ND_3 MITOCHONDRIAL POLIMORPHISM SIGNIFICANTLY ASSOCIATED WITH ELEVATED SERUM 8-OHdG CONCENTRATION AND INCREASED RISK OF MALIGNANT BEHAVIOR IN BREAST CANCER

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INTRODUCTION

- Breast cancer is the second most prevalent disease in women after cervical cancer.
- It also associated with significant amount of mortality annually surpassed only by cervical cancer.
- Breast cancer also possess a challenge for surgical oncologists especially in Bali because most patients come with advanced stage (Clinical Stage III-IV) and there is still no known methodology to predict the invasiveness of breast cancer.
- Polymorphisms in mitochondrial especially ND_3 genes had been proved to be associated with the occurrence of breast cancer.

- The genes that encode the protein involved in the electron transport chain are particularly important because the defect in this process could result in increased production of ROS.
- ND3 gene: The gene that encodes NADH dehydrogenase of the complex-1 of the electron transport chain.
- ND3 gene had been associated with several kinds of solid cancer namely bladder, prostate, and thyroid cancer.
- However, its association with breast cancer is still controversial.

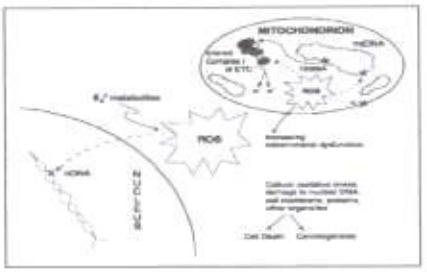
- The presence of the polymorphism especially A10389G clearly result in the alteration of mitochondrial complex-1 function.
- However, several studies that evaluate its association in the population showed non-conclusive findings.
- Meta-analysis conducted by Mao et.al found that A-10398G polymorphism may not be the risk factor of breast cancer while Grzybowska-Szatkowska et.al found that the polymorphism is associated with breast cancer in Polish population.

- polymorphisms of ND_3 gene had been associated with increased risk of breast cancers.
- Czarneka et.al reported that the ND_3 polymorphism A10398G was significantly associated with sporadic breast cancer in Poland.
- Then, Canter et.al found that the same polymorphism might also contribute to the risk of breast cancer in African-American women (OR: 1.6; 95%CI: 1.10-2.31).
- Finally, Jiang et.al also supported those findings, stated that the A10398G ND_3 polymorphism also contributed significantly to the breast cancer risk among Chinese Han women (OR: 1.49; 95%CI: 1.05-2.11).
- However, meta-analysis conducted by Mao et.al showed that this polymorphism may not be the risk factor of breast cancer.

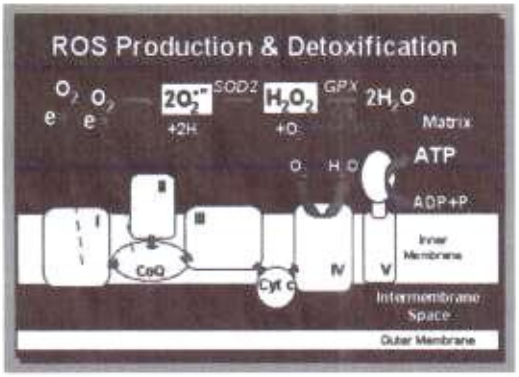
- However, the role of ND_3 polymorphism in breast cancer progression is not yet evaluated.
- Considering the important role of ROS in cancer progression and the already-establish association of ND_3 polymorphism with increased free radical formation, it is important to evaluate the true nature of the association of ND_3 polymorphism with cancer progression.

- Oxidative stress: is undeniably plays important role in initiating carcinogenesis and cancer progression.
- Inside the cell, mitochondria are the primary site where most free radical form especially when there is an alteration in the electron transport chain process.
- Normally, the free radicals produced would be neutralized by endogenous antioxidant systems.

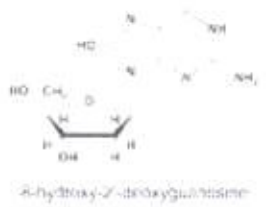
ROS PRODUCTION AND DNA DAMAGE



Jeffrey Carter 2005



STRUCTURE OF 8OHdG



METHOD

- **A case-control** study was conducted in Faculty of Medicine Udayana University and Sanglah General Hospital
- Case Group: Breast cancer patient with LVI(+) and High histological grade (grade III).
- Control: Breast cancer with LVI (-) and low grade
- The blood samples were obtained to evaluate the polymorphism and serum 8-OHdG concentration.

- ***DNA Isolation and Sequencing***
- The DNA was isolated using Promega Blood DNA Isolation Kit, the isolated DNA was subsequently amplified by PCR. The amplified gene product was sent to Eijkman Institute for sequencing process.
- ***Examination of Serum 8-OHdG Concentration***
- Blood 8-OHdG concentration was examined by Enzyme link Immunosorbent Assay (ELISA) Kit.

- **STATISTICAL ANALYSIS**
- All of the data obtained were analyzed descriptively to obtain the proportion of each variable in each group and the mean of 8-OHdG.
- Then, analytical study were conducted using one-way annova to evaluate the difference concentration of 8-OHdG between case and control group.
- Risk assessment was conducted using chi-square test by classifying the 8-OHdG concentration into high and low and assess whether the presence of ND_3 polymorphism increased the risk of invasive morphology in breast cancer.

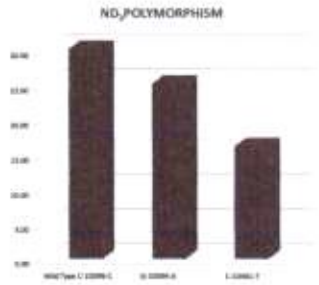
RESULT

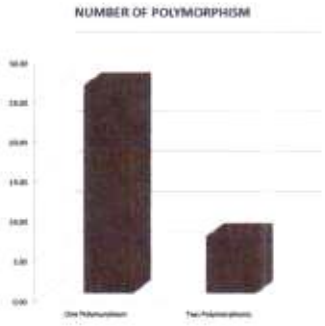
- 70 SUBJECTS WERE ENROLLED IN THIS STUDY WITH 35 SAMPLES FOR EACH GROUP.

Variables	Mean
Age	Overall : 40.93±9.204 Control : 34.31±5.88 Case : 47.54±6.874
8-OH-dG	Overall : 474.129±253.27 ng/mL Control : 393.357±198.875 ng/mL Case : 554.9±277.839 ng/mL
ND_3 Polymorphism	Wild Type C-10399-C : 30 (42.9%) G-10399-A : 25 (35.7%) C-10401-T : 16 (22.9%)
Number of Polymorphism	One Polymorphism : 26 (37.1%) Two Polymorphisms: 7 (10%)

Comparison Variables	Serum 8-OHdG Concentration	Statistical Analysis
Polymorphism		
Wild Type	375.37±208.56 ng/mL	P < 0.05
G-10399-A	610.65±271.9 ng/mL	
C-10401-T	636.18±287.75 ng/mL	
Number of Polymorphism:		
No Polymorphism	391.45±210.97 ng/mL	P < 0.05
One Polymorphism	487.52±227.18 ng/mL	
Two Polymorphism	861.36±253.27 ng/mL	

THE PRESENCE OF POLYMORPHISM INCREASED THE RISK OF MORE MALIGNANT CANCER BEHAVIOR (**OR: 4.792**; 95%CI: 1.741 – 13.188).





CONCLUSION

- G-10399A and C-10401T ND_3 gene polymorphism were associated with increased oxidative damage of the DNA by ROS and increased the risk of more malignant phenotype in breast cancer.
- However, the findings need to be validated with further research to elucidate the true association and the mechanism of malignancy enhancement caused by these two polymorphisms.

THANK YOU