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The Genetic Defect in Multiple Endocrine Neoplasia Type 2A Maps Next to the Centromere of Chromosome 10

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Summary

Multiple endocrine neoplasia type 2A (MEN2A) is a rare cancer syndrome that is inherited in an apparently autosomal dominant fashion. Previous linkage studies had assigned the MEN2A locus to chromosome 10 in the pericentromeric region. We recently have described several new easily scorable RFLPs for the chromosome 10–specific alpha satellite DNA (the D10Z1) locus that is known, on the basis of previous in situ hybridization experiments, to lie at the centromere. We report here tight linkage between MEN2A and D10Z1, as demonstrated by a maximum lod score of 12.02 at the recombination frequency of zero (1-lod-unit support interval 0–4 cM), indicating that the genetic defect in MEN2A lies in the immediate vicinity of the centromere. By means of a set of ordered polymorphic DNA markers from the pericentromeric region, multipoint as well as pairwise linkage analyses place the MEN2A locus at the middle of a small region (~11 cM) bracketing the centromere with FNRB (at 10p11.2) and RBP3 (at 10q11.2) on either side, providing further support for the centromeric location of the MEN2A locus. Marked sex difference in recombination frequencies exists in this pericentromeric region: significantly ($P < .01$) more female than male crossovers were observed across all of the adjacent intervals D10S24–FNRB, FNRB–D10Z1, and D10Z1–RBP3. However, a sex difference was not seen in the 7-cM interval from RBP3 to D10S5, suggesting that large variation in the sex difference in recombination can occur over small chromosomal regions. Proper clinical application of these DNA markers in genetic counseling to determine the genotypes at the disease locus for those at-risk members in informative afflicted families must therefore take into account the implications imposed by the large and highly significant sex effect on recombination in the MEN2A region.

Introduction

The multiple endocrine neoplasia type 2A (MEN2A) syndrome is characterized in at-risk individuals by a propensity to hyperplasia and tumor development in three endocrine glands, resulting in medullary thyroid carcinoma (MTC), phenchromocytomas (PHEOs), and/or hyperparathyroidism, of which MTC is both the most frequent and most aggressive defect. The genetic defect predisposing to the syndrome had been previously localized to the pericentromeric region of chromosome 10 (Mathew et al. 1987; Simpson et al. 1987). The clinical penetrance of the disease gene seems to be far from complete (Easton et al. 1989), but early detection of MTC can often be reliably achieved by demonstration of a characteristic rise in blood calcitonin level induced by calcium infusion and/or pentagastrin stimulation (Wells et al. 1978; Gagel et al. 1982). Thyroidectomy performed on those who are positive for this diagnostic test but have no clinical manifestation usually greatly improves the prognostic outcome of these patients

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(Gagel et al. 1988). Accurately identifying at-risk individuals (the disease-allele carriers) is therefore very important for clinical management of members of the afflicted families: those with the normal genotype at the MEN2A locus could be freed of unpleasant regular screening tests, and those inheriting the disease allele could be put under more stringent scrutiny for early detection and subsequent treatment.

Polymorphic DNA markers that are closely linked to—and, ideally, flank—the gene for a genetic disease have proved to be powerful means for determining the genotypes at the disease-gene locus of potentially at-risk members in afflicted families (Farrer et al. 1988; Beaudet et al. 1989). In an attempt to identify closely linked genetic markers for MEN2A, we have tested—and, in some cases, also characterized—numerous chromosome 10 RFLP loci, an effort which resulted both in the construction of a fine-resolution linkage map for the pericentromeric and proximal long-arm region of that chromosome and in identification of flanking markers on either side of the MEN2A locus (Wu et al. 1988*b*, 1989*b*; Myers at al. 1989). We report here the linkage analyses, done in the MEN2A families as well as in non-MEN2A families, that demonstrate that the genetic defect for MEN2A is tightly linked to the D10Z1 locus and located in the middle of a small region delimited by FNRB and RBP3.

Material and Methods

Family Resources

Nine MEN2A families and six non-MEN2A families with a total of 1,092 individuals were used in the present study. All of these families are from North America and are of mixed European ancestry. Families R, C, S, W, B, MEN2A-N, MEN2A-H, MEN2A-OHIO, MEN2A-ORE, OA, OOA, TSORE, and TSCAN have been described elsewhere (Simpson et al. 1987; Wu et al. 1989*a*). TSK and TSM are two Tourette disorder families newly collected by K. Kidd and collaborators. The diagnoses of the MEN2A families R (designated K in Simpson et al. 1987), C, S, W, B, and MEN2A-N (designated N in Simpson et al. 1987) have been well established and were used for analyzing MEN2A linkage to other chromosome 10 markers. In contrast, the diagnoses for some members of the other three MEN2A families are still not certain; these families were therefore used, along with other non-MEN2A reference families, only for linkage mapping of the pericentromeric DNA markers.

DNA Markers and RFLP Typing

Eight chromosome 10 markers were used in the present study; they are p7A9(D10S24), pGEM-32(FNRB), pα10RP8(D10Z1), H.4IRBP(RBP3), pMCK2(D10S15), p9-12A(D10S5), pTB10.163(D10S22), and CDC2H (CDC2). The centromeric marker D10Z1 and its RFLPs have been described recently (Devilee et al. 1988; Wu and Kidd, in press), and the RFLP properties associated with all the other seven markers have been summarized by Smith and Simpson (1989). Standard procedures as described by Wu et al. (1989*a*) were used for human genomic DNA restriction-enzyme digestion, agarose gel electrophoresis, blotting, hybridization with [α-^{32}P]-dCTP-labeled probes, and autoradiography.

Linkage Analyses

Pairwise and multipoint linkage analyses were done by means of the programs LIPED (Ott 1976) and LINKMAP (from the LINKAGE package; Lathrop et al. 1984), respectively. For each of the above-mentioned markers except D10S15, D10S5 and D10S22, haplotypes were constructed from two or more RFLPs and used in the analyses. The frequency of the disease allele was assumed to be .0005, and an assumption of incomplete and age-dependent penetrance of the MEN2A gene as described by Farrer et al. (1987) was incorporated in the lod-score calculation involving MEN2A. Homogeneity of recombination frequencies in males and females was evaluated using a likelihood-ratio χ^2 test (Ott 1985).

Results

The recently described *Pst*I, *Eco*RV, and *Hin*cII polymorphisms (Wu and Kidd, in press) detected with the probe pα10RP8, a representative clone of the chromosome 10 alpha satellite DNA (Devilee et al. 1988), are quite frequent and could be scored unambiguously in the complex hybridization patterns usually seen with repetitive sequence–containing probes, thereby making an excellent marker for the D10Z1 locus. With these polymorphisms, tight linkage between D10Z1 and MEN2A was demonstrated in the four informative MEN2A kindreds (C, S, W, and MEN2A-N): pairwise linkage analyses indicate no crossovers between the two loci and the maximum lod score of 12.02 was obtained at a recombination frequency (θ) of zero (1-lod-unit support interval 0–4 cM) (table 1). Since this probe has been previously shown to hybridize specifically to the centromeric region of chromosome 10 under high-strin-

Table 1

Pairwise Linkage of Chromosome 10 Percentromeric Markers and MEN2A

Locus Pair	Lod Scores for θ Values ($\theta_m = \theta_f$)								Peak Lod Scores[a] and Corresponding θ Values					
	.000	.001	.010	.050	.100	.200	.300	.400	$\theta_m = \theta_f$	Peak Lod Score	θ_m	θ_f	Peak Lod Score	P[b]
D10S24–FNRB	−∞	−20.9	1.0	14.0	17.0	15.6	10.7	4.9	.135	17.7	.056	.217	19.5	<.01
D10S24–RBP3	−∞	−50.8	−13.3	10.2	17.1	17.8	12.9	6.1	.163	18.2	.035	.348	25.5	<.01
FNRB–RBP3	−∞	−5.0	17.1	29.0	30.3	25.2	16.7	7.3	.100	30.3	.000	.180	36.3	<.01
FNRB–D10Z1	−∞	24.1	27.7	28.1	25.6	18.9	11.7	4.9	.037	28.2	.000	.071	30.3	<.01
RBP3–D10Z1	−∞	24.7	28.5	29.3	27.1	20.3	12.4	5.1	.044	29.3	.000	.074	31.1	<.01
RBP3–D10S15	−∞	43.9	45.2	43.2	39.2	29.8	19.1	8.3	.024	45.9	.029	.004	46.6	>.05
RBP3–D10S5	−∞	11.5	16.4	18.7	18.1	14.6	9.8	4.6	.067	19.0	.066	.067	19.0	>.05
MEN2A–D10S24	−11.0	−6.3	−0.5	4.3	5.7	5.7	4.2	2.1	.145	5.9	.135	.179	6.0	>.05
MEN2A–FNRB	5.6	8.0	11.2	13.1	12.9	10.6	7.3	3.3	.063	13.2	.059[c]	.085	13.2	>.05
MEN2A–D10Z1	12.0	12.0	11.8	10.7	9.4	6.6	3.9	1.5	.000	12.0	.000	.000	12.0	>.05
MEN2A–RBP3	9.5	11.6	12.7	13.1	12.3	9.7	6.5	2.9	.045	13.1	.000	.097	14.1	<.05
MEN2A–D10S15	11.3	14.0	16.4	17.3	16.3	13.0	8.7	3.8	.054	17.3	.054	.054	17.3	>.05
MEN2A–D10S5	.2	1.7	4.6	6.7	7.1	6.1	4.3	2.2	.106	7.1	.049	.149	7.5	>.05
MEN2A–D10S22	−7.8	−3.1	2.6	6.8	7.7	6.9	4.9	2.3	.127	7.8	.070	.166	8.1	>.05
MEN2A–CDC2	−24.1	−19.5	−9.6	−.1	3.5	5.3	4.5	2.4	.218	5.4	.190	.245	5.4	>.05

Note.—Lod scores involving the MEN2A locus were calculated using the program LIPED (Ott 1976) modified by a straight line age-at-onset correction (Farrer et al. 1987; Hodge et al. 1979); the penetrance of MEN2A was considered to increase linearly from a value of 0 at age of 3 years or younger to a maximum of .99 at age of 35 years or older. The frequency of the disease allele used was .0005, and that of phenocopies was .001.

[a] Quadratically interpolated using the QUAD2 program (Pakstis et al. 1986).

[b] For statistical test for sex differences in recombination (Ott 1985).

[c] No obligate crossovers exist in the data; however, the possibility of some crossovers exists, resulting in this nonzero estimate for recombination.

gency conditions (Devilee et al. 1988), our results indicate that the MEN2A defect also maps to the immediate vicinity of the centromere, although it cannot be determined whether it is on the short-arm or long-arm side.

Also shown in table 1 are the pairwise results of MEN2A versus the other seven pericentromeric markers, as well as some marker-marker comparisons. Several of these markers deserve some mention here. RBP3 has been one of the most extensively studied loci in MEN2A linkage analyses and until now has remained one of the closest markers for that syndrome. The physical location of RBP3 previously had been reported to be at 10p11.2-q11.2 (Liou et al. 1987) and recently was refined to 10q11.2 (table 2; Smith and Simpson 1989). D10S15 (Nakamura et al. 1988*a*) has been reported to be very close to RBP3, and the position of this locus relative to RBP3 on the chromosome 10 linkage map had not been resolved in the recently reported linkage studies (Nakamura et al. 1988*b*, 1989; White et al., in press). We, however, observed two obligate crossovers between these two loci, and the segregation patterns of the alleles of their adjacent markers suggest that D10S15 is distal to RBP3 on the long-arm side (fig. 1). The D10S24 *Taq*I and *Msp*I polymorphisms, detected by the probe p7A9, previously had been recharacterized and shown by multipoint linkage analysis to be linked to, and about 17% recombination units away from, RBP3 on the MEN2A side (Wu et al. 1988*a*).

We recently also discovered multiple RFLPs associated with the human fibronectin receptor β subunit gene locus (FNRB) by using the probe pGEM-32 (Wu et al. 1989*a*), and the PIC for the haplotypes of its *Ban*II, *Hin*fI, and *Kpn*I RFLP alleles is as high as .71, making this locus an excellent marker for linkage studies. To position FNRB relative to the other pericentromeric markers that have been previously ordered as pter–D10S24–RBP3–D10S5–qter (Simpson et al. 1987; Wu et al. 1988*a*), multipoint linkage analysis, using the LINKMAP program (Lathrop et al. 1984) and moving the FNRB locus across the fixed interval D10S24–RBP3, was conducted. The result indicates that FNRB is located between D10S24 and RBP3 (fig. 2), with odds of 5.37×10^{12} and 7.08×10^{2} against the alterna-

Table 2

Flanking DNA Markers for MEN2A

Symbol	Probe	Physical Location	Restriction Enzymes Revealing RFLPs	Highest PIC Reported	References
D10S24	p7A9	10p12.2-p13	*Msp*I and *Taq*I	.55[a]	Smith and Simpson 1989; Wu et al. 1988*a*
FNRB.....	pGEM-32	10p11.2	*Ban*II, *Hin*fI, *Kpn*I, *Bgl*II and *Sac*I	.71[b]	Goodfellow et al. 1989; Wu et al. 1989*a*
	pB/R2	10p11.2	*Msp*I		
D10Z1	pα10RP8	10cen	*Pst*I, *Eco*RV, and *Hin*cII	.50[c]	Devilee et al. 1988; Wu and Kidd, in press
RBP3	H.41RBP	10q11.2	*Bgl*II and *Msp*I	.34[d]	Smith and Simpson 1989; Liou et al. 1987
D10S15	pMCK2	10q11.2	*Pvu*II and *Rsa*I	.35[e]	Smith and Simpson 1989; Nakamura et al. 1988*a*

[a] For haplotypes of *Msp*I and *Taq*I RFLP alleles.

[b] For haplotyes of *Ban*II, *Hin*fI, and *Kpn*I RFLP alleles.

[c] Calculated heterozygosity for the *Pst*I 1.1-kb morph; *Msp*I and *Bcl*I were also reported (Devilee et al. 1988) to reveal RFLPs with pα10RP8, but they were difficult to score in large kindreds because of high complexities of hybridization patterns.

[d] For *Msp*I RFLP.

[e] For *Pvu*II RFLP; this probe also detects the same VNTR polymorphisms with *Pst*I and *Msp*I.

tive locations outside the interval on the D10S24 or RBP3 side, respectively. This order of D10S24–FNRB–RBP3 is entirely consistent with the physical location data for these three loci (table 2). Analyses with other markers distal to RBP3 strengthen the conclusion that FNRB is not distal to RBP3 (data not shown).

The location of the MEN2A relative to FNRB and RBP3 was also tested by multipoint linkage analyses. Because of the large and complex structures of the six MEN2A kindreds used in the present study, only three-point analyses were feasible on our VAX/8800 computer. Assuming incomplete and age-dependent penetrance of the disease gene and moving the MEN2A locus across the fixed map of FNRB–RBP3, we found that the peak lod score occurred when the MEN2A locus was positioned between FNRB and RBP3 (fig. 2). Positioning the MEN2A gene within the FNRB–RBP3 interval is also supported by the pairwise (marker–marker and marker–MEN2A) linkage relationship between MEN2A and a set of DNA markers from the pericentromeric region that have been shown to form a continuous linkage group (Wu et al. 1989*b*); the tightest linkage to MEN2A was found for FNRB and RBP3 (not counting D10Z1), and a clear-cut linkage distance gradient was found for markers distal to this region in either orientation (table 1). Since the FNRB–RBP3 interval brackets the centromere, these results provide some independent support for the centromeric location of the MEN2A locus.

Similar three-point analyses moving D10Z1 across the fixed FNRB–RBP3 interval were carried out to locate D10Z1 in relation to these ordered loci already on the genetic linkage map. The results place D10Z1 approximately in the middle of the interval (about 12 and 14 cM, on the female map, away from RBP3 and FNRB respectively), with odds of about 10,000 to 1 and 1,000 to 1 against its being located outside the interval on the FNRB and RBP3 sides, respectively. These and pair-

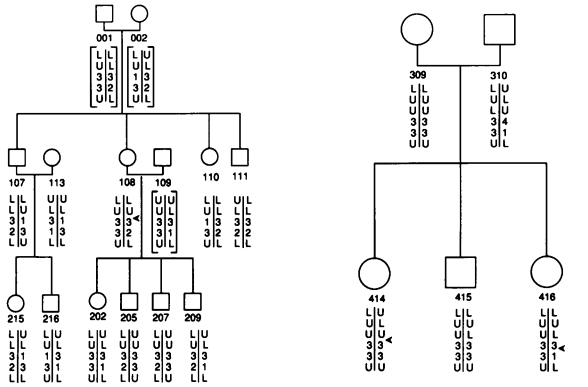

Figure 1 Part of TSORE family (left) and S family (right), each showing a crossover between RBP3 and D10S15. The most parsomonious explanation of the segregation patterns of the RFLP markers flanking the RBP3–D10S15 interval is that individuals 108 (TSORE) and 414 (S) are single crossovers for D10S15–RBP3 and that D10S15 therefore is distal to RBP3 on the 10q side. Marker loci used are (from top to bottom) FNRB (pGEM-32/*Ban*II), RBP3 (H.4IRBP/*Msp*I), D10S15 (pMCK2/*Pvu*II), D10S22 (pTB10.163/*Msp*I), and CDC2 (CDC2H/*Taq*I) for TSORE and FNRB (pGEM-32/*Ban*II), FNRB (pGEM-32/*Hin*fI), RBP3 (H.4IRBP/*Msp*I), D10S15 (pMCK2/*Pvu*II, D10S20 (OS-3/*Taq*I), and OAT (R5/*Rsa*I) for S. The genotypes for each family member represent the haplotypes of the above-mentioned markers. The genotypes in brackets are inferred from their offspring, but for 001 and 002 the two distinct genotypes cannot be assigned to specific individuals. U, L, 1, 2, 3, and 4 denote alternative alleles of the RFLP systems used.

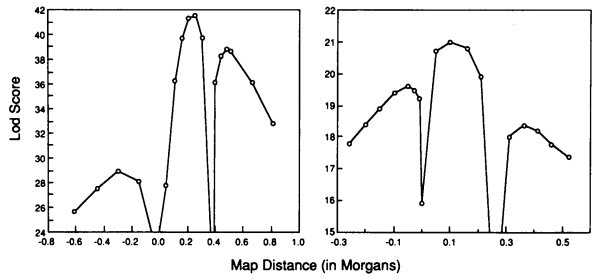

Figure 2 Multipoint analyses of the FNRB (*left panel*) and MEN2A (*right panel*) loci when the LINKMAP program is used. A constant female-to-male genetic distance ratio of 10:1 was assumed for both analyses, and the results are presented using female map distances. The 10:1 sex ratio was chosen on the basis of the pairwise linkage results that, in all the adjacent intervals D10S24–FNRB, FNRB–D10Z1, and D10Z1–RBP3, the values of θ_f are much larger than those of θ_m and that there is a 10-fold sex difference in recombination frequency estimates for D10S24-RBP3 (table 1). The position of FNRB (*left panel*) was determined by moving it across the fixed map of D10S24 (arbitrarily set at .00) and RBP3 (set at 3.6 cM in the male map and at 36 cM in the female map). The peak lod score (41.68) occurs when FNRB is positioned one-third of the distance from RBP3 to D10S24. The location of the MEN2A gene (*right panel*) was tested against a fixed RBP3–FNRB interval, with RBP3 arbitrarily set at .00 and with FNRB set at 2.6 cM in the male map. The age-dependent penetrance of MEN2A was defined as a step function for six age groups (Farrer et al. 1987), and the steps were chosen to give a close approximation to the linear age-at-onset correction used in LIPED. The maximum lod score (21.00) places the MEN2A locus within the RBP3–FNRB interval in a position about 40% of the distance from RBP3 to FNRB. This position is favored by odds of 25 to 1 and 417 to 1 over the two other peaks, which are distal to RBP3 in the long arm and distal to FNRB in the short arm, respectively.

wise linkage results involving D10Z1 (table 1) are not only consistent with and supportive of its centromeric location suggested by in situ hybridization but also contributory to the conclusion that MEN2A is located next to the centromere, since both loci appear to be roughly in the middle of the FNRB–RBP3 interval.

Marked sex differences in θ values were observed in the region from D10S24 to RBP3 (table 1). At least seven chromosomes recombinant for FNRB and RBP3 could be unambiguously identified in the families used in the present study, and they were all products of female meioses, giving maximum-likelihood estimates (MLEs) of θ values between FNRB and RBP3 of .18 for female and .00 for male. The subintervals of the FNRB–RBP3 region, FNRB–D10Z1, D10Z1–RBP3, and RBP3–MEN2A also showed dramatic sex differences in θ values ($P < .05$). However, in the RBP3–D10S5 interval, which is immediately adjacent to the FNRB–RBP3 region on 10q, there appears to be no sex effect on recombination (table 1). By interpolation from the sex-specific grids actually calculated for each interval, we estimated the likelihoods for constant sex ratios applied to both regions. The likelihood surface is quite flat, varying the sex ratio (f:m) from 5:1 to 30:1 but peaks at roughly 20:1 with a Log likelihood of just under 52.6. The calculated maximum of the $\log_{10}$ likelihood when separate sex ratios are allowed is 55.2. The likelihood ratio of $10^{2.6} = 398$ translates into a $\chi^2_1 = 12.0$, $P \approx .0005$. This probability is sufficiently small that we feel safe in concluding that the sex ratios for the two adjacent intervals are significantly different from each other, even though we had to assume that our estimates for the two regions are independent. Thus, it appears that there is a significant change in the sex ratio in θ values over this small chromosome region.

Discussion

Both the demonstrated tight linkage of MEN2A to D10Z1 and the successful localization of the genetic defect within a well-defined small region as reported here will not only facilitate further efforts for more detailed molecular studies of the genetic defect predisposing to MEN2A but will also have immediate clinical value for genetic counseling. A recently completed study also gave evidence localizing the MEN2A defect to a small region bounded by RBP3/D10S15 and another short-arm marker, D10S34 (Nakamura et al. 1989). The availability of close flanking markers on either side of the MEN2A gene allows highly accurate diagnoses of the disease-allele carriers in families in whom markers on both sides are informative. The high heterozygosity values that could be achieved for the loci D10S24, FNRB, D10Z1, RBP3, and D10S15 would make it possible to predict genotypes at the MEN2A locus for those at-risk individuals in most of the afflicted families. The marked excess of female over male recombination in the vicinity of the MEN2A locus would indicate that the genotype determinations made on the offspring of affected males would be much more accurate than those made on offspring of affected females. The difference is so large that proper genetic counseling must take it into account.

Previous studies also observed overall higher frequencies of female than male recombination around the chromosome 10 pericentromeric region in Centre d'Etude de Polymorphisme Humain families (Lathrop et al. 1988; Nakamura et al. 1988*b*; White et al., in press). However, it is difficult to compare directly these results with ours, since different RFLP markers (except for

RBP3, D10S15, and D10S5) were used to define the intervals in this region and since two of these studies (Lathrop et al. 1988; Nakamura et al. 1988*b*) did not report results of statistical tests for the sex difference for the individual intervals defined. Consistent with our findings of a large sex difference in recombination for the region in the immediate vicinity of the centromere, Nakamura et al. also noted similar differences in the intervals of D10S34–D10S15 (Nakamura et al. 1988*b*) (which should overlap extensively the FNRB–RBP3 region), MEN2A–D10S34, and MEN2A–RBP3 (Nakamura et al. 1989); however, the differences in the latter two intervals did not reach statistical significance (Nakamura et al. 1989), and whether the difference in the D10S34–D10S15 interval is significant was not reported (Nakamura et al. 1988*b*).

It should also be noted that, although the possibility of etiological heterogeneity of the MEN2A syndrome has not been fully excluded, evidence accumulated so far seems to be against such a possibility: both the six MEN2A families used in the present study and most of the 12 other previously reported families (each with published lod scores) from several different geographical and ethnic origins (Mathew et al. 1987; Simpson et al. 1987; Sobol et al. 1988; Yamamoto et al. 1989) showed linkage to the chromosome 10 pericentromeric markers when informative, and none excluded linkage to this region. The same locus near the chromosome 10 centromere appears to be responsible for predisposing to MEN2A in most (possibly even all) affected families, but a more definite conclusion requires that many more MEN2A families be tested. It is interesting that positive lod scores have also been found between MEN2B and several chromosome 10 pericentromeric markers, leading Jackson et al. (1988) to suggest that a locus in the same general centromeric area as the one for MEN2A may be involved in MEN2B.

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