DIPLOMA THESIS

Expression of genes coding for G protein-coupled inwardly-rectifying potassium channels (GIRKs) in breast cancer

Submitted by

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Affidavit

I, hereby, declare that the following diploma thesis has been written only by the undersigned and without any assistance from third parties. Furthermore, I confirm that no sources have been used in the preparation of this thesis other than those indicated in the thesis itself.

Graz, June 2014 *Armin Andreas Sokolowski*

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Abstract

Background

Among the many molecular players in cancer, G-protein coupled receptors have recently been identified to play an important role in the process of cancer progression and metastasis. GIRK1 protein, known for being overexpressed in breast cancer tissue, and a high GIRK1 mRNA expression have been shown to correlate with lymph node metastases in studies with small patient numbers. The aim of this study was to validate these findings by analyzing GIRK1 mRNA expression using large breast cancer patient sets.

Methods

The GIRK1 mRNA expression levels of 914 invasive breast carcinoma samples available at TCGA were downloaded from the cBio portal with the corresponding clinical data from the UCSC Cancer Genomics Browser. In addition, mRNA expression levels of 105 healthy tissue samples corresponding to 105 of the patients mentioned above were downloaded from the TCGA Data Portal. The mRNA data and the clinical data were combined in Microsoft Excel 2010® and the further analysis was performed in SigmaPlot v12.5®.

Results

Analysis of the TCGA data showed that GIRK1 is significantly overexpressed in breast cancer compared to normal tissue ($p<0.001$). Furthermore, GIRK1 expression is significantly higher in estrogen receptor positive tumors ($p<0.001$) and in lymph node positive tumors ($p<0.001$) than in estrogen receptor negative and lymph node negative tumors, respectively. Survival analysis of the TCGA data set showed that the hazard rate is significantly affected by GIRK1 mRNA expression ($p<0.034$).

Conclusion

Our analysis of TCGA data indicates that GIRK1 overexpression is associated with lymph node positive as well as with estrogen receptor positive breast cancer and confirms previous findings. In addition we could show that GIRK1 overexpression has a negative effect on the overall survival, especially in estrogen receptor positive breast cancer.

Zusammenfassung

Hintergrund

Unter den vielen molekularen Mitspielern der Kanzerogenese sind kürzlich G-Protein-gekoppelte Rezeptoren als wichtige Faktoren im Prozess der Tumorprogression und Metastasierung identifiziert worden. In vorhergehenden Studien an kleineren Kollektiven zeigte sich eine GIRK1 Protein-Überexpression in Brustkrebsgewebe. Eine hohe GIRK1 mRNA-Expression wurde mit Lymphknotenmetastasen assoziiert. In dieser Studie wollen wir diese Feststellung durch die Analyse von GIRK1 mRNA-Expressionsdaten an einem größeren Kollektiv untersuchen.

Methoden

Die GIRK1 mRNA Expressionsdaten von 914 Mammakarzinom-Gewebeproben, wurden über das cBio Portal mit den entsprechenden klinischen Daten aus dem UCSC Cancer Genomics Browser entnommen. Außerdem wurden mRNA Expressionsdaten von gesunden Geweben von 105 der oben erwähnten Patientinnen über das TCGA Portal heruntergeladen. Die mRNA- Daten und die klinischen Daten wurden mit Microsoft Excel 2010® verarbeitet und mit SigmaPlot v12.5® analysiert.

Ergebnisse

Die Analyse der TCGA Daten zeigte, dass GIRK1 in Brusttumoren im Vergleich zu Normalgewebe deutlich überexpremiert ist ($p<0,001$). Weiters ist GIRK1 in Östrogenrezeptor positiven Tumoren ($p<0,001$) und in Lymphknoten positiven Tumoren ($p<0,001$) im Vergleich zu Östrogenrezeptor negativen und Lymphknoten negativen Tumoren signifikant erhöht. Die Überlebensanalyse der TCGA Daten zeigte, dass die Hazardrate von GIRK1 signifikant beeinflusst wird ($p<0,034$).

Konklusion

Unsere Analyse der TCGA Daten zeigt, dass die Überexpression von GIRK1 mit Lymphknoten positivem als auch mit Östrogenrezeptor positivem Brustkrebs assoziiert ist und bestätigt damit frühere Erkenntnisse. Darüber hinaus konnten wir zeigen, dass eine GIRK1 Überexpression besonders bei Östrogenrezeptor positivem Brustkrebs einen negativen Einfluss auf das Gesamtüberleben hat.

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1 INTRODUCTION

Tumor growth and metastasis consist of multiple biological processes and are subject to complex molecular mechanisms (Steeg, 2006). It is the challenge and the goal of modern cancer research to explore and understand these processes in order to develop new therapeutic targets in the fight against cancer. Among the many molecular players G-protein coupled receptors have recently been identified to play an important role in the process of cancer progression and metastasis (Dorsam, et al., 2007).

A study presented by Stringer has indicated that an overexpression of mRNA that encodes a G-protein coupled inwardly-rectifying potassium channel (GIRK1) correlates with axillary lymph node metastasis in primary invasive breast carcinomas (Stringer, et al., 2001). Several further studies have shown that GIRK1 is involved in cellular signaling in breast cancer cells and that it is an integral part in cell proliferation and tumor progression (Dhar, et al., 2006; Hance, et al., 2008; Takanami, et al., 2004).

The aim of the study presented in this thesis was to evaluate gene-expression data available on The Cancer Genome Atlas, to investigate the influence of GIRK on breast cancer in a larger cohort of patients.

1.1 Breast cancer

Breast cancer is a malignant epithelial tumor of the mammary gland parenchyma, which is responsible for about 20% of cancer-related deaths in women. One in eight women in the western world develop breast cancer during their lifetime, with the peak incidence in the 5th and 6th decade of life (Boecker, et al., 2012). Therefore, breast cancer is the most common malignant tumor in women, with a proportion of over 29% of all new malignant cancer cases (Wayerstahl, et al., 2013).

Hereinafter the various methods of breast cancer classification will be discussed, as far as they are important for the understanding of the present study.

1.1.1 Classification according to histology

Almost all tumor types develop from noninvasive, ductal or lobular precursors, the so called ductal carcinoma in situ (85-90 %) and lobular carcinoma in situ (10-15 %) (Kaufmann, et al., 2007). The most common invasive tumor type of breast cancer is an adenocarcinoma called invasive ductal carcinoma, with 80 % of all breast cancer types. The second most common histologic subtype, with about 10-15 %, is an invasive lobular carcinoma. About 10 % are various special forms, such as the tubular, medullary or mucinous carcinoma (Kaufmann, et al., 2007; Wayerstahl, et al., 2013; Boecker, et al., 2012).

1.1.2 TNM staging system

The TNM classification is based on the American Joint Committee on Cancer (AJCC) TNM system and describes tumor size and spread (T), the number of involved lymph nodes (N) and of possible distant metastases (M) (American Cancer Society, 2013; Sinn, et al., 2010; American Joint Committee on Cancer, 2010). The following table shows a summary of the TNM classification:

Primary tumor (T)		Nearby lymph nodes (N)		Distant metastasis (M)	
Tx	Primary tumor cannot be assessed	Nx	Nearby lymph nodes cannot be assessed	Mx	Distant spread cannot be assessed
T0	No evidence of primary tumor	N0	Cancer has not spread to nearby lymph nodes	M0	No distant spread is found
Tis	Carcinoma in situ	N1	Cancer has spread to 1 to 3 axillary lymph nodes	M1	Cancer has spread to distant organs
T1	includes T1a, T1b, and T1c, Tumor is 2 cm or less across	N2	Cancer has spread to 4 to 9 axillary lymph		
T2	Tumor is more than 2 cm but not more than 5 cm across	N3	10 or more axillary lymph nodes and/or lymph nodes under the clavicle, with at least one area of cancer spread greater than 2mm		
T3	Tumor is more than 5 cm across				
T4	includes T4a, T4b, T4c, and T4d, Tumor of any size growing into the chest wall or skin, includes inflammatory breast cancer				

Table 1 - TNM classification of breast cancer

The tumor stage is determined based on the findings of the TNM classification. The following table summarizes the guidelines of the stage classification by The American Joint Committee on Cancer (AJCC) (American Cancer Society, 2013) (American Joint Committee on Cancer, 2010).

Stage	T	N	M
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIA	T0, T1	N1	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0, T1, T2	N2	M0
	T3	N1, N2	M0
Stage IIIB	T4	N0, N1, N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

Table 2 - Stage classification by The American Joint Committee on Cancer (2010)

1.1.3 Hormone-receptor and Her2-Status

Another way to classify breast cancer is by its hormone-receptor and Her2-receptor status. It is assessed for all breast cancers as it is of great prognostic and predictive value (Cianfrocca, et al., 2004; Lebeau, et al., 2014). Furthermore, it determines the patient's suitability for a targeted treatment such as Tamoxifen or Trastuzumab (den Hollander, et al., 2013). Tamoxifen is an antagonist of the estrogen receptor and it is used for the treatment of estrogen receptor positive breast cancer. Trastuzumab is a monoclonal antibody that binds on the Her2 receptor and is used for the treatment of breast cancer types that overexpress Her2, which has significantly improved the prognosis for the patients (Romond, et al., 2005).

Breast cancer with no positive receptors (triple negative cancer) is lacking these targeted treatments, thus patients suffering from that type have a comparatively poor prognosis (Dent, et al., 2007; Anders, et al., 2008; Engebraaten, et al., 2013).

1.1.4 Molecular Classification

Based on the gene expression profile, obtained from the tumor tissue with DNA microarrays, five different molecular subtypes of breast cancer can be distinguished: hormone receptor positive tumors with lower or higher aggressiveness (luminal A and luminal B), Her2-positive tumors (Her2-enriched) and hormone receptor - and Her2-negative carcinomas with or without basal cell characteristics (basal-like and normal-like phenotypes) (Hyams, 2010; Reis-Filho, et al., 2011; Parker, et al., 2009; Perou, et al., 2000; Neve, et al., 2006). The knowledge of these intrinsic subtypes may predict distinct clinical outcomes for patients. Further they may be used as genomic predictors of clinical response to therapy and of the survival rate in future (Carey, et al., 2006; Reis-Filho, et al., 2011; Neve, et al., 2006; Al Saeed, et al., 2013).

1.2 Genes of interest

In this study, mRNA expression data of genes coding for GIRK1, GRIK2, GIRK3, GIRK4, TrpC3, TrpC6, Piezo1, Piezo2 and ANO1 is investigated. Hereinafter, the genes mentioned above will be described in detail and their functions briefly explained.

1.2.1 G-protein activated potassium channels (GIRKs)

The G protein-coupled inwardly-rectifying potassium channels (GIRKs) belong to the family of inwardly-rectifying potassium ion channels activated by ligand-stimulated G protein-coupled receptors, followed by a cascade of signal transduction (Dascal, 1997). The subtypes of the GIRK family are the proteins GIRK1 (corresponding gene KCNJ3), GIRK2 (corresponding gene KCNJ6), GIRK3 (KCNJ9) and GIRK4 (KCNJ5) (Weigl, et al., 2001). The GIRK channels play an essential role in many physiological and pathophysiological processes, for instance in the cardiovascular physiology (heart rate, hypertension), neurological

functions (including nociception, memory/learning, anxiety, and schizophrenia), thermoregulation, energy homeostasis, et cetera (Luján, et al., 2014; Dascal, et al., 1993). Furthermore, GIRK channels function as targets of neurotransmitters and clinically relevant drugs like volatile anesthetics (Luján, et al., 2014; Weigl, et al., 2001). Numerous recent studies have shown that GIRK channels, especially the subtype GIRK1 are found in breast cancer cells (Brevet, et al., 2008). They are involved in cellular signaling pathways in breast cancer cells and are an integral part in cell proliferation and tumor progression (Stringer, et al., 2001; Dhar, et al., 2006; Plummer, et al., 2004; Hance, et al., 2008; Wagner, et al., 2010; Takanami, et al., 2004; Brevet, et al., 2008; Plummer, et al., 2005). Stringer et al. 2001 used an expression profiling technique to identify genes with an aberrantly increased expression in invasive breast carcinomas and compared them to adjacent normal breast tissue from the same individual. Among these genes they identified GIRK1 as the one whose overexpression correlated with axillary lymph node metastasis (Stringer, et al., 2001).

1.2.2 TrpC3, TrpC6

TRPC3 and TRPC6 are members of the transient receptor potential cation channels, subfamily C (The letter C stands for ‘canonical’). They function as ion-channels, non-selectively permeable to cations activated by phospholipase C or diacylglycerol. They play an important role in various calcium-mediated physiological processes such as in the erythropoietin modulation of calcium influx in erythroid cells (Hirschler-Laszkiewicz, et al., 2009; Tong, et al., 2008).

Recent studies suggest that TrpC3 and TrpC6 play an important role in the progression of the cancerogenesis in ovarian cancer (TrpC3), gastric cancer (TrpC6) and in head and neck squamous cell carcinomas (TrpC6) (Tao, et al., 2013; Cai, et al., 2012; Cai, et al., 2009; Bernaldo de Quirós, et al., 2013). Guilbert et al. found that TrpC6 channels are highly expressed in breast cancer epithelial cells (Guilbert, et al., 2008). Furthermore Aydar et al. identified TrpC3 and TrpC6 as significantly up regulated in breast cancer tissues when compared to normal breast tissue (Aydar, et al., 2009).

1.2.3 Piezo1, Piezo2

Piezo1 and its close homolog Piezo2 (also referred to as FAM38A andFAM38B) are mechanosensitive ion channel proteins, that are expressed in regions where mechanosensation has an important biological role (Coste, et al., 2012; Coste, et al., 2010).

1.2.4 ANO1

ANO1 is located within the 11q13 amplicon, a very often amplified chromosomal region in human cancer (Britschgi, et al., 2013). It encodes for the Anoctamin-1 protein, which is a member of the transmembrane 16 (TMEM16) protein family and functions as a calcium dependent chloride channel. Anoctamin-1 plays an important role in several physiological processes, such as in vascular smooth muscles or in transepithelial anion transports (Manoury, et al., 2010; Scuderi, et al., 2011).

In our study ANO1 is used as a reference gene, since it has been identified as amplified and overexpressed in breast cancer and associated with increased tumor progression in a previous work (Britschgi, et al., 2013).

1.3 Online Gene-expression sources

Clinical annotation and mRNA-expression levels (RNAseq) for breast cancer tissue samples were obtained from The Cancer Genome Atlas (TCGA, https://tcga-data.nci.nih.gov/tcga/) using the Data Matrix functionality or through the cBio Portal for Cancer Genomics (www.cbioportal.org) and The UCSC Cancer Genomics Browser (https://genome-cancer.ucsc.edu/).

In the following, these sources will be described and explained in detail as the origin of the data used in this study.

1.3.1 TCGA Data Portal (https://tcga-data.nci.nih.gov/tcga/)

The Cancer Genome Atlas begun in 2006 as a three-year project to catalogue genetic mutations in cancer by the means of genome analysis technologies such as genome sequencing and bioinformatics. Today it is an integrated network of

hundreds of researchers, with the goal to explore the molecular basis of cancer and its genomic changes, and to enable new possibilities of cancer diagnosis, treatment and prevention. It is financed by the National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI). By making its data freely available online, this project enables researchers all around the world to make further investigations and important discoveries. The data sets generated by TCGA are accessible through the TCGA Data Portal platform online (TCGA Data Portal, https://tcga-data.nci.nih.gov).

How TCGA works (The Cancer Genome Atlas, http://cancergenome.nih.gov/)

1.) Tissue Processing

The biospecimens (a portion of tumor and eventually normal tissue) are donated from patients undergoing a surgery for tumor excision. The samples and clinical metadata are collected by a Tissue Source Site and are then sent to a Biospecimen Core Resource. Next the samples are processed to meet the TCGA biospecimen criteria and prepared for further genomic analysis

2.) Research and Discovery

Tumor and normal tissues from hundreds of patients are analyzed by TCGA researchers to produce a complete genomic profile for selected cancer types. For instance genetic changes, gene expression changes and changes in DNA sequence are analyzed.

3.) Data Sharing

To share the data with a broader community, information generated by the TCGA is processed and entered into public databases allowing scientists all around the world to search, access, download and finally analyze the datasets through the TCGA Data Portal.

4.) Community Research and Discovery

TCGA enables the Cancer community to do research that could not be possible without it. The TCGA data has a multiplier effect on the scope and quality of research. The goal is to enable better therapeutic care for patients suffering from cancer.

1.3.2 cBio Portal for Cancer Genomics

The cBio portal for Cancer Genomics is an open-access online analysis tool which stores genomic data from large scale, cancer genomic data sets from TCGA. It allows data analysis and downloads of small data slices, such as user-defined gene and sample sets, without the need to download entire large scale data sets (cBioPortal for Cancer Genomics, http://www.cbioportal.org; Cerami, et al., 2012; Gao, et al., 2013).

1.3.3 UCSC Cancer Genomics Browser

The UCSC Cancer Genomics Browser (https://genome-cancer.ucsc.edu/) is an open-access online analysis tool to display, investigate and analyze cancer genomics data and its associated clinical information, provided by The Cancer Genome Atlas Research Network (Goldman, et al., 2013; Cline, et al., 2013).

The public datasets available on the Cancer Genomics Browser include data sets from TCGA, the Cancer Cell Line Encyclopedia, and some other data sets from the literature. For each data set, there is a “Dataset details” link available that gives more information about the data origin. For the TCGA data sets used in this study, the data was downloaded from the TCGA Data Coordination Center (DCC) (Cline, et al., 2013).

The Cancer Browser is developed and maintained by the University of California Santa Cruz Cancer Genomics Group, led by David Haussler and Josh Stuart. (UCSC Cancer Genomics Browser, https://genome-cancer.ucsc.edu/)

2 OBJECTIVES OF THE STUDY

As part of the thesis, the validity of the findings of Stringer's et al. study, as described in the introduction, should be verified on a larger data set (Stringer, et al., 2001).

This study is part of a research project supported by the Austrian Science Fund FWF. The title of the project is 'Predictive and prognostic value of GIRK in breast cancer' and it is performed at the Division of Oncology, Department of Internal Medicine at the Medical University of Graz under the supervision of Prof. Dr. Thomas Bauernhofer (project number KLI 182). This project is designed to investigate the influence of GIRK expression on the clinical course. In a screening cohort, the subtypes of breast cancer showing a GIRK overexpression should be identified.

Within the scope of this study, the following study objectives have been formulated:

GIRK mRNA expression profiles should be correlated with the histological subtype, the axillary lymph node status, disease-free survival and overall survival as well as with four different breast cancer subtypes, depending on the hormone receptor status (ER, PR) and the expression of Her2.

Furthermore the various population subgroups of female breast cancer patients should be analyzed for significant differences in mRNA expression of the genes mentioned in the introduction (GIRK1, GIRK2, GIRK3, GIRK4, TrpC3, TrpC6, Piezo1, Piezo2, ANO1). Also any possible differences in the gene expression between tumor and corresponding normal tissue should be detected.

In particular, the following questions should be answered by performing this study:

Is there a difference in the mRNA expression of the genes of interest between:

- lymph-node negative and lymph-node positive patients?
- distant metastasis negative and distant metastasis positive patients?
- the four breast cancer groups depending on the ER/PR and Her2 status as defined in the ‘Material and Methods’ section?
- the intrinsic molecular breast cancer subtypes luminal A, luminal B, Her2-enriched, basal-like and normal-like?
- breast tissue and corresponding normal tissue?

To clarify the question whether GIRK1 or the other above-mentioned genes have an influence on the clinical outcome (disease free survival, overall survival), survival analyzes should be carried out.

3 MATERIALS AND METHODS

3.1 Data acquisition and study population

The cohort for this study consists of patient-data available on TCGA through the cBio portal (www.cbioportal.org). The **mRNA expression levels** (RNA Seq V2 RSEM) of the breast cancer biospecimens were acquired via the 'Download Data'-functionality, found on the cBio portal- homepage on the 4th of august 2013. The selected cancer study was 'Breast Invasive Carcinoma (TCGA, Provisional)' from the TCGA Breast Cancer project, which contained the mRNA expression levels (RNA Seq V2 RSEM) of 915 BRCA cases. The selected genomic profile was 'mRNA expression (RNA Seq V2 RSEM)', the patient/case set was 'All Tumors', the entered gene set was a user-defined list with the Genes 'ANO1, KCNJ3, KCNJ5, KCNJ6, KCNJ9, PIEZO1, PIEZO2, TRPC3, TRPC6'. The options were submitted and a transposed data matrix acquisitioned. The results were exported and saved as a local file (*cBioportal_genexpression*). The numeric values were gene expression profiles measured experimentally using the Illumina HiSeq 2000 RNA Sequencing platform established by the University of North Carolina TCGA genome characterization center. The data set shows the gene-level transcription estimates, as in RSEM normalized count (TCGA DCC description; UCSC Cancer Genomics Browser, https://genome-cancer.ucsc.edu/). Level 3 data (gene-level data) is created using two different methods: The original one follows the 'Reads Per Kilobase of exon model per Million mapped reads' – method of quantitation (RPKM), whereas the newer version 2 data 'RNA Seq V2', introduced in May 2012, uses a combination of MapSplice (to do the alignment) and RSEM (to perform the quantitation) to determine the expression levels (normalized results for expression of a gene, respectively a measure in terms of transcripts per million (TPM)) (NCI Wiki, https://wiki.nci.nih.gov; Wang, et al., 2010; Li, et al., 2011; Li, et al., 2010).

The associated **clinical data** was downloaded from the website of the UCSC Cancer Genomics Browser (https://genome-cancer.ucsc.edu) (UCSC Cancer Genomics Browser, https://genome-cancer.ucsc.edu/). The data set used, was

the ‘BRCA gene expression (IlluminaHiSeq)’ from the ‘TCGA breast invasive carcinoma (BRCA) gene expression by RNAseq’ source. The resulting file with the relevant information was named ‘clinical_data’.

The **normal tissue samples mRNA expression levels** (RNA Seq V2 RSEM) were downloaded as individual data files for each sample through the Data Matrix functionality on TCGA (TCGA Data Portal, https://tcga-data.nci.nih.gov).

3.2 Statistical parameter

For this study the following data were used:

1.) mRNA Expression levels of ANO1, KCNJ3, KCNJ5, KCNJ6, KCNJ9, TrpC3, TrpC6, Piezo1, Piezo2

The selected genomic profile was ‘mRNA expression (RNA Seq V2 RSEM)’ showing the gene-level transcription estimates, as in RSEM normalized count.

2.) Age_at_Initial_Pathologic_Diagnosis_nature2012

The patient’s age at the time of the diagnosis in years.

3.) HER2_Final_Status_nature2012

The ‘HER2 Final Status’ was delivered by HER2 calls following the ASCO/CAO guidelines (American Society of Clinical Oncology/College of American Pathology), supplementing the missing calls with FISH results in the second step, and finally in the third and last step supplementing them with copy number calls. The exact procedure is described in the supplementary information of the TCGA Nature study 2012 (The Cancer Genome Atlas Network, 2012).

4.) PAM50_mRNA_nature2012

The samples were subtyped by the 50-gene PAM50 predictor. PAM50 is a 50-gene classifier (Prediction Analysis of Microarray) that was developed to measure expression profiles for 50 genes and to identify the intrinsic breast cancer subtypes Luminal A, Luminal B, HER2-enriched and basal-like. Based on the intrinsic subtypes a Risk of Recurrence (ROR) score is generated, which is used to provide more detailed prognostic information by

combining molecular subtype data with standard clinical variables (histologic grade, tumor size, node status, ER status) (Parker, et al., 2009; Gnant, et al., 2013; Leach, 2013; Bastien, et al., 2012). The PAM50-gene test can be carried out in routine hospital pathology laboratories (Gnant, et al., 2013).

5.) _EVENT

The event is ‘1’ in cases of death and ‘0’ in censored cases.

6.) _OS

The overall survival is the time of survival in days after the initial diagnosis. It is based on death from any cause.

7.) breast_carcinoma_estrogen_receptor_status

Describes the estrogen receptor status of the breast carcinoma.

8.) breast_carcinoma_progesterone_receptor_status

Describes the progesterone receptor status of the breast carcinoma.

The current guideline by ASCO/CAP, effective January 2010, recommends to call a breast tumor ER or PR positive if >= 1% of tumor cell nuclei are immunoreactive (Hammond, et al., 2010). Prior to 2010 local hospitals used their own thresholds (such as 5% or 10%) for their clinical practices, lacking a universal standard. So the determination of the clinical status for ER and PR followed a mixture of different thresholds in the samples collected prior to 2010 (The Cancer Genome Atlas Network, 2012).

9.) menopause_status

‘Premenopause’ was defined as <6 months since the last menstrual period AND no prior bilateral ovariectomy AND not on estrogen replacement). ‘Postmenopause’ was defined as prior bilateral ovariectomy OR >12 months since last menstrual period with no prior hysterectomy.

If neither the pre- or postmenopausal definition was correct the menopause_status was ‘Indeterminate’ (The Cancer Genome Atlas Network, 2012).

10.) pathologic_M

The pathologic metastasis status M0 and M1 as described in the introduction.

11.) pathologic_N

The pathologic lymph node status N0 and N1/N2/N3 without a further distinction in the sub-classifications as described in the introduction.

12.) pathologic_T

The pathologic tumor status T1, T2, T3 and T4, without a further distinction in the sub-classification as described in the introduction.

13.) pathologic_stage

The breast cancer stages I, II, III, and IV, without a further distinction in the sub-classification as described in the introduction.

3.3 Data processing and management

The files with the relevant data (cBioportal_genexpression for the mRNA expression levels and clinical_data for the patient related clinical informations) were imported into Microsoft Excel 2010 (Microsoft Corporation, Albuquerque, New Mexico, USA) and combined into one table. The final worksheet contained the sample IDs in the first column followed by the mRNA expression values for the genes of interest and finally the complete clinical fields. So one row contained the tissue sample ID followed by all the tissue and associated patient information necessary to carry out the study.

Next some data cleaning was done. Male patients (n=9) and patients with missing expression levels for all relevant genes were excluded. In this study only primary tumors were analysed in the cancer samples group so all samples unequal ‘Primary Tumor’ as ‘sample_type’ (‘Metastatic’ and ‘Solid tissue Normal’) were removed. Finally all ‘age’ values of ‘0’ were replaced with ‘NaN’ for ‘Not a Number’.

The samples were then sorted in a second table regarding the different clinical groups. The title of the clinical group (for instance ‘LN-‘ for lymph node negative patients) was entered in the first row. A sample corresponding to a group showed the mRNA expression value of the gene to be examined. A sample which did not correspond to the specific group showed the value ‘NaN’.

The study population was divided into the following subgroups to investigate eventual statistical differences in the mRNA expression:

1.) Gr.1 vs. Gr.2 vs. Gr.3 vs. Gr.4

Group 1 describes breast cancer with positive hormone receptor status (either estrogen or progesterone-receptor positive) and negative Her2 status. Group 2 describes breast cancer with negative hormone receptor status (both estrogen and progesterone receptor negative) and positive Her2 status. Group 3 is breast cancer with positive hormone receptor status (either estrogen or progesterone-receptor positive) and also positive Her2 status. And finally group 4 is a triple negative cancer with negative hormone receptor (both estrogen and progesterone) and negative Her2 status.

2.) LumA vs. LumB vs. Basal vs. Her2 vs. Normal

As described above, these are five different molecular subtypes of breast cancer as identified by the 50-gene PAM50 predictor. (Luminal A, Luminal B, Basal-like, Her2-enriched and Normal-like)

3.) ER+PR+ vs. ER+PR- vs. ER-PR+ vs. ER-PR-

These groups are distinguished by the particular hormone receptor status (estrogen and progesterone) regardless of the Her2 status.

4.) ER- vs. ER+

These groups are distinguished by the estrogen receptor status regardless of the progesterone receptor or Her2 status.

5.) PR- vs. PR+

These groups are distinguished by the progesterone receptor status regardless of the estrogen receptor or Her2 status.

6.) <35a vs. >=35a prem. vs. >=35a postm.

Further division of the patients by age and menopausal status. The first group describes patients under the age of 35 years at the time of the diagnosis. The second group describes patients age 35 or higher at the time of the diagnosis and a premenopausal status as described above. The third group includes patients age 35 or higher at the time of the diagnosis but a postmenopausal status.

7.) T1 vs. T2 vs. T3 vs. T4

The pathologic tumor status T1, T2, T3 and T4, without a further distinction in the sub-classification as described in the introduction.

8.) Stage I vs. Stage II vs. Stage III vs. Stage IV

The breast cancer stages I, II, III, and IV, without a further distinction in the sub-classification as described in the introduction.

9.) LN neg. vs. LN pos.

Lymph node negative versus Lymph node positive breast cancer, as defined by the pathologic lymph node status N0 and N1/N2/N3 without a further distinction in the sub-classifications as described in the introduction.

10.) M neg. vs. M pos.

Distant metastasis negative versus distant metastasis negative positive breast cancer, as defined by the pathologic metastasis status M0 and M1 described in the introduction.

The resulting table was then copied into a Sigmaplot worksheet for each gene separately.

For the graphs in logarithmic scale, the '0 'values were replaced by ‘0.1’ to avoid a graphical distortion of the results. The values '0' correspond to a gene expression under the detection limit whereas 0.1 corresponds to approximately one-third of the lowest detectable gene expression. The statistical analysis was performed on the original data regardless of this change.

To investigate the differences between tumor and corresponding healthy tissue, the patients’ data with available expression values of both sample types was studied in a separate table. Further, the ratio of tumor and normal sample was calculated by dividing the tumor sample’s expression values by the normal sample’s expression values.

The population of patients with available mRNA expression values of both tumor and normal samples was divided into the following investigation groups for the tumor vs. normal tissue analysis:

1.) Normal vs. Tumor

The mRNA expression levels of tumor tissue and corresponding healthy tissue.

2.) Tumor LN- vs. Tumor LN+

The mRNA expression levels in lymph node negative and lymph node positive tumor tissue of patients with available normal tissue samples.

3.) Normal LN- vs. Normal LN+

The mRNA expression levels in normal tissue samples of lymph node negative and lymph node positive patients.

4.) Quotient LN- vs. Quotient LN+

The ratio of tumor to normal sample calculated by dividing the tumor sample's mRNA expression values by the normal sample's mRNA expression values.

5.) ER- vs. ER+

mRNa expression levels in estrogen receptor negative versus estrogen receptor positive patients.

6.) PR- vs. PR+

mRNa expression levels in progesterone receptor nagative versus progesterone receptor positive patients.

7.) LN- ER- vs. LN+ ER-

mRNa expression levels in lymph node negative versus lymph node positive tumor in estrogen receptor negative patients only.

8.) LN- ER+ vs. LN+ ER+

mRNa expression levels in lymph node negative versus lymph node positive tumor in estrogen receptor positive patients only.

9.) LN- PR- vs. LN+ PR-

mRNa expression levels in lymph node negative versus lymph node positive tumor in progesterone receptor negative patients only.

10.) LN- PR+ vs. LN+ PR+

mRNa expression levels in lymph node negative versus lymph node positive tumor in progesterone receptor positive patients only.

3.4 Statistical analysis

The statistical analysis was performed with the software ‘Sigmaplot für Windows v12.5’ (Systat Software Inc.).

The following statistical tests have been used for the analysis:

The ‘Shapiro-Wilk normality test’ was performed to test for a normally distributed population. The test failed for all populations so the ‘Mann-Whitney Rank Sum Test’ was used to see if the medians of two samples were significantly different (Systat Software). For testing more than two different groups the ‘Kruskal-Wallis One Way Analysis of Variance on Ranks’ was used, followed by ‘All Pairwise Multiple Comparison Procedures (Dunn's Method)’.

For testing normal tissue versus corresponding tumor tissue mRNA expression values of the same patient, the ‘Wilcoxon Signed Rank Test’ was used.

For the survival analysis the ‘Cox Regression - Proportional Hazards Model’ was used with the age and the mRNA expression levels as ‘Numeric Covariates’ and the estrogen receptor status as ‘Categorical Covariate’.

The survival plot was created in the *R*® 3.0.3. statistical environment (www.r-project.org) using a script developed by Györffy et al. (Györffy, et al., 2010). The original script was adapted to use the acquired patient data from TCGA. In total there were 735 patient samples with overall survival data available.

4 RESULTS

4.1 Study population

The number of all patient samples containing used for the analysis was 905. The Age ranged from 26 to 90 with a median of 58. The 25%-percentile was 48 and the 75%-percentile was 67.

	Min	Max	Median	25%	75%
Age	26	90	58	48	67

Table 3 - Age distribution of breast cancer patients

The following tables show the number of patients in the various study groups.

Gr.1 ++ -	Gr.2 -- +	Gr.3 ++ +	Gr.4 -- -
487	30	74	119

Table 4 - Number of patients in the various study groups

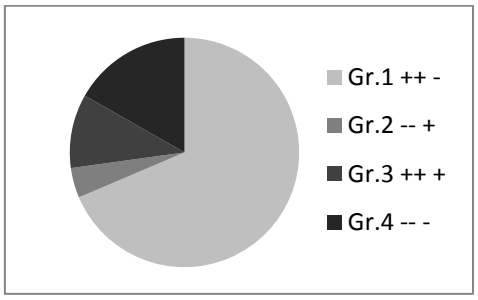


Figure 1 - Distribution of patients in relation to the clinical groups

The number of patients was 487 in group 1, 30 in group 2, 74 in group 3 and 119 in group 4.

LumA	LumB	Basal	Her2	Normal
228	121	96	57	7

Table 5 - Number of patients in relation to PAM50 classification

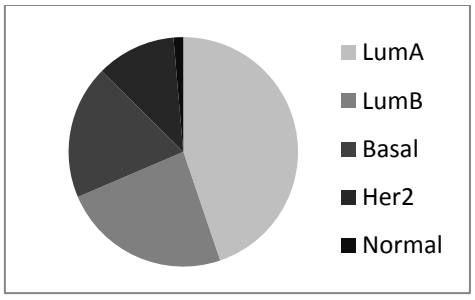


Figure 2 - Distribution of patients in relation to PAM50 classification

There were 228 LuminalA breast cancer patients, 121 LuminalB, 96 Basal-like, 57 Her2-enriched and 7 Normal-like breast cancer patients.

ER+PR+	ER+PR-	ER-PR+	ER-PR-
547	97	13	179

Table 6 - Number of patients in relation to hormone receptor status combinations

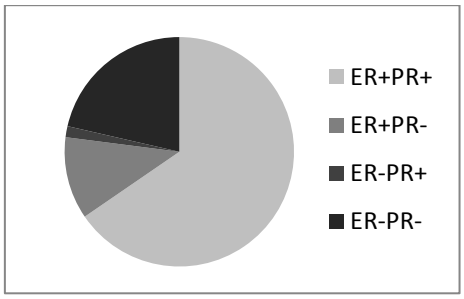
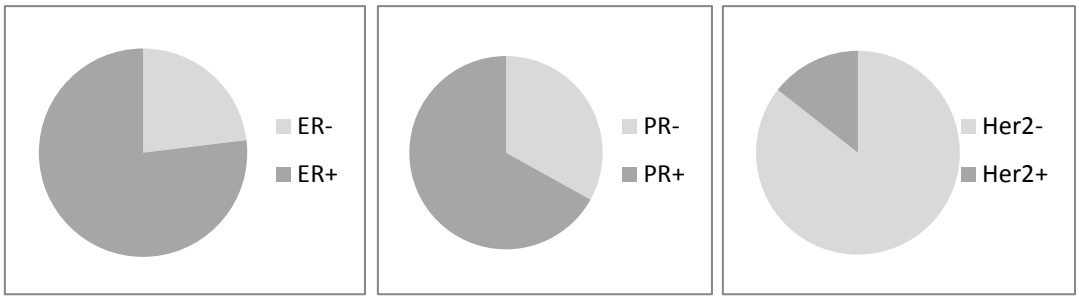


Figure 3 - Distribution of patients in relation to hormone receptor status

547 tumor samples were estrogen receptor and progesterone receptor positive, 97 samples were estrogen receptor positive and progesterone receptor negative, 13 samples were estrogen receptor negative and progesterone receptor positive and 179 samples were hormone receptor negative.

ER-	ER+	PR-	PR+	Her2-	Her2+
194	647	277	561	635	107

Table 7 - Number of patients in relation to hormone receptor and Her2 status



Figures 4A, 4B and 4C - Distribution of patients in relation to estrogen, progesterone or Her2 status

194 tumor samples were estrogen receptor negative and 647 positive, 277 samples were progesterone receptor negative and 561 positive, 635 samples were Her2 negative and 107 Her2 positive.

<35	>=35 prem	>=35 postm
22	164	510

Table 8 - Number of patients in relation to age group

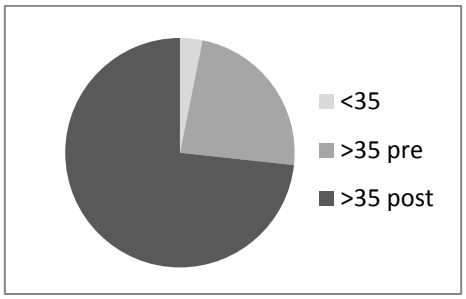


Figure 5 - Distribution of patients in relation to age group

22 patients were under the age of 35, 164 were 35 years or older and premenopausal and 510 patients were 35 years or older and postmenopausal.

T1	T2	T3	T4
235	509	102	33

Table 9 - Number of patients in relation T status

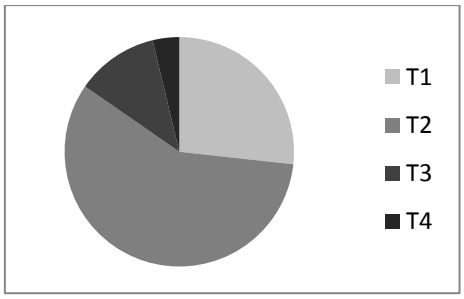


Figure 6 - Distribution of patients in relation to T-status

235 tumor samples corresponded to T1, 509 T2, 102 T3 and 22 T4.

Stage I	Stage II	Stage III	Stage IV
149	505	194	15

Table 10 - Number of patients in relation to Stage

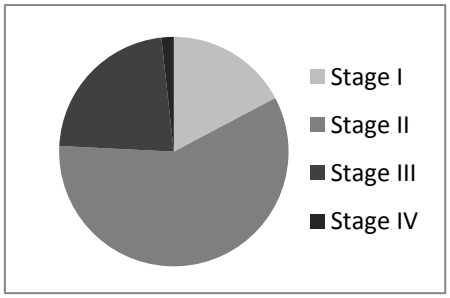
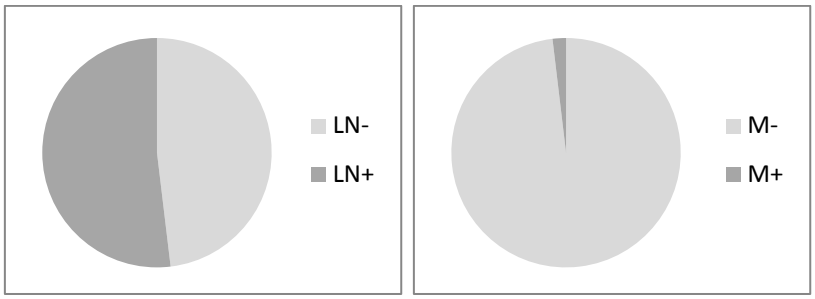


Figure 7 - Distribution of patients in relation to tumor Stage

149 samples corresponded to Stage I, 505 Stage II, 194 Stage III and 15 Stage IV.

LN-	LN+	M-	M+
417	450	780	15

Table 11 - Number of patients in relation lymph node and distant metastasis status



Figures 8A and 8B - Distribution of patients in relation to lymph node and distant metastasis status

417 samples were lymph node negative, 450 were lymph node positive, 780 were distant metastasis negative and 15 were distant metastasis positive.

4.2 Comparison of Gene-expression between various clinical breast cancer subpopulations

All following figures show mRNA expression levels (in TPM) in logarithmical scale in different clinical subpopulations. The median is indicated by a solid line within a box, upper and lower borders indicate the 75% and 25% percentile, error bars indicate 10% and 90% percentiles, respectively. RNA levels below the 10% percentile and above the 90% percentile are shown individually by a cross. The dotted line indicates the arithmetic mean value. The number of patients in each group is shown in parenthesis above the respective box. Any existing significances are shown between the respective groups by the corresponding p-values ($p<0.05$, $p<0.01$ or $p<0.001$).

4.2.1 KCNJ3 mRNA expression in different populations of breast cancer patients

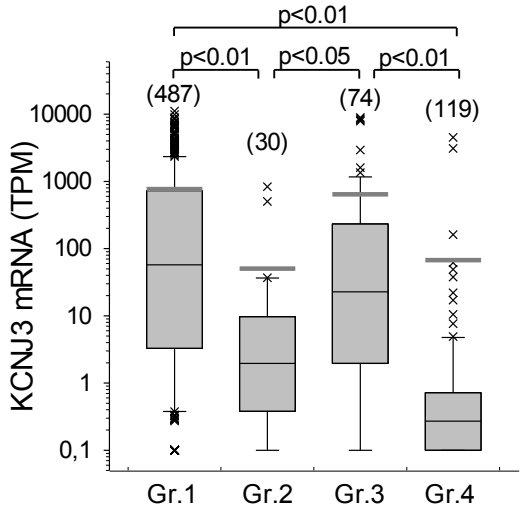


Figure 9A - KCNJ3 mRNA in relation to clinical classification group

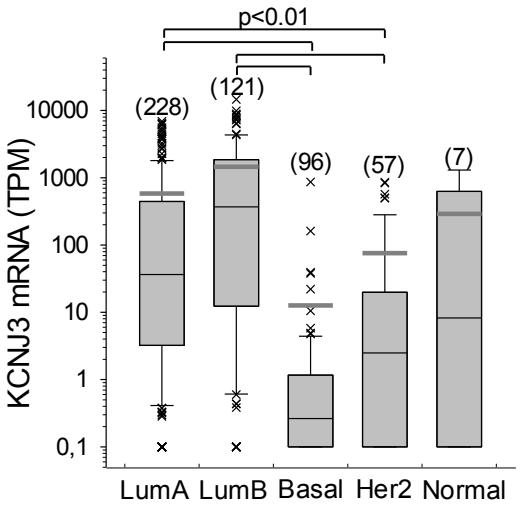


Figure 9A - KCNJ3 mRNA in relation to clinical classification group- KCNJ3 mRNA in relation to PAM50 classification

In Figure 9A the KCNJ3 mRNA levels (in TPM) are shown in logarithmical scale in four different clinical groups as described in the 'Material and Methods' section (Gr. 1: positive ER or PR status and a negative Her2 status, Gr. 2: both ER and PR negative status but positive Her2 status, Gr. 3: positive ER or PR status and also positive Her2 status, Gr. 4: triple negative status). The mRNA expression levels differ statistically significant in Groups 1 and 2 ($p<0.01$), 2 and 3 ($p<0.05$), 3 and 4 ($p<0.01$) and 1 and 4 ($p<0.01$). The expression levels are highest in Groups 1 and 3, which correspond to hormone receptor positive breast cancer patients.

On the other hand Figure 9B shows the mRNA expression levels in the intrinsic breast cancer subtypes Luminal A, Luminal B, basal-like, HER2-enriched and normal-like. The differences between LumB and Basal, LumA and Basal, LumB and Her2 and LumA and Her2 are significant ($p<0.01$). The highest levels are found in Luminal A and Luminal B cancer subtypes that represent estrogen receptor positive breast cancer.

To restrict the association of high KCNJ3 mRNA expression to a specific hormone receptor, the expression between different hormone receptor status combinations was analyzed.

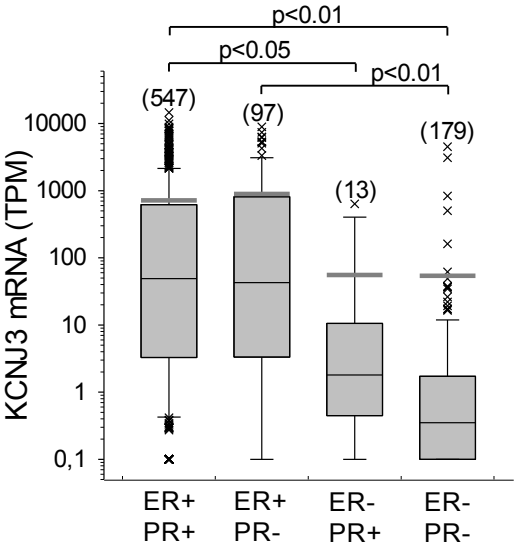


Figure 10A - KCNJ3 mRNA in relation to hormone receptor status

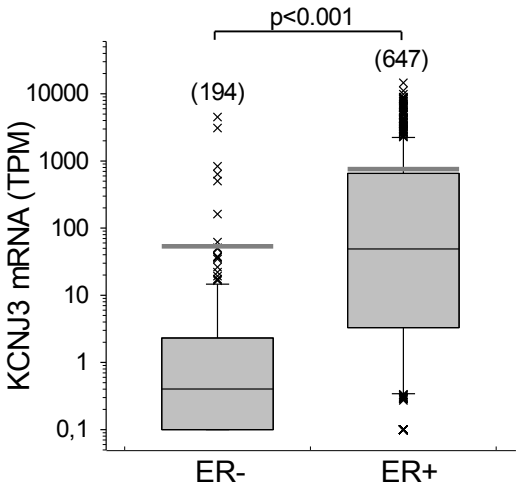


Figure 10B - KCNJ3 mRNA in relation to estrogen receptor status.

Figure 10A shows the results of different combinations of estrogen receptor and progesterone receptor. The differences are significant between ER+ PR+ and ER- PR+ ($p<0.05$), ER+ PR- and ER- PR- ($p<0.01$) and ER+ PR+ and ER- PR- ($p<0.01$) suggesting that the mRNA expression of KCNJ3 is associated with estrogen receptor status. Figure 10B shows the mRNA expression levels of KCNJ3 in relation to estrogen receptor status. The KCNJ3 mRNA expression is significantly higher in estrogen receptor positive breast cancer as compared to estrogen receptor negative breast cancer ($p<0.001$). Next it was tested, whether the KCNJ3 expression is higher in breast cancer of younger women or premenopausal women. The three age/menopause status groups are based on a clinical relevant classification.

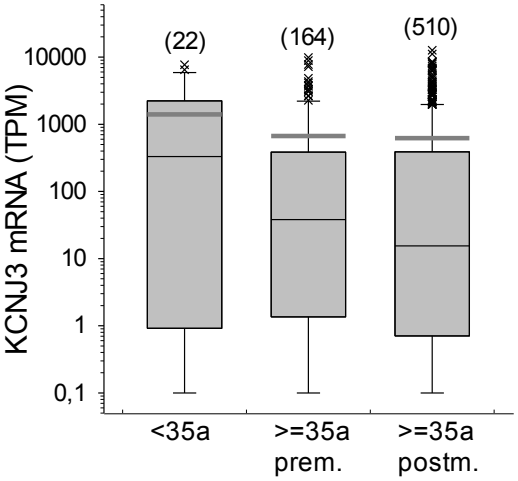


Figure 11A - KCNJ3 mRNA in relation to the 3 age/menopause status groups

Figure 11A shows the mRNA expression levels of KCNJ3 in the three different age/menopause status groups as defined in the 'Methods' section. The KCNJ3 mRNA expression tends to be higher in younger and premenopausal women, although there are no statistically significant differences between the groups, possibly due to the relatively low number of women below the age of 35. We further checked if there are any differences between the age/menopause status groups when limited to estrogen receptor negative or positive breast cancer patients only.

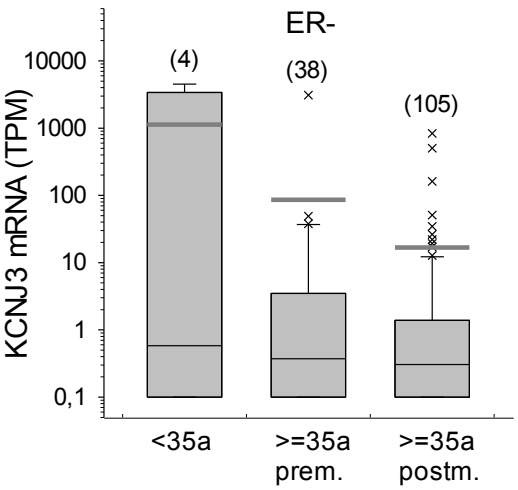


Figure 11B - KCNJ3 mRNA in relation to the 3 age/menopause status groups in ER- patients

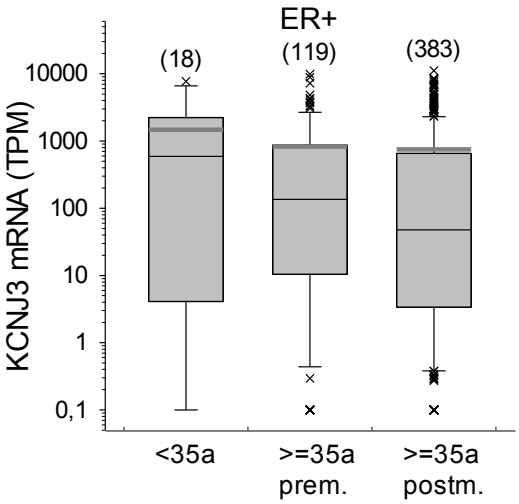


Figure 11C - KCNJ3 mRNA in relation to the 3 age/menopause status groups in ER+ patients

The Figure 11B represents the estrogen receptor negative patients, whereas Figure 11C represents the estrogen receptor positive patients only, divided into the 3 age groups. The differences between estrogen receptor negative and positive patients are huge, as shown before but there are no significant differences within a certain estrogen receptor status.

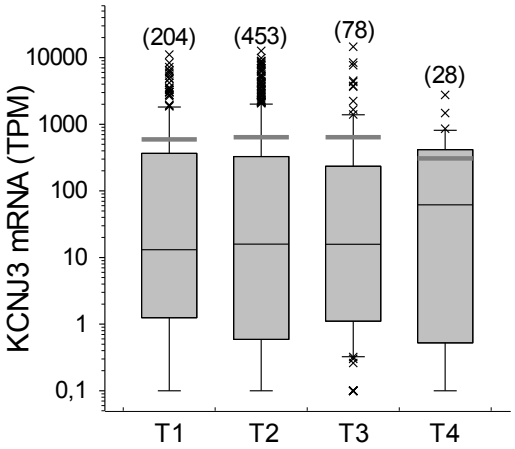


Figure 12A - KCNJ3 mRNA in relation to Tumor Status

Figure 12A shows the mRNA expression levels of KCNJ3 in relation to the tumor status with a non-significantly higher expression in T4 cancer.

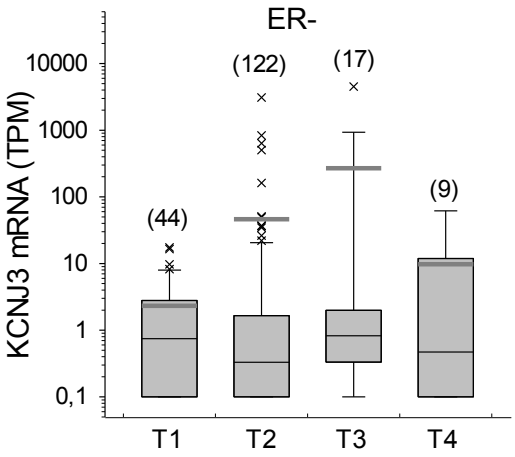


Figure 12B - KCNJ3 mRNA in relation to Tumor Status in ER- patients

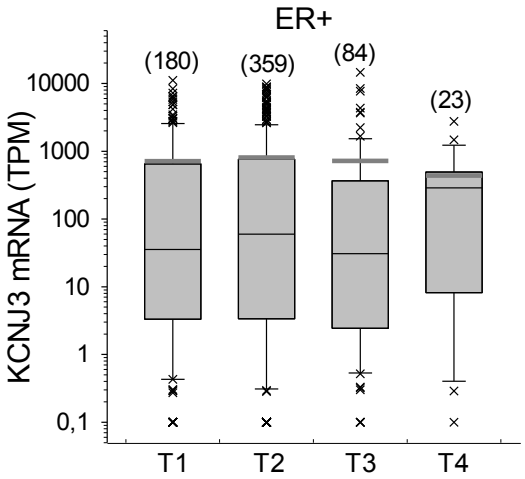


Figure 12C - KCNJ3 mRNA in relation to Tumor status in ER+ patients

The Figure 12B represents the estrogen receptor negative patients, whereas Figure 12C represents the estrogen receptor positive patients only, divided into the 4 different tumor status. Again, the expression is higher in T4 cancer but the number of T4 cancer patients is rather low thus showing no significance.

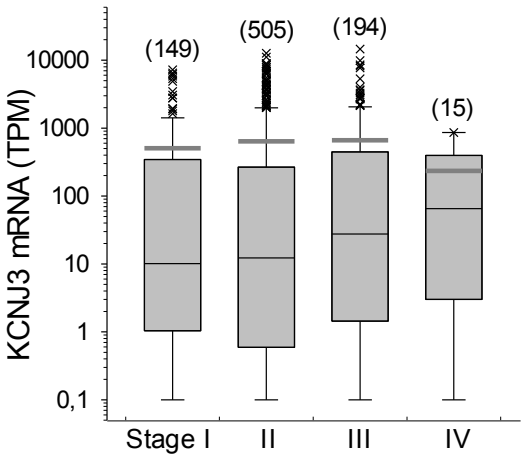


Figure 13A - KCNJ3 mRNA in relation to the Stage

Figure 13A shows the mRNA expression levels of KCNJ3 in relation to the breast cancer stage. The expression seems to rise with a more advanced tumor stage but the results are not significant.

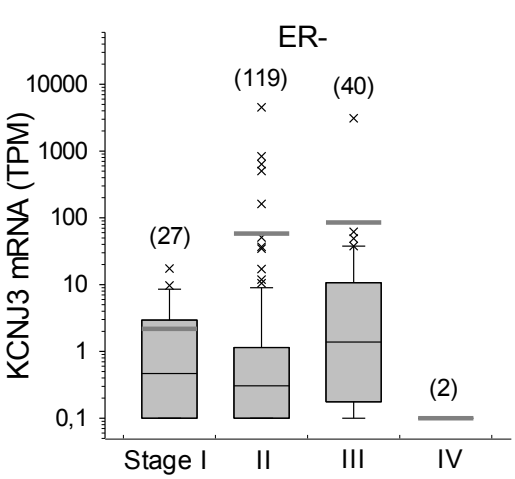


Figure 13B - KCNJ3 mRNA in relation to the Stage in ER- patients

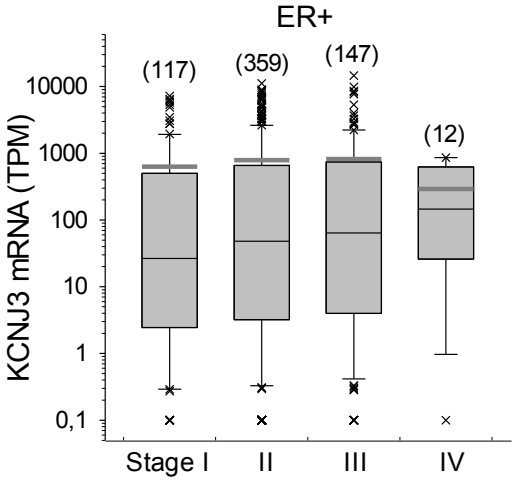


Figure 13C - KCNJ3 mRNA in relation to the Stage in ER+ patients

The Figure 13B represents the estrogen receptor negative patients, whereas Figure 13C represents the estrogen receptor positive patients only, divided into the 4 different breast cancer stages. Again, the expression seems to rise with a more advanced tumor stage in the estrogen receptor positive population but the results are not significant.

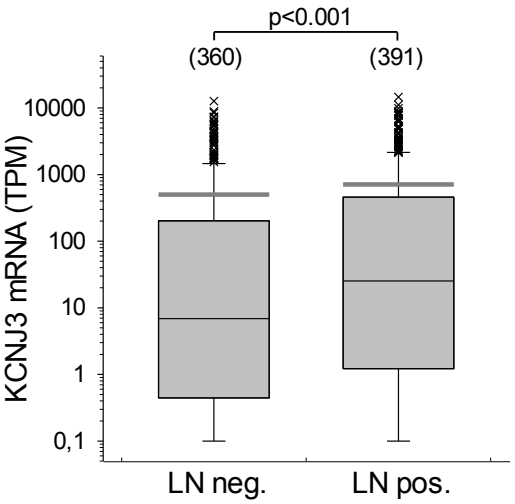


Figure 14A - KCNJ3 mRNA in relation to the Lymph node Status

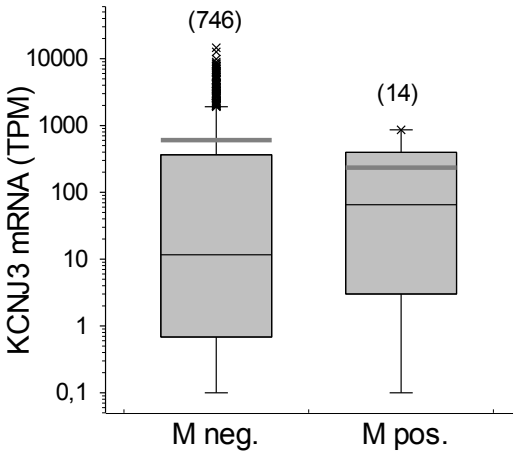


Figure 17B - KCNJ3 mRNA in relation to distant Metastasis Status

Figure 14A shows the mRNA expression levels of KCNJ3 in relation to the lymph node status (negative versus positive). Figure 14B shows the mRNA expression levels of KCNJ3 in relation to the distant metastasis status (negative versus positive). The KCNJ3 expression is significantly higher in lymph node positive breast cancer compared to lymph node negative cancer ($p<0.001$). The KCNJ3 expression in distant metastasis positive cancer is higher than in distant metastasis negative cancer, however no statistically significance can be found, possibly due to the relatively low number of patients with distant metastasis.

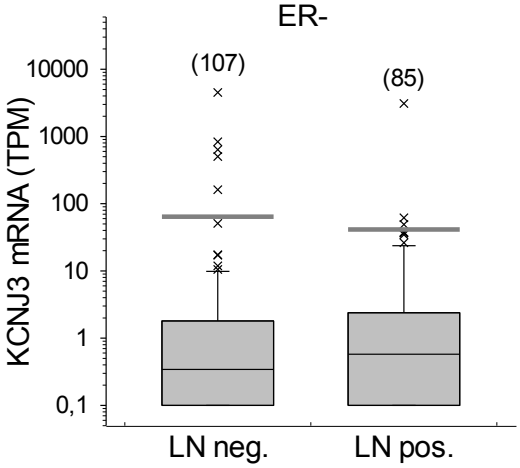


Figure 15A - KCNJ3 mRNA in relation to Lymph node status in ER- patients

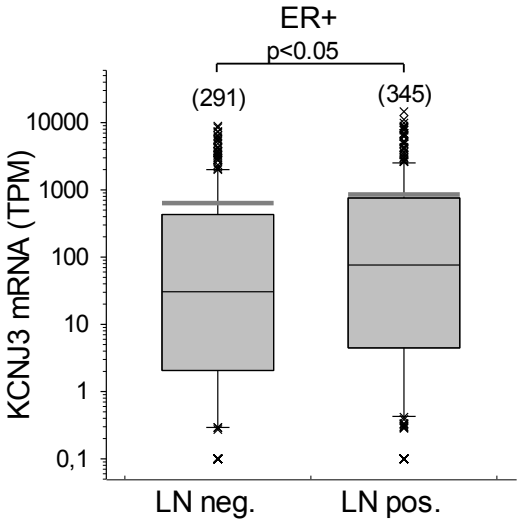


Figure 15B - KCNJ3 mRNA in relation to Lymph node status in ER+ patients

Figure 15A represents the estrogen receptor negative patients, whereas Figure 15B represents the estrogen receptor positive patients only, divided into lymph node negative and lymph node positive breast cancer patients. The difference between lymph node negative and lymph node positive patients is significant ($p<0.05$) in the population of estrogen receptor positive patients.

4.2.2 KCNJ5 mRNA expression in different populations of breast cancer patients

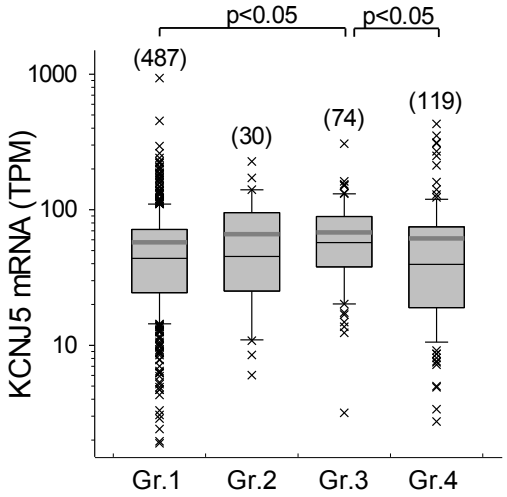


Figure 16A - KCNJ5 mRNA in relation to clinical classification group

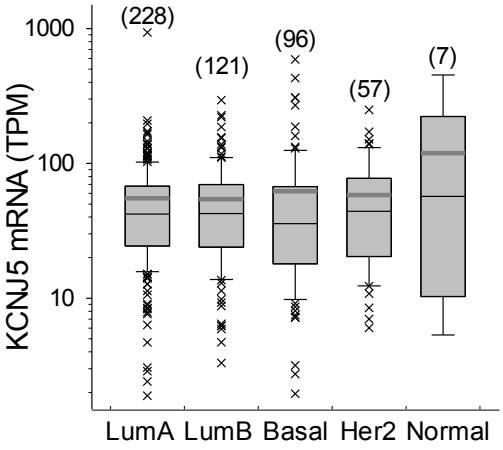


Figure 16B - KCNJ5 mRNA in relation to PAM50 classification

In Figure 16A the KCNJ5 mRNA levels (in TPM) are shown in logarithmical scale in four different clinical groups as described in the 'Material and Methods' section. There is a significant difference between the mRNA expression levels of Group 1 and 3 ($p<0.05$) and 3 and 4 ($p<0.05$). The expression level is highest in Group 3, which corresponds to her2 positive breast cancer patients.

On the other hand Figure 16B shows the mRNA expression levels in the intrinsic breast cancer subtypes Luminal A, Luminal B, basal-like, HER2-enriched and normal-like. No significant differences can be found between these Groups.

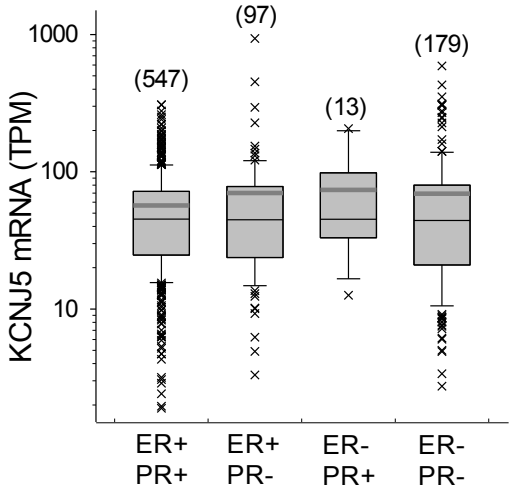


Figure 17A - KCNJ5 mRNA in relation to hormone receptor status.

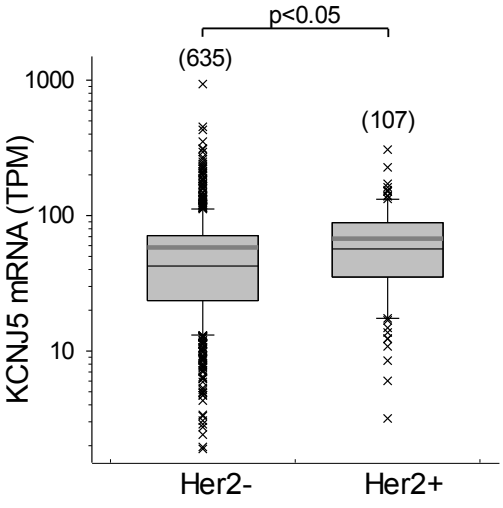


Figure 17B - KCNJ5 mRNA in relation to Her2 receptor status.

Figure 17A shows the results of various combinations of estrogen receptor and progesterone receptor without any significant differences. The results suggest that the mRNA expression of KCNJ5 is associated with Her2 receptor status.

Figure 17B shows that the mRNA expression levels of KCNJ5 are significantly higher in Her2 receptor positive breast cancer compared to Her2 receptor negative breast cancer ($p<0.05$). Next, it was tested, whether the KCNJ5 expression is higher in breast cancer of younger women or premenopausal women. The three age/menopause status groups are based on a clinical relevant classification.

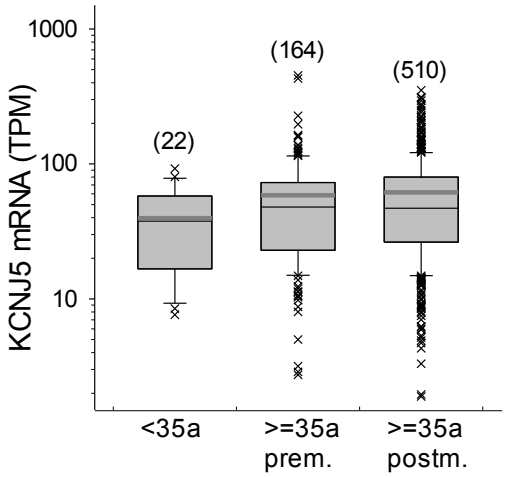


Figure 18A - KCNJ5 mRNA in relation to the 3 age/menopause status groups

Figure 18A shows the mRNA expression levels of KCNJ5 in the three different age/menopause status groups as defined in the 'Methods' section. The KCNJ5 mRNA expression tends to be higher in older and postmenopausal women, although there are no statistically significant differences between the groups, possibly due to the relatively low number of women below the age of 35. We further checked if there are any differences between the age/menopause status groups when limited to Her2 receptor negative or positive breast cancer patients only.

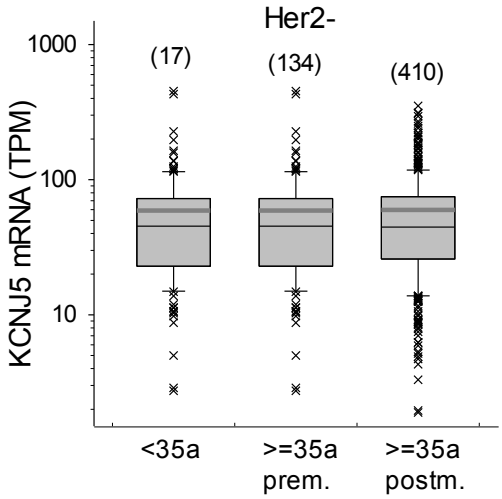


Figure 18B - KCNJ5 mRNA in relation to the 3 age/menopause status groups in HER2- patients

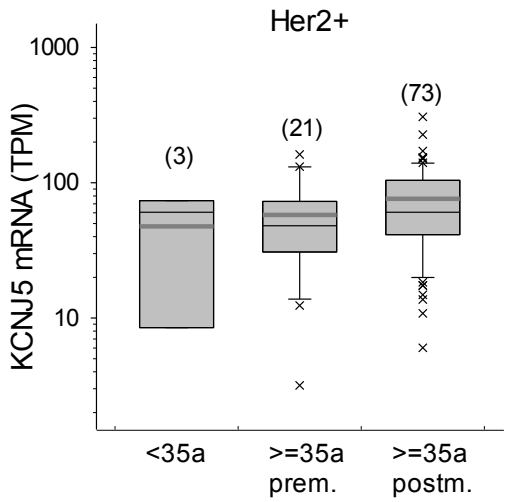


Figure 18C - KCNJ5 mRNA in relation to the 3 age/menopause status groups in HER2+ patients

The Figure 18B represents the Her2 receptor negative patients, whereas Figure 18C represents the Her2 receptor positive patients only, divided into the 3 age groups. There are no significant differences within a certain Her2 receptor status.

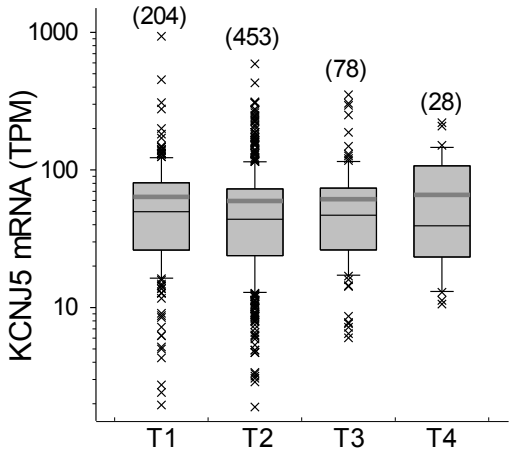


Figure 19A - KCNJ5 mRNA in relation to Tumor Status

Figure 19A shows the mRNA expression levels of KCNJ5 in relation to the tumor status with no significant differences.

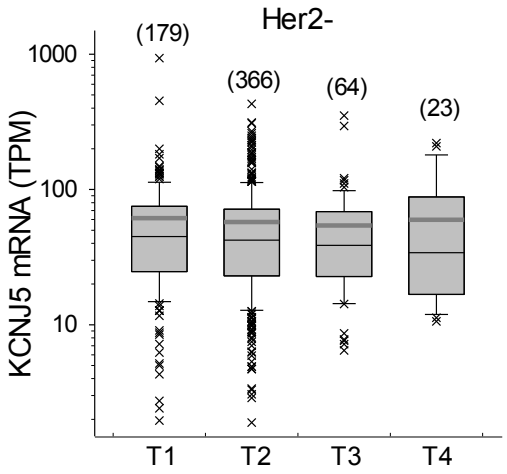


Figure 19B - KCNJ5 mRNA in relation to Tumor Status in HER2- patients

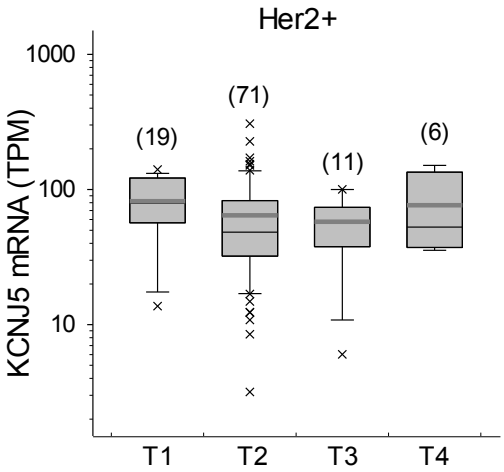


Figure 19C - KCNJ5 mRNA in relation to Tumor status in HER2+ patients

The Figure 19B represents the Her2 receptor negative patients, whereas Figure 19C represents the Her2 receptor positive patients only, divided into the 4 different tumor status.

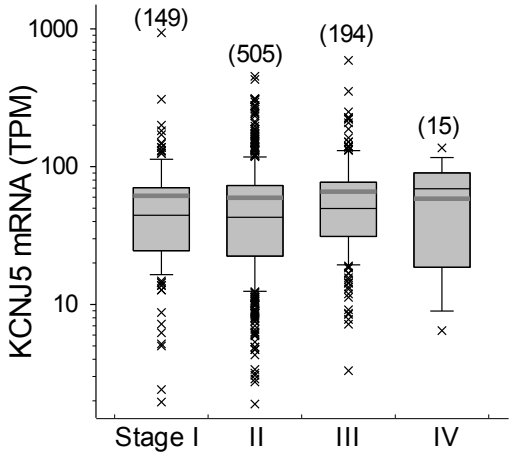


Figure 20A - KCNJ5 mRNA in relation to the Stage

Figure 20A shows the mRNA expression levels of KCNJ5 in relation to the breast cancer stage. The expression seems to rise with a more advanced tumor stage but the results are not significant.

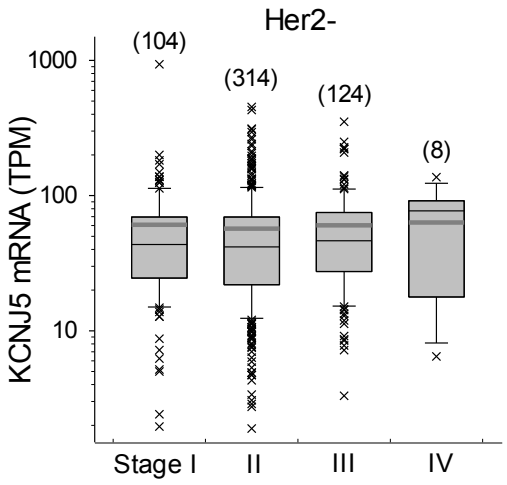


Figure 20B - KCNJ5 mRNA in relation to the Stage in HER2- patients

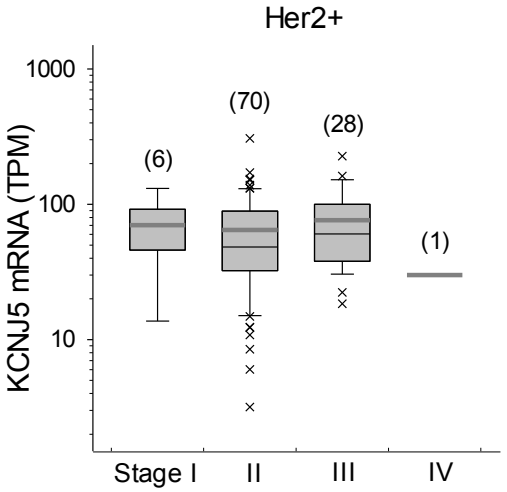


Figure 20C - KCNJ5 mRNA in relation to the Stage in HER2+ patients

The Figure 20B represents the Her2 receptor negative patients, whereas Figure 20C represents the Her2 receptor positive patients only, divided into the 4 different breast cancer stages. Again, the expression seems to rise with a more advanced tumor stage in the Her2 receptor negative population but the results are not significant.

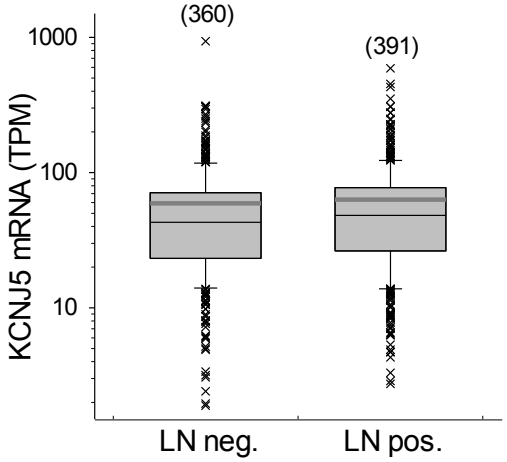


Figure 21A - KCNJ5 mRNA in relation to the Lymph node Status

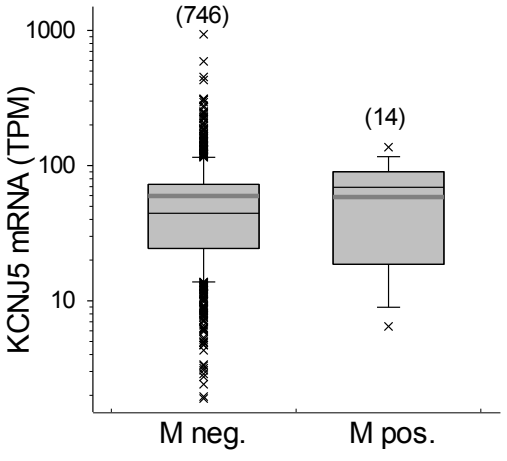


Figure 21B - KCNJ5 mRNA in relation to distant Metastasis Status

Figure 21A shows the mRNA expression levels of KCNJ5 in relation to the lymph node status (negative versus positive). Figure 21B shows the mRNA expression levels of KCNJ5 in relation to the distant metastasis status (negative versus positive). There are no significant differences.

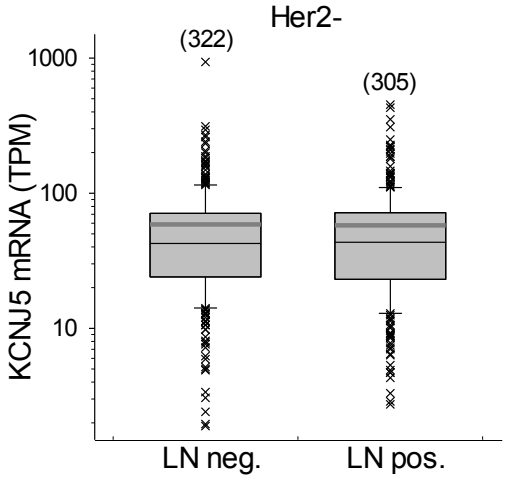


Figure 22A - KCNJ5 mRNA in relation to Lymph node status in HER2- patients

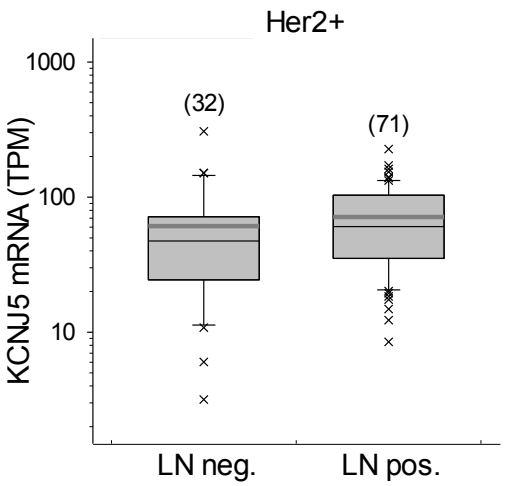


Figure 22B - KCNJ5 mRNA in relation to Lymph node status in HER2+ patients

The Figure 22A represents the Her2 receptor negative patients, whereas Figure 22B represents the Her2 receptor positive patients only, divided into lymph node negative and lymph node positive breast cancer patients. There are no significant differences.

4.2.3 KCNJ6 mRNA expression in different populations of breast cancer patients

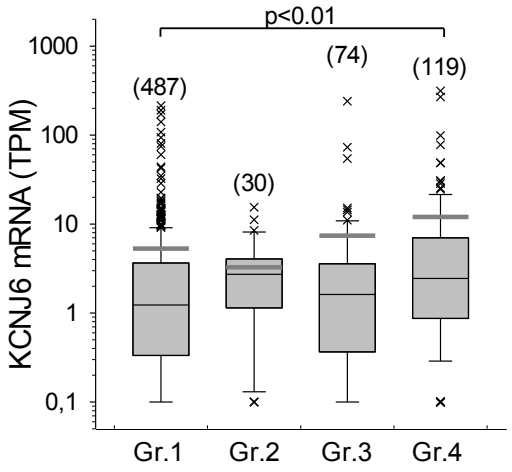


Figure 23A - KCNJ6 mRNA in relation to clinical classification group

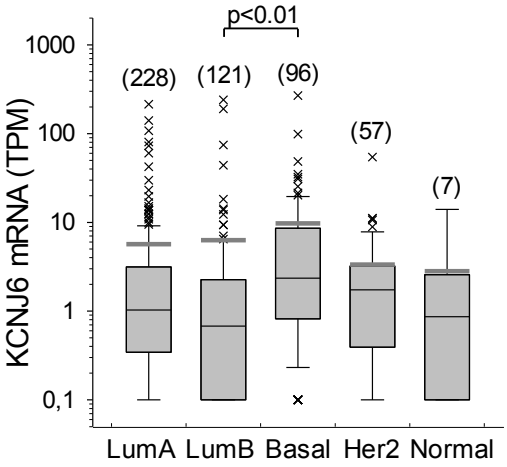


Figure 23B - KCNJ6 mRNA in relation to PAM50 classification

In Figure 23A the KCNJ6 mRNA levels (in TPM) are shown in logarithmical scale in four different clinical groups as described in the 'Material and Methods' section. The difference between the mRNA expression levels of Groups 1 and 4 is statistically significant ($p<0.01$).

On the other hand Figure 23B shows the mRNA expression levels in the intrinsic breast cancer subtypes Luminal A, Luminal B, basal-like, HER2-enriched and normal-like. The difference between LumB and Basal is significant ($p<0.01$).

To restrict the association of high KCNJ6 mRNA expression to a specific hormone receptor, the expression between different hormone receptor status combinations was analyzed.

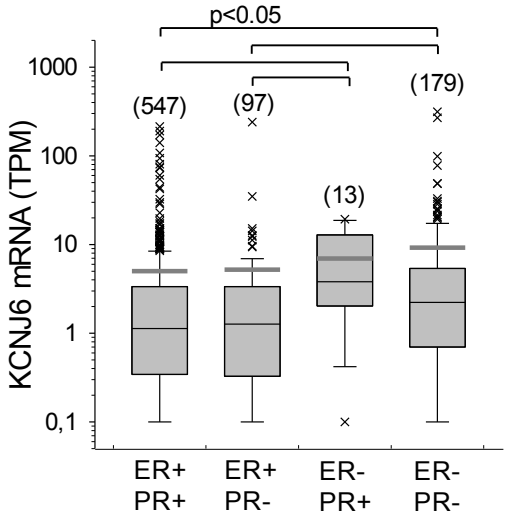


Figure 24A - KCNJ6 mRNA in relation to hormone receptor status.

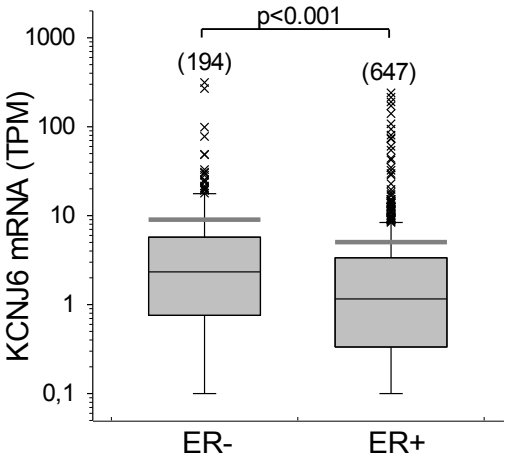


Figure 24B - KCNJ6 mRNA in relation to estrogen receptor status.

Figure 24A shows the results of different combinations of estrogen receptor and progesterone receptor. The differences are significant between ER+ PR- and ER- PR+, ER+ PR+ and ER- PR+, ER+ PR- and ER- PR- and ER+ PR+ and ER- PR- ($p<0.05$) suggesting that the mRNA expression of KCNJ6 is associated with estrogen receptor status. Figure 24B shows that the mRNA expression level of KCNJ6 is significantly higher in estrogen receptor negative breast cancer compared to estrogen receptor positive breast cancer ($p<0.001$). Next, it was tested, whether the KCNJ6 expression is higher in breast cancer of younger women or premenopausal women. The three age/menopause status groups are based on a clinical relevant classification.

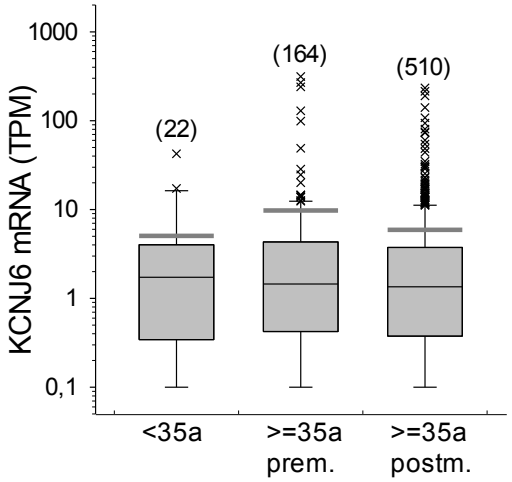


Figure 25A - KCNJ6 mRNA in relation to the 3 age/menopause status groups

Figure 25A shows the mRNA expression levels of KCNJ6 in the three different age/menopause status groups as defined in the 'Methods' section. There are no significant differences.

Further it was checked if there are any differences between the age/menopause status groups when limited to estrogen receptor negative or positive breast cancer patients only.

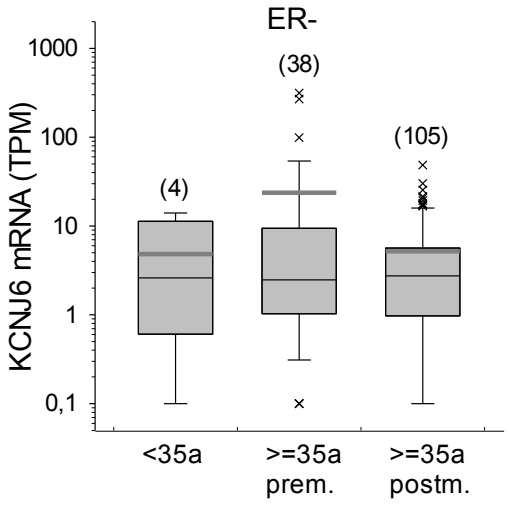


Figure 25B - KCNJ6 mRNA in relation to the 3 age/menopause status groups in ER- patients

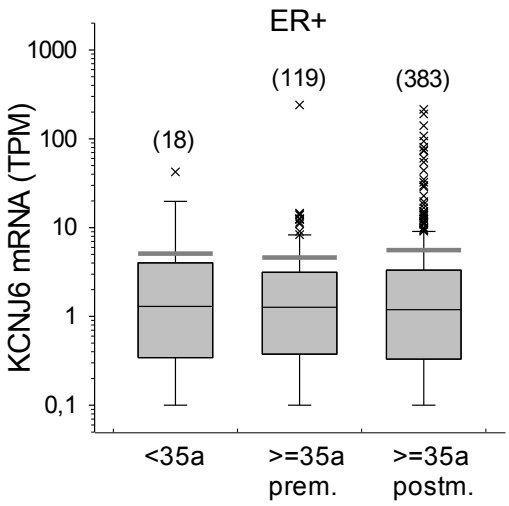


Figure 25C - KCNJ6 mRNA in relation to the 3 age/menopause status groups in ER+ patients

The Figure 25B represents the estrogen receptor negative patients, whereas Figure 25C represents the estrogen receptor positive patients only, divided into the 3 age groups. There are no significant differences within a certain estrogen receptor status.

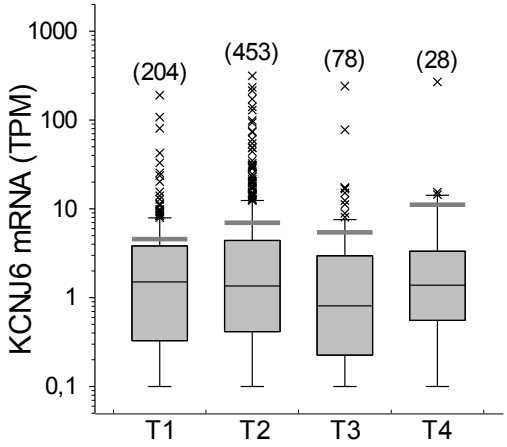


Figure 26A - KCNJ6 mRNA in relation to Tumor Status

Figure 26A shows the mRNA expression levels of KCNJ6 in relation to the tumor status with no significant results.

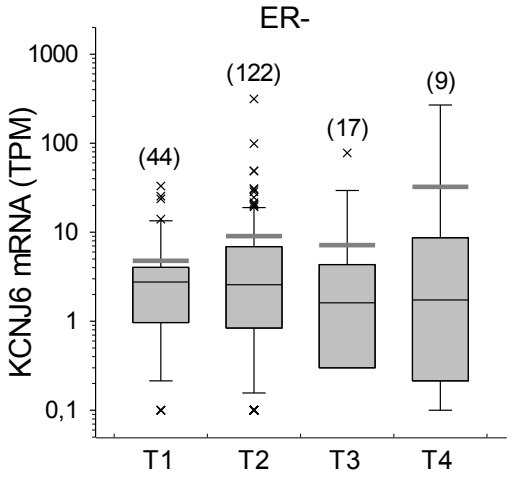


Figure 26B - KCNJ6 mRNA in relation to Tumor Status in ER- patients

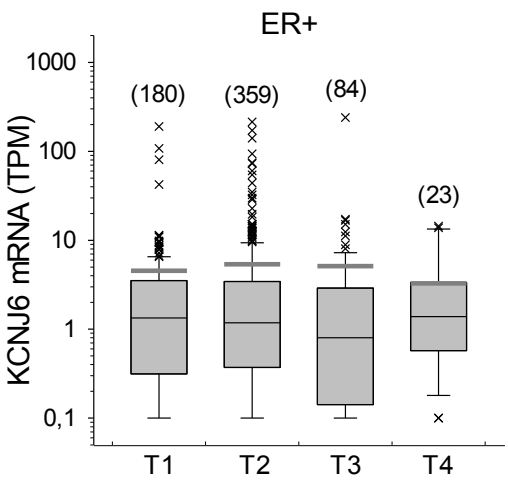


Figure 26C - KCNJ6 mRNA in relation to Tumor status in ER+ patients

The Figure 26B represents the estrogen receptor negative patients, whereas Figure 26C represents the estrogen receptor positive patients only, divided into the 4 different tumor status.

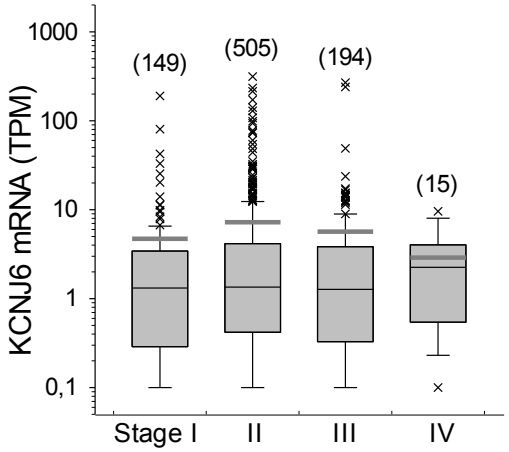


Figure 27A - KCNJ6 mRNA in relation to the Stage

Figure 27A shows the mRNA expression levels of KCNJ6 in relation to the breast cancer stage.

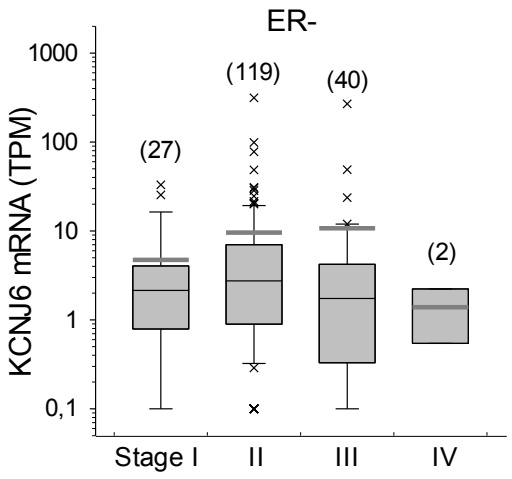


Figure 27B - KCNJ6 mRNA in relation to the Stage in ER- patients

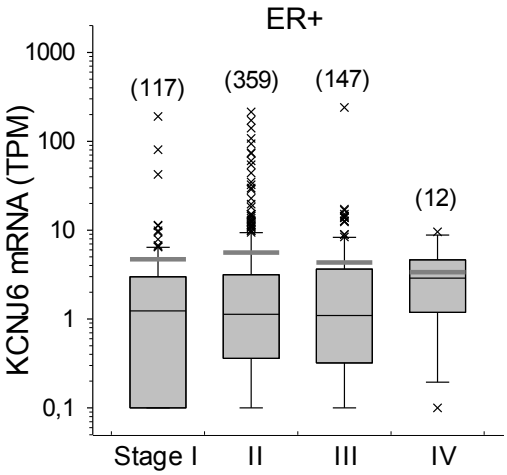


Figure 27C - KCNJ6 mRNA in relation to the Stage in ER+ patients

The Figure 27B represents the estrogen receptor negative patients, whereas Figure 27C represents the estrogen receptor positive patients only, divided into the 4 different breast cancer stages.

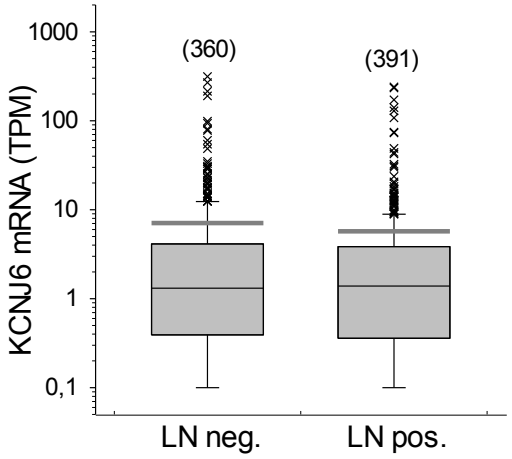


Figure 28A - KCNJ6 mRNA in relation to the Lymph node Status

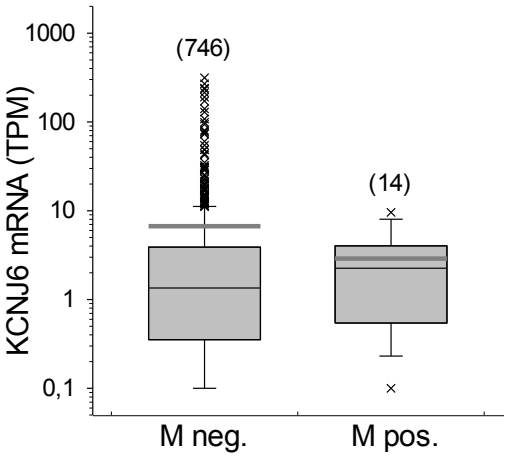


Figure 28B - KCNJ6 mRNA in relation to distant Metastasis Status

Figure 28A shows the mRNA expression levels of KCNJ6 in relation to the lymph node status (negative versus positive). Figure 28B shows the mRNA expression levels of KCNJ6 in relation to the distant metastasis status (negative versus positive).

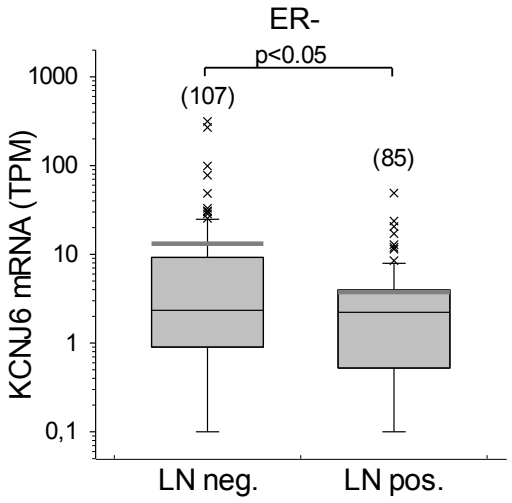


Figure 29A - KCNJ6 mRNA in relation to Lymph node status in ER- patients

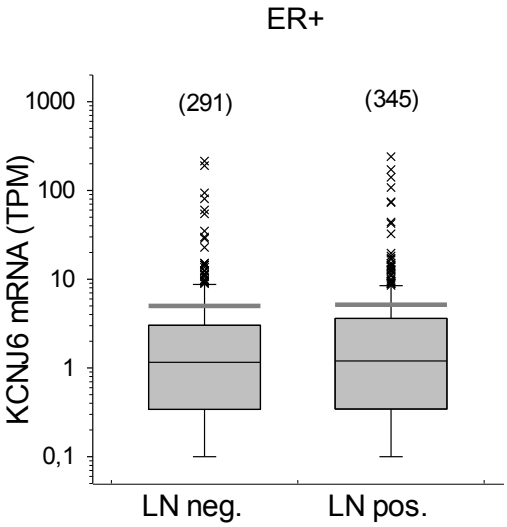


Figure 29B - KCNJ6 mRNA in relation to Lymph node status in ER+ patients

The Figure 29A represents the estrogen receptor negative patients, whereas Figure 29B represents the estrogen receptor positive patients only, divided into lymph node negative and lymph node positive breast cancer patients. The difference between lymph node negative and lymph node positive patients is only in the population of estrogen receptor negative patients significant ($p<0.05$).

4.2.4 KCNJ9 mRNA expression in different populations of breast cancer patients

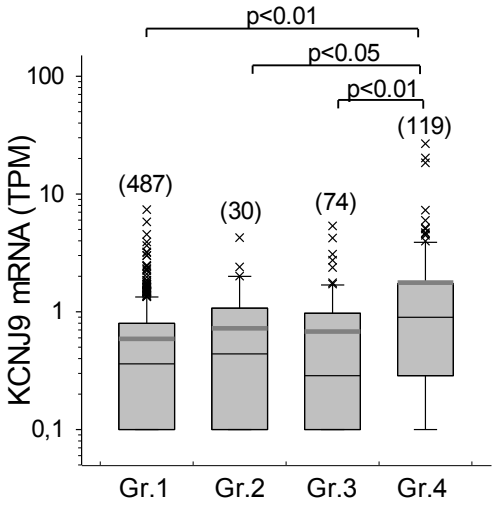


Figure 30A - KCNJ9 mRNA in relation to clinical classification group

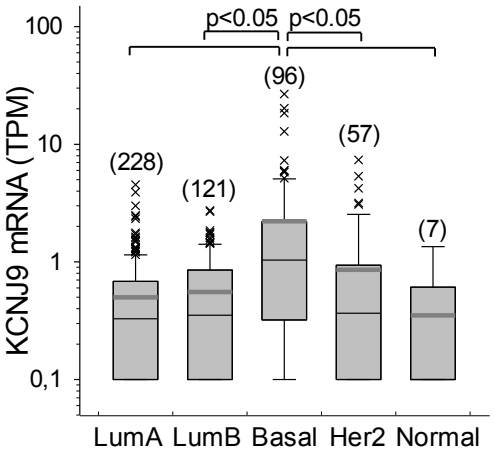


Figure 30B - KCNJ9 mRNA in relation to PAM50 classification

In Figure 30A the KCNJ9 mRNA levels (in TPM) are shown in logarithmical scale in four different clinical groups as described in the 'Material and Methods' section. There is a statistically significant difference between the mRNA expression levels of Groups 3 and 4 ($p<0.01$), 2 and 4 ($p<0.05$) and 1 and 4 ($p<0.01$).

On the other hand Figure 30B shows the mRNA expression levels in the intrinsic breast cancer subtypes Luminal A, Luminal B, basal-like, HER2-enriched and normal-like. The differences between LumA and Basal, Basal and Normal, LumB and Basal and Basal and Her2 are significant ($p<0.05$).

To restrict the association of high KCNJ9 mRNA expression to a specific hormone receptor, the expression between different hormone receptor status combinations was analyzed.

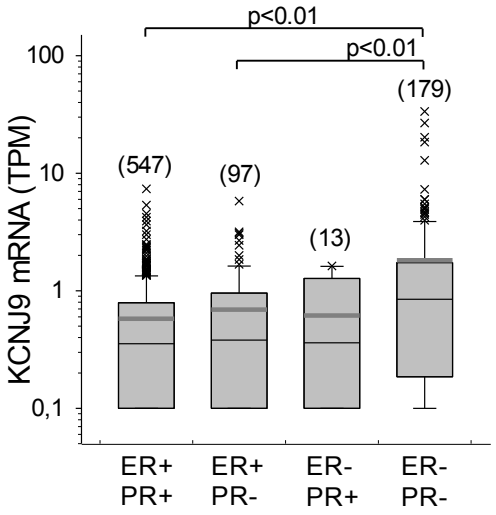


Figure 31A - KCNJ9 mRNA in relation to hormone receptor status.

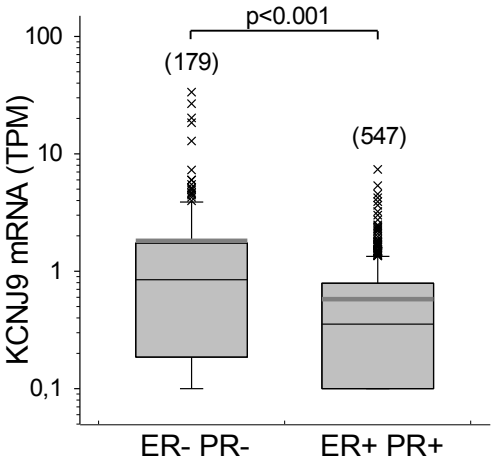


Figure 31B - KCNJ9 mRNA in relation to hormone receptor status.

Figure 31A shows the results of different combinations of estrogen receptor and progesterone receptor. The differences are significant between ER+ PR- and ER- PR- and ER+ PR+ and ER- PR- ($p<0.01$) suggesting that the mRNA expression of KCNJ9 is associated with an hormone receptor negative receptor status. Figure 31B shows that the mRNA expression levels of KCNJ9 is significantly higher in hormone receptor negative breast cancer compared to hormone receptor positive breast cancer ($p<0.001$). Next, it was tested, whether the KCNJ9 expression is higher in breast cancer of younger women or premenopausal women. The three age/menopause status groups are based on a clinical relevant classification.

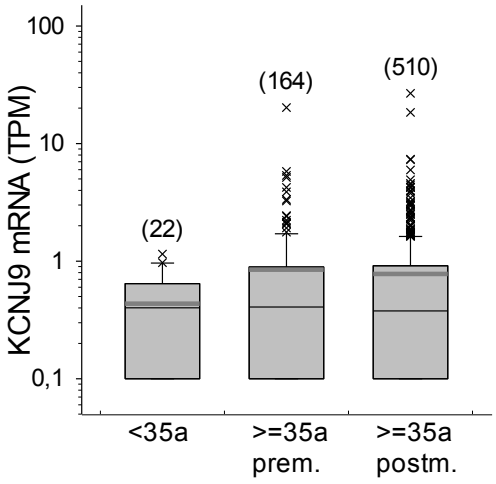


Figure 32A - KCNJ9 mRNA in relation to the 3 age/menopause status groups

Figure 32A shows the mRNA expression levels of KCNJ9 in the three different age/menopause status groups as defined in the 'Methods' section.

We further checked if there are any differences between the age/menopause status groups when limited to estrogen receptor negative or positive breast cancer patients only.

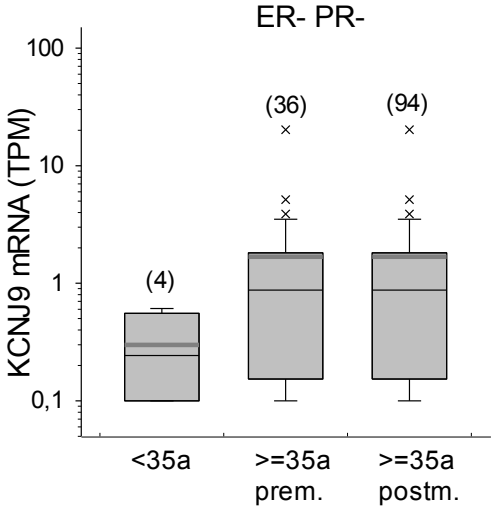


Figure 32B - KCNJ9 mRNA in relation to the 3 age/menopause status groups in ER- PR- patients

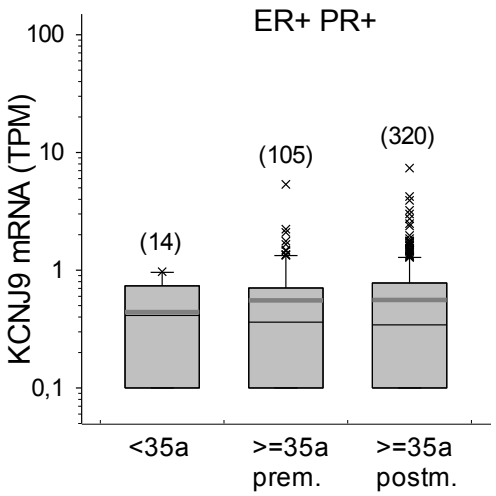


Figure 32C - KCNJ9 mRNA in relation to the 3 age/menopause status groups in ER+ PR+ patients

The Figure 32B represents the estrogen and progesterone receptor negative patients, whereas Figure 32C represents the estrogen and progesterone receptor positive patients only, divided into the 3 age groups. There are no significant differences within a certain hormone receptor status.

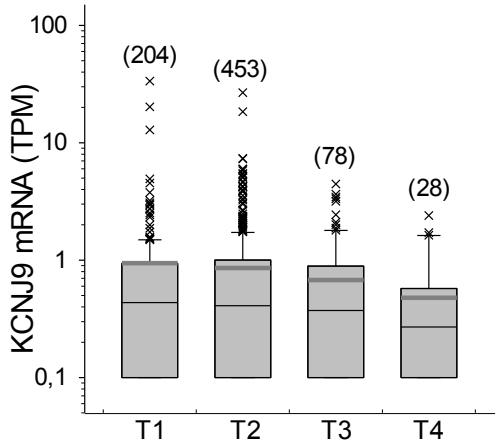


Figure 33A - KCNJ9 mRNA in relation to Tumor Status

Figure 33A shows the mRNA expression levels of KCNJ9 in relation to the tumor status with no significant differences.

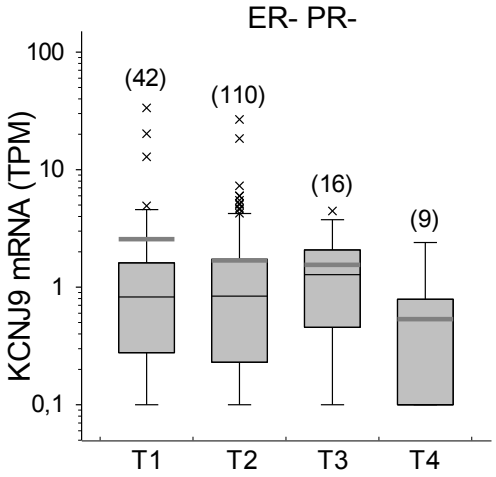


Figure 33B - KCNJ9 mRNA in relation to Tumor Status in ER- PR- patients

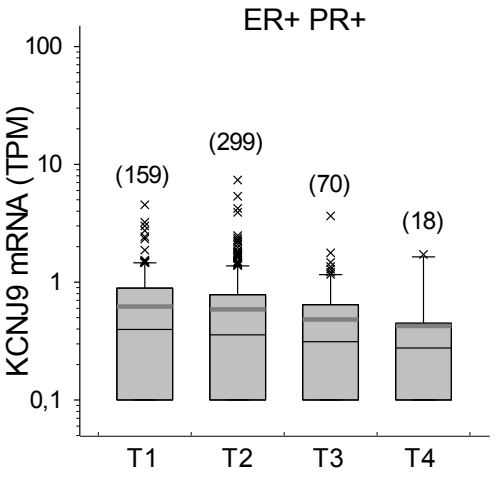


Figure 33C - KCNJ9 mRNA in relation to Tumor status in ER+ PR+ patients

The Figure 33B represents the estrogen and progesterone receptor negative patients, whereas Figure 33C represents the estrogen and progesterone receptor positive patients only, divided into the 4 different tumor status.

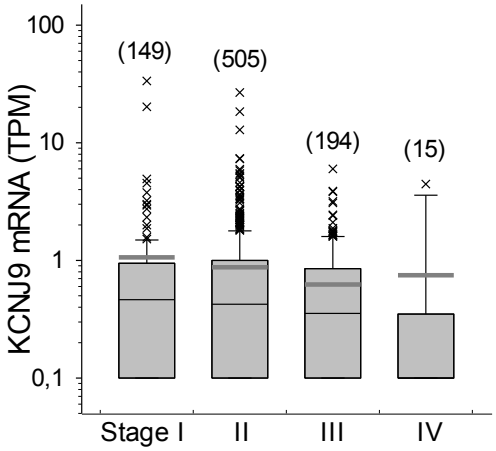


Figure 34A - KCNJ9 mRNA in relation to the Stage

Figure 34A shows the mRNA expression levels of KCNJ9 in relation to the breast cancer stage.

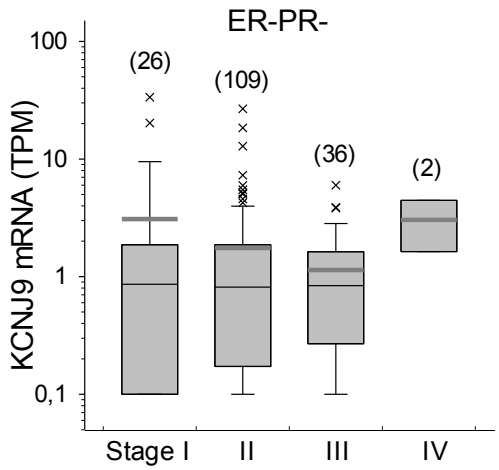


Figure 34B - KCNJ mRNA in relation to the Stage in ER- PR- patients

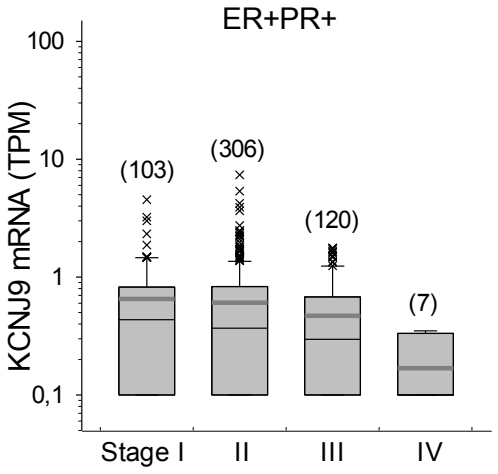


Figure 34C - KCNJ9 mRNA in relation to the Stage in ER+ PR+ patients

The Figure 34B represents the estrogen and progesterone receptor negative patients, whereas Figure 34C represents the estrogen and progesterone receptor positive patients only, divided into the 4 different breast cancer stages.

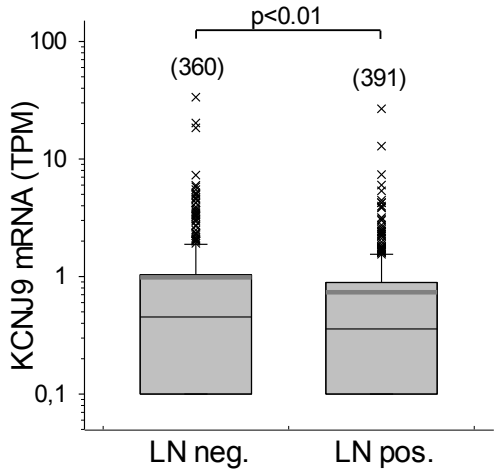


Figure 35A - KCNJ9 mRNA in relation to the Lymph node Status

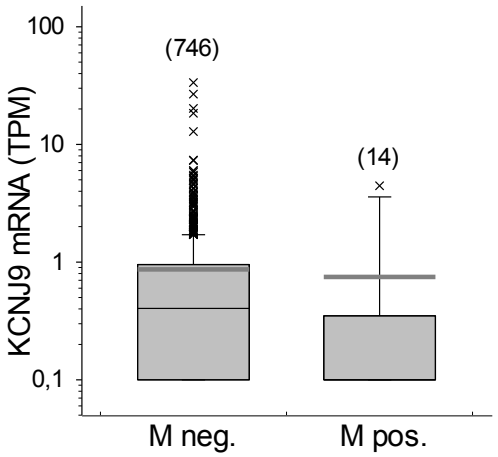


Figure 35B - KCNJ9 mRNA in relation to distant Metastasis Status

Figure 35A shows the mRNA expression levels of KCNJ9 in relation to the lymph node status (negative versus positive). Figure 35B shows the mRNA expression levels of KCNJ9 in relation to the distant metastasis status (negative versus positive). The KCNJ9 expression is significantly higher in lymph node negative breast cancer compared to lymph node positive cancer ($p<0.001$).

The number of patients with distant metastasis is just 14 and too low to make a statement about the difference to M negative patients.

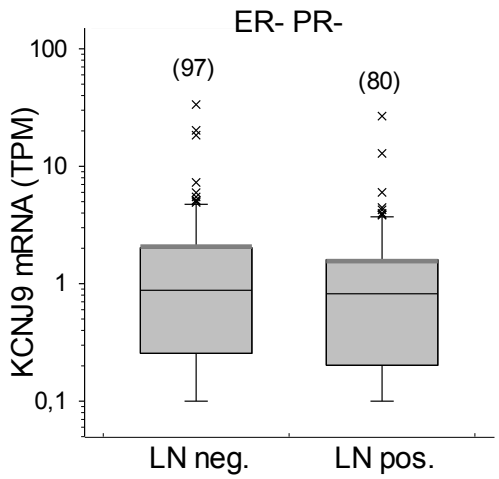


Figure 36A - KCNJ9 mRNA in relation to Lymph node status in ER- PR- patients

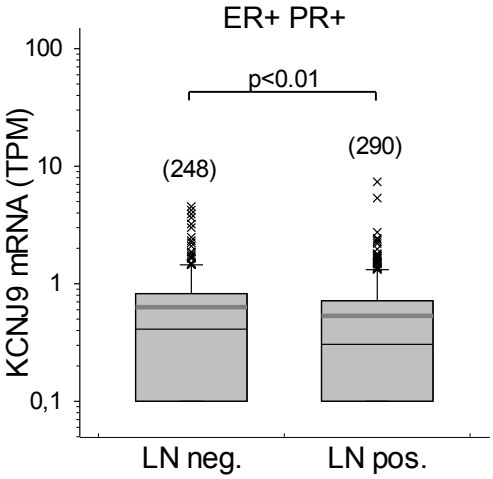


Figure 36B - KCNJ9 mRNA in relation to Lymph node status in ER+ PR+ patients

The Figure 36A represents the estrogen and progesterone receptor negative patients, whereas Figure 36B represents the estrogen and progesterone receptor positive patients only, divided into lymph node negative and lymph node positive breast cancer patients. The difference between lymph node negative and lymph node positive patients is only in the population of estrogen and progesterone receptor positive patients significant ($p<0.01$) with a higher expression in lymph node negative patients.

4.2.5 Piezo1 mRNA expression in different populations of breast cancer patients

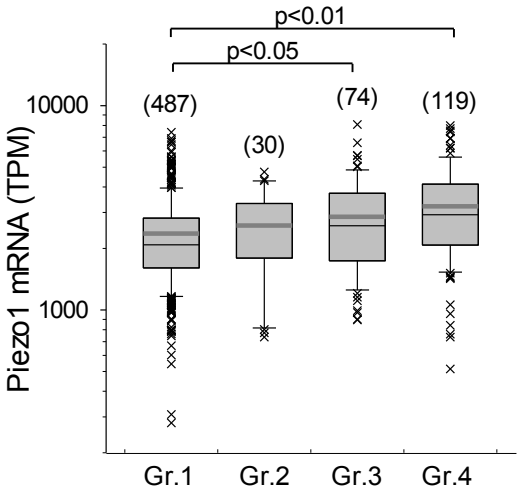


Figure 37A - Piezo1 mRNA in relation to clinical classification group

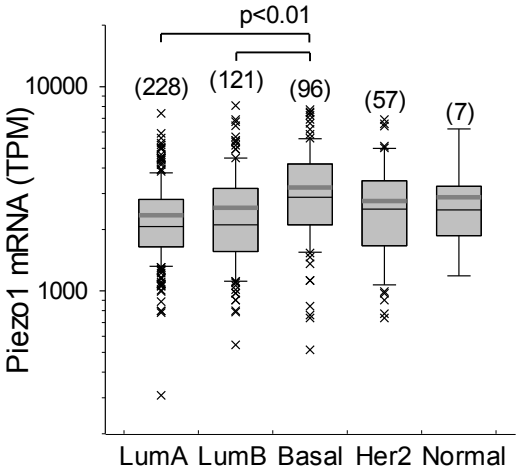


Figure 37B - Piezo1 mRNA in relation to PAM50 classification

In Figure 37A the Piezo1 mRNA levels (in TPM) are shown in logarithmical scale in four different clinical groups as described in the 'Material and Methods' section. The difference is significant between the mRNA expression levels of Groups 1 and 3 ($p<0.05$) and 1 and 4 ($p<0.01$).

On the other hand Figure 37B shows the mRNA expression levels in the intrinsic breast cancer subtypes Luminal A, Luminal B, basal-like, HER2-enriched and normal-like. The differences between LumB and Basal and LumA and Basal are significant ($p<0.01$). To restrict the association of Piezo1 mRNA expression to a specific hormone receptor, the expression between different hormone receptor status combinations was analyzed.

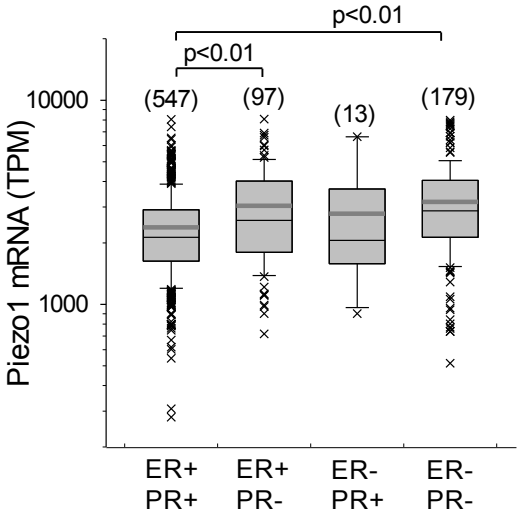


Figure 38A - Piezo1 mRNA in relation to hormone receptor status.

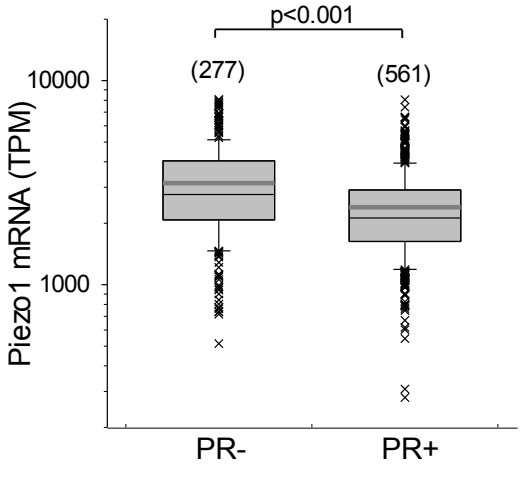


Figure 38B - Piezo1 mRNA in relation to progesterone receptor status.

Figure 38A shows the results of different combinations of estrogen receptor and progesterone receptor. The differences are significant between ER+ PR+ and ER+ PR- and ER+ PR+ and ER- PR- ($p<0.01$) suggesting that the mRNA expression of Piezo1 is associated with progesterone receptor status. Figure 38B shows that the mRNA expression levels of Piezo1 is significantly higher in progesterone receptor negative breast cancer compared to progesterone receptor positive breast cancer ($p<0.001$). Next, it was tested, whether the Piezo1 expression is higher in breast cancer of younger women or premenopausal women. The three age/menopause status groups are based on a clinical relevant classification.

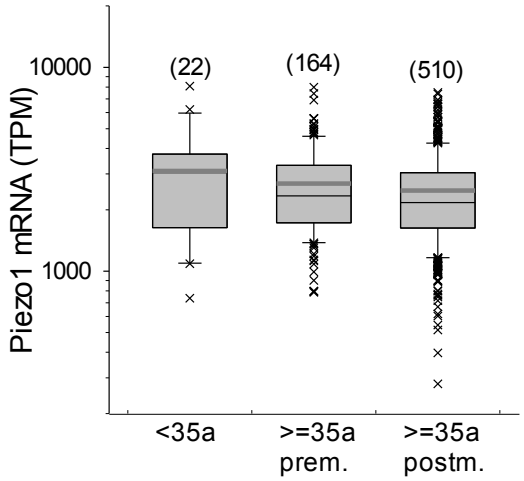


Figure 39A - Piezo1 mRNA in relation to the 3 age/menopause status groups

Figure 39A shows the mRNA expression levels of Piezo1 in the three different age/menopause status groups as defined in the 'Methods' section. We further checked if there are any differences between the age/menopause status groups when limited to progesterone receptor negative or positive cancer only.

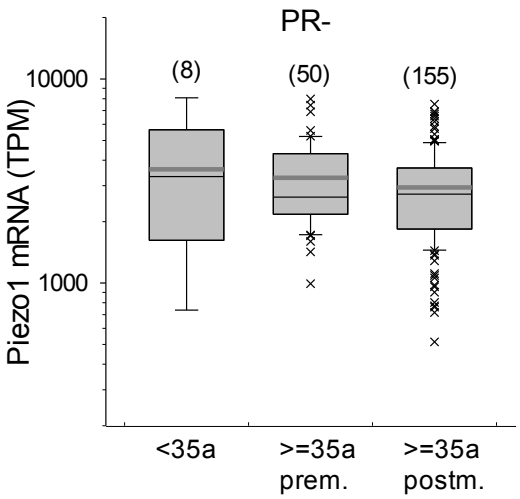


Figure 39B - Piezo1 mRNA in relation to the 3 age/menopause status groups in PR- patients

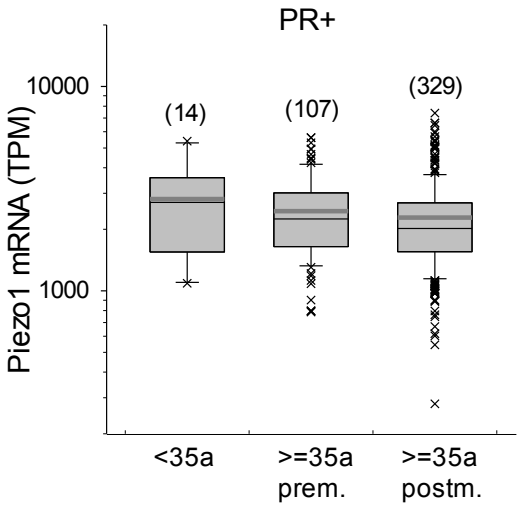


Figure 39C - Piezo1 mRNA in relation to the 3 age/menopause status groups in PR+ patients

The Figure 39B represents the progesterone receptor negative patients, whereas Figure 39C represents the progesterone receptor positive patients only, divided into the 3 age groups. There are no significant differences within a certain progesterone receptor status.

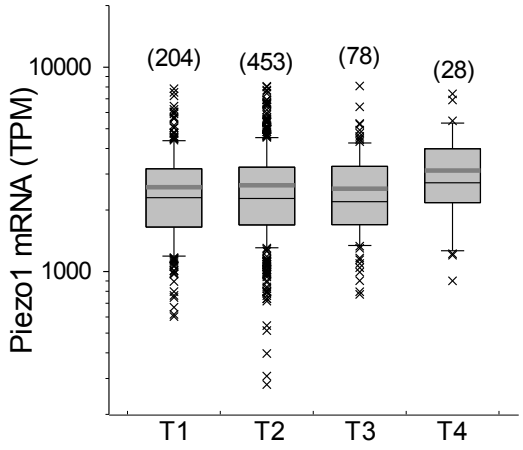


Figure 40A - Piezo1 mRNA in relation to Tumor Status

Figure 40A shows the mRNA expression levels of Piezo1 in relation to the tumor status with no significant differences.

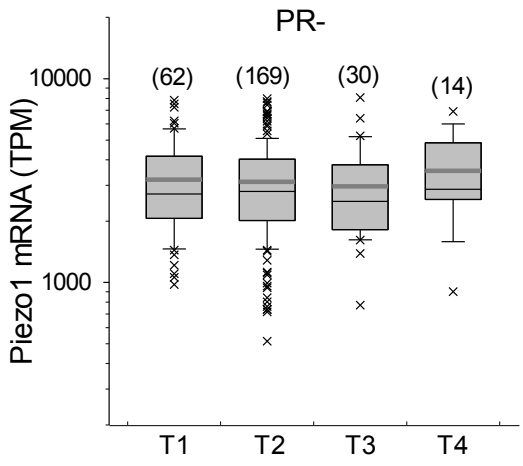


Figure 40B - Piezo1 mRNA in relation to Tumor Status in PR- patients

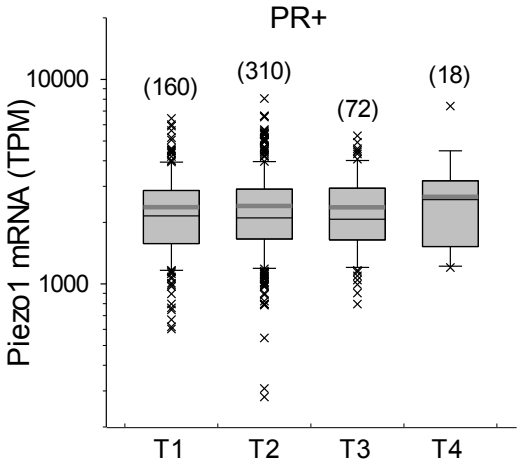


Figure 40C - Piezo1 mRNA in relation to Tumor status in PR+ patients

The Figure 40B represents the progesterone receptor negative patients, whereas Figure 40C represents the progesterone receptor positive patients only, divided into the 4 different tumor status.

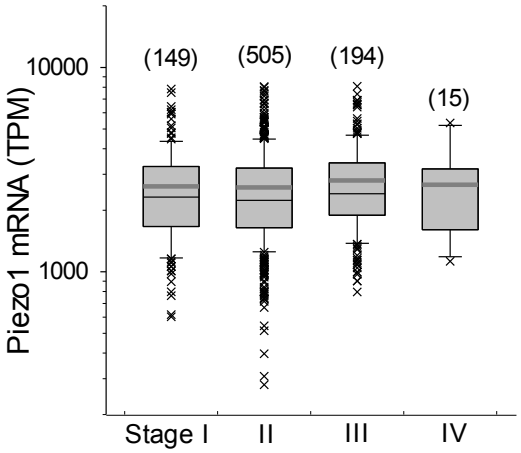


Figure 41A - Piezo1 mRNA in relation to the Stage

Figure 41A shows the mRNA expression levels of Piezo1 in relation to the breast cancer stage.

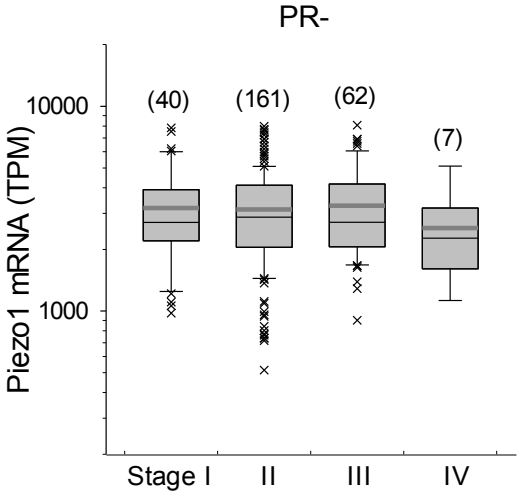


Figure 41B - Piezo1 mRNA in relation to the Stage in PR- patients

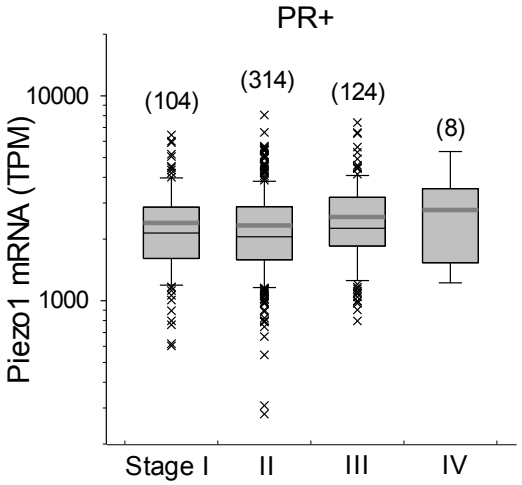


Figure 41C - Piezo1 mRNA in relation to the Stage in PR+ patients

The Figure 41B represents the progesterone receptor negative patients, whereas Figure 41C represents the progesterone receptor positive patients only, divided into the 4 different breast cancer stages.

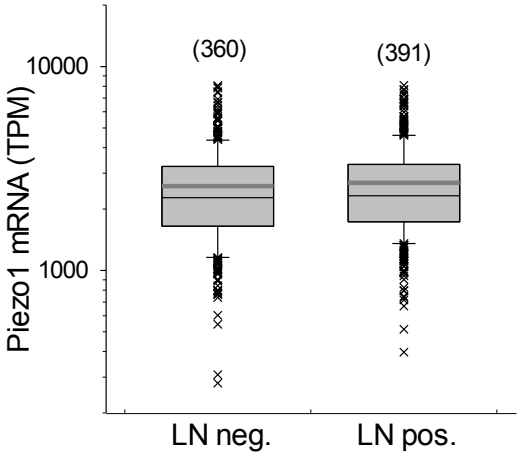


Figure 42A - Piezo1 mRNA in relation to the Lymph node Status

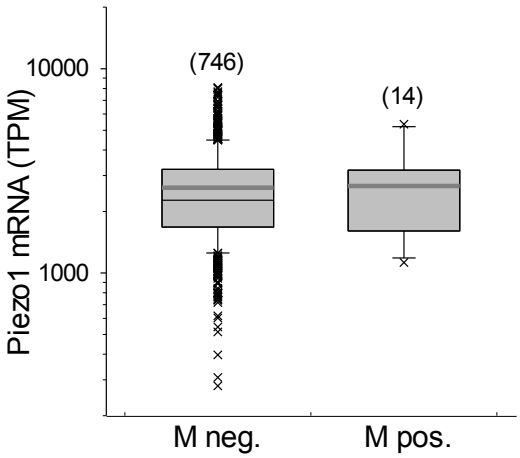


Figure 42B - Piezo1 mRNA in relation to distant Metastasis Status

Figure 42A shows the mRNA expression levels of Piezo1 in relation to the lymph node status (negative versus positive). Figure 42B shows the mRNA expression levels of Piezo1 in relation to the distant metastasis status (negative versus positive). The Piezo1 expression is not associated with lymph node status.

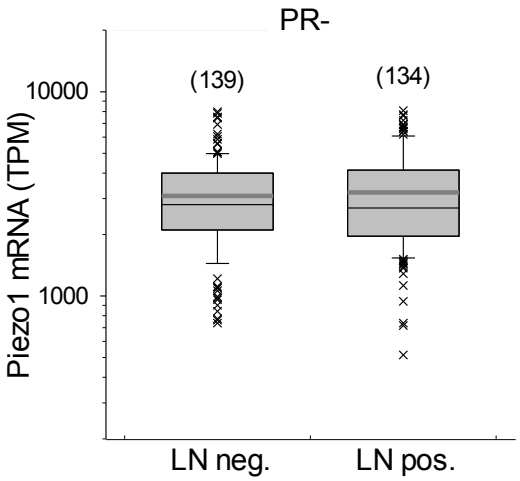


Figure 43A - Piezo1 mRNA in relation to Lymph node status in PR- patients

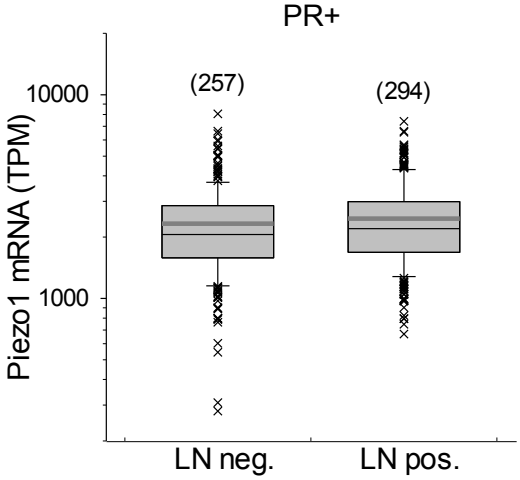


Figure 43B - Piezo1 mRNA in relation to Lymph node status in PR+ patients

The Figure 43A represents the progesterone receptor negative patients, whereas Figure 43B represents the progesterone receptor positive patients only, divided into lymph node negative and lymph node positive breast cancer patients.

4.2.6 Piezo2 mRNA expression in different populations of breast cancer patients

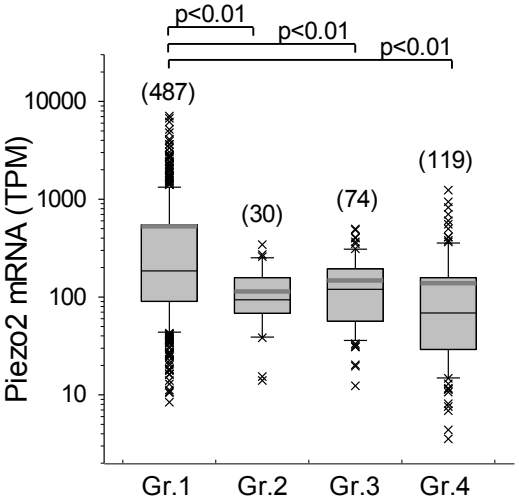


Figure 44A - Piezo2 mRNA in relation to clinical classification group

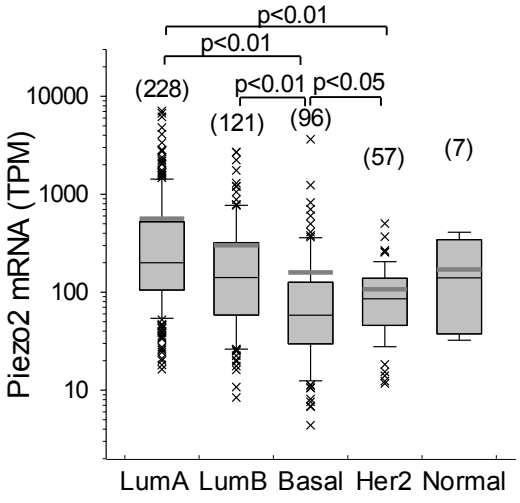


Figure 44B - Piezo2 mRNA in relation to PAM50 classification

In Figure 44A the Piezo2 mRNA levels (in TPM) are shown in logarithmical scale in four different clinical groups as described in the 'Material and Methods' section. The difference between the mRNA expression levels of Groups 1 and 2, 1 and 3 and 1 and 4 is significant ($p<0.01$).

On the other hand Figure 44B shows the mRNA expression levels in the intrinsic breast cancer subtypes Luminal A, Luminal B, basal-like, HER2-enriched and normal-like. The differences between LumB and Basal ($p<0.01$), Basal and Her2 ($p<0.05$), LumA and Basal ($p<0.01$) and LumA and Her2 ($p<0.01$) are significant. The highest levels are found in Luminal A and Luminal B cancer subtypes which represent estrogen receptor positive breast cancer.

To restrict the association of high Piezo2 mRNA expression to a specific hormone receptor, the expression between different hormone receptor status combinations was analyzed.

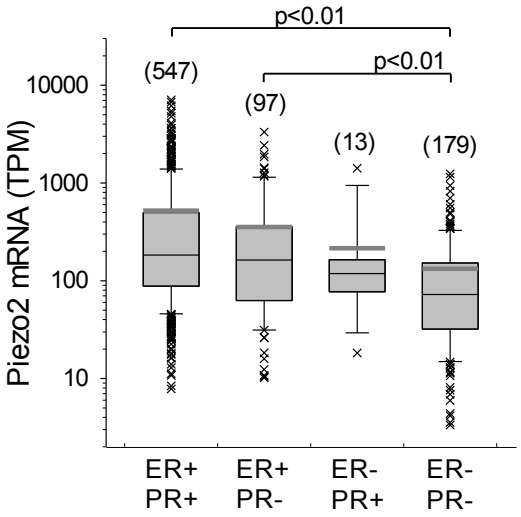


Figure 45A - Piezo2 mRNA in relation to hormone receptor status.

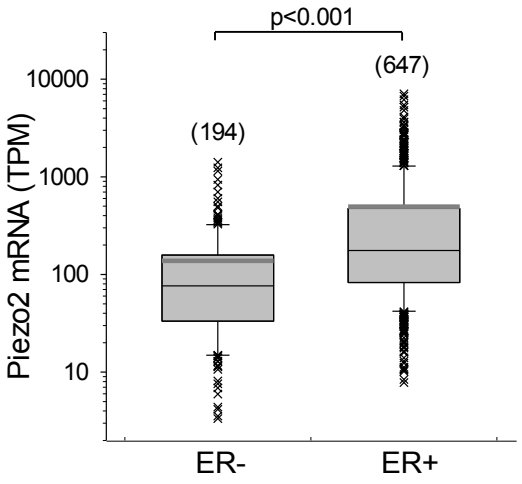


Figure 45B - Piezo2 mRNA in relation to estrogen receptor status.

Figure 45A shows the results in different combinations of estrogen receptor and progesterone receptor. The differences are significant between ER+ PR- and ER- PR- and ER+ PR+ and ER- PR- ($p<0.01$) suggesting that the mRNA expression of Piezo2 is associated with estrogen receptor status. Figure 45B shows that the mRNA expression levels of Piezo2 are significantly higher in estrogen receptor positive breast cancer compared to estrogen receptor negative breast cancer ($p<0.001$). Next, it was tested, whether the Piezo2 expression is higher in breast cancer of younger women or premenopausal women. The three age/menopause status groups are based on a clinical relevant classification.

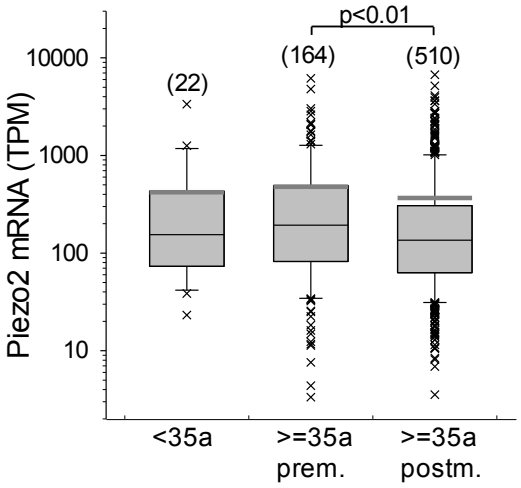


Figure 46A - Piezo2 mRNA in relation to the 3 age/menopause status groups

Figure 46A shows the mRNA expression levels of Piezo2 in the three different age/menopause status groups as defined in the 'Methods' section. The Piezo2 mRNA expression tends to be higher in 35 years or older and premenopausal women ($p<0.01$). Further it was checked if there are any differences between the age/menopause status groups when limited to estrogen receptor negative or positive breast cancer patients only.

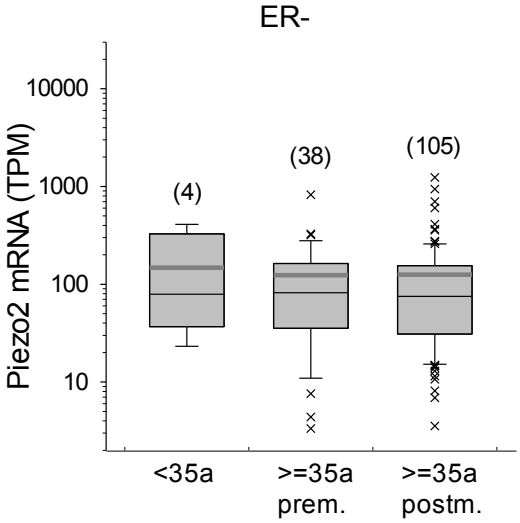


Figure 46B - Piezo2 mRNA in relation to the 3 age/menopause status groups in ER- patients

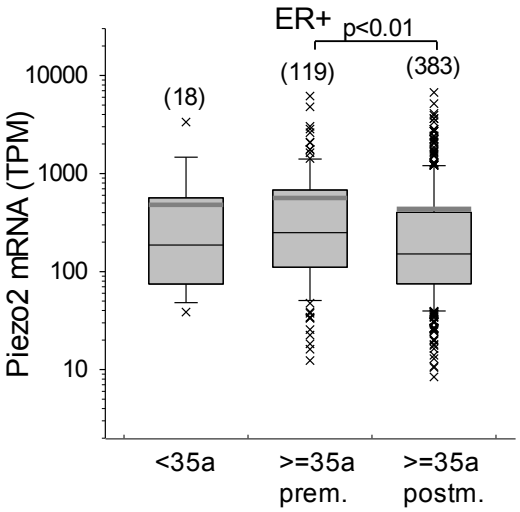


Figure 46C - Piezo2 mRNA in relation to the 3 age/menopause status groups in ER+ patients

The Figure 46B represents the estrogen receptor negative patients, whereas Figure 46C represents the estrogen receptor positive patients only, divided into the 3 age groups. There is a significant difference between premenopausal and postmenopausal women over the age of 35 within the estrogen receptor positive group ($p<0.01$).

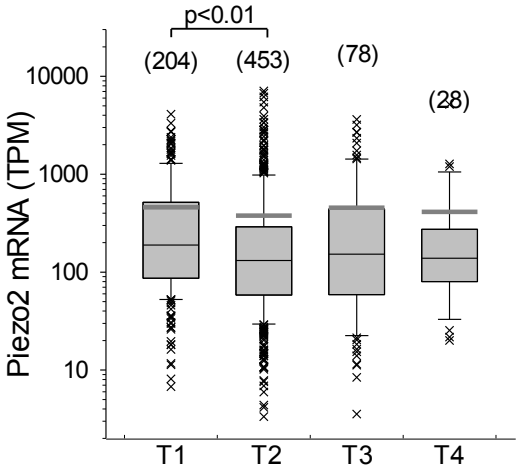


Figure 47A - Piezo2 mRNA in relation to Tumor Status

Figure 47A shows the mRNA expression levels of Piezo2 in relation to the tumor status with a significant higher expression in T1 cancer compared to T2 cancer ($p<0.01$).

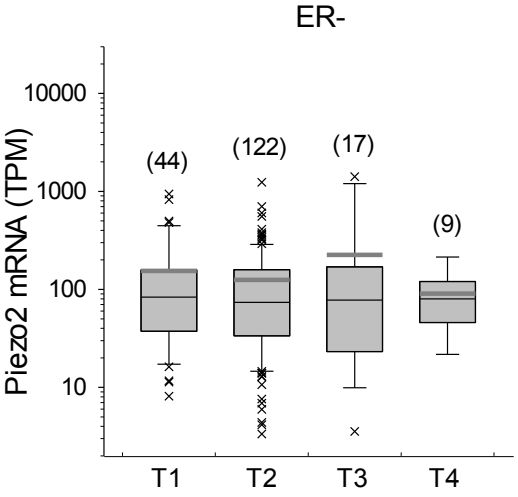


Figure 47B - Piezo2 mRNA in relation to Tumor Status in ER- patients

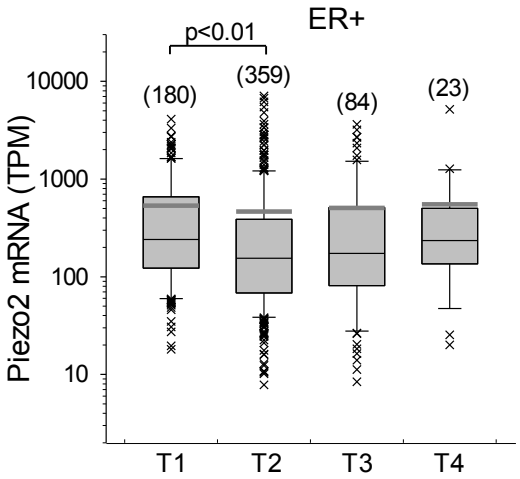


Figure 47C - Piezo2 mRNA in relation to Tumor status in ER+ patients

The Figure 47B represents the estrogen receptor negative patients, whereas Figure 47C represents the estrogen receptor positive patients only, divided into the 4 different tumor status. Again, the expression is higher in T1 cancer compared to T2 cancer within the estrogen receptor positive group ($p<0.01$).

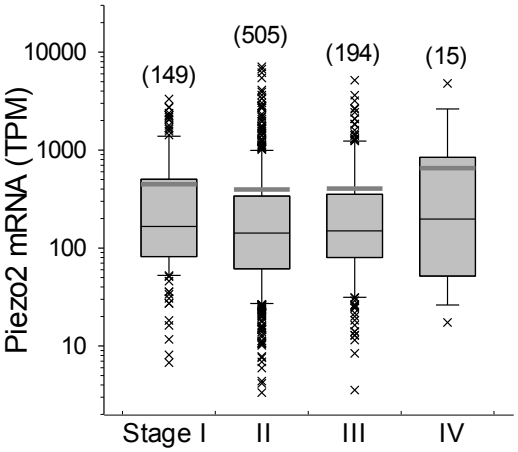


Figure 48A - Piezo2 mRNA in relation to the Stage

Figure 48A shows the mRNA expression levels of Piezo2 in relation to the breast cancer stage.

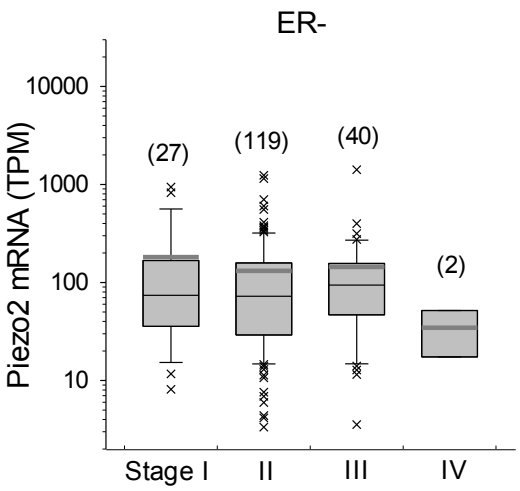


Figure 48B - Piezo2 mRNA in relation to the Stage in ER- patients

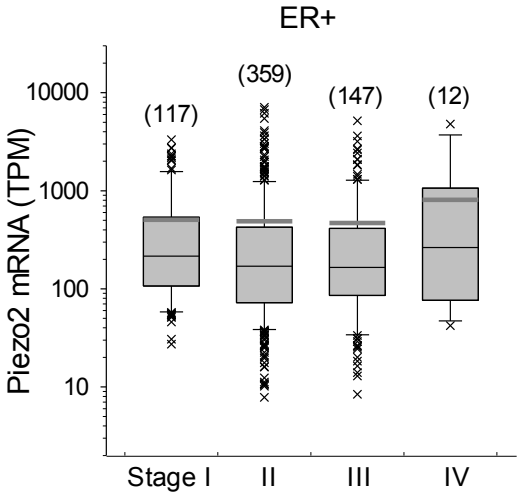


Figure 48C - Piezo2 mRNA in relation to the Stage in ER+ patients

The Figure 48B represents the estrogen receptor negative patients, whereas Figure 48C represents the estrogen receptor positive patients only, divided into the 4 different breast cancer stages.

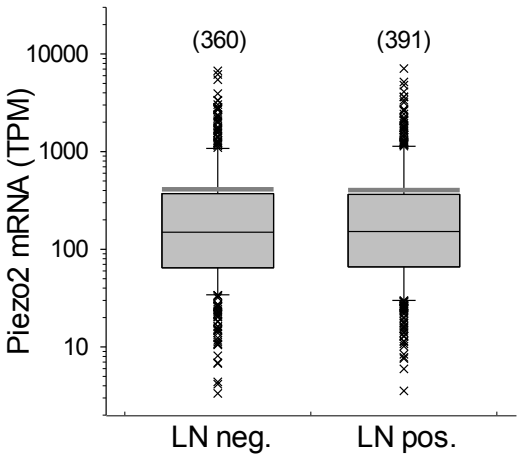


Figure 49A - Piezo2 mRNA in relation to the Lymph node Status

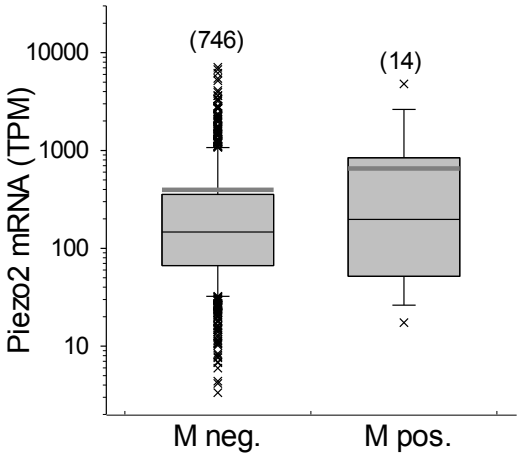


Figure 49B - Piezo2 mRNA in relation to distant Metastasis Status

Figure 49A shows the mRNA expression levels of Piezo2 in relation to the lymph node status (negative versus positive). Figure 49B shows the mRNA expression levels of Piezo2 in relation to the distant metastasis status (negative versus positive). The Piezo2 expression is not associated with lymph node status.

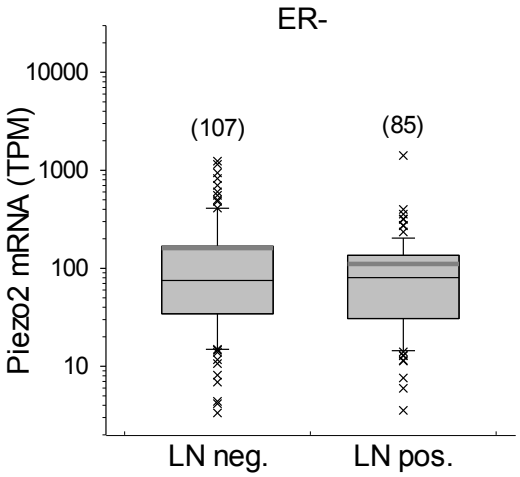


Figure 50A - Piezo2 mRNA in relation to Lymph node status in ER- patients

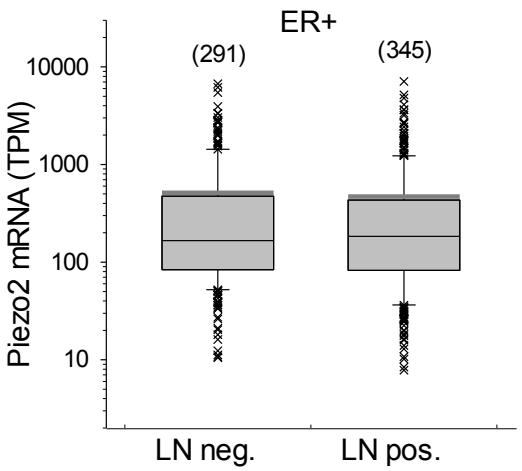


Figure 50B - Piezo2 mRNA in relation to Lymph node status in ER+ patients

The Figure 50A represents the estrogen receptor negative patients, whereas Figure 50B represents the estrogen receptor positive patients only, divided into lymph node negative and lymph node positive breast cancer patients.

4.2.7 TrpC3 mRNA expression in different populations of breast cancer patients

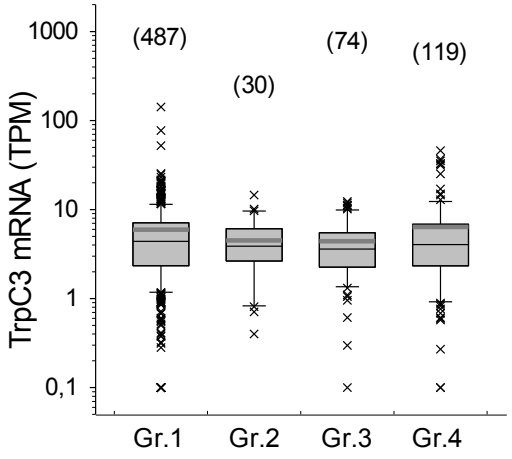


Figure 51A - TrpC3 mRNA in relation to clinical classification group

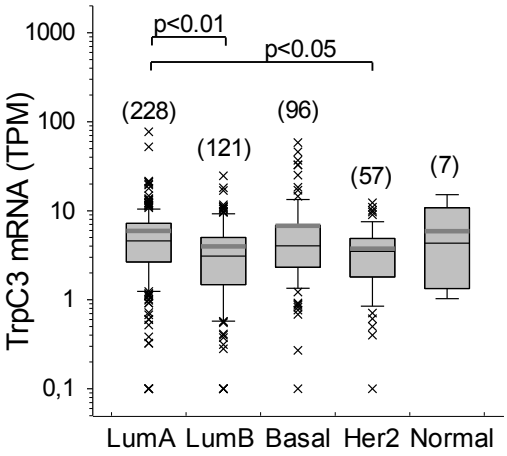


Figure 51B - TrpC3 mRNA in relation to PAM50 classification

In Figure 51A the TrpC3 mRNA levels (in TPM) are shown in logarithmical scale in four different clinical groups as they are described in the 'Material and Methods' section.

Figure 51B shows the mRNA expression levels in the intrinsic breast cancer subtypes Luminal A, Luminal B, basal-like, HER2-enriched and normal-like. The differences between LumA and Her2 ($p<0.05$) and LumA and LumB ($p<0.01$) are significant.

To find an association of TrpC3 mRNA expression to a specific hormone receptor, the expression between different hormone receptor status combinations was analyzed.

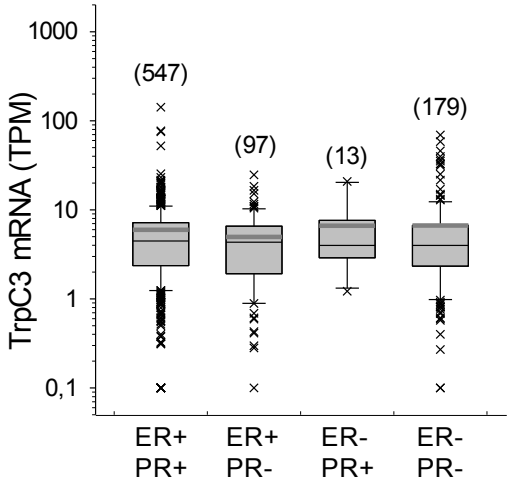


Figure 52A - TrpC3 mRNA in relation to hormone receptor status.

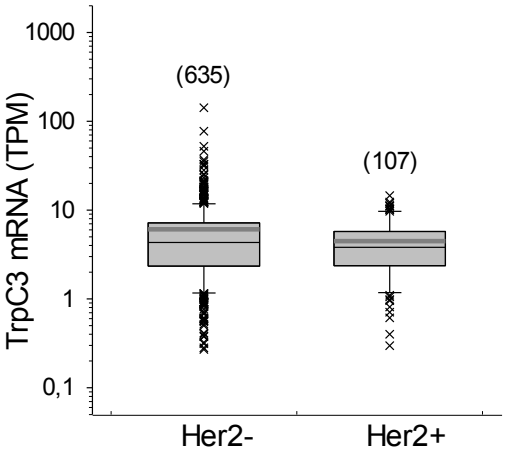


Figure 52B - TrpC3 mRNA in relation to estrogen receptor status.

Figure 52A shows the results in different combinations of estrogen receptor and progesterone receptor whereas Figure 52B shows the results in Her2 negative and Her2 positive cancer samples. There is no statistically significant association of TrpC3 mRNA expression with any tested receptor status.

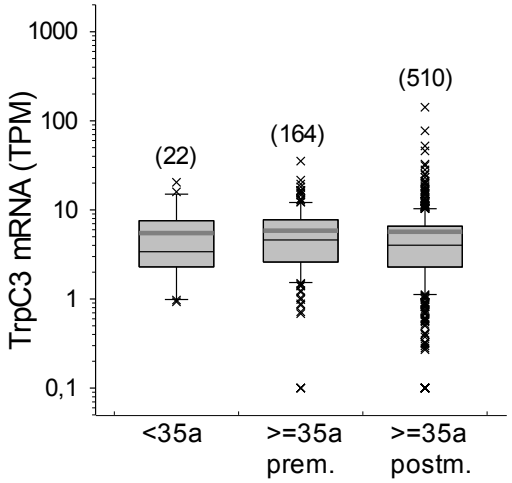


Figure 53 - TrpC3 mRNA in relation to the 3 age/menopause status groups

Figure 53 shows the mRNA expression levels of TrpC3 in the three different age/menopause status groups as defined in the 'Methods' section.

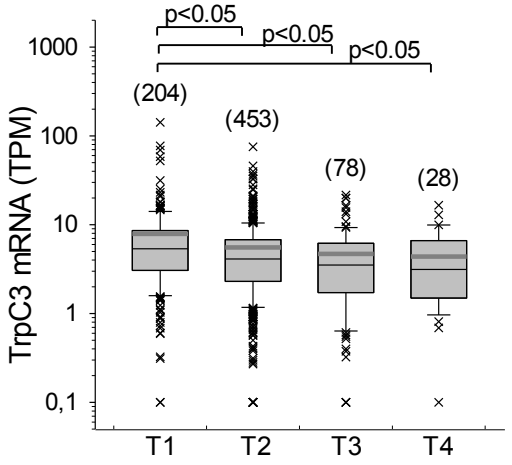


Figure 54 - TrpC3 mRNA in relation to Tumor Status

Figure 54 shows the mRNA expression levels of TrpC3 in relation to the tumor status with a significant higher expression in T1 cancer compared to each other cancer ($p<0.05$).

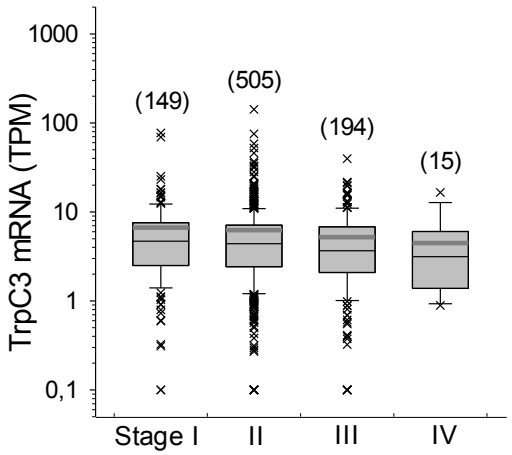


Figure 55 - TrpC3 mRNA in relation to the Stage

Figure 55 shows the mRNA expression levels of TrpC3 in relation to the breast cancer stage.

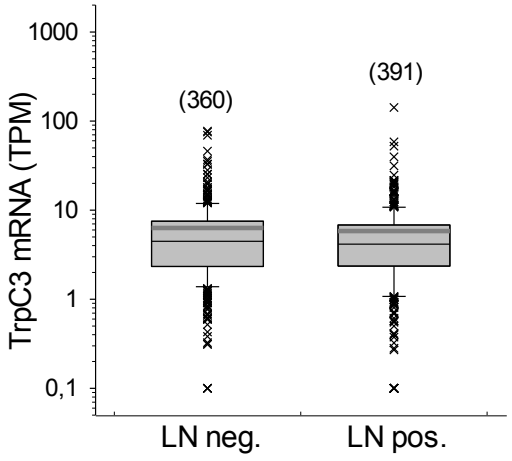


Figure 56A - TrpC3 mRNA in relation to the Lymph node Status

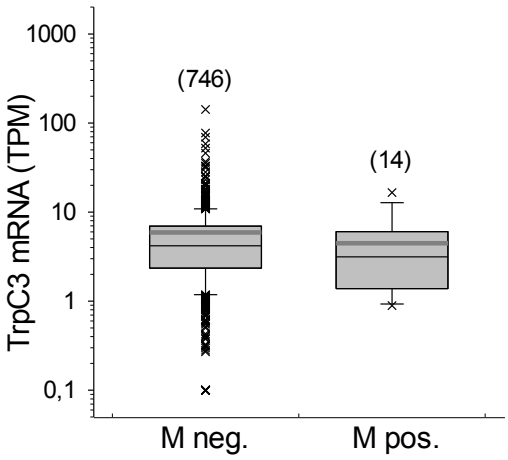


Figure 56B - TrpC3 mRNA in relation to distant Metastasis Status

Figure 56A shows the mRNA expression levels of TrpC3 in relation to the lymph node status (negative versus positive). Figure 56B shows the mRNA expression levels of TrpC3 in relation to the distant metastasis status (negative versus positive). The TrpC3 expression is not associated with lymph node status.

4.2.8 TrpC6 mRNA expression in different populations of breast cancer patients

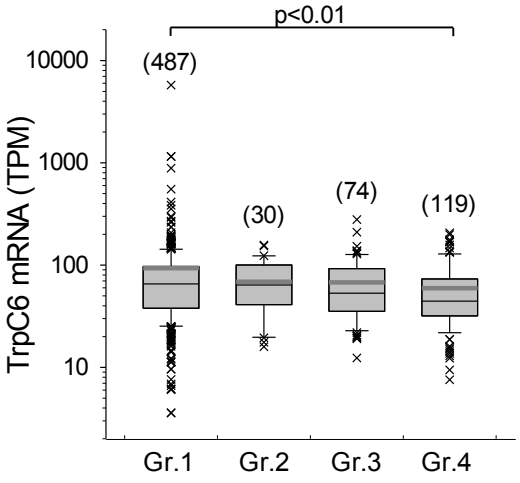


Figure 57A - TrpC6 mRNA in relation to clinical classification group

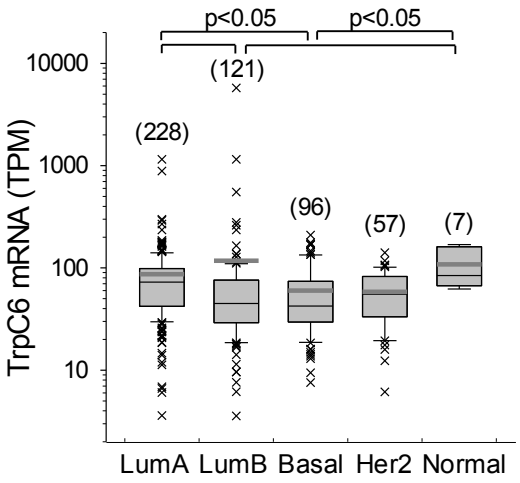


Figure 57B - TrpC6 mRNA in relation to PAM50 classification

In Figure 57A the TrpC6 mRNA levels (in TPM) are shown in logarithmical scale in four different clinical groups as described in the 'Material and Methods' section. Figure 57A shows a significant difference between the mRNA expression levels of groups 1 and 4 ($p<0.01$).

On the other hand Figure 57B shows the mRNA expression levels in the intrinsic breast cancer subtypes Luminal A, Luminal B, basal-like, HER2-enriched and normal-like. The differences between LumA and LumB, LumB and Normal, LumA and Basal and Basal and Normal are significant ($p<0.05$).

To restrict the association of high TrpC6 mRNA expression to a specific hormone receptor, the expression between different hormone receptor status combinations was analyzed.

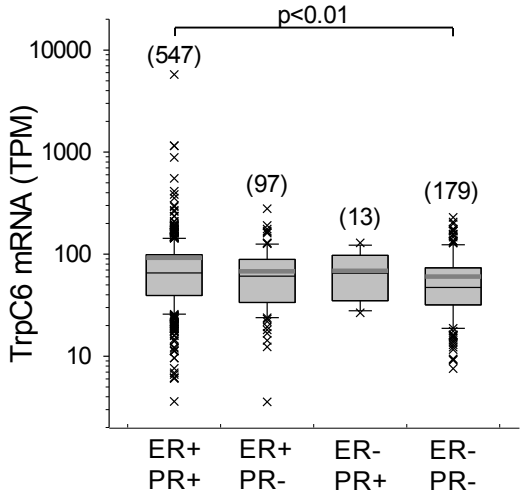


Figure 58 - TrpC6 mRNA in relation to hormone receptor status.

Figure 58 shows the results in different combinations of estrogen receptor and progesterone receptor. The difference is significant between ER+ PR+ and ER- PR- ($p<0.01$) suggesting that the mRNA expression of TrpC6 is associated with estrogen and progesterone receptor positive status.

Next, it was tested, whether the TrpC6 expression is higher in breast cancer of younger women or premenopausal women. The three age/menopause status groups are based on a clinical relevant classification.

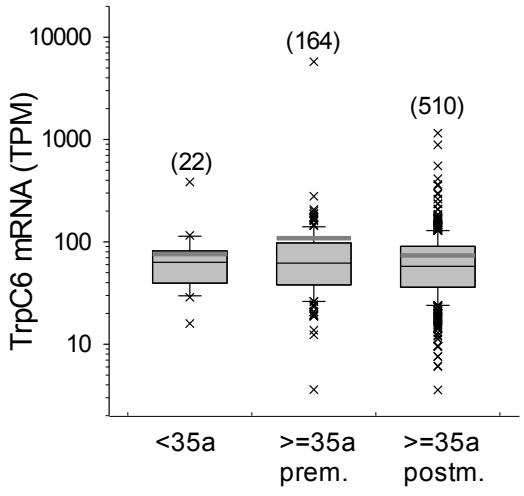


Figure 59A - TrpC6 mRNA in relation to the 3 age/menopause status groups

Figure 59A shows the mRNA expression levels of TrpC6 in the three different age/menopause status groups as defined in the ‘Methods’ section.

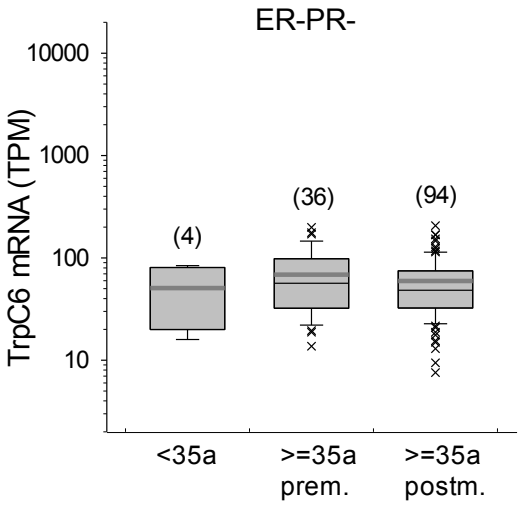


Figure 59B - TrpC6 mRNA in relation to the 3 age/menopause status groups in ER- PR- patients

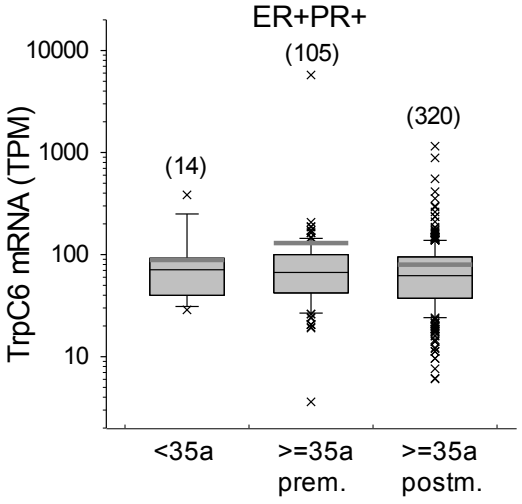


Figure 59C - TrpC6 mRNA in relation to the 3 age/menopause status groups in ER+ PR+ patients

The Figure 59B represents the estrogen and progesterone receptor negative patients, whereas Figure 59C represents the estrogen and progesterone receptor positive patients only, divided into the 3 age groups.

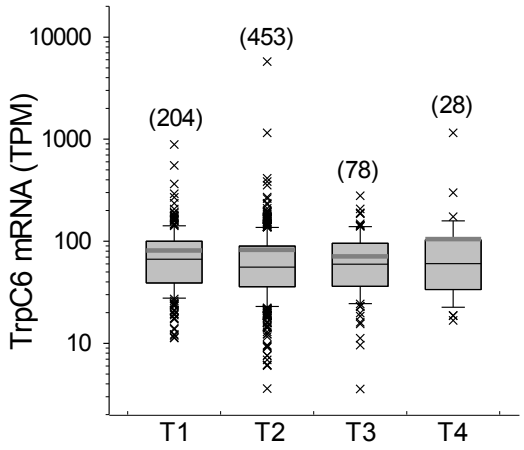


Figure 60A - TrpC6 mRNA in relation to Tumor Status

Figure 60A shows the mRNA expression levels of TrpC6 in relation to the tumor status.

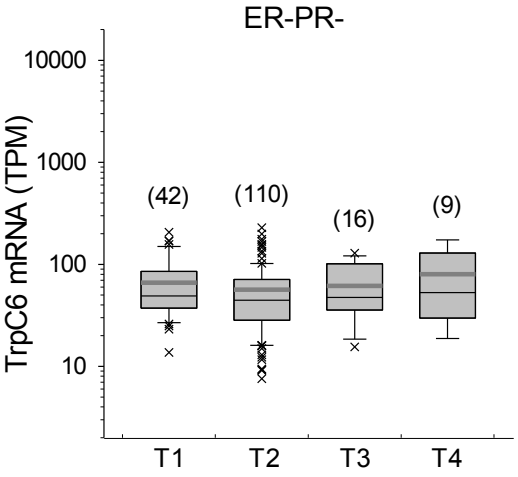


Figure 60B - TrpC6 mRNA in relation to Tumor Status in ER- PR- patients

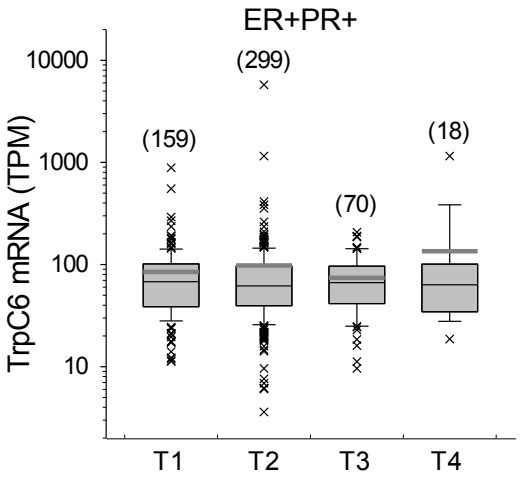


Figure 60C - TrpC6 mRNA in relation to Tumor status in ER+ PR+ patients

The Figure 60B represents the estrogen and progesterone receptor negative patients, whereas Figure 60C represents the estrogen and progesterone receptor positive patients only, divided into the 4 different tumor status.

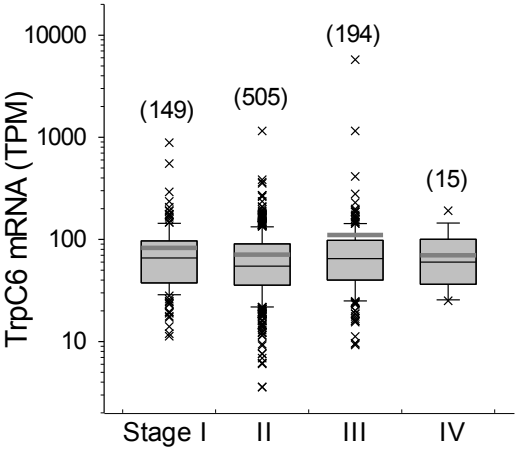


Figure 61A - TrpC6 mRNA in relation to the Stage

Figure 61A shows the mRNA expression levels of TrpC6 in relation to the breast cancer stage.

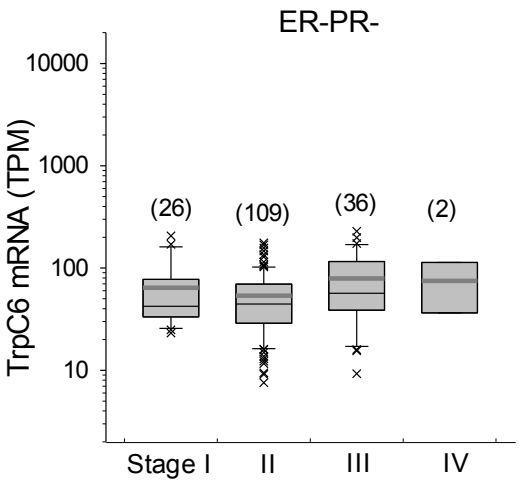


Figure 61B - TrpC6 mRNA in relation to the Stage in ER- PR- patients

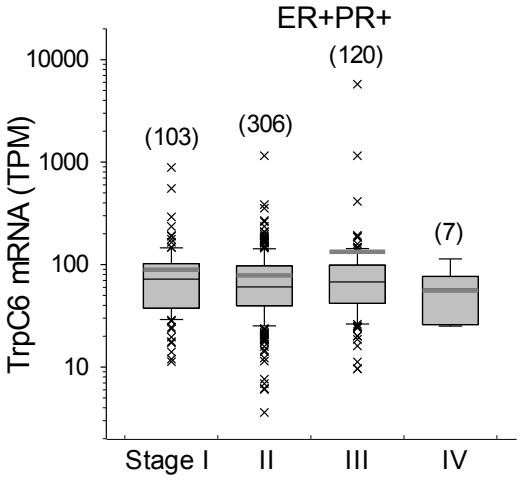


Figure 61C - TrpC6 mRNA in relation to the Stage in ER+ PR+ patients

The Figure 61B represents the estrogen and progesterone receptor negative patients, whereas Figure 61C represents the estrogen and progesterone receptor positive patients only, divided into the 4 different breast cancer stages.

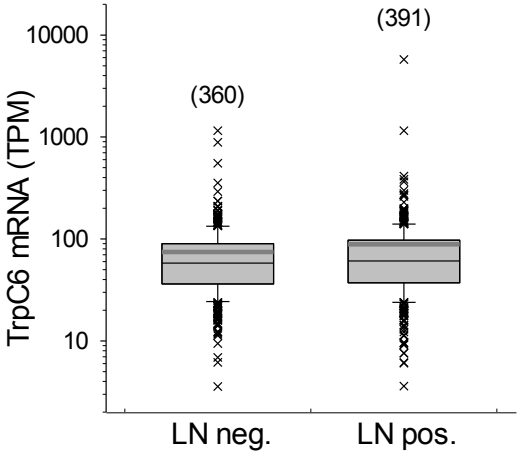


Figure 62A - TrpC6 mRNA in relation to the Lymph node Status

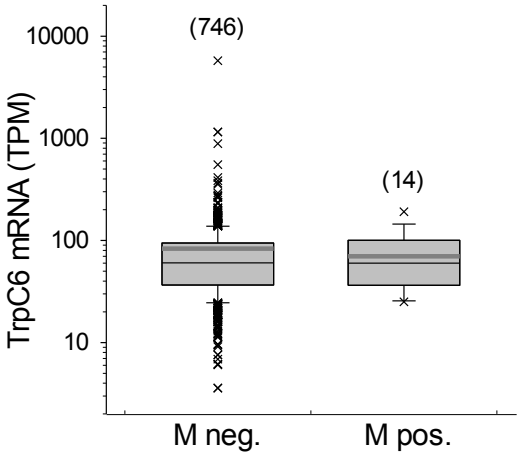


Figure 62B - TrpC6 mRNA in relation to distant Metastasis Status

Figure 62A shows the mRNA expression levels of TrpC6 in relation to the lymph node status (negative versus positive). Figure 62B shows the mRNA expression levels of TrpC6 in relation to the distant metastasis status (negative versus positive). TrpC6 is not associated with lymph node metastasis.

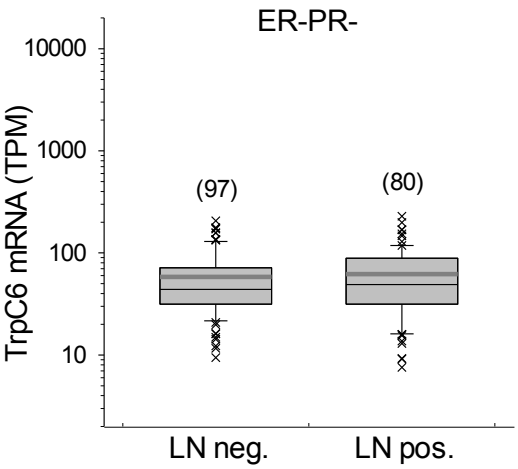


Figure 63A - TrpC6 mRNA in relation to Lymph node status in ER- PR- patients

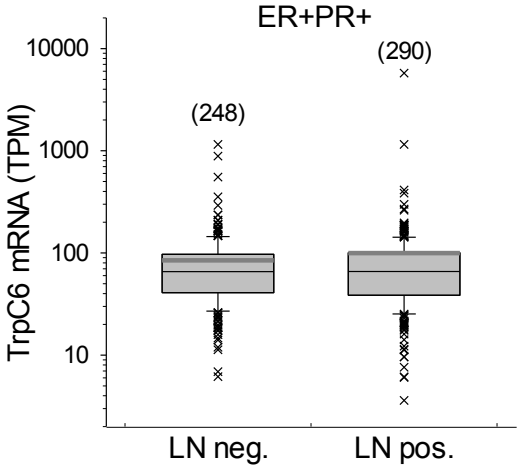


Figure 63B - TrpC6 mRNA in relation to Lymph node status in ER+ PR+ patients

The Figure 63A represents the estrogen and progesterone receptor negative patients, whereas Figure 63B represents the estrogen and progesterone receptor positive patients only, divided into lymph node negative and lymph node positive breast cancer patients.

4.2.9 ANO1 mRNA expression in different populations of breast cancer patients

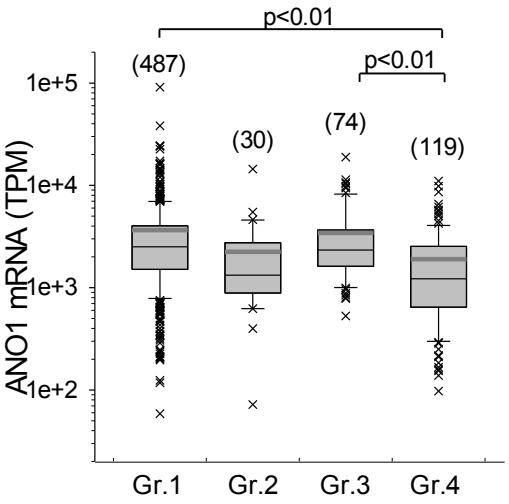


Figure 64A - ANO1 mRNA in relation to clinical classification group

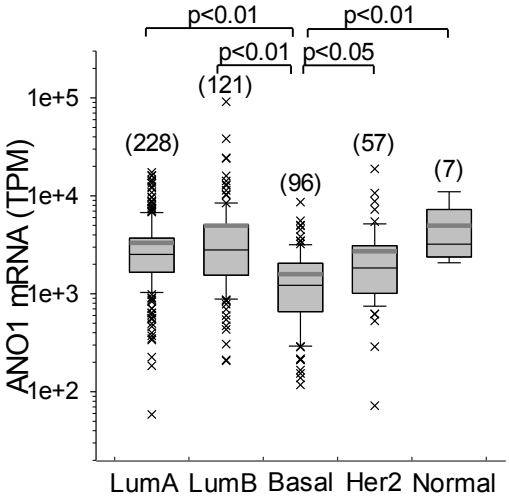


Figure 64B - ANO1 mRNA in relation to PAM50 classification

In Figure 64A the ANO1 mRNA levels (in TPM) are shown in logarithmical scale in four different clinical groups as described in the 'Material and Methods' section. There is a statistically significant difference between the mRNA expression levels of Groups 3 and 4 and 1 and 4 ($p<0.01$).

On the other hand Figure 64B shows the mRNA expression levels in the intrinsic breast cancer subtypes Luminal A, Luminal B, basal-like, HER2-enriched and normal-like. The differences between LumB and Basal ($p<0.01$), Basal and Her2 ($p<0.05$), LumA and Basal ($p<0.01$) and Basal and Normal ($p<0.01$) are significant.

To restrict the association of high ANO1 mRNA expression to a specific hormone receptor, the expression between different hormone receptor status combinations was analyzed.

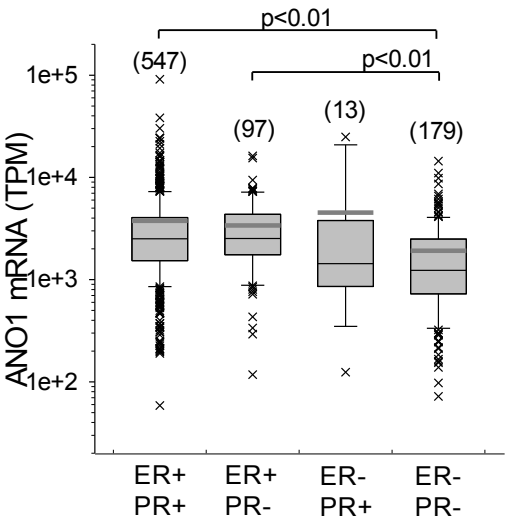


Figure 65A - ANO1 mRNA in relation to hormone receptor status.

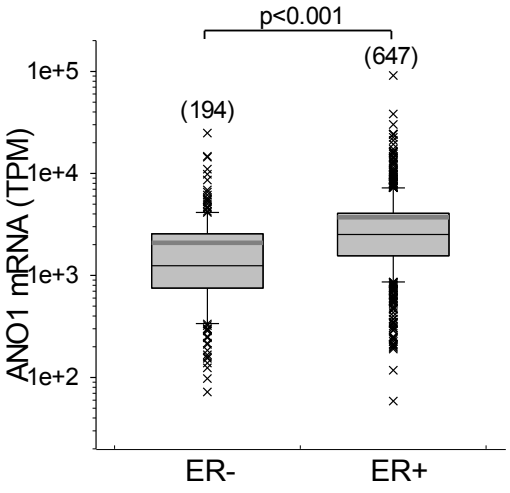


Figure 65B - ANO1 mRNA in relation to estrogen receptor status.

Figure 65A shows the results in different combinations of estrogen receptor and progesterone receptor. The differences are significant between ER+ PR- and ER- PR- and ER+ PR+ and ER- PR- ($p<0.01$) suggesting that the mRNA expression of ANO1 is associated with estrogen receptor status. Figure 65B shows the mRNA expression levels of ANO1 in relation to estrogen receptor status. The mRNA expression levels of ANO1 is significantly higher in estrogen receptor positive breast cancer as compared to estrogen receptor negative breast cancer ($p<0.001$). Next, it was tested, whether the ANO1 expression is higher in breast cancer of younger women or premenopausal women. The three age/menopause status groups are based on a clinical relevant classification.

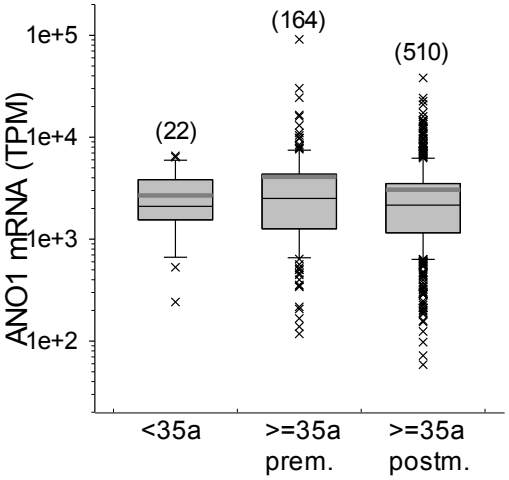


Figure 66A - ANO1 mRNA in relation to the 3 age/menopause status groups

Figure 66A shows the mRNA expression levels of ANO1 in the three different age/menopause status groups as defined in the 'Methods' section. There are no statistically significant differences between the groups. Further it was checked, if there are any differences between the age/menopause status groups when limited to estrogen receptor negative or positive breast cancer patients only.

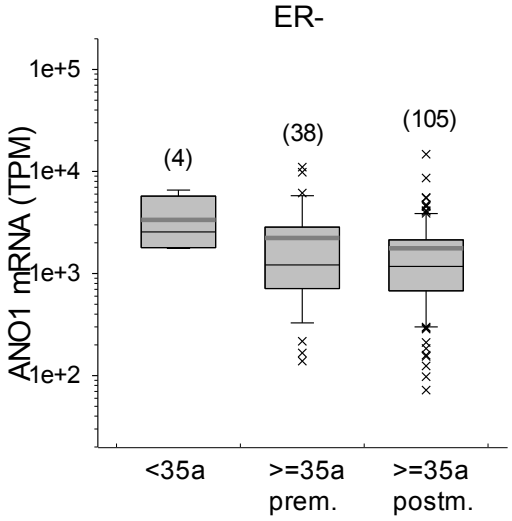


Figure 66B - ANO1 mRNA in relation to the 3 age/menopause status groups in ER- patients

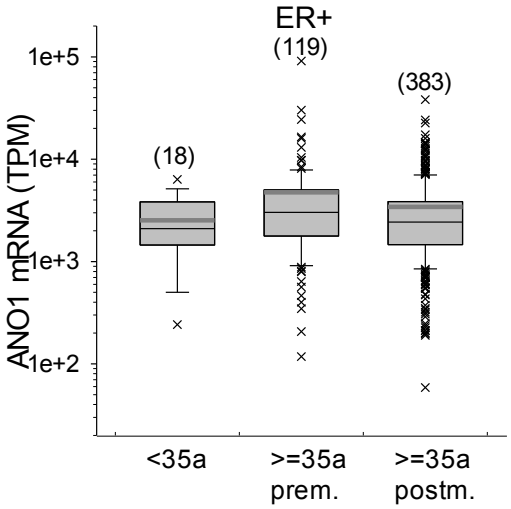


Figure 66C - ANO1 mRNA in relation to the 3 age/menopause status groups in ER+ patients

The Figure 66B represents the estrogen receptor negative patients, whereas Figure 66C represents the estrogen receptor positive patients only, divided into the 3 age groups.

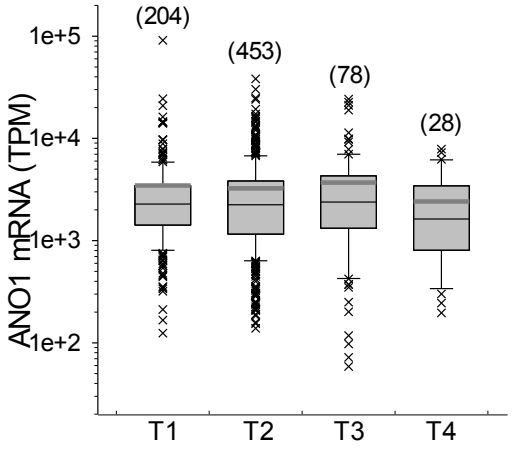


Figure 67A - ANO1 mRNA in relation to Tumor Status

Figure 67A shows the mRNA expression levels of ANO1 in relation to the tumor status.

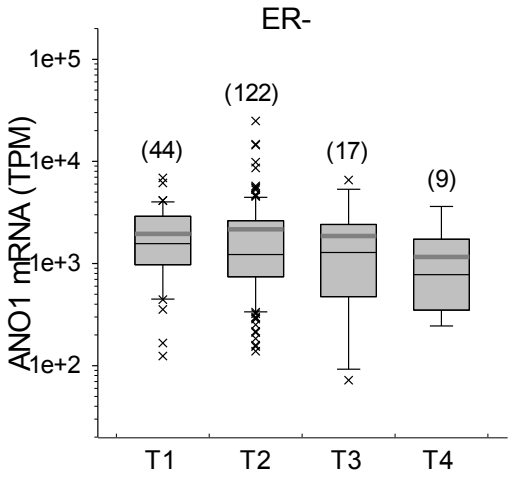


Figure 67B - ANO1 mRNA in relation to Tumor Status in ER- patients

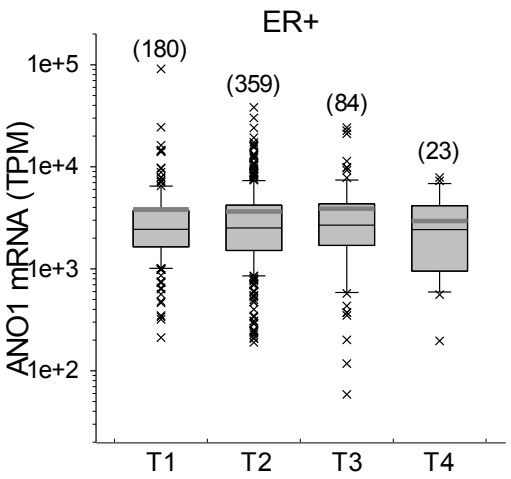


Figure 67C - ANO1 mRNA in relation to Tumor status in ER+ patients

The Figure 67B represents the estrogen receptor negative patients, whereas Figure 67C represents the estrogen receptor positive patients only, divided into the 4 different tumor status.

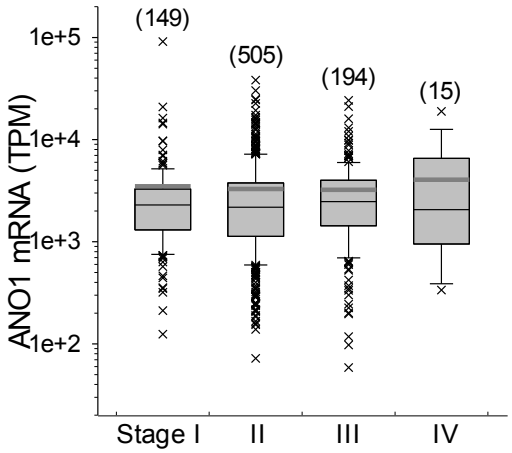


Figure 68A - ANO1 mRNA in relation to the Stage

Figure 68A shows the mRNA expression levels of ANO1 in relation to the breast cancer stage.

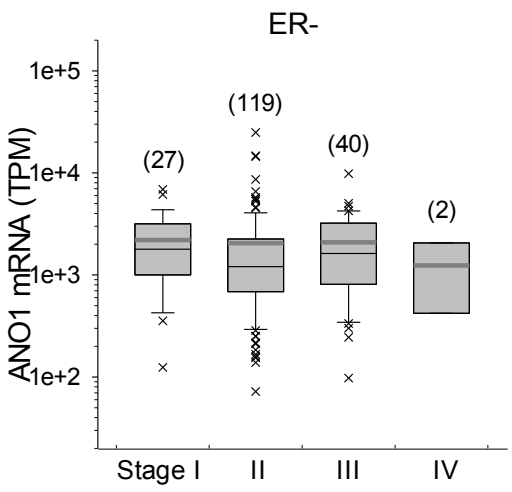


Figure 68B - ANO1 mRNA in relation to the Stage in ER- patients

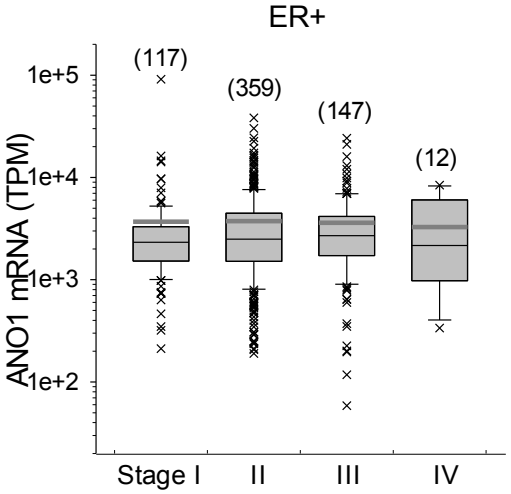


Figure 68C - ANO1 mRNA in relation to the Stage in ER+ patients

The Figure 68B represents the estrogen receptor negative patients, whereas Figure 68C represents the estrogen receptor positive patients only, divided into the 4 different breast cancer stages.

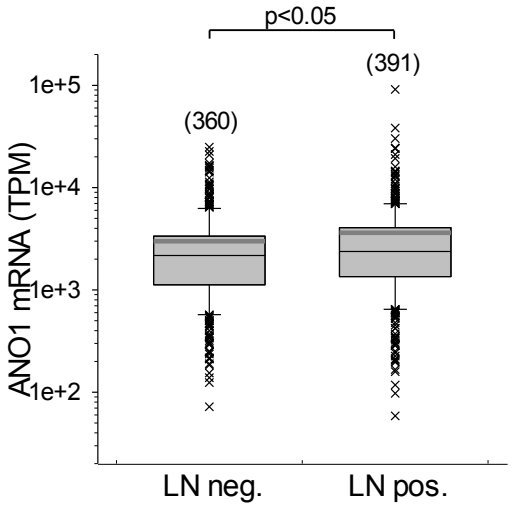


Figure 69A - ANO1 mRNA in relation to the Lymph node Status

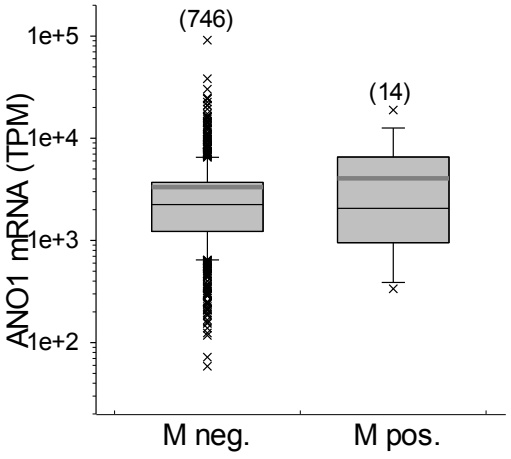


Figure 69B - ANO1 mRNA in relation to distant Metastasis Status

Figure 69A shows the mRNA expression levels of ANO1 in relation to the lymph node status (negative versus positive). Figure 69B shows the mRNA expression levels of ANO1 in relation to the distant metastasis status (negative versus positive). The ANO1 expression is significantly higher in lymph node positive breast cancer compared to lymph node negative cancer ($p<0.05$).

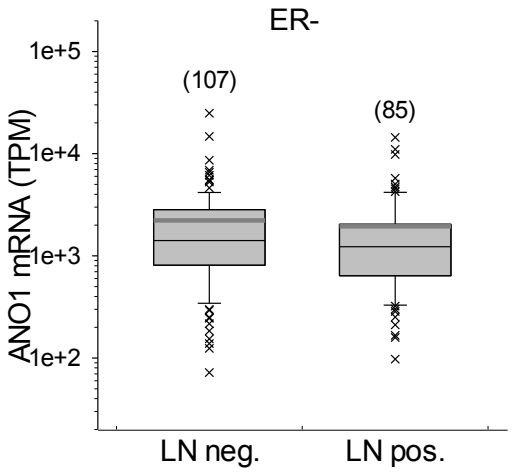


Figure 70A - ANO1 mRNA in relation to Lymph node status in ER- patients

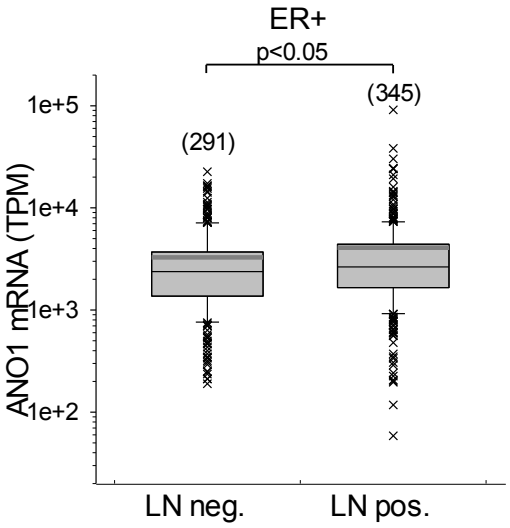


Figure 70B - ANO1 mRNA in relation to Lymph node status in ER+ patients

The Figure 70A represents the estrogen receptor negative patients, whereas Figure 70B represents the estrogen receptor positive patients only, divided into lymph node negative and lymph node positive breast cancer patients. The difference between lymph node negative and lymph node positive patients is only in the population of estrogen receptor positive patients significant ($p<0.05$).

4.3 Comparison of Gene-expression between tumor and normal tissue

The following figures show the results in the mRNA expression in patients with available tumor samples and normal samples.

4.3.1 KCNJ3 mRNA expression in tumor and normal tissue

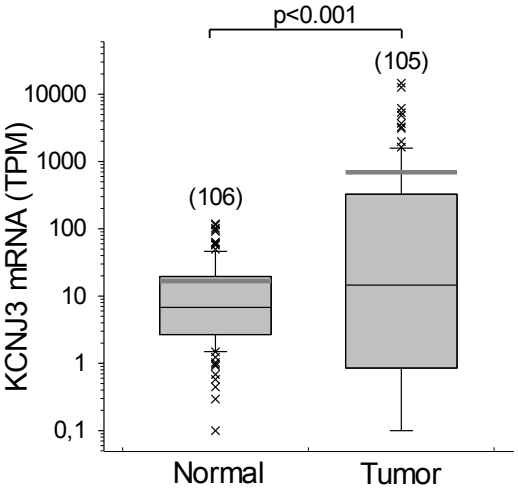


Figure 72 - mRNA in normal tissue and tumor tissue

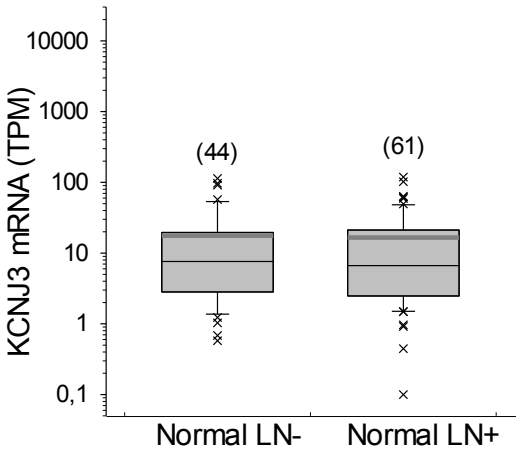


Figure 71A - KCNJ3 mRNA in normal tissue of lymph node negative and lymph node positive breast cancer patients

Figure 71 shows the mRNA expression levels of KCNJ3 in normal tissue and tumor tissue. The expression levels are higher in tumor tissue compared to normal/healthy tissue. The difference is statistically significant ($p<0.001$). Figure 72A shows the mRNA expression levels of KCNJ3 in normal tissue of lymph node negative and lymph node positive breast cancer patients. The levels are approximately equal in both groups.

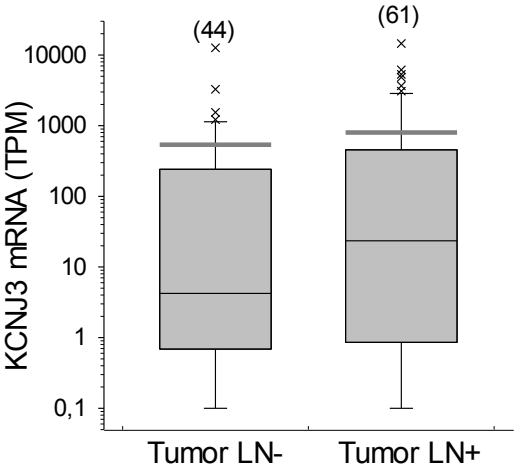


Figure 71B - KCNJ3 mRNA in relation to lymph node status in tumor tissue samples

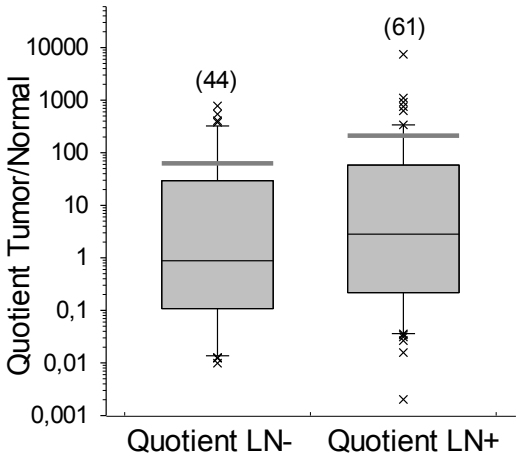


Figure 71C - ratio of KCNJ3 mRNA expression between tumor and corresponding normal sample in relation to lymph node status

Figure 72B shows the mRNA expression levels of KCNJ3 in tumor tissue of lymph node negative and lymph node positive breast cancer patients. As shown above the KCNJ3 expression is higher in lymph node positive patients. This can also be found in the smaller collective of tumor patients with available normal tissue data, however no significance can be found.

Figure 72C shows the ratio of KCNJ3 mRNA expression between tumor and corresponding normal sample. As the difference between the mRNA expression of KCNJ3 in lymph node negative and lymph node positive patients can only be found in tumor tissue, there is also a non-significantly higher ration in the lymph node positive group.

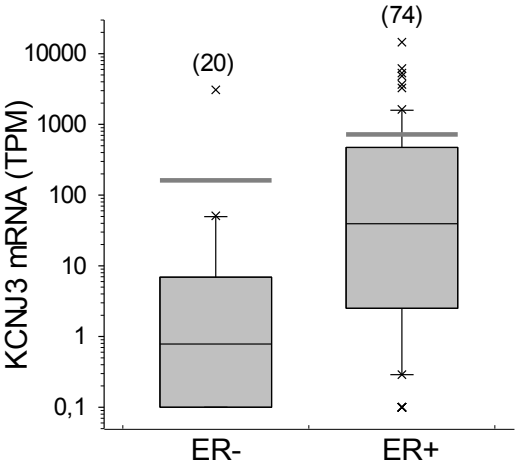


Figure 73A - KCNJ3 mRNA in relation to estrogen receptor status

Figure 73A shows the mRNA expression levels of KCNJ3 in estrogen receptor negative and estrogen receptor positive breast cancer tissue. As shown above the KCNJ3 expression is much higher in estrogen receptor positive patients. This can also be found in the smaller collective of tumor patients with available normal tissue data, however no significance can be found.

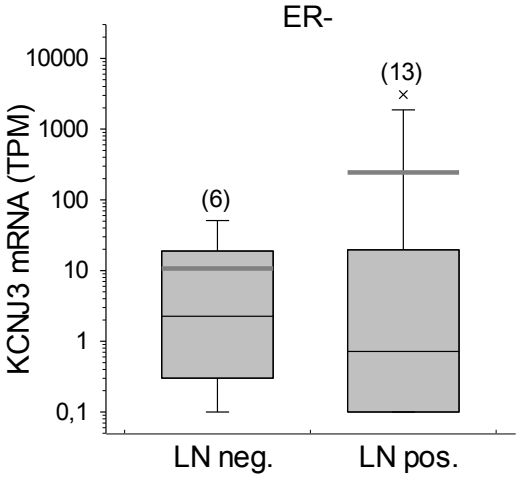


Figure 73B - KCNJ3 mRNA in relation to lymph node status in ER negative patients

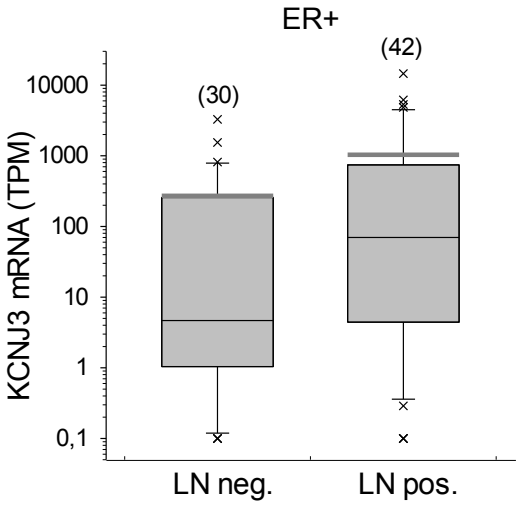


Figure 73C - KCNJ3 mRNA in relation to lymph node status in ER positive patients

In analogy to the upper analyzes on cancer tissue data Figure 73B shows the mRNA expression levels of KCNJ3 in estrogen receptor negative, Figure 73C in estrogen receptor positive breast cancer only, divided into lymph node negative and positive patients.

4.3.2 KCNJ5 mRNA expression in tumor and normal tissue

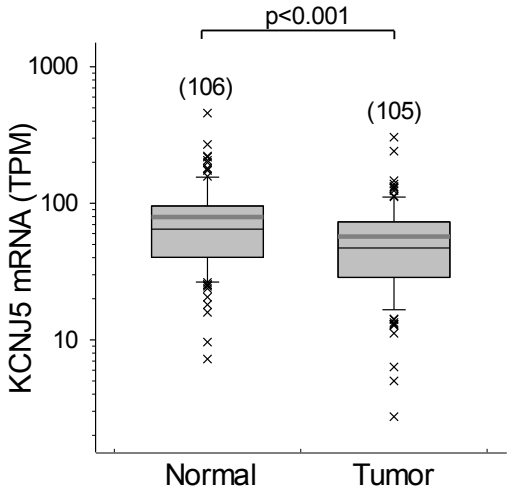


Figure 74 - KCNJ5 mRNA in normal tissue and tumor tissue

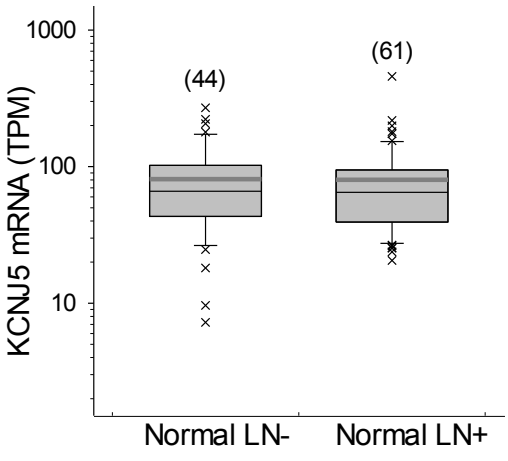


Figure 75A - KCNJ5 mRNA in normal tissue of lymph node negative and lymph node positive breast cancer patients

Figure 74 shows the mRNA expression levels of KCNJ5 in normal tissue and tumor tissue. The expression levels are higher in normal tissue compared to tumor tissue. The difference is statistically significant ($p<0.001$). Figure 75A shows the mRNA expression levels of KCNJ5 in normal tissue of lymph node negative and lymph node positive breast cancer patients. The levels are approximately equal in both groups.

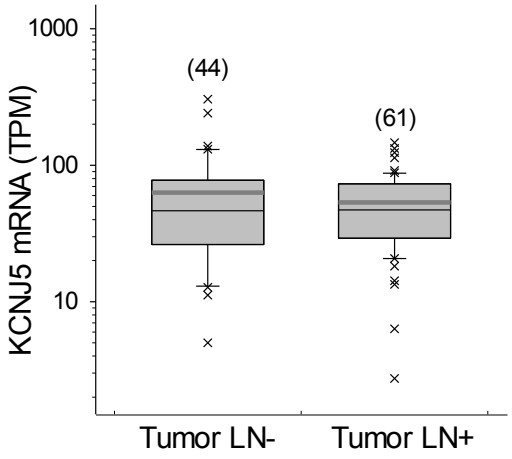


Figure 75B - KCNJ5 mRNA in relation to Lymph node status

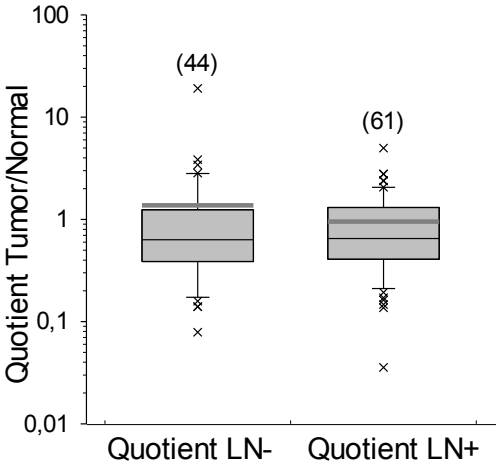


Figure 75C - ratio of KCNJ5 mRNA expression between tumor and corresponding normal sample in relation to lymph node status

Figure 75B shows the mRNA expression levels of KCNJ5 in tumor tissue of lymph node negative and lymph node positive breast cancer patients. As shown above the KCNJ5 expression is higher in lymph node positive patients. Figure 75C shows the ratio of tumor to normal sample KCNJ5 mRNA expression level.

4.3.3 KCNJ6 mRNA expression in tumor and normal tissue

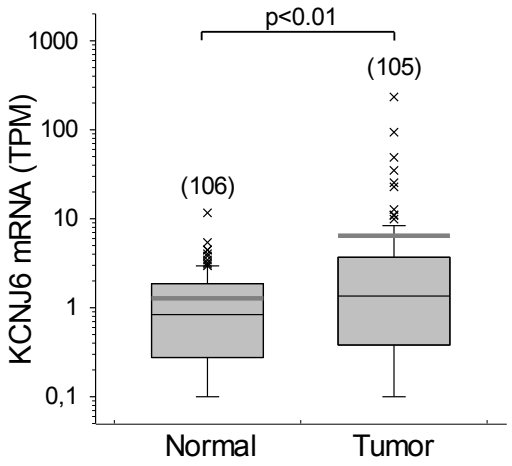


Figure 77 - ratio of KCNJ6 mRNA expression between tumor and corresponding normal sample in relation to lymph node status

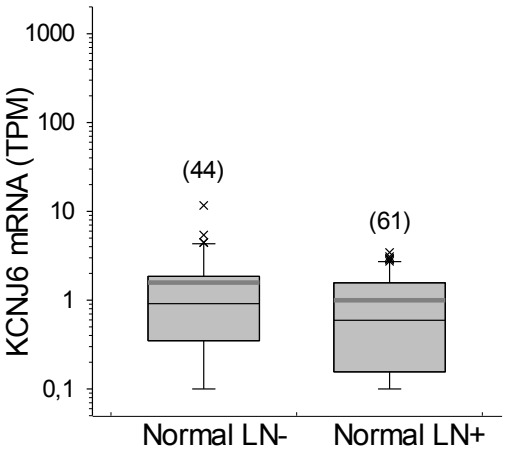


Figure 76A - KCNJ6 mRNA in normal tissue and tumor tissue

Figure 76 shows the mRNA expression levels of KCNJ6 in normal tissue and tumor tissue. The expression levels are higher in tumor tissue compared to normal/healthy tissue. The difference is statistically significant ($p<0.01$). Figure 77A shows the mRNA expression levels of KCNJ6 in normal tissue of lymph node negative and lymph node positive breast cancer patients. The levels are approximately equal in both groups.

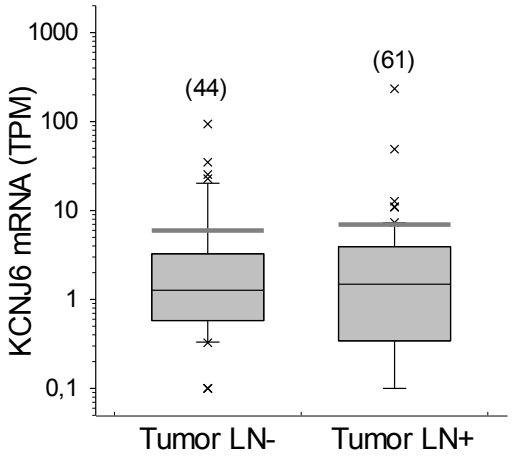


Figure 76B - KCNJ6 mRNA in relation to Lymph node status

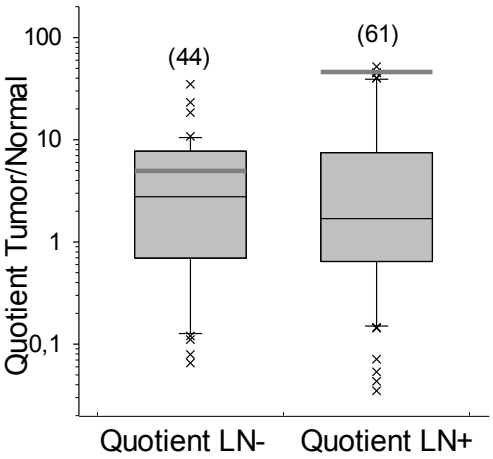


Figure 76C - KCNJ6 mRNA in relation to Lymph node status

Figure 77B shows the mRNA expression levels of KCNJ6 in tumor tissue of lymph node negative and lymph node positive breast cancer patients. Figure 77C shows the ratio of tumor to normal sample KCNJ6 mRNA expression level.

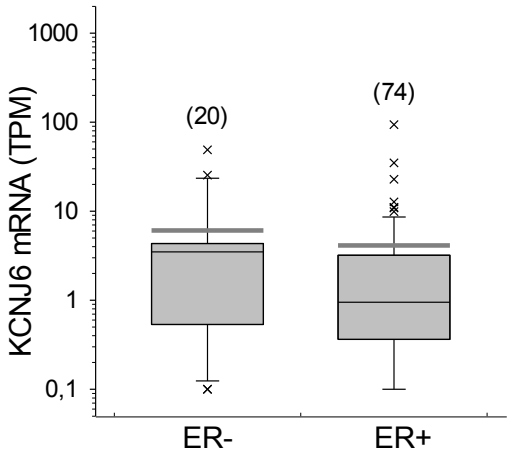


Figure 78 - KCNJ6 mRNA in relation to estrogen receptor status

Figure 78 shows the mRNA expression levels of KCNJ6 in estrogen receptor negative and estrogen receptor positive breast cancer tissue.

4.3.4 KCNJ9 mRNA expression in tumor and normal tissue

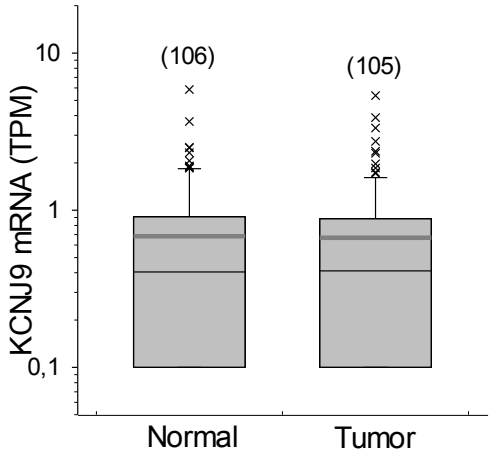


Figure 79 - KCNJ9 mRNA in normal tissue and tumor tissue

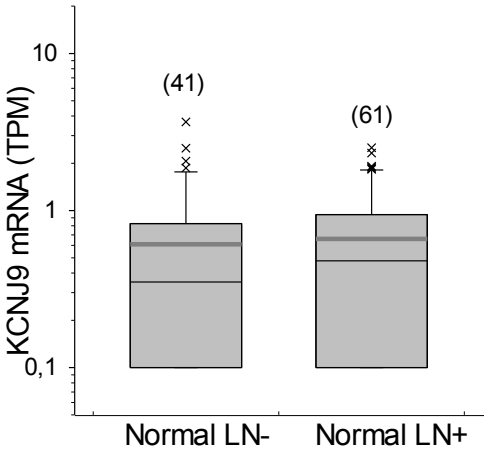


Figure 80A - KCNJ9 mRNA in normal tissue of lymph node negative and lymph node positive breast cancer patients

Figure 79 shows the mRNA expression levels of KCNJ9 in normal tissue and tumor tissue. Figure 80A shows the mRNA expression levels of KCNJ9 in normal tissue of lymph node negative and lymph node positive breast cancer patients. In both Figures the levels are approximately equal in both groups.

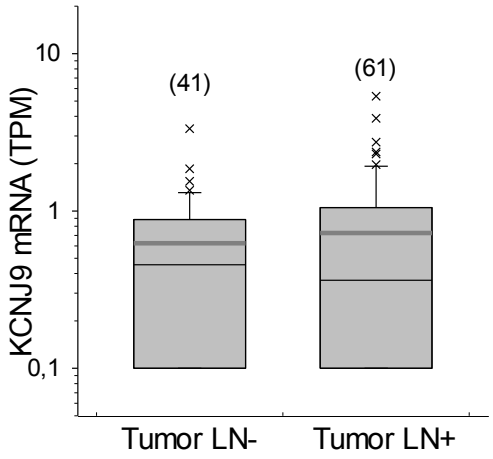


Figure 80B - KCNJ9 mRNA in relation to Lymph node status

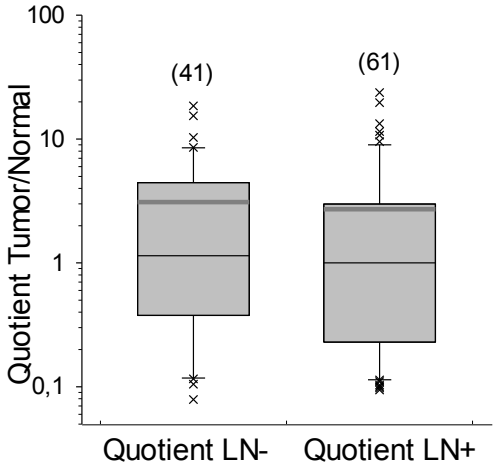


Figure 80C - ratio of KCNJ9 mRNA expression between tumor and corresponding normal sample in relation to lymph node status

Figure 80B shows the mRNA expression levels of KCNJ9 in tumor tissue of lymph node negative and lymph node positive breast cancer patients. Figure 80C shows the ratio of tumor to normal sample KCNJ9 mRNA expression level.

4.3.5 TrpC3 mRNA expression in tumor and normal tissue

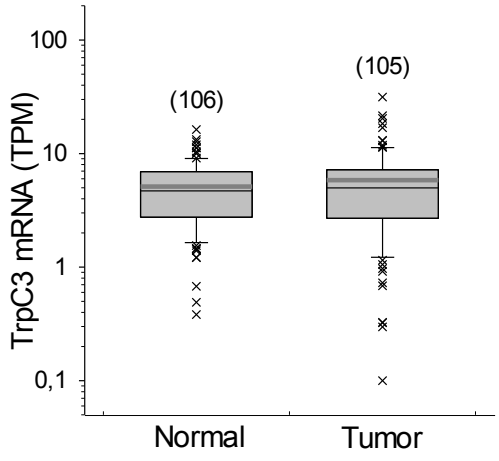


Figure 82 - TrpC3 mRNA in normal tissue and tumor tissue

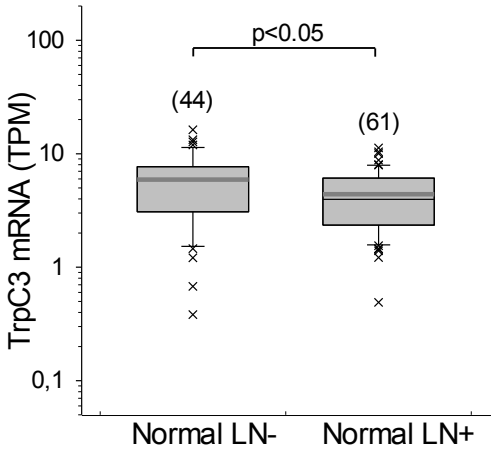


Figure 81A - TrpC3 mRNA in normal tissue of lymph node negative and lymph node positive breast cancer patients

Figure 81 shows the mRNA expression levels of TrpC3 in normal tissue and tumor tissue. The levels are approximately equal in both groups. Figure 82A shows the mRNA expression levels of TrpC3 in normal tissue of lymph node negative and lymph node positive breast cancer patients. The expression levels are significantly higher in the lymph node negative group ($p<0.05$).

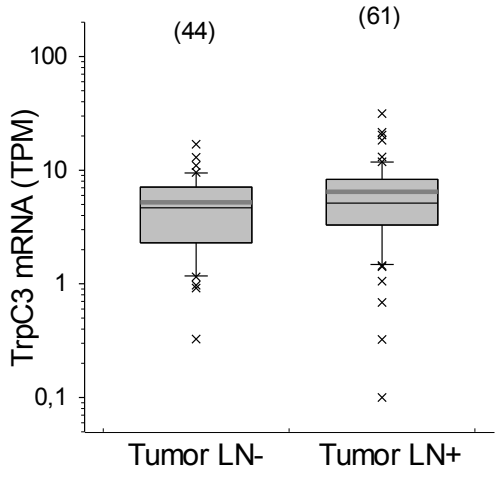


Figure 81B - TrpC3 mRNA in relation to Lymph node status

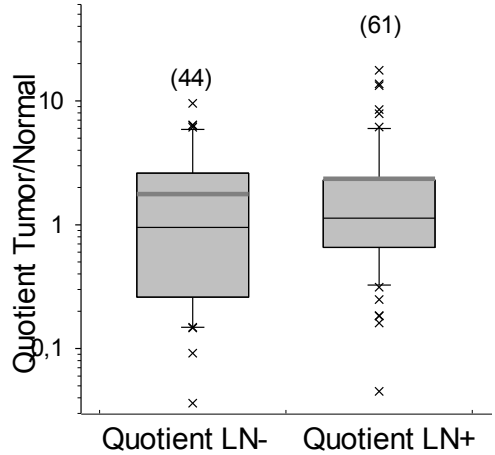


Figure 81C - ratio of TrpC3 mRNA expression between tumor and corresponding normal sample in relation to lymph node status

Figure 82B shows the mRNA expression levels of TrpC3 in tumor tissue of lymph node negative and lymph node positive breast cancer patients. Figure 82C shows the ratio of tumor to normal sample TrpC3 mRNA expression level.

4.3.6 TrpC6 mRNA expression in tumor and normal tissue

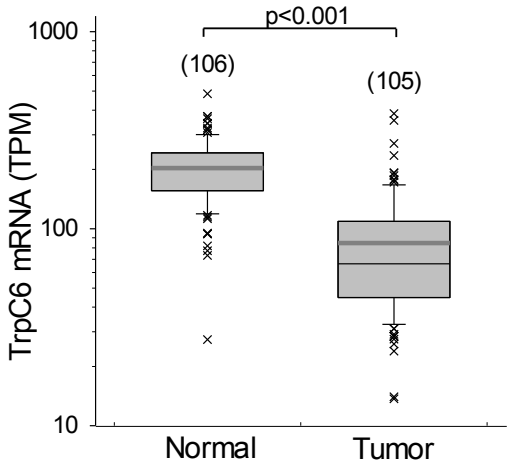


Figure 84 TrpC6 mRNA in normal tissue and tumor tissue

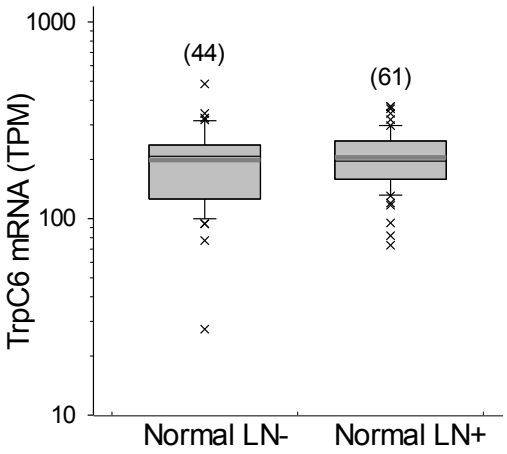


Figure 83A - TrpC6 mRNA in normal tissue of lymph node negative and lymph node positive breast cancer patients

Figure 83 shows the mRNA expression levels of TrpC6 in normal tissue and tumor tissue. The expression levels are higher in normal tissue compared to tumor tissue. The difference is statistically significant (p<0.001). Figure 84A shows the mRNA expression levels of TrpC6 in normal tissue of lymph node negative and lymph node positive breast cancer patients. The levels are approximately equal in both groups.

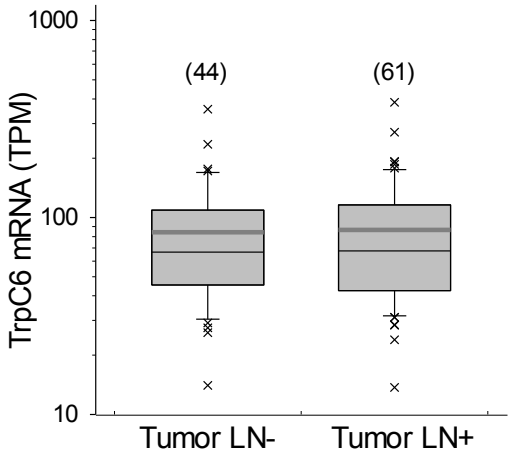


Figure 83B - TrpC6 mRNA in relation to Lymph node status

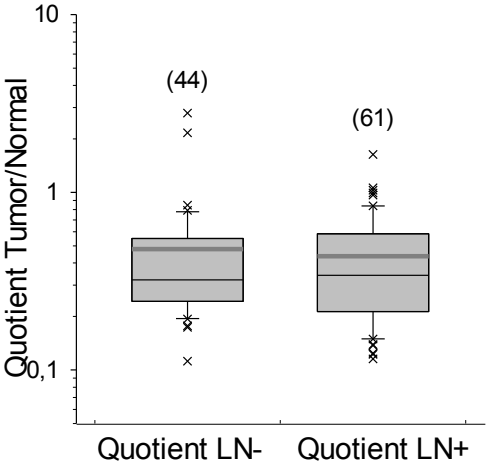


Figure 83C - ratio of TrpC6 mRNA expression between tumor and corresponding normal sample in relation to lymph node status

Figure 84B shows the mRNA expression levels of TrpC6 in tumor tissue of lymph node negative and lymph node positive breast cancer patients. Figure 84C shows the ratio of tumor to normal sample TrpC6 mRNA expression level.

4.3.7 ANO1 mRNA expression in tumor and normal tissue

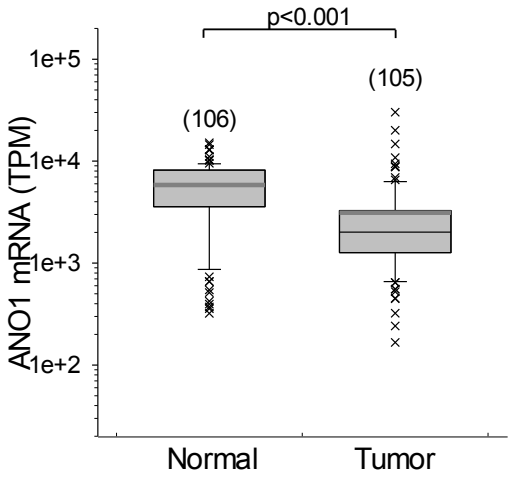


Figure 85 - ANO1 mRNA in normal tissue and tumor tissue

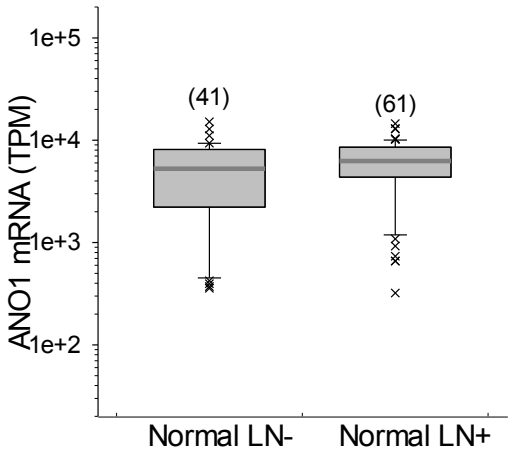


Figure 86A - ANO1 mRNA in normal tissue of lymph node negative and lymph node positive breast cancer patients

Figure 85 shows the ANO1 expression levels of ANO1 in normal tissue and tumor tissue. The expression levels are higher in normal tissue compared to tumor tissue. The difference is statistically significant ($p<0.001$). Figure 86A shows the mRNA expression levels of ANO1 in normal tissue of lymph node negative and lymph node positive breast cancer patients. The levels are approximately equal in both groups.

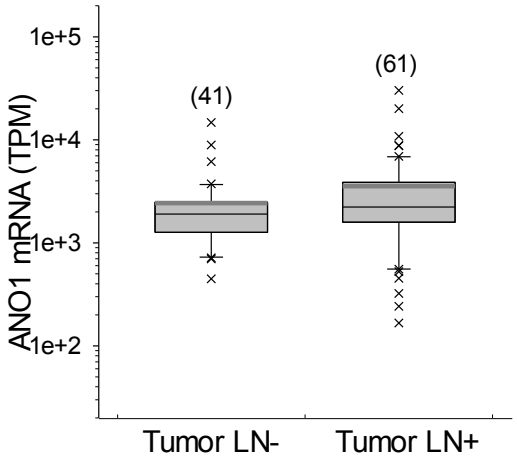


Figure 86B - ANO1 mRNA in relation to Lymph node status

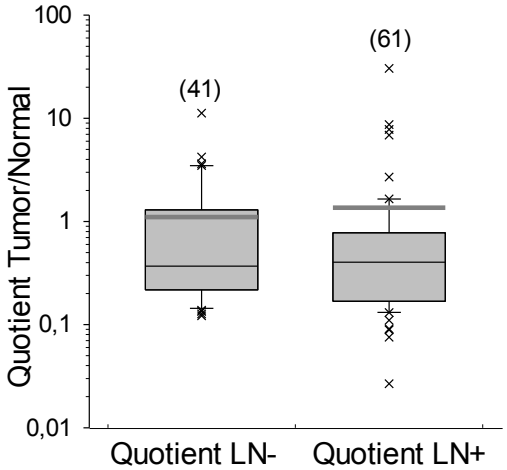


Figure 86C - ratio of ANO1 mRNA expression between tumor and corresponding normal sample in relation to lymph node status

Figure 86B shows the mRNA expression levels of ANO1 in tumor tissue of lymph node negative and lymph node positive breast cancer patients. Figure 86C shows the ratio of tumor to normal sample ANO1 mRNA expression level.

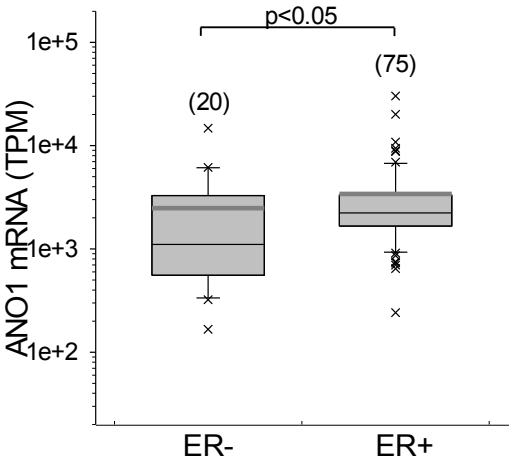


Figure 87A - ANO1 mRNA in relation to estrogen receptor status

Figure 87A shows the mRNA expression levels of ANO1 in estrogen receptor negative and estrogen receptor positive breast cancer tissue. The ANO1 expression is higher in estrogen receptor positive patients ($p<0.05$)

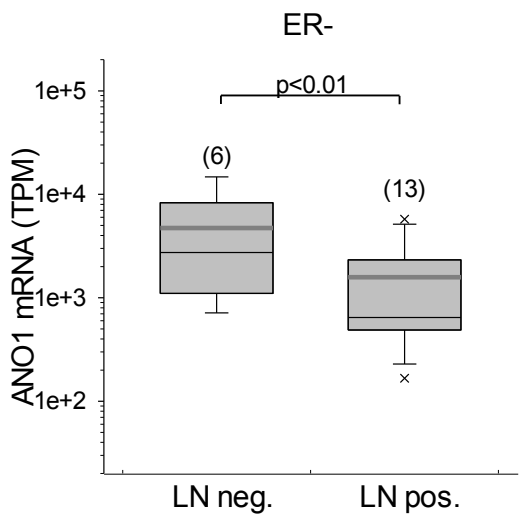


Figure 87B - ANO1 mRNA in relation to Lymph node status in ER- samples

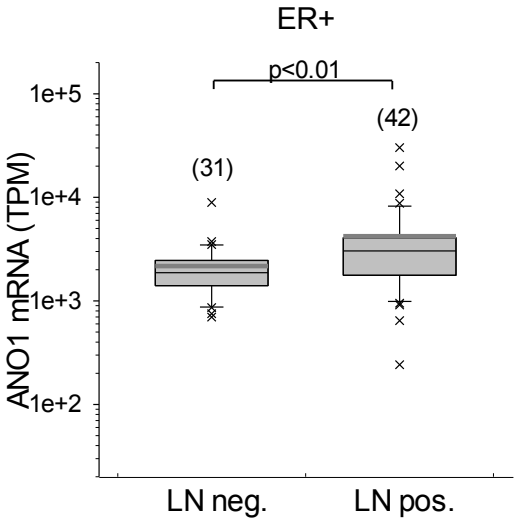


Figure 87C - ANO1 mRNA in relation to Lymph node status in ER+ samples

In analogy to the upper analyzes on cancer tissue data Figure 87B shows the mRNA expression levels of ANO1 in estrogen receptor negative, Figure 87C in estrogen receptor positive breast cancer only, divided into lymph node negative and positive patients. ANO1 shows a higher expression in lymph node negative samples in estrogen receptor negative cancer and a higher expression in lymph node positive samples in estrogen receptor positive cancer ($p<0.01$). However the validity of these results is limited due to the relatively low number of patients in the subgroups.

4.4 Survival analysis

Table 12 shows the results of the Cox Regression - Proportional Hazards Model analysis, performed with all genes and with patients' age as covariates. The hazard rate is significantly affected by age (p<0.001) and by KCNJ3 only (p=0.047). The other genes which have been analyzed in this study do not show a significant influence on the hazard ratio.

Covariate	Coefficient	StdErr	Wald Chi-Square	P Value
Age	0,0272	0,00807	11,392	<0,001
ANO1	0,0000308	0,0000231	1,781	0,182
KCNJ3	**0,00014**	**0,0000702**	**3,953**	**0,047**
KCNJ5	0,00203	0,0021	0,934	0,334
KCNJ6	0,00276	0,00256	1,162	0,281
KCNJ9	0,0224	0,0666	0,113	0,737
PIEZO1	0,0000807	0,0000784	1,058	0,304
PIEZO2	0,0000418	0,000143	0,0849	0,771
TRPC3	0,00352	0,0158	0,0495	0,824
TRPC6	-0,0000325	0,000224	0,021	0,885

Table 12 - Cox Regression - Proportional Hazards Model analysis, performed with all genes and with patients' age as covariates

When the analysis is performed on KCNJ3 and age as the only covariates, KCNJ3 shows an even more significant influence on the hazard ratio (p=0.034).

Covariate	Coefficient	StdErr	Wald Chi-Square	P Value
Age	0,0261	0,00774	11,325	<0,001
KCNJ3	0,000123	0,0000584	4,476	0,034

Table 13 - Cox Regression - Proportional Hazards Model analysis, performed on KCNJ3 and age as covariates

The significance for KCNJ3 affecting the hazard ratio increases further when the estrogen receptor status is added as a third covariate (p=0.011). Table 14 shows the results in detail.

Covariate	Coefficient	StdErr	Wald Chi-Square	P Value
Age	0,029	0,00821	12,434	<0,001
KCNJ3	0,000151	0,0000596	6,391	0,011
ER_positive	-0,483	0,249	3,775	0,052

Table 14 - Cox Regression - Proportional Hazards Model analysis, performed on KCNJ3, age and estrogen receptor status as covariates

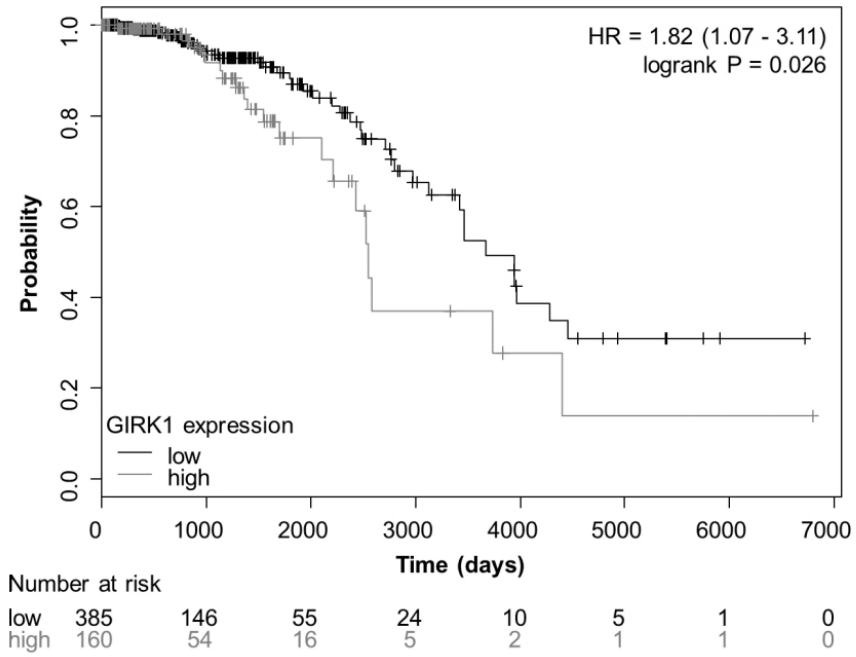


Figure 88 - Survival of estrogen receptor positive breast cancer patients with high and low GIRK1 expression levels

Figure 88 shows the survival plot of 545 estrogen receptor positive breast cancer patients from TCGA, containing overall survival data. The statistical analysis revealed that estrogen receptor positive breast cancer patients with higher GIRK1 expression have a worse overall survival than estrogen receptor positive breast cancer patients with low GIRK1 expression ($p<0.05$, HR=1.82).

5 DISCUSSION

Analysis of the open-access TCGA data revealed several interesting findings concerning the mRNA expression levels of GIRK (and the other genes) in breast cancer tissue. In the following the most important results will be summarized and discussed in the context of the literature.

The main objective of this study was to analyze the GIRK mRNA expression levels in different clinical subgroups of breast cancer. By analyzing the TCGA data we were able to validate previous observations that the expression of GIRK1 is significantly higher in breast cancer as compared to healthy breast tissue (Brevet, et al., 2008; Stringer, et al., 2001). Furthermore, the level of GIRK1 expression in tumor tissue correlates with lymph node positive breast cancer as shown previously (Stringer, et al., 2001). In the context of the clinical classification used in a current research project at the Medical University of Graz (FWF KLI 182) GIRK1 mRNA expression is highest in Groups 1 and 3. Within the different PAM50 molecular subtypes the GIRK1 mRNA expression is highest in the Luminal A and Luminal B breast cancer subtypes. Another interesting result of the present study is the finding that a high GIRK1 expression is associated with estrogen receptor positive breast cancer. This result was also found in a recent study by Ko et al. (Ko, et al., 2013)

The GIRK2 mRNA expression is also higher in tumor tissue as compared to normal tissue but it seems to correlate with a negative estrogen receptor status. The expression of GIRK2 is highest in the triple negative clinical Group 4. Dhar and Plummer have shown previously, that GIRK2 is overexpressed in various cancer cell lines (Dhar, et al., 2006; Plummer, et al., 2004; Plummer, et al., 2005).

The present study did not show a statistically significant difference of the GIRK3 mRNA expression between tumor and normal tissue. Although an overexpression in the clinical Group 4, representing triple negative breast cancer was detected, future studies are needed to clarify the involvement of GIRK3 in breast cancer development and progression (Plummer, et al., 2004).

On the other hand the GIRK4 mRNA expression levels were lower in tumor tissue when compared with normal tissue. They tended to be higher in the clinical Group 3 and in Her2 positive tumor tissue.

Piezo1 mRNA expression was associated with triple negative breast cancer, in particular with progesterone receptor negative tumor tissue. Secondly, Piezo2 tends to be overexpressed in estrogen receptor positive tumor tissue including Luminal A and Luminal B subtypes and the clinical Group 1.

An interesting result concerning TrpC3 was the finding that the mRNA expression levels are highest in T1 breast cancer and seem to get lower with a higher T-status. TrpC3 did not show a significant difference in the mRNA expression between tumor and normal tissue, whereas TrpC6 mRNA expression was much lower in tumor tissue when compared to normal tissue in our population. This is in contrast to the findings of Aydar et al. who revealed TrpC3 and TrpC6 as significantly up regulated in breast cancer biopsy tissue of five mice and some breast cancer epithelial cell lines (Aydar, et al., 2009). Furthermore Ko et al. have found a lower expression of TrpC6 and also a lower expression of ANO1 in lung adenocarcinoma tissues when compared to normal tissues (Ko, et al., 2014).

ANO1 was also lower expressed in breast tumor tissue as compared to normal breast tissue in the present study. This is in contrast to the findings delivered by Britschgi et al. who identified ANO1 as amplified, overexpressed and associated with a poor prognosis in breast cancer (Britschgi, et al., 2013). Moreover, similar to the results concerning GIRK1, ANO1 was found to be overexpressed in the clinical Groups 1 and 3, in estrogen receptor positive tumors and lymph node positive tumors. This is in accordance with the findings of Ko et al. showing a higher ANO1 expression in estrogen receptor positive breast cancer (Ko, et al., 2013).

The survival analysis in our study has shown that GIRK1 overexpression indeed seems to indicate a negative effect on the overall survival in the Cox-regression model. We could show that estrogen receptor positive breast cancer patients seem to have a worse prognosis when GIRK1 is overexpressed.

6 CONCLUSION

Numerous previous studies have shown that GIRK1 is involved in cellular signaling pathways and that it is an integral part in cell proliferation and tumor progression, especially in breast cancer (Stringer, et al., 2001; Dhar, et al., 2006; Plummer, et al., 2004; Hance, et al., 2008; Wagner, et al., 2010; Takanami, et al., 2004; Brevet, et al., 2008). Brevet et al. have shown, that GIRK1 protein is overexpressed in breast cancer tissue compared to normal tissue (Brevet, et al., 2008). Also Stringer et al. 2001 attained some remarkable results in their study when they identified GIRK1 as the one gene whose overexpression correlated with axillary lymph node metastasis (Stringer, et al., 2001). The results of these studies encouraged us to examine their findings on a larger collective with the help of newer, even more modern methods.

To achieve the goals set for this work, The Cancer Genome Atlas proved to be a suitable source for expression data and the patients' clinical information. The data acquisition from TCGA is surprisingly simple and allows researchers a cost-effective access to a huge genome database. In summary it can be stated that The Cancer Genome Atlas is an amazing and mighty tool which offers new possibilities in the study of effects of gene expressions.

As far as the effects of a high GIRK1 expression are concerned, there is indeed a significant association between GIRK1 overexpression and breast cancer, particularly lymph node positive and also estrogen receptor positive breast cancer.

A positive association does not necessarily imply a causal relationship. The question of cause and impact of the associations found in this study needs to be explored in further studies. Thus no definite statement about the clinical relevance of these findings can be made. However, the results of this study support the hypothesis of an influential relationship between GIRK1 and tumor progression and may help to improve molecular diagnostics and a more targeted therapy of breast cancer in the future.

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8 FIGURES

9 TABLES

10 ABBREVIATIONS

35a	35 years
AJCC	American Joint Committee on Cancer
ANO1	Anoctamin-1, also known as Transmembrane member 16A
ASCO	American Society of Clinical Oncology
BRCA	breast cancer
CAO	College of American Pathology
DCC	Data Coordinating Center
ER	estrogen receptor
ER-	estrogen receptor negative
ER+	estrogen receptor negative
FWF	Fonds zur Förderung der wissenschaftlichen Forschung
GIRK	G protein-coupled inwardly-rectifying potassium channel
Gr.	group
Her2	human epidermal growth factor receptor 2
HR	hazard ratio
KCNJ3	Potassium inwardly-rectifying channel, subfamily J, member 3, also known as GIRK1
KCNJ5	Potassium inwardly-rectifying channel, subfamily J, member 5, also known as GIRK4
KCNJ6	Potassium inwardly-rectifying channel, subfamily J, member 6, also known as GIRK2
KCNJ9	Potassium inwardly-rectifying channel, subfamily J, member 9, also known as GIRK3
LN	lymph node
LN neg	lymph node negative
LN pos	lymph node positive
LumA	Luminal A
LumB	Luminal B
M neg	distant metastasis negative

M pos	distant metastasis positive
mRNA	messenger ribonucleic acid
NaN	Not a Number
NCI	National Cancer Institute
NHGRI	National Human Genome Research Institute
OS	overall survival
PAM50	prediction analysis of microarray 50 (PAM50) subtype predictor, Breast Cancer Intrinsic Classifier
Piezo1	Piezo-type mechanosensitive ion channel component 1
Piezo2	Piezo-type mechanosensitive ion channel component 2
postm	postmenopausal
PR	progesterone receptor
PR-	progesterone receptor negative
PR+	progesterone receptor positive
prem	premenopausal
RNA	Ribonucleic acid
RNA-Seq	RNA Sequencing, also called ‘Whole Transcriptome Shotgun Sequencing‘
ROR	Risk of Recurrence
RPKM	Reads Per Kilobase per Million mapped reads
RSEM	software package for estimating gene and isoform expression levels from RNA-Seq data
TCGA	The Cancer Genome Atlas
TNM	Tumor Node Metastasis
TPM	transcripts per million
TrpC3	Transient receptor potential cation channel, subfamily C, member 3
TrpC6	Transient receptor potential cation channel, subfamily C, member 6
UCSC	University of California, Santa Cruz