Published in final edited form as:

Clin Cancer Res. 2009 March 1; 15(5): 1770–1778. doi:10.1158/1078-0432.CCR-08-1030.

Predicting Relapse in Favorable Histology Wilms Tumor Using Gene Expression Analysis: A Report from the Renal Tumor Committee of the Children's Oncology Group

Chiang-Ching Huang¹, Samantha Gadd², Norman Breslow³, Colleen Cutcliffe², Simone T. Sredni², Irene B. Helenowski¹, Jeffrey S. Dome⁴, Paul E. Grundy⁵, Daniel M. Green⁶, Michael K. Fritsch⁷, and Elizabeth J. Perlman²

¹Department of Preventive Medicine, Northwestern University's Feinberg School of Medicine and Robert H. Lurie Cancer Center, Chicago, Illinois

²Department of Pathology, Northwestern University's Feinberg School of Medicine and Robert H. Lurie Cancer Center, Chicago, Illinois

³Department of Biostatistics, University of Washington and Fred Hutchinson Cancer Research Center, Seattle, Washington

⁴Division of Oncology, Children's National Medical Center, Washington, District of Columbia

⁵Departments of Pediatrics and Oncology, Cross Cancer Institute and University of Alberta, Edmonton, Alberta, Canada

⁶Department of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, Memphis, Tennessee

⁷Department of Pathology, University of Wisconsin-Madison, Madison, Wisconsin

Abstract

Purpose—The past two decades has seen significant improvement in the overall survival of patients with favorable histology Wilms tumor (FHWT); however, this progress has reached a plateau. Further improvements may rely on the ability to better stratify patients by risk of relapse. This study determines the feasibility and potential clinical utility of classifiers of relapse based on global gene expression analysis.

Experimental Design—Two hundred fifty FHWT of all stages enriched for relapses treated on National Wilms Tumor Study-5 passed quality variables and were suitable for analysis using oligonucleotide arrays. Relapse risk stratification used support vector machine; 2- and 10-fold cross-validations were applied.

Results—The number of genes associated with relapse was less than that predicted by chance alone for 106 patients (32 relapses) with stages I and II FHWT treated with chemotherapy, and no further analyses were done. This number was greater than expected by chance for 76 local stage III patients. Cross-validation including an additional 68 local stage III patients (total 144 patients, 53 relapses) showed that classifiers for relapse composed of 50 genes were associated with a median sensitivity of 47% and specificity of 70%.

Requests for reprints: Elizabeth J. Perlman, Children's Memorial Hospital, 2300 Children's Plaza, Box 17, Chicago, IL 60614. EPerlman@childrensmemorial.org.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Disclosure of Potential Conflicts of Interest: No potential conflicts of interest were disclosed.

Conclusions—This study shows the feasibility and modest accuracy of stratifying local stage III FHWT using a classifier of <50 genes. Validation using an independent patient population is needed. Analysis of genes differentially expressed in relapse patients revealed apoptosis, Wnt signaling, insulin-like growth factor pathway, and epigenetic modification to be mechanisms important in relapse. Potential therapeutic targets include FRAP/MTOR and CD40.

Wilms tumor is the most common urogenital malignancy in children, with ~500 new cases per year in North America. Several national and international cooperative group clinical trials have optimized the therapy resulting in an increase in the overall survival rate to ~90%. The current therapeutic approach for Wilms tumor is based on histologic subtype (favorable versus unfavorable histology) and tumor stage (1). The majority of Wilms tumor has favorable histology, defined as the absence of anaplasia, and these represent the focus of the current study. Patients with anaplasia are treated differently than those with favorable histology Wilms tumor (FHWT) and are beyond the scope of this study.

In recent years, the improvement in relapse-free and overall survival for FHWT at each stage has reached a plateau. Some patients are not successfully treated initially, resulting in relapse and less frequently death. Of equal importance, many patients may receive more therapy than needed; this is particularly true for patients with stage III disease (2,3). Further improvements in outcome will rely in part on the ability to identify markers associated with relapse, with the hope of better stratifying patients. This goal represented a major focus of the National Wilms Tumor Study-5 clinical protocol, which included a large-scale effort aimed at tumor banking and molecular analysis. These efforts showed that loss of heterozygosity (LOH) for both chromosomes 1p and 16q was associated with poor outcome (4). However, LOH is able to detect only a very small subset of FHWT patients who have an increased risk of relapse and death. Additional efforts are therefore required to further define markers of relapse. In this study, we analyzed gene expression patterns to identify such markers and to investigate the feasibility of developing classifiers able to predict patients at high risk for relapse.

Translational Relevance

This article evaluates gene expression signatures to predict relapse in patients registered on the National Wilms Tumor Study-5 cooperative group protocol using stage and treatment-specific analyses. This will enable independent validation using samples from patients registered in the ongoing Children's Oncology Group protocols. Successful signatures will be able to be used for therapeutic stratification during protocols estimated to open in 2012. Signatures with 50 genes were associated with relapse in stage III tumors (sensitivity of 47% and specificity of 70%). Existing markers for relapse currently used for stratification (1p and 16q loss of heterozygosity) have a sensitivity of 8% and specificity of 96%. Analysis of specific genes associated with relapse revealed apoptosis, Wnt signaling, and the insulin-like growth factor pathway to be important. These pathways will be separately validated at the protein level within the current protocol. Importantly, all the above-identified pathways have been previously targeted for developmental therapies in the current literature. Two additional potential therapeutic targets identified in this study were FRAP/MTOR and CD40. MTOR inhibitors and anti-CD40 antibodies are currently used in clinical trials. The Children's Oncology Group Renal Tumor Committee has an active Developmental Therapeutics Subcommittee that is able to bring such promising agents forward.

Materials and Methods

Patient samples

On June 25, 2003, all 1,451 patients with FHWT registered on National Wilms Tumor Study-5 from August 1995 to June 2002 who had available pretreatment tumor tissues were identified. Six hundred patients, consisting of all those known to have relapsed and a random sample including ~30% of the remainder, were randomly divided into two groups of 300 patients each (groups A and B), both enriched for relapse.

The National Wilms Tumor Study-5 protocol was approved by the review boards of institutions that registered patients. Parents or guardians signed informed consent for collection and banking of biological samples. Histologic diagnosis and local stage were confirmed by central review. Patients who did not receive the therapy consistent with the diagnosis and stage found on central review were rejected from the analysis. Chest X-ray, abdominal ultrasound, and computed tomography studies were required for staging. Tumor samples were obtained at initial diagnosis before the initiation of therapy and were snap frozen. A frozen section was evaluated to confirm at least 80% viable tumor cellularity.

Gene expression analysis

RNA was extracted and hybridized to Affymetrix U133A arrays, scanned, and subjected to quality-control variables according to the previously described protocol (5).

Quantitative reverse transcription-PCR

RNA levels of five genes showing a range of fold changes and overall expression levels were further analyzed by quantitative reverse transcription-PCR using TaqMan Gold and the ABI Prism 7700 Sequence Detection System (Applied Biosystems). β -Actin was used as the endogenous control. Primer and probe sequences are provided in Supplementary Table S1.⁸ Each threshold cycle (C_T) was determined and the C_T for β -actin was subtracted from this for normalization.

Data analysis

Positional-dependent nearest-neighbor model software⁹ was used to translate the scanned images into expression analysis files and to normalize the data across all arrays (6). Genes with maximum expression less than a log scale of 6 across all tumors and Affymetrix control genes were excluded, resulting in 20,931 probe sets for analysis. Support vector machine was chosen for relapse risk stratification (7). The following procedure was used to construct 150 different classifiers for each randomly drawn training set of tumors. For each gene, the t statistic comparing expression between cases and controls and the associated P value were calculated using the Welch method. The K genes (1-150 genes) with the smallest P values were selected to construct a n support vector machine model as developed by Chang and Lin¹⁰ and implemented in a R software package, e1071 (7). This model was then applied to categorize tumors within the complementary test set as having high or low risk for relapse.

Validation procedures

Cross-validations (2- and 10-fold) were used to investigate the ability of classifiers established in a training set to predict relapse in an independent test set. For 2-fold cross-validation, the data set was randomly divided 500 times into training and corresponding test sets of equal size,

⁸Supplementary tables can be found at <http://www.childrensmrc.org/perلمان/>.

⁹<http://odin.mdacc.tmc.edu/~zhangli/PerfectMatch/>

¹⁰<http://www.csie.ntu.edu.tw/~cjlin/libsvm>

each including half the patients who relapsed. A classifier for relapse was identified for each training set and used to assign tumors in the corresponding test set to low-risk and high-risk categories. The training and test sets were then swapped. The number of top K genes in each classifier evaluated ranged from 1 to 150. Therefore, 150,000 different classifiers were developed, one for each value of K from 1 to 150 for each of the 1,000 (500×2) training sets. For 10-fold cross-validation, the data set was randomly divided 500 times into 10 groups of approximately equal size. Each group included approximately the same number of relapses. For each such group, a classifier was built with the remaining 9 of 10 of the samples and then used to categorize tumors in the group as low or high risk; the process was repeated until all tumor samples were categorized as low or high risk. For all the cross-validation procedures, to avoid gene-selection bias, classifiers were completely rebuilt in each cross-validation iteration (8).

Results

Patient information and quality control

Group A—Ninety of the 300 tumors from group A were rejected. Following verification of the stage, relapse status, clinical follow-up, and diagnosis, 1 tumor (with diffuse hyperplastic perilobar nephroblastomatosis) was excluded for diagnosis, 14 tumors with clinical follow-up <3 years were excluded, 29 tumors were rejected due to <80% viable tumor cellularity, 34 tumors were rejected due to A_{260}/A_{280} ratios < 1.8, and 12 tumors failed to meet Affymetrix-recommended hybridization quality-control standards. Illustrated in Table 1 is the stage and relapse status of group A. The tumors were categorized based on local (abdominal) and overall stage. Stage I tumors (49) were confined to the kidney. Stage II tumors (73) showed infiltration beyond the kidney with negative surgical margins and regional lymph nodes; 6 of these patients had distant metastases (local stage II, overall stage IV). Of 76 local stage III patients, 12 presented with distant metastasis (local stage III, overall stage IV). Six patients presented with bilateral disease (stage V) and 6 unilateral tumors were biopsied and treated before the subsequent removal of the kidney following therapy (“biopsy only”).

This study grouped together for analysis patients who received the same therapy. First, there were 10 stage I patients (3 relapses) registered on a treatment arm within National Wilms Tumor Study-5 that included patients ages ≤ 24 months with stage I disease whose kidney and tumor had a combined weight of ≤ 550 g; these patients were treated without initial chemotherapy as reported previously (9). These patients are not further considered in this particular study due to their small numbers. The remaining patients with stage I and those with overall stage II disease were treated with nephrectomy, vincristine, and dactinomycin (106 patients, 32 relapses); they formed a second group referred to below as stage I/II tumors. The third group, referred to collectively as stage III tumors, includes those patients with local and overall stage III disease (64 patients) and those with local stage III, overall stage IV disease (12 patients). Careful consideration was given to the inclusion of patients with distant metastasis (stage IV) to this group due to their potential biological differences. Because the goal of the study was to define clinically applicable signatures, it was considered to be optimal to group all patients receiving the same therapy. Patients with stages III/III and III/IV disease both receive nephrectomy, vincristine, actinomycin, doxorubicin, and abdominal radiation therapy. In addition, both stages III and IV tumors have known or presumed residual disease, and one of the most significant causes for relapse is resistance to chemotherapy, which does not change with stage. To ensure that the stage IV patients did not significantly alter the results, all analyses of the stage III tumors were also performed, leaving the patients with overall stage IV disease out. Six patients with local stage II, overall stage IV disease were excluded from the stage I/II group because they received additional adjuvant therapy; they were also excluded from the stage III group because they did not receive abdominal radiation. Six tumors biopsied and

treated before removal of the kidney were not included nor were 6 patients with bilateral disease (stage V) due to the individualized nature of their therapy. In total, gene expression analysis from 182 tumors from group A was used in these analyses.

Group B—The only patients analyzed from group B were the 99 appropriately diagnosed and treated stages III/III and III/IV tumors as defined above. The same quality-control variables outlined above were applied. Nine tumors were rejected following histology review, 5 had <3 years of follow-up, 8 had poor RNA quality, and 9 failed to meet quality-control variables following hybridization, leaving 68 tumors for analysis. Clinical stage and outcome for all 144 stage III tumors within the entire group of 600 tumors are provided in Table 2.

Classifiers that identify relapse risk: initial feasibility assessment

Group A was used to assess the feasibility of developing classifiers that predict risk of relapse within the above groups. To accomplish this, the expression of each probe set in the tumors that relapsed was compared with the tumors that did not relapse using the *t* test, and a nominal *P* value was established for this association. The distribution of *P* values within the stage I/II tumors failed to show more probe sets with $P < 0.05$ than would be expected by chance alone, indicating that further investigation would be associated with high false discovery rates. Similar results were found with the separate analysis of stage II tumors alone. The distribution of *P* values for stage III tumors, however, showed a higher frequency of probe sets at low *P* values than would be expected by chance alone (1,445 probe sets with $P < 0.05$). This analysis was repeated excluding stage IV patients with similar results. This indicates that a gene signature associated with relapse may exist. The remainder of the study therefore focused on the stage III tumors.

Experimental verification

Five genes with *P* values < 0.05 were analyzed using quantitative reverse transcription-PCR. These included *COL2A1*, *DNMT1*, *ENPP2*, *GCNT*, and *LY6G6D*. In all cases, the gene expression data from array analysis and from quantitative reverse transcription-PCR were comparable (data not shown). Replicate analysis beginning with RNA extraction done on a subgroup of 10 tumors confirmed reproducibility of the expression patterns.

Developing and validating classifiers that predict relapse in stage III FHW

The original design was to identify classifiers based on the association of gene expression with time to relapse using the 300 tumors in group A and to then apply these classifiers in a blinded fashion to tumors in group B. Following this design, a classifier was developed from stage III tumors in group A using the association between gene expression and time to relapse to select the top genes. This classifier was applied to stage III tumors within group B in a blinded fashion. Six of 10 tumors placed in the high-risk category relapsed and 23 of 63 tumors placed in the low-risk category relapsed, resulting in an odds ratio measure of association of 2.6 ($P = 0.18$, Fisher's exact test). Due to concerns regarding the small number of relapses and the use of time to relapse in addition to fact of relapse to select genes, we also analyzed the results using a case-control design including all stage III patients. Three years had elapsed during the laboratory analysis, allowing for extended follow-up for the 600 patients. Lastly, it is increasingly recognized that small sample sizes may hinder the reliability of gene classifiers, particularly those for which outcome prediction is the goal (10). Therefore, for the remaining analyses, a case-control design was applied. All stage III patients within both groups A and B who were known to have relapsed as of February 2007 were defined as cases; those who had not relapsed and who had been followed >3 years were defined as controls. The risk of relapse after 3 years is low, with only 3 of 213 (1.4%) patients relapsing beyond 3 years in National Wilms Tumor Study-5 (4).

The 144 stage III tumors from groups A and B include 53 relapses (cases) and 91 nonrelapses (controls; Table 2). The distribution of P values for all 144 tumors again showed a higher frequency of probe sets at low P values than would be expected by chance alone (2896 probe sets with $P < 0.05$). (When the stage IV tumors were left out, 2,259 probe sets had a $P < 0.05$.) Cross-validations (2- and 10-fold) were repeated 500 times, varying the number of genes in the classifiers from 1 to 150. The specificity, sensitivity, and overall error rate were determined for each classifier and are illustrated in Fig. 1. The error rate improved markedly when the number of genes in the classifier increased from 5 to 50 but only very slowly with increasing number of genes thereafter. With 10-fold cross-validation, when 50 genes were used in the classifier, the median sensitivity was 47% (range, 35-58%), the median specificity was 70% (range, 60-78%), and the median overall error rate was 38% (range, 31-45.8%) in this mix of cases and controls. The 10-fold cross-validation analysis was repeated leaving the stage IV patients out, and the median sensitivity was 35% and the median specificity was 78%.

Performance of classifiers within individual tumors

To evaluate the robustness of the classifiers when applied to individual tumor samples, we observed the 10-fold cross-validation analyses, selecting only the 50-gene classifiers. Every tumor was assessed 500 times for relapse risk using a 50-gene classifier and the success of every classification was tracked for each individual tumor. The percentage of classifiers that categorized each tumor as high risk is illustrated in Fig. 2. Fifteen (28%) relapsed tumors were consistently and correctly classified as high risk using a stringent cut-point requiring $>90\%$ of the classifiers to predict relapse (*top dashed line*). Similarly, 47 (52%) nonrelapsed tumors were consistently and correctly classified as low risk using a cut-point requiring $<10\%$ of the classifiers to predict relapse (*bottom dashed line*). However, a large number of tumors fell in between these cut-points, and 18 (34%) of the tumors that relapsed were consistently but incorrectly placed in the low-risk group even when using the least stringent cut-point of 10%.

Genes associated with relapse in stage III FHWI

Using the 144 stage III tumors, 109 genes were identified with $P < 0.001$ and are listed in Supplementary Table S2¹ (false discovery rate of 11.6%; ref. 11). The entire gene expression data and description of the experiment using the MIAME format are available.¹¹ Of the 20,931 genes analyzed, a total of 1,626 genes were represented in at least 1 of the 5,000 50-gene classifiers used in the 10-fold cross-validation: 1,004 genes were selected <10 times, 579 genes were selected >50 times, 244 genes were selected >500 times, and 55 genes were selected $>1,000$ times. The number of times each of the top 100 genes was used and its ranking within the 5,000 50-gene classifiers is provided within Supplementary Table S2. The P value correlated highly with the number of times each gene was selected.

Among the top 109 genes with $P < 0.001$, approximately one-quarter are known to function in metastasis, tumorigenesis, tumor progression, or tumor suppression; these are listed in Table 3. The chromosomal location of the 109 top genes (provided in Supplementary Table S2) was also analyzed to determine consistent areas of gain or loss of expression. No genes located on chromosomes 1p or 16q were in the top gene list and down-regulated. However, 16 of the top genes were located in the region 1p31-1pter and were up-regulated and 6 genes were located on distal 1q and were up-regulated in relapsed patients. These include CHC1, FRAP/MTOR, PTGER, and UBE4B from 1p and CDC42BPA and TRIM17 from 1q (Table 3).

¹¹<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=pnknysekesyuly&acc=GSE10320>

Discussion

We report the gene expression analysis of a large group of FHWT consistently treated within a cooperative group setting. The goals were to determine the feasibility of using gene expression classifiers to identify patients who could benefit from increased or decreased adjuvant chemotherapy and to identify specific biological markers associated with relapse in patients with FHWT.

Ability to predict relapse in FHWT using gene expression varies with tumor stage

Efforts to establish an association between gene expression and relapse were not successful for stages I and II tumors yet were moderately successful for stage III tumors. The clinical endpoint of relapse in FHWT relies on two distinctly different features, the tumor's invasiveness (the ability to achieve lymphovascular access and distant growth, resulting in occult or overt residual disease) and the responsiveness of any residual disease to the therapy provided. The outcome of stage I FHWT <550 g in patients <24 months would suggest that most low-stage FHWT do not achieve lymphovascular access and are able to be completely treated by surgery alone. The majority of FHWT at all stages are exceedingly responsive to chemotherapy. As a result, our failure to detect gene expression signatures correlating with relapse in stage I to II patients treated with chemotherapy is not surprising. The majority of such tumors that are biologically "high risk" due to unresponsiveness to therapy are completely resected and the majority of those that are not completely resected (due to occult metastases, for example) are responsive to therapy. Conversely, stage III tumors by definition have evidence of residual disease. Therefore, the gene expression profile of the residual disease would be expected to correlate with responsiveness to therapy. It should be noted that the current study analyzes a random sample of the tumor, which is only an imperfect approximation of the residual disease itself.

Best classifiers in stage III FHWT will only be able to detect a subset of high-risk tumors

Our analysis indicates that not all stage III tumors that will go on to relapse can be correctly and consistently classified as high risk by global gene expression using Affymetrix U133A arrays. There are several possible explanations, all of which likely play a role:

- Global gene expression analysis performs well when detecting large differences in genes expressed at moderate to high levels. It is more difficult to detect reliable expression differences with genes that are tightly regulated with small differences in expression or genes that are expressed and regulated at very low levels.
- Drug resistance may develop during the administration of chemotherapy and the causes of this may not be detectable in the original sample.
- Clonal evolution may result in a more invasive or less responsive tumor clone. If this clone is not sampled, the tumor will not be correctly classified.
- Significant heterogeneity for the intrinsic causes of relapse likely exists, requiring a very large number of samples to define each cause.
- Wilms tumors have a wide spectrum of histologic appearances and varied lines of differentiation. The resulting spectrum of gene expression may obscure the identification of genes specifically associated with relapse.
- Some stage III tumors will have margins that are technically positive, but with no residual viable disease, or will have positive regional lymph nodes, with completely resected tumor. Therefore, it is expected that some stage III tumors would be identified based on gene expression as high risk that would not relapse.

Clinical utility of classifiers predicting relapse in stage III FHWI

In order for a biological marker to be clinically useful, it must be sufficiently robust and must have acceptable sensitivity and specificity to address the particular clinical needs of the patients being addressed. In this study, the classifiers developed using 50 genes resulted in a median sensitivity of 47% (25 of 53) and specificity of 70% (64 of 91). This compares with a sensitivity of 8% and specificity of 96% for LOH of both 1p and 16q in stages III and IV FHWI (4). To put this information into further perspective, the sensitivity and specificity of anaplasia for predicting death in Wilms tumor are 30% and 96%, respectively (12). Although the data suggest that the reliability of classifiers based on gene expression will be limited, strategies can be envisioned that propose either increased adjuvant therapy for those tumors at high risk or decreased adjuvant therapy for those tumors at low risk.

This study represents the first of a multistep process. The second step is the identification of a specific set of genes and the establishment of a mathematical rule that will be optimal for prospective prediction of individual tumors. This gene selection process may use the sensitivity and specificity of each individual gene, the overall absolute expression levels and differences in median expression levels (variables that take into account robustness of expression), the biological functions of the genes, and the interactions of expression within the top genes. The final and most critical step is the validation of the proposed classifier when applied in a blinded fashion to an independent patient population. This process is not currently possible and will require the analysis of patients in the current therapeutic protocols. In the absence of an ability to perform an independent validation currently, the proposal of a specific classifier may result in erroneous and premature conclusions. Therefore, we have chosen not to propose a specific classifier.

Comparison of gene expression data with prior publications

Three publications have reported differences in gene expression associated with clinical outcome in Wilms tumor (13–15). Zirn et al. analyzed 67 samples of all stages and of favorable and unfavorable histology collected following preoperative chemotherapy (13). Of their top genes associated with relapse, 3 were concordantly regulated and found in our Supplementary Table S2: *IL11RA*, *POSTN*, and *SMPDL3A*. *IL11RA* and *SMPDL3A* have been shown to be up-regulated in common adult cancers (16,17). Both genes were down-regulated in FHWI that relapsed. *POSTN*, periostin, is up-regulated in many cancers and may activate the Akt/protein kinase B pathway and promote anchorage-independent growth (18,19). Conversely, other studies have shown *POSTN* to be down-regulated in several human tumors and to inhibit metastasis and anchorage-independent growth (20). *POSTN* was down-regulated in relapses in FHWI. Williams et al. compared 27 pre-chemotherapy FHWI samples of all stages, of which 13 relapsed (14). None of their 15 top genes were identified in our Supplementary Table S2. Li et al. used a custom cDNA array and analyzed 26 pre-chemotherapy tumor samples (12 relapses) of all stages and of both favorable and unfavorable histology (15). Four top genes were reported that were capable of identifying tumors that relapsed: *C/EBPB*, *p21*, *H4FG*, and cDNA *CF542255* (*CLK1*). The *P* values of the associations of these genes with relapse in our study were all >0.1. In summary, the available current literature associating gene expression with relapse in Wilms tumor includes studies with small sample sizes and significant clinical heterogeneity. Our study has the advantage of increased numbers as well as the analysis of only stage III tumors of favorable histology treated using a single chemotherapeutic regimen.

Natrajan et al. performed array-based comparative genomic hybridization analysis to define regions of genetic gain or loss that correlate with relapse in 76 FHWI of all stages, half of which relapsed (21). Genes within these chromosomal regions that were concordantly differentially expressed with *P* < 0.01 in our study are listed in Table 4. (No genes meeting these criteria were found within regions 13q31-13q33, 14q32, 18q21, 21q22, and 1q32.)

Several genes were found to be both gained and overexpressed on chromosome 1q. *ADAM15* may function in metastasis (22), *TGFB2* has an established role in tumorigenesis and cancer progression (23), *CDC42BPA* (*MRCK*) is a protein kinase implicated in tumor cell invasion (24), and *SETDB1* and *SMYD2* are methyltransferases up-regulated in relapses, and the latter suppresses the proapoptotic action of p53 (25). Genes gained and overexpressed in the 16p13.2-13.2 region include *MAPK8IP3* whose up-regulation is associated with cell invasion and brain tumor malignancy (26). Lastly, 4 genes in 12q24.13-24.31 were down-regulated in our analysis including *DIABLO*, involved in apoptosis as described further below.

Specific genes involved in relapse of stage III FHWT

Those genes within Supplementary Table S2 with known or speculated biological function are provided in Table 3. There are several genes that merit special attention. *PEG3* (down-regulated in FHWT relapse) is induced after DNA damage by a p53-dependent mechanism and contributes to the Bax translocation necessary for apoptosis (27). *PEG3* is silenced by methylation in several gynecologic cancer cell lines (28). *DIABLO*, likewise down-regulated in FHWT relapse, is released from the mitochondrial membrane into the cytosol during apoptotic signaling, where it contributes to caspase activation (29). Loss of function mutations and down-regulation of *DIABLO* are associated with tumor aggressiveness and drug resistance (30, 31). Because *DIABLO*, *PEG3*, and *SMYD2* (mentioned earlier) all operate in the p53/Bax apoptotic pathway, this pathway may be of significance in relapse of FHWT. *TUSC3* is a potential tumor suppressor gene with decreased expression in advanced ovarian cancer (32). As with *PEG3*, down-regulation of *TUSC3* occurs via methylation in glioblastoma (33). *RECK* acts as a suppressor of tumor invasion and metastasis by inhibiting matrix metalloproteinases (34). Down-regulation of *RECK* in colon cancer via promoter methylation results in increased cell invasion, and these actions are reversed by DNMT inhibition (35). Therefore, down-regulation by methylation of several top genes in our study and up-regulation of two x-methyltransferases (*SETDB1* and *SMYD2*) suggest that epigenetic changes may play a role in outcome within stage III FHWT.

Several biologically significant genes were up-regulated with relapse in our study. *FRAP1/MTOR* is a serine/threonine kinase involved in signal transduction, translation initiation, and elongation. It exerts cell growth and survival effects via the Akt/PTEN pathway. *FRAP1* has a well-documented role in cancer (reviewed in ref. 36) and is a promising therapeutic target (37). Another up-regulated gene that is a potential therapeutic target is CD40, a member of the tumor necrosis factor family. CD40 induces antiapoptotic genes (including Bcl-2 and its family members), early angiogenesis, and activation of cell proliferation signaling pathways (38). Both *BCL2* and *BLC2L11*, a Bcl-2-like gene, were significantly up-regulated in our data ($P = 0.008$ and 0.0001 , respectively). The up-regulation of CD40 and Bcl-2 provides further support to the importance of apoptosis in FHWT relapse. *LY6D*, a GPI-anchored protein involved in cell-cell adhesion, is associated with high-risk lung cancer and with colorectal cancer invasion (39,40).

FGF18, up-regulated in FHWT relapse, is also up-regulated in colon cancer (41). *FGF18* is regulated at the transcriptional level by TCF-CTNNB1 responsive elements in its promoter region (41). *FGF18* induces nuclear accumulation of CTNNB1 by down-regulation of GSK3B activity at the protein level (42). CTNNB1 has been shown to undergo mutational activation in 10% to 15% of Wilms tumor, and there is a strong association between WT-1 mutation or deletion and CTNNB1 mutation (43). In addition, the putative tumor suppressor gene, WTX, which is mutated in ~30% of Wilms tumors, was recently shown to induce degradation of CTNNB1 (44,45). Our data therefore suggest that aberrant Wnt signaling may be involved in FHWT relapse.

Insulin-like growth factor (IGF)-I and its receptor, IGFR1, have been inversely associated with WT-1 in studies showing repression of IGFR1 by WT-1 and repression of WT-1 by IGF-I (46). Copy number gain at chromosome 15q23.5 (the location of IGFR1) has been shown to correlate with FHWT relapse, and gain of expression of *IGFR1* was confirmed by reverse transcription-PCR (47). Analysis of our expression data showed no significant increases within relapse samples of IGFR1, IGFR2, or IGF2, whereas IGF-I was significantly down-regulated in association with relapse. Although high levels of IGF-I have been implicated in oncogenesis in several different cancers, exogenous IGF-I was reported to induce apoptosis in a Wilms tumor cell line (48), suggesting that IGF-I may in certain situations induce cell death. Therefore down-regulation of IGF-I in relapses, or up-regulation in nonrelapses, may affect cell survival.

LOH of 1p and of 16q have been associated with relapse in FHWT (4). Only 3 of the 53 stage III relapses in our study were predicted as high risk by LOH of both 1p and 16q, consistent with the reported data that LOH detects only a small subset of relapses in FHWT. Rather than provide support for the association of loss of expression on 1p with relapse, our data show that 16 of the top 100 genes were located on 1p and were overexpressed, including several biologically important genes such as *PTGER3*, *UQCRH*, and *FRAP1*. It has been suggested that the critical event resulting in 1p LOH may involve gain of 1q rather than loss of 1p (49). This hypothesis is supported by Natrajan et al., as discussed above, which showed increased copy number of 1q to be associated with relapse (21). Our data provide additional support of this hypothesis by showing 6 of the top genes to be located on 1q, all of which were overexpressed.

In conclusion, this study provides some evidence supporting an association between gene expression and relapse in tumors of patients with stage III FHWT. The sensitivity of 47% and specificity of 70% moderate enthusiasm for direct clinical translation of a classifier based on gene expression, although this remains a potential interest if validation on an independent patient population is successful. Several different hypotheses are raised or further supported by these data and merit further investigation in the attempt to find potentially more robust methods of stratifying patients with FHWT. These include the relationship between relapse and apoptotic pathways, IGF-I signaling, the Wnt/ β -catenin pathway, 1q gain, and epigenetic modification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant support: NIH grants U10CA42326 (N. Breslow, D.M. Green, and E.J. Perlman), U10CA98543 (J.S. Dome, P.E. Grundy, and E.J. Perlman), and U01CA88131 (E.J. Perlman).

References

1. Green DM. The treatment of stages I-IV favorable histology Wilms tumor. *J Clin Oncol* 2004;22:1366–72. [PubMed: 15084612]
2. Breslow NE, Ou SS, Beckwith JB, et al. Doxorubicin for favorable histology, stage II-III Wilms tumor: results from the National WilmsTumor Studies. *Cancer* 2004;101:1072–80. [PubMed: 15329918]
3. Grundy RG, Hutton C, Middleton H, et al. Outcome of patients with stage III or inoperable WT treated on the second United Kingdom WT protocol (UKWT2); a United Kingdom Children's Cancer Study Group (UKCCSG) study. *Pediatr Blood Cancer* 2004;42:311–9. [PubMed: 14966826]
4. Grundy PE, Breslow NE, Li S, et al. Loss of heterozygosity for chromosomes 1p and 16q is an adverse prognostic factor in favorable-histology Wilms tumor: a report from the National WilmsTumor Study Group. *J Clin Oncol* 2005;23:7312–21. [PubMed: 16129848]

5. Huang CC, Cutcliffe C, Coffin C, Sorensen PH, Beckwith JB, Perlman EJ. Classification of malignant pediatric renal tumors by gene expression. *Pediatr Blood Cancer* 2006;46:728–38. [PubMed: 16425275]
6. Zhang L, Miles MF, Aldape KD. A model of molecular interactions on short oligonucleotide microarrays. *Nat Biotechnol* 2003;21:818–21. [PubMed: 12794640]
7. Vapnik, VN. Statistical learning theory. New York (NY): John Wiley and Sons; 1998.
8. Simon R, Radmacher MD, Dobbin K, McShane LM. Pitfalls in the use of DNA microarray data for diagnostic and prognostic classification. *J Natl Cancer Inst* 2003;95:14–8. [PubMed: 12509396]
9. Green DM, Breslow NE, Beckwith JB, et al. Treatment with nephrectomy only for small, stage I/ favorable histology Wilms tumor: a report from the National Wilms Tumor Study Group. *J Clin Oncol* 2001;19:3719–24. [PubMed: 11533093]
10. Michiels S, Koscielny S, Hill C. Prediction of cancer outcome with microarrays: a multiple random validation strategy. *Lancet* 2005;365:488–92. [PubMed: 15705458]
11. Storey JD. A direct approach of false discovery rates. *JR Stat Soc Ser B* 2002;64:479–98.
12. Beckwith JB, Palmer NF. Histopathology and prognosis of Wilms tumors: results from the First National WilmsTumor Study. *Cancer* 1978;41:1937–48. [PubMed: 206343]
13. Zirn B, Hartmann O, Samans B, et al. Expression profiling of Wilms tumors reveals new candidate genes for different clinical parameters. *Int J Cancer* 2006;118:1954–62. [PubMed: 16287080]
14. Williams RD, Hing SN, Greer BT, et al. Prognostic classification of relapsing favorable histology Wilms tumor using cDNA microarray expression profiling and support vector machines. *Genes Chromosomes Cancer* 2004;41:65–79. [PubMed: 15236318]
15. Li W, Kessler P, Yeger H, et al. A gene expression signature for relapse of primary Wilms tumors. *Cancer Res* 2005;65:2592–601. [PubMed: 15805255]
16. Hanavadi S, Martin TA, Watkins G, Mansel RE, Jiang WG. Expression of interleukin 11 and its receptor and their prognostic value in human breast cancer. *Ann Surg Oncol* 2006;13:802–8. [PubMed: 16614887]
17. Wright KO, Messing EM, Reeder JE. Increased expression of the acid sphingomyelinase-like protein ASML3a in bladder tumors. *J Urol* 2002;168:2645–9. [PubMed: 12442002]
18. Kudo Y, Ogawa I, Kitajima S, et al. Periostin promotes invasion and anchorage-independent growth in the metastatic process of head and neck cancer. *Cancer Res* 2006;66:6928–35. [PubMed: 16849536]
19. Bao S, Ouyang G, Bai X, et al. Periostin potently promotes metastatic growth of colon cancer by augmenting cell survival via the Akt/PKB pathway. *Cancer Cell* 2004;5:329–39. [PubMed: 15093540]
20. Kim CJ, Yoshioka N, Tambe Y, Kushima R, Okada Y, Inoue H. Periostin is down-regulated in high grade human bladder cancers and suppresses *in vitro* cell invasiveness and *in vivo* metastasis of cancer cells. *Int J Cancer* 2005;117:51–8. [PubMed: 15880581]
21. Natrajan R, Williams RD, Hing SN, et al. Array CGH profiling of favourable histology Wilms tumours reveals novel gains and losses associated with relapse. *J Pathol* 2006;210:49–58. [PubMed: 16823893]
22. Kuefer R, Day KC, Kleer CG, et al. ADAM15 disintegrin is associated with aggressive prostate and breast cancer disease. *Neoplasia* 2006;8:319–29. [PubMed: 16756724]
23. Bellone G, Carbone A, Tibaudi D, et al. Differential expression of transforming growth factors- β 1, - β 2 and - β 3 in human colon carcinoma. *Eur J Cancer* 2001;37:224–33. [PubMed: 11166150]
24. Wilkinson S, Paterson HF, Marshall CJ. Cdc42-MRCK and Rho-ROCK signalling cooperate in myosin phosphorylation and cell invasion. *Nat Cell Biol* 2005;7:255–61. [PubMed: 15723050]
25. Huang J, Perez-Burgos L, Placek BJ, et al. Repression of p53 activity by Smyd2-mediated methylation. *Nature* 2006;444:629–32. [PubMed: 17108971]
26. Takino T, Nakada M, Miyamori H, et al. JSAP1/JIP3 cooperates with focal adhesion kinase to regulate c-Jun N-terminal kinase and cell migration. *J Biol Chem* 2005;280:37772–81. [PubMed: 16141199]
27. Deng Y, Wu X. Peg3/Pw1 promotes p53-mediated apoptosis by inducing Bax translocation from cytosol to mitochondria. *Proc Natl Acad Sci U S A* 2000;97:12050–5. [PubMed: 11050235]

28. Dowdy SC, Gostout BS, Shridhar V, et al. Biallelic methylation and silencing of paternally expressed gene 3 (PEG3) in gynecologic cancer cell lines. *Gynecol Oncol* 2005;99:126–34. [PubMed: 16023706]
29. Kim R. Recent advances in understanding the cell death pathways activated by anticancer therapy. *Cancer* 2005;103:1551–60. [PubMed: 15742333]
30. Bonavida B, Huerta-Yepez S, Goodglick L, Mizutani Y, Miki T. Can we develop biomarkers that predict response of cancer patients to immunotherapy? *Biomarkers* 2005;10:S69–76. [PubMed: 16298914]
31. Tirro E, Consoli ML, Massimino M, et al. Altered expression of c-IAP1, survivin, and Smac contributes to chemotherapy resistance in thyroid cancer cells. *Cancer Res* 2006;66:4263–72. [PubMed: 16618750]
32. Pils D, Horak P, Gleiss A, et al. Five genes from chromosomal band 8p22 are significantly down-regulated in ovarian carcinoma: N33 and EFA6R have a potential impact on overall survival. *Cancer* 2005;104:2417–29. [PubMed: 16270321]
33. Li Q, Jedlicka A, Ahuja N, et al. Concordant methylation of the ER and N33 genes in glioblastoma multiforme. *Oncogene* 1998;16:3197–202. [PubMed: 9671399]
34. Noda M, Oh J, Takahashi R, Kondo S, Kitayama H, Takahashi C. RECK: a novel suppressor of malignancy linking oncogenic signaling to extracellular matrix remodeling. *Cancer Metastasis Rev* 2003;22:167–75. [PubMed: 12784995]
35. Cho CY, Wang JH, Chang HC, Chang CK, Hung WC. Epigenetic inactivation of the metastasis suppressor RECK enhances invasion of human colon cancer cells. *J Cell Physiol* 2007;213:65–9. [PubMed: 17443689]
36. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev* 2004;18:1926–45. [PubMed: 15314020]
37. Faivre S, Kroemer G, Raymond E. Current development of mTOR inhibitors as anticancer agents. *Nat Rev Drug Discov* 2006;5:671–88. [PubMed: 16883305]
38. Dallman C, Johnson PW, Packham G. Differential regulation of cell survival by CD40. *Apoptosis* 2003;8:45–53. [PubMed: 12510151]
39. Lu Y, Lemon W, Liu PY, et al. A gene expression signature predicts survival of patients with stage I non-small cell lung cancer. *PLoS Med* 2006;3:e467. [PubMed: 17194181]
40. Reichling T, Goss KH, Carson DJ, et al. Transcriptional profiles of intestinal tumors in Apc(Min) mice are unique from those of embryonic intestine and identify novel gene targets dysregulated in human colorectal tumors. *Cancer Res* 2005;65:166–76. [PubMed: 15665292]
41. Shimokawa T, Furukawa Y, Sakai M, et al. Involvement of the FGF18 gene in colorectal carcinogenesis, as a novel downstream target of the β -catenin/T-cell factor complex. *Cancer Res* 2003;63:6116–20. [PubMed: 14559787]
42. Katoh M, Katoh M. Cross-talk of WNT and FGF signaling pathways at GSK3 β to regulate β -catenin and SNAIL signaling cascades. *Cancer Biol Ther* 2006;5:1059–64. [PubMed: 16940750]
43. Maiti S, Alam R, Amos CI, Huff V. Frequent association of β -catenin and WT1 mutations in Wilms tumors. *Cancer Res* 2000;60:6288–92. [PubMed: 11103785]
44. Rivera MN, Kim WJ, Wells J, et al. An X chromosome gene, WTX, is commonly inactivated in Wilms tumor. *Science* 2007;315:642–5. [PubMed: 17204608]
45. Major MB, Camp ND, Berndt JD, et al. Wilms tumor suppressor WTX negatively regulates WNT/ β -catenin signaling. *Science* 2007;316:1043–6. [PubMed: 17510365]
46. Bentov I, LeRoith D, Werner H. The WT1 Wilms tumor suppressor gene: a novel target for insulin-like growth factor-I action. *Endocrinology* 2003;144:4276–9. [PubMed: 12960088]
47. Natrajan R, Reis-Filho JS, Little SE, et al. Blastemal expression of type I insulin-like growth factor receptor in Wilms tumors is driven by increased copy number and correlates with relapse. *Cancer Res* 2006;66:11148–55. [PubMed: 17145858]
48. Granerus M, Johannisson A, Ekblom P, Engstrom W. Insulin-like growth factors I and II induce cell death in Wilms tumour cells. *Mol Pathol* 2001;54:30–5. [PubMed: 11212886]
49. Lu YJ, Hing S, Williams R, Pinkerton R, Shipley J, Pritchard-Jones K. Chromosome 1q expression profiling and relapse in Wilms tumour. *Lancet* 2002;360:385–6. [PubMed: 12241781]

Fig. 1.

Cross-validation with 144 stage III FHWTS: all 144 stage III FHWTS were randomly divided into 2 and 10 groups. For each training set composed of all but one group, a classifier was developed using from 1 to 150 genes; each classifier was then applied to the group withheld to define low-risk and high-risk tumors. This was repeated until all groups were categorized. This entire process was repeated 500 times. *Left and right*, results of the 2- and 10-fold cross-validations, respectively. For each, five curves are illustrated representing the 5th, 25th, 50th, 75th, and 95th percentiles. *Red curves*, 50% percentile. *X axis*, number of genes used in the classifier ($K = 1-150$). *A*, specificity: among tumors that did not relapse, the percentage categorized using gene expression as low risk for relapse. *B*, sensitivity: among tumors that

relapsed, the percentage categorized as high risk for relapse. *C*, error rate/accuracy: percentage of tumors incorrectly classified.

Fig. 2. Success of the 10-fold cross-validation method using 50-gene classifiers for each individual tumor. *Y axis*, percentage of the 500 50-gene classifiers that categorized each tumor as high risk for relapse; *X axis*, arbitrary tumor number, with relapses (*red dots to the left of the vertical line*) having low numbers and nonrelapses having higher numbers (*black dots*).

Table 1

Categorization of 300 samples in group A by stage

Local stage/overall stage	No. (relapses)	Rejected (relapses)	Final (relapses)
I/I	69 (10)	20 (0)	49 (10)
II/II	103 (30)	36 (5)	67 (25)
II/IV	7 (1)	1 (0)	6 (1)
III/III	85 (22)	21 (5)	64 (17)
III/IV	16 (9)	4 (2)	12 (7)
V	9 (4)	3 (1)	6 (3)
Biopsy only	11 (6)	5 (3)	6 (3)
Total	300 (82)	90 (16)	210 (66)

Table 2

Clinical characteristics of 144 stage III tumors

	Cases (n = 53)	Controls (n = 91)	Total
Stage			
III/III	37	77	114
III/IV	16	14	30
Reason for stage III			
Positive margin or rupture/spillage	15	36	51
Lymph node involvement	19	41	60
Positive margin and lymph node involvement	19	14	33
Median (range) time to relapse (d)	284.5 (76-1940)		
Median (range) disease-free follow-up (d)		2,162 (1,124-3,788)	
Site of relapse			
Lung only	26		
Abdomen	13		
Liver	5		
Other	9		
LOH for 1p and 16q	3 of 53	5 of 89	8 of 142

Table 3

Categorization of selected genes of interest

		Chromosomal location	Unigene ID
Metastasis and tumorigenesis			
CD40	Up	chr20q12-q13.2	Hs.118832
CDC42BPA	Up	chr1q42.11	Hs.326691
CHC1	Up	chr1p36.1	Hs.84746
EWSR1	Up	chr22q12.2	Hs.129953
FGF18	Up	chr5q34	Hs.49585
FRAP1/MTOR	Up	chr1p36.2	Hs.250723
IGF-I	Down	chr12q22-q23	Hs.85112
Ly6D	Up	chr8q24-qter	Hs.3185
MARK2	Up	chr11q12-q13	Hs.157199
MMP26	Up	chr11p15	Hs.204732
PDE3A	Up	chr12p12	Hs.777
POSTN	Down	chr13q13.3	Hs.136348
PPFIA1	Up	chr11q13.3	Hs.183648
PTGER3	Up	chr1p31.2	Hs.170917
PTPRH	Up	chr19q13.4	Hs.179770
TRIM17	Up	chr1q42	Hs.121748
UBE4B	Up	chr1p36.3	Hs.24594
UQCRH	Up	chr9q31	Hs.152601
Tumor suppressor			
DIABLO	Down	chr12q24.31	Hs.169611
PEG3	Down	chr19q13.4	Hs.139033
RECK	Down	chr9p13-p12	Hs.29640
RECQL	Down	chr12p12	Hs.235069
TUSC3	Down	chr8p22	Hs.71119.0

NOTE: Among the top 109 genes (sorted by *P* value and provided in Supplementary Table S2), approximately one-quarter were selected as genes of interest based on their known genetic and molecular functions.

Table 4

Genes concordantly differentially expressed within reported genetic regions of gain or loss in relapses

Probe set ID	Unigene	Gene symbol	Chromosomal location	P
Gains at 1q				
202337_at	Hs.94446	PMF1	chr1q12	0.0095375
214197_s_at	Hs.20991	SETDB1	chr1q21	0.0016913
217778_at	Hs.7854	SLC39A1	chr1q21	0.0044798
212147_at	Hs.4990	SMG5	chr1q21.2	0.0024339
208100_x_at	Hs.516316	SEMA6C	chr1q21.2	0.0029472
202596_at	Hs.111680	ENSA	chr1q21.2	0.0051370
217007_s_at	Hs.92208	ADAM15	chr1q21.3	0.0029510
219373_at	Hs.110477	DPM3	chr1q22	0.0049546
219125_s_at	Hs.292154	RAG1AP1	chr1q22	0.0085894
205789_at	Hs.1799	CD1D	chr1q22-q23	0.0023229
216885_s_at	Hs.110707	WDR42A	chr1q22-q23	0.0096002
205253_at	Hs.155691	PBX1	chr1q23	0.0079297
217797_at	Hs.177507	Ufc1	chr1q23.3	0.0063011
201482_at	Hs.77266	QSCN6	chr1q24	0.0011956
207350_s_at	Hs.102664	VAMP4	chr1q24-q25	0.0017361
215109_at	Hs.127338	KIAA0492	chr1q25.1	0.0074330
218253_s_at	Hs.274151	LGTN	chr1q31-q32	0.000072
208270_s_at	Hs.283667	RNPEP	chr1q32	0.0078793
212165_at	Hs.17481	TMEM183A	chr1q32.1	0.0064554
202186_x_at	Hs.155079	PPP2R5A	chr1q32.2-q32.3	0.0048414
212922_s_at	Hs.66170	SMYD2	chr1q41	0.0082410
220406_at	Hs.169300.1	TGFB2	chr1q41	0.000893
207025_at	Hs.100072	GJA12	chr1q41-q42	0.0042464
220279_at	Hs.121748	TRIM17	chr1q42	0.0000805
213595_s_at	Hs.326691	CDC42BPA	chr1q42.11	0.000582
215583_at	Hs.287529	TMEM63A	chr1q42.12	0.0042900
221582_at	Hs.28777	HIST3H2A	chr1q42.13	0.0000185
212155_at	Hs.144904	RNF187	chr1q42.13	0.0052750
205689_at	Hs.31438	PCNXL2	chr1q42.2	0.000767
208934_s_at	Hs.4082	LGALS8	chr1q42-q43	0.0063052
203581_at	Hs.119007	RAB4A	chr1q42-q43	0.0074064
204678_s_at	Hs.79351	KCNK1	chr1q42-q43	0.0094796
220661_s_at	Hs.23617	ZNF692	chr1q44	0.0026939
Gains at 16p13.3-16p13.2				
222009_at	Hs.274361	CEMP1	chr16p13.3	0.0017931
206416_at	Hs.592088	ZNF205	chr16p13.3	0.0022295
218969_at	Hs.16089	Magmas	chr16p13.3	0.0052089
207134_x_at	Hs.294158	TPSB2	chr16p13.3	0.0087975
213177_at	Hs.88500	MAPK8IP3	chr16p13.3	0.0094881

Probe set ID	Unigene	Gene symbol	Chromosomal location	<i>P</i>
Losses at 2q36.3-2q37.1				
201370_s_at	Hs.78946	CUL3	chr2q36.3	0.0072559
218091_at	Hs.171545	HRB	chr2q36.3	0.0097753
Losses at 12q24.13-12q24.31				
219350_s_at	Hs.169611	DIABLO	chr12q24.31	0.000952
213153_at	Hs.154525	SETD1B	chr12q24.31	0.0042973
201632_at	Hs.78592	EIF2B1	chr12q24.31	0.0081670
218175_at	Hs.288909	CCDC92	chr12q24.31	0.0099578

NOTE: Listed are genes differentially expressed ($P < 0.01$) in the current study corresponding within the genetic regions showing loss or gain in association with relapse in the study of Natrajan et al. (21).