Genetic landscape of breast cancer

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Breast cancer encompasses heterogeneous group of tumors with different histological, biological and clinical behavior. Intra- and inter--tumor heterogeneity as a result of genetic and non-genetic factors has considerable impact on different responses to anticancer therapy and prognosis. Introduction of new methods such as next-generation sequencing or comparative genome hybridization uncovered complexity of genetic nature of breast cancer. This article summarizes some of the new findings on this field.

Key words: **breast cancer, comparative genome hybridization, next-generation sequencing, copy number variations**

Genetické pozadie nádorov prsníka

Nádory prsníka tvoria heterogénnu skupinu nádorov s rozdielnymi histologickými, biologickými a klinickými prejavmi. Vplyvom genetických a negenetických faktorov vzniká rozdielnosť nielen medzi nádormi pacientov, ale aj v rámci jedného nádoru, čo má značný vplyv na liečbu a prognózu. Zavedenie nových metodík, ako sú sekvenovanie novej generácie a komparatívna genómová hybridizácia, odhalilo zložitosť genetického pozadia nádorov prsníka. Predkladaný článok sumarizuje niektoré z nových zistení na tomto poli.

Kľúčové slová: **nádory prsníka, komparatívna genómová hybridizácia, sekvenovanie novej generácie, zmeny počtu kópií**

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Introduction

Mammary gland is a unique organ that undergoes remarkable changes during the different stages of lifetime. During each menstrual cycle, the mammary gland passes through the waves of proliferation and apoptosis. Proliferation of the mammary epithelial cells is mainly driven by cyclic fluctuations of the hormonal factors such as estrogen and progesterone. Apoptosis controlled form of cell suicide is regulated by both hormonal and non-hormonal factors, but this process is still not well elucidated. Pregnancy also has great impact on breast morphology and function since it leads to extensive ductal branching and alveogenesis (1).

Breast tissue gladly responses to any hormonal or structural changes which makes it favorable target for tumorigenesis. Cancer can originate from any cell in the breast that has undergone tumorigenic transformation, mainly from epithelial tissue. This process can be partly explained as a progression from premalignant disease (e.g., hyperplasia, ductal carcinoma in situ) through invasive carcinoma to metastases. The whole process is accompanied and managed by accumulation of distinct genetic abnormalities. The common targets for breast cancer metastases are bone, lung, liver and brain. The prominent target organ is bone (2). The reason for this might lie in the fact that this tissue expresses higher level of hyaluronan and osteopontin. Hyaluronan interacts with osteopontin and serves as a specific ligand for CD44. This attachment complex is involved in breast cancer adhesion, migration and invasion (3). In addition, bone is an estrogen rich organ which might create generous environment for breast cancer cells (4).

Nowadays, two models are trying to explain the origin of cancer in general. First is based on the initial transformed cell that represents the cell-of-origin for the tumor. Accumulation of mutations in the regulatory genes, tumor suppressors or/and oncogenes gives such a cell growth advantage over the others what results to growth acceleration of some cell population and tumor formation. Probably, the best example illustrating the multistage neoplastic process „from one cell to tumor" is the well--known model of colorectal tumorigenesis (5).

The second theory considers the possibility of existence of self-renewing cancer cells called cancer stem cells (CSC). CSCs are subpopulation of cancer cells with high proliferative and metastatic potential. Breast cancer CSCs were isolated and identified as a CD44+/CD24- phenotype cells (6). Some introduced other markers of CSCs including ALDH1, CD49f and CD61 (7, 8, 9). This theory is trying to explain resistance to conventional therapies by the CSCs' ability of enhanced transmembrane transport outward by ABC transporters, specific mechanisms of DNA repair, ability to maintain specific signaling pathways involving key transcription factors, level of tumor suppressors and collaborative interactions among cancer stem cells to maintain specific microenvironment (10). Of course, each mentioned model has its strengths and weaknesses which are discussed elsewhere (11).

Classification of breast cancer

Breast cancer is classified into different subtypes that are associated with different patient survival outcomes. For many years, breast cancer has been classified based on clinicopathological features such as tumor type, tumor size, lymph node status and histological grade. One of the classification system is the Nottingham histologic score system. It is a grading system which utilizes features including tubule formation (similarity with normal breast duct structures), nuclear features (size and shape) and mitotic activity. According to this classification, low grade tumors (grade 1) tend to be less aggressive then the high grade tumors (grade 3). Despite not optimal reproducibility, grading system is the key point in the diagnosis and management of the disease (12).

Another classification of breast cancer is based on the expression of Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal growth factor Receptor (HER2). Best survival rate was observed in the

case of ER+/PR+HER2- subtype, the difference in survival was less than 1 % between ER+/PR+/HER- and ER+/PR+/HER2+ subtypes. All of the ER+ subtypes have better survival and adjusted mortality than all of the ER- subtypes. This suggests that ER may be a more important factor in survival than HER2 (13). ER, PR and HER2 status are predictive factors of tumor response to ER, PR, and HER2 targeted drugs.

In 2000, a classification system for the breast tumors based on variations in gene expression patterns was described. It was later revised and redivided into four subtypes: luminal A, luminal B, HER2-enriched, and basal-like (14).

Luminal A type of cancers have a low histological grade and are predominantly ER-positive. Luminal B group cancers are predominantly ER-positive with intermediate to high histological grade. Prognosis is good in the case of luminal A tumors, whereas luminal B type has intermediate prognosis with high risk of relapse. Both types of cancer are suitable for hormonal therapy.

Third type – HER2-enriched – is characteristic by high histological grade, ER- and PR-negative, HER2-positive with poor prognosis. Therapy uses drugs aimed against HER2 (e.g. trastuzumab).

Tumors that belong to the basal type or triple negative for ER, PR and HER2 group are of high histological grade with poor prognosis. Treatment of these cancer types is difficult (12).

Mutations in familial breast cancer

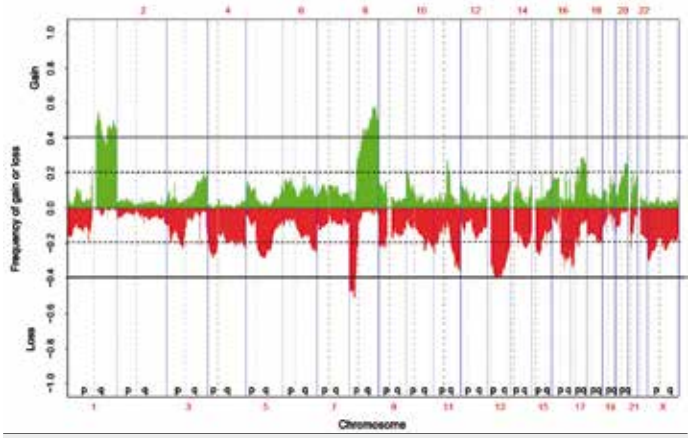
Genome-wide scanning revealed genetic variation in over 75 loci significantly associated with familial breast cancer. Despite progress on this field, currently known risk alleles explain only about 40 % of familial bound breast cancer (15). Up to date, *BRCA1* and *BRCA2* remain the two most significant genes linked to familial breast and ovarian cancer and account for about 20 % of familial breast cancer (16).

Traditional view on the subject suggests the key role of *BRCA1/2* in familial breast and ovarian cancer onset. Accordingly, mutation in one of these genes significantly increases cancer risk. However, things are not so easy. There are some reports out there claiming that some *BRCA1/2* mutations increase breast cancer risk only moderately (e.g. *BRCA1* c.1966Gln) (17), or even mean low risk for the affected person (e.g. *BRCA2* p.Lys3326*) (18). Spectrum of mutations found in *BRCA1* and *BRCA2* is extremely wide. Some of the mutations have been found one time only, whereas some have been found recurrently, often in enclosed ethnic groups, for example Askhenazi Jews (e.g. *BRCA1* c.66_67delAG; *BRCA2* c.5946delT) (19).

Both BRCA1 and BRCA2 play critical roles in DNA repair, cell cycle checkpoint control and maintenance of genomic stability. Thus, searching for other genes involved in corresponding networks led to discovery of genes related to breast cancer, including *ATM*, *CHEK2*, *BRIP1*, *PALB2* and *NBS1*. Mutations in these genes increase breast cancer risk approximately twice. Another group of genes with influence on familial breast cancer is composed of genes such as *FAM175A*, *BARD1*, *RAD51C*, *MRE11*, *RAD50* (16).

Familial syndromes, such as Li-Fraumeni syndrome (*TP53*), Cowden syndrome (PTEN Hamartoma Tumour Syndrome) (*PTEN*), Peutz-Jeghers syndrome (*STK11*), and hereditary diffuse gastric cancer syndrome (*CHD1*) are known to be one of the prominent diagnostic marks of breast cancer.

Figure 1. Frequency of copy number anomalies in the overall population. Courtesy of Dr. Andre (Andre et al., 2009)



Gained regions are green, lost are red. Two regions, 1q and 8q are the most frequent gained regions in the population. Lost regions are spread all over the genome more equally.

Families affected by breast cancer seldom carry mutations in these genes but person with mutation in one of these genes has 2- to 10-fold increased risk of getting breast cancer early during the lifetime (16).

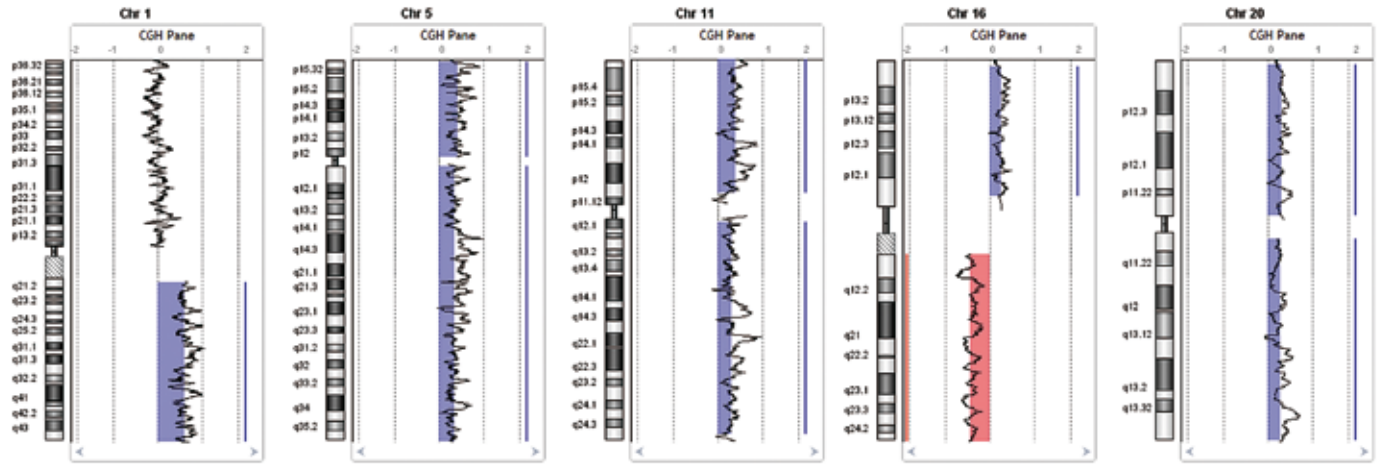
Modern screening methods such as next generation sequencing (NGS) revealed rare alleles associated with breast cancer risk, including *FANCM*, *BLM*, *FANCC*, *XRCC2* and *MCPH1/BRIT1* (20). The *MCPH1* c.904_916del mutation was genotyped in 1 370 breast cancer cases (145 familial cases, 75 young cases diagnosed below the age of 40 years, and 1 150 cases unselected for a family history of cancer or age at disease onset) and 1 160 healthy geographically matched controls. The highest prevalence for *MCPH1* c.904_916del was observed among the familial cases (5/145, 3.4 %), whereas only 5 of the 1 160 healthy controls (0.4 %) carried the mutation (20).

Castéra et al. used NGS 69 to detect germline deleterious alterations within *BRCA1* and *BRCA2* in 708 patients, 4 *TP53* mutations in 468 patients and also 36 variations inducing either a premature stop codon or a splicing defect among other genes: 5/708 in *CHEK2*, 3/708 in *RAD51C*, 1/708 in *RAD50*, 7/708 in *PALB2*, 3/708 in *MRE11A*, 5/708 in *ATM*, 3/708 in *NBS1*, 1/708 in *CDH1*, 3/468 in *MSH2*, 2/468 in *PMS2*, 1/708 in *BARD1*, 1/468 in *PMS1* and 1/468 in *MLH3* (21).

Aloraifi et al. screened *BRCA1/2* negative families with breast cancer. As expected, they identified mutations in several well-known high-susceptibility and moderate-susceptibility genes, including *ATM* (~ 5 %), *RAD50* (~ 3 %), *CHEK2* (~ 2 %), *TP53* (~ 1 %), *PALB2* (~ 1 %), and *MRE11A* (~ 1 %). They also identified novel pathogenic variants in 30 other genes: *MAP3K1*, *CASP8*, *RAD51B*, *ZNF217*, *CDKN2B-AS1* and *ERBB2* including a splice site mutation which, as they predict, would generate a constitutively active HER2 protein (22).

Chrupek et al. screened 289 African American women for inherited mutations. African Americans have a disproportionate burden of aggressive young-onset breast cancer. Of patients with mutations, 80 % (52/65) carried mutations in *BRCA1* and *BRCA2* genes and 20 % (13/65) carried mutations in *PALB2, CHEK2, BARD1, ATM, PTEN,* or *TP53*. The mutational allelic spectrum was highly heterogeneous with 57 different mutations in 65 patients (23).

Figure 2. Example of the comparative genome hybridization (aCGH)



Result of the aCGH in our laboratory. DNA was isolated from the tumor of patient with breast cancer. Gain regions are blue (1q, 16p, and 5, 11 and 20 both arms), lost region (16q) is red.

A total of 133 patients were enrolled in Li`s study. Total 30 patients (22.6 %) were found to carry germline deleterious mutations: 9 in *BRCA1, 11* in *BRCA2, 2* in *RAD50, 2* in *TP53* and one each in *ATM, BRIP1, FANCI, MSH2, MUTYH,* and *RAD51C* (24).

These data clearly support the idea to implement NGS into clinical management of familial breast cancer. It is highly probable that near future will bring routine usage of panels containing all known breast cancer associated loci for mutation screening in affected families.

Copy number variations in breast cancer

During last two decades, our understanding of cancer related loci has increased. The comparative genome hybridization (CGH) is tightly connected with this progress, despite its limitations. CGH is used for mapping losses and gains of DNA in the cancer genomes. These experiments revealed some loci that are commonly affected in breast cancer. Most frequently gained regions are localized on the chromosomal arms 1q, 8q, 17q, as well as 11q and 20q. On the contrary, most frequently lost regions were observed on 8p, 11q and 16q (25,26,27).

Closer view on the frequency plots shows two most prevalent regions with high gain alterations: 1q and 8q (figure 1). Andre et al. observed the gain of the 153 Mb region at 1q in 55 % cases out of 106 breast cancer patients. The second most frequently observed region of gain was spanning throughout the 116.7 and 127.5 Mb of the 8q. These gains were shown in 58 % of all cases (27). Interestingly, long arm of chromosome 1 is almost exclusively affected by gain of genetic material in breast tumors. On the other hand, its short arm is mainly involved in loss (28).

On the contrary, loss of genetic material is more or less equally distributed throughout the genome. However, at least a 24.2 Mb region at 8p is more frequently lost in 51 % cases, followed by a 47.8 Mb region at 13q seen in 41 % cases (27). Similar findings were spotted by others as well (25). Some patients show more complex anomalies in their tumor genome (figure 2).

Several studies have shown positive correlation between copy number alteration and gene expression level. For example, Andre et al. identified 3 007 such genes by Affimetrix U133A probes, including *MYC*, *FOXA1*, *FGF3*, *FGF4*, CCND1, *PAK1.* In addition, amplification with positive gain/loss and gene expression were observed in two amplicons (8p11-12 and 17q11-21) where genes *PROSC*, *GPR124*, *ADRB3*, as well as genes for trans-membrane tyrosine kinases -*ERBB2*, *FGFR1* are localized (27).

On the other hand, regions that were shown to be lost and highly correlated with changes in gene expression contain genes such as *BRCA1*, *STAT3*, *STAT5A*, *STAT5B*, and *MAPT* genes, as well as genes encoding chemokines (*CCL3*, *CCL4*, *CCL5*, *CCL14*, *CCL15*, *CCL16*, *CCL18*, *CCL23*) (27).

According to the accepted theory, cancerogenesis is a multistep process leading from primary cancer cells through tumor formation to metastasis. According to this theory, one can expect differences in genetic variations within different stages of tumor. Navin et al. dissected primary breast cancer tumors into sections and analyzed them by flow cytometry and CGH. Based on their observations, breast carcinomas divided into two groups: (1) monogenomic and (2) polygenomic. Monogenomic tumors contained a single major clonal subpopulation with a highly stable chromosome structure. Polygenic tumors contained only a few, no more than three major tumor subpopulations. They do not found any series of gradual intermediates which can confirm the multistage carcinogenesis (29).

In this context, it is not surprising that differences between primary tumors and metastasis are not so significant. Comparison of known driver oncogenes located within regions frequently amplified in breast cancer showed 100 % concordance for *ERBB2* (17q12) and *FGFR1* (8p11.23), 96 % for *CCND1* (11q13.3) and *PAK1* (11q14.1), and 88 % for *MYC* (8q24) (25). Mutational status of primary tumors and metastases by NGS showed tumor-metastases concordance of variants 92 % for recurrent mutations (*AKT1*, *ERBB2*, *PIK3CA*, *TP53*) and 73 % for non-recurrent variants (25). High level of similarity was found by others as well, for instance Vollebergh (30).

However, genetic footprint of the primary tumors and metastases is not always in perfect concordance. For example, an ATM-containing region was found to be deleted in metastases, but in primary tumors it was usually amplified (25).

Thus, it is evident that primary tumors and metastases share higher level of copy number variation, but a little less concordance is found in mutational status. This suggests that metastasis formation does not require much more genetic events as was thought. To elucidate the carcinogenesis in general, further investigation will need information from the other fields, for example proteomics (25).

Conclusion

Breast cancer is the most common cancer in women worldwide. Despite improvements in the diagnostics and treatment in the past decade, our knowledge of this disease is still limited. Differences on the gene level observed not only between two different patients, but even among the cells of the same tumor cause problems which makes treatment and prognosis more challenging. Treatment regime which is successful in one patients might not be of any value for another. Therefore, involvement of new techniques such as CGH might be a good way towards personalized medicine from which each patient can benefit the most.

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References

1. Navarrete MA, Maier CM, Falzoni R, et al. Assessment of the proliferative, apoptotic and cellular renovation indices of the human mammary epithelium during the follicular and luteal phases of the menstrual cycle. *Breast Cancer Res.* 2005;7(3):306–313.
2. Jin X, Mu P. Targeting breast cancer metastases. *Breast Cancer (Auckl.).* 2015;9(Suppl 1):23–24.
3. Cook AC, Chambers AF, Turley EA, et al. Osteopontin induction of hyaluronan synthase 2 expression promotes breast cancer malignancy. *J Biol Chem.* 2006;281(34):24381–24389.
4. Nakamura T, Imai Y, Matsumoto T, et al. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. *Cell.* 2007;130(5):811–823.
5. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell.* 1990;61(5):759–767.
6. Al-Hajj M, Wicha MS, Benito-Hernandez A, et al. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A.* 2003;100(7):3983–3988.
7. Ginestier C, Hur MH, Charafe-Jauffret E, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell.* 2007;1(5):555–567.
8. Charafe-Jauffret E, Ginestier C, Bertucci F, et al. ALDH1-positive cancer stem cells predict engraftment of primary breast tumors and are governed by a common stem cell program. *Cancer Res.* 2013;73(24):7290–300.
9. Vaillant F, Asselin-Labat ML, Shackleton M, et al. The mammary progenitor marker CD61/beta3 integrin identifies cancer stem cells in mouse models of mammary tumorigenesis. *Cancer Res.* 2008;68(19):7711–7717.
10. Bozorgi A, Khazaei M, Khazaei MR. New findings on breast cancer stem cells: A review. *J Breast Cancer.* 2015;18(4):303–312.
11. Rycaj K, Tang DG. Cell-of-Origin of Cancer versus Cancer Stem Cells: Assays and Interpretations. *Cancer Res.* 2015;75(19):4003–4011.
12. Rosa M. Advances in the molecular analysis of breast cancer: Pathway toward personalized medicine. *Cancer Control.* 2015;22(2):211–219.
13. Parise CA, Caggiano V. Breast Cancer Survival Defined by the ER/PR/HER2 Subtypes and a Surrogate Classification according to Tumor Grade and Immunohistochemical Biomarkers. *J Cancer Epidemiol.* 2014;2014. Article ID 469251, doi:10.1155/2014/469251.
14. Sørlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA.* 2001;98(19):10869–10874.
15. Lalloo F, Evans DG. Familial breast cancer. *Clin Genet.* 2012;82(2):105–114.
16. Hilbers FS, Vreeswijk MP, van Asperen CJ, et al. The impact of next generation sequencing on the analysis of breast cancer susceptibility: a role for extremely rare genetic variation? *Clin Genet.* 2013;84(5):407–414.
17. Spurdle AB, Whiley PJ, Thompson B, et al. BRCA1 R1699Q variant displaying ambiguous functional abrogation confers intermediate breast and ovarian cancer risk. *J Med Genet.* 2012;49(8):525–532.
18. Michailidou K, Hall P, Gonzalez-Neira A, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nat Genet.* 2013;45(4):353–361
19. Cierniková S, Tomka M, Kovác M, et al. Ashkenazi founder BRCA1/BRCA2 mutations in Slovak hereditary breast and/or ovarian cancer families. *Neoplasma.* 2006;53(2):97–102.
20. Mantere T, Winqvist R, Kauppila S, et al. Targeted Next-Generation Sequencing Identifies a Recurrent Mutation in MCPH1 Associating with Hereditary Breast Cancer Susceptibility. *PLoS Genet.* 2016;12(1):e1005816.
21. Castéra L, Krieger S, Rousselin A, et al. Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. *Eur J Hum Genet.* 2014;22(11):1305–1313.
22. Aloraifi F, McDevitt T, Martiniano R, et al. Detection of novel germline mutations for breast cancer in non-BRCA1/2 families. *FEBS J.* 2015;282(17):3424–3437.
23. Churpek JE, Walsh T, Zheng Y, et al. Inherited predisposition to breast cancer among African American women. *Breast Cancer Res Treat.* 2015;149(1):31–39.
24. Lin PH, Kuo WH, Huang AC, et al. Multiple gene sequencing for risk assessment in patients with early-onset or familial breast cancer. *Oncotarget.* 2016;7(7):8310–8320.
25. Bertucci F, Finetti P, Guille A, et al. Comparative genomic analysis of primary tumors and metastases in breast cancer. *Oncotarget.* 2016; doi: 10.18632/oncotarget.8349. [Epub ahead of print].
26. Bekhouche I, Finetti P, Adelaïde J, et al. High-resolution comparative genomic hybridization of inflammatory breast cancer and identification of candidate genes. *PLoS One.* 2011;6(2):e16950.
27. Andre F, Job B, Dessen P, et al. Molecular characterization of breast cancer with high-resolution oligonucleotide comparative genomic hybridization array. *Clin Cancer Res.* 2009;15(2):441–451.
28. Orsetti B, Nugoli M, Cervera N, et al. Genetic profiling of chromosome 1 in breast cancer: mapping of regions of gains and losses and identification of candidate genes on 1q. *Br J Cancer.* 2006;95(10):1439–1447.
29. Navin N, Krasnitz A, Rodgers L, et al. Inferring tumor progression from genomic heterogeneity. *Genome Res.* 2010;20(1):68–80.
30. Vollebergh MA, Klijn C, Schouten PC, et al. Lack of Genomic Heterogeneity at High-Resolution aCGH between Primary Breast Cancers and Their Paired Lymph Node Metastases. *PLoS One.* 2014; 9(8):e103177.

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