Loss of Chromosome 16 From Renal Epithelial Cells in Humans

Jeffrey P. Gardner, Xiao-Yan Yang, Joan Skurnick, Patricia D. Wilson, Hana Aviv, Smita Patel, Amy L. Davidow, Michael Gutkin, Abraham Aviv

Abstract—This work explores the notion that low-frequency, acquired aneuploidy may play a role in complex genetic traits such as essential hypertension. To this end, renal epithelial cells in urinary sediments and in renal cysts were examined by fluorescent in situ hybridization with DNA probes specific for the heterochromatic and centromere regions of chromosomes 16 and 1. Chromosome 16 was probed because it harbors variant genes causing monogenic hypertension. These genes have also been investigated for their role in essential hypertension. Chromosome 1 was also probed as an internal control. Higher proportions of renal epithelial cells in the urinary sediments showed monosomy of chromosome 16 than monosomy of chromosome 1 (P<0.001). We also observed in epithelial cells of renal cysts a preponderance of monosomy for chromosome 16 over monosomy for chromosome 1 (P<0.024). Low-frequency loss of heterozygosity that results from acquired monosomy of chromosome 16 and perhaps other chromosomes may contribute to expression of complex genetic traits such as essential hypertension, in which the diverse phenotypic manifestations are poorly understood. (Hypertension. 2002;40:928-933.)

Key Words: hypertension, essential □ epithelium □ kidney

As a complex genetic trait, essential hypertension is thought to be caused by several or perhaps multiple variant genes. Each of these genes may contribute little to the phenotypic expression of the trait, making the variant genes hard to detect. In addition, the ultimate expression of variant genes may depend on the ever-changing milieu within which genes operate. One factor that might contribute to changes in genetic background within the lifetime of an individual is acquired chromosomal aneuploidy (loss or gain of chromosomes) in somatic cells.

Chromosomal aneuploidy is a common feature of cancer,1,2 but it also occurs in a small percentage of somatic cells from apparently normal persons.3–7 The underlying causes for and the collective impact of acquired, low-frequency aneuploidy in noncancerous tissues are largely unknown. Low-frequency aneuploidy may, however, exert a considerable effect through loss of heterozygosity (LOH) if aneuploidy involves chromosomes that harbor variant genes with important functions. Such a concept is in line with the “second hit” model, originally proposed for certain types of cancers.8

Cytogenetic analyses in metaphase cells have revealed that the sporadic loss of small chromosomes is more common than that of large chromosomes.9–13 Chromosome 16 is a relatively small chromosome harboring variant genes that cause monogenic forms of hypertension in human beings.14–16 We therefore examined the frequency of loss (or gain) of chromosome 16 from renal epithelial cells in urinary sediments, testing the hypothesis that the loss of chromosome 16 from renal epithelial cells is not an exceedingly rare event. To this end, we used fluorescent in situ hybridization (FISH) to monitor for aneuploidy of chromosome 16 in renal epithelial cells in urinary sediments and in renal cysts. Chromosome 1, the largest human chromosome, was used as an internal control.

Methods

Subjects
Urinary sediments from female subjects frequently show an abundance of squamous and stratified epithelium from the external urogenital tract. This makes it difficult to precisely evaluate other cells by using the FISH technique. For this reason, we restricted our analysis to urine obtained from white and black male subjects, ages 5 to 73 years. The subjects were in a good health without known kidney disease, including urinary tract infection. Approval and informed consents were obtained from all subjects; the protocol was approved by the Institutional Review Board of UMDNJ-New Jersey Medical School. Of the 49 subjects studied, the ages of 5 subjects were 5 to 10 years. General characteristics of the remaining adult subjects are described in Table 1. Of the 44 adult subjects, body mass index (BMI) was measured in 42 individuals and blood pressure parameters were measured in 37 individuals.

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Preparation of Urinary Sediment
Since the morphology of urinary cells progressively deteriorates with increasing pH and storage,19 freshly voided morning urine samples were collected while the donors were fasting (no food or fluids after dinner). The first urine voided after awakening was discarded, and the second urinary specimen was collected and immediately processed. All urine samples were tested with chemstrips (Boehringer Mannheim Corp) and were negative for leukocytes, nitrates, protein (except for one subject, showing 2+ protein), glucose, and blood. Samples were centrifuged at 500g for 10 minutes. The sediment was treated with alcoholic carbowax (3 g carbowax in 100 mL 60% ethanol) as a prefixative18 and 2% paraformaldehyde as a final fixative. Two slides precoated with 3-aminopropyltriethoxysilane were made from each specimen. The cells were aged at room temperature for 24 hours before hybridization with the two chromosomal probes.

Fluorescent In Situ Hybridization
Directly labeled fluorescent DNA probes specific for human satellite II and α satellite DNA sequences at heterochromatic and centromere regions of chromosomes 16 and 1 (Vysis Inc) were used for FISH. Cells were simultaneously cohybridized with equal amounts of probes for the D1Z5 α satellite (SpectrumOrange label, chromosome 1) and D16Z3 satellite II (SpectrumGreen label, chromosome 16). The samples and probes were codenatured at 90°C for 2 minutes with the use of Vysis Hybrite Hybridization System, followed by overnight hybridization of probes at 37°C, and counterstaining with DAPI-II (Vysis, Inc). Cells on both slides were scored and the results pooled.

Selection of Urinary Cells for Chromosomal Scoring
The following criteria were used to differentiate and then exclude from the scoring analysis subsets of cells from the lower urinary tract. The squamous cells could be easily identified by their large size and irregular polygonal form; these cells were not scored. The transitional epithelial cells varied in size, though in general they were smaller than the squamous cells. The larger transitional cells, composed of oval, rhombic, and cubic cells, were also not scored. Small cells with uniformly round, fairly large nuclei and with somewhat granular cytoplasm were selected for scoring. In preliminary investigations, attempts were made to identify the origin of FISH-scored cells, using various antibodies, including anti-human dipeptidyl peptidase (CD26) and anti-human Tamm-Horsfall glycoprotein antibodies. This approach was abandoned, because fixation for immunostaining interfered with the FISH for chromosomal identification. We note, however, that the size and morphology of cells positively stained with the renal cell antibodies were the main criteria for selecting cells for the FISH scoring (Figure 1). A feature that facilitated the identification of these cells was the complete dissolution of their cytoplasm by the hybridization procedure (ie, the fixation and subsequent incubation for 2 minutes at 90°C and overnight incubation at 37°C). In contrast, the cytoplasm of the urothelial cells was to a large extent preserved (Figure 1).

Preparations of Renal Epithelial Cells From Renal Cysts for Chromosomal Scoring
To further ascertain the source of cells and whether aneuploidy observed in epithelial cells of the urine is representative of the chromosomal pattern in the renal epithelium in situ, we FISH-probed renal epithelial cells from renal cysts using a touch preparation approach. This was done by pressing positively charged silanized slides against the interior of the cysts. By this method, epithelial cells were transferred to the glass surface of the slides. The renal cysts were derived from one dysplastic kidney, one hydronephrotic kidney, and two polycystic kidneys from patients who did not have the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (n=44)</td>
<td>48.4 ± 15.1</td>
<td>23–74</td>
</tr>
<tr>
<td>SBP (n=37)</td>
<td>119.5 ± 14.4</td>
<td>97–163</td>
</tr>
<tr>
<td>DBP (n=37)</td>
<td>77.8 ± 9.1</td>
<td>63–103</td>
</tr>
<tr>
<td>BMI (n=42)</td>
<td>26.9 ± 4.1</td>
<td>19.3–42.6</td>
</tr>
</tbody>
</table>

Age in years, SBP (systolic blood pressures) and DBP (diastolic blood pressure) in mm Hg, BMI (body mass index) in kg/m².
heritable forms of polycystic kidney disease (PKD). We used the touch preparation technique in renal cysts rather than using histological sections of kidneys because sectioning of renal tissue yields multilayers of renal epithelial cells with transected nuclei, which confound FISH analysis. In contrast, the touch preparation provides monolayers of intact nuclei. The same approach was undertaken in our previous work examining aneuploidy in the human vascular endothelium.7 As shown in Figure 1, the nuclei of renal epithelial cells in situ exhibited an identical morphology and size as the nuclei scored in the urinary sediment. Thus, the urinary cells used for the FISH scoring were almost certainly derived from the renal tubular epithelium.

Chromosome 1 scoring served as an internal control for that of chromosome 16. Only well-defined nuclei exhibiting distinct fluorescent signals were scored. The following signals were scored for each chromosome: 1 signal (monosomy), 2 signals (disomy), 3 signals (trisomy), and 4 signals (tetrasomy). The superimposition of signals from chromosomes 16 (green) and 1 (orange) generates a white fluorescent signal and indicates chromosome fusion. This was occasionally observed in several urinary specimens and in renal cysts (illustrated in Figures 1A and 1B, 2B, and 3B). To examine the presence or absence of the p arm of chromosome 16 in the fused chromosomes, an additional set of probes for the subtelomere region of the p arm of chromosome 16 (Vysis, Inc) was included with centromere probes for chromosomes 1 and 16. In these studies, 50% of the manufacturer’s recommended amount of probe was labeled with SpectrumOrange and 50% was labeled with SpectrumGreen. This combination of subtelomeric probes resulted in small, achromatic (white) fluorescent signals adjacent to generally larger fluorescence signals associated with the centromeric probe for chromosome 16 (Figure 2).

Chromosomal scoring was performed by an experienced cytogeneticist who was not aware of the working hypothesis and was not given any information about the donors. Slides were visualized with a ×100 objective and an Ultraprobe Cytovision Imaging System (Applied Imaging). Urinary specimens with <100 scored cells were excluded.

Statistical Methods
The proportions of scored cells that showed monosomy and aneuploidy for chromosomes 1 and 16 were calculated for each urinary specimen. The signed rank test was used to compare the proportion with monosomy for chromosome 1 to the proportion with monosomy for chromosome 16 within each specimen, by using the specimen as the analysis unit. Similarly, the signed rank test was used to compare the proportions with monosomy and trisomy for chromosome 1 within specimens and the proportions with monosomy and trisomy for chromosome 16. Possible association of aneuploidy with subject characteristics (age, BMI, systolic and diastolic blood pressure) was evaluated by means of Spearman’s rank correlation coefficient. Association of race with measures of chromosome loss was tested by means of Wilcoxon’s rank sum test. SAS version 8.2 was used for these analyses. All probability values are 2-tailed.

Monosomy for chromosomes 1 and 16 observed within 4 renal cyst samples were recorded as mutually exclusive events on a per cell basis because no cell exhibited monosomy for both chromosomes 1 and 16. A multinomial model was used to test the hypothesis that monosomy for chromosome 1 and monosomy for chromosome 16 occur at different rates. The model included separate parameters for each cyst. Conditional binomial tests of greater monosomy on chromosome 16 than on chromosome 1 were performed for each cyst. The four 1-sided probability values were transformed to $\chi^2$. 

Figure 2. Illustrations of chromosomal signals for 1, 16, and the subtelomere region of the p arm of chromosome 16 in renal epithelial cells. A, Example of a normal cell with 2 signals for chromosome 1, 2 signals for chromosome 16, and 2 signals (white) for the p arm of chromosome 16. B, Example of chromosome 1 and 16 fusion and presence of 2 signals for the subtelomere region of chromosome 16. Small and large white signals adjacent to each other represent the subtelomere region of the p arm of chromosome 16 and fusion of chromosomes 1 and 16, respectively.

Figure 3. Illustrations of chromosomal signals in scored cells from urinary sediment. A, Cell showing 2 signals each for chromosomes 1 and 16. B, Cell with fusion of chromosomes 16 and 1, shown in 43% of the scored cells of the urine donor. C, Two cells with monosomy of chromosome 16. D, Cell with monosomy of chromosome 1. E, Cell showing rare tetrasomy (4 signals) for chromosome 16. F, Two cells, one of which shows trisomy (3 signals) for chromosome 1.
variates with 2 df and combined into a composite $\chi^2$ with 8 df. The probability value for the composite $\chi^2$ test was doubled to represent the probability of 4 results in the opposite direction (greater monosomy on chromosome 1 than on chromosome 16). The criterion for statistical significance was $P<0.05$.

Results

Chromosomal Scores in Epithelial Cells of Urinary Sediments

The number of scored cells per each urinary specimen ranged from 103 to 759. The proportion of cells that showed aneuploidy for chromosome 16 ranged from 0.6% to 8.6% (median 1.7%). Fifteen urinary sediment specimens had no cells with aneuploidy for chromosome 1; the maximum proportion was 3.2%, and the median was 0.6%. Figure 3 illustrates scored cells with different forms of aneuploidy for chromosomes 16 and 1. Monosomy for chromosome 16 accounted for the majority of cells with aneuploidy for this chromosome; only 5 specimens showed trisomy for chromosome 16. The excess of monosomy over trisomy for chromosome 16 was statistically significant at $P<0.001$ (signed rank test). Trisomy for chromosome 1 was observed in 17 specimens, affecting a maximum of 2.9% of cells. Nevertheless, monosomy for chromosome 1 was more prevalent than trisomy ($P=0.04$). One specimen exhibited tetrasomy for chromosome 1 in 2 cells.

Figure 4 illustrates the predominance of aneuploidy (A) and monosomy (B) for chromosome 16 versus aneuploidy and monosomy for chromosome 1 in renal epithelial cells of urinary sediments. Difference is significant at $P<0.001$ for both A and B.

Figure 5. Illustrations of cells in urinary sediment with apparent micronuclei. A, Cell with 2 signals for chromosome 1 and 2 signals for chromosome 16. B, Cell showing apparent micronucleus without detectable signal. C, Cell with apparent micronucleus with a signal for chromosome 16. D, Cell showing apparent micronucleus with signal for chromosome 1.

Aneuploidy for chromosome 16 was significantly more prevalent than aneuploidy for chromosome 1 (within specimens signed rank test $P<0.001$; Figure 4A). Of the 49 specimens, 42 had a higher proportion with aneuploidy for 16 than for chromosome 1, and only 2 had a higher proportion with aneuploidy for chromosome 1. The preeminence of monosomy for chromosome 16 over monosomy for chromosome 1 was even greater (Figure 4B): 46 specimens had a higher proportion with monosomy for chromosome 16, and only 1 had a higher proportion for chromosome 1 monosomy ($P<0.001$).

The initial scoring of urine specimens from 3 subjects identified fusion (ie, a white signal) in 43%, 26%, and 21% of scored cells, indicating fusions of chromosomes 1 and 16. An additional specimen identified fusion in 43% of 4 additional urine specimens obtained from another subject. The subject originally showing 43% fusion demonstrated 30% fusion in a subsequent urine sample. The subject originally showing 26% fusion showed 13%, 8%, 18%, and 14% fusion on subsequent urine samples taken several months after the original sample.

Eight of the urinary sediments showed cells that exhibited what appeared to be “micronuclei,” that is, round nuclear extensions of which some appear to be separated from the larger nuclear structure (illustrated in Figure 5). The highest number of micronuclei per specimen was 3, with a total of 13 for all scored cells (0.1%). Two of the micronuclei contained the signal for chromosome 16 and 2 contained the signal for chromosome 1. The remaining micronuclei showed no chromosomal signal.

There were no associations between proportions of cells exhibiting aneuploidy with blood pressure, BMI, or race.

Chromosomal Scores of Epithelial Cells of Renal Cysts

Table 2 shows preponderance of monosomy for chromosome 16 over monosomy for chromosome 1, which was significant at $P=0.024$ ($\chi^2, 8$ df = 19.6). In addition, 2.3% of the cells showed fusion of chromosome 1 and 16. To further explore the nature of this fusion, we used subtelomeric fluorescent probes for chromosome 16, which assesses the integrity of the
p arm of chromosome 16. All fused cells that were probed with the use of this approach showed two subtelomeric signals (Figure 2), indicating that the fusion of chromosome 1 and 16 in renal epithelial cells was not associated with the loss of the p arm of chromosome 16.

Discussion

Our results indicate that the rate of loss of chromosome 16 from renal epithelial cells far exceeded that of chromosome 1. Moreover, monosomy of chromosome 16 was not a rare event, either in epithelial cells of the urinary sediment or cells of renal cysts. The loss of chromosome 16 signal was unlikely to be an artifact resulting from the superimposition of 2 signals from chromosome 16 or from damage to the nucleus because our scoring system included chromosome 1 as an internal control. If the loss of chromosome 16 was due to technical reasons or to chance, one would expect that the frequency of loss of chromosome 16 would not differ from that of chromosome 1, given that the signal intensities of the probes for chromosomes 1 and 16 were roughly the same.

Fusion of chromosomes 16 and 1 was reproducibly observed in the urinary sediments from 2 of 42 adults and was also present in a third subject. In addition, fusion of chromosomes 16 and 1 was found in renal cysts. This fusion was not associated with the loss of the p arm of chromosome 16. It has been shown that the fusion of chromosomes 16 and 1 may result from hypomethylation of the main satellite DNA associated with pericentric heterochromatin of these chromosomes. The cause of this fusion in subsets of epithelial cells in our subjects is unknown.

On rare occasions, we found what appeared to be micronuclei, a subset of which exhibited the signals for chromosome 16 or chromosome 1. The presence of micronuclei has been proposed as an index of chromosomal damage. These nuclear bodies carry whole chromosomes or eccentric chromosome fragments. It is noteworthy, however, that the visualization of micronuclei was done without the cyto-kinetic-block technique. Thus, it is possible that the structures we observed are not true micronuclei, which are usually found in cells shortly after cell division. Some of these cells may represent the precursors to aneuploid cells exhibiting loss of chromosomes 1 and 16.

Taken together, a sizeable proportion of renal epithelial cells in the urinary sediment exhibited aneuploidy, chromosomal fusion, and perhaps micronuclei. We cannot exclude the possibility that cells that express these changes were in fact cells that were eliminated because of their chromosomal abnormalities. However, we also observed monosomies of chromosomes 16 and 1 and fusion of these chromosomes in renal epithelial cysts. These findings suggest that aneuploidy is a feature of renal epithelial cells in situ. Furthermore, aneuploidy is seen in cultured mammalian cells, indicating that subsets of these cells, which have gained or lost chromosomes, are viable. It follows that even if only a few cells that develop aneuploidy are retained by the kidneys, their potential effect might be considerable. We did not observe age-dependent increase in aneuploidy of chromosomes 16 and 1 in the urinary sediment; given that this is a cross-population rather than a longitudinal study, one cannot determine with certainty whether observed aneuploidy increases with age for individual subjects. The results suggest, however, that at any given time a portion of renal epithelial cells exhibit aneuploidy.

The greater frequency of monosomy for chromosome 16 than chromosome 1 agrees with the concept that the survival potential of cells that lose chromosomal material is inversely proportional to the amount of lost DNA. The loss of a small chromosome would therefore be more compatible with an extended time period of cell survival and a better chance of detection of the loss, whereas the loss of a large chromosome would promptly lead to cell death, thereby diminishing the chance of detection of aneuploidy. It has also been proposed that the high frequency of observed loss of small chromosomes in metaphases might be artifactual. This concern, however, does not apply to our research, which was performed with FISH in interphase cells.

Aneuploidy and LOH can theoretically contribute to diseases other than cancer, including PKD, in which LOH was observed in renal cysts. The majority of patients with PKD harbor a variant gene, namely PKD1, on the p arm of chromosome 16. However, whether aneuploidy and LOH may be determinants in the diverse phenotypic expressions of complex genetic traits such as essential hypertension is unclear at present.

The multigenic nature of essential hypertension suggests that although the effects of variant genes that cause this disorder are individually weak, in the aggregate they can substantially raise blood pressure. For instance, Liddle’s syndrome is a monogenic form of human hypertension that occurs because of an augmented sodium reabsorption by the kidney caused by variant genes of α- and γ-subunits of the amiloride-sensitive, epithelial sodium channel, which were mapped to chromosome 16. Attempts to link the same and other variants of these genes to essential hypertension, a

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**TABLE 2. FISH Analysis of Renal Cysts Using Chromosomes 1 and 16 Centromeric Probes**

<table>
<thead>
<tr>
<th>Renal Origin of Cyst</th>
<th>Chromosome 1</th>
<th>Chromosome 16</th>
<th>Chromosome 16 Fusion</th>
<th>Other*</th>
<th>Chromosome 1 and 16 Disomy</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydronephrotic</td>
<td>1 (0.3)</td>
<td>4 (1.3)</td>
<td>7 (2.3)</td>
<td>—</td>
<td>291 (96.0)</td>
<td>303</td>
</tr>
<tr>
<td>Dysplastic</td>
<td>4 (1.7)</td>
<td>7 (2.9)</td>
<td>1 (0.4)</td>
<td>—</td>
<td>226 (95.0)</td>
<td>238</td>
</tr>
<tr>
<td>Polycystic (non-PKD)</td>
<td>2 (0.7)</td>
<td>10 (3.4)</td>
<td>9 (3.1)</td>
<td>1 (0.3)</td>
<td>270 (92.5)</td>
<td>292</td>
</tr>
<tr>
<td>Polycystic (non-PKD)</td>
<td>2 (1.6)</td>
<td>8 (6.5)</td>
<td>5 (4.0)</td>
<td>1 (0.8)</td>
<td>108 (87.1)</td>
<td>124</td>
</tr>
<tr>
<td>Total</td>
<td>9 (0.9)</td>
<td>29 (3.0)</td>
<td>22 (2.3)</td>
<td>2 (0.2)</td>
<td>895 (93.5)</td>
<td>957</td>
</tr>
</tbody>
</table>

*Other includes chromosomes 16 disomy/1 trisomy.

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One cyst from each patient was studied. Data represents the number of cells scored per cyst and percentage (%).
complex genetic trait in human beings, have been mixed.26–30 One potential explanation for this lack of consensus is that the contribution of variant genes that are present on chromosome 16, as well as on other chromosomes in the general population can be expressed only with LOH. New genetic paradigms will therefore be necessary to incorporate the role of the "second hit" in essential hypertension.

Acknowledgments
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References

Gardner et al Aneuploidy of Chromosome 16 933
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