

## Short communication

Variable expressivity of *KMT2B* variants at codon 2565 in patients with dystonia and developmental disorders

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## ABSTRACT

**Introduction:** Variable expressivity is an emerging characteristic of *KMT2B*-related dystonia. However, it remains poorly understood whether variants reoccurring at specific sites of lysine-specific methyltransferase-2B (*KMT2B*) can drive intra- and interfamilial clinical heterogeneity. Our goal was to ascertain independent families with variants affecting residue Arg2565 of *KMT2B*.

**Methods:** Whole-exome/genome sequencing, multi-site recruitment, genotype-phenotype correlations, and DNA methylation episignature analysis were performed.

**Results:** We report four individuals from two families harboring the variant c.7693C > G, p.Arg2565Gly. In an additional patient, a *de-novo* c.7693C > T, p.Arg2565Cys variant was identified. The observed phenotypic spectrum ranged from childhood-onset dystonia (N = 2) over unspecific intellectual disability syndromes (N = 2) to undiagnosed behavioral symptoms in adulthood (N = 1). Samples bearing p.Arg2565Gly had a *KMT2B*-typical episignature, although the effect on methylation was less pronounced than in carriers of loss-of-function *KMT2B* variants.

**Conclusions:** We established the existence of a *KMT2B* missense-mutation hotspot associated with varying degrees of disease severity and expression, providing information for patient counseling and elucidation of pathomechanisms.

## 1. Introduction

Heterozygous variants in *KMT2B* are responsible for *KMT2B*-related

dystonia (*DYT-KMT2B*) [1,2]. Non-motor neurodevelopmental abnormalities are part of the phenotypic spectrum of this condition [3–5]. Although many hereditary dystonias are characterized by variable

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expressivity [6], the phenomenon is less well understood in DYT-KMT2B. The relationship between KMT2B variants and clinical presentations has been mostly examined in individuals ascertained as a result of specific, often movement disorder-focused phenotyping [3,4,7], potentially introducing a bias towards overestimation of disease severity. A few pedigrees have been described in which affected members either had progressive dystonia or milder neurodevelopmental disorders with no signs of dystonic features [1,8]. Moreover, a recent cohort study reported nine sporadic patients with KMT2B missense or loss-of-function variants who presented mild-to-profound developmental disturbances with no signs of dystonia [7]. No genotype-phenotype correlations explaining this variability in clinical expression have been identified [4], and it is unknown whether variants clustering at certain sites of lysine-specific methyltransferase-2B (KMT2B) are linked to only one or multiple phenotypic outcomes. A better delineation of the relationship between individual KMT2B genotypes and the observable set of associated disease characteristics is crucial to improving variant interpretation, family counseling, and the study of molecular mechanisms.

Herein, we highlight variable expressivity of a recurrently mutated amino-acid residue of KMT2B. We assembled a group of five patients with heterogeneous dystonic and non-dystonic features who each carried a missense variant at codon 2565 of KMT2B. Variant causality was confirmed by application of sequence classification guidelines [9] and DNA methylation analysis [10].

## 2. Patients and methods

Motivated by the identification of a KMT2B p.Arg2565Gly alteration in a family (family-A) from our Munich dystonia research program [10–12], we proposed a collaborative study for recruitment of patients with variants occurring exclusively at residue Arg2565 of KMT2B. A-II-1 from family-A has been included in the publication by Mirza-Schreiber and colleagues [10] without comprehensive clinical characterization, whereas A-I-1's phenotypic abnormalities have not been reported so far. For details of the case-identification strategy and the involved centers and cohorts, see flow chart in Supplementary Figure. Existing exome-/genome-sequencing data and genetic testing reports were screened. Two other new patients (family-B) were identified by scrutiny of a repository of 500 neurodevelopmental disorder genome-sequencing datasets from the Institute for Clinical Genetics, Technical University Dresden, Germany. An additional previously unreported carrier (family-C) was enrolled from the outpatient clinic of the Center for Rare Diseases of University Hospital Cologne, Germany, following external genetic testing (see Supplementary Figure). Written informed consent was collected from all participants. The families underwent genetic investigation by exome or genome sequencing using family-based analysis designs (parent-child trio-exome sequencing in families-A/C, singleton genome sequencing followed by targeted sequencing of a twin brother in family-B) [11,13–15]. Prioritization of variants based on minor allele frequency and predicted impact were conducted as described [11–13,15,16]. Genome-wide peripheral blood DNA methylation profiles were generated with Illumina MethylationEPIC v2 BeadChip arrays. Data preprocessing, quality control, and derivation of methylation M-values were done using SeSAMe (<https://git.bioconductor.org/packages/sesame>) [17]. The KMT2B epismutation scores of the samples were calculated using the previously published SVM classifier [10], but restricted to 97 of 113 CpG-sites overlapping between the EPIC v1 and v2 arrays. The pathogenicity of reported variants was evaluated according to American-College-of-Medical-Genetics-and-Genomics (ACMG) interpretation criteria [9], using the varsome interface (<https://varsome.com/>) [18].

## 3. Results

### 3.1. Molecular findings

Four individuals from families-A/B were found to share the recurrent transversion c.7693C > G (NM\_014727.3), causing an arginine-to-glycine substitution at amino-acid position 2565 of KMT2B (p.Arg2565Gly) (Table 1; Fig. 1A). In the affected child from family-C, we identified a KMT2B *de-novo* c.7693C > T transition, resulting in an arginine-to-cysteine alteration at the same residue (p.Arg2565Cys) (Table 1; Fig. 1A). Variants affecting Arg2565 in KMT2B have not been listed in gnomAD v4.1.0 (neither missense/indel nor synonymous variants). The altered residue was phylogenetically conserved and localized in a highly mutation-intolerant sequence stretch (amino acids 2560–2576) [19] within the functionally important C-terminal region of KMT2B containing the FY-rich/C and SET domains [1] (Fig. 1B). Both variants were predicted to be deleterious according to machine-learned and other classifiers (p.Arg2565Gly: CADD = 24.6, REVEL = 0.8, AlphaMissense = 0.998; p.Arg2565Cys: CADD = 23.6, REVEL = 0.672, AlphaMissense = 0.997) [20–22]. To further proof a damaging effect of p.Arg2565Gly, we examined leukocyte DNA methylation in two patients from family-B (B-II-1, B-II-2; Fig. 1A). Indeed, both individuals showed a global increase in DNA methylation (not shown) and typical aberrations at the CpG sites of the DYT-KMT2B epismutation [10] (Fig. 1C). The methylation aberrations in these subjects were more moderate than in samples bearing KMT2B loss-of-function mutations (Fig. 1C), consistent with previous results for family-A [10] and the observation that KMT2B missense variants sometimes cause 'milder' aberrations of epismutation DNA methylation profiles [10]. Following ACMG rules [9], p.Arg2565Gly and p.Arg2565Cys were each categorized as 'likely pathogenic'.

### 3.2. Clinical phenotypes

Carrier individuals from family-A have been mentioned in a prior study but without comprehensive phenotype reports [10]. A-II-1 was a 13-year-old boy with normal developmental history, known for early-onset dystonia. Just before the age of 7, he started experiencing hand spasms and arm tremor, soon followed by progression to dystonic posturing of the trunk and all four limbs. At age 9, he additionally manifested laryngeal dystonia causing profound dysphonia. Testing of intellectual functioning (Wechsler Intelligence Scale for Children) provided results consistent with average cognitive ability. The remainder of the neurological examination was without significant findings, and there was no notion of physical abnormalities. In particular, there were no facial dysmorphic features or congenital anomalies. His brain MRI was normal. At last examination, he displayed generalized isolated dystonia with mild-to-moderate response to anticholinergic therapy. His 44-year-old father A-I-1 did not report any symptoms that impacted his daily life. There was no movement disorder. He had a history of learning difficulties and suspected ADHD-like features in childhood. In adulthood, he continued to have deficits in social motivation and episodes of anxiety.

In family-B, B-II-1 and his twin brother B-II-2 (15 years old) were under medical surveillance due to delayed psychomotor development and intellectual impairment. They had been placed in foster care. Family history was positive for dystonia on the maternal side but detailed information was unavailable. Clinical assessment at 15 months of age showed neurodevelopmental delays of both motor and language skills. Over time, mild dysmorphic facial features were noted in both individuals, including synophrys, smooth philtrum, broad nasal tip, and thin upper lip. B-II-1's brain MRI at 6 years of age did not reveal any intracranial abnormalities. To date, the twins presented mild intellectual disability (Kaufman-Assessment-Battery-for-Children score of 60 for B-II-1) and speech abnormalities. Further, B-II-1 had cryptorchidism, plagiocephaly, and congenital talipes. In several examinations, there

**Table 1**  
Clinical summary of patients with variants at codon 2565 of KMT2B.

Family/Patient	Family-A/A-II-1	Family-A/A-I-1	Family-B/B-II-1	Family-B/B-II-2	Family-C/C-II-1
<i>KMT2B</i> variant	NM_014727.3: c.7693C > G, NP_055542.1: p.Arg2565Gly	NM_014727.3: c.7693C > G, NP_055542.1: p.Arg2565Gly	NM_014727.3: c.7693C > G, NP_055542.1: p.Arg2565Gly	NM_014727.3: c.7693C > G, NP_055542.1: p.Arg2565Gly	NM_014727.3: c.7693C > T, NP_055542.1: p.Arg2565Cys
Gender	male	male	male	male	female
Current age	13 years	44 years	15 years	15 years	9 years
Developmental delay	no	not reported	yes – mild motor and cognitive delay	yes – mild motor and cognitive delay	yes – mild motor and cognitive delay
Dystonia age of onset	6 years	N/A	N/A	N/A	around 2–3 years
Dystonia site of onset	left upper extremity	N/A	N/A	N/A	lower extremities
Dystonia progression	yes	N/A	N/A	N/A	yes/variable
Dystonia sites involved (last exam)	upper and lower extremities, trunk, larynx	N/A	N/A	N/A	upper and lower extremities
Dystonia pattern	persistent	N/A	N/A	N/A	action-dependent, aggravated by voluntary movements
Dystonia treatment	anticholinergics, under evaluation for deep brain stimulation	N/A	N/A	N/A	anticholinergics
Hypotonia	no	not reported	not reported	not reported	yes – during early childhood
Intellectual impairment	no	borderline – learning difficulties in childhood	yes – mild, K-ABC score: 60	yes – mild	yes – mild
Speech impairment	yes – dysarthria	no	yes – mild expressive speech impairment	yes – mild expressive speech impairment	no
Dysmorphia	no	no	yes – synophrys, smooth philtrum, broad nasal tip, thin upper lip	yes – synophrys, smooth philtrum, thin upper lip	no
Behavioral deficits	no	yes – ADHD features in childhood, anxiety	no	no	autism spectrum disorder (Asperger)
Visual abnormalities	no	no	no	strabismus	no
Brain MRI abnormality	no	not performed	no	no	no
Other features	no	no	yes – cryptorchidism, plagiocephaly, clubfoot	no	potential episodes of epileptic activity, EEG normal

ADHD, attention deficit hyperactivity disorder; EEG, electroencephalography; K-ABC, Kaufman Assessment Battery for Children; *KMT2B*, lysine-specific methyltransferase-2B MRI, magnetic resonance imaging; N/A, not applicable.

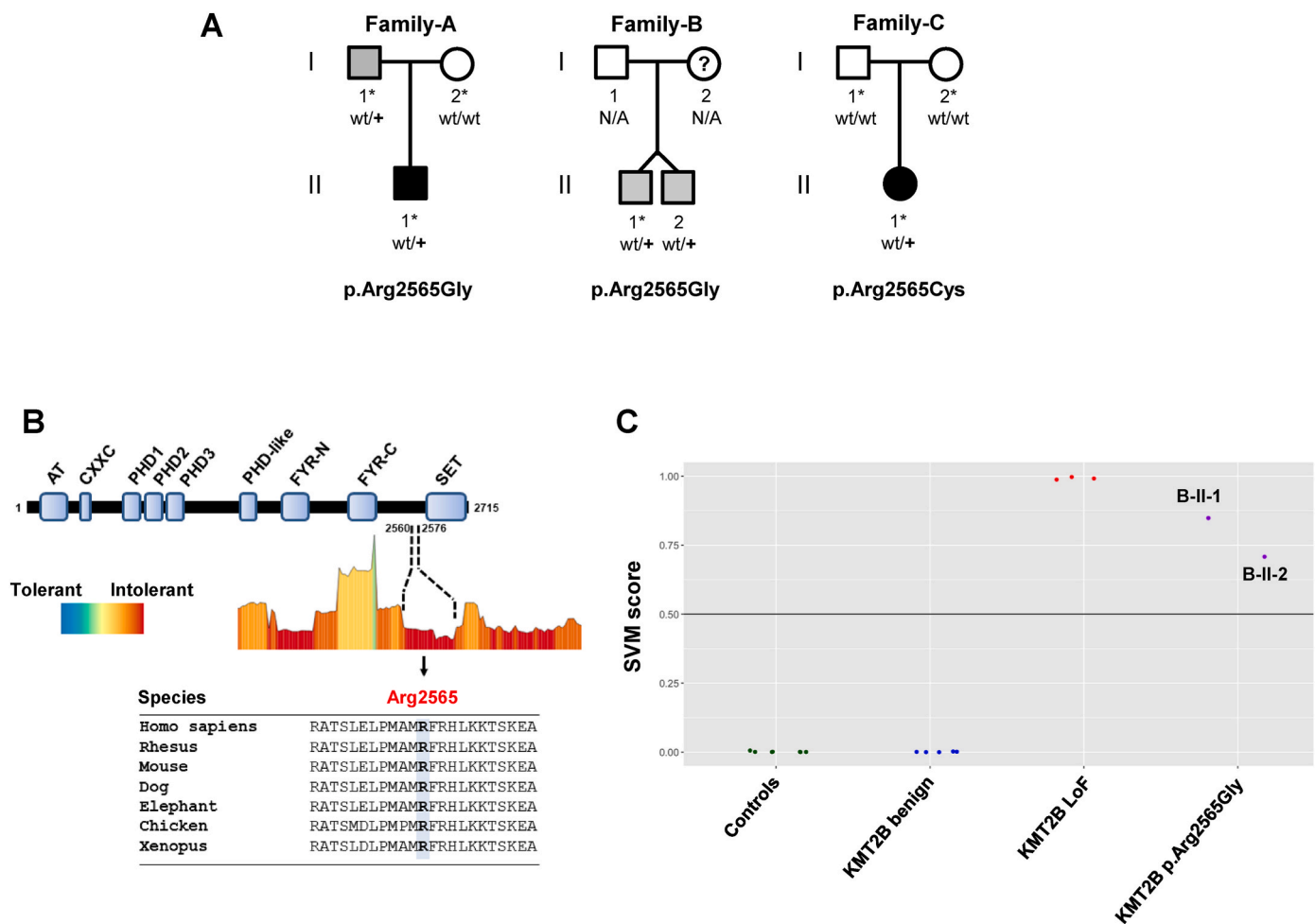
were no signs of dystonia.

Patient C-II-1 was a 9-year-old girl with action-induced dystonia, mild intellectual disability, and suspected autism spectrum disorder. Her motor milestones and language development were delayed. Muscular hypotonia in infancy was also documented. Brain MRI conducted at 1 year of age was normal. Between her second and third birthday, her right foot started to turn inward while walking. At the age of 4, she came to medical attention because of increasing exercise intolerance. Detailed neurological gait assessment revealed dystonic postures of both legs worsened by action, which was interpreted as an exacerbation of dystonic features from baseline. There were no clinical signs of neuromuscular disease and creatine kinase was normal. Additional cardiac evaluation and pulmonary function tests did not reveal any abnormalities. Over the next few months, her parents noticed dystonia of upper and lower extremities with diurnal fluctuations. She was started on anticholinergic treatment with moderate effect. Currently, the girl's examination showed mild dystonic posturing of the limbs mostly triggered by voluntary movements, without any impact on motor function.

#### 4. Discussion

For the present study we collected patients carrying disease-causing variations at an identical amino-acid position of *KMT2B* and investigated the variability of their phenotypes. Likely pathogenic/pathogenic *KMT2B* variants published to date were mostly unique to single affected individuals, with high rates of non-recurrent *de-novo* mutations [3,4,7,23]. We have successfully identified a series of subjects from unrelated families with missense substitutions affecting only one residue (Arg2565) of *KMT2B*, establishing a previously unrecognized

mutational hotspot for *DYT-KMT2B*. Despite the identical variant position, the phenotypic spectrum observed in the carrier individuals was unexpectedly broad, ranging from different types of dystonia (two patients) to non-dystonic neurodevelopmental conditions (two patients) and undiagnosed behavioral-disorder symptoms (one patient). The phenotype of A-II-1 (family-A) was consistent with 'classical', that is, *DYT-TORIA/DYT-THAP1*-like isolated generalized dystonia [6], whereas patient C-II-1 (family-C) demonstrated more atypical dystonic features with multifocal distribution including signs not previously highlighted for *DYT-KMT2B* such as prominent exercise intolerance. By contrast, the twins from family-B had developmental-disease phenotypes similar to those previously reported in a few *KMT2B*-mutated cases without dystonia, such as variable intellectual disability and unspecific craniofacial morphological abnormalities [5,7]. We note that some aspects of the observed dysmorphic features such as smooth philtrum and thin upper lip are uncommon in *DYT-KMT2B*, at least according to current knowledge of this rare disease; on the other hand, the results of our molecular analyses with demonstration of variant recurrence at Arg2565 and an epigraphic pattern consistent with the pathogenicity of this variant are clearly indicative of a diagnosis of *DYT-KMT2B* in the twins. Finally, in case of patient A-II-1 (family-A) with isolated dystonia, the father was initially evaluated as clinically unaffected [10], but later found to manifest adult behavioral disturbances. Although an association of these abnormalities with the familial *KMT2B* variant seems to be likely, we cannot rule out that some clinical features are coincidental given that learning disorders and ADHD are among the most common unspecific neurodevelopmental traits in the general population [24]. The heterogeneous clinical spectrum seen in the herein investigated families is consistent with prior results from a large cohort of patients



**Fig. 1.** *KMT2B* variants occurring exclusively at codon 2565 in patients with variable dystonic and non-dystonic phenotypes. (A) Pedigrees of three families with missense variants affecting Arg2565 of *KMT2B*. Patients with dystonia are indicated with filled black symbols and patients with neurodevelopmental symptoms but no dystonia with gray symbols. Phenotype of the mother in family-B was unknown (affected twins placed in foster care), with reported history of dystonia in a maternal relative. Individuals who underwent exome or genome sequencing are highlighted with asterisks. wt/+, variant carrier; wt/wt, individual homozygous for wild-type allele; N/A, genotyping unavailable. (B) Depiction of the domain structure of *KMT2B* and relative location of residue Arg2565 in the functionally important C-terminal part of the protein [1]. The magnified view illustrates the mutation intolerance of Arg2565 and its surrounding amino acid sequence, as defined by Wiel et al. [19]. Arginine at position 2565 was conserved in all organisms tested. (C) Results of epesignature analysis [10,25] for samples carrying the variant p.Arg2565Gly. SVM probability scores of the individuals B-II-1 and B-II-2 from family-B with this variant (*KMT2B* p.Arg2565Gly) were determined and compared with the scores for samples with pathogenic *KMT2B* *de novo* loss-of-function mutations (*KMT2B* LoF; N = 3), benign *KMT2B* missense variants (*KMT2B* benign; N = 5), and controls, that is, individuals without a rare variant in *KMT2B* (N = 7). Technically, a score >0.5 indicates that a sample is more likely to carry a disease-causing *KMT2B* variant than not to carry such an alteration. Previous analyses showed that in individuals with missense variants scores may be between control and LoF levels and correlate with the age of dystonia onset [10,25]. Abbreviations: *KMT2B*, lysine-specific methyltransferase-2B; SVM, support vector machine.

with *KMT2B* variants described by Cif and colleagues [7], which reported variably expressed comorbid features in *DYT-KMT2B* such as cognitive difficulties in 48 % and dysmorphic features in 52 %. The results of our present study have several implications. First, our findings provide strong evidence for the causal character of the variants p.Arg2565Gly and p.Arg2565Cys, facilitating future interpretation of sequence changes that will be identified at the hotspot Arg2565 of *KMT2B*. Notably, p.Arg2565Gly was first regarded as a variant of uncertain significance according to ACMG criteria [9], but the insights from our epesignature study allowed us to reclassify this variant as likely pathogenic. Second, the marked heterogeneity in phenotypic outcomes that we describe in families with variants affecting Arg2565 informs clinicians about the necessity of individualized care planning and careful counseling to ensure optimal management of affected families. We suggest to examine seemingly healthy parents and other relatives of patients with *KMT2B* Arg2565-involving variation for subtle signs of movement and developmental abnormalities. Third, the discovery of a mutation hotspot associated with diverse disease presentations can offer

important possibilities to explore mechanisms of variable expressivity in *DYT-KMT2B*. While p.Arg2565Gly was associated with an epesignature score that is diagnostic for *DYT-KMT2B* (family-B, see Fig. 1C, and family-A [10]), samples carrying this variant had lower scores than carriers of loss-of-function variants. This is in line with the existence of allelic series in *DYT-KMT2B* [10,25], and reminiscent of what has been described in some other monogenic disorders characterized by variable expressivity [26,27]. On the other hand, heterogeneity of dystonic and non-dystonic manifestations has also been observed within families positive for *KMT2B* loss-of-function variants and extreme epesignature scores [1,10], suggesting that profiling of blood methylation alone will not always be sufficient to predict outcomes. Mosaicism is another possible cause of variable expressivity but is not typical of *DYT-KMT2B* [25]. Indeed, the patients in the present study also were unlikely to be mosaics since their variants were inherited or suspected to be inherited and the one *de novo* change had a variant allele frequency close to 50 %. Other potential causes of variability include differential effects of missense variants on protein stability and turnover [28], modifier genes,

sex, parent-of-origin, varying environmental influences, and random effects in early development [29]. Moreover, it is possible that variants specifically clustering in the pre-SET domain region (see Fig. 1B) may confer a differential biological effect that is associated with heterogeneous outcomes. Additional research efforts are needed to better define how *KMT2B* variants, resulting methylation alterations, and downstream regulatory disruptions determine disease states and the associated severity of symptoms.

In summary, our present data underscore the notion that variable expressivity likely plays a greater than expected role in families with dominantly transmitted *KMT2B* variants. Inherited *KMT2B* variants, especially those reoccurring at specific sites, should not be disregarded in clinical curation, as they may represent under-appreciated causes of pleiotropic familial movement- and developmental-disorder phenotypes.

#### CRedit authorship contribution statement

**Antonia M. Stehr:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Jan Fischer:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Nazanin Mirza-Schreiber:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Katerina Bernardi:** Writing – review & editing, Formal analysis, Data curation. **Joseph Porrmann:** Writing – review & editing, Formal analysis, Data curation. **Philip Harrer:** Writing – review & editing, Formal analysis, Data curation. **Frank Kaiser:** Writing – review & editing, Formal analysis, Data curation. **Rami Abou Jamra:** Writing – review & editing, Formal analysis, Data curation. **Juliane Winkelmann:** Writing – review & editing, Formal analysis, Data curation. **Robert Jech:** Writing – review & editing, Formal analysis, Data curation. **Anne Koy:** Writing – review & editing, Formal analysis, Data curation. **Konrad Oexle:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Michael Zech:** Writing – original draft, Formal analysis, Data curation, Conceptualization.

#### Disclosures

None of the authors report disclosures concerning the present manuscript.

#### Declaration of competing interest

The authors state that they have nothing to declare.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.parkreldis.2025.107319>.

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