



## Research Article

# Unveiling the Prognostic Significance of Long Non-Coding RNA (lncRNA) PCAT1 in Invasive Breast Carcinoma

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### Abstract

**Objective:** Prostate cancer-associated transcript 1 (PCAT1) is a long non-coding RNA (lncRNA), composed of more than 200 nucleotides which expression controls the proliferation, migration, invasion, and metastasis of different cancers. In this current study, we have analyzed the possible role of PCAT1 as a prognostic biomarker for invasive breast cancer (IBC).

**Method:** Online web genomic portal cBioportal, GEPIA, PhosphoSitePlus, IGV, GENEMANIA, Ensembl, and ENCORI were used for this analysis.

**Result:** Our analysis demonstrated that PCAT1 expression was higher in BRCA-mutated BC patients cells compared to normal cells (fold change ~ 1.56). Over time, patients with greater PCAT1 expression had a significantly lower overall survival rate ( $p = 5.539 \times 10^{-5}$ ). Besides, there was significant alteration of PCAT1 in PR ( $p = 1.672 \times 10^{-6}$ ), HER2 ( $p = 3.920 \times 10^{-4}$ ) negative, and ER ( $p = 3.190 \times 10^{-6}$ ) positive primary IBC samples. In addition, a correlation was also found with the alteration of PCAT1 and the histologic grade of the IBC ( $p > 10^{-10}$ ). Moreover, the co-occurrence of PCAT1 with the oncogenes of CASC family, i.e., CASC8, CASC11, and CASC19 in IBC was identified. The PCAT1, CASC8, CASC11, and CASC19 have genomic location, named chr8q24, which contains the loci responsible for different cancers including BC.

**Conclusion:** These findings indicate the possible association of PCAT1 expression with poor clinical outcomes and co-occurrence with previously established oncogenes as well as biomarkers suggests its usefulness as a prognostic biomarker in IBC.

**Keywords:** Prostate cancer-associated transcript 1, long non-coding RNA, invasive breast carcinoma, estrogen receptor, cancer susceptibility candidate

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One of the leading factors of mortality for women is breast cancer (BC) worldwide. The American Cancer Society (ACS) estimates that BC alone is responsible for 15% of deaths due to cancer and around 30% of all newly diagnosed female malignancies.<sup>[1]</sup> Depending on its molecular profile, breast cancer can be classified as invasive

or non-invasive; the former category accounts for 90–95% of the incidences of the disease.<sup>[2]</sup> It is estimated that between the two principal molecular categories of invasive breast cancer (IBC), approximately 80% corresponds to invasive ductal carcinoma (IDC) and roughly 10% to invasive lobular carcinoma (ILC).<sup>[3]</sup> Non-invasive BC, also called

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in situ or pre-cancer, usually remains between the ducts or milk lobules of the breast without growing in the normal cells, whereas for IBC, normal breast cells become affected through the lymph system and bloodstream.<sup>[4]</sup> This makes IBC a heterogeneous anomaly that causes constitutional alteration in tumor cells that develop secondary tumors.<sup>[5]</sup> The capacity to diagnose the illness and determine the best course of action at an early stage is a constant requirement for managing BC patients.<sup>[6]</sup> When compared to traditional diagnostic methods, biomarkers can be more effective clinical and diagnostic tools and are crucial for the early detection and diagnosis of BC.<sup>[7,8]</sup> Estrogen receptors (ER), progesterone receptors (PR), and human epidermal receptor proteins (HER2) are all well-known hormonal biomarkers for IBC.<sup>[9]</sup> Beyond these hormonal biomarkers, various new biomarkers like Ubiquinone Oxidoreductase core subunit S3 (NDUFS3),<sup>[10]</sup> RNA polymerase III specific TFIIIB subunit BRF2,<sup>[11]</sup> nerve growth factor (p75NGFR),<sup>[12]</sup> microRNA-320a (miR-320a),<sup>[13]</sup> heterogeneous nuclear ribonucleoproteins (hnRNPs),<sup>[14]</sup> etc. have been reported for their ability to predict and identify IBC.

Long non-coding RNAs (lncRNA), comprising more than 200 nucleotides, are known to control cell cycle regulation, lipid metabolism, cell differentiation, and innate immune response.<sup>[15]</sup> Although there is a lack of information regarding the number of molecular subtypes of lncRNAs, it is clear that major portions belong to "genic" (overlap a protein-coding transcript) and "intergenic" (unlikely to overlap a protein-coding transcript) in humans.<sup>[16]</sup> Besides, open reading frames (ORFs), which monitor the protein-coding gene expression (both through cis and trans mechanism), are absent within these lncRNAs.<sup>[17]</sup> lncRNAs may accelerate the transcription by attaching to the transcriptional factors.<sup>[18]</sup> They can also play a negative role in the transcription process by impeding the initiation, elongation, or termination of another gene. Moreover, lncRNAs hinder the transcription factors to commute in the cytoplasm aiming to restrain them from targets.<sup>[18]</sup> Consequently, the variety of lncRNAs' modes of activity indicates that these are the indispensable controllers of transcription.<sup>[17,18]</sup>

There is a scientific connection between lncRNAs and cancer.<sup>[19]</sup> lncRNAs are responsible for cancer development for their abnormal expression and act as oncogenes in BC.<sup>[20]</sup> As an example, HOX transcript antisense RNA (HOTAIR) and BC-200 (brain cytoplasmic RNA 1) are two well-known lncRNAs that have significant roles in BC progression as well as are prominent biomarkers. HOTAIR, situated at the HOX loci which experiences deregulation at the time of BC development, shows overexpression in primary BC. HOTAIR plays an indicator

for metastatic BC along with death. Another lncRNA, BC-200 reveals in neurons rather than somatic cells and expressed excessively in various cancers with BC. Moreover, BC-200 is highly identical in IBC to normal tissues and benign tumors. It is clear that lncRNAs act as prognostic biomarkers in IBC.<sup>[18]</sup>

In this study, we investigated the role of prostate cancer-associated transcript 1 (PCAT1) in IBC. PCAT1 was previously reported to up-regulate proliferation, migration, invasion, metastasis, and apoptosis resistance in BC.<sup>[21]</sup> The mode of action of PCAT1 in cancer development demonstrates the involvement of several pathways including, i) participation of PCAT1 in double-stranded DNA break (DSBs) repair through regulation of (Breast Cancer Gene 2) BRCA2, ii) impairment of post-translational modifications by the complex of PCAT1 and polycomb repressive complexes 2 (PRC2), iii) interlinkage of PCAT1 with c-MYC, iv) association of PCAT1 with single nucleotide polymorphisms (SNPs), and v) inhibition of microRNA to their targets molecules.<sup>[22]</sup> The expression analysis of PCAT1 identified the overexpression of PCAT1 in patients with BC.<sup>[21]</sup> Moreover, up-regulated PCAT1 exacerbated hypoxia-associated BC advancement of interaction with the receptor of activated protein C kinase-1 (RACK1).<sup>[23]</sup> PCAT1 was dramatically up-regulated in hepatocellular carcinoma and could be a biomarker.<sup>[24-26]</sup> In colorectal cancer, it was also found to be significantly up-regulated in tumor tissues (~ 64%) compared to normal tissues.<sup>[27]</sup> Research on PCAT1 expression in gastric cancer showed that the growth of surrounding non-tumorous tissues was significantly less than the overexpression of this gene in malignant cells.<sup>[28,29]</sup> Overexpression of PCAT1 was also found in esophageal cancer,<sup>[30,31]</sup> osteosarcoma,<sup>[32,33]</sup> non-small cell lung cancer,<sup>[34]</sup> bladder cancer,<sup>[35]</sup> cervical cancer,<sup>[36]</sup> and multiple myeloma.<sup>[37]</sup> Besides, PCAT1 enhanced the development of osteosarcoma through miR-508-3p/zinc finger E-box binding homeobox 1 (ZEB1)<sup>[38]</sup> and fostered the proliferation of prostate cancer cells by regulating the BRCA2 tumor suppressor protein negatively.<sup>[37]</sup> In addition, PCAT1 induced the development of cholangiocarcinoma by sponging miR-216a-3p<sup>[39]</sup> and of clear cell renal cell carcinoma through the up-regulation of YAP by miR-656 and miR-539.<sup>[40]</sup> Moreover, PCAT1/miR-129/ABCB1 axis has been reported to have chemo-resistance ability in non-small cell lung cancer. In this study, we analyzed the prognostic potential of PCAT1 in IBC by patient data-derived meta-analysis. As a lncRNA, our findings demonstrated a potential prognostic ability of PCAT1 and proposed its clinical importance as a biomarker for IBC for the very first time.

## Methods

### Search and Inclusion/Exclusion Criteria

cBioportal, GEPIA, STRING, PhosphoSitePlus, IGV, GENEMANIA, Ensembl, and ENCORI genomic web portals were used for this meta-analysis. Further, five selected web genomic portals were used to extract the data for the completion of this study. The inclusion criteria were: 1) availability of information regarding the PCAT1 gene, and 2) availability of the relevant data. After the selection of the databases, the statistical significance of each of the datasets was calculated (Fig. 1).

### TCGA Pancancer Atlas and cBioPortal for Gene Alteration Analyses

Experts in bioinformatics and cancer computational biology are responsible for maintaining cBioPortal.<sup>[11]</sup> We initially considered 12 studies (4807 samples) from cBioPortal (<https://www.cbioportal.org/>), to look at PCAT1 alterations in IBC, including Breast Cancer (CPTAC, Cell 2020),<sup>[41]</sup> Breast Cancer (METABRIC, Nature 2012 & Nat Commun 2016),<sup>[42]</sup> Metastatic Breast Cancer Project (Provisional, February 2020),<sup>[43]</sup> Breast Invasive Carcinoma (TCGA, cell 2015)<sup>[44]</sup>, Metastatic Breast Cancer (INSERM, PLoS Med 2016)<sup>[45]</sup>, Breast Cancer (MSK, Clinical Cancer Res 2020)<sup>[46]</sup>, Breast Cancer (SMC 2018)<sup>[47]</sup>, Breast Cancer Xenografts (British Columbia, Nature 2015)<sup>[48]</sup>, Breast Cancer (MSKCC, NPJ Breast Cancer 2019)<sup>[49]</sup>, Breast Invasive Carcinoma (British Columbia, Nature 2012)<sup>[50]</sup>, Breast Invasive Carcinoma (Broad, Nature 2012)<sup>[51]</sup>, and Breast Invasive Carcinoma (Sanger, Nature 2012)<sup>[52]</sup>. In the following step, we performed a comprehensive analysis using CPTAC<sup>[41]</sup> and METABRIC<sup>[42]</sup> (including 2,631 samples). These studies included the patients suffering from primary BC and reported copy num-

ber alterations (CNA) and gene expressions as well as long-term clinical follow-up data<sup>[41,42,53,54]</sup>. Moreover, we explored the INSERM<sup>[45]</sup> data set (where the whole-exome sequencing was used to identify the metastasis) (includes 216 patients) for the analysis of patients with metastatic cancer<sup>[45]</sup>. In our current analysis of PCAT1 alterations in IBC, the frequency of genetic alterations, overall survival rates, ER, PR, and HER2 statuses with PCAT1 alterations, the relationship between the age of patients and PCAT1 alterations, and the co-occurrence of the PCAT1 gene with other oncogenes were assessed using the database of cBioPortal for Cancer Genomics.

### GEPIA Dataset for Gene Expression Analysis

Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/index.html>) is an interactive web server used for estimating mRNA expression data based on 9,736 tumors and 8,587 normal samples in the Cancer Genome Atlas (TCGA) and the Genotype-tissue Expression dataset projects. The main interactive and configurable features of GEPIA include comparable gene detection, dimensionality reduction analysis, patient survival analysis, differential expression analysis, profiling, and graphing.<sup>[55]</sup> We used GEPIA for the gene expression profile across all tumor samples and paired normal tissues to analyze the expression of the PCAT1 gene in different tumor samples.

### Integrative Genomics Viewer to Determine Gene Location

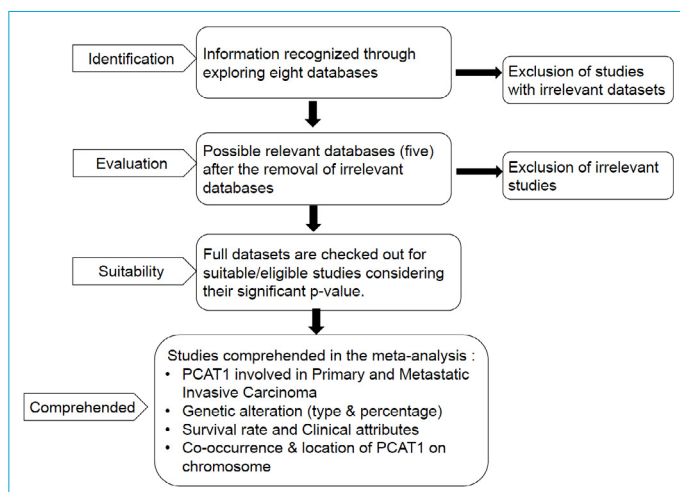
A genome-based database called IGV (<https://igv.org/app/>) contains a variety of data from large genomic data sets. The information includes copy number outlines, the expression profile of coding and non-coding RNAs, the sequences of exomes and genomes, etc. This database can be used to gain a thorough understanding of the genome and establish links with different disorders.<sup>[56]</sup> Here, we utilized this data set to identify the genomic location of PCAT1, CASC8, CASC11, and CASC19 genes.

### Ensembl for Gene Location Overlap

A database called Ensembl (<https://www.ensembl.org>) has genome annotation and can combine and summarize exploratory data with subject genomes. It performs three tasks: interpreting the genome, annotating the vertebrate subphylum, and carrying out the analysis in accordance with predetermined guidelines.<sup>[57]</sup> Using this data set, we analyzed the genes which overlap with the PCAT1 gene.

### ENCORI Data Set for lncRNA-RNA Interaction

ENCORI, formerly known as starBase v2.0, is a database that



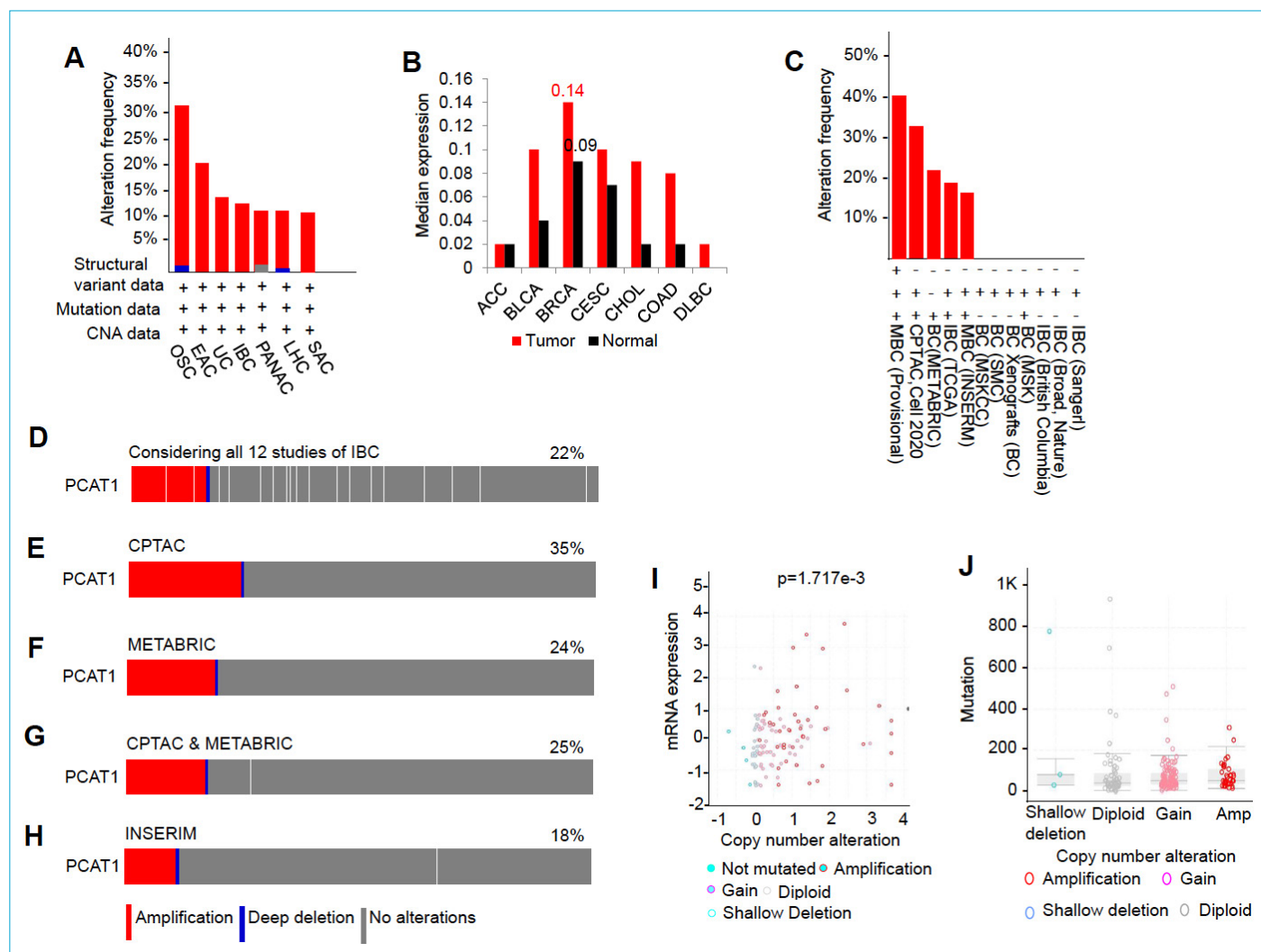
**Figure 1.** Flow chart showing the selection procedure of comprehended studies.

can assist in indicating the relationships between 37 distinct studies and RNA-RNA (sncRNA-RNA, lncRNA-RNA, and mRNA-RNA) and protein-RNA interaction networks, pan-cancer analysis, and miRNA-target interaction.<sup>[58]</sup> We used this database to analyze the co-expression of PCAT1 with CASC8, CASC11, and CASC19.

### Human Protein Atlas to Analyze the Histopathology

Beginning in 2003, the Human Protein Atlas ([https://www.](https://www.proteinatlas.org/)

proteinatlas.org/) project aimed to provide a comprehensive picture of all human proteins found in cells, tissues, and organs by combining multiple omics techniques, including transcriptomics, mass spectrometry-based proteomics, antibody-based imaging, and systems biology. It is divided into ten sections, each concentrating on a distinct aspect of the genome-wide investigation of human proteins.<sup>[59]</sup> We explored this program to understand the histopathology of grade 1, grade 2, and grade 3 IDC cells.



**Figure 2.** Alteration frequency and gene expression profile for different types of cancers. **(a)** Alteration frequency of PCAT1 in detailed cancer types from TCGA pan-cancer atlas dataset. Here, OSC: Ovarian Serous Cystadenocarcinoma, EAC: Esophageal Adenocarcinoma, UC: Uterine Carcinosarcoma, IBC: Invasive Breast Carcinoma, PANAC: Pancreatic Adenocarcinoma, LHC: Liver Hepatocellular Carcinoma, SAC: Stomach Adenocarcinoma. **(b)** The median PCAT1 expression profile over all tissue samples of different cancer types and normal tissues of respective organs. Here, ACC: Adrenocortical carcinoma, BLCA: Bladder urothelial carcinoma, BRCA: Breast invasive carcinoma, CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma, CHOL: Cholangio carcinoma, COAD: Colon carcinoma, DLBC: Lymphoid neoplasm diffuse large B-cell lymphoma **(c)** Alteration frequency of PCAT1 from 12 studies. **(d)** Percentage of PCAT1 gene alteration in the Invasive Breast Cancer patients. **(e)** Percentage of PCAT1 gene alteration from OncoPrint considering CPTAC. **(f)** Percentage of PCAT1 gene alteration from OncoPrint considering METABRIC **(g)** Percentage of PCAT1 gene alteration from OncoPrint considering combined study of CPTAC and METABRIC **(h)** Percentage of PCAT1 gene alteration from OncoPrint considering INSERM dataset. **(i)** The relation of the z-scores of mRNA expression and copy number alteration(CAN) of PCAT1 and **(j)** CNA of PCAT1 among patients with metastatic breast cancer.

## Results

### Genetic Alterations and Expression Analysis of the PCAT1 Gene in Multiple Cancers

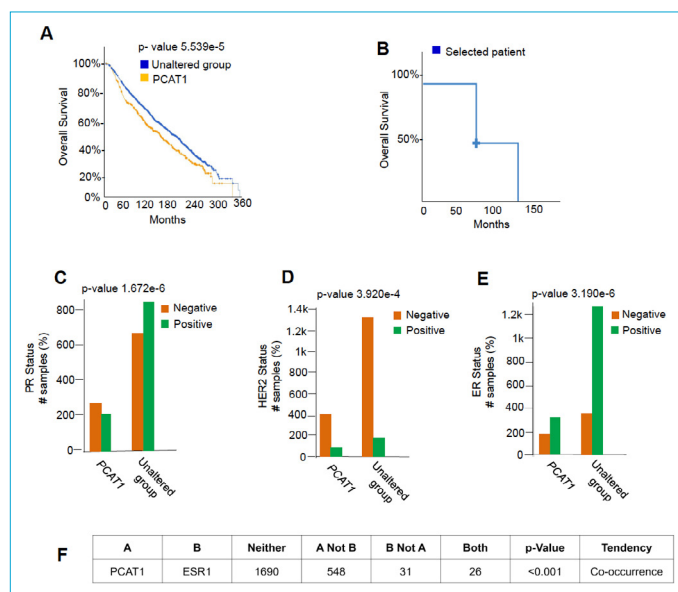
We investigated the genetic modification of PCAT1 using cBioportal. When a pan-cancer atlas dataset comprising 32 distinct cancer types was examined, it was found that 13.38% of IBC patients had genetically modified PCAT1 (Fig. 2A, here the first 7 data sets are shown in the image). A significant copy number alteration (CNA) i.e., amplification, was noticed throughout the data sets of different cancers. We have further utilized GEPIA to explore the transcriptional expression of PCAT1 in BRCA-mutated BC patients. Data revealed that PCAT1 was highly expressed (1.56-fold; BRCA-0.14, normal cell- 0.09) compared to normal cells (Fig. 2B). Based on these findings, we considered the PCAT1 for further analysis in IBC.

### Genetic Alterations of PCAT1 in IBC

We analyzed 12 human studies (4746 samples) (Fig. 2C) of IBC from cBioportal which were Provisional<sup>[43]</sup>, CPTAC<sup>[41]</sup> METABRIC<sup>[42]</sup>, TCGA<sup>[44]</sup>, INSERM<sup>[45]</sup>, MSK<sup>[46]</sup>, SMC 2018<sup>[47]</sup>, British Columbia<sup>[48]</sup>, MSKCC<sup>[49]</sup>, British Columbia<sup>[50]</sup>, Broad<sup>[51]</sup>, and Sanger<sup>[52]</sup> to calculate the percentage of patients having a PCAT1 alteration. We found that PCAT1 amplified in a considerable number of patients in the first five studies which were 39.58%, 35.25%, 24.39%, 19.71%, and 17.59% respectively. Besides, in the Oncoprint data of 12 studies, (Fig. 2D) the PCAT1 gene showed amplification in 22% of IBC patients. Among the first five of the 12 studies, we considered two studies, i.e., METABRIC<sup>[42]</sup> and CPTAC<sup>[41]</sup> (2,631 samples) where patients were diagnosed with primary BC<sup>[41,42]</sup>. Besides, we investigated INSERM<sup>[45]</sup> data which considered only metastatic tumors<sup>[45]</sup>. We analyzed the genetic alterations, survival rates, clinical outcomes, and the tendency of co-occurrence of PCAT1 with other oncogenes in patients with primary and metastatic BC.

### PCAT1 Amplified in Primary and Metastatic BC

CPTAC<sup>[41]</sup> and METABRIC<sup>[42]</sup> studies demonstrated that PCAT1 amplified in 35% (Fig. 2E) and 24% (Fig. 2F) of primary BC patients, respectively. Additionally, considering these two studies (Fig. 2G) at a time we found amplification of PCAT1 in 25% of patients. Besides, 18% of metastatic BC patients showed amplification (Fig. 2H) upon analyzing the INSERM<sup>[45]</sup> data set. The Z-score of the mRNA expression data (Fig. 2I) from the CPTAC<sup>[41]</sup> and METABRIC<sup>[42]</sup> datasets represented a significant ( $p$ -value  $1.717 \times 10^{-3}$ ) relation of CNA of the PCAT1 gene. Moreover, the INSERM<sup>[45]</sup> data represented the CNA of PCAT1 among patients with metastatic BC (Fig. 2J).



**Figure 3.** (a) The overall survival (months) curve for altered and unaltered group considering CPTAC and METABRIC, combined studies. (b) The Kaplan-Meier curve of overall survival (months) of selected patients with PCAT1 alteration. (c) Relation of PCAT1 gene expression with progesterone receptor (PR) status, (d) Relation of PCAT1 gene expression with human epidermal growth factor receptor 2 (HER2) status, and (e) Relation of PCAT1 gene expression with estrogen receptor (ER) status in Invasive Breast Cancer patients considering CPTAC and METABRIC combined studies. (f) Represented the mutual exclusivity of ESR1 with PCAT1 in CPTAC and METABRIC combined studies.

### Poor Survival Rate Observed in Patients, Diagnosed with Primary BC with an Alteration of PCAT1

We compared the overall survival curves for patients with amplified PCAT1 genes and patients without PCAT1 alterations (Fig. 3A). The patients who were suffering from primary BC with amplified PCAT1 gene had a median survival of 132.33 months ( $p$  value  $5.539 \times 10^{-5}$ ) as compared to a median survival of 167.93 months of patients without PCAT1 alterations. This data indicates that the amplification of PCAT1 significantly reduced the overall survival rate of patients with primary BC. The Kaplan-Meier plot showed a downward trend of survival rate with a progression of months while PCAT1 was overexpressed (Fig. 3B). However, the survival rate in the case of metastatic BC was not investigated in INSERM<sup>[45]</sup> data set.

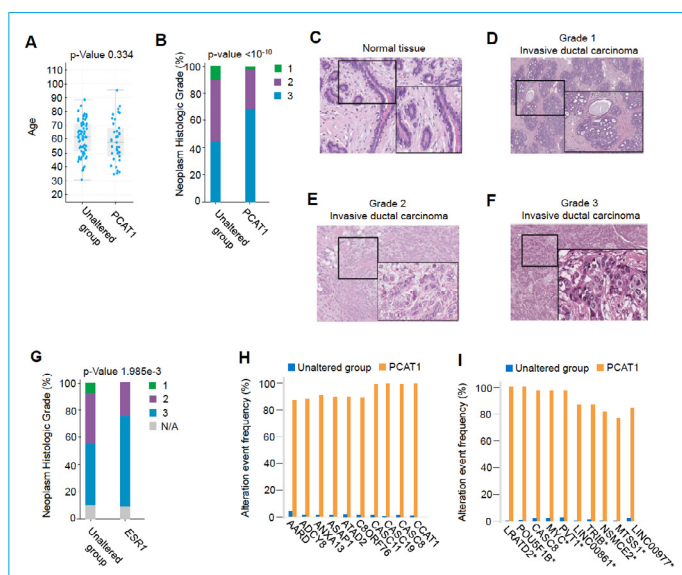
### PCAT1 Gene Expression is Related to ER, PR, and HER2 Expression of Patients

The clinical attributes from cBioportal revealed that the PCAT1 significantly altered in PR ( $p$ -value  $1.672 \times 10^{-6}$ ) (Fig. 3C) and HER2 ( $p$ -value  $3.920 \times 10^{-4}$ ) (Fig. 3D) negative as well as ER ( $p$ -value  $3.190 \times 10^{-6}$ ) (Fig. 3E) positive primary BC samples. However, similar clinical attributes were not present in the

INSERM Cancer [45] dataset for cases of metastatic tumors. PCAT1 expressions co-occur significantly ( $p < 0.001$ ) with Estrogen Receptor 1 (ESR1) expressions (Fig. 3F). The co-occurrence of PCAT1 with ESR1 was not anticipated and led us to investigate the relationship of this lncRNA related to patient age and histologic grade.

### PCAT1 Was Not Correlated with Patient Age While Showing a Positive Correlation with Neoplasm Histologic Grade of Tumor

While the investigation of the primary BC data set was conducted using cBioportal, there is no significant correlation of PCAT1 expression with patient age (Fig. 4A). However, this relation was not evaluated for the INSERM [45] dataset. In addition, we analyzed the correlation between the PCAT1 expressions with neoplasm histologic grades 1, 2, and 3 which can differentiate between normal and cancerous cells. Also, it was found that, in primary BC samples with different grades of tumor, the PCAT1 amplified significantly (68.42%,  $p$ -value  $> 10^{-10}$ ) in grade 3 cancer compared to grade 2 and 1 tumors (28.85% and 2.73% expression, respectively; Fig. 4B). These results indicated that the PCAT1 gene altered mostly in grade 3 histological grade of cancer cells. (Fig. 4C-4F) represented the histopathology of normal

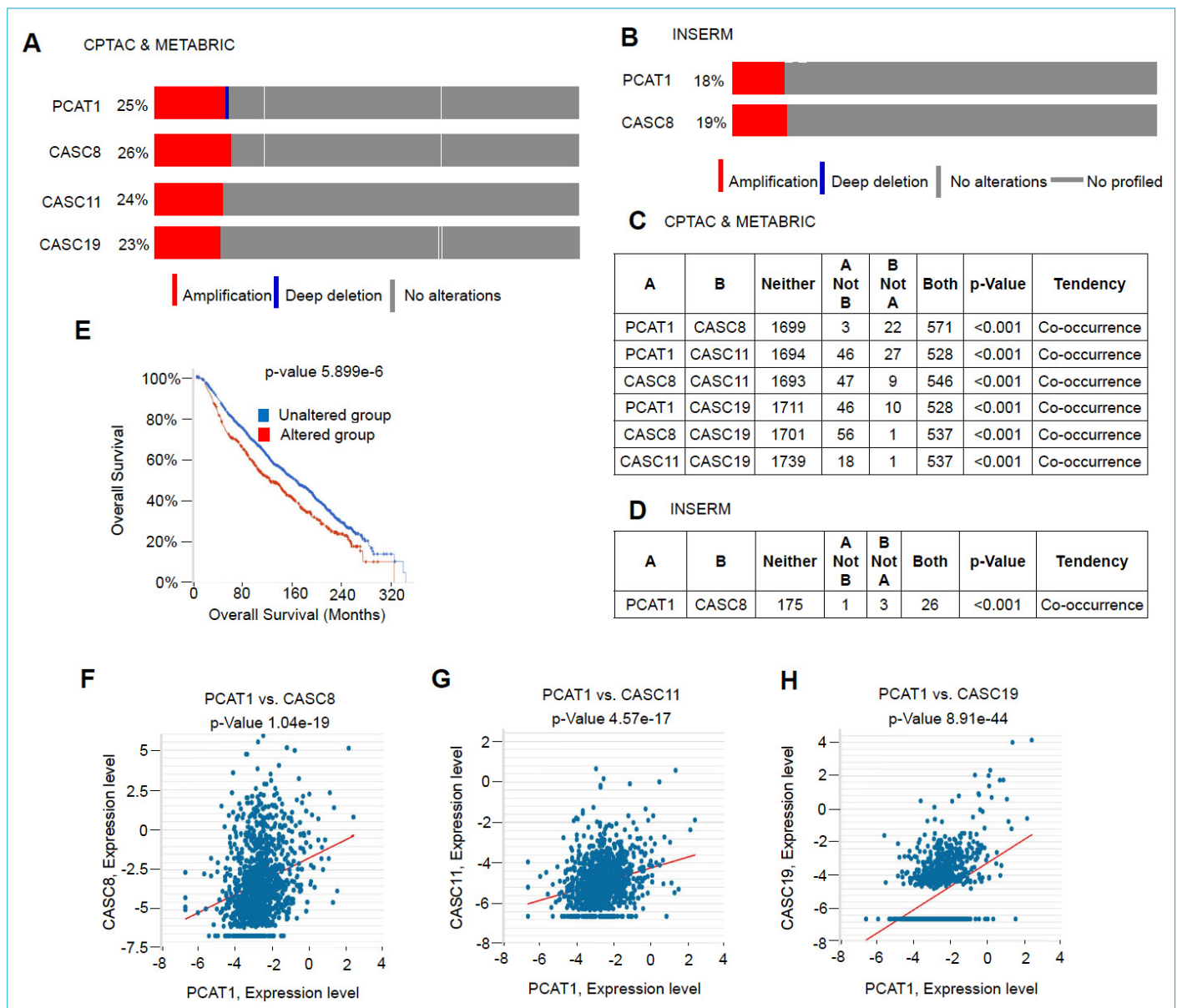


**Figure 4.** (a) Relation between PCAT1 gene expression and age of patient considering CPTAC and METABRIC (b) Relation between Neoplasm histologic grade and alteration of PCAT1 gene considering CPTAC and METABRIC. (c-f) The histopathology of normal tissue, grade 1, grade 2, and grade 3 Invasive ductal carcinoma cells respectively. (g) Relation between Neoplasm histologic grade and alteration of PCAT1 gene. (h) Analysis of global genes associated with alteration of PCAT1 gene in primary breast cancer considering CPTAC and METABRIC combined studies. (i) Analysis of global genes associated with alteration of PCAT1 gene in metastatic breast cancer considering INSERM data set.

tissue, grade 1, grade 2, and grade 3 invasive ductal carcinoma cells respectively. We also investigated the relation of ER expression to the neoplastic histologic grade of primary BC from the CPTAC [41] and the METABRIC [42] datasets. As shown in Fig. 4G in primary BC samples with grade 3, 2, and 1 tumors, the ESR1 amplified significantly ( $p = 1.985 \times 10^{-3}$ ) in 45.08 %, 37.40%, and 7.77 % samples respectively. These results indicated that similar to PCAT1, ER also altered in the highest percentage of samples with grade 3 cancer cells. This finding supported the notion of the co-occurrence of PCAT1 and ER in IBC.

### PCAT1 Gene Amplification Co-occurs with CASC8, CASC11, and CASC19 Oncogenes

To find the co-occurrence of PCAT1 expression with other oncogenes, we analyzed cBioportal for the genes with the highest genetic alterations (considering genes with most significant  $p$ -values) in primary and metastatic BC patients. Our findings demonstrated that CASC8 altered in both primary and metastatic BC patients ( $\sim 100\%$ ) (Fig. 4H, Fig. 4I). In primary BC patients, the other two subgroups of the CASC family (CASC11 and CASC19) showed the highest alterations (100%). Moreover, genetic amplifications of PCAT1 (25%), CASC8 (26%), CASC11 (24%), and CASC19 (23%) were observed from Oncoprint with the same data set of primary BC (Fig. 5A). Similarly, we explored the alteration of CASC8 (Fig. 5B) for metastatic cancer patients where the percentage for PCAT1 and CASC8 were 18% and 19% respectively. Concurrent genetic alteration of genes indicates that they may be associated with distinctive cancer subtypes [11]. To identify mutual exclusivity and co-occurrence of the CASC subfamily (CASC8, CASC11, and CASC19) and the PCAT1 gene we queried the same data set of primary and metastatic BC from cBioportal. CASC8, CASC11, and CASC19 are previously known to have a relation with BC as well as worked as biomarkers for different types of cancers including colorectal, and esophageal [60-63]. The CASC8, CASC11, and CASC19 significantly ( $p < 0.001$ ) co-occurred with PCAT1 (Fig. 5C) in primary BC. Similarly, the CASC8 co-occurs significantly ( $p < 0.001$ ) with PCAT1 in metastatic BC (Fig. 5D). In addition, we investigated the overall survival of primary BC patients with concurrent alteration in PCAT1, CASC8, CASC11, and CASC19. Figure 5E shows. It can be observed that the patients with alterations of these genes have a median survival period of 125.60 months ( $p = 5.8 \times 10^{-6}$ ) as compared to the median survival period ( $\sim 168.97$  months) of patients without alterations of these genes. This is indicative that the co-occurring alterations of



**Figure 5.** Mutual exclusivity and co-occurrence among PCAT1 and some other genes involved in Primary Breast Cancer. **(a)** Alteration frequency of PCAT1, CASC8, CASC11 and CASC19 genes involved in primary breast cancer considering combined studies CPTAC and METABRIC. **(b)** Alteration frequency of PCAT1 and CASC8 genes involved in metastatic breast cancer considering INSERM data set. **(c, d)** represents the mutual exclusivity of genes in primary (PCAT1, CASC8, CASC11 and CASC19) and metastatic cancer (PCAT1 and CASC8) respectively. **(e)** The overall survival (months) curve for unaltered and altered group of PCAT1, CASC8, CASC11, and CASC19 genes. Co-expression of PCAT1 gene with CASC8, CASC11, and CASC19 genes using ENCORI data set. **(f)** Co-expression of PCAT1 gene with CASC8. **(g)** Co-expression of PCAT1 gene with CASC11. **(h)** Co-expression of PCAT1 gene with CASC19.

PCAT1 with CASC8, CASC11, and CASC19 significantly reduced the overall survival rate of patients with BC.

Moreover, the ENCOR dataset was used for the analysis of the co-expression of PCAT1 with CASC8, CASC11, and CASC19. The finding represented the significant co-expression of PCAT1 with CASC8 (Fig. 5F, p value 1.04e-19), CASC11 (Fig. 5G; p value 4.57e-17), and CASC19 (Fig. 5H,

p value 8.91e-44). The genome location of PCAT1, CASC8, CASC11, and CASC19 was identified using the Integrative Genomics Viewer (IGV) data (Sup. Fig. A-D). Coincidentally, they are located in the same chromosome (chr8q24) which is known to have BC risk regions [64]. Besides, the Ensembl data was used to extract a graph for the PCAT1 location which indicates the overlapping tendency of PCAT1 with CASC8 and CASC19 (Sup. Fig. A-D). These in-

vestigations lead to an interesting conclusion of the co-occurrence of PCAT1 with the other three oncogenes and biomarkers of cancers (CASC8, CASC11, and CASC19). The coincidental similarity of the gene location PCAT1 to the other three oncogenes is suggestive of having the highest possibility of PCAT1 acting as a prognostic biomarker in IBC.

## Discussion

Cancer statistics show that cancer has been a deadly disease from earlier times to the present.<sup>[1,65]</sup> Gene alterations ultimately result in alterations in genetic expression, which then triggers the emergence of aggressive malignancies. Furthermore, the primary cause of both invasiveness and metastasis in BC is the variable genetic expression of the transcriptome at each stage of the disease.<sup>[66]</sup> This interrelation of alternative expression of a gene within various cases characterizes the prognostic biomarkers.<sup>[66]</sup> A lncRNA called PCAT1 is responsible for several processes that lead to the growth of malignancies. Prostate cancer, breast cancer, hepatocellular carcinoma, colorectal cancer, gastric cancer, esophageal cancer, osteosarcoma, non-small cell lung cancer, bladder cancer, cervical cancer, multiple myeloma, and other cancers are known to be associated with aberrant expressions of PCAT1.<sup>[22]</sup> The goal of this analysis was to quantify the expression, genetic alteration, and correlation of PCAT1 with CASC family members to prove the possibility as a prognostic biomarker in IBC using various genomic tools. We discovered that a significant proportion of patients with IBC had PCAT1 amplification by examination of the TCGA pan-cancer atlas. The GEPIA dataset indicates that PCAT1 expression was higher in BC cells with a BRCA mutation than in normal cells. However, the overexpressed PCAT1 gene is known to cause invasion, migration, proliferation, metastasis, and resistance to apoptosis in BC, similar to other malignancies, suggesting a worse prognosis for patients.<sup>[21]</sup> In our analysis, we explored the percentage of alteration of PCAT1 in IBC patients using the cBioportal data, where significant amplification had been identified in five studies including both primary and metastatic cancer patients. The amplification of PCAT1 is not the only variable responsible for the total process of IBC development. Given the PCAT1 gene status at different stages of cancer, it may be used as a biomarker for BC before the metastatic stage.<sup>[21]</sup> Therefore, we analyzed the patients with primary BC from the CPTAC<sup>[41]</sup> and METABRIC<sup>[42]</sup> datasets for genetic alterations of PCAT1, the overall survival rate, correlation with clinical outcomes like ER, PR, HER2 status, neoplasm histologic grade, and age of patients. Moreover, we found genetic alterations in patients with metastatic BC from IN-

SERM.<sup>[45]</sup> We also used the Human Protein Atlas dataset<sup>[59]</sup> to represent the histopathology of three grades of IDC cells.

A significant proportion of patients with primary BC had elevated PCAT1 expression, and those with modified PCAT1 gene had a much lower overall survival rate. While PCAT1 modification was strongly correlated with different tumor grades, with the highest percentage of patients with grade 3 tumors showing PCAT1 alterations, there was no significant link detected between the age of patients in the altered and unaltered groups. Three grades of cancer cells, named grade 1, grade 2, and grade 3, can be helpful to distinguish between normal and cancerous cells. There is less difference between the grade 1 (grows slowly) cancer cells and normal cells, but the difference is higher in the case of grade 2 (grows faster than grade 1) cancer cells. However, the grade 3 (expands more destructively) cancer cell shows the highest difference between normal and cancer cells.<sup>[4]</sup> PCAT1 alternations have been reported to have a correlation with pathological grade and tumor size in BC patients through in vivo studies.<sup>[23]</sup> Additionally, in our analysis, the expression of PCAT1 was significantly higher in patients with ER positive, PR, and HER2 negative patients, where PCAT1 showed a significant co-occurrence tendency with alteration of ER. Moreover, the PCAT1 revealed considerable amplification in metastatic BC patients, as understood from cBioportal.

We also investigated the correlation and co-occurrence of PCAT1 expression with three genes in the cancer susceptibility candidate (CASC) family, CASC8, CASC11, and CASC19. These three genes are known to be related to BC and act as biomarkers of different cancers.<sup>[60-63]</sup> Analyzing the same dataset, we discovered from the Oncoprint analysis that a significant proportion of patients with primary BC had amplification of CASC8, CASC11, and CASC19 with PCAT1. PCAT1 and CASC8 amplification was also present in a considerable number of patients with metastatic BC. Moreover, PCAT1 tends to co-occur significantly ( $p < 0.001$ ) with CASC8, CASC11, and CASC19 in primary cancer patients as well as CASC8 in metastatic BC patients. The overall survival rate in primary BC patients decreased significantly ( $p = 5.899e-6$ ) having amplification of the mentioned multiple genes. CASC8 is another lncRNA located in the 8q24 region and performs a crucial role in the cMyc regulation. Here, single nucleotide polymorphisms (SNPs) like rs7837328, rs6983267, and rs7014346 are mainly responsible for various cancer development, e.g., prostate, breast, colorectal, and gastric cancers.<sup>[60,61]</sup> CASC11 and CASC19 are also located in the same chromosomal region (8q24) as CASC8 and show identical mechanisms for BC progression.<sup>[62,63]</sup> Interestingly, the location of PCAT1 is also identified in the region of 8q24 of the chromosome.<sup>[35]</sup> Previous studies



revealed that risk loci for different epithelial cancers (i.e., colon, breast, and prostate) are situated in the 8q24 region, and various enhancer elements of these risk loci interact with MYC resulting in cancer development.<sup>[64]</sup> In our study, the Ensembl data showed the overlapping of the PCAT1 gene with the CASC8 and CASC19 genes. In addition, the expression of PCAT1 and CASC8, CASC11, and CASC19 co-occurred according to the ENCORI data. Considering these findings, it can be stated that PCAT1 has the potential to become a prognostic biomarker for IBC patients, and further studies are required to understand the role of PCAT1 in BC progression and its co-occurrence with members of the CASC family.

## Conclusion

This analysis serve as pivotal tools in consolidating evidence from multiple studies, enabling a comprehensive assessment of PCAT1's prognostic significance in breast carcinoma. These analyses involve pooling data from various research cohorts, applying statistical models to evaluate consistency, and determining the overall impact of PCAT1 expression on clinical outcomes. The current analysis investigating PCAT1 in invasive breast carcinoma highlight its potential as a prognostic biomarker. However, continued research efforts aimed at unraveling its molecular mechanisms and conducting robust clinical validations are essential before considering its integration into routine clinical practice for improved patient management.

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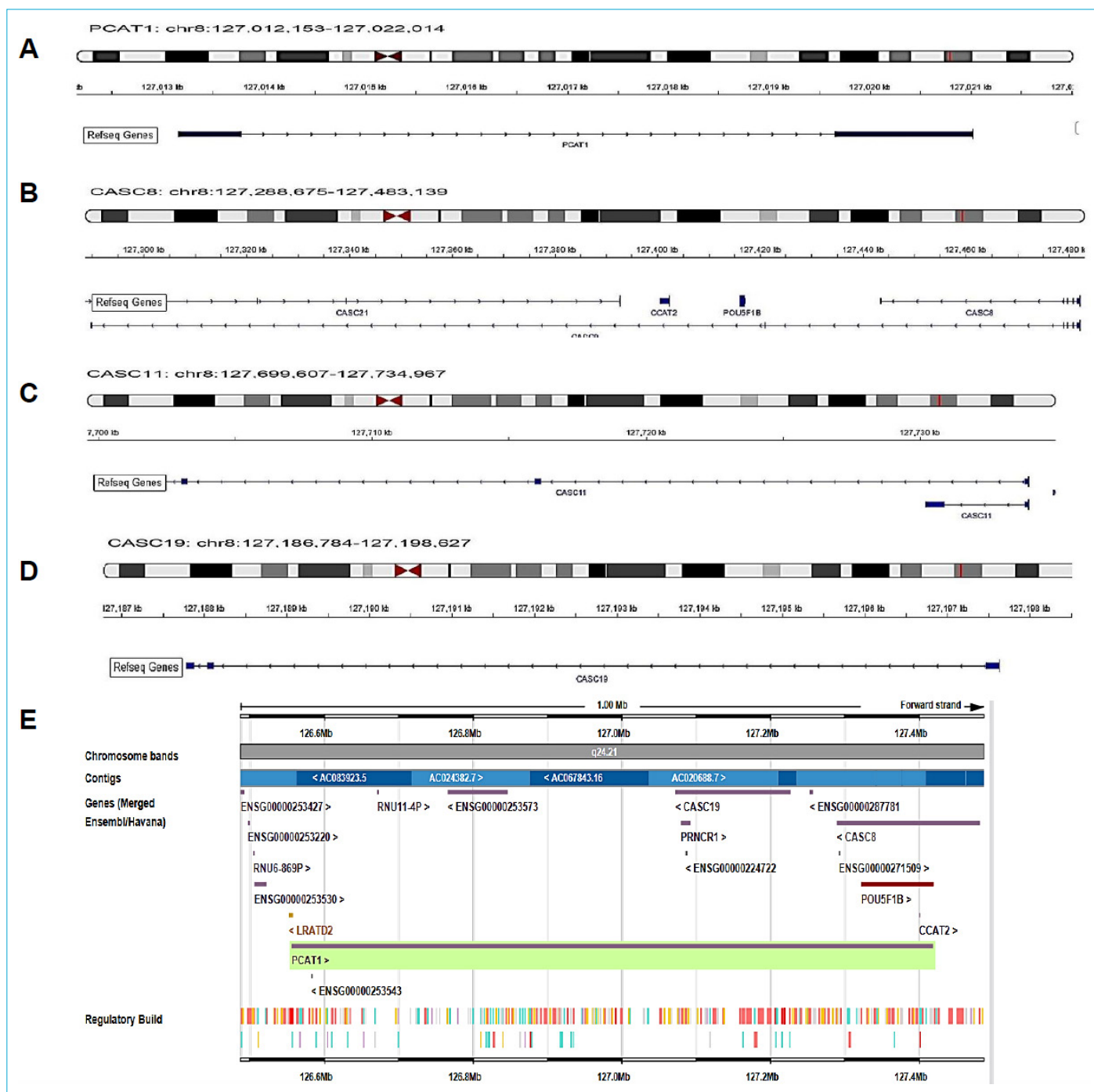
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**Supporting Figure 1.** The Genome location of PCAT1, CASC8, CASC11, and CASC19 genes using IGV and Ensembl data. (a) Genome location of PCAT1 using IGV data. (b) Genome location of CASC8 using IGV data. (c) Genome location of CASC11 using IGV data. (d) Genome location of CASC19 using IGV data. (e) The overlapping of CASC8 & CASC19 genes together with PCAT1 gene using Ensembl data.