ESR1 Pvull polymorphism: from risk factor to prognostic and predictive factor of the success of primary systemic therapy in advanced breast cancer

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Abstract

Background: The ESR1 gene encodes Estrogen Receptor alpha (ERα), which plays a role in the tumorigenesis of breast cancer. A single nucleotide polymorphism (SNP) in intron 1 of this gene called ESR1 Pvull (rs2234693) has been reported to increase the risk of breast cancer. This study aimed to investigate the ESR1 Pvull polymorphism as a prognostic and predictive factor guiding the choice of therapy for advanced breast cancer.

Methods: This retrospective study was conducted in 104 advanced breast cancer patients at Dharmais Cancer Hospital from 2011 to 2018. The ESR1 Pvull polymorphism was analysed by Sanger sequencing of DNA from primary breast tumour samples.

Results: The percentages of patients with ESR1 Pvull genotypes TT, TC, and CC were 42.3, 39.4, and 18.3%, respectively. Looking at prognosis, patients with ESR1 Pvull TC + CC had shorter overall survival than those with the TT genotype [HR = 1.79; 95% CI 1.05–3.04; p = 0.032]. As a predictive marker, TC + CC was associated with shorter survival (p = 0.041), but TC + CC patients on primary hormonal therapy had a median overall survival longer than TC + CC patients on primary chemotherapy (1072 vs 599 days).

Conclusion: The ESR1 Pvull TC + CC genotypes confer poor prognosis in advanced breast cancer, but these genotypes could be regarded as a good predictor of the therapeutic effect of hormonal treatment.

Keywords: ESR1 Pvull, Breast cancer, Hormonal, Chemotherapy, Indonesia

Background

Breast cancer is the most common cancer in women and is a heterogeneous disease based on several molecular subtypes by immunohistochemistry, epidemiological risk, and response to treatment [1]. In each individual with breast cancer, there is a set of genetic aberrations that can be informative in identifying their risk, choosing their therapy, and making a prognosis. Information on genetic aberrations in cancer will lead to more precise treatments [2].

Over two-thirds of breast cancers express estrogen receptor α protein (encoded by ESR1) which plays a role in the tumorigenesis of breast cancer [3, 4]. Recent, retrospective analyses of ESR1 mutations in circulating tumour DNA suggested that the occurrence of the mutations was associated with poor overall survival and resistance to hormonal treatment in patients with metastatic...
disease [5]. The majority of these mutations are located in exon 8, within the ligand-binding domain (LBD), and create a ligand-free constitutively activated ER, which has been associated with a worse outcome and could be considered a predictive marker guiding therapeutic decision making [6, 7].

An intronic polymorphism in the ESR1 gene (rs2234693), also called ESR1 PvuII, is associated with an increased risk of breast cancer and decreased estrogen receptor (ER) expression [3]. Recent data from several studies have garnered interest in investigating the potential role of ESR1 mutational status as a predictive marker and a tool to guide clinicians in choosing therapies, but there are many limitations to developing predictive biomarkers [6]. In this study, we investigated ESR1 PvuII as a prognostic and predictive factor for the selection of therapy in advanced breast cancer.

Methods
Study design and patients
This was a retrospective study that included 104 consecutive advanced breast cancer patients who had been treated between 2011 and 2018 in Dharmais Cancer Center Hospital. Advanced breast cancer included both locally advanced disease (stage 3B and 3C) and metastatic breast cancer with distant metastases to other organs, commonly the skeleton, lung, brain, and liver [8]. Patients were included who met the inclusion criteria and had complete data on both tissue characteristics and follow-up status. Fresh tissue was taken before primary systemic treatment. Patients with complete treatment were those who were given primary hormonal therapy for 6 months or primary chemotherapy within 6 cycles.

Therapeutic options were chosen based on the treatment protocol in the NCCN guidelines [9]. The agents available to the primary hormonal therapy group were Aromatase Inhibitor (AI) and Tamoxifen for postmenopausal patients and premenopausal patients, respectively. The patients, received Tamoxifen only or bilateral salpingo-oophorectomy (BSO) plus AI/Tamoxifen, or if patients rejected BSO they were given Gonadotropin-Releasing Hormone Analogue (GnRHa) and AI/Tamoxifen for 6 months. The AI was Letrozole, Anastrozole, or Exemestane.

The primary chemotherapy group received FAC (5-Fluourouracil, Adriamycin, and Cyclophosphamide) which was given for 6 cycles. In this study, patients with HER2+ cancer did not receive anti-HER2 agents. The patients provided written informed consent to participate in the study, which was approved by the Ethics Committee of Dharmais Hospital (ethical clearance numbers 049/PEP/08/2011 and 199/KEPK/XI/2019).

Mutational analysis
DNA samples from primary breast tumours were processed by Polymerase Chain Reaction (PCR) using the ESR1 forward primer TGT AAA ACG ACG GCC AGT TCA CGC AGT CTG GAG TTG TC and reverse primer CAG GAA ACA GCT ATG ACC AGA CCA ATG CTC ATC CCA AC. The total product was 519bp, which was sequenced by Sanger sequencing with BigDye v3.1 reagent [Applied Biosystems]. Sequence data were analysed using Bioinformatics Software [Seqscape] and combined with clinicopathological data. The sequenced of ESR1 PvuII Polymorphism (Fig. 1) was divided into wild-type (TT variant), TC variant, and CC variant.

Statistical analysis
Statistical analysis was performed using IBM SPSS 21. Associations between ESR1 PvuII polymorphism and clinicopathological variables were assessed by the chi-square test ($\chi^2$ test). All analyses were hypothesis-driven, and $P < 0.05$ was considered statistically significant. Overall survival (OS) was defined as the time from diagnosis until death from any cause. OS rates were estimated using the Kaplan-Meier method. A Cox proportional hazards model was used to estimate the prognostic value of ESR1 PvuII Polymorphism on overall survival (OS). To estimate the predictive factor of genotypes on OS, hazard ratios (HRs) with 95% confidence interval (CIs) were calculated for primary hormonal therapy vs. primary chemotherapy in the TT and TC + CC groups.

Results
Correlation of ESR1 PvuII genotype with survival
Correlations type of ESR1 PvuII polymorphism with treatment effect and survival
In the TT group, patients who underwent primary hormonal therapy had a median OS of 1375 days (95% CI, 983–1766 days) compared with 951 days for patients who underwent primary chemotherapy (Fig. 3A). There was a significant difference in survival in the TC + CC variant group (Fig. 3B), as these patients survived longer after primary hormonal therapy than primary chemotherapy (1072 vs. 599 days).

Discussion
Breast cancer in Indonesia, as in other developing countries, is disregarded and mostly diagnosed late, at stages 3 and 4, at which time the patient has a low life expectancy [10]. The persisting issue in a clinical setting of advanced breast cancer is the type of therapy give. Currently, oncologists routinely apply the clinical TNM staging system and detect the ER, progesterone receptor (PR), and
Her2 proteins in the tumour cells [9]. Most clinicians tend to give chemotherapy as soon as possible and ignore the ER/PR as the hormonal status, whose expression remains the lead function in ER/PR positive cases, and but this approach seems to give no extra consideration to the underlying obstacles and benefits [11]. The Cochrane meta-analysis for advanced breast cancer showed no difference in survival between chemotherapy or hormonal therapy and chemotherapy worsened quality of life [12]. Our study found that there was a statistically significant difference survival between patients given hormonal therapy and patients given chemotherapy in the TC + CC genotype group, where hormonal therapy yielded a longer survival than chemotherapy.

Only few prospective studies have compared primary chemotherapy with primary hormonal therapy in advanced breast cancer [12–17]. Treatment selection of advanced breast cancer is based on hormonal receptors at the protein levels rather than at the genotype level, according to the NCCN guidelines HR-positive patients will be given hormonal therapy whereas HR-negative or Her2+ and visceral metastasis patients will receive chemotherapy [9]. Chemotherapy for advanced stages hormone receptor-positive cases breast cancer with visceral metastasis and Her2+ positivity does not prolong life expectancy [12]. Severe side effects of chemotherapy have become a reason to minimize the administration this treatment to patients. A new parameter is needed that can predict which patients should receive chemotherapy or hormonal therapy.

*ESR1* SNPs are associated with tumour carcinogenesis, cell proliferation, metastasis, and prognostic [18–20]. Every woman with breast cancer has the *ESR1* gene but in only 70–80% of breast tumours is ERα expressed, as shown by immunohistochemistry [21]. Several mechanisms have been shown to silence ER expression, such as *ESR1* mutations, polymorphisms, epigenetic events, and posttranslational modification events [22, 23]. Immunohistochemical detection of hormone receptor expression is often a problem in clinical practice (Table 1) [24].

In general *ESR1* Pvull (rs2234693) changes the proteins detectable in blood and has been used as an inherited risk factor for Asian people [25]. This paper does not discuss breast cancer risk factors, but we will discuss further how this SNP is present in tumour tissue and can be used as a predictor of the best therapy. This translational study is the first analysis of a novel genetic predictor that could help us choose between chemotherapy or
hormonal therapy as the primary treatment for advanced breast cancer.

As a prognostic factor, the TT genotype was correlated with longer survival than the TC and CC genotypes (Fig. 2A & B). The risk of death in the TC+CC genotype group was higher than that in the TT genotype group, and the highest risk of death was in the CC genotype subgroup (Table 2). Blood detectable ESR1 mutations in exon 8 after AI failure have been associated with a worse prognosis for overall survival than wild-type ESR1 [6, 26].

This study found a statistically significant difference between the survival of patients given different therapies in the TC+CC genotype group, where the hormonal therapy subgroup had a longer survival than the chemotherapy subgroup (Fig. 3B). Giving chemotherapy to the TC+CC variant group brought a risk of death 2.01 times higher than giving hormonal therapy (Fig. 3B). These finding are in line with Kou's study, which found that the ESR1 PvuII rs2234693 T/T genotype vs. C/T had a better OS when the patients were not given adjuvant chemotherapy [27]. This result is slightly different from others, which have shown that there are no specific benefits of chemotherapy or hormonal therapy in patients with circulating ESR1 exon mutated cells [6, 28].

ESR1 mutations in circulating tumour cells have been used as a predictive factor for breast cancer patients after failure of hormonal therapy [28]. One strength of the current study was genotyped ESR1 before applying the therapy to the primary tissue. The weakness of this study is that the results shown are still lacking in precision,

Table 1  Correlations between ESR1 PvuII polymorphisms and clinicopathological features

| Characteristics          | ESR1 PvuII Polymorphism | P-value |
|--------------------------|-------------------------|---------|
|                          | TT genotype (n = 44)    | TC + CC genotype (n = 60) |
| **Age at biopsy**        |                         |         |
| Mean (± SD)              | 47.5 (9.5)              | 48.1 (10.9) |
| Median (range)           | 48.5 (28–68)            | 47 (22–75) |
| **Grade**                |                         |         |
| Low                      | 19 (36.5)               | 33 (63.5) |
| High                     | 25 (48.1)               | 27 (51.9) |
| **Hormonal Receptor**    |                         |         |
| Negative                 | 9 (34.6)                | 17 (65.4) |
| Positive                 | 35 (44.9)               | 43 (55.1) |
| **ER (Estrogen Receptor)**|                        |         |
| Negative                 | 13 (40.6)               | 19 (59.4) |
| Positive                 | 31 (43.1)               | 41 (56.9) |
| **PR (Progesteron Receptor)**|                       |         |
| Negative                 | 13 (41.9)               | 18 (58.1) |
| Positive                 | 31 (42.5)               | 42 (57.5) |
| **Her2 status**          |                         |         |
| Negative                 | 33 (44.6)               | 41 (55.4) |
| Positive                 | 11 (36.7)               | 19 (63.3) |
| **Histology**            |                         |         |
| Ductal                   | 41 (43.6)               | 53 (56.4) |
| Lobular                  | 3 (30.0)                | 7 (70.0) |
| **Therapy**              |                         |         |
| Primary hormonal therapy | 24 (54.5)               | 29 (48.3) |
| Primary chemotherapy     | 20 (45.5)               | 31 (51.7) |

P value: * = Pearson Chi Square; ** = Independent sample T test

P value: 0.093

**Fig. 2** Kaplan-Meier curves for Overall Survival (OS) according to ESR1 PvuII Polymorphism genotypes. Comparing overall survival between all three genotypes individually (A). Probability of Overall Survival for TT vs TC + CC variants (B)
because confidence intervals (CIs) were quite wide and the number of samples was small, so it will be necessary to investigate these issues in a larger, prospective study. Further study of the mechanisms underlying the better prognosis of patients with different genotypes PvuII rs2234693 is warranted [27].

**Conclusion**

In general, TC + CC variants have a worse prognosis than TT variants. However, hormonal therapy will provide a longer survival rate than chemotherapy to the former subgroup. Our analyses provide compelling evidence that ESR1 PvuII is a novel prognostic marker in breast cancer and is also highly predictive of anticancer therapy outcomes. It could become a predictive factor for first-line hormonal treatment outcomes because the genotype might predict which kind of therapy is expected to be more effective.

**Table 2** Overall Survival by ESR1 PvuII polymorphism alleles

| Group          | No. | Events | OS, Median (95% CI), days | Hazard Ratio (95% CI) | P-value |
|----------------|-----|--------|---------------------------|-----------------------|---------|
| TT variant     | 44  | 22     | 1375 (965–1784) days      | NA                    | NA      |
| TC variant     | 41  | 28     | 730 (237–1222) days       | 1.77 (1.01–3.1)       | 0.046   |
| CC variant     | 19  | 10     | 644 (601–688) days        | 1.85 (0.85–4.01)      | 0.117   |
| TC + CC variants | 60  | 34     | 654 (449–858) days        | 1.79 (1.05–3.04)      | 0.032   |

Abbreviation: NA not applicable

**Abbreviations**

AI: Aromatase inhibitor; BSO: Bilateral salpingo-oophorectomy; CIs: Confidence interval; ER: Estrogen receptor; FAC: 5-fluorouracil, adriamycin, and cyclophosphamide; GnRHa: Gonadotropin-releasing hormone analogue; HRs: Hazard ratios; LBD: Ligand binding domain; NA: Not applicable; NACT: Neoadjuvant chemotherapy; NAHT: Neoadjuvant hormonal therapy; OS: Overall survival; PCR: Polymerase chain reaction; PR: Progesterone receptor; SD: Standard deviation.

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**Authors’ contributions**

R.K conceptualized and designed the study, and was a major contributor to data interpretation and manuscript writing. S.J.H, B. K, W.A.H, and T. A contributed manuscript writing. Y. P coordinated the data input and did a significant amount of the work on statistical analysis, data interpretation, and manuscript writing. All authors read, contributed to, and approved the final manuscript.

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Availability of data and materials
The datasets for this manuscript are not publicly available because they contain personal patient information and the data belong to the Dharmais Cancer Hospital. Requests for data must be directed to [Ramadhan Karsono, ramadhan@dharmais-surgonc.com].

Declarations

Ethics approval and consent to participate
All procedures performed in studies involving human participants were performed per the ethical standards of the institution with written and verbal informed consent. The number of ethical clearances was 049/PEP/08/2011 and 199/KEPK/XI/2019 by Dharmais National Cancer Center Hospital. Hospital administrative permission is required to access raw data, this is included in the application for ethical approval. Ethical approval was given by the chairman and the ethics review team of Dharmais National Cancer Center Hospital, where this research was known and approved by the head of research and development and the main director of the hospital.

Consent for publication
Not Applicable.

Competing interests
The authors declare no conflict of interest.

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References
1. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn H-J, et al. Strategies for subtypes-dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol Off J Eur Soc Med Oncol [Internet]. 2011 Aug [cited 2019 Apr 15];22(8):1736–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21709140.
2. Roychowdhury S, Iyer MK, Robinson DR, Lonigro RJ, Wu Y-M, Cao X, et al. Personalized oncology through integrative high-throughput sequencing: a pilot study. Sci Transl Med 2011;3(111):1–20.
3. Markiewicz A, Welnicka-Jaśkiewicz M, Skokowski J, Jaśkiewicz J, Szade J, Jassene J, et al. Prognostic Significance of ESRI Amplification and ESRI PvuII, CYP2C19P, UGT2B15*2 Polymorphisms in Breast Cancer Patients. Medeiros R, editor. PLoS One [Internet]. 2013 Aug 8 [cited 2019 Apr 16];8(e272219. Available from: https://dx.doi.org/10.1371/journal.pone.0072219.
4. Robinson DR, Wu Y-M, Vats P, Su F, Lonigro RJ, Cao X, et al. Activating ESRI mutations in hormone-resistant metastatic breast cancer. Nat Genet. 2013;45(12):1446–51.
5. Jeselsohn R. Are we ready to use ESRI mutations in clinical practice? Breast Care [Internet]. 2012;7(5):309–13 Available from: https://www.karger.com/Article/FullText/481428.
6. Reinert T, Saad ED, Barrios CH, Bines J. Clinical implications of ESRI mutations in hormone receptor-positive advanced breast cancer. Front Oncol. 2017;7(March):1–9.
7. Niu J, Andres G, Kramer K, Kundsanda M, Alvarez R, Klimant E, et al. Incidence and clinical significance of ESRI mutations in heavily pretreated metastatic breast cancer patients. Onco Targets Ther [Internet]. 2015 Nov 11 [cited 2019 Apr 18];8:3323. Available from: https://www.dovepress.com/incidence-and-clinical-significance-of-esri-mutations-in-heavily-pretreated-breast-cancer-an-update-to-systemic-thera.
8. Carson E, Dear R. Advanced breast cancer: An update to systemic therapy. Aust J Gen Pract [Internet]. 2019 May 1 [cited 2020 May 11];48(5):278–83. Available from: https://www1.racgp.org.au/apgg/2019/may/advanced-breast-cancer-an-update-to-systemic-thera.
9. NCCN. NCCN Clinical Practice Guidelines in Oncology: Breast Cancer. Netw Natl Compr Cancer. 2018;1.
10. Wido I, Dwianingsih EK, Triningsih E, Utomo T, Seroptio. Clinicopathological features of Indonesian breast cancers with different molecular subtypes. Asian Pacific J Cancer Prev. 2014;15. Spring LM, Gupta A, Reynolds KL, Gadda MA, Ellisen LW, Isakov SJ, et al. Neoadjuvant endocrine therapy for estrogen receptor-positive breast cancer: a systematic review and meta-analysis. JAMA Oncol. 2016;2(11):1477–86.
11. Semiglazov VF, Semiglazov VW, Dashyan GA, Ziltsova EK, Ivanov VG, Bozhok AA, et al. Phase 2 randomized trial of primary endocrine therapy versus chemotherapy in postmenopausal patients with estrogen receptor-positive breast cancer. Cancer. 2007;110(2):244–54.
12. Akhsan S, Aryanando T. Prognostic factors of locally advanced breast cancer patients receiving neoadjuvant and adjuvant chemotherapy. Asian Pac J Cancer Prev. 2010;11(3):759–761. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20139049.
13. Wilcken N, Hornbuckle J, Ghersi D. Chemotherapy alone versus endocrine therapy alone for metastatic breast cancer. Cochrane Database Syst Rev. 2003(2):CD002747. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12804433.
14. Alba E, Calvo L, Albanel J, De la Haba Jr, Arcusa Lanoza A, Chacon J, et al. Preoperative Neoadjuvant hormonal therapy and Neoadjuvant chemo-therapy for stage 3B and 4 breast Cancer patients in Dharmais hospital-National Cancer Center, Indonesia: a cohort study. Indones J Cancer. 2019;12(4):109.
15. Bozhok AA, Levine K, Santana-Santos L, Benos PV, Wang P, Andersen C, et al. Preoperative Neoadjuvant hormonal therapy and Neoadjuvant chemotherapy for luminal breast cancer patients: results from the GEICAM/2006-03, a multicenter, randomized, phase-II study. Ann Oncol. 2012;23(12):3069–74.
16. Saad ED, Katz A, Buyse M. Overall Survival and Post-Progression Survival in Advanced Breast Cancer: A Review of Recent Randomized Clinical Trials. 2018;28(11).
17. Lu H, Chen D, Hu L-P, Zhou L-L, Xu H-Y, Bai Y-H, et al. Estrogen receptor alpha gene polymorphisms, and breast Cancer risk: a case-control study with Meta-analysis combined. Asian Pacific J Cancer Prev. 2014;14(11):6743–9.
18. Hertz DL, Henry NL, Kidwell KM, Thomas D, Goddard A, Azzouz F, et al. Personalized chemotherapy and activity. Genome Med [Internet]. 2016 [cited 2020 May 11];8(128):1–11. Available from: http://www.gpmrr.pitt.edu/gpmrr.
19. Toy W, Weir H, Razavi P, Lawson M, Goepfert AU, Mazzola AM, et al. Activating ESRI mutations differentially affect the efficacy of ER antagonists. Cancer Discov. 2017;7(3):277–87.
20. Billam M, Witt AE, Davidson NE. The silent estrogen receptor: can we make it speak? Cancer Biol Ther. 2009(86):485–95.
21. Hertz DL, Henry NL, Kidwell KM, Thomas D, Goddard A, Azzouz F, et al. Estrogen receptor alpha gene polymorphisms, and breast cancer risk: a case-control study with Meta-analysis combined. Asian Pacific J Cancer Prev. 2014;14(11):6743–9.
27. Kuo S, Yang S, You S, Lien H, Lin C-H, Lin P-H, et al. Polymorphisms of ESR1, UGT1A1, HCN1, MAP3K1, and CYP2B6 are associated with the prognosis of hormone receptor-positive early breast cancer. Oncotarget [Internet]. 2017 Mar 28;8(13):20925–38 Available from: http://www.oncotarget.com/fulltext/14995.

28. Clatot F, Perdrix A, Augusto L, Beaussire L, Delacour J, Calbrix C, et al. Kinetics, prognostic and predictive values of ESR1 circulating mutations in metastatic breast cancer patients progressing on aromatase inhibitor. Oncotarget [Internet]. 2016 Nov 15 [cited 2019 May 9];7(46):74448–59. Available from: http://www.oncotarget.com/fulltext/12950

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