A Kindred Exhibiting Cosegregation of an Overlap Connective Tissue Disorder and the Chromosome 16 Linked Form of Autosomal Dominant Polycystic Kidney Disease

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ABSTRACT
Autosomal dominant polycystic kidney disease (ADPKD) is a disorder of adult onset manifested by bilaterally enlarged cystic kidneys frequently associated with progressive renal failure. The mutated gene (PKD1) responsible for 85 to 95% of cases has been localized to a small segment on the distal tip of the short arm of chromosome 16. A clinical spectrum of heritable connective tissue disorders that remain unclassifiable under the present nosology but that contain elements of the Marfan's syndrome have previously been described. The genetic localization and molecular basis of such overlap connective tissue disorders (OCTD) have not been elucidated. In this report, a kindred in which ADPKD and OCTD appear to cosegregate is described. The connective tissue phenotype in this family includes aortic root dilation, aortic and vertebral artery aneurysms with dissection, and aortic valve incompetence, as well as pectus abnormalities, pes planus, joint laxity, arachnodactyly, scoliosis, dolichostenomelia, and high arched palate. ADPKD was manifest primarily as bilateral renal cysts with or without renal failure. The DNA of all living family members was studied with markers recognizing polymorphic loci flanking the PKD1 region (3'HVR and O90a), as well as markers from the loci of chromosomes 15 and 5, associated with fibrillin genes FBN1 and FBN2, respectively. In this kindred of 20 family members traced through five generations, cosegregation of ADPKD and the OCTD phenotype was observed in 12 of 12 males and 3 of 3 phases known. Both markers for PKD1 were tightly linked to both ADPKD and OCTD, whereas there was no evidence for linkage with either fibrillin locus. In this family, the ADPKD and OCTD mutations are genetically linked. The presence of OCTD with ADPKD identifies a group of patients at significantly greater risk for sudden death from aortic root and other vascular aneurysmal dissection and rupture.

Key Words: Overlap connective tissue disorder, autosomal dominant polycystic kidney disease, PKD1 chromosome 16, genetic, aneurysm

Autosomal dominant polycystic kidney disease (ADPKD) is the most common form of inherited renal disease, occurring with a frequency of 1 in 1,000 live births. It is transmitted as an autosomal dominant trait, manifested primarily by multiple bilateral renal cysts, frequently resulting in progressive renal failure.

The association of ADPKD with a number of extra-renal manifestations affecting both the gastrointestinal and cardiovascular systems has led to the hypothesis that ADPKD is a systemic disorder (1). The extrarenal cystic manifestations most commonly involve the liver but may also include cysts in the pancreas, spleen, testes, and ovaries. Cerebral vascular aneurysms occur more often in ADPKD patients, but the precise prevalence is difficult to determine (2, 3). When they occur, such aneurysms are a cause of considerable morbidity and mortality.
Cardiovascular involvement in ADPKD has also included a range of valvular defects, including mitral valve prolapse; mitral, tricuspid, and aortic valvular incompetence (4); annuloaortic ectasia (5); as well as aneurysms of the abdominal aorta (6–8). A retrospective analysis of 11 of 62 ADPKD patients with cardiovascular abnormalities revealed aortic root dilation with valvular incompetence in 7 (9). The observed frequency of dissecting aortic aneurysms coexisting with ADPKD in an autopsy series was found to be 7.3 times greater than would be expected with a chance association (10). It is uncertain if such findings are simply related to ESRD, irrespective of cause (11). These associations, as well as evidence of disordered synthesis of the extracellular matrix, have led authors to hypothesize that a defect in connective tissue may underlie the pathogenesis of ADPKD (4).

One of the difficulties in sorting out the true relationship of these pleiotropic manifestations to the underlying gene defect in ADPKD has been the sporadic nature of many of these extrarenal manifestations, even within families (12). In particular, none of the studies describing cardiovascular abnormalities in ADPKD patients, exclusive of cerebral vascular aneurysms, have noted a familial component to these extrarenal manifestations.

It has been estimated that up to 50% of patients referred for evaluation of heritable connective tissue disorders have enough clinical signs to suggest a systemic defect in the extracellular matrix, even though they do not fit the diagnostic criteria for a recognized syndromal pattern (13). The term “overlap” heritable connective tissue disorder (OCTD) has been suggested for describing such phenotypes that lie on the continuum between Marfan’s syndrome at one extreme and isolated mitral valve prolapse at the other (13). Here, we describe a family in whom an OCTD phenotype cosegregates with classic, chromosome 16-linked ADPKD.

METHODS
The Family
A kindred with ADPKD and an OCTD was identified by interviewing the proband, IV.5 (Figure 1). During a consultation for renal disease, she volunteered an extensive family history of aortic aneurysm as well as polycystic kidney disease. The entire kindred was subsequently analyzed with respect to both ADPKD and OCTD.
Connective Tissue Disorder

Complete medical histories were obtained, and physical examinations were performed on all living members in the kindred by two of the authors (G. Rutecki, A. Cugino) (Table 1). Inpatient and outpatient hospital records were reviewed, as were pathological data where available. All living members except for III.4 underwent routine echocardiography, with particular attention to aortic root dimensions and valvular function. Findings in individuals II.1 and III.2 were based on hospital records and autopsy data.

The diagnosis of OCTD was determined by the presence or absence of 16 of the original 18 clinical and echocardiographic signs for each subject (13): striae atrophicae, joint hypermobility, pes planus, metatarsus varus, high arch palate, height greater than the 95th percentile, arm span to height ratio of more than 1.03, upper to lower body segment ratio of less than the 5th percentile for age, ectopia lentis, mitral valve prolapse, and aortic root diameter to predicted aortic root diameter ratio of more than 2 or 3 SD above 1.0, respectively. We decided to include mitral valve prolapse, despite its common accompaniment of ADPKD per se (4). Because of the young age of many of the subjects evaluated in the fifth generation, we chose not to get x-rays to evaluate the family for thoracic lordosis. Clinically, this finding was not present in any family member. Because myopia is a common finding in the general population, we decided to eliminate this category of evaluation for this family. Glesby and Pyeritz (13) have found that the mean number of signs (±SE) for OCTD was 6.1 (±2.9), whereas normal controls had 1.3 (±1.3). In this study, individuals with six or more signs (8 ± 0.7) or those with aortic dissection plus any other sign were classified as positive for OCTD, whereas those with three or fewer signs (1.8 ± 0.4) and no aortic dissection were classified as negative.

ADPKD

All living members of the kindred underwent renal ultrasonography. The criteria used for the diagnosis of polycystic kidney disease were multiple cysts in both kidneys and bilateral kidney enlargement (14).

DNA-Based Analyses

The DNA probes for the PKD1 region have been described previously (15, 16). Briefly, 3'HVR (D16S85) is a highly polymorphic noncoding segment located 8 kilobases (kb) 3' to the end of the α-globin genes. A variable number tandem repeat element accounts for the heterozygosity of 95% observed at this locus. In this kindred, nine alleles were identified. The probe O90a (D16S45) recognizes alleles of 23 and 15 kb in EcoRI-digested genomic DNA; the heterozygosity at this locus is ~50%. The loci recognized by 3'HVR and O90a have been shown to flank the PKD1 locus (16).

To evaluate the relationship of the OCTD to Marfan's syndrome and to a phenotypically related syndrome, congenital contractual arachnodactyly, this family was also studied with polymorphic markers from chromosomes 15 and 5, which are tightly linked with the FBN1 and FBN2 members of the fibrillin gene family, respectively (17). MF-13 (gift of Dr. F. Ramirez; 17) is a 1.6-kb cDNA for FBN1 that recognizes a TaqI restriction fragment length polymorphism with allele sizes of 5 and 6 kb. DNA probe hybridizations were carried out as previously described on panels of PvuII-, EcoRI-, and TaqI-digested genomic DNA for 3'HVR, O90a, and MF-13, respectively (16).

Genotyping by use of the polymerase chain reaction (PCR) with primers flanking simple sequence repeats was also performed for both the FBN1 and FBN2 loci. Primers described by Lee et al. (17) were used: FBN1: CCTGGCTACATTCAACTCCCC and GAGTACATAGGTGTATAGGG; FBN2: AAGGTGTTCTTTGCATGTTCACC (23 mer) and GTAATGTGTACCCTCTTTCTCTAGTTCAAC (25 mer). These pairs flank (TAAAA)n and (GT)n repeats, respectively. PCR reactions (25 μL) contained 50 to 100 ng of genomic template, 200 μM dNTP, 1.5 mM MgCl2, 0.625 U of Taq DNA polymerase with standard reaction buffer, and 2 μCi of [32P]dCTP per reaction. FBN1-linked primers were used at 1 μM each, whereas FBN2-linked primers were used at 0.1 μM (23 mer) and 1.0 μM (25 mer), respectively, to decrease "slippage." Temperature cycling after an initial 5-min 94°C incubation was for 1 min each at 94, 65, and 72°C for 10 cycles, followed by 25 cycles with 60°C annealing. There was a final 10-min 72°C extension.

Alleles were analyzed by the running of 1 μL of the PCR products in sequencing "stop" buffer (USB, Cleveland, OH) on standard 5% denaturing sequencing gels. This kindred had four alleles of the FBN2-linked dinucleotide repeat and two alleles of the FBN1-linked pentanucleotide repeat.

Linkage Analysis

Linkage analysis was performed with version 5.1 of the LINKAGE programs compiled for DOS (18). The locus order for the chromosome 16 studies was set as centromere—O90a—PKD1—3'HVR—telomere. The sex-specific recombination fractions between PKD1 and the markers O90a and 3'HVR were fixed as previously described (16): sex-averaged recombination fractions were used with the nonchromosome
TABLE 1. Phenotypic features of individual members of the kindred<sup>a</sup>

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<th>Aortic Dissection</th>
<th>Pectus Deformity</th>
<th>Dollcho-stenomaenia</th>
<th>Arachnoidactyly</th>
<th>High Arched Palate</th>
<th>Joint Laxity</th>
<th>Pes Planus</th>
<th>Positive Thumb Sign</th>
<th>Arm Span &gt;1.03</th>
<th>Mitral Valve Prolapse</th>
<th>Height &gt;95th Percentile</th>
<th>Upper Segment/Upper Segment &lt;5th %</th>
<th>Aortic Root OCTD</th>
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<sup>a</sup> +, present; 0, absent; U, unknown; MVP.

<sup>b</sup>Vertebral artery aneurysm.
16 markers. The frequency of the PKD1 allele was set at 0.001 and that of the OCTD was set at 0.0001. We used marker allele frequencies from previous studies (19) for Q90a and assigned equal allele frequencies for 3’HVR, MF13, and the FBN2 dinucleotide repeat. The FBN1 pentanucleotide repeat alleles were in complete disequilibrium with MF-13 and were not used further in the linkage analysis.

Individuals were assigned to one of three liability classes for the diagnosis of ADPKD on the basis of age: >30 yr, 20 to 30 yr, and 15 to 20 yr, with respective penetrances of 0.95, 0.72, and 0.32 (14). Unaffected family members younger than 15 yr old were eliminated from the calculations. OCTD was assumed to have penetrance of 0.95 at an age of over 30 yr, and unaffected individuals under 30 were excluded from the analysis.

RESULTS

Clinical

The OCTD manifestations in this kindred are listed in Table 1. The most striking cardiovascular manifestation noted was thoracic aortic aneurysm with dissection. One instance of vertebral artery aneurysm with dissection was also seen. Only signs of OCTD present in the kindred are listed in Table 1. Of note, there were no lenticular ocular findings suggestive of classical Marfan’s syndrome and no members of the kindred had scoliosis, striae atrophicae, or wrist sign. The mean number of signs for patients evaluated with OCTD (and ADPKD) was 8 ± 0.7. In the family members who had neither disease, the mean number of signs of OCTD was 1.8 ± 0.4.

In the first generation, I.1 was reported by family members to have an asthenic build and to have died in her early 40s of a “heart attack.” No other information is available, so her disease status is unknown. A dissecting aortic aneurysm and polycystic kidneys were described at autopsy in patient II.1, and historical data suggested asthenic build and joint laxity. Therefore, we included her as positive for both OCTD and ADPKD.

In the third generation, we examined III.1, III.4, and III.5 and found no evidence of either disease. Hospital records of patient III.2 indicated that he was dialysis dependent when he had repair of a dissecting ascending aortic aneurysm with aortic valve replacement 5 years before his death at age 43. He had bilaterally enlarged, tender kidneys along with several skeletal features of OCTD.

The proband, patient IV.5, has polycystic kidney disease by renal ultrasound criteria and several skeletal manifestations of OCTD. Patient IV.3 is illustrative of the major cardiovascular complications seen in this kindred:

A 31-yr-old white man was admitted to the hospital in 1985 for midsternal chest pain of 1 day’s duration. A physical examination demonstrated a blood pressure of 150/90 mm Hg with a transient episode of hypotension. A cardiac examination revealed a diastolic II/VI decrescendo murmur at the aortic area transmitted to the left sternal border and a two-component pericardial friction rub.

A radiograph of the chest was normal, and an electrocardiogram showed sinus rhythm with left axis deviation. Initial laboratory studies included a creatinine of 141.1 μmol/L, urea nitrogen of 6.8 mmol/L, and hematocrit of 33%. A cardiac echogram showed ascending aortic dilatation, moderately severe aortic insufficiency, mild left ventricular hypertrophy, and mitral valve prolapse. Cardiac catheterization studies revealed a markedly dilated proximal aorta and anatomically normal aortic cusps with aortic insufficiency secondary to ascending aortic dissection.

He underwent emergency aortic arch surgery for graft replacement of the ascending aorta with retention of the native aortic valve. A pathologic examination revealed the aortic root above the coronary ostia to be 34 mm in diameter with cystic medial necrosis of the vessel wall. There was evidence for two healed areas of previous dissection.

Additional findings on physical examination included marked joint instability (fingers, wrists, and hips), pes planus, arachnodactyly, dolichostenomelia (span to height ratio, 1.04), a positive “thumb sign,” and a high arched palate. Abdominal ultrasound showed bilaterally cystic kidneys as well as hepatic cysts. Progressive renal insufficiency ensued. In 1991, a severe headache led to a selective angiographic study and a diagnosis of a left vertebral artery dissecting aneurysm opposite C1. This has been managed conservatively and has led to no further morbidity.

Genetics

In the first four generations of the pedigree (Figure 1), OCTD coexists with polycystic kidney disease in every case in which a diagnosis of either disease can be made. In these four generations, there are four individuals with both diseases (II.1, III.2, IV.3, IV.5), five individuals at risk for both diseases but having neither disease, one individual with unknown risk having neither disease, and no individual having one disease without the other.

In assessing the fifth and youngest generation, one
must consider that, because the age-dependent penetration of either disease has not been established in this age group, the probability of correctly determining whether an individual carries a disease mutation from clinical investigation alone is not known. In the case of ADPKD, it is clear from previous reports that a false-negative clinical diagnosis is not uncommon in the first two decades of life (14).

With these caveats in mind, an inspection of generation five reveals that the two diseases cosegregate in at least two individuals (V.2 and V.3) and the phenotypic defects of either disease are absent in three individuals (V.4, V.5, V.6). The clinical picture of OCTD is seen in the absence of ultrasonographic evidence of ADPKD in a 5-yr-old individual, V.1. DNA analysis, described below, suggests that V.1 is a carrier for the PKD1 mutation, whereas the other three unaffected members of this generation are not.

This kindred was studied with six probes from the PKD1 region, only two of which (3'HVR and O90a) were informative (Figure 1). Linkage analysis results are expressed as the odds of the likelihood of linkage at a given recombination fraction \( \theta < 0.5 \) versus the likelihood of no linkage \( \theta = 0.5 \). The logarithm of the odds ratio is termed the LOD score. A LOD score of more than 3.0 \( (>10:1 \text{ odds in favor of linkage}) \) between two loci is usually taken as proof of linkage in the absence of prior knowledge about their location.

Multipoint analysis of ADPKD with respect to 3'HVR and O90a in this kindred yielded a LOD score of 2.36 at the previously defined sex-specific recombination fractions (16). Because the prior probability of linkage between these markers and ADPKD is between 85 and 95\% (20-22), this LOD score is highly significant, and in any individual where both flanking markers segregate together, the polycystic kidney disease status of that individual can be predicted with a certainty of more than 99.9\% (16). Thus, this family has the PKD1 form of ADPKD and individual V.1 has the ADPKD mutation, whereas V.4, V.5, and V.6 are unaffected.

In order to test whether the apparent association between ADPKD and OCTD in this kindred is merely a chance finding, two-point and multipoint linkage analyses were performed. Two-point analysis indicated that OCTD was tightly linked to 3'HVR, O90a, and ADPKD (Table 2). The maximum LOD scores for each locus pair occurred at \( \theta = 0 \), indicative of the fact that there were no recombinations between OCTD and the three loci studied. The odds for linkage to 3'HVR, O90a, and PKD1 were 355.1, 30:1, 912:1, corresponding to LOD scores of 2.55, 1.48, and 2.96, respectively. The latter almost achieved the 1,000:1 odds often accepted as proof of linkage.

Although these data suggest tight linkage between OCTD and the PKD1 locus, with a most likely recombination fraction of zero, it should be noted that too few meioses were available for study to allow an accurate estimation of the true recombination fraction. The 99\% confidence limit for the sex-averaged recombination fraction is 0.17.

Multipoint analysis allows linkage of all four loci to be considered simultaneously. The position of OCTD is varied along a fixed genetic map of PKD1 and its two flanking markers, on the basis of sex-specific recombination fractions from previous studies (16). The maximum LOD score obtained is 3.23. Although this value corresponds to the condition in which the OCTD locus is at the same point as the PKD1 locus, there are insufficient crossovers in this family to position the OCTD locus precisely. The corresponding odds in favor of linkage is 1.698:1.

Two inherited connective tissue disorders, Marfan's syndrome and congenital contractual arachnodactyly, have been linked to the genes for fibrillin (FBN1, chromosome 15) and its homolog (FBN2, chromosome 5), respectively (17). To formally exclude these two disorders from consideration as a cause of the OCTD in this family, we performed linkage analysis with markers tightly linked to each locus. Both showed recombination with OCTD in this family (Figure 2) and generated LOD scores of -5.07 and -10.49, respectively, at \( \theta = 0 \). These markers have never shown recombination with their respective disease entities (17, 23).

An analysis of the metaphase chromosomes of the proband, IV.5, and her son, V.3, revealed normal 46,XX and 46,XY karyotypes, respectively, under standard Giemsa banding analysis.

<table>
<thead>
<tr>
<th>Locus 1</th>
<th>Locus 2</th>
<th>LOD Score at Recombination Fractions (( \theta )) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>OCTD*</td>
<td>3'HVR</td>
<td>2.55</td>
</tr>
<tr>
<td>OCTD</td>
<td>O90a</td>
<td>1.48</td>
</tr>
<tr>
<td>OCTD</td>
<td>PKD1</td>
<td>2.96</td>
</tr>
</tbody>
</table>

* Female recombination/male recombination (\( \delta_t/\delta_m \)) = 0.14.
DISCUSSION

Booth et al. (24) were the first to describe a case of ADPKD occurring in an individual with a connective tissue disease phenotype—possibly Marfan's syndrome without evidence of aortic disease. This was followed by two reports of individuals in whom polycystic kidneys and a "forme fruste" of Marfan's syndrome coexisted (25, 26), a case report of polycystic kidney disease, Marfan's syndrome without ocular findings and spina bifida occulta (27), and a recent report of dissecting thoracic aortic aneurysm and polycystic kidneys (28). In reviewing the clinical data in these reports, it is likely that most, if not all, represent points along the continuum of heritable connective tissue disorders proposed by Giesby and Pyeritz (13) and are not classic Marfan syndrome as defined in the Berlin nosology (29).

In one instance (25), several family members were examined and one sibling of the proband had polycystic kidneys without evidence of a connective tissue disorder, whereas the father died at age 68 of a ruptured cerebral aneurysm with only the diagnosis of arterial hypertension. In all of the other cases reported, the family histories were not discussed.

In this report, we present a clinically significant connective tissue disorder (OCTD) segregating as an apparent autosomal dominant trait in a kindred with ADPKD. The two disorders are genetically linked and colocalize to the PKD1 region on chromosome 16 with a high degree of certainty. In no instance did the two disease phenotypes segregate independently within this family.

The observed genetic linkage between the two disorders can be explained in a number of ways. It is possible that a single mutation affecting two or more members of a set of contiguous genes is responsible for both diseases. The normal karyotype observed in the two individuals makes large-scale alterations less likely. Molecular analyses using genomic and cDNA probes from the ADPKD region (G. Germino, Johns Hopkins University, personal communication) have not shown evidence of submicroscopic alterations affecting contiguous genes in this family (S. Somlo, unpublished data).

A second possibility is that the diseases are produced by independent but genetically linked mutations, possibly affecting different genes. It is possible that the two disease genes are linked at a finite recombination fraction and that no recombination was observed because the number of meioses is small. We have excluded linkage to Marfan's syndrome and congenital contractual arachnodactyly loci in this family, but there is evidence that related disorders, e.g., Marfan syndrome without ocular findings (30), map to independent loci, of which 16p13.3 may be one. Familial aortic aneurysm with dissection but no recognized disorder of connective tissue (31, 32) may bear consideration in this respect.

The frequent observation of cardiovascular abnormalities (4, 5, 9) in ADPKD raises a third possibility: that the disease in this family is an extreme form of ADPKD and that the skeletal and cardiac abnormalities we observed are an "extended" phenotype of a mutation in the PKD1 gene. Although this may be a likely interpretation, previous studies of the range of clinical features found in ADPKD do not allow us to extend the definition of this disease to include all of the cardiac and skeletal features seen in this family. Thus, it is not possible to exclude this family from consideration as part of the spectrum of clinical connective tissue disorders that remain unclassifiable and have been termed "overlap" syndromes. These several possibilities are unlikely to be resolved until the molecular basis for each aspect of the phenotype seen in this family is determined.

With all of the attendant caveats considered, this study is the first in which a family with inherited predisposition to aortic aneurysm and skeletal features of connective tissue disorder as well as ADPKD was studied from both a clinical and a genetic perspective. From a genetic standpoint, it would seem appropriate to consider the possibility of mutation in this chromosomal region in families, given the pleotropic, and likely heterogeneous, diagnosis of OCTD. From a clinical perspective, given the possibility that this is an extreme manifestation of ADPKD, we feel that it is critical to identify this subset of ADPKD patients. The suggestion of a coexisting OCTD in a family with ADPKD should lead to screening echocardiography, with particular attention to the ascending aortic root.

REFERENCES

1. Gabow PA: Autosomal dominant polycystic kid-