The Renin - Angiotensin System Gene Polymorphisms and Clinicopathological Correlations in IgA Nephropathy

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Abstract

Genetic variability in the renin-angiotensin system (RAS) may modify renal responses to injury and disease progression. We examined whether RAS alleles affect severity of IgA nephropathy. These genetic variants include angiotensin I converting enzyme deletion polymorphism in intron 16 (ACE I/D), a point mutation in the angiotensinogen (AGT) gene resulting in a methionine to threonine substitution at residue 235 (M235T) and an angiotensin receptor type I (ATR) A to C transition at bp 1166 (A 1166 C). A total of 53 patients with biopsy-proven IgA nephropathy and 80 normal control subjects were recruited for study. These patients were classified into two groups according to serum creatinine at renal biopsy. Group 1 patients (n = 40) had normal renal function, serum creatinine $\leq$ 1.5 mg/dl and group 2 patients (n =13) had renal insufficiency with serum creatinine > 1.5 mg/dl. The blood pressure and urinary protein of group 2 patients were higher than group 1 ($p < 0.01$). The mean scores of histological parameters including mesangial proliferation, glomerular sclerosis (global and segmental), the interstitial fibrosis and crescent formation in group 2 patients were significantly higher than in group 1 patients ($p < 0.05$). The most frequent genotype in IgA patients was ID (47%) genotype, followed by II (45%) and DD (8%) genotype of ACE gene. The mean serum ACE activity in the DD group was significantly higher than in the II group ($p < 0.05$) but was not significantly different from that of the ID group. No statistically significant differences were found with respect to allele frequencies between IgA group 1, group 2, or between controls and all IgA patients. Furthermore, no significant difference in AGT alleles, ATR alleles frequencies was detected between groups of IgA patients, although a trend for a higher frequency of DD genotype and AGT-TT genotype were noted in IgA group 2. The combined analysis of the ACE-DD and AGT-TT genotypes did not show any genetic influence on the risk of the disease susceptibility. To resolve the true role of ACE genotype and any dependent effect on progression, larger collaborative studies are required.

Key word : RAS Gene Polymorphism, IgA Nephropathy, Clinicopathology

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Angiotensin-converting enzyme (ACE) is a dipeptidyl carboxypeptidase which converts angiotensin I to angiotensin II in the renin-angiotensin system, and inactivates kinin, a vasodilator[1]. The renin-angiotensin system is active not only in the circulating blood, but also in local tissues[2]. Recently, the tissue renin-angiotensin system has been shown to be important in the regulation of blood pressure[3] and vascular cell proliferation[4] via a mechanism involving some protooncogenes and growth factors[5]. In the kidney, angiotensin II enhances the efferent arteriolar tone of the glomerulus[6] and results in glomerular hypertension[7]. It also appears to have a hypertrophic effect on mesangial cells[8]. Therefore, this system may play an important role in the pathogenesis or progression of renal damage and development of end stage renal disease.

The human ACE gene has 26 axons[9] and its locus is at 17q 23. A 287 bp insertion/deletion (I/D) polymorphism has been detected in intron 16 of this gene[10]. Serum ACE activity has been shown to be higher in people with the D allele than in those without the D allele[10]. A recent study found that the DD genotype was more frequent in patients with myocardial infarction than in controls [11]. This polymorphism has been also demonstrated to be related to left ventricular hypertrophy [12]. These data suggest that ACE polymorphism is linked with a defect in the ACE gene itself or with other genes near the ACE gene which can affect the renin-angiotensin system.

The significance of ACE gene polymorphism varies among different organs or tissues, depending on the local availability of angiotensin I for conversion to angiotensin II by ACE. It has been shown that the angiotensin II /angiotensin I ratio is markedly high in the kidney; this ratio averaged 0.3 and ~ 2.0 in the plasma and kidney, respectively[13]. It is possible that the ACE I/D polymorphism, may be particularly relevant at the renal tissue level, such that renal tissue injury is under a greater modulatory influence by the ACE I/D polymorphism, when compared to its influence on blood pressure or other organs.

IgA nephropathy is recognized as the most common form of glomerulonephritis in various parts of the world. Prognosis is poorer than initially predicted, and nearly half of the patients end up with chronic renal failure. The important risk factors for renal failure currently known include severe proteinuria, impaired renal function and hypertension at the time of presentation[14]. Since these risk factors are common to practically all forms of chronic renal disease, it is suggested that several key pathophysiological mechanisms independent of the pathogenesis of IgA nephropathy per se may operate in the progression of IgA nephropathy. Locally increased angiotensin II activity has also been suggested to affect the progression of IgA nephropathy [15]. Moreover, specific pharmacologic agents are now available to lower ACE activity, identification of such potential risk factors could enable us to devise a possible therapeutic intervention.

In this study, the authors determined the gene polymorphism in IgA nephritis patients, the correlation of ACE gene and serum ACE activity and also the association between these genes, clinical parameters and renal histology.

MATERIAL AND METHOD

Subjects

The subjects included 53 patients, 18 males and 35 females aged 18 to 61 years with biopsy-proven IgA nephropathy. Diagnosis of IgA nephropathy was based on established pathological criteria, including mesangial expansion and the diagnostic presence of IgA predominant immunoglobulin[16]. Patients with other disease entities who had mesangial IgA deposition were excluded. Since treatment was not standardized, the potential impact of treatment on patient outcome could not be evaluated. Hypertension was defined as systolic blood pressure > 140 and/or diastolic blood pressure > 90 mmHg. Eighty normal control subjects (34 males, 46 females) with mean age 40 $\pm$ 10 years were randomly selected among the individuals attending a medical center for routine annually check up. The control subjects did not have hypertension or other diseases with normal urinalysis and serum creatinine.

Groups

All the patients (n = 53) whose serum creatinine at the time of renal biopsy $\leq$ 1.5 mg/dl (group 1) were compared with those whose creatinine was > 1.5 mg/dl (group 2).

Methods

Serum ACE activity[17,18]

Serum ACE activity was measured by spectrophotometric method developed by Cushman and

Cheung employing hippuryl-L-histidyl-L-leucine (HHL) as substrate which measured the formation of free hippuric acid by the action of ACE.

DNA Extraction

Nuclear DNA was isolated from peripheral leukocytes in 5 ml EDTA blood samples by guanidine-HCl extraction method. The genomic DNA was precipitated with absolute ethanol and suspended in TE buffer pH 8.0.

Determination of ACE genotypes[19]

Polymerase chain reaction (PCR) was used to detect the two alleles of 490 and 190 bp corresponding to the insertion (I) and deletion (D) fragments. The sense oligonucleotide primer was 5' CTG GAG ACC ACT CCC ATC CTT TCT 3' and the antisense primer was 5' GAT GTG GCC ATC ACA TTC GTC AGA T 3'. The DNA was amplified for 30 cycles with denaturation at 94°C for one minute, annealing at 58°C for one minute and extension at 72°C for 2 minutes.

Determination of angiotensinogen M235T genotypes[20]

The exon 2 region, which covered the M235T polymorphic site of the angiotensinogen gene was amplified by PCR. The sense oligonucleotide primer was 5'-GAT GCG CAC AAG GTC CTG TC-3' and the antisense primer was 5'-CAG GGT GCT GTC CAC ACT GGA CCC C-3'. The PCR product was digested with 5 U Tth 111-I by restriction endonuclease digestion method at 65°C for 3 hours.

Determination of angiotensin II type 1 receptor A1166 C genotype[21]

Amplification of an 856 bp sequence encompassing the A1166 C polymorphism was performed with the sense oligonucleotide primer : 5'-AAT GCT TGT AGC CAA AGT CAC CT-3' and the antisense oligonucleotide primer : 5'-GGC TTT GCT TTG TCT TGT TG-3'. Restriction endonuclease digestion of the PCR product was continued with 3U Dde I at 37°C for 1 hour.

Agarose gel electrophoresis for analysis of DNA fragments

The method to separate DNA fragments was gel electrophoresis which separated DNA molecule according to their size. Amplification products were separated by 2.5 per cent agarose gel electrophoresis and visualized under ultraviolet light after ethidium bromide staining.

Clinical and histopathological analysis

Blood pressure, 24 hour urinary protein excretion, serum albumin, creatinine, and creatinine clearance at the time of renal biopsy were used for the clinical assessment.

Using the scoring system previously described[22] with some modification, each glomerulus was examined for the presence of mesangial proliferation, matrix expansion, segmental or global sclerosis and hyalinosis. Each parameter was given a score between 0-3 : 0 = absence of that parameter, 1-3 = involvement of less than 30 per cent, 30-60 per cent and over 60 per cent of glomerular area, respectively. The mean glomerular score was then calculated from the total glomerular score divided by the number of glomeruli. For the tubulointerstitial lesions, score 0 = no involvement, score 1-3 = involvement of less than 20 per cent, 20-40 per cent and over 40 per cent of the cortical interstitium. Vascular changes, arteriosclerosis and arteriolosclerosis were scored from 0-3 according to the degree of intimal thickening. Score 0 = no involvement, score 1-3 = intimal thickening less than 1/3, 1/3-2/3 and over 2/3 of tunica media, respectively. The mean score was then calculated by the same method used for glomerular lesions. The crescent lesions were also recorded.

Statistical analysis

The results were expressed as the mean ± standard deviation. The statistical significance of differences between two means was assessed with the unpaired Student t - test, and the differences in the genotype or allele frequency between each group was compared by the chi-square test. Subgroup comparisons were made by Fisher's exact test.

RESULTS

Clinical features of the patients

The clinical and laboratory features of the two groups of patients at the time of renal biopsy are summarized in Table 1. Means of age and sex distributions were not distinguishable between the patient groups. Also the mean period of duration from the initial discovery of urinary abnormalities to this renal biopsy was similar in both groups.

Table 1. Comparisons of the clinical features between patients at the time of renal biopsy.

	Group 1 (Serum Cr ≤ 1.5 mg/dl)	Group 2 (Serum Cr > 1.5 mg/dl)
n	40	13
Age (yr)	37.3 ± 10.5	34.8 ± 10.6
Sex (M : F)	11 : 29	7 : 6
Duration (months)	21.25 ± 5.08	15.00 ± 6.30
Systolic BP (mmHg)	123.5 ± 20.3	153.8 ± 22.9*
Diastolic BP (mmHg)	80.4 ± 11.8	103.1 ± 16.5*
Urinary protein (g/D)	2.5 ± 2.6	4.8 ± 4.1**
Serum creatinine (mg/dl))	1.0 ± 0.3	4.0 ± 4.1**
Ccr (ml/min)	83.8 ± 33.6	50.1 ± 20.5*

* $p < 0.001$, ** $p < 0.05$

Table 2. Distribution of renin angiotensin system (RAS) genotype frequencies in normal controls and IgA patients ($p > 0.05$).

Parameter	Controls n = 80	%	IgA patients n = 53	%
ACE genotype (D/I)				
DD	5	6	4	8
ID	30	38	25	47
II	45	56	24	45
x^2	-		1.54	
p	-		0.46	
AGT genotype (M 235T)				
TT	41	51	34	64
MT	36	45	18	34
MM	3	4	1	12
x^2	-		2.27	
p	-		0.32	
ATR genotype (A 1166 C)				
CC	1	1	0	0
AC	10	13	7	13
AP	69	80	46	87
x^2	-		0.68	
p	-		0.71	

The renin-angiotensin system component genes

Genotype and allele frequencies are shown in Table 2. The common ACE gene polymorphism in IgA patients were of ID and II type which was different from the Japanese[25] and Caucasians[26] as demonstrated in Table 3. However, there was no significant difference in the frequency of each genotype between the patients, and control subjects.

Serum ACE activities

In patients with IgA nephropathy, the serum ACE activities were 5.9 ± 2.7, 6.9 ± 3.5, and 10.2 ± 1.9 u/ml for the II, ID and DD genotype groups, respectively. While the activities in the control subjects were 6.3 ± 2.5, 7.3 ± 3.0 and 10.5 ± 3.8 u/ml respectively. The mean serum ACE activity in the DD group was significantly higher than in the II group ($p < 0.05$), but was not significantly different from that of the ID group. For controls, the mean ACE activity in the DD group was significantly higher than in the ID and II groups ($p < 0.05$).

Table 3. Genotype of the ACE gene in different countries in the patients with IgA nephropathy.

Genotype	Japan[25] % (n = 48)	Caucasian[26] % (n = 168)	Thailand % (n = 53)
DD	16.6	33	7.5
ID	27.1	48	47.2
II	56.3	19	45.3

Table 4. Histopathological parameters in IgA patients.

	Group 1 (n = 40)	Group 2 (n = 13)	p value
1. Glomerular lesion (%)			
Mesangial proliferation	21.82 ± 1.33	39.5 ± 5.08	0.0001
Glomerular sclerosis			
global	6.91 ± 1.71	34.6 ± 5.9	< 0.0001
segmental	1.88 ± 1.30	17.14 ± 5.46	0.0016
2. Vascular (%)			
Hyaline arteriolosclerosis	0.06 ± 0.04	0.10 ± 0.07	NS
Fibroelastic hyperplasia	0.15 ± 0.06	0.25 ± 0.10	NS
Fibrinoid necrosis	0	0.05 ± 0.05	NS
3. Interstitium (%)			
Interstitial fibrosis	11.21 ± 2.65	39.0 ± 5.9	< 0.0001
Tubular atrophy	11.06 ± 2.3	36.2 ± 5.9	< 0.001
4. Crescent formation	0.3 ± 0.1	5.7 ± 2.6	0.01

There was no significant difference in the mean activity between the patients and control subjects for each genotype.

We found that the distribution of genotypes for ACE genes, angiotensinogen (AGT) genotypes and the angiotensin II receptor (ATR) genotypes was similar between IgA group 1 and group 2. Although there was a trend of higher frequencies of DD genotype and AGT-TT genotype in IgA-group 2, there was no statistical significance.

To evaluate the genetically high risk group (group 2) in IgA patients, the authors combined classification according to the genotypes of ACE-DD and AGT-TT but the result did not show any statistical significance in this study.

Histopathological analysis

The histological findings were compared between two groups of IgA patients as illustrated in Table 4. All histological parameters including mesangial proliferation glomerular sclerosis both global and segmental, the interstitial fibrosis and tubular atrophy, and crescent formation were significantly higher in group 2 than in group 1 patients ($p < 0.001$), while vascular changes seemed to have no influence in this study.

The Clinical and pathological features in relation to ACE genotype

The clinical and pathological data from 53 IgA patients were analysed according to ACE genotype. There were no statistically significant differences among the three groups in terms of blood pressure, urinary protein excretion, serum creatinine, creatinine clearance, and histological parameters.

DISCUSSION

In this study, no difference was found in the gene frequencies for the I/D allele between 53 IgA patients and control subjects which agreed with the study of Harden[23], Schmidt[24] and Yorioka

(25). This indicates that the risk of developing IgA nephropathy is not related to the I/D polymorphism of the ACE gene. The distribution of genotypes in IgA patients also differs from the Japanese and Caucasians(25,26). The DD:ID:II genotype was 7.5:47.2:45.3 per cent in Thai IgA patients compared to 33:48:19 per cent in Caucasians and 16.6:27.1:56.3 percent in Japanese. These datas suggest that, I and D alleles may possess a racial difference.

Levels of tissue and circulating ACE activity are under tight genetic control. Tiret et al(21), using segregation and linkage analysis, suggested that the ACE I/D polymorphism, which accounted for 28 per cent of the total variance of plasma ACE in their Caucasian subjects, is a marker in strong linkage disequilibrium with a functional variant in the ACE gene. In their analysis, this major gene effect accounted for 44 per cent of interindividual variability in circulating ACE levels.The study confirmed that the ACE D allele is associated with elevated ACE activity in plasma.

The results suggest that polymorphisms in ACE was not predictive of prognosis of IgA nephropathy. As an indirect approach to access susceptibility to renal disease versus susceptibility to progression, the study analyzed separately patients with stable renal function (group 1 patients) and with renal insufficiency at renal biopsy in another group (group 2 patients). Although the clinical and histopathological parameters between the two groups of IgA patients represent significant difference. There were no significant association between ACE DD genotype in patients with stable renal function (group 1) and patients with renal insufficiency at renal biopsy (group 2). IgA nephropathy might be the result of the genetic interaction and environmental components. It is well documented that the incidence of disease increases additively with an increase in the number of nephropathy risk factors, such as hypertension, proteinuria. It is possible that these two groups of patients were not homogeneous, eg; the pathogenesis of progression were not uniform. Damage of the kidney due to inflammation, damage resulting from proteinuria, and damage resulting from hypertension, etc, might have its own roles in destroying the kidney.

The observation by Hunley et al(28) showed that the rate of progression was significantly worse in IgA nephropathy patients with DD genotype than the others. Moreover, when patients presenting with the known risk factors of hypertension and/or heavy proteinuria were excluded, the difference became even greater. Several reports(27-29) agree with the notion that, while the ACE I/D genotype does not affect the incidence of IgA nephropathy, the genotype has an appreciable impact on the progression of the nephropathy, D allele and DD genotype promote poor prognosis in patients with IgA nephropathy. With respect to the renal risk in IgA nephropathy, this study does not prove an association with ACE gene polymorphism; however, a trend for overpresentation of individuals with DD genotype was noted in patients with IgA nephropathy with renal insufficiency at renal biopsy.

The tissues renin-angiotensin system is distributed throughout many tissues such as the vascular endothelium, and the epithelia of the lungs, kidneys and intestines(30). In the kidney, this system is present not only in the proximal tubules but also in the glomeruli(31). Two types of ACE are known, one is the soluble type and the other is a membrane bind type(32). This latter ACE may be important for the tissue RAS. Our results and previous reports have shown that the ACE polymorphism is related only to the serum ACE activity, but not with the membrane binding ACE activity. In our study we found that ACE of DD genotype had the highest serum ACE activity, followed by ID and II types respectively both in patients and normal controls. It is necessary to search for the relationship between ACE polymorphism and the membrane bound form of ACE to clarify the influence of this polymorphism in IgA nephropathy increased generation of angiotensin II(29). Although the functional significance of polymorphism in the ACE gene is not yet established, increased local production of angiotensin II may have a direct hypertrophic action on the glomerular cells and may bring about glomerular hypertrophy, an important factor in the induction of sclerosis(33,34). Furthermore, angiotensin II has been shown to cause tubular and mesangial cell proliferation and matrix production in animal models of progressive renal disease(35,36). Thus, angiotensin II might play an important role in the progression of IgA nephropathy. This hypothesis is supported by the recent clinical observation that ACE inhibitors reduce proteinuria and attenuate the progressive decline in renal function in IgA nephropathy(37).

In this study, the authors compared patients of group 1 with normal renal function to patients of

group 2 with more severe clinical and laboratory features to find the association of ACE genotype and the severity of the disease. The results showed no association of ACE genotype of both groups. These findings were similar to the study of Schmidt et al[24] and Pei et al[26] but did not support the previous reports[25,27,28] who found the frequency of the D allele in patients with declining renal function and with risk factors for progression. More recently, Pei et al[26] examined the role of other polymorphisms with the RAS. The M235T polymorphism in angiotensinogen was significantly associated with severity of disease. An excess of angiotensinogen MT and TT genotypes was found in the moderate and fast progressor groups of IgA patients[38,39]. The authors believed that the angiotensinogen T235 variant might be associated with an increase in intrarenal angiotensinogen production. For this reason, combined genotype analysis was also performed. In this analysis, the AGT TT genotype in M235T polymorphism did not enhance the risk of impaired renal function in patient with the DD genotype. In the IgA patients group, there was no CC genotype of A1166C polymorphism and the frequency of C allele was only 4 per cent, a level substantially lower than that reported for Japanese (8.5%) and Caucasians (30%). This study, therefore, did not find any significant impact of A1166C polymorphism in ATR gene on the impaired renal function in IgA nephropathy in Thai patients. These negative results may be attributed to the extremely low frequency of C allele, or CC genotype in Thai population. Nevertheless, the results indicate that, overall, the A1166C polymorphism in ATR gene does not have a major impact on in the impaired renal function of IgA nephritis in Thai population. In our study although there was a trend of higher numbers of IgA patients group 2 who carried ACE of DD genotype and angiotensinogen gene of TT type than in group 1 patients with normal renal function, and statistical significance could not be demonstrated between both groups. The possible role of angiotensin II receptor genes was also evaluated but no relation was found in this study.

This reflects the small numbers of patients studied in this common disease, a larger collaborative studies will be required to resolve the true role of RAS gene polymorphisms and any independent effect on the severity.

Concerning the renal histology, we found the following parameters: mesangial proliferation, glomerular sclerosis, interstitial fibrosis, tubular atrophy, and crescent formations were highly statistically significant in group 2 ($p<0.01$) compared to group 1 but could not find the correlation in vascular changes ($p>0.05$). When ACE genotype was considered to compare the different ACE genotype and the degree of histopathological scores, the association was not detected. This may be due to the small number of patients in group 2 ($n = 13$) in comparison with patients of group 1 ($n = 40$). Previous reports indicated the ACE gene polymorphism may not influence the extent of mesangial proliferation and crescents that were acute lesions but ID/DD genotypes were associated with chronic lesions such as capsular adhesions or glomerulosclerosis[25,40].

In this study, the authors found that the ACE deletion polymorphism detected by PCR analysis appears to be associated with serum ACE activity, but no difference of ACE genotype distribution and allele frequencies between patients with IgA-GN and the general population was seen. Moreover, gene polymorphisms of other components of the RAS, specifically the M235T polymorphism of AGT gene and the A1166C polymorphism in ATR gene, were not associated with IgA-GN. A trend for higher frequency of ACE-DD genotype and AGT-TT genotype were noted in IgA-GN group 2 with renal insufficiency at renal biopsy.

In summary, these data indicated that among the three genes of the renin angiotensin system studied, only angiotensin converting enzyme and angiotensinogen gene polymorphism had a trend of association with severe form of IgA nephropathy. Future studies with larger patient sample size will be needed to confirm both the angiotensinogen 235T and ACE D/D associations. Moreover, controlled clinical trials are also required to prove whether the clinical course of patients with these genetic risk markers can be modified by pharmacologic interventions such as treatment with an ACE inhibitors or angiotensin II receptor antagonist.

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คณะผู้รายงานได้ศึกษาผู้ป่วยไอจีเอ 53 ราย เปรียบเทียบกับคนปกติ 80 ราย ศึกษาลักษณะทางคลินิกพยาธิสภาพทางชิ้นเนื้อไตและชนิดของยีน ACE แองจิโอเทนซิโนเจน (AGT) และแองจิโอเทนซิโนเจนรีเซ็พเตอร์ (ATR) แบ่งผู้ป่วยตามระดับครีอะตินีน โดยกลุ่มที่ 1 มีครีอะตินีนเท่ากับหรือน้อยกว่า 1.5 มก/ดล กลุ่มที่ 2 มีครีอะตินีนมากกว่า 1.5 มก/ดล ผู้ป่วยกลุ่มที่ 2 มีความดันโลหิตและค่าโปรตีนในปัสสาวะสูงกว่ากลุ่มแรก อย่างมีนัยสำคัญทางสถิติ ($p<0.01$) ลักษณะทางพยาธิสภาพก็รุนแรงกว่า ($p < 0.05$) ส่วนการศึกษายีน ACE พบว่าชนิด DD มี 8%, ชนิด ID มี 47% และชนิด II มี 45% ยีน ACE ของผู้ป่วยไอจีเอไม่ต่างจากคนปกติ, activity ของ ACE ในผู้ที่มี DD จีโนทัยท์จะสูงกว่าชนิด II ($p < 0.05$) เมื่อเปรียบเทียบผู้ป่วยกลุ่มที่ 1 และกลุ่มที่ 2 ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างยีน ACE, ATR และ AGT กับลักษณะทางคลินิก และพยาธิสภาพทางไต ซึ่งอาจเกิดจากการที่มีผู้ป่วยในกลุ่ม DD มีจำนวนน้อยมาก การศึกษาจึงจำเป็นต้องเพิ่มจำนวนผู้ป่วยให้มากขึ้นเพื่อจะได้พิสูจน์ความสำคัญของปัจจัยเสี่ยงของยีนชนิดต่างๆ ในระบบเรนินแองจิโอเทนซินในผู้ป่วยโรคไตชนิดไอจีเอได้ชัดเจนยิ่งขึ้น

คำสำคัญ : ยีนในระบบเรนิน-แองจิโอเทนซิน, โรคไตชนิดไอจีเอ, ลักษณะทางคลินิกและพยาธิสภาพ

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