Null Results in Brief

No Evidence For Large-scale Germline Genomic Aberrations in Hereditary Bladder Cancer Patients with High-Resolution Array-Based Comparative Genomic Hybridization

Lambertus A. Kiemeney,[1,2,4] Roland P. Kuiper,[3] Rolph Pfundt,[3] Simon van Reijmersdal,[3] Mark P. Schoenberg,[5] Katja K. Aben,[2,4] Martinus F. Niermeijer,[3] J. Alfred Witjes,[1] and Eric F.P.M. Schoenmakers[3]

Departments of [1]Urology, [2]Epidemiology and Biostatistics, and [3]Human Genetics, Radboud University Nijmegen Medical Centre; [4]Comprehensive Cancer Center IKO, Nijmegen, the Netherlands; and [5]Department of Urology, Johns Hopkins Hospital, Baltimore, Maryland

Introduction

Linkage studies in high-risk families have led to the identification of several important susceptibility genes for hereditary cancer. Unfortunately, such studies offer limited possibilities in the search for high-penetrance bladder cancer genes, as extended bladder cancer families are very rare. Traditional karyotyping or conventional comparative genomic hybridization (CGH) may reveal constitutional chromosomal anomalies that point to the location of susceptibility genes (1). Both these techniques, however, are hampered by their limited resolution (~3 Mb with CGH). The recent development of array-based CGH has increased this resolution to ~100 kb. Using a 32,447 bacterial artificial chromosome array with close to complete coverage of the entire human genome (2, 3), we recently identified several novel submicroscopic interstitial chromosomal abnormalities in patients with unexplained mental retardation (4). In this study, we have used these tiling resolution genomic microarrays to investigate the presence of copy number abnormalities in 10 patients, representing 10 nonrelated Dutch bladder cancer families.

Materials and Methods

In a large study on familial bladder cancer, we identified 95 patients who had at least one first-degree relative with bladder cancer (5). Using arbitrary criteria [at least (*a*) three male cases in the first degree; or (*b*) two affected first-degree relatives diagnosed before the age of 45 years; or (*c*) two female first-degree relatives with an aggressive form of bladder cancer], we identified eight families suggestive for hereditary bladder cancer (Fig. 1). Two additional high-risk families did not fulfill one of these criteria but were included because they sought counsel for hereditary bladder cancer. After informed consent, one index case from each family (Fig. 1, *arrows*) donated a blood sample for the investigations described here.

The preparation of a tiling resolution microarray consisting of 32,447 overlapping bacterial artificial chromosome clones selected to cover the entire sequenced human genome (2, 3) has been described previously (4, 6). Genomic DNA (500 ng) was isolated from blood leucocytes using routine procedures, labeled, and hybridized against a sex-mismatched reference pool as previously described (4). In case of ''suspicious'' findings, hybridizations were done in duplicate in a so-called dye swap experiment. Spot identification and two-color fluorescence intensity measurements were obtained using the Genepix 5.0 software package, and all data were entered into a database for subsequent automated data normalization and analysis. Data normalization and copy number detection were done according to previously reported methodology (4). All identified genomic copy number alterations were compared with both public and private databases of known disease-unrelated large-scale copy number variations (refs. 4, 7-9 and http://projects.tcag.ca/variation/).

Results

In all 10 patients selected for this study, we detected one or more genomic copy number alterations that varied in size from 0.25 to 5.8 Mb. All of these alterations, however, have previously been categorized as disease-unrelated large-scale copy number variations. In total, 41 aberrations were detected representing 21 different large-scale copy number variations. As an example, Fig. 2 shows the genome profile from one of the male patients, which was hybridized against a female reference pool. Four regions of neighboring clones with aberrant $\log_2$ ratios are visible, which are located on 9p13.2-p11.2 (5.8 Mb), 16p11.2 (1.7 Mb), 17q21.31 (0.29 Mb), and Xp22.31 (0.39 Mb). After analyzing the data from all 10 patients, only two areas of genomic loss (at 8q23 and 21q21, each in one of the 10 patients) seemed to be new initially but could not be confirmed in a duplicate, dye-swapped experiment.

Discussion

Well-known risk factors for urinary bladder cancer include cigarette smoking and occupational exposure to aromatic amines and polycyclic hydrocarbons. Because of the strong oncogenic effect of these exogenous factors and because multipatient bladder cancer families are rare, bladder cancer is generally considered to occur almost exclusively as sporadic, nonhereditary, cases. The frequent occurrence of bladder cancer among survivors of bilateral retinoblastoma (10) indicate, however, that *Rb1* mutation carriers run a high risk of bladder cancer. Additional evidence for the existence of a

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Requests for reprints: Lambertus A. Kiemeney, Department of Epidemiology and Biostatistics, Radboud University Nijmegen Medical Centre, P.O. Box 9101, NL-6500 HB Nijmegen, the Netherlands. Phone: 31-24-361-3745; Fax: 31-24-361-3505. E-mail: b.kiemeney@epib.umcn.nl

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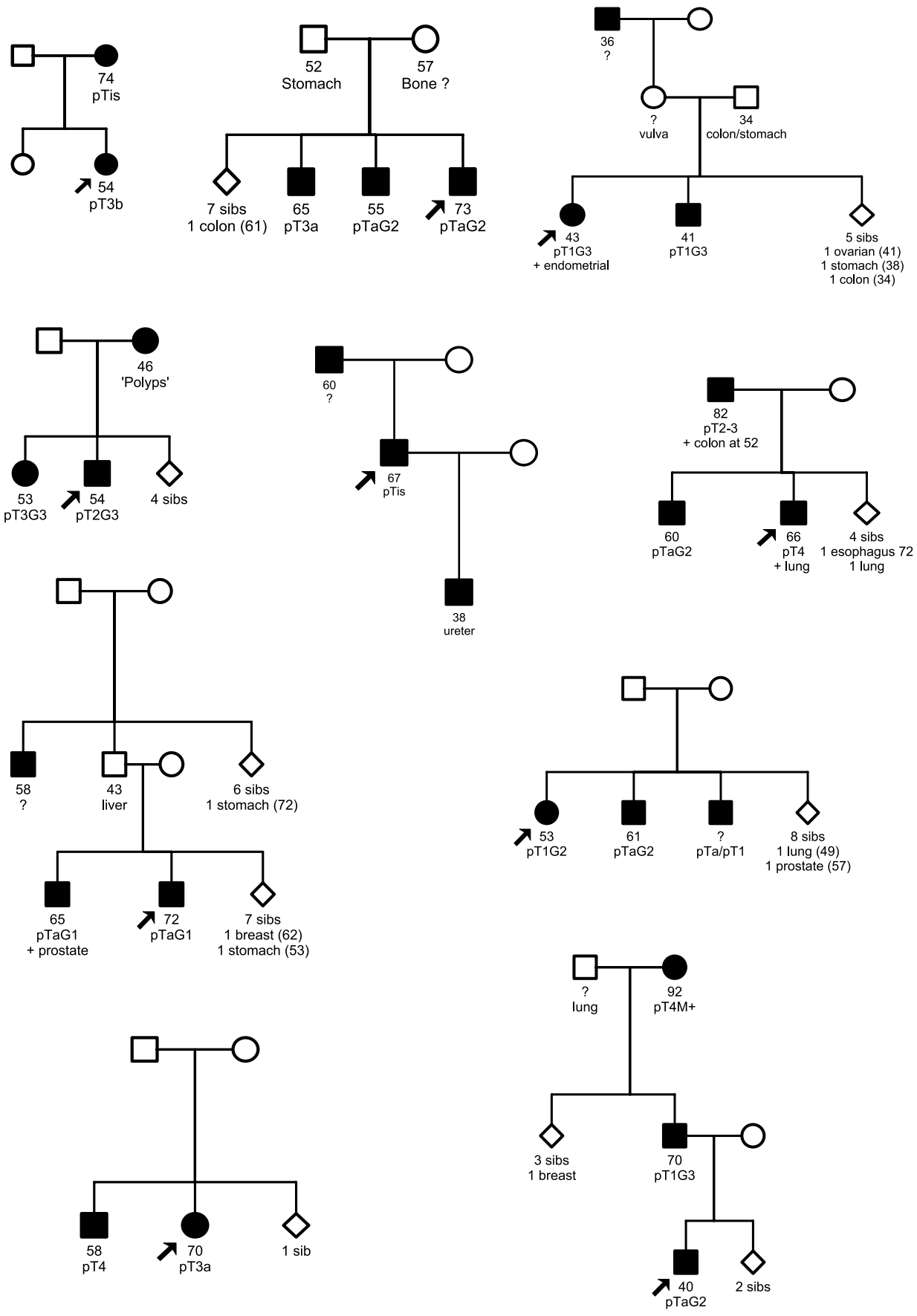

Figure 1. Pedigree drawings of the 10 selected familial bladder cancer patients. Patients with urothelial cell carcinoma are presented as black squares (men) or circles (women). Age at diagnosis and disease stage (tumor-node-metastasis) and grade (WHO) are presented for each patient. The arrows point to the index patients (“probands”).

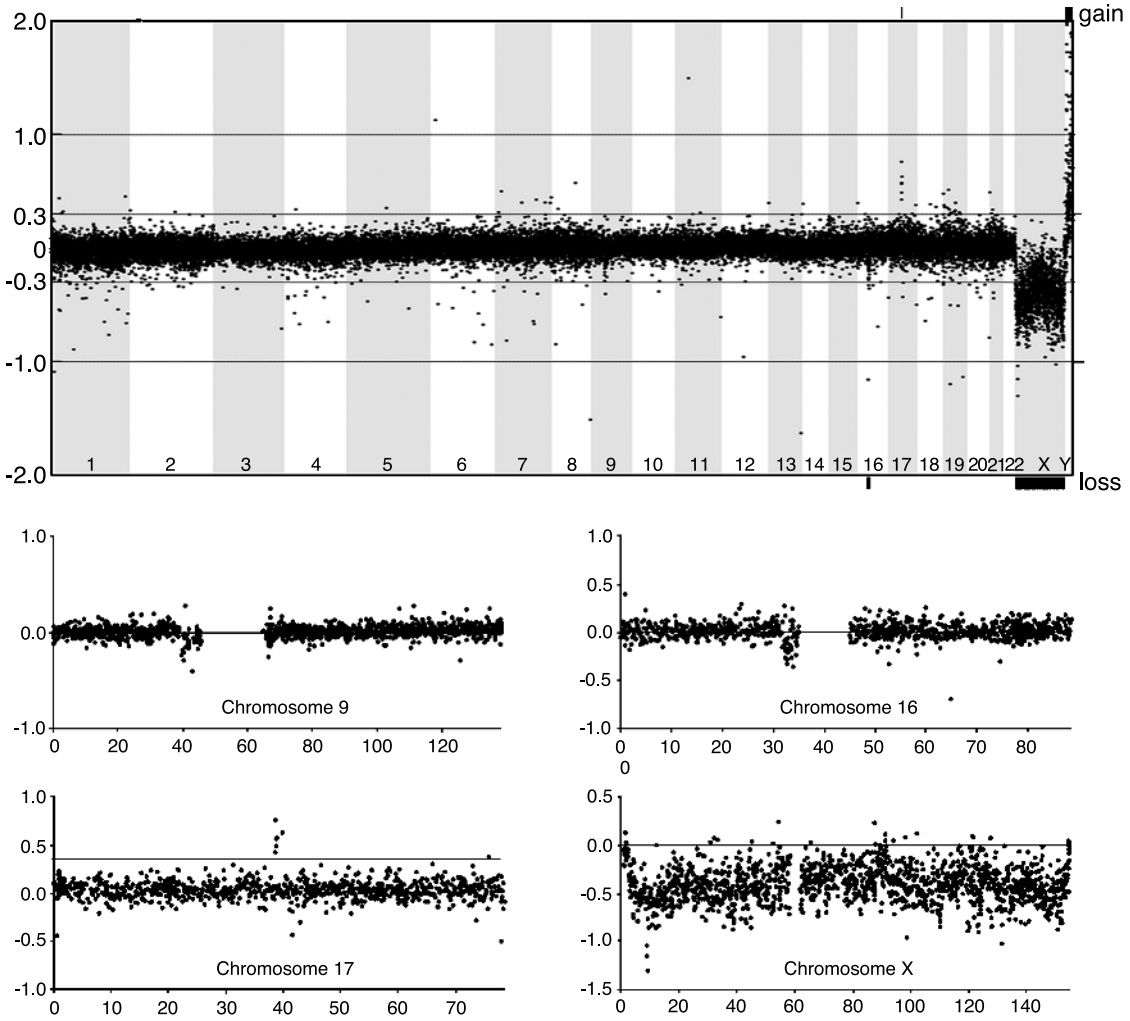

Figure 2. Genomic profiles of one of the patients with hereditary bladder cancer. Array containing 32,447 human bacterial artificial chromosome clones (indicated by small circles representing the $\log_2$-transformed and normalized test-over-reference intensity ratios), genomically ordered from 1pter to Yqter in the genome profile, and for individual chromosomes from pter to qter, all based on the physical mapping positions obtained from the May 2004 freeze of the University of California Santa Cruz genome browser. All copy number gains and losses that were determined by use of a Hidden Markov Model (4) are indicated as bars above and below the profile diagram, respectively. Clones of the X and Y chromosomes show aberrant $\log_2$ ratios due to sex-mismatched hybridizations. Three losses (Xp22.31, 9p13.2-p11.2, and 16p11.2) and one gain (17q21.31), of which the latter two were detected with Hidden Markov Model, are large-scale copy number variations.

Mendelian subtype of bladder cancer comes from numerous case reports of patients with striking family histories (11). In one of these families, we found a constitutional balanced translocation t(5;20)(p15;q11), the first bladder cancer-related germline abnormality ever described (12). Further focus on the translocation breakpoints eventually resulted in the identification of the *CDC91L1* gene, residing at 20q11 (1). This gene encodes CDC91L1, also called phosphatidylinositol glycan class U, which is known to function in the glycosylphosphatidylinositol anchoring pathway. The translocation resulted in overexpression of the gene and presumably to both bladder cancers observed in this pedigree. Protein altering mutations in the gene were not identified.

In this study, we used genome-wide tiling resolution array CGH to screen probands with a strong family history of bladder cancer for large-scale genomic copy number alterations. The resolution of the technique we used is ~100 times higher than that of conventional karyotyping (~5 to 10Mb). Our finding of 41 known large-scale copy number variations in this series of 10 patients, ranging in size from 0.25 to 5.8 Mb, confirms this resolution. Unfortunately, we did not find any candidate region for a gene that may predispose to the development of bladder cancer. Obviously, germline point mutations in tumor suppressor or DNA repair genes were below our detection limit. Such mutations followed by allelic loss of the second copy of the genes are the cause of most inherited cancer syndromes. In other words, a positive finding, even in one patient only, might have been an enormous step forward, but a negative finding bears limited information. Still, it has been shown recently, although not yet in the field of cancer research, that high-resolution CGH is a powerful technique for the mapping of high-penetrance genes (13). The alternative of positional cloning through classic linkage mapping will only become possible with an international collaborative effort to identify additional, preferably extended high-risk bladder cancer families.

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