Investigation of the *IL7Ra* Gene Polymorphism rs6897932 and the Expression Levels of the *CDH1*, *TTPAL*, and *FHIT* Genes in Patients with Breast Cancer

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**Objective:** The aim of this study was to determine the expression levels of *CDH1*, *FHIT*, and *TTPAL* genes and to determine the genotype and allele frequencies of the *IL7Ra* gene polymorphism rs6897932 in patients with breast cancer.

**Methods:** The expression levels of genes and the distribution of the *IL7Ra* gene polymorphism rs6897932 were analyzed by real-time polymerase chain reaction.

**Results:** No differences in genotype ratios or allele frequencies were observed between the 2 groups for the *IL7Ra* gene polymorphism rs6897932. The frequency of the *IL7Ra* rs6897932 T risk allele was found to be similar between breast cancer patients and controls. *CDH1* messenger RNA (mRNA) levels decreased (0.714-fold and 0.834-fold, respectively), and *TTPAL* mRNA levels increased (2.675-fold [P < .05] and 1.169-fold, respectively) in tumor tissues and peripheral blood samples. *FHIT* mRNA levels decreased (0.559-fold) in tumor tissue samples and increased (2.21-fold) in peripheral blood samples.

**Conclusion:** Our results are compatible with those reported in the literature. It can be suggested that the upregulation observed in the *TTPAL* gene might be a marker for breast cancer. The downregulation of *CDH1* and *FHIT* gene expression has been validated in our study. An increase in the copy numbers of *FHIT* mRNA in blood samples and a decrease in the tumor samples can also be considered an abnormal condition. [P R Health Sci J 2023;42(4):283-290]

**Key words:** *CDH1*, *FHIT*, *TTPAL*, *IL7Ra*, rs6897932, Breast cancer

Breast cancer was responsible for one-quarter of the cancers seen in women in the past and took third place after lung and colorectal cancers in cancer-related deaths (1). According to the latest data, it has become the most common cancer in women today and has assumed first place in that population in terms of cancer-related deaths (2). Today, with the contributions of developments in the molecular field, breast cancer is considered a heterogeneous disease, having differences in morphological structure, biological behavior, and response to treatment. A new molecular classification was developed for breast cancer by extracting gene expression profiles from tumor tissue samples.

Interleukins (IL) are key regulators of the immune response. Genetic variations in IL genes may affect breast cancer risk and mortality, given the roles of such genes in the regulation of cell growth, angiogenesis, and the inflammatory process (3). Interleukin-7 plays an important role in the development and maintenance of lymphoid cells. By activating the JAK1/3-STAT5 and PI3K/AKT signaling pathways, IL-7 stimulates the growth (in culture) of tumor cells in breast cancer. In addition, poor prognoses have been associated with the expression of IL-7 signaling components. A polymorphism in exon 6 of the *IL7Ra* (IL-7 receptor alpha) gene (rs6897932; Thr244Ile) shifts the balance between membrane-bound and soluble *IL7Ra* variants (4).

The epithelial-mesenchymal transition (EMT) process is associated with the decreased expression of epithelial markers such as e-cadherin (*CDH1*) (5). The loss of *CDH1* during the EMT procedure leads to the destruction of cell–cell adhesion, increased cell motility, and advanced stages of cancer (6). A member of a family of homophilic transmembrane glycoproteins, *CDH1*, which is expressed in almost all epithelial tissues, promotes calcium-dependent cell–cell adhesion. This protein is also significant in terms of cell signaling as well as conserving...
normal tissue morphology, directing cellular differentiation, and establishing and maintaining cellular polarity (7, 8, 9). Some studies have reported that the dysfunction of CDH1 is caused by allelic deletions and mutations (10, 11). In addition, CDH1 inactivation leads to the activation of β-catenin transcription (12); its constitutively activated form has been found in several types of human cancer, and up to 80% of tumors in colon cancer exhibit nuclear β-catenin accumulation (13). Mutations in CDH1 have been linked not only to breast cancer but also to ovarian, gastric, colorectal, and thyroid cancer. Further, when this gene ceases functioning, proliferation, invasion, and metastasis can increase, contributing to cancer progression (14). A P1-P3-bis(5'-adenosyl) triphosphate hydrolase, the protein encoded by FHIT, is involved in purine metabolism. Additionally, FRA3B (on chromosome 3), a common fragile region, is included in FHIT; it is a frequent site of carcinogen-induced damage and has a role in both chromosomal translocation and the generation of aberrant transcripts. Genomic changes within the FHIT gene have been reported to occur in a variety of human cancers, including, lung, colon, breast, esophageal, head and neck, and stomach (15–18). Replication stress and DNA damage result from the loss of the encoded protein’s activity, which is to act as a tumor suppressor (19).

It has been reported that high TTPAL (tocopherol [alpha] transfer protein-like) messenger RNA (mRNA) expression is significantly associated with low survival rates in patients with multiple cancer types (20). In 2014, Tuupanen et al. described TTPAL as a novel gene exhibiting hot spot mutations in validation set samples (21). It is likely that TTPAL plays a role in the invasion and metastasis of colorectal cancer (22). In addition, after having performed genome-wide copy-number analysis in primary colorectal tumor tissues, Gou et al. (23) reported that TTPAL was the most amplified gene in colorectal cancer. The copy-number gain of TTPAL has been shown to lead to gene overexpression in colorectal cancer.

The aim of this study was to determine the expression levels of CDH1, FHIT, and TTPAL genes in the tumor tissues and blood samples of breast cancer patients. A second aim was to determine the genotype and allele frequencies of the IL7Ra gene polymorphism rs6897932 in patients with breast cancer.

Methods

Sampling

Twenty-five breast cancer patients and 25 healthy control samples were included in our study. Genotype analysis was performed with DNA from blood samples from all 50 samples. Of the 25 controls, 5 were selected as controls for the gene expression analysis (Tissue samples were obtained from women suspected of having breast cancer. We confirmed via histopathology that the samples from the 5 cases were cancer free). Tumor tissues were obtained during breast operations performed at Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of General Surgery, from June 2018 though June 2020. This study was approved by the Ethics Committee of Afyonkarahisar Health Sciences University (September 11, 2020/420), and all the patients provided informed consent.

DNA extractions from peripheral blood samples and Sanger sequencing analysis

Genomic DNA was extracted from peripheral blood using the relevant DNA isolation procedures (Invitrogen PureLink Genomic DNA Mini Kit, Cat. No. K182002, USA), and the amount and purity were determined with the Promega Quantifluor E6090 (Promega, Madison, WI, USA) and stored at -20 °C until use.

Genotyping analysis of the IL7Ra gene polymorphism rs6897932

The genotyping study of the IL7Ra gene polymorphism rs6897932 was performed using the Applied Biosystems 3130XL Genetic Analyzer (USA). We used the following protocol: 95 °C for 3 minutes (initial denaturation), followed by 35 cycles at 95 °C for 30 seconds, 60 °C for 30 seconds, 72 °C for 30 seconds, and 72 °C for 10 minutes and hold at 4 °C. MyTaq™ HS DNA Polymerase (Bioline, Meridian Bioscience, TN, USA) was used in the reaction mixture, and the relevant primer were designed by Sentebiolab (Ankara, Turkey).

RNA extraction and real-time PCR analysis

The EZ-RNA Total RNA extraction kit (Biological Industries, Cat. No. 20-400-100, Israel) was used for extracting RNA from tissues and peripheral blood samples (25 breast cancer patients and 5 controls). The NanoDrop ND-1000 Spectrophotometer V3.7 was used for determining RNA amounts and purity. Complementary DNA was obtained from 1 µg of total RNA using iScript Reverse Transcription Supermix (Bio-Rad, USA, Cat. No. 170884). The expression levels of CDH1, FHIT, and TTPAL genes were analysed by Rotor-Gene Q (QIAGEN, Hilden, Germany). The reaction mix was prepared with Taq Universal SYBR Green Supermix (Bio-Rad, USA, Cat. No. 1725122) and oligonucleotide primers (CDH1, TTPAL, FHIT, and TBP designed by Genometry Biotechnology, Izmir, Turkey; and GAPDH by Oligomere Biotechnology, Ankara, Turkey). Primers were designed using the following sequences:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDH1</td>
<td>5'-CCCTTCCTCAAAACACACTCC-3'</td>
<td>5'-TGCGGAGCTGTGTCCTCAGAACATC-3'</td>
</tr>
<tr>
<td>FHIT</td>
<td>5'-GGGAGCTTCTGCTTCTTCTGAGA-3'</td>
<td>5'-GCGGTGCTCTCACAACAGTGTCCTGCCA-3'</td>
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<tr>
<td>TTPAL</td>
<td>5'-CCACTCCATCTCCTCAATCAAC-3'</td>
<td>5'-CTCCACAGACCTTCACCTTACC-3'</td>
</tr>
<tr>
<td>TBP</td>
<td>5'-CTTATCCCACACTCAGTCTGTCAGCC-3'</td>
<td>5'-GTCTTCTCCTTCCTTCTTATCC-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-CATGGCCCTCAAGGACACCCTT-3'</td>
<td>5'-GGTTGGGCTCAGGGTCTTACTCC-3'</td>
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</table>

We used the following real-time polymerase chain reaction (PCR) protocol for CDH1 and GAPDH: 95 °C for 30 seconds
(initial denaturation), followed by 40 cycles at 95 °C for 5 seconds and at 60 °C for 30 seconds; for FHIT, TTPAL, and TBP, it was 95 °C for 3 minutes (initial denaturation), followed by 35 cycles at 95 °C for 5 seconds, 58 °C for 10 seconds, and 72 °C for 20 seconds. To confirm single-product amplification, a melting curve analysis was performed at the end of the PCR, with the temperature gradually increasing (in 0.5 °C increments) from 65 °C to 95 °C, at 5 seconds/step. Each run was performed in triplicate.

Statistical analysis
The REST 2009 (v2.0.13) and IBM SPSS Statistics for Windows, 19.0, software programs (24) were used for assessing the relative expression levels. The allele and genotype frequencies of the studied gene polymorphism were analyzed with the chi-square test. A \( P \)-value lower than .05 was considered significant.

Results

Cases
Information on prognostic parameters such as stage, histological subtype, and immunohistochemical estrogen and progesterone receptor (ER, PR) and HER2 staining results were obtained from patient pathology reports. Table 1 shows the surgical stage, histological grade, genotype status, and immunohistochemical status of all the breast cancer patients.

Genotype data
The genotype ratios and allele frequencies of the \( IL7Ra \) gene polymorphism rs6897932 were analyzed in genomic DNA isolated from the peripheral blood of 25 breast cancer patients and 25 healthy controls. The \( IL7Ra \) gene polymorphism rs6897932 exists in humans in 3 different genotypes: CC (wild type), CT (heterozygous), and TT (mutant) (Figure 1A, B, & C, respectively). The T risk allele sometimes appears as an A base. However, there were no cases in our study with the CA or AA genotype. The partial sequence in which the rs6897932 polymorphism exists is as follows:

\[
\text{AATGCAAAGCACCCTGAGACCCTACCCCCACTGCAT} \\
\text{GGCTACTTGATGTCACCCAATCTATTTGCTTT} \\
\text{CCAGGGGAGATGGGATCCTATCTTACTAA} [\text{C/A/T}] \text{CA} \\
\text{TCAGCAATTTGTGACTTTTTTCTCTGTCGCTGTT} \\
\text{GTCACTCCTGGCCCTGTGTTATGGAAAAAGGTG} \\
\text{ACCTTTCTTCAACTAATAAAGGGGTGAT}
\]

Genotype ratios of \( IL7Ra \) gene polymorphism rs6897932
In the control group, the number of cases with the CC genotype was 15 (57.76%), the number of cases with the CT genotype was 8 (36.48%), and the number of cases with the TT genotype was 2 (5.76%). In the breast cancer patient group, the number of cases with the CC genotype was 16 (64%), the number of cases with the CT genotype was 8 (32%), and the number of cases with the TT genotype was 1 (4%) (Table 1). When the patient and control groups were compared statistically using the \( \chi^2 \) test, no difference was observed in terms of genotype ratios (\( P = .8329 \)).

Allele frequencies of the \( IL7Ra \) gene polymorphism rs6897932
In the control group, the frequency of the C allele was 38 (76%) and the frequency of the T allele was 12 (24%); in the breast cancer patient group, the frequency of the C allele was 40 (80%) and the frequency of the T allele was 10 (20%). The C and T allele frequencies of the \( IL7Ra \) gene polymorphism rs6897932 were analyzed using the \( \chi^2 \) test. There was no difference between the allelic frequencies of the 2 groups (\( P = .6315; 95\% \text{ CI: } 0.3063−2.0449 \)).

mRNA analysis of \( CDH1, TTPAL, \) and \( FHIT \) genes expressed in tumor tissues and peripheral blood of breast cancer patients
Alterations in mRNA levels of \( CDH1, TTPAL, \) and \( FHIT \) genes expressed in tumor tissues and the peripheral blood of patients with breast cancer were determined compared to the pertinent controls. The mRNA levels of the \( CDH1 \) gene were downregulated in tumor tissues and peripheral blood samples (0.714-fold and 0.834-fold, respectively) (Figure 2; fold changes are shown on a Log10 scale). The mRNA levels

<table>
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<th>Surgical stage</th>
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<th>Genotype</th>
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<td>2</td>
<td>II A</td>
<td>I</td>
<td>CT</td>
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<td>8</td>
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<td>CC</td>
<td>6</td>
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<td>I</td>
<td>CC</td>
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</table>

Table 1. Clinical data and genotype distribution of the breast cancer patients

Abbreviations: ER, estrogen receptor; PR, progesterone receptor
Gene Expressions in Breast Cancer

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The mRNA levels of the FHIT gene were downregulated in tumor tissue samples (0.559-fold) and upregulated in peripheral blood samples (2.21-fold) (Figure 4). Discussion

As stated above, interleukins are key regulators of the immune response. Considering the role of genetic variation in IL genes on the regulation of cell growth, angiogenesis, and the inflammatory process, it has been suggested that such variation may affect breast cancer risk and mortality (3). It has been shown that IL7Ra is critical for the IL-7 response for certain stages in shaping T cell development, thymus maturation, and survival (25, 26, 27). IL7Ra is a cytokine receptor whose expression is regulated during the development and lifetime of the lymphoid. Balasubramanian et al. (28) found a weak association in their meta-analysis of IL6 rs1800795, whereas Yu et al. (29) observed no such relationship in their study. In another study, this one conducted, a very low correlation was observed for IL6 rs2069861 (30). It has been suggested that most of the interactions exist between proinflammatory cytokines to change the risk and that IL genes work together in the carcinogenic process (3). Vitiello et al. (4) reported that a polymorphism in exon 6 of the IL7Ra gene (rs6897932; Thr244Ile) shifts the balance between membrane-bound and soluble IL7Ra variants (4). This team indicated that “IL7Ra is involved in breast cancer, and that [the] IL7RA polymorphism may play distinct roles in breast carcinogenesis according to breast cancer subtype, pointing to this genetic variant as an interesting marker for breast carcinogenesis.” But in our study, when the genotype ratios of IL7Ra gene polymorphism rs6897932 were examined in 25 breast cancer patients and 25 healthy controls, no significant differences were observed in terms of wild-type, heterozygous, or mutant genotype ratios. There was no difference between patients and controls in terms of the T risk allele. Also there were 3 triple-negative (ER[-], PR[-], and HER2[-]) patients; 2 of them had the CC genotype and just 1 the CT. However, the number of patients and controls in this study was very low. With the results we obtained here, we cannot comment on the relationship between IL7Ra gene polymorphism rs6897932 and breast cancer. Although this polymorphism has been associated with autoimmune diseases, studies of cancer including breast cancer are rare.

The CDH1 gene expresses the e-cadherin protein. E-cadherin, one of the most important intercellular epithelial cell-adhesion proteins, regulates epithelial cell–cell interaction through the calcium-dependent homophilic interaction of its extracellular domain; the reduction or loss of e-cadherin protein has been reported in invasive cancer cells and the primary sites of cancerous cells (31, 32). The reduction or loss of CDH1 has been proposed as a biomarker for colorectal cancer (33). Decreased CDH1 expression levels have been associated with advanced and poorly differentiated cancers (34).
In our study, \textit{CDH1} gene expression was found to be decreased in both the blood and tissues of breast cancer patients compared to control tissues. Tamura et al. \cite{35} examined \textit{CDH1} protein expression and found a loss or significant decrease in its expression in 12 gastric carcinomas. Additionally, there were downregulations in 51 tumor tissues of colorectal cancer patients in our former study \cite{36}.

In this study, the mRNA levels of the \textit{TTPAL} gene were found to be increased in tissue and blood samples. Specifically, this upregulation is statistically significant in tumor tissues of breast cancer patients. No study was found on \textit{TTPAL} gene expression and breast cancer in the literature review. The findings of a number of studies support the notion that the upregulation of the \textit{TTPAL} gene, as observed in breast cancer, may also be observed in other forms of cancer. Gou et al.'s report \cite{23} that the \textit{TTPAL} expression was detected in some cancer cells (at both mRNA and protein levels) mirrors our own. This team also observed significantly upregulated \textit{TTPAL} mRNA expression in primary colorectal cancer tumors compared to adjacent normal tissues. Moreover, Liu et al. \cite{37} revealed that \textit{TTPAL} expression was significantly increased in gastric cancer tissues compared to non-tumor tissues. In addition, Liu and team observed that \textit{TTPAL} expression levels were higher in the members of their sample group having increased DNA copy numbers, leading to the determination that DNA copy number was positively correlated with \textit{TTPAL} expression level. An analysis (from The Cancer Genome Atlas studies) of \textit{TTPAL} copy number in cancers showed that \textit{TTPAL} is amplified preferentially and more frequently in colorectal cancers than in other cancer types, suggesting that the gene plays a specific role in colorectal cancer \cite{23}. Additionally, in our former study, mRNA levels of the \textit{TTPAL} gene increased in tissues at all surgical stages (I–IV) of colorectal cancer \cite{36}. Whether \textit{TTPAL} gene expression plays a role that is in addition to those played by the major factors in the formation of breast cancer or whether it is a consequence of the formation of breast cancer should be clarified with more extensive studies.

The \textit{FHIT} gene is a putative tumor suppressor gene thought to be involved in the carcinogenesis of breast cancer. The loss of \textit{FHIT} expression has been observed in up to 72% of breast cancers and has been associated with increased p53, a high proliferation index, and increased tumor size and grade \cite{38}. Approximately half of all cancers of the colon, stomach, and esophagus possess abnormal \textit{FHIT} gene transcripts, and in many different types of cancer, this particular gene is reported to be inactive. Moreover, the loss of the activity of the encoded protein results in DNA damage and replication stress, with this inactivation seeming to occur at later stages of cancer progression, especially to neoplasms of a more aggressive nature \cite{18}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{CDH1.png}
\caption{Alterations of \textit{CDH1} gene expression in tumor tissues and peripheral blood samples of breast cancer patients compared to controls. \textit{GAPDH} was used as the reference gene for normalization.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{TTPAL.png}
\caption{Alterations of \textit{TTPAL} gene expression in tumor tissues and peripheral blood samples of breast cancer patients compared to controls. \textit{TBP} was used as the reference gene for normalization (*P < .05).}
\end{figure}
In the past decades, many studies have evaluated changes in the FHIT gene in breast cancer. The loss of heterozygosity in the FHIT gene is one of those changes (39–41). The deletion of the FHIT gene has also been observed in preneoplastic lesions and has been suggested to occur early in breast carcinogenesis (40). Altered transcription is often caused by deletions in FHIT, and point mutations are extremely rare (42). One of the mechanisms by which the loss of expression may occur in breast cancer is hypermethylation of FHIT (43). Although altered FHIT transcripts have been reported in 20% to 38% of primary breast carcinomas (18, 44, 45), the reduction or absence of FHIT protein has been reported in up to 72% of breast carcinoma samples (44). The precise clinicopathological significance of the loss of FHIT expression in breast cancer is unknown; however, several studies have shown that it may be associated with poor clinical outcomes (44, 46–48). In our study, FHIT gene expression was found to be downregulated in the tumor tissues of patients with breast cancer (0.559-fold decrease compared to what was seen in the controls). While the results we obtained directly from tumor tissue were in line with this, the situation in blood was the opposite: a 2,210-fold increase compared to what was seen in the controls. Additionally, we reported that the mRNA levels of the FHIT gene increased in tumor tissues and decreased in blood samples from colon cancer patients in our previous study (36). Thiagalingam et al. (49) reported very high mRNA levels of the FHIT gene in cell lines, but Kapitanovic et al. (50) reported finding significantly decreased mRNA levels in colon tumors.

These results perhaps support the suggestion that FHIT inactivation appears to be a relatively later event in cancer development and is probably associated with progression to more aggressive neoplasms (18). The inactivation observed directly in the cells of the tumor tissue may not have occurred yet in the cells in the blood, and to the contrary, the increase in copy number can be considered an abnormal condition. In addition, the relationship between FHIT expression and other potential prognostic markers in breast cancer is unknown.

It was determined that CDH1 gene expression decreased in tumor tissue and blood samples, TTPAL gene expression increased in tumor tissue and blood samples, and FHIT gene expression decreased in tumor tissues, only. These results were found to be in agreement with those of the literature. In addition, in this study, it was determined that FHIT gene expression increased in blood samples. Several studies have noted that the upregulation of the TTPAL gene might be a marker for breast cancer. Whether, in addition to the major factors, TTPAL gene expression plays a role in the formation of breast cancer or whether it is a consequence of the formation of breast cancer should be clarified with more extensive studies. In addition, the downregulation of CDH1 and FHIT gene expressions has been reported in many studies in the literature and has been validated in ours. A simultaneous increase in the copy numbers of FHIT mRNA in blood samples and a decrease of same in tumors can also be considered to be an abnormal condition. The frequency of the IL7Ra rs6897932 polymorphism T risk allele was found to be similar between breast cancer patients and controls. Although this polymorphism has been associated with autoimmune diseases, studies of cancer, including breast cancer, are rare.

**Resumen**

Objetivo: El objetivo de este estudio fue determinar los niveles de expresión de los genes CDH1, FHIT, TTPAL y determinar las frecuencias genotípicas y alélicas del polimorfismo rs6897932 del gen IL7Ra en pacientes con cáncer de mama. Métodos: Se analizaron los niveles de expresión de los genes y la distribución del gen IL7Ra rs6897932 mediante el método de reacción en cadena de la polimerasa en tiempo real. Resultados: No se observaron diferencias en las proporciones de genotipos y las frecuencias alélicas entre los dos grupos para el polimorfismo rs6897932 del gen IL7Ra. Se observó que la frecuencia del alelo de riesgo IL7Ra rs6897932 T era similar entre los pacientes con cáncer de mama y los controles. El nivel de mRNA de CDH1 disminuyó (0.714 veces y 0.834 veces, respectivamente) y el nivel de mRNA de TTPAL aumentó [2.675 veces (P<.05) y 1.169 veces, respectivamente] en tejidos tumorales y muestras de sangre periférica. El nivel de mRNA de FHIT disminuyó...
(0.559 veces) en las muestras de tejido tumoral y aumentó (2.21 veces) en las muestras de sangre periférica. Conclusión: Nuestros resultados son compatibles con la literatura. Se puede sugerir que la regulación positiva observada en el gen TTPAL también puede ser un marcador en el cáncer de mama. La regulación a la baja de las expresiones génicas de CDH1 y FHIT ha sido validada en nuestro estudio. Un aumento del número de copias del mRNA de FHIT en las muestras de sangre y una disminución en las muestras tumorales también puede considerarse una condición anormal.

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