Neurofibromatosis type 1: New developments in genetics and treatment

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Neurofibromatosis type 1 is the most common neurocutaneous syndrome, with a frequency of 1 in 2500 persons. Diagnosis is paramount in the pretumor stage to provide proper anticipatory guidance for a number of neoplasms, both benign and malignant. Loss-of-function mutations in the *NF1* gene result in truncated and nonfunctional production of neurofibromin, a tumor suppressor protein involved in downregulating the RAS signaling pathway. New therapeutic and preventive options include tyrosine kinase inhibitors, mTOR inhibitors, interferons, and radiofrequency therapy. This review summarizes recent updates in genetics, mutation analysis assays, and treatment options targeting aberrant genetic pathways. We also propose modified diagnostic criteria and provide an algorithm for surveillance of patients with neurofibromatosis type 1. (J Am Acad Dermatol 2021;84:1667-76.)

Key words: interferon; mTOR inhibitor; mutation analysis; neurofibromatosis; radiofrequency therapy; RAS; tyrosine kinase inhibitor.

Neurofibromatosis (NF) type 1 is the most commonly inherited neurocutaneous syndrome.[1,2] Variable expressivity, even within the same family, makes anticipatory guidance and prenatal counseling difficult. Common cutaneous manifestations that may suggest this diagnosis include axillary freckling, café au lait macules, and neurofibromas (Figs 1 and 2).

HISTORY AND EPIDEMIOLOGY

The earliest reports of individuals with NF date from 1000 CE.[3] In 1881, von Recklinghausen coined the name *neurofibroma*. In the late 1900s, the condition was classified into 2 types, NF1 and NF2. In 1988, the National Institutes of Health Consensus Development Conference assembled diagnostic criteria for NF1 that remain in use.[4]

NF1 is relatively common, with a birth incidence from 1 in 2500 to 1 in 5000.[5,6] Ethnic differences may exist in penetrance and expressivity. For instance, individuals of African and Asian descent are less likely to develop pediatric brain tumors than those of European lineage because of the difference in frequency of risk alleles.[7] Although NF1 is inherited in an autosomal dominant fashion, up to 50% of cases are due to de novo mutations.[8] In addition, the disease displays complete penetrance with age, with 50% showing characteristic clinical features by age 1 year and 97% by age 8 years.[9]

PATHOGENESIS

The *NF1* gene is localized to chromosome locus 17q11.2.[10,11] Its gene product is neurofibromin, a tumor suppressor of the Ras-mediated signaling pathway.[12,13] By deactivating RAS-GTP, neurofibromin downregulates pathways involved in cell proliferation, including mitogen activated protein kinase (MAPK), MAPK/extracellular signal–regulated kinase (ERK), and cyclic adenosine monophosphate (cAMP)–mediated protein kinase A pathways.[14,15] Patients with NF1 lack a properly functioning neurofibromin, resulting in uninhibited proliferation of RAS-GTP pathways. In addition, there is upregulation of the mTOR pathway, which facilitates malignancy development.[16,17] These pathways are current treatment targets.

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Mutations

Because of the large size of the *NF1* gene, spanning 350 kilobase pairs of genome, it is prone to a variety of mutations, of which 85% to 90% are point mutations, 5% to 10% are microdeletions, and 2% are exon deletions or duplications[18]; 80% of the 1485 described mutations resulted in premature termination codons and truncated neurofibromin.[2,16,19-21] Recently, more genotype-phenotype correlations have been identified and are listed in Table I.[22-29] For instance, clustering of mutations at the 5′ end of the *NF1* gene may predispose individuals to optic nerve gliomas.[30,31] This finding suggests that closer mutational analysis can determine which individuals need more thorough and frequent ophthalmologic examinations.

CAPSULE SUMMARY

- Neurofibromatosis type 1, a neurocutaneous syndrome characterized by benign and malignant neoplasms, has had expanded treatment options due to updates in mutation analysis assaying, facilitating direct targeting of overactive genetic pathways.
- Modified diagnostic criteria and an algorithm for surveillance and treatment are provided to assist in diagnosis and health care maintenance.

Penetrance, expressivity, and mosaicism

Although this autosomal dominant disorder is completely penetrant by adulthood, variable expressivity, even within the same family, is a common phenomenon, likely due to the vast array of mutations in the *NF1* gene. Variable expressivity has also been attributed to allelic heterogeneity, epistasis, and epigenetic factors such as methylation.[32]

Mosaicism in NF1 results in the classification of individuals as having mild generalized disease, localized disease, and gonadal mosaicism. Those with mild generalized disease are likely haplo-insufficient in a small number of cells, resulting in a mild phenotype. Those with localized disease have symptoms only in a segment of their bodies, resulting in difficult genetic mutational analysis and risk counseling. Those with localized disease may occasionally have gonadal mosaicism with *NF1* mutations in germline cells. Although surveillance of these individuals can be relaxed compared to those with generalized NF1, their offspring are at greater risk than the general population of developing classic NF1.[33] Diagnosis of these milder genotypes is facilitated by mutational analysis of Schwann cells from affected areas.[34]

CLINICAL PRESENTATION

Cutaneous signs and symptoms

NF1 is marked by the development of benign and malignant tumors, with the highest risk of developing symptomatic visceral tumors within the first 6 years of life.[35] Café au lait macules (CALMs) and overall cutaneous hyperpigmentation are the earliest signs, appearing within the first 2 years of life (Fig 1).[15,36] CALMs in the skin folds resemble freckles and are referred to as Crowe's sign, the most specific sign for NF1.[37] These 1- to 3-mm, well-circumscribed macules, ranging from light to dark brown in color, commonly appear between ages 3 and 10 years. CALMs may occur in the axillae, groin, or inframammary region; on the neck and trunk; or around the lips.[38,39] Additionally, they grow proportionately to body growth. Mutational analyses of melanocytes from CALMs show 2-hit *NF1* mutations and consequent upregulation in the Ras signaling pathway.[15,40]

Cutaneous neurofibromas usually occur in children older than 7 years and are the most common cutaneous manifestations in NF1 that are responsible for disfigurement, pain, and paresthesia. Thus, they may be the most distressing physical finding.[41] They tend to increase in number during puberty and pregnancy and are first evident as slow-growing nodules that may become pedunculated. Classically, they may demonstrate the buttonhole sign and rarely reach a size greater than 3 cm. Subcutaneous neurofibromas may cause more tenderness and paresthesia than their cutaneous counterparts.[42,43] They require closer monitoring because of an enhanced potential for malignant transformation.

Plexiform neurofibromas (PNs) are found in one third of patients with NF1. Diffuse PNs tend to occur in early childhood, whereas deep nodular PNs develop in adolescence. Most form along nerve trunks or spinal roots; however, some can infiltrate soft tissue, causing disfigurement and pain (Fig 2). They also are commonly associated with pigmentary changes and hypertrichosis in the overlying skin.

Although most plexiform neoplasms are clinically indolent, the loss of the *CDKN2A* locus on chromosome 9p21.3 is associated with atypical neurofibromatous neoplasms of uncertain biologic potential that progress to malignant peripheral nerve sheath tumors with high penetrance.[44] Early delineation of rapidly growing plexiform neurofibromas is essential, because this behavior serves as a sign of possible malignant neurofibroma transformation. PNs have a 15% lifetime incidence of transformation to highly aggressive sarcomas called malignant peripheral nerve sheath tumors (MPNSTs).[45]

Abbreviations used:

CALM:	café au lait macules
cAMP:	cyclic adenosine monophosphate
MAPK:	mitogen activated protein kinase
MEK:	MAPK/ERK kinase
MPNST:	malignant peripheral nerve sheath tumor
NF:	neurofibromatosis
PN:	plexiform neurofibromas

Early diagnosis in the pretumor stage is particularly important because children with NF1 are at increased risk of certain visceral neoplasms, including Wilms tumor, duodenal carcinoids, and pheochromocytoma. A cohort study following 167 childhood cancer survivors with NF1 and 1541 childhood cancer survivors without NF1 reported a 20-year cumulative incidence of subsequent neoplasms in childhood cancer survivors with NF1 of 7.3%, compared with 2.9% in the childhood cancer survivors without NF1 (P = .003); NF1-affected individuals had a 2.4-fold higher risk of subsequent neoplasms.

Common dermoscopic features in neurofibromas include peripheral pigment network, peripheral halo of brown pigmentation, and fingerprint-like structures. Isolated neurofibromas demonstrate dermoscopic features of pink-red structureless areas, blood vessels, and scar-like areas. Fissures and irregular linear crypts are observed in both types of neurofibromas.[46]

Malignant peripheral nerve sheath tumors

MPNST, a rare, aggressive soft-tissue sarcoma originating from Schwann cells, lacks the mature Schwann cell S-100 marker in approximately 50% of cases because of dedifferentiation.[47,48] Approximately half of MPNSTs arise in patients with NF1, and MPNSTs develop in 8% to 13% of patients with NF1.[45,49] This transformation, which usually metastasizes early, generally occurs in patients ages 20 to 25 years old and is evident as pain, swelling, and increased size. The most common metastatic sites of MPNSTs include the lung, soft tissue, bone, and liver, with metastasis occurring in 39% of the patients with and 16% of the patients without NF1.[45] Long-term survival is possible if the sarcoma is completely excised with wide margins, which is often unfeasible.[45,50-52]

Less common cutaneous manifestations

Blue-red macules and pseudoatrophic macules represent rare variants of neurofibromas. Blue-red macules appear as bluish, soft, dome-shaped to irregular contoured macules developing before puberty, usually on the trunk. Histologic analysis shows neural tissue infiltration into thickened blood vessels.[37,53] Pseudoatrophic macules are evident as depressed plaques with grayish coloration and an atrophic appearance. Histologic analysis shows neural tissue replacing collagen and infiltrated with Schwann cells and fibroblasts.[54] Glomus tumors are subungual benign neoplasms found in approximately 5% of patients with NF1.[55] Xanthogranulomas may be associated with chronic myeloid leukemia (xantholeukemia).[6,56] Nevus anemicus has been anecdotally associated with NF1.[57-59] Cutaneous melanoma are rarely found in patients with NF1.[59-62]

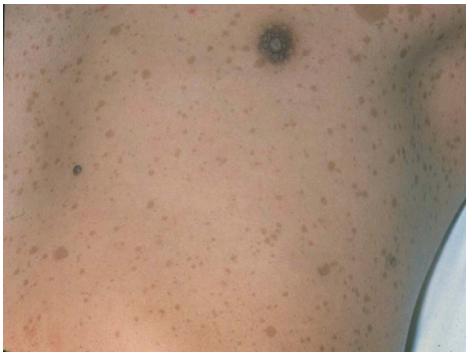

Fig 1. Café au lait macules with axilla freckles.

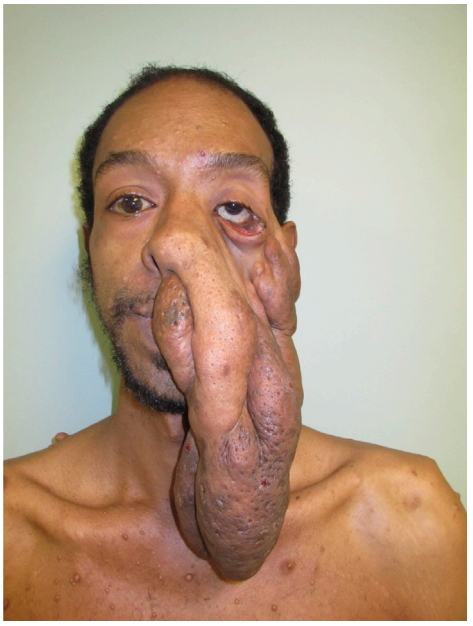

Fig 2. An extreme example of pendulous plexiform neurofibromas.

Systemic manifestations

Systemic manifestations are myriad and sometimes debilitating (Table II[63-77]). Of particular interest is the increased rate of neurocognitive delays and skeletal abnormalities found in patients with NF1.

Table I. Genotype-phenotype correlations

Subtype	Mutation	Clinical features
1[21,30-32]	Microdeletion chromosome locus 17q11.2, maternal chromosome	Affects 5% of patients with NF1 Increased number of dermal fibromas, more severe learning disabilities, higher risk of cardiovascular and malignant peripheral nerve sheath tumors Craniofacial dysmorphism Tend to be taller than average
2[19,34,101]	3-base pair deletion in exon 17 (AAT)	Pigmentary disorders and pulmonic stenosis Lower frequency of all other complications without clinically evident cutaneous or plexiform neurofibromas
3[24]	Splice site germline mutations	Increased tendency to develop malignant peripheral nerve sheath tumors and CNS gliomas
4[25-27]	In-frame duplication in exon 28, 80-kb deletion	Also known as Watson syndrome Multiple café au lait spots Pulmonic stenosis and intellectual disability Lower frequency of Lisch nodules and neurofibromas
5[28,29]	*SPRED1*, chromosome 15	Also known as Legius syndrome Presence of café au lait spots and freckling but no peripheral nerve tumors or eye findings Macrocephaly and lipomas more common

CNS, Central nervous system; *NF1*, neurofibromatosis type 1.

Congenital pseudoarthrosis of the tibia is closely related to NF1.[78-83] NF1-deficient osteoblasts promote the activation of osteoclasts through the secretion of cytokines such as osteopontin.[82,84,85] Sphenoid dysplasia, a facial skeletal alteration involving the big and/or small wings, is another recently documented skeletal manifestation of NF1.[86] Furthermore, haploinsufficiency of neurofibromin leads to premature apoptosis of osteoblasts and alteration in proliferation/differentiation of osteoprogenitor cells.[87-89] Up to 81% of children with NF1 show moderate to severe impairment in at least 1 cognitive domain; almost 40% fulfill diagnostic criteria for attention deficit/hyperactivity disorder.[90] The NF1-associated cognitive sequelae and their etiology have not been fully elucidated.[91-96]

DIAGNOSIS

A patient meeting 2 of the 7 diagnostic criteria is considered to have NF1[4]:

- 6 or more CALMs with a diameter greater than 5 mm in prepubescent individuals and greater than 15 mm in postpubescent individuals,
- 2 or more neurofibromas of any type or 1 plexiform neurofibroma,
- freckling in the axillary or inguinal regions,
- optic nerve glioma,
- 2 or more iris Lisch nodules,
- a distinctive osseous lesion (eg, sphenoid wing dysplasia, long bone cortical thinning with or without pseudoarthrosis),
- a first-degree relative who meets the criteria for NF1.

We suggest that mutational analysis demonstrating mutation in *NF1* gene should be added to the diagnostic criteria. The presence of 1 criterion should alert physicians to the potential diagnosis, because earlier intervention allows for better outcome. In particular, in children with 6 CALMs, 95% will develop NF1.[97] In patients with both cutaneous neurofibromas and skin freckling, we suggest that mutational analysis be considered in future diagnostic criteria before confirming a diagnosis of NF1, because axillary and inguinal freckling represent small CALMs. The presence of RNA mutations in patients with presumed NF1 has high sensitivity.[98] Furthermore, negative results on analysis of the *NF1* gene provide strong reassurance that children with 6 or more CALMs are unlikely to have NF1.[98]

Imaging may be desirable, particularly if the patient becomes symptomatic or is unable to cooperate with a full ophthalmologic examination. MRI for optic pathway glioma, bone scans, and echocardiography are recommended when patients become symptomatic.[99] Examination with near infrared confocal ophthalmoscopy may be used to evaluate for choroidal abnormalities.[90] Positron emission tomography with computed tomography scan may be performed if there is suspicion of transformation of PNs in0to MPNSTs, particularly in patients with microdeletions who are more prone to developing MPNSTs (Table II).

Table II. Other manifestations in NF1

Organ systems	Specific manifestations
Ophthalmologic[35,63,64]	Optic pathway glioma with frequency up to 20%, characterized by proptosis, strabismus, diminished visual acuity, poor color vision, and pale optic discs
	Lisch nodules (iris hamartomas)
	Sphenoidal wing dysplasia causing exophthalmos
Neurologic[65-70]	Macrocephaly due to increased white matter volume
	Epilepsy
	Sensorineural hearing loss
	Cerebrovascular disease
	Aqueduct stenosis causing increased intracranial pressure
	Spinal cord compression from plexiform neurofibromas or scoliosis
	Intellectual disability in up to 60% of individuals
	Autism
	Attention deficit hyperactivity disorder in up to 50% of individuals
	Poor social skills
Endocrine[69,71-73]	Precocious puberty
	Short stature in 40%
	Growth hormone excess leading to tall stature in 46%
	Pheochromocytoma
Skeletal[6,8,31]	Pseudoarthrosis, especially of the tibia
	Scoliosis
	Sphenoid dysplasia at birth
	Nonossifying fibromas
	Decreased bone mineralization, causing pathologic fractures
Other tumors/ conditions[37,74-77,107]	Congenital heart disease
	Pulmonary stenosis
	Coarctation of the aorta
	Renal artery stenosis
	Teratoma
	Laryngeal neurofibromas
	Juvenile myelomonocytic leukemia
	Gastrointestinal stromal tumors
	Rhabdomyosarcoma

NF1, Neurofibromatosis 1.

THE IMPORTANCE OF MUTATIONAL ANALYSIS

NF1 mutational analysis is usually performed in patients with high clinical suspicion for NF1, particularly in individuals seeking prenatal counseling or children younger than 8 years. Genetic counseling of patients with typical NF1 requires emphasis on the difficulty of accurately predicting phenotype because of variable expressivity. For children with sporadic NF1, mutation analysis may be useful to determine if the known genotype-phenotype subtypes or SPRED 1 genotypes are found. In particular, prenatal counseling proves challenging.[97,100-102]

DIFFERENTIAL DIAGNOSIS

The major differential diagnosis for NF1 includes Legius syndrome, Noonan syndrome, and NF2. Each of these can be distinguished from NF1 with genetic testing.[6,8,103-105]

TREATMENT

Physical destruction

Cutaneous neurofibromas are benign and do not require removal unless they are symptomatic. Rapid growth, particularly in a subcutaneous or plexiform neurofibroma, may mandate biopsy or excision, because that may signify malignant degeneration.[106] Surgical excision, laser, and electrocautery have been commonly used. However, surgical resection is often difficult because neurofibromas lack a well-defined capsule and show high tumor vascularity. Tumor recurrence is more common in patients younger than 10 years and those with incomplete surgical resection.[107] For individuals with a high number of cutaneous neurofibromas, excision may not be feasible. Carbon dioxide laser treatment under general anesthesia has been proposed for small to medium-sized neurofibromas. One drawback to carbon dioxide ablation is the development of depigmented, atrophic scars.[108-110]

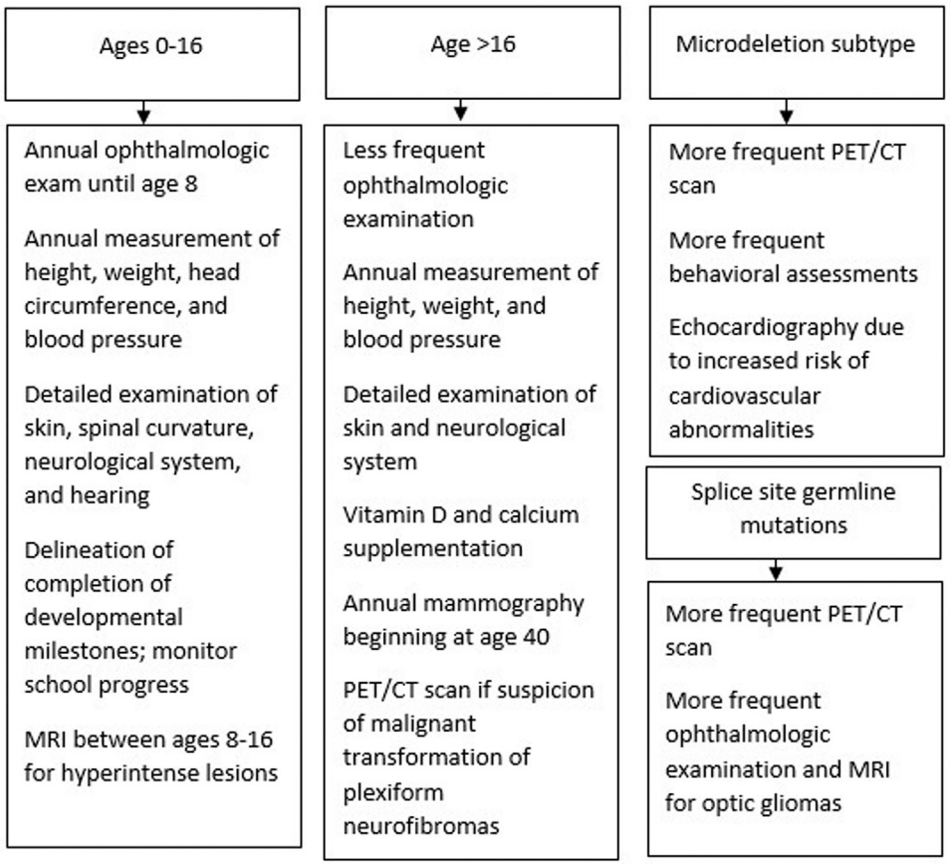

Fig 3. Approach to diagnosis, treatment, and surveillance of patients with NF1.[128-133] *CT,* Computed tomography; *MRI,* magnetic resonance imaging; *PET,* positron emission tomography.

Treatment of PNs is more difficult because of the involvement of nerves. Surgical resection is the criterion standard of treatment but is often impractical. For inoperable tumors, other modalities have been used. Radiofrequency therapy has been employed with some success; however, radiation therapy is contraindicated because of the potential for inducing MPNSTs.[111,112] Examination with magnetic resonance imaging and positron emission tomography scans can be used to monitor the progression of PNs and determine if transformation to MPNSTs has occurred, although histologic analysis is necessary for confirmation.

Interestingly, in the setting of MPNSTs, adjuvant radiation is occasionally provided to reduce the risk of local recurrence and as a limb-salvaging strategy. Adjuvant chemotherapy has a role in advanced or metastatic disease, but prognosis remains poor even with treatment.[50,113] The novel glutamine antagonist prodrug JHU395 has also shown promising antitumor activity in malignant peripheral nerve sheath tumors.[114]

Optic pathway gliomas are the most common type of gliomas in NF1, are generally asymptomatic, and are treated with carboplatin (with or without vincristine) chemotherapy.[12,113] Radiation is typically avoided.[115]

Targeted genetic treatment

Sirolimus, an immunosuppressant, has been successfully used to delay PN progression and decrease associated pain. This drug inhibits the mTOR pathway, which is commonly implicated in tumor growth in NF1.[61,116] Sirolimus is generally well tolerated.[61] Tipifarnib blocks RAS signaling by inhibiting the farnesylation of RAS, thus downregulating this pro-oncogenic pathway. Although it does not prevent PN progression, tipifarnib improves the emotional domain of quality of life compared to placebo, perhaps by acting on hippocampal neurons.[117,118] Pirfenidone, an inhibitor of fibroblasts, has also been shown to inhibit disease progression, with the most common adverse effect being gastrointestinal discomfort.[119] However, it does not cause tumor regression. The use of pegylated interferons

because of their antiproliferative and antiangiogenic properties has been shown to induce tumor regression and decrease pain levels.[120]

Imatinib, a tyrosine kinase inhibitor, has also been used for PNs. In contrast to sirolimus, this drug may result in tumor regression, with a median reduction of 26.5% in tumor volume, in addition to halting tumor progression.[121,122] However, its use is limited by adverse effects, including edema, skin rash, pain, weight gain, aminotransferase elevation, and neutropenia. More recently, the use of nilotinib in vivo has also led to tumor size reduction with fewer adverse effects compared to imatinib.[123] Phase 2 clinical trials on the use of selumetinib, cabozantinib, and PD-0325901 are currently being held to discover other options for inoperable PNs.

Direct tyrosinase inhibitors, such as kojic acid, have been suggested to target the hyperpigmentation and CALMs in NF1.[15] Other genetic pathway inhibitors include MEK inhibitor PD032059 and protein kinase A—cAMP pathway inhibitor HA1004.[15,124] More established treatments for CALMs include pulsed radiofrequency therapy and topical vitamin D3.[125,126] In addition, because vitamin D levels tend to be significantly lower in patients with NF1, use of ultraviolet B irradiation has been proposed to increase vitamin D levels and potentially reduce hyperpigmentation.[127]

CONCLUSION

Surveillance is salient. In Fig 3,[128-133] we highlight recommended examinations in patients aged 0 to 16 years and older than 16 years and important evaluations in patients with the microdeletion or splice site germline mutation subtypes of NF1. We recommend that genetic confirmation be added to the diagnostic criteria for NF1.

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