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Missense *MED12* variants in 22 males with intellectual disability: From nonspecific symptoms to complete syndromes

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Abstract

We describe the phenotype of 22 male patients (20 probands) carrying a hemizygous missense variant in *MED12*. The phenotypic spectrum is very broad ranging from

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nonspecific intellectual disability (ID) to the three well-known syndromes: Opitz–Kaveggia syndrome, Lujan–Fryns syndrome, or Ohdo syndrome. The identified variants were randomly distributed throughout the gene ($p = 0.993$, χ^2 test), but mostly outside the functional domains ($p = 0.004$; χ^2 test). Statistical analyses did not show a correlation between the *MED12*-related phenotypes and the locations of the variants ($p = 0.295$; Pearson correlation), nor the protein domain involved ($p = 0.422$; Pearson correlation). In conclusion, establishing a genotype–phenotype correlation in *MED12*-related diseases remains challenging. Therefore, we think that patients with a causative *MED12* variant are currently underdiagnosed due to the broad patients' clinical presentations.

KEYWORDS
intellectual disability, MED12, phenotype, genotype

1 | INTRODUCTION

Intellectual disability (ID) is characterized by impaired intellectual functioning and adaptive behavior arising before the age of 22 (Schalock et al., 2021). ID is one of the most common neurodevelopmental conditions with a prevalence of about 1%–3% (Patel et al., 2018) and X-linked ID (XLID) accounts for about 5%–10% ID in males (Lubs et al., 2012).

MED12 (OMIM no. *300188) is an X-linked gene that encodes for the Mediator of RNA polymerase II transcription subunit 12 protein that is part of the kinase module of the Mediator complex, together with CDK8, MED13, and Cyclin C, which is involved in the regulation of gene expression through the RNA polymerase II. MED12 comprises 2177 amino acid (aa) residues and contains three functional domains: Med12 (103–161 aa), LCEWAV (289–757 aa), and PQL-catenin-binding (1616–2051 aa) (Polla et al., 2020). Pathogenic variants in the kinase module, including MED12, have been shown to disrupt developmental and oncogenic signaling pathways, such as Wnt, Sonic Hedgehog, Epidermal Growth Factor, and Notch pathways (Clark et al., 2015).

MED12 germline variants are known to cause syndromic or non-syndromic forms of XLID. Syndromic forms include (i) Opitz–Kaveggia syndrome (also known as FG syndrome 1 [FGS1], OMIM no. 305450) which is characterized by ID, hypotonia, distinct facial features, relative macrocephaly, broad thumbs and halluces, corpus callosum abnormalities, and gastrointestinal complications (Risheg et al., 2007); (ii) Lujan–Fryns syndrome (OMIM no. 309520) which partially overlaps with FGS1 but shows several distinctive features, such as a tall stature, hypernasal voice, and hyperextensible digits (Schwartz et al., 2007) and (iii) Ohdo syndrome (OMIM no. 300895) which shows particular features, as ptosis and blepharophimosis (Vulto-van Silfhout et al., 2013). These *MED12*-related syndromes have been assigned to different regions of the protein: variants in leucine-rich and serine-rich region (LS, 500–1616 aa) have been associated with FGS1 and Lujan syndrome, while variants in LS and PQL-catenin-binding domain have been associated with Ohdo syndrome (Srivastava et al., 2019). Notwithstanding, other nonspecific clinical presentations were also described (Bouazzi et al., 2015; Prontera et al., 2016; Yamamoto & Shimojima, 2015), where the three corresponding missense variants were also randomly distributed along *MED12*.

In the past decade, next-generation sequencing (NGS) has revealed several unique variants in *MED12* in male patients with ID, and there is an urgent need to assess whether the phenotype of those patients is compatible with either FGS1, Lujan–Fryns or Ohdo syndromes. Nevertheless, the more variants are identified in this gene, the more difficult is the patients' classification into the different related syndromes due to the high phenotypic variability related to *MED12* variants (Charzewska et al., 2018). Because of this phenotypic variability in the increasing number of *MED12* variants that are found by NGS, it is essential to gain more knowledge about the different effects of *MED12* variants and possible phenotypes. This could result in more accurate clinical counseling of the involved families, and facilitate the challenge of distinguishing pathogenic variants from non-pathogenic variants in the *MED12* gene.

In this study, we focus on 22 male patients (20 probands) with variants in the *MED12* gene. We provide an overview of the clinical features and discuss their differences and similarities with the previously reported *MED12* mutational spectrum.

2 | PATIENTS AND METHODS

2.1 | Patient cohort

We report a series of male patients with ID who harbor a variant in *MED12*. The patients were gathered from an international collaboration through Genematcher (Au et al., 2015; Sobreira et al., 2015). In total, we have managed to recruit 22 male patients from 10 different hospitals/research institutes. Of these, 20 are index male patients. All reported patients were evaluated by their respective clinical geneticists and DNA sequencing studies (either

targeted or exome sequencing) were performed for diagnostic purposes. The severity of ID was determined with different tests: WISC-III, WISC-IV, and WAIS-III, by checking developmental milestones, or by performing a complete neuropsychological evaluation depending on the age of the individuals. Written informed consent has been obtained from all the legal representatives of the participants to publish clinical data and photographs of the included patients.

2.2 | Statistical analyses

For the purposes of this study, statistical analyses, namely chi-square (χ^2) and Pearson's chi-squared test (Pearson's χ^2), have been performed by using SPSS Statistics 26.0 (IBM-SPSS, Chicago, Illinois). A *p*-value of <0.05 was considered statistically significant. The variables tested included variant exon location, protein domain involved, affected amino acid residue, clinical features, and syndrome classification.

3 | RESULTS

Here, we describe 22 male patients (20 probands) carrying a missense variant in the *MED12* gene. Most of the probands were found through exome sequencing (11/20) and the other patients by single gene testing (5/20), targeted ID (2/20), or X-linked ID (1/20) panels (Table 1 and Table S1). Individuals, who had undergone single gene testing, reported with a phenotype that fitted a number of the characteristics of the *MED12*-related phenotypes. In total, 13 of these variants were maternally inherited and 4 occurred de novo. The mothers of three remaining patients were unavailable for testing. Patient 11 also had a maternally-inherited pathogenic variant c.913C > T (p.Arg305*) in *RPS6KA3* pointing to a diagnosis of Coffin–Lowry syndrome.

TABLE 1 Molecular data of *MED12* patients

Classification (syndrome)	Patient	Genomic (NM_005120.2)	Protein (NP_005111.2)	Domain	Mother carrier	Diagnostic methodology	Variant classification (ACMG/AMP guidelines)
Opitz-Kaveggia	1	c.2881C > T	p.(Arg961Trp)	No	Yes	Exome sequencing	P
	2	c.2881C > T	p.(Arg961Trp)	No	No	Targeted variant analysis	P
	3	c.3020A > G	p.(Asn1007Ser)	No	Yes	Single gene testing	LP
	4	c.3469A > G	p.(Asn1157Asp)	No	No	Single gene testing	VUS
	5	c.3883C > T	p.(Arg1295Cys)	No	Yes	Single gene testing	P
Lujan–Fryns	6	c.67G > A	p.(Asp23Asn)	No	NT	Exome sequencing	VUS
	7	c.4879C > T	p.(Arg1627Cys)	PQL	Yes	Targeted ID panel	LP
Ohdo	8	c.1054 T > G	p.(Cys352Gly)	LCEWAV	Yes	Exome sequencing	LP
	9	c.3064A > G	p.(Met1022Val)	No	No	Exome sequencing	LP
	10	c.4147G > A	p.(Ala1383Thr)	No	No	Exome sequencing	LP
	11	c.5965C > T	p.(Arg1989Cys)	PQL	Yes	Exome sequencing	LP
Unclassified or unknown	12	c.745C > G	p.(Leu249Val)	No	Yes	Trio exome sequencing	VUS
	13	c.887G > A	p.(Arg296Gln)	LCEWAV	Yes	Exome sequencing	LP
	14	c.1546C > T	p.(Arg516Cys)	LCEWAV	Yes	Trio exome sequencing	LP
	15	c.1862G > A	p.(Arg621Gln)	LCEWAV	Yes	Single gene testing	LP
	16	c.2639 T > G	p.(Met880Arg)	No	No	Exome sequencing	LP
	17	c.3797G > A	p.(Arg1266His)	No	Yes	Single gene testing	B
	18	c.3884G > A	p.(Arg1295His)	No	NT	Unknown	P
	19	c.3946C > G	p.(Gln1316Glu)	No	Yes	Exome sequencing	VUS
	20	c.5009C > T	p.(Ser1670Phe)	PQL	Yes	Targeted XLID panel	VUS
	21	c.5009C > T	p.(Ser1670Phe)	PQL	Yes	Targeted XLID panel	VUS
	22	c.6371C > T	p.(Ala2124Val)	No	NT	Targeted ID panel	VUS

Abbreviations: ACMG, American College of Medical Genetics; AMP, Association for Molecular Pathology; B, Benign; ID, Intellectual disability; LCEWAV, Mediator complex, subunit Med12 LCEWAV-domain; LP, Likely pathogenic; NA, not assessed; P, Pathogenic; PQL, Mediator complex, subunit Med12, catenin-binding domain; VUS, Variant of uncertain significance; XLID, X-linked intellectual disability.

The majority of the variants are located outside currently recognized functional domains (13/20; $p = 0.004$, χ^2 test), despite the variants being randomly located among the 45 *MED12* exons ($p = 0.993$, χ^2 test). Arginine showed to be the most frequently mutated amino acid (9/20; $p = 0.013$, χ^2 test) (Tables 2 and 3), which is in line with previous studies (Khan & Vihinen, 2007).

The phenotypic spectrum of our cohort is very broad, in terms of clinical features and of severity, which is expected as *MED12* is a gene associated with a large phenotypic variability (Table 4 and Table S1). Our patients present from borderline to severe ID. While autism is rare (5/22 patients), behavioral problems are common (15/22), ranging from problems with concentration to aggressivity. Delay or absence of speech is reported in 20/22 patients. MRI was performed in 19/22 patients, 12 of which showed structural brain abnormalities, such as corpus callosum agenesis. Eyes/vision involvement is present in 11/22 patients and hearing impairment in 5/22 patients. One patient had dysplasia of his semicircular canals. This could be an underreported feature in individuals with a *MED12* variant as a T2-weighted MRI specifically directed at finding this aberration needs to be performed. We have collected two uncle/nephew families: Patients 1 and 2, and Patients 20 and 21, respectively. The phenotype is relatively consistent among the two individual family members. The main noticeable difference is vision impairment (Table 4), which occurs in one case the younger (Patient 1), and the other case the older family member (Patient 20).

The presence of facial dysmorphic features is also diverse (Table 4, Figure 1, and Table S1). They range from absence of dysmorphisms (β) to multiple congenital anomalies such as severe prenatal craniosynostosis, tall prominent forehead, hypertelorism, downslanting palpebral fissures, high narrow palate, maxillary hypoplasia, low-set ears, and a cloverleaf skull (Patient 1).

Most of the patients described in this cohort could not be classified into any of the reported syndromes. However, some patients were classified as FGS1 (5/22), Lujan–Fryns syndrome (2/22), or Ohdo syndrome (4/22). One patient (Patient 11) also has a maternally inherited pathogenic variant c.913C > T (p.Arg305*) in *RPS6KA3* pointing to a diagnosis of Coffin–Lowry syndrome in addition to Ohdo–FG spectrum. No significant correlation was observed between the variants' genomic localization and *MED12*-related syndromes (Tables 2 and 3; exon: $p = 0.295$; protein domain: $p = 0.422$ Pearson's χ^2). However, a statistically significant correlation was observed between the affected domain and cardiac defects ($p = 0.010$; Pearson's χ^2). Furthermore, statistical significance was observed in the correlation analysis between hearing impairment and cardiac defects

TABLE 2 Statistical results of the identified variants in all individuals with a *MED12* variant except for individual 11 who also has a pathogenic variant in *RPS6KA3*

χ^2 test	
Null hypothesis	***p*-value**
The variants are randomly distributed over the different exons	0.993
The variants affect randomly the different protein domains	**0.004**
The wild-type amino acid residues are equally affected	**0.013**

Note: A χ^2 test was calculated to determine if there was correlation between the different phenotype–genotype characteristics. Depicted in bold are the correlations with a $p < 0.05$.

TABLE 3 Statistical results of the identified variants in all individuals with a *MED12* variant except for individual 11 who also has a pathogenic variant in *RPS6KA3*

Pearson's χ^2 test							
	Syndrome[a]	Intellectual disability[b]	Autism	Facial dysmorphisms[c]	Eyes/vision involvement	Hearing impairment	Cardiac defects
Exon[d]	0.295	0.568	0.288	0.162	0.872	0.330	0.184
Domain	0.422	0.242	0.242	0.059	0.129	0.410	**0.010**
Syndrome[a]		0.798	0.523	0.138	0.059	0.238	0.904
Intellectual disability[b]			0.703	0.145	0.355	0.296	0.319
Autism				0.407	0.058	0.329	0.439
Facial dysmorphisms[c]					0.286	0.293	0.454
Eye involvement						0.548	0.291
Hearing impairment							**0.034**
Cardiac defects							

Note: A Pearson correlation was calculated to determine if there was correlation between the different phenotype–genotype characteristics. Depicted in bold are the correlations with a $p < 0.05$.

[a]*MED12*-related phenotypes: FGS1; LFS; Ohdo; nonspecific; no clinical information.

[b]Intellectual disability: developmental delay; borderline; mild; moderate; severe; profound; no information.

[c]Facial dysmorphisms: number of clinical features.

[d]Domains: (i) Mediator complex, subunit Med12; (ii) LCEWAV-domain; (iii) catenin-binding domain; and (iv) outside important domains.

TABLE 4 Summary of the clinical data of *MED12* patients

		Neurodevelopment					Dysmorphic features						Other features		
Classification (Syndrome)	Patient	ID	ASD	Behavioral disturbances	Speech impairment	MRI findings	Face shape	Eyes	Nose	Mouth and chin	Ears	Extremities abnormalities	Eyes/vision impairment	Hearing Impairment	Cardiac defects
Opitz-Kaveggia	1	Severe	−	NA	NA	+	+	+	−	+	+	+	+	−	+
	2	Moderate	−	+	+	+	+	+	−	+	+	+	−	−	+
	3	Mild	−	+	+	+	+	+	+	+	+	+	−	−	NA
	4	Mild	+	+	+	−	+	+	+	+	+	+	−	NA	−
	5	Moderate	−	−	+	+	+	+	+	+	+	+	+	−	−
Lujan–Fryns	6	Borderline	−	+	+	−	+	+	+	+	+	+	+	−	−
	7	+	+	+	+	−	+	−	NA	−	−	+	−	−	NA
Ohdo	8	Mild	−	+	+	+	+	+	+	−	+	+	+	−	+
	9	+	−	+	+	+	+	+	+	+	+	+	+	+	−
	10	+	NA	+	+	−	+	+	+	+	+	−	+	−	−
	11	Severe	NA	NA	+	+	+	+	+	+	+	−	−	−	NA
Unclassified or unknown	12	DD	−	+	+	+	NA	+	+	+	+	+	+	NA	+
	13	DD	−	−	+	−	+	+	+	+	+	+	+	−	+
	14	Moderate	−	+	+	−	−	−	−	−	−	+	−	−	−
	15	Moderate	NA	NA	+	NA	+	−	−	+	−	+	+	+	+
	16	Severe	NA	+	+	+	+	−	−	−	−	−	+	+	−
	17	Mild	−	NA	+	NA	+	+	−	+	−	+	−	+	+
	18	Mild	NA	+	NA	+	+	+	−	+	+	+	−	−	NA
	19	Mild	+	+	+	+	+	+	+	+	+	+	−	+	+
	20	Borderline	NA	+	+	+	−	+	+	+	−	+	+	NA	NA
	21	NA	+	NA	+	NA	−	−	+	+	−	+	−	NA	NA
	22	Severe	+	+	+	−	+	−	−	−	+	+	−	−	−

Abbreviations: ASD, autism spectrum disorder; DD, developmental delay; ID, intellectual disability; MRI, magnetic resonance imaging; NA, not assessed.

FIGURE 1 Clinical aspects of patients with a *MED12* variant. Clearly visible are the different phenotypes. For instance, Patient 21 has a completely normal face, whereas Patient 1 presented with multiple congenital anomalies such as severe prenatal craniosynostosis, tall prominent forehead, hypertelorism, down-slanting palpebrae, high narrow palate, maxillary hypoplasia, low-set ears, and a cloverleaf skull.

($p = 0.034$; Pearson's χ^2). Taken together, these data suggest that when a patient presents hearing impairment, the clinician should also look for hidden cardiac defects.

4 | DISCUSSION

In this study, we describe a cohort of 22 male patients carrying missense *MED12* variants, most of which could not be classified into one of the three known syndromes. The phenotypic spectrum presented by the patients is very broad, with different levels of severity. In fact, trying to establish a genotype–phenotype correlation in *MED12*-related diseases remains challenging despite the fact that more than 80 MED12 variants in both males and females have been identified (Figure 2).

Eight of the variants carried by the patients reported herein are described in the literature, and the majority are related to different phenotypes. For instance, the variant c.887G > A (p.Arg296Gln) carried by the Patient 13, is described as associated with clinical variability, even intrafamilial (Martinez et al., 2017; Patil et al., 2017). Schwartz et al. (2007) described "*the original Lujan syndrome family*" carrying the variant c.3020A > G (p.Asn1007Ser) that is the same variant as found in our Patient 3, who is classified as FGS1. The variant c.3883C > T (p.Arg1295Cys) identified in Patient 5, was previously reported in patients classified as FGS1 (Graham et al., 1999; Hu et al., 2016). Interestingly, a different substitution affecting the same amino acid residue p.(Arg1295His) (c.3884G > A), carried by Patient 18, was also reported in patients with a mild phenotype (Callier et al., 2013; Srivastava et al., 2019). Finally, two siblings carrying the variant c.4147G > A (p.Ala1383Thr) previously described as Ohdo syndrome (Langley et al., 2015), shared clinical features with our Patient 10, who carried the same variant, except for the cleft palate, hearing impairment, and cardiac defects. This indicates that the MED12 phenotype can vary quite a bit even in patients with the same variants, which can be the result of the presence genetic modifiers of the phenotype in the patient's genome or certain stochastic effects.

Arginine is the most mutated residue among the missense variants described herein. This amino acid is the only one containing CpG

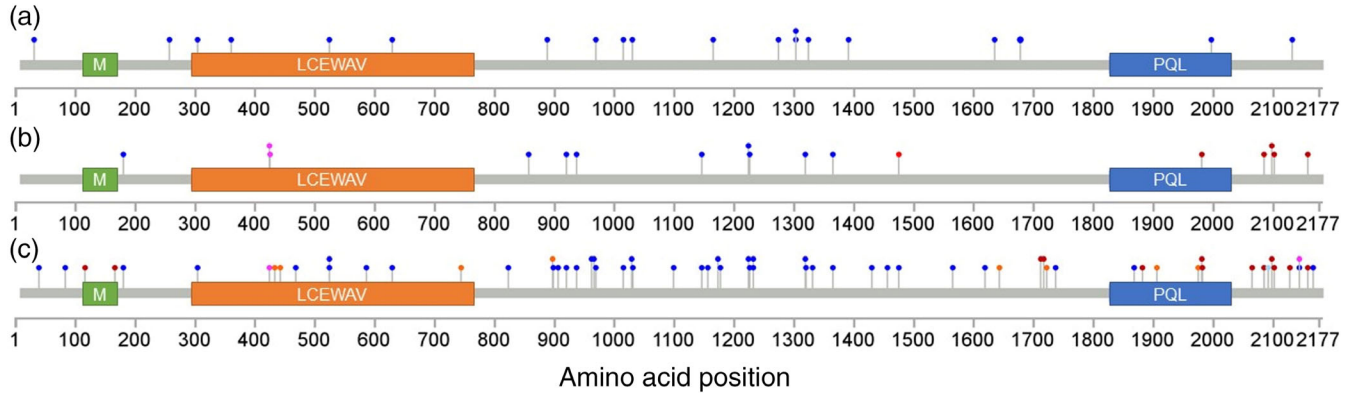

FIGURE 2 MED12 protein structure and reported ID-causing variants. (a) Variants found in this study. (b) Variants reported in females by Polla et al., 2020. (c) All pathogenic and likely pathogenic variants were reported to ClinVar as of September 19, 2021. The latter include the variants by Polla et al. M, Med12 domain. Blue dots represent the missense variants; in red the nonsense variants are given; pink dots indicate the splice site variants; and orange dots signify frameshift variants. Gene diagrams were created using lollipops v1.5.3 using UniProt ID Q93074.

nucleotides in the first and second codon position (CGA, CGG, CGT, and CGC), conferring a high mutability resulting from the spontaneous deamination either to TG or CA dinucleotides (Shen & Ji, 2015). Arginine positive charge and structure contribute for general protein stability. The replacement of an arginine residue, especially by neutral or negatively charged residues, occurs in seven out of the nine variants. This could impair protein stability by disruption of electrostatic or surface charge–charge interactions (Strickler et al., 2006). Furthermore, DNA-binding proteins such as transcription factors may use arginine to recognize and bind specific DNA sites (Lin & Guo, 2019; Zhou & Pang, 2018), and the loss of this residue can compromise this protein function.

In this cohort, no protein truncating variant was identified in contrast with the study of Polla et al. (2020) in which 18 female patients with ID carried a de novo *MED12* variant. Seven of these variants were protein truncating: two splice site changes and five nonsense variants. In this study, females carrying protein truncating variants clearly showed a more severe phenotype than those with missense variants (Polla et al., 2020). This observation led us to propose that *MED12* is intolerant to haploinsufficiency. Based on this assumption, and taking into account other X-linked genes, such as *OFD1*, *SMC1A*, and *NSDHL*, among others (Migeon, 2020), we hypothesize that protein truncating variants drastically compromise the protein function, causing severe or lethal outcomes in males. Unfortunately, X-chromosome inactivation (XCI) cannot be used as an additional criterion as we show that while a skewed XCI pattern was demonstrated in 3 mothers, 11 showed a random XCI pattern in blood cells (Table S1). A higher number of patients, as well as the study of other relevant tissues, could contribute to disclose the mechanism underlying XCI skewing in *MED12* variants carriers.

This study, by describing the genotype and phenotype of *MED12* patients, gives new insights into the molecular and clinical diversity associated with this gene. It highlights the importance of a detailed clinical characterization for the establishment of accurate genotype–phenotype correlations, and for disclosing the pathophysiological mechanism underlying the disease. For example, the data suggest that when a patient shows hearing impairment, the clinician should also look for hidden cardiac defects. Taken together, understanding the molecular and phenotypic spectrum associated with a given gene results in a decrease of misdiagnosed patients, especially (i) in patients that might go underdiagnosed due to presenting clinical features not typically associated with these genes, or (ii) when patients carry misclassified variants, for instance by in silico predictions.

AUTHOR CONTRIBUTIONS

Nuno Maia and Nekane Ibarluzea were involved in the design of the study, the acquisition of data, the analysis and interpretation of data, and in drafting the article. Mala Misra-Isrie was involved in the conception and design of the study and in the analysis and interpretation of data. Daniel C. Koboldt was involved in the analysis and interpretation of data, and in drafting the article. Isabel Marques, Gabriela Soares, Rosário Santos, Carlo L.M. Marcelis, Riikka Keski-Filppula, Miriam Guitart, Elisbeth Gabau Vila, April Lehman, Scott Hickey, Mari Mori, Paulien Terhal, Irene Valenzuela, Amaia Lasa-Aranzasti, Anna Ma Cueto-González, Brian H. Chhouk, Rebecca C. Yeh, Jennifer E. Neil, Bassam Abu-Libde, Tjitske Kleefstra, Mariet W. Elting, Andrea Császár, Judit Kárteszi, and Beáta Bessenyei were involved in the analysis and interpretation of data. Hans van Bokhoven, Paula Jorge, and Johanna M. van Hagen were involved in the conception and design of the study. Arjan P. M. de Brouwer was involved in the conception and design of the study, the acquisition of data, the analysis and interpretation of data, and in drafting the article.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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