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A Novel 1297-1304delGCCTGCCA Mutation in the Exon 10 of the Thyroid Hormone Receptor β Gene Causes Resistance to Thyroid Hormone

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Abstract

Introduction: Resistance to the thyroid hormone (RTH) is an inherited syndrome of reduced tissue responsiveness to hormonal action caused by mutations located in the ligand-binding domain and adjacent hinge region of the thyroid hormone receptor β (*TR*β) gene.

Patient: The patient in this study, a 42-year-old Caucasian male, came to medical attention because he experienced atrial fibrillation. Clinical evaluation showed a small and diffuse goiter and biochemical tests revealed markedly elevated concentrations of total T_4, total T_3, and free T_4, normal thyroid-stimulating hormone (TSH) values and slightly increased I^{131} thyroid uptake at 24 hours. The thyroperoxidase, thyroglobulin, and TSH receptor antibodies were positive. He was treated with cabergoline plus methimazole. This treatment was stopped because of the inconsistent response, monotherapy with tri-iodothyroacetic acid (TRIAC) was then prescribed after molecular diagnosis confirmed RTH syndrome.

Methods: The exons 9 and 10 of the *TR*β gene, including splicing signals and the flanking intronic regions of each intron, were amplified with PCR. DNA sequences from each amplified fragment were performed with the Taq polymerase-based chain terminator method and using the specific *TR*β forward and reverse primers.

Results: Direct sequence analysis of the exons 9 and 10 of the *TR*β gene revealed an eight basepair deletion, 1297-1304delGCCTGCCA in exon 10. The mutation produces a frameshift at amino acid 433 and introduces a stop codon TGA at position 461, 85 nucleotides downstream from deletion. This alteration was not detected in either the father or mother of the patient, suggesting a *de novo* mutation that was confirmed by DNA fingerprint analysis.

Conclusions: In the present study we have identified a novel sporadic mutation corresponding to 1297-1304delGCCTGCCA deletion in the activating function 2 (AF-2) region of *TR*β. To our knowledge, this is the first time that the presence of a partial deletion of eight nucleotides in the *TR*β has been reported.

The syndrome of resistance to thyroid hormone (RTH) is characterized by variable tissue hyposensitivity to the thyroid hormone.[1] Biochemically, this genetic disorder is defined by increased thyroid hormone levels with inappropriately normal or elevated level of thyroid-stimulating hormone (TSH) reflecting a resistance to the normal negative feedback mechanisms within the hypothalamus and pituitary glands.[1] Clinical presentation is highly variable and ranges from isolated abnormal findings on routine thyroid testing to a combination of features that include goiter, variable symptoms of hyper- and hypothyroidism, delayed bone

maturation, short stature, and attention deficit-hyperactivity disorder.[2,3] The incidence of RTH is probably 1 in 50 000 live births.

RTH is linked to the thyroid hormone receptor β (*TR*β) gene located on chromosome 3.

Thyroid hormone receptors are nuclear hormone receptors that regulate the transcription of target genes by binding to thyroid hormone response elements (TREs) in their promoter regions.[4] Thyroid hormone receptors bind to TREs as homodimers or heterodimers with retinoid X receptors (RxRs).

In the absence of ligand thyroid hormone receptors, homodimers and heterodimes are associated with the corepressors (CoRs) NcoR and SMRT, repressing the basal transcription of genes positively regulated by T_3. The subregion of thyroid hormone receptor that interacts with CoRs is located in the hinge region between the DNA-binding domain (DBD) and ligand-binding domain (LBD).[5-7]

Ligand-dependent activation requires the cellular coactivators (CoAs) SRC-1, TIF2, RIP 140, p/CIP, RAC3/ACTR/TRAM-1/AIB1,[8-14] and the intact activation domain residing at the carboxyl terminus of the LBD (known as activating function 2 [AF-2]).[15-18]

All mutations linked to RTH are located in the functionally relevant LBD and adjacent hinge region of the *TR*β gene. Three mutational clusters have been identified with intervening cold regions.[19-29] Almost all subjects identified as RTH are heterozygous for the mutant *TR*β allele, consistent with the autosomal dominant pattern of inheritance. Therefore, mutant *TR*βs interfere with the function of the wild-type receptor by a dominant negative mechanism.[30-32] Transmission was clearly recessive in only one family. In this case, a complete deletion of the protein-coding region of the *TR*β gene was identified.

In the present study we identified the 1297-1304delGCCTGCCA mutation in exon 10 of the *TR*β gene, in a patient with RTH and persistent atrial fibrillation. His parents had only the wild-type sequences, indicating a *de novo* mutation. To our knowledge, this is the first time that the presence of a partial deletion of 8-nucleotides (nt) in the *TR*β has been reported.

Materials and Methods

Clinical Data

Patient CB, a 42-year-old Caucasian male, was admitted to the cardiology department of the Central Military Hospital with atrial fibrillation, he was anticoagulated with acenocoumarol, and then referred on to the endocrinology department. On evaluation it was established that the propositus was delivered normally, but presented with erythroblastosis fetalis requiring exchange transfusion. His IQ was determined to be 60 and there was a history of attention deficit-hyperactivity disorder.

On physical examination, the patient was tachycardic (104 beats/min, irregular) and normotensive (with blood pressure [BP] of 105/60mm Hg). He was 1.64m tall, complained of recent weight loss, and had a body mass index (BMI) of 21.7. The patient was apathetic and did not present any other symptom suggestive of hyperthyroidism. Examination of the thyroid gland identified a small and diffuse goiter (30g) with a slightly irregular surface.

Laboratory tests indicated that he had elevated total T_4 (TT_4), total T_3 (TT_3), and free T_4 (FT_4) concentrations, but normal TSH levels (table I). The evaluation of anti-thyroid antibodies showed an anti-thyroperoxidase (anti-TPO) level of 3.9 U/mL (normal levels <0.5), an anti-thyroglobulin (anti-TG), 1/100 (normal levels <1/100), and thyroid-stimulating hormone receptor antibody (TRAb) of 24% (normal levels <15%). His I^{131} thyroid uptake over 1 hour was 8% (normal range 5–15%), and over 24 hours it was 47% (normal range 20–45%). A thyroid scintigraphic scan revealed that the thyroid gland was enlarged, predominantly in the right lobe, with the uniform distribution of the tracer. Electrocardiography examination confirmed atrial fibrillation and an incomplete right bundle branch block. A two-dimensional echocardiography was performed and showed mild left atrial dilation and a slight dilation of the right cavities.

The presence of a TSH-producing pituitary adenoma was ruled out by means of magnetic resonance imaging (MRI) with gadolinium. Hormonal testing showed prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and cor-

Table I. Thyroid function tests and thyroid weight from the patient

	Total T_3 (ng/mL)	Total T_4 (μg/dL)	Free T_4 (ng/dL)	TSH (μUI/mL)	Thyroid weight
Normal range	0.87–1.78	4.5–12	0.58–1.63	0.49–4.6	
Level at presentation	2.10	23.95	4.5	1.23	30g
Methimazole + cabergoline	3.22	22.94	5.32	35.05	80–85g
Tri-iodothyroacetic acid	3.75	35.4	5.5	2.80	25g

TSH = thyroid stimulating hormone.

tisol concentrations within normal values. Based on a presumptive diagnosis of resistance to thyroid hormones, therapy with cabergoline plus methimazole was indicated. The patient was treated with cabergoline 0.5 mg/week plus methimazole 20 mg/day for a month, which was then increased to cabergoline 1 mg/week plus methimazole 25 mg/day for an additional 2 months. This treatment was stopped because TSH and goiter increased (table I) and the patient persisted with atrial fibrillation. A cardioversion was carried out, with a good clinical response. Monotherapy with tri-iodothyroacetic acid (TRIAC) 2.1 mg/day was then prescribed after molecular diagnosis confirmed RTH. Three months after the start of TRIAC therapy, the thyroid gland appeared normal in size, the levels of thyroid hormones were increased with the decrease of TSH level to a normal range (table I), the patient returned to the atrial fibrillation, he was treated with atenolol 100 mg/day, propaphenone 300 mg/day, and anticoagulated with warfarin. TRIAC administration was interrupted and then continued with the cardiological drugs described above. However, the patient persisted with atrial fibrillation.

Thyroid Function Tests

TT_4 concentrations were determined using fluorescence polarized immunoassay (FPIA; AxSYM®, Abbot GmbH & Co. KG, Wiesbaden, Germany). TT_3 concentrations were determined by microparticle immunoassay (MEIA; AxSYM®, Abbot GmbH & Co. KG, Wiesbaden, Germany).

FT_4 concentrations were determined using electrochemiluminescence immunoassay (Elecsys®, Roche Diagnostics, Penzberg, Germany). Serum TSH levels were measured by MEIA (AxSYM®, Abbott GmbH & Co. KG, Wiesbaden, Germany). Anti-TPO antibodies were determined by direct binding using an TPO-antibodies coated tube assay (RSR Ltd, Cardiff, UK). Anti-microsomal and anti-TG antibodies were measured by the agglutination method (Serodia-AMC, Fujirebio Inc., Tokyo, Japan). TRAb were measured using radioreceptor method (Assay for thyrotrophine receptor autoantibodies; RSR Ltd, Cardiff, UK).

Genomic DNA Isolation

After the project was approved by the institutional review board and written informed consent had been obtained from the patient, peripheral blood samples were collected. Genomic DNA was isolated from white blood cells by the SDS-proteinase K method.

DNA Amplification

The exons 9 and 10 of the *TR*β gene, including splicing signals and the flanking intronic regions of each intron, were amplified with PCR. The PCR amplifications were performed in 100μL, using a standard PCR buffer (Invitrogen Life Technologies, Carlsbad, CA, USA), containing 100–300ng of genomic DNA, 2.5 mmol/L $MgCl_2$, 200 μmol/L each dNTP (dATP, dCTP, dTTP, and dGTP), 4% dimethylsulfoxide, 2U Taq polymerase (Invitrogen Life Technologies), and 50 pmoL of each forward and reverse primers. Forward and reverse intronic primers were specially designed for exons 9 and 10 (exon 9: forward: 5′-ctggcatttt-gaattcgttctttgctg-3′; reverse: 5′-aaagctctttggatccccactaacgag-3′; exon 10: forward: 5′-ccttccatctctgcagcaatgtccatc-3′; reverse: 5′-gaattatgagaatgaattcagtcagt-3′).

Samples were denatured at 95°C for 3 minutes followed by 40 cycles of amplification. Each cycle consisted of denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, and primer extension at 72°C for 1 minute. After the last cycle, the samples were incubated for an additional 10 minutes at 72°C to ensure that the final extension step was complete.

The amplified products were analyzed in 2% agarose gels. Purification of PCR fragments for sequence reactions was performed with Concert™ rapid gel extraction system (Invitrogen Life Technologies, Carlsbad, CA, USA).

DNA Sequencing

DNA sequences from each amplified fragment were performed with the Taq polymerase-based chain terminator method (*f*mol® DNA Cycle Sequencing System, Promega, Madison WI, USA) and using the specific forward and reverse primers used in the DNA amplification. The results were analyzed using the PC gene (Intelligenetics, Geneva, Switzerland), DNASTAR Lasergene (DNASTAR Inc., Madison, WI, USA), and Nucleotide BLAST (http://www.ncbi.nlm.nih.gov/BLAST) software programs.

Microsatellite Analysis

The TGrI29 and TGrI30 microsatellites, localized in intron 29 and 30 of the human thyroglobulin gene, respectively, were amplified using the primers and PCR conditions described previously.[33] PCR products were resolved by electrophoresis in 6% polyacrylamide-denaturing gels.

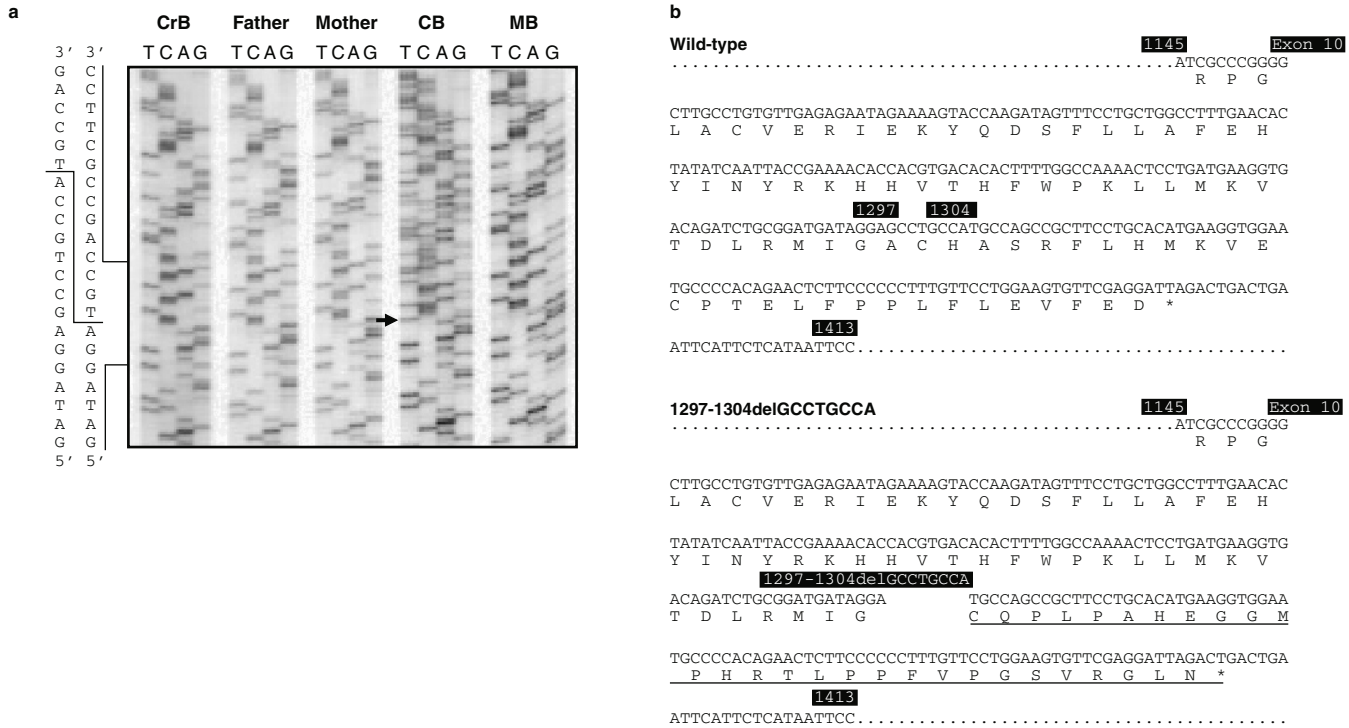

Fig. 1. Identification of the 1297-1304delGCCTGCCA mutation in exon 10 of the thyroid hormone receptor β (*TR*β) gene. (**a**) Partial DNA sequence (coding strands) of the exon 10 PCR-amplified fragment from the genomic DNA of the patient (CB). The arrow points to the 1297-1304delGCCTGCCA mutation in the propositus. Wild-type alleles co-exist with the mutated allele. The father, mother, and sisters (CrB and MB) had only the wild-type sequences at this position. (**b**) Partial nucleotide and deduced amino acid sequences of exon 10 from wild-type and mutant 1297-1304delGCCTGCCA *TR*β gene. The exon maps between nucleotides 1145 and 1413 of the thyroid hormone receptor mRNA. The 28 altered amino acids produced by the 8-nucleotide deletion are underlined and the site of the new stop codon is indicated by an asterisk. The nucleotide sequence is given in the upper line and amino acid translation (represented by single-letter code) is given below their respective codons.

Results

Thyroid Function Studies

The results of thyroid function test of patient are shown in table I; thyroperoxidase, thyroglobulin, and TSH receptor antibodies were positives. The patients' parents were clinically and biochemically normal. These findings are suggestive of the diagnosis of RTH associated with thyroid autoimmunity.

Sequence Analysis of Thyroid Hormone Receptor β Gene

Exons 9 and 10 of the *TR*β gene were analyzed along with the flanking regions of each intron. The GT-AG splicing consensus sequences are conserved. We identified an eight base deletion, 1297-1304delGCCTGCCA in exon 10 (figure 1). This 8-nt deletion produces a frameshift at amino acid 433 and introduces a stop codon TGA at position 461, 85 nt downstream from deletion (figures 1 and 2). The wild-type stop codon was at position 462. This mutation was not detected in the father, mother, and sisters, suggesting that it was a *de novo* mutational event. This finding was confirmed by DNA fingerprint analysis. The haplotypes between TGrI29 and TGrI30 genotypes for all family members are shown in figure 3. The propositus inherited the haplotype TGrI29.2/TGrI30.8 from his mother and the haplotype TGrI29.1/TGrI30.1 from his father.

Discussion

Our patient presented with markedly elevated thyroid hormones levels, unsuppressed TSH concentrations, atrial fibrillation, a small and diffuse goiter, positive TPO, TG, and TSH receptor antibodies, and a slightly increased I^{131} thyroid uptake at 24 hours. These findings suggested a diagnosis of RTH associated with thyroid autoimmunity. RTH has multiple clinical manifestations and is characterized by elevated serum levels of thyroid hormones and normal, or slightly increased, serum TSH concentration that respond to thyrotropin-releasing hormone (TRH).[1] While most individuals are clinically euthyroid, some individuals with RTH appear to be hypothyroid while others appear thyrotoxic. This had

led investigators to classify RTH based on a subjective clinical presentation. Those that present with eumetabolic or hypothyroid are categorized as generalized RTH (GRTH) and those with hypermetabolic as pituitary RTH (PRTH);[34] GRTH and PRTH are phenotypic variants of the same genetic disorder.

Mutations in the *TR*β gene[19-29] result in a receptor with a dominant negative effect on the mutated receptor protein on normal *TR*β function.[31-33]

The identification and characterized of an increasing number of natural mutations has provided important insights into structure-function relationships in this receptor. The patient had a deletion at 1297-1304delGCCTGCCA in the AF-2 region of the *TR*β (figure 2). The AF-2 domain is an activation function region located within helix 12 of the LBD in the carboxy-terminal extreme which is conserved among nuclear hormone receptors.[35] Based on the x-ray crystal structure of the *TR*β, it has been suggested that the AF-2 domain induced conformational changes after binding to the ligand.[36-38] The major change is that the AF-2 domain in helix 12 comes into close contact with helices 3, 5, and 6. The apposition of helix 12 with these other regions of the receptor creates a small hydrophobic cleft that is a binding site for transcriptional CoAs.[39] The conformational changes cause the disruption in CoRs binding, the helix 12 of the thyroid hormone receptor acts as a dual functional domain. After the binding of T_3, its conformation changes, causing the disruption of CoRs and recruitment of CoAs.[32]

Mutations in helix 12, particularly mutations that truncate the receptor,[40] appear to cause a particularly severe form of hormone resistance, such as that observed in our patient. Several mutations

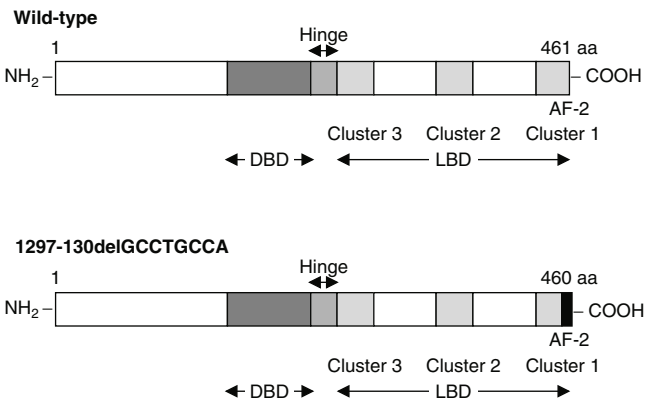

Fig. 2. Schematic representation of the DNA-binding domain (DBD) and ligand-binding domain (LBD) in the wild-type and putative mutant 1297-1304delGCCTGCCA thyroid hormone receptor β proteins. The mutational clusters 1, 2, 3, and hinge region are all indicated. The dark box represents the 28 altered amino acids and a new stop codon due to the frameshift. **AF-2** = activating function 2.

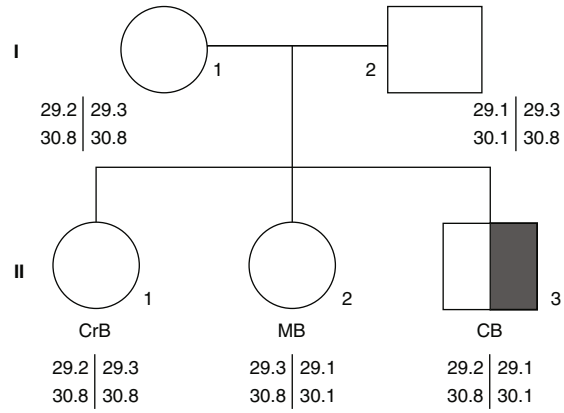

Fig. 3. The pedigree of the patients' family showing haplotype analysis using the two thyroglobulin short tandem repeats (TGrI29 and TGrI30). All data are aligned with each individual symbol on the pedigree. The half-filled symbol denote the heterozygous 1297-1304delGCCTGCCA mutation. Note that the propositus (II-3, CB) had inherited the haplotype TGrI29.2/TGrI30.8 from his mother (I-1) and haplotype TGrI29.1/TGrI30.1 from his father (I-2).

have been reported in the AF-2 domain in RTH patients who show normal T_3 binding, DNA binding, and heterodimerization. However, these mutations are transcriptionally inactive in the presence of ligand, as a result of defective binding to CoAs, and have potent dominant-negative activity in co-transfection studies.[41,42]

Other *TR*β mutants are defective in ligand-induced release of CoRs and have been shown to have strong dominant-negative activity.[43] When the transcriptional and hormone-binding properties of natural mutant receptors associated with RTH are considered, three types of mutants have been described: type I mutants exhibit reduced transactivation in keeping with their altered ligand binding, but higher T_3 concentrations elicit full activation; type II mutants have disproportionately reduced transactivation relative to their altered ligand binding at both low and high T_3 concentrations; type III mutants neither bind ligand nor transactivate. The activation profile of one of the mutants (L454V) in the AF-2 domain is most consistent with type II properties.[41] A possible cause of impaired transactivation is the alteration in receptor DNA binding or dimerization functions. Additional studies of the AF-2 domain mutants indicate that this region also plays a critical role in CoRs. Although the AF-2 domain mutants L454S, L454A, and E457A, still bind T_3 and fail to interact with CoAs, their interactions with NcoR were very different.[32]

The various forms of RTH could represent a continuum of the same defect with variable expression in different tissues. In the present case, atrial fibrillation with increased heart rate response resulted from increased sensitivity to the high levels of thyroid hormones. On the two occasions that atrial fibrillation was de-

tected, we observed increased levels of TT_4 and TT_3. Atrial fibrillation has been observed in RTH patients, but it is unclear whether this is more common than in the general population. Brucker-Davis et al.[3] have reported that 16% of patients with GRTH have an increased HR response when compared to 12.5% of patients without resistance. This may be due to the predominance of *TR*α versus *TR*β in atrial tissue.

No treatment is available to fully correct the defect causing RTH.[34] The currently accepted therapies for patients with signs of hypermetabolic include β-blockers, anxiolytic drugs, dopaminergic agonist, somatostatin analogs, D-T_4,[44] T_3,[45] and TRIAC.[46,47]

In this patient we administered a novel combination of cabergoline and methimazole. However, atrial fibrillation was not prevented. Moreover, when the dose of both drugs was increased, the level of TSH and goiter increased. This resulted in the discontinuation of treatment with cabergoline and methimazole after three months. Monotherapy with TRIAC was prescribed but this therapy was not effective in preventing atrial fibrillation.

In our opinion, experimental therapies such as GC-1, a TRβ-specific agonist, could be useful in the current case. GC-1 would allow TSH suppression through $TR\beta_2$ and reduce thyroid hormone levels that act through TRα on the heart. Thus, further clinical and molecular trials are needed before we will be able to fully understand this condition.

Conclusions

This is the identification of a novel sporadic mutation corresponding to 1297-1304delGCCTGCCA deletion in the AF-2 region of the *TR*β. The mutation produces a frameshift at amino acid 433 and introduces a stop codon TGA at position 461. Atrial fibrillation is the predominant clinical feature of the case associated with thyroid autoimmunity. Conventional therapy with antiarrythmic drugs, methimazole with cabergoline and TRIAC, were ineffective in atrial fibrillation. Experimental therapies may be useful in cases like this.

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