# Thyroid Hormones and Vitamin D in Patients with Breast Cancer with Mutations in *BRCA1* or *BRCA2* Genes

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**Abstract.** *Aim: The thyroid hormones free triiodothyronine (fT3), free thyroxine (fT4), thyroid-stimulating hormone (TSH) and vitamin D seem to be involved in the process of differentiation and proliferation of breast tissue. Little is known about these factors in breast cancer 1 and 2 (BRCA1/BRCA2)-mutation carriers with breast cancer (BC). The purpose of this investigation was to evaluate the association of thyroid gland function and vitamin D with BC in patients with BRCA mutations. Patients and Methods: At the Department of Hereditary Breast and Ovarian Cancer of the Ludwig Maximilian University Hospital of Munich, 40 patients with BC (10 patients with mutations in the BRCA1 gene, 10 with mutations in the BRCA2 gene, and 20 without mutations, as control group) were selected for analysis of the following parameters: fT3, fT4, TSH and vitamin D. The primary diagnosis was made between 21 and 62 years of age. The patients were matched by age. Anamnestic data were evaluated concerning disorders of the thyroid gland and primary BC diagnosis. Results: In patients with BC, BRCA mutations are not associated with thyroidal dysfunctions. A significantly increased level of vitamin D in BRCA2-mutation carriers compared to those without mutation (p=0.02) was detected. The grade of the tumors in the BRCA2 group was better than in those with mutation. BRCA1-mutation carriers had an increased incidence of primary BC diagnosis during pregnancy (30% vs. 0%) in comparison to those without mutation. Conclusion: No association between the thyroid hormones and BC in BRCA1/2-mutation carriers was found. Vitamin D was significantly elevated in BRCA2-mutation carriers and the observation of a better tumor grade in this group could be consistent with the ability of vitamin D to inhibit growth and induce differentiation.*

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Breast cancer (BC) is the most frequent type of cancer among women worldwide, with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). About 5-10% of the cases are assumed to be due to mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* (1).

The effects of the thyroid hormones free triiodothyronine (fT3) and free thyroxine (fT4) are varied. In general, they play an important role in the regulation of metabolism (2).

There have been many studies focusing on thyroid hormones and thyroid dysfunction as clinical markers for patients with BC. The results remain divergent. Some studies showed increased serum levels of thyroid-stimulating hormone (TSH), with subclinical or manifest hypothyroidism in 10.0-19.7% of patients with BC (3, 4).

A significant association between benign thyroid disease, *i.e.* thyroid dysfunction, Hashimoto thyroiditis and Graves disease, and the prevalence of BC was demonstrated, patients with BC had a higher prevalence of benign thyroid disease (5).

In a previous prospective case–control study, our group showed that the blood levels of fT3 and fT4, as well as antibodies against thyroid peroxidase and the TSH receptor were significantly elevated at the time of primary diagnosis

of BC (*i.e.* before the start of any therapeutic regimen: surgery, radiation, chemo- or endocrine therapy) (6).

Vitamin D has an important role in the metabolism of calcium and phosphate. Furthermore, the active form of vitamin D is known to be an inhibitor of growth and is able to induce apoptosis (7-9). Its ability to inhibit growth and induce differentiation was demonstrated in a variety of cancer cells including myeloid, breast and prostate (10-13).

A large cohort study of postmenopausal women focusing on their vitamin D intake showed a possible association of an intake of >800 IU/d with a decrease in the risk of BC (14). Recent data support this finding. Dietary vitamin D supplementation appears to reduce the risk of BC development or relapse (15). The functions of the thyroid hormones and vitamin D have been studied in patients with BC in general. However, a search of the literature on PubMed did not reveal any data on the role of these factors in the subgroup of patients with BC who carry a mutation in one of the two breast cancer susceptibility genes *BRCA1* and *BRCA2*.

The purpose of this investigation was to evaluate the association of thyroid gland function and vitamin D with BC in patients with BRCA mutations.

## Patients and Methods

*Patients*. At the Department of Hereditary Breast and Ovarian Cancer of the Ludwig Maximilian University Hospital of Munich, 40 patients who had a histologically confirmed diagnosis of BC were selected for our study. The cohort comprised 10 patients with mutations in the *BRCA1* gene, 10 patients with mutations in the *BRCA2* gene, and 20 patients without mutations in either of these two genes as a control group. The primary diagnosis of BC was made between 21 and 62 years of age.

Blood serum samples were obtained from the patients when they presented to the Department of Hereditary Breast and Ovarian Cancer of the Ludwig Maximilians University Hospital of Munich. Details of the patients' medical history were obtained from their health records. The patients of the control group were matched by age in regard to the time of blood withdrawal. The mean interval from diagnosis of BC to blood withdrawal was 33.5 months (range=0-180 months).

The study was approved by the Human Institutional Review Board of the Ludwig Maximilians University (no. 48-08). Signed informed consent was obtained from the participants, allowing analysis of all clinical and laboratory data mentioned here.

*Clinical chemistry*. Whole blood was centrifuged in a Hettich Rotixa RP Centrifuge (Hettich Company, Andreas Hettich GmbH & Co.KG, Tuttlingen, Baden-Wuerttemberg, Germany) at 1700×g at 20˚C. The serum supernatant was transferred to Nunc vials (1.8 ml, Cryotube, Thermo Scientific, Waltham, MA, USA) and frozen to -20˚C. Within 7 days the vials were transferred to a freezer at −80˚C where they were stored until further analysis.

Serological determination of thyroid hormones and TSH was performed using chemiluminescent immunoassays according to the manufacturer's instructions: fT4 (normal range=0.9-1.7 ng/dl), fT3 (2.3-4.2 pg/ml) and TSH (0.44-3.80 μU/ml) (Siemens Healthcare, Erlangen, Bavaria, Germany); 25-hydroxyvitamin D (25-OH-VitD; 20.0-100.0 ng/ml) (Immunodiagnostic Systems, Frankfurt, Hesse, Germany).

TSH values >3.80 μU/ml were considered to indicate hypothyroidism, from 3.8-10.0 μU/ml latent hypothyroidism and values >10.0 μU/ml manifest hypothyroidism; values <0.44 μU/ml were considered to indicate hyperthyroidism. Vitamin D values <20.0 ng/ml were considered to indicate vitamin D deficiency.

*Statistical analysis*. Parametrically distributed data are expressed as the mean±standard deviation (SD) (minimum, maximum). Means were analyzed by the *t*-test. *p*-Values of less than 0.05 were regarded as statistically significant. Data were examined with Microsoft Excel and SPSS for Windows (version 16.0; SPSS Inc., Chicago, IL, USA).

## Results

An overview of the patient characteristics is given in Table I.

The tumors in the *BRCA1*-mutation group tended to be of larger size compared to the non-mutation group: 60% of the tumors were larger than 2 cm, whereas the tumor size in the coontrol group exceeded 2 cm in only 12.5% of cases ($p$=0.066). This tendency was also seen in the *BRCA2*-mutation carriers in comparison to the non-mutation group: 30% of the tumors in the *BRCA2*-mutation group were larger than 2 cm compared to 20% in the control group (not significant) (Table I).

Lymph node involvement was observed more often in the *BRCA1*-mutation carriers: 40% in the *BRCA1*-mutation group had lymph node metastasis, whereas no nodal involvement was detected in the control group ($p$=0.087). Concerning the group with *BRCA2* mutation, nodal involvement was observed more frequently than in the corresponding cotrol group (40% *vs*. 30%; not significant). Only one of the patients in the *BRCA2* control group had distant metastasis, while all patients of the other groups in this study were free of distant metastasis.

A total of 89% of tumors in the BRCA1-mutation group were poorly differentiated in compared to 75% in the control group ($p$=0.576). The tumors in the BRCA2-mutation group showed a tendency for a better tumor grade compared to those in the non BRCA2-mutation group: 87.5% of the BRCA2-mutated tumors were moderately or well-differentiated, while in the control group, only 50% had a grade lower than G3 ($p$=0.282) (Table I).

Hormone receptor (HR) status was classified as positive if at least one of the two receptors for estrogen or progesterone was positive. In the *BRCA1*-mutation group, 20% of the tumors had a positive HR status compared to the non-mutation group, where HR positivity was 67% ($p$=0.07). HER2 status was positive in 14% of the *BRCA1*-mutation cases compared to 50% in the non *BRCA1*-mutation group ($p$=0.282). The majority of tumors in the *BRCA1*-mutation

Table I. *Patients' characteristics*.

| Characteristic | *BRCA1* mutation | No *BRCA1* mutation | *BRCA2* mutation | No *BRCA2* mutation |
|---|---|---|---|---|
| Mean±SD age at primary diagnosis, years | 36±8.8 | 36.2±8.1 | 41.8±11.1 | 44.6±8.7 |
| Mean±SD age at blood testing, years | 38.2±8.0 | 37.8±7.7 | 46.8±10.4 | 46.8±10.0 |
| pT Stage, % (n/N) | | | | |
| T0 | 20% (2/10) | 0% (0/8) | 0% (0/10) | 0% (0/10) |
| Tis | 0% (0/10) | 0% (0/8) | 0% (0/10) | 10% (1/10) |
| T1a | 10% (1/10) | 25% (2/8) | 0% (0/10) | 10% (1/10) |
| T1b | 0% (0/10) | 37.5% (3/8) | 40% (4/10) | 30% (3/10) |
| T1c | 10% (1/10) | 25% (2/8) | 30% (3/10) | 30% (3/10) |
| T2 | 50% (5/10) | 12.5% (1/8) | 30% (3/10) | 20% (2/10) |
| T3 | 10% (1/10) | 0% (0/8) | 0% (0/10) | 0% (0/10) |
| T4 | 0% (0/10) | 0% (0/8) | 0% (0/10) | 0% (0/10) |
| pN Stage, % (n/N) | | | | |
| Nx | 20% (2/10) | 0% (0/9) | 0% (0/10) | 10% (1/10) |
| N0 | 40% (4/10) | 100% (9/9) | 60% (6/10) | 60% (6/10) |
| N1 | 40% (4/10) | 0% (0/9) | 40% (4/10) | 20% (2/10) |
| N2 | 0% (0/10) | 0% (0/9) | 0% (0/10) | 0% (0/10) |
| N3 | 0% (0/10) | 0% (0/9) | 0% (0/10) | 10% (1/10) |
| Metastasis, % (n/N) | | | | |
| Mx | 20% (2/10) | 67% (6/9) | 62% (5/8) | 43% (3/7) |
| M0 | 80% (8/10) | 33% (3/9) | 38% (3/8) | 43% (3/7) |
| M1 | 0% (0/10) | 0% (0/9) | 0% (0/8) | 14% (1/7) |
| Differentiation grade, % (n/N) | | | | |
| G1 | 0% (0/9) | 0% (0/8) | 12.5% (1/8) | 12.5% (1/8) |
| G2 | 11% (1/9) | 25% (2/8) | 75% (6/8) | 37.5% (3/8) |
| G3 | 89% (8/9) | 75% (6/8) | 12.5% (1/8) | 50% (4/8) |
| HR status, % (n/N) | | | | |
| HR-positive | 20% (2/10) | 67% (6/9) | 100% (10/10) | 78% (7/9) |
| HER2-positive | 14% (1/7) | 50% (4/8) | 50% (4/8) | 6% (6/9) |
| Triple-negative | 60% (6/10) | 22% (2/9) | 0% (0/10) | 11% (1/9) |
| Diagnosis in pregnancy, % (n/N) | 30% (3/10) | 0% (0/10) | 0% (0/10) | 0% (0/10) |

BRCA1/2: Breast cancer gene; SD: standard deviation; SD: standard deviation; HR: hormone receptor.

group were triple-negative BC (60%). In the control group, 22% were negative for all three receptors ($p$=0.17). All tumors in the *BRCA2*-mutation group were HR-positive (100%), while 78% were HR-positive in the control group. No triple-negative BC occurred in the *BRCA2*-mutation group, while there were 10% in the corresponding control group. In the *BRCA1*-mutation group, 30% of cases were first diagnosed during pregnancy, whereas none of the remaining patients was diagnosed with BC during pregnancy (Table I).

The analysis of the thyroid hormones (fT3, fT4) and TSH revealed no significant differences between the subgroups. Hypothyroidism was common but was not significantly more often found in any specific subgroup (Table II).

The level of vitamin D was similar in the *BRCA1*-mutation group compared to the control group. In the *BRCA2*-mutation group, a significant elevation of vitamin D was detected compared to the control group ($p$=0.02). In general, the vitamin D levels remained within the normal range (20.0-100.0 ng/ml) but they were at the lower end, with a maximum mean for the *BRCA2*-mutation group of 31.7 ng/ml (Table II).

## Discussion

Although many studies have focused on the function of the thyroid gland and its association with BC, the results remain controversial (16-20). While Jsikra *et al*. showed increased blood levels of TSH indicating hypothyroidism in almost 20% of cases (4), Muller and Pinchera *et al*. found higher serum levels of the thyroid hormones fT3 and fT4 in patients with BC (5). This is consistent with a recent meta-analysis that analyzed the results of eight cross-sectional studies with 4,189 participants. Overall the pooled results showed significantly increased levels of fT3 and fT4 in pateints with BC compared to healthy controls (21).

In a previous study, our group found significantly increased levels of fT3 and fT4 in patients with BC compared to a control group, although these values remained within the normal ranges (6). A possible explanation for this finding is

Table II. *Thyroid function and serum vitamin D level.*

| | *BRCA1* mutation | No *BRCA1* mutation | *p*-Value | *BRCA2* mutation | No *BRCA1* mutation | *p*-Value |
|---|---|---|---|---|---|---|
| % (x/n) | | | | | | |
| Hypothyroidism w/RT | 30 (3/10) | 33 (3/9) | | 30 (3/10) | 11 (1/9) | |
| Hypothyroidism w/o RT | 10 (1/10) | 0 (0/10) | | 0 (0/10) | 0 (0/10) | |
| Hyperthyroidism | 0 (0/10) | 0 (0/10) | | 0 (0/10) | 10 (1/10) | |
| Mean±SD | | | | | | |
| TSH (0.44-3.80 μU/ml) | 1.75±0.88 | 1.61±0.8 | 0.73 | 1.56±0.32 | 1.23±0.59 | 0.15 |
| fT3 (2.3-4.2 pg/ml) | 3.12±0.37 | 3.05±0.13 | 0.6 | 3.05±0.24 | 3.05±0.37 | 1 |
| T4 (0.9-1.7 ng/dl) | 1.2±0.22 | 1.13±0.19 | 0.49 | 1.17±0.14 | 1.23±0.17 | 0.42 |
| Vitamin D (20.0-100.0 ng/ml) | 28.1±5.7 | 27.6±15.3 | 0.78 | 31.7±4.6 | 23.1±9.1 | 0.02 |

w/: With; w/o: without; RT: replacement therapy; TSH: thyroid-stimulating hormone; fT3: triiodothyronine; fT4: thyroxine.

that fT3 and fT4 might exert an estrogen-like effect on breast tissue in terms of stimulation of proliferation (22).

In a mouse model, mammary gland development was found to be regulated by thyroid hormones (23). In contrast to these findings, we did not find any alterations of the thyroid hormones in our cohort of patients suffering from hereditary BC. The results from the comparison with groups of patients with BC without *BRCA1/2* mutations did not demonstrate any thyroid dysfunction either. This might be due to the small sample size of our cohort or be a first hint that the tumors of patients with BRCA mutations are not sensitive to alterations of thyroid hormones.

To our knowledge, there are no data in the literature focusing on thyroid function in the subgroup of patients with BC with BRCA mutations.

In our cohort, we confirmed that the tumors of *BRCA1*-mutation carriers have worse histopathological features associated with a poorer prognosis (24). The tumors in this subgroup were of larger size. Lymph node involvement was also more frequent, which is in contrast to a study that ruled-out a higher prevalence of nodal involvement in BRCA mutation carriers (25). Another observation of our study consistent with the literature is that these tumors appeared to have a very high rate of triple negative receptor status (26), furthermore, they were found to have a higher tumor grade in most cases (27). All of these characteristics are known risk factors for a worse overall prognosis.

Concerning the tumors in the *BRCA2*-mutation group, we demonstrated that these tumors tended to be better differentiated than the corresponding tumors in the control group. Furthermore, we found a significantly higher vitamin D level in the BRCA2-mutation group. The better tumor grade in the group with *BRCA2* mutation might be associated with the higher levels of vitamin D detected in this group compared to the group of patients without mutation. An explanation for this finding could be that vitamin D is believed to be associated with better differentiation status due to its regulatory effects on malignant breast tissue in terms of differentiation and proliferation (28). Furthermore, vitamin D is believed to down-regulate estrogen receptor abundance, thereby inhibiting the promotory effects of estrogen (29).

Another pathway by which vitamin D might exert its antiproliferative effects on BC cells might be the induction of *BRCA1* gene expression (30). Very recent data describe the potential of vitamin D to restore sensitivity to inhibitors of poly(ADP-ribose) polymerase in triple-negative or BRCA1-deficient tumors (31).

Overall, the levels of vitamin D were at the lower end of the normal range in all subgroups, which supports the theory of increased risk of BC in the case of a deficiency or low levels of vitamin D.

In the group of *BRCA1*-mutation carriers 30% of primary diagnoses of BC were made during pregnancy. This surprising observation might be explained by the fact that carriers of *BRCA1/2* mutations tend to develop BC at a younger age, *i.e.* childbearing age, than patients with sporadic BC. Cullinane *et al.* observed a decrease of BC risk in patients with *BRCA1* mutation who had four or more births (32). Another group showed that the survival of patients with *BRCA1/2* mutations was not impacted by pregnancy, neither if diagnosed during pregnancy nor if the patients became pregnant thereafter (33).

The most important limiting aspect of our approach was the fact that blood draw took place at different times relative to the primary diagnosis of BC. This led to an inhomogeneity in terms of the therapies (surgery, radiation, chemo- or endocrine therapy) that the patients had received at the time when the blood tests were performed. This might have had an effect on our investigations since the (auto)immune system can be influenced by surgery, and chemotherapy (34), which may trigger or worsen autoimmune disease (35-37). Since the levels of the parameters mentioned above had already changed in patients

before the initiation of any therapy in other studies (38, 39), the assumption of an autoimmune reaction induced by chemo- or endocrine therapy which might induce a change in thyroid function was less probable in this case.

Other limitations of this study were its retrospective nature and the small number of patients included. Despite these limitations, some interesting tendencies became apparent which should now be examined further in a prospective approach with greater patient numbers and a set time for blood tests before the start of any therapy.

We showed an elevated risk for patients with *BRCA1* mutation to be diagnosed with BC during pregnancy. No difference in survival in mutation carriers who were diagnosed with BC in pregnancy or shortly thereafter has been demonstrated in the literature.

Overall, we did not demonstrate any significant differences concerning the thyroid hormones, but we showed a tendency for vitamin D to be elevated in the group with BRCA2 mutation, and this might reach significant levels in a larger cohort. Therefore, these findings should now be investigated in a larger group of *BRCA* mutation carriers. Further studies are planned in order to help correlate these laboratory blood test findings with the expression of thyroid and vitamin D receptors.

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