A large TSC2 and PKD1 gene deletion is associated with renal and extrarenal signs of autosomal dominant polycystic kidney disease

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Abstract

Background. The renal lesions in tuberous sclerosis complex (TSC) consist in multiple angiomyolipomas, often associated with cysts of variable size. Recently a few TSC patients with early-onset renal cysts resembling the autosomal dominant polycystic kidney disease (ADPKD) have been described. Virtually all of them showed deletions of both TSC2 and PKD1 genes.

Methods. Two unrelated families in which TSC and PKD co-segregate were investigated. 16p13.3-linked haplotype segregation, Southern blot, pulsed field gel electrophoresis, and loss of heterozygosity analyses were performed in both affected and unaffected family members.

Results. The proband from family 1 was first recognized as presenting typical neurological signs and skin lesions of TSC and multiple renal cysts at 12 years of age. Haemodialysis became necessary at age 28. CT and MRI scans revealed multiple cysts in the liver and an asymptomatic, 3–4 mm aneurysm of the middle cerebral artery. His mother, who died at 47 of breast cancer, had ADPKD and reached the ESRD at 42. She showed facial angiofibromas. Both patients carried a submicroscopic germline deletion spanning the entire TSC2 gene and the large majority of PKD1 coding sequence. In the proband from family 2, the TSC diagnosis was made at 4 years. Enlarged polycystic kidneys causing end-stage renal failure at 19 years were observed. This patient carried a large germline, de novo deletion involving the entire TSC2 and PKD1 genes. In addition we could show in a renal hamartoma from this subject the loss of heterozygosity of markers spanning the TSC2 and PKD1 genes from the residual, normal chromosome 16 of paternal origin.

Conclusions. The presence of a deletion involving both TSC2 and PKD1 genes should be considered in the clinical assessment of TSC children with an early-onset polycystic kidney disease, and more generally in all ADPKD patients who develop end-stage renal failure prior to the fourth or fifth decade of life. Finally, the occurrence of typical renal and extrarenal signs of ADPKD in a PKD1 hemizygote individual seems to support the concept that a somatic inactivation of the residual PKD1 gene is required for the development of the cysts.

Key words: autosomal dominant polycystic kidney disease; contiguous TSC2 and PKD1 gene syndrome; loss of heterozygosity analysis; tuberous sclerosis complex

Introduction

TSC is an autosomal dominant hamartomatosis with multisystem involvement [1–3]. Renal lesions are multiple angiomyolipomas often associated with cysts of variable size [3–6]. End-stage renal failure is rare in TSC patients and only occasionally occurs before the third decade of life [7–12]. Recently a few sporadic TSC infants with early and multiple renal cysts, commonly seen in the advanced stages of ADPKD, have been described. The molecular defect in these patients appears to be a deletion disrupting the genes causing TSC and ADPKD, known as TSC2 and PKD1 respectively [13,14]. These two genes are located only a few nucleotides apart, in a tail-to-tail orientation, on chromosome 16p13.3 [15]. Here we report the clinical and molecular investigation of three patients from two families in which both TSC and ADPKD appear to co-segregate.

Subjects and methods

Patients

The patients with both TSC and PKD belonged to two unrelated Italian families. Family 1 was investigated in Brescia, family 2 in Bolzano. TSC diagnosis was according to the Gomez criteria [1]. The diagnosis of ADPKD was supported by the typical images at ultrasonography and/or computed tomography [16]. All available first-degree relat-
The *TSC2* and *PKD1* contiguous gene syndrome

DNA from the probands and their family members was extracted from peripheral blood leukocytes and from histological sections of renal angiomylipomas (patient II-8 of family 1 and patient II-1 of family 2) and amplified by PCR as previously described [17]. For the segregation analysis, 16p13.3-linked markers and two new intragenic insertion/deletion polymorphisms (paper in preparation), provisionally named *TSC2*-IVS2 and *TSC2*-IVS8, located in introns 2 and 8 of *TSC2* respectively, were used (Figures 1 and 2). The genomic location of the probes and the ClaI and MluI restriction site map is shown in Figure 5. Loss of heterozygosity (LOH) analysis was performed by comparing the genotypes found in the lesions with those present in the surrounding normal tissue and/or in peripheral blood leukocytes.

**Results**

**Clinical data**

The proband, a 30-year-old man (subject III-1 in the pedigree shown in Figure 1), was noted to have enlarged and polycystic kidneys at age 12, when first investigated for cerebral (epilepsy and moderate mental retardation) and skin (facial angiofibromas) stigmata of TSC. At 28 years, haemodialysis became necessary. Subependymal calcifications and cortical tubers were...
Fig. 2. Segregation of TSC2- and PKD1-linked polymorphic markers in family 2. The haplotypes found in a renal angiomyolipoma (AML) of the proband II-1 are shown at the left. \( \text{nd, not determined; ni, not informative.} \) According to PFGE data summarized in Figure 5, D16S259 and D16S665 markers are probably included in the deletion, but this evidence cannot be obtained by microsatellite analysis since the patient is not informative at those loci (asterisk). See note to Figure 1 for additional details of the symbols used.

![CT scans](image)

**Fig. 3a, b.** Abdominal CT scans. **a,** Enlarged polycystic kidneys; **b,** multiple liver cysts.

found at the CT scan of the brain (not shown). CT scan of the abdomen revealed, in addition to enlarged polycystic kidneys, multiple liver cysts (Figure 3a, b). Magnetic resonance angiography and cerebral arteriography showed a single asymptomatic aneurysm of 3-4 mm in diameter, located at the middle cerebral artery bifurcation (not shown). The mother (II-8) was diagnosed to have PKD at age 34, upon a urographic examination because of symptoms of renal insufficiency. Eight years later she began haemodialysis. At 44 years of age she underwent bilateral nephrectomy for urinary sepsis. Bilateral angiomyolipomas and enlarged cysts were found in her kidneys. She died 3 years later of breast cancer. No neurological symptom was reported, but only mild signs of facial angiofibromatoma. The father (II-9) died after a road accident at age 45. He never suffered any renal disease. Autopsy was not performed.

The other available family members (Subjects II-1, II-2, II-3, II-4, II-5, II-6, II-7) did not reveal any sign or symptom of either TSC or PKD.

### Molecular data

The haplotype segregation of eight 16p13.3-linked polymorphisms revealed that the proband did not receive any maternal allele at three closely linked loci, located in intron 2 and intron 8 of TSC2 (TSC2-IVS2 and TSC2-IVS8) and on the 3′UT tail of PKD1 (KG8) respectively (Figure 1). The family analysis indicated that a deletion on the maternal chromosome 16 of the d haplotype was transmitted to the proband, and that the mother carried the same defect. Indeed, the three maternal sibs (II-1, II-2 and II-3) showing the bd haplotype combination are heterozygous at KG8 (A4/A5), whereas the mother (II-8) who carries the same haplotypes shows only the A5 marker of the bd haplotype. Although TSC2-IVS2 and -IVS8 polymorphisms are not informative in the maternal sibs, these data strongly suggest that the 3′UT tail of PKD1 and probably the entire TSC2 gene have been lost from the d haplotype both in the mother and in the son.

PFGE analysis on the proband leukocytes allowed us to identify a 175 kb deletion within the 190 kb region flanked by two MluI fragments of normal size, i.e. a 75-kb, telomeric fragment, revealed by the CMM65 probe, and a 60-kb, centromeric fragment, detected by the SM6 probe (Figures 4 and 5). The H2 probe did not reveal any abnormal band, suggesting that it lies within the deletion. On the contrary, additional bands of 165 and 305 kb (the latter being a

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![Figure 2](image)
The TSC2 and PKD1 contiguous gene syndrome

**Fig. 4.** PFGE analysis of the deletions in the two probands (the DNA codes 107.3 and 40.3 here used correspond to patient III-1 of Figure 1, and II-1 of Figure 2 respectively). The probes and restriction enzymes used are indicated above and below the blots respectively. Lanes C contain control DNA from unrelated, healthy subjects. The proband 107.3 shows two novel ClaI fragments of 165 and 305 kb (asterisks) with the CMM65 and SM6 probes. As discussed in the results, the 305- and 480-kb bands present both in patients and controls are due to partial ClaI digestion. The H2 probe, being totally included in the 107.3 deletion, reveals only the germline MluI and ClaI fragments from the normal chromosome 16. The patient 40.3 does not show any abnormal fragment with either probe and restriction enzyme combination, suggesting that the 40.3 deletion includes the CMM65 and SM6 DNA segments (the physical extents of the two deletions are shown in Figure 5).

**Fig. 5.** Schematic representation of the TSC2 and PKD1 gene deletions. A map of the TSC2 and PKD1 region showing the size of the MluI and ClaI restriction enzyme fragments detected by the three probes used (solid boxes) is shown at the top (kb, kilobase; tel., telomere; centr., centromere). This restriction map is consistent with the genomic maps previously reported [13,20,21]. The transcriptional orientation of TSC2 and PKD1 is indicated by arrows. Due to partial digestion of the second ClaI site, a 480-kb (140 + 340) partially digested ClaI fragment appeared at PFGE. The black bars below the map represent the genomic extent of the germline deletions identified in the two families here described. The deletion present in the proband of family 1 (here coded 107.3) maps within the 190-kb fragment shown above. The telomeric and centromeric ends of the deletion found in the proband of family 2 (coded 40.3) are unknown. However the minimum length of this deletion should be approximately 260 kb, i.e. the physical distance between CMM65 and SM6 probes (see text for details).

partially digested product; see notes to Figures 4 and 5) appeared in ClaI blots, hybridized with CMM65 or SM6 probes. Subtracting the novel 165-kb fragment from the germline 340-kb fragment we obtained the approximate size of the deletion, i.e. 175 kb. Since only 15 kb of the 190 kb region mentioned above are spared by the deletion (190–175), no more than 2% of the PKD1 coding sequence should have been preserved. Indeed, on the basis of the PKD1 genomic structure [22,23], one may predict that all exons, with the exception of exon 1, should be located within the deletion. As shown in Figures 4 and 5, the PFGE analysis confirmed that that the entire TSC2 gene was lost from the deleted chromosome. Finally, four renal angiomyolipomas from the affected mother were tested for loss of heterozygosity at 16p13.3 markers, but no difference from the expected germline genotypes was observed (not shown).
Family 2

The proband, a 34-year-old man (subject II-1 in the pedigree shown in Figure 2), had the diagnosis of TSC at 4 years of age for the presence of epilepsy, mental retardation, facial angiofibromas and hypomelanotic maculae. At the age of 19 he developed end-stage renal failure requiring peritoneal dialysis. Ultrasonography revealed bilateral polycystic kidneys, and the brain CT scan showed calcified subependymal nodules (not shown). At age 21, he underwent renal transplantation. Both native kidneys were removed at age 28, due to retroperitoneal haemorrhage caused by rupture of a renal cyst. Multiple renal cysts and angiomyolipomas were found at the histological examination. The other family members (I-1, I-2, II-2 and II-3) were negative for both TSC and PKD signs and symptoms.

The first evidence for a gene deletion in the proband was the defective segregation of the TSC2-IVS8 marker. As shown in Figure 6, he received only the paternal allele (A1) and did not show any maternal contribution (A2 or A3 alleles). The segregation of additional 11 16p13.3-linked markers revealed that he had lost from the maternal haplotype (haplotype c in Figure 2) also the A1 and A2 alleles of the TSC2-IVS2 and KG8 loci respectively. This is consistent with a de novo deletion involving both TSC2 and PKD1 genes.

No abnormal bands appeared at PFGE, following the MluI and ClaI digestion and the three-probe hybridizations shown in Figure 4. These findings suggest that the probes should map entirely within the deleted region, whose minimum size should correspond to the approximate distance between CMM65 and SM6, i.e. 260 kb (Figure 5). The cytogenetic analysis on peripheral blood did not reveal any abnormality. As previously reported [17], one renal angiomyolipoma of the proband showed loss of heterozygosity at multiple 16p13.3 markers (HBAP1, D16S521, KG8, D16S291 and D16S663; Figure 2). The heterozygosity reappeared at D16S423. The alleles lost belonged to the paternal haplotype, the one not carrying the germline deletion described above. These findings indicate that the paternal chromosome 16 in the angiomyolipoma has acquired a somatic deletion within the terminal 16p13.3 band, spanning the two residual TSC2 and PKD1 genes, the other two alleles being germline deleted from the maternal chromosome.

Discussion

Renal disease is a major and often neglected complication of TSC, representing the commonest cause of death in patients over the age of 30 [24]. The renal impairment is a consequence of the gradual substitution of the normal parenchyma by multiple hamartomas (angiomyolipomas), which tend to grow both in number and size with age. In addition, isolated or multiple renal cysts are present in a third of the patients [5,25–27]; the latter lesions do not seem to expand with age [5]. Occasionally, end-stage renal disease may occur, but it is exceptional before the third decade of life [1,5]. In our personal series of 130 unrelated TSC probands, four reached the ESRD before their thirties; two of these probands and their first-degree relatives could be investigated both clinically and at the DNA level and their findings are the subject of this report.

Here we show that a large deletion spanning both TSC2 and PKD1 genes is present in these patients. Deletions involving these two genes have been recently found in a small subset of TSC patients showing early-onset, multiple renal cysts and/or an early decline of glomerular filtration rate [13,14]. This peculiar phenotype has been ascribed to a novel TSC2 and PKD1 contiguous gene syndrome [13]. Indeed, our probands from family 1 and 2, in addition to typical TSC skin and brain lesions, presented with an early-onset, polycystic kidney disease, resembling the classical picture of ADPKD. They entered dialysis at 28 and 19 years respectively. The mother of one proband (family 1) who died of breast cancer. FIG. 6. Lack of segregation of any maternal allele at the TSC2-IVS8 locus in the patient II-1 from family 2. Note that II-1 and II-3 carry the same haplotypes (see Figure 2). Ethidium bromide staining of PCR products containing the TSC2-IVS8 allelic variants, run in 2% agarose gel. The first lane on the left shows the size-markers (MspI-cut pBR322).
cancer, started dialysis at 42 years due to polycystic kidneys. She too carried the TSC2 and PKD1 gene deletion found in her son. Indeed, she was diagnosed as ADPKD. She had a mild facial angiofibroma, which was overlooked, and no apparent neurological symptoms. The correct TSC diagnosis was eventually made at the age of 44, on the basis of renal histology following nephrectomy for urinary sepsis.

Only a few examples of parent-to-offspring transmission of TSC2 and PKD1 gene deletions have been described so far [14]. In these cases, the polycystic disease in the parent was less severe than in the affected child and the renal function was preserved. Indeed, the transmitting parents were genetic mosaics for similar large deletions. The affected mother mentioned above showed a slightly less severe PKD phenotype compared to the son, but nevertheless she progressed to ESRD. The evidence for a germline deletion and against a somatic mosaicism is the total lack of the KG8 allele A4 on the d haplotype (see Figure 1), obtained by PCR on paraffin-embedded tissues from normal skin and lymph nodes, collected during breast cancer surgery. Given the high sensitivity of the PCR, we may conclude that the fraction of a putative normal, i.e. undeleted, cell population cannot be higher than 0.001, at least in the tissues examined. This could be the first report of a segregation of the TSC2 and PKD1 contiguous gene syndrome from a putative ‘germline deleted’ parent.

Interestingly, one of our patients with a large deletion (the proband of family 1) exhibited the full ADPKD phenotype, i.e. a cerebral aneurysm and multiple liver cysts. Extrarenal signs of ADPKD in the TSC2 and PKD1 contiguous gene syndrome have not been described so far. The latter findings expand our knowledge on the phenotype and pathogenesis of this rare syndrome, and more generally give us a clue on the possible genetic mechanism at the basis of the ADPKD phenotype.

The first point we would like to discuss is the reason that polycystic kidney disease is more severe in the TSC2 and PKD1 contiguous gene syndrome, as compared to the classical ADPKD. The simplest explanation is that of an additive or synergistic effect of deleterious mutations of two genes, TSC2 and PKD1, whose products play a crucial role in the development and probably in the maintenance of the renal architecture. Accordingly, any PKD1 mutation able to generate the ADPKD phenotype might add a deleterious effect in a carrier of a TSC2 mutation. The reason that most PKD1 defects in the contiguous gene syndrome are large deletions and not point mutations, as it commonly happens in ADPKD, is probably statistical: two independent point mutations in TSC2 and PKD1 have a lower chance of hitting the same cell than a single mutation involving both genes. If this is true, one can predict that (a) concomitant point mutations in both genes should occasionally occur. Indeed, a minority of TSC patients with early-onset PKD, not showing a contiguous gene deletion does exist [14]; (b) large or complete deletions of PKD1, like the ones found in the two families here described and in the TSC2-PKD1 contiguous gene syndromes reported by others [13,14], should be able to produce both the renal and extrarenal signs of ADPKD, analogously to the PKD1 point mutations so far identified in the classical ADPKD phenotype.

Though concomitant defects at the two contiguous loci should result in a worst prognosis, the extent of such an effect might vary in different individuals, even in the presence of the same TSC2 and/or PKD1 mutation. Indeed, dramatic intrafamilial variations of clinical phenotypes are known to occur in TSC [28] and in ADPKD as well, ranging in the latter disease from rare instances of death in utero [29,30] to asymptomatic >70-year-old subjects. The clinical data of the TSC2 and PKD1 contiguous gene syndromes so far reported are sparse [13]. Nevertheless, the progression toward the ESRD appears to vary significantly between the patients. In particular, the two affected members of the same family here reported started haemodialysis at 28 and 42 years respectively. In this context, one should not be surprised to see patients carrying a TSC2 and PKD1 defect showing no dramatic anticipation in the age of onset of ESRD. One such case may be the TSC patient who allowed the isolation of PKD1 [21]. This subject did not show a particularly severe polycystic kidney disease although he had lost one TSC2 allele and disrupted one PKD1 allele, due to an unbalanced chromosomal translocation.

Whether the dominant effect of the PKD1 mutation requires the expression of an abnormal protein which interferes with the function of the normal product expressed by the second PKD1 allele has been a matter of debate. It has been argued that most PKD1 mutations of the adult variety of PKD are compatible with the expression of an abnormal polycystin (dominant negative effect) [21,31–33]. However, the PKD1 mutations so far identified do not represent an unbiased sample, since virtually all map in the not repetitive 3' end of the gene, the only portion of PKD1 presently amenable to mutation analysis [33].

Our findings suggest that a large germline deletion of PKD1, yielding no abnormal polycystin, or a polycystin-derived N-terminal peptide probably too short for any biological significance (i.e. less than 2% of the size of mature polycystin), is sufficient to generate the renal and extrarenal signs of ADPKD.

It is tempting to speculate whether the ADPKD lesions, analogously to the TSC hamartomas [17,34], need the functional inactivation of both alleles. One allele would be hit by a germline mutation, the other would be inactivated by a somatic mutation in the post-zygotic life. As reported in a variety of tumour-suppressor-based genetic diseases which fit the ‘two hit’ model [35], a somatic point mutation is only one of the mechanisms that inactivate the second allele. Somatic recombination, deletion, or gene conversion events might as well result in the loss of the second allele and these phenomena can be revealed by the LOH of linked markers. Recently, LOH in individual cysts for two closely linked markers within the PKD1
gene has been reported [36]. In one renal angiomyolipoma from the proband of family 2, we could show the loss of 16p13.3–16pter alleles of paternal origin. Since the TSC2 and PKD1 genes had been deleted from the maternal chromosome due to a de novo, germline deletion, it follows that the hamartomatous lesion examined should not express any TSC2 or PKD1 gene product. We are at present testing whether polycystin-free cysts do exist in these kidneys. The patchy distribution of TSC and PKD lesions in kidney, liver and a variety of other tissues and the dramatic intra-familial phenotypic variability of both diseases, seem to support the two-hit mutation mechanism also for ADPKD.

We would like to emphasize the need for a vigilant search for TSC signs in individuals with ADPKD. We suggest that in the clinical assessment of young children with ADPKD and of adult ADPKD patients who develop end-stage renal failure at an earlier age than expected [37], the possibility of a contiguous TSC2–PKD1 gene syndrome should be considered. An early diagnosis may have important implications for patient management, in view of the increased risk of a retroperitoneal spontaneous rupture of a bloody cyst located in proximity to subcortical angiomyolipomas [3,9,10].

Finally, nephrologists need to be more aware of the risk of renal complications in TSC. Two major reasons support this view. First, the condition seems to be more widespread than once thought: there is an increasing recognition of affected adults who have neither seizures nor mental retardation [2,6]. Second, since the better management of neurological complications should improve the life expectancy of the TSC patients, renal involvement might become the major medical complication of TSC [5,6].

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