals inluding with 1444 cases with breast cancer in Turkey Short Title: BRCA profiling of breast-cancer patients in Turkey

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Abstract

BACKGROUND

Breast Cancer is the most common cancer type in women, second among the all cancers, and inherited with autosomal dominant pattern. The clinical diagnosis of BC relies on the published diagnostic criteria, and two genes have been identified as the main causative for breast cancer which are *BRCA1* and *BRCA2*.

OBJECTIVE

We aimed to compare the index cases with the diagnostic features to describe the genotype/demographic information association in breast cancer.

METHODS

We performed mutational analyses for the *BRCA1* and *BRCA2* genes on 2475 individuals from collobrative centers across Turkey, whom 1444 of them were ascertained index cases.

RESULTS

We identified mutations in 17% (421/2475) of all individuals while its almost the same 16.6% (239/1444) in 1444 index cases. Mutations in *BRCA1*/*BRCA2* genes were detected in 17.8% (131/737) of familial cases and 12% (78/549) of sporadic breast cancer, with 4.9% of mutations in the *BRCA1* and 12% in the *BRCA2*.

CONCLUSIONS

Genotype variation and demographic information patterns were analyzed of all observed breast cancer findings in probands. We showed that patients with *BRCA2* mutations have significantly been identified more than *BRCA1* mutations. We also observed that sporadic breast cancer cases without familial history have less mutation positivity in BRCA1/BRCA2 genes, and the results were consistent with other studies in the Mediterranean region. On performing meta-analyses of our data and the other limited studies of the Mediterranean region in the literature, we found significant correlations that individual studies did not have sufficient power to conclude. Correlating genotypes with demographic information should facilitate the disease management of BC both the familial and non-familial cases.

Background

Breast Cancer (BC) is an autosomal dominant condition affecting ~ 2 million people per year globally. The incidence is estimated 1:8 in women and 1:833 in men (1). The clinical diagnosis of BC relies on the published diagnostic criteria (2). Two genes have been identified as the main causative for BC but not limited with that. The *BRCA1* gene, located on chromosome 17, codes for breast cancer type 1 susceptibility protein, named for its association with BC. This gene has 22 exons distributed over approximately 110 kb of genomic DNA. In contrast with the *BRCA1* gene, the *BRCA2* gene has 27 exons approximately 84.2 kb of genomic DNA on chromosome 13 (3). To date, more than 3242 disease-causing mutations have been identified in either the *BRCA1* or *BRCA2* genes (4). The remaining individuals probably have mutations on other cancer related genes or large gene deletions, somatic mosaic mutations, and mutations in unanalyzed gene noncoding regions of *BRCA1* and *BRCA2* genes.

Of interest is whether the phenotypic presentation of BC differs by whether the disease results from mutations in *BRCA1* or *BRCA2*. Early studies from the Mediterranean countries even including the population based ones reporting genotype/phenotype correlations did not find any evidence for phenotypic differences between patients with *BRCA1* mutations and patients with no mutation identified or patients with *BRCA1* versus *BRCA2* mutations (3, 5–8). These studies, however, tended to have smaller sample sizes. The largest studies published to date found *BRCA2* more frequent in individuals with BC in the region. The main study primarily included the patients without family history is also limited with the low number of index cases in the study group but with relatively higher frequency in the Mediterranean region of Turkey when compared with other international studies (9, 10).

In this study, we performed mutational analysis for the *BRCA1* and *BRCA2* genes on 2475 individuals whom 1444 of them were ascertained index cases and compared the index cases with the diagnostic features to describe the genotype/demographic information association in BC. The majority of our patients have a mutation in the *BRCA2* gene. We found that these patients generally have a breast cancer phenotype than those with *BRCA1* mutations. Our study reports the findings and extends the genotype/demographic information association to the BC features. We also compared mutation type (protein truncation [PT] vs. missense [MS]) with phenotypic features, as well as with the familial probands. These latter comparisons were made to determine whether there is additional prognostic information that can be provided to families based on genetic test results or mode of inheritance.

Methods

1.1. Patients’ Characteristics

Patients with a diagnosis of BC and healthy individuals with family history of breast cancer were enrolled between 2013 and 2020 with informed written consents approved by the institutional reviews at all the participated University and ethics board at Cukurova University. All the cases were diagnosed with invasive ductal breast cancer with no other types of cancers or any other precancerous conditions. Similarly, individuals that were studied for screening were not affected with any other malignancies. For the familial studies, we have included the individuals who had family history of invasive ductal breast

cancer. Patient selection was made accordingly with American Society of Clinical Oncology (ASCO) guidelines (11). Patients enrolled were evaluated by all our collaborators from Turkey including Mediterranean, Aegean, Black Sea, Central Anatolia, Marmara, Eastern Anatolia, Southeastern Anatolia Regions, and also from Northern Cyprus. The goal was to identify the genetic cause of BC in these patients in terms of *BRCA1* and *BRCA2* genes. For familial BC cases enrolled, we included only the index patients were used for phenotypic analyses.

Subjects enrolled in our research protocol through these centers in Turkey: Cukurova University AGENTEM (Adana Genetic Diseases Diagnosis and Treatment Center), Adana (n = 1141); Uludag University Faculty of Medicine, Bursa (n = 602); Medical Genetics Department of Erciyes University, Kayseri (n = 598); Bezmialem Vakıf University Medical Faculty, İstanbul (n = 73); Uskudar University Faculty of Medicine, İstanbul (n = 45); and in the Northern Cyprus Near East University Faculty of Medicine (n = 16). A total of 1444 (58.3%) affected BC individuals were included. Clinical information was not available for every feature of BC on every participant. Some patients were referred and enrolled in the mutation screening process without sending sufficient clinical information to determine diagnostic status. Among 1444 BC patients, 737 (51%) of them had positive family history while 549 (49%) cases had no invasive ductal breast cancer in their family. The rest of the patient's (n = 158) family history of BC were unknown. Patients who had no information such as familial history were not included in the demographic analysis. We have, however, included them in the description of the mutations. Under 18 years of age individuals who were all index cases were included to the study. Also, patients who were under 30 years of age and carry TP53 mutations were excluded due to the purpose of our study.

1.2. Screening and Classification of Genetic Variations

DNA was extracted from peripheral blood lymphocytes of both healthy individuals and cases. Next generation sequencing was performed for all coding exons and exon-intron junctions of the *BRCA1* and *BRCA2* genes. In addition, Multiplex Ligation-dependent Probe Amplification (MLPA) was performed for 591 AGENTEM's primer index patients as the national reference center for *BRCA1/BRCA2*. MLPA assay was not applied in other collaborative centers due to the absence of patients reported with positive MLPA results in our country. Nucleotide change was considered as pathogenic, a polymorphism or a variant of unknown significance (or unclassifiable variant) when it was novel and parents were unavailable for study. American College of Medical Genetics and Genomics (ACMG) criteria was followed for variant classification. The variations that were not identified in Human Gene Mutation Database (HGMD) and The Single Nucleotide Polymorphism Database (dbSNP) or any other clinical databases such as ClinVar and VarSome the Human Genomic Variant Search Engine (VarSome) were assed as novel changes. Novel variants were then investigated through *in silico* analysis for variant classification.

1.3. Statistical Analysis

The BC disease features for the following groups were compared: (1) gene loci mutated *BRCA1* versus *BRCA2* and (2) familial versus sporadic. We analyzed our patient clinical findings by grouping our patients according to gender, familial or sporadic, mutation in *BRCA1* or *BRCA2*. Because patients came

from different sources and may not have all demographic criteria assessed, the numbers for each analysis varied. We used information from patients with a definite diagnosis for our statistical analyses.

1.4. Populational Comparison

GnomAD v2.1.1 data set (GRCh37/hg19) was used for the population comparison which spans 125,748 exome sequences and 15,708 whole-genome sequences from unrelated individuals.

It is the largest publicly available population data to date, and categorizes the populations as follows; African/African-American, Amish, Latino/Admixed American, Ashkenazi Jewish, East Asian, South Asian, Middle Eastern, European (Finnish), European (non-Finnish) and other. However, the proportion of the gnomAD population did not cluster with any of the Mediterranean populations and was classified as other which is likely to include individuals of mixed background as in Turkey.

The MAF cut-off of 0.001 that is recommended for variant discovery in dominant inherited mendelian diseases, was used to classify variants as rare frequency (MAF $\leq$ 0.001) supporting variants' pathogenic effect, and common frequency (MAF $\geq$ 0.001) which are unlikely to be the causative.

Results

1.5. Patients' Characteristics

BRCA1 and BRCA2 mutational analysis were performed in 2475 subjects. However, we were unable to use regression analysis to correct the data for phenotypic features. Also, not all subjects in our study were interviewed for familial history, therefore we include 1444 cases contributing to results. Among patients with a definite diagnosis, identification of a genetic alteration for familial patients is higher (54.8%; 131/239) than for patients with sporadic BC (32.6%; 78/239), and this is statistically significant. The rest of the variation positive patients (n = 30) were the individuals with unknown familial history of BC.

The median age for all index patients (n = 1444) was 51.5 years, and the average age was 48.6 years (age range 15–88 years). Figure 1 details the demographic characteristics of our study population.

Figure 1.The distribution of age and sex.

1.6. Mutation Analysis

We identified pathogenic mutations for 218 individuals and variants of unknown significance for 139 in affected BC cases 114 of them had pathogenic and 85 cases had VUSs. Total variants, their pathogenicity, and internal frequencies were given in supplementary data (Supplementary Table 1). No genetic change could be identified for 2054 patients (82.9%) in total, and 1205 (83.5%) in BC cases. Among 737 BC cases with positive family history, 36 cases (4.9%) have variations in *BRCA1* and 95 cases (12.89%) have variations in *BRCA2*, and 6 (4.6%) patients have genetic alterations in both genes resulting in a *BRCA1*:*BRCA2* ratio of 1/2.6. Twenty-seven of 549 patients (4.9%) without family history

have variants in *BRCA1* and 51 patients (9.2%) have variants in *BRCA2*, resulting in a *BRCA1*:*BRCA2* ratio of approximately 1/2.

The mutations identified in *BRCA1* and *BRCA2* genes in all 2475 individuals are distributed as follows: 51.3% pathogenic, 15.5% likely pathogenic and 33% variant of uncertain significance (Table 1). Moreover, variant classifications for affected BC cases were given in Table 1.

Table 1
Overall distribution of variant classification in *BRCA1* and *BRCA2* genes for both healthy individuals with BC diagnosed cases (n = 2475) and affected BC cases (n = 1444).

		Pathogenic	Likely Pathogenic	VUS[1]
Both healthy individuals and BC diagnosed cases	**BRCA1**	70.1% (103/147)	11.5% (17/147)	18.4% (27/147)
	BRCA2	41.6% (118/283)	17.6% (50/283)	40.6% (115/283)
	Total	221(51.3%)	67(15.5%)	142(33%)
Affected BC cases	**BRCA1**	71.4% (50/71)	11.3% (8/71)	18.3% (13/71)
	BRCA2	37.9% (66/174)	19.5% (34/174)	42.5% (74/174)
	Total	116	42	87

[1]VUS: Variant of uncertain significance.

We also examined the most frequent variants that were detected on both *BRCA1* and *BRCA2* genes listed in Table 2. The most frequent variants were distributed equally in both genes. In the perspective of pathogenicity, pathogenic variants were presented relatively more frequent with 9 variants. Novel genetic variations in both BRCAs were listed in Table 3. In contrast with frequent variant list, *BRCA2* gene is the dominant for the 14 novel variants versus 1 novel variant in *BRCA1* gene.

Table 2
The most frequent detected variants in *BRCA1* and *BRCA2* genes.

Gene	Variant	Impact	Class.[1]	Freq.[2] (%)
BRCA2	c.7689delC p.H2563Qfs*85	Frameshift	P[3]	3.92 (n = 17)
BRCA1	c.1444_1447delATTA p.I482*	Frameshift	P	3.46 (n = 15)
BRCA1	c.2800C > T p.Q934*	Nonsense	P	3 (n = 13)
BRCA1	c.4327C > T p.R1443*	Nonsense	P	3 (n = 13)
BRCA1	c.5266dupC p.Q1756Pfs*74	Frameshift	P	3 (n = 13)
BRCA2	c.1909 + 22delT	Inframe del	VUS[4]	2.07 (n = 9)
BRCA2	c.3836A > G p.N1279S	Missense	LP[5]	2.07 (n = 9)
BRCA2	c.9097dupA p.T3033fs*11	Frameshift	P	2.07 (n = 9)
BRCA2	c.3318C > G p.S1106R	Missense	LP	1.61 (n = 7)
BRCA2	c.3751dupA p.T1251fs*14	Frameshift	P	1.38 (n = 6)
BRCA2	c.4169delT p.L1390fs*20	Frameshift	P	1.38 (n = 6)
BRCA2	c.67 + 1G > A	Intronic	P	1.38 (n = 6)
BRCA2	c.8881G > A p.G2961S	Missense	VUS	1.38 (n = 6)

[1]Class.: Classification, [2]Freq.: Frequency, [3]P: Pathogenic, [5]LP: Likely pathogenic, [4]VUS: Variant of uncertain significance

Table 3
Detected novel variants in *BRCA1* and *BRCA2* genes.

Gene	Variant	Impact	Class.[1]
BRCA1	c.5152 + 23C > T	Intronic	VUS[3]
BRCA2	c.1519delA p.R507fs*2	Frameshift	LP[2]
BRCA2	c.1854C > A p.A618A	Synonymous	VUS
BRCA2	c.5647A > T p.K1883*	Nonsense	LP
BRCA2	c.5697T > A p.D1899E	Missense	VUS
BRCA2	c.6609T > A p.V2203V	Synonymous	VUS
BRCA2	c.6934G > C p.D2312H	Missense	LP
BRCA2	c.7645T > G p.C2549G	Missense	LP
BRCA2	c.7700A > G p.Y2567C	Missense	VUS
BRCA2	c.8020_8021dupAA p.I2675fs*2	Frameshift	LP
BRCA2	c.8021A > G p.K2674R	Missense	LP
BRCA2	c.8487 + 39T > C	Intronic	VUS
BRCA2	c.9370_9381delAACCTCCAGTGG p.N3124_W3127del	Inframe del	LP
BRCA2	c.9370_9383delAACCTCCAGTGGCGinsCT p.R3128delinsL	Missense	LP
BRCA2	c.9772G > A p.E3258K	Missense	VUS

[1]Class.: Classification, [2]LP: Likely pathogenic, [3]VUS: Variant of uncertain significance

1.7. Overall clinical features

The phenotypic characteristics for BC patients in this study are listed in Table 4.

Table 4
Gender and family history distribution of cases.

	Family history (+)	Family history (-)	Unknown family history
Female	729	537	158
Male	8	12	0
Total	737	549	158

1.8. Demographic comparisons

We have collected the clinical information on 1444 diagnosed BC index patients. We compared phenotypes of these patients by gender and their mutations. Observed frequencies of clinical features listed in Table 5 for BC patients in this study. 1.39% (n = 20) of 1444 diagnosed BC index patients were male. We observed pathogenic variations in *BRCA2* genes of two (10%) of these 20 male BC patients.

Table 5
Phenotypic comparison of variant between genders in cases.

	Pathogenic	Likely Pathogenic	VUS[1]
Female	113	43	87
Male	2	0	0
Total	115	43	87

[1]VUS: Variant of uncertain significance

1.9. Family history positive patients versus sporadic BC cases

We investigated the phenotypic effects of mutation between the *BRCA1* gene and the *BRCA2* gene, BC features for 737 familial index patients and 549 sporadic BC patients were analyzed according to the effects of gene mutated. The median age was 52 years for familial index patients and 48.5 years for sporadic BC patients with average ages of 43.3 and 43.5 years, respectively. Comparison of the disease features of these two groups do not show any significant difference. We also noted that familial patients have a higher proportion of *BRCA1*/2 gene mutations than *BRCA1*/2 gene mutations compared with sporadic BC patients.

1.10. Gene mutation effect on clinical features of BC

We examined the effect of gene mutation on invasive ductal carcinoma features including 70 patients with *BRCA1* mutations and 172 patients with *BRCA2* mutations. The median ages were 50.5 and 50 years, and the average ages were 41 and 45.3 years, respectively.

To determine whether gene mutation (*BRCA1* versus *BRCA2*) showed different effects in familial BC cases, disease features from a total of 737 familial index patients, including 36 patients with mutations in *BRCA1* and 95 patients with mutations in *BRCA2*, were analyzed. The median ages were 50 and 50 years, and the average ages were 44.6 and 43.9 years, respectively.

When comparing sporadic BC cases only, we analyzed the disease features of 27 patients with *BRCA1* mutation compared with 51 patients with *BRCA2* mutation. The median ages were 50.5 and 48 years, and the average ages were 39.9 and 42.9, respectively.

1.11. Impact of Mutation Types

The type of mutations in many genetic related disorders affects disease severity. To evaluate the effect of the type of mutations on the presence of BC features, we compared features of patients.

The percentages of the mutations types detected were listed in Table 6.

Table 6
Overall distribution of genetic variation types.

	Frameshift	Missense	Nonsense	Intronic	In-frame dup	In-frame del
BRCA1	34	43	40	11	0	1
BRCA2	76	128	24	27	2	6
Total	110	171	64	38	2	7

1.12. Allel Frequency Comparison

Among total of 220 different types of detected variations, 190 (86.4%) of them had higher allel frequencies than their aggregated gnomAD allel frequency. With a 0.001 MAF cut-off, 134 (60.9%) of the 220 variants were evaluated as rare which all of them showed higher frequency in our study and considered as more likely to be pathogenic. In addition, 73.7% (56/76) globally common variants (MAF ≥ 0.001) were more frequent in our study while 20 of them (26.3%) showed lesser frequencies than aggregated gnomAD. Distribution of BRCAs common (MAF ≥ 0.001) and rare variants (MAF ≤ 0.001) by gnomAD population and the aggregated gnomAD a were given as supplementary data (Supplementary Table 2).

The frequencies of pathogenic variants and VUSs were compared across several ethnic groups [African/African-American, Amish, Latino/Admixed American, Ashkenazi Jewish, East Asian, South Asian, Middle Eastern, European (Finnish), European (non-Finnish) and other] and the local whole exome sequencing databases.

The analysis showed that out of 28 pathogenic variants located in *BRCA1*, 31 occurred as a higher frequency than aggregated gnomAD data and distinctive populational gnomAD data. Details were given in supplementary data (Supplementary Table 2).

Discussion

We tested for mutations in all coding exons and exon-intron junctions of the *BRCA1* and *BRCA2* genes in DNA from 2475 diagnosed and screening patients from Turkey; we identified 221(51.3%) pathogenic mutations which some of these mutations have been previously reported, 142 (33%) VUS and 15 (3.7%) novel while the overall the *BRCA1* and *BRCA2* mutation detection rate is 9.9%.

As noted in previous studies, the mutation detection rate varies from 2.7–19% for patients with positive family history but without clinical information in different populations (8, 9, 12).

One of the main focuses of this study is to pool a nationwide Mediterranean country dataset that will increase the power of further analysis for clinical interpretations both in the familial and non-familial cases and the cases with *BRCA1* and *BRCA2* mutations.

In multifactorial disorders such as cancers, the genotype variation and demographic information correlation is not as understood as it is in Mendelian disorders. Analysis and interpretation of genetic test results should be considered with the patient's clinical and family history. This study also showed that the significant percentage of *BRCA1* and *BRCA2* variations are still classified as VUS. Thus, the improvement of genetic variation databases is crucial for the correct diagnosis. Therefore, this study contributes the growing list of reported mutations databases for breast cancer. In the light of the fact that the genotype and phenotype correlation of breast cancer is still controversial, the present outcome can enhance our knowledge on this complicated, common and severe condition.

It was also observed that the most common mutations in the *BRCA1* and *BRCA2* genes in Turkish population were not among the first 10 mutations that were reported in a study includes all continents. *BRCA1* c.1444_1447delATTA p.I482* and *BRCA2* c.7689delC p.H2563Qfs*85 mutations can be considered to be founder mutations for Turkish population and a screening program can be planned for early diagnosis of breast cancer (13).

We demonstrated the importance of looking at each variants' frequency per specific ethnic groups as opposed to the overall gnomAD frequency. Our analysis pointed out 56 pathogenic variants that had MAF ≤ 0.001 (Minor Allel Frequency) in the aggregated gnomAD population but were common in our study. Furthermore, when a more stringent MAF cut-off value (≤ 0.0001) is used, 123 pathogenic variants should be re-classified as more frequent that might be speculated as a founder affect for our population. In brief, these data suggesting that still a number of variants classified as pathogenic are not truly disease causing or the variants with the higher observed frequency are not truly benign.

Conclusion

The overall mutation detection rate for patients with BC in Turkey was 9.9% in our study. The proportion of BRCA1 to BRCA2 mutation is approximately 2 to 2.5 for BC cases, and sporadic BC cases without familial history have more mutation positivity. In sporadic BC, BRCA1 mutation accounted for 34.6% and BRCA2 mutations accounted for 65.4%.

Our study summarizes the evidence for interpretation process using the most important criteria as ACMG guidelines, gene specific databases for analysis of the variant frequency in the largest available population together with local datasets and results of the computational predictions.

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of from Cukurova University Ethical Committee (102-2 and 07/08/2020).

All participants were informed, and signed written consent/permissions for this research in accordance with Helsinki declaration.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request. Table S1: The total detected variant list. Table S2. Distribution of common (MAF≥0.001) and rare variants (MAF≤0.001) in BRCAs by aggregated and population specific gnomAD data.

Competing interests

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Author's contributions

Conception: A.B.

Interpretation or analysis of data: methodology: I.B., A.H., C.M., C.R., O.S., E.A., N.B., N.C., C.O.E, A.A., and B.D.; investigation, L.A., E.A., N.B., N.C., B.Du. and S.T.B.; resources, S.O.S., L.A., N.D., S.Y.O., M.C.E., C.K.P, O.Y., K.D., T.E., S.C., E.C., A.D., O.B., S.T., S.G., B.Du., D.T., F.O., M.De., M.C., O.D., P.O. and S.T.B.; data curation, I.B., A.H., C.M., C.R., O.S., L.A., E.A., N.B., N.C., C.O.E, A.A., and B.D.;

Preparation of the manuscript: writing—original draft preparation, A.B., S.G.T., M.C.E. and M.Du.; writing—review and editing, A.B., S.G.T. and M.Du.; visualization, I.B., A.H., C.M., C.R., and O.S.

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Figures

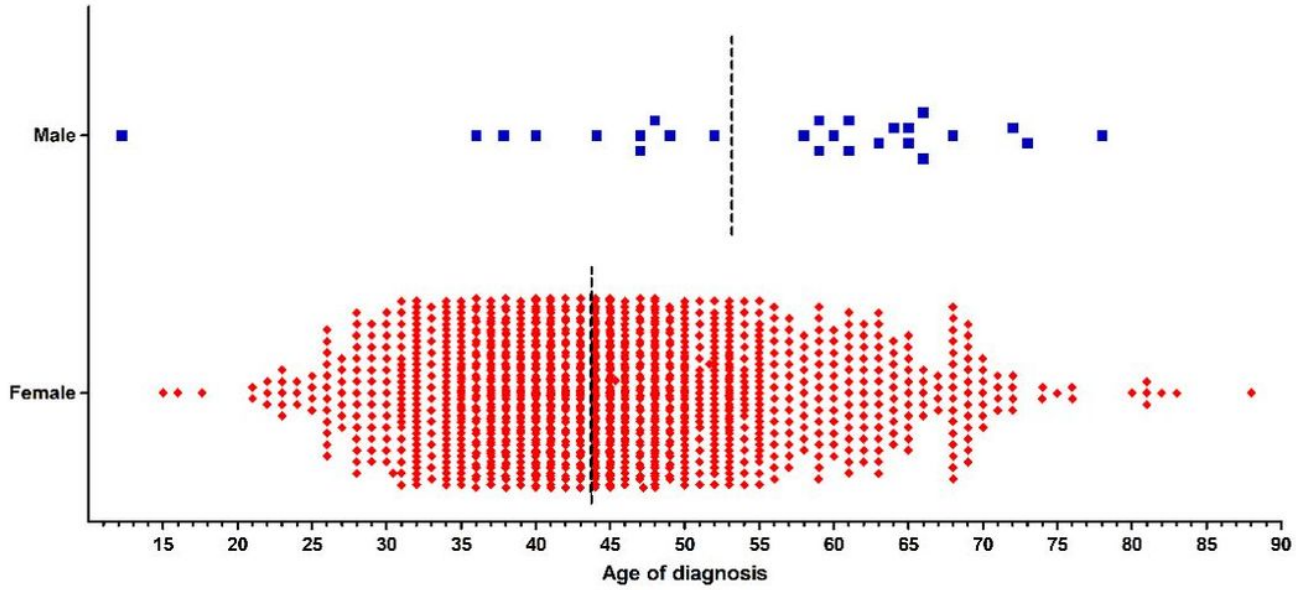

Figure 1

The distribution of age and sex.

Supplementary Files

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