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ARTICLE

Digenic inheritance of *STUB1* variants and *TBP* polyglutamine expansions explains the incomplete penetrance of SCA17 and SCA48

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ABSTRACT

Purpose: This study aimed to unravel the genetic factors underlying missing heritability in spinocerebellar ataxia type 17 (SCA17) caused by polyglutamine-encoding CAG/CAA repeat expansions in the *TBP* gene. Alleles with >49 CAG/CAA repeats are fully penetrant. Most patients, however, carry intermediate $TBP_{41\text{-}49}$ alleles that show incomplete penetrance.

Methods: Using next-generation sequencing approaches, we investigated 40 SCA17/$TBP_{41\text{-}54}$ index patients, their affected (n = 55) and unaffected (n = 51) relatives, and a cohort of patients with ataxia (n = 292).

Results: All except 1 (30/31) of the index cases with $TBP_{41\text{-}46}$ alleles carried a heterozygous pathogenic variant in the *STUB1* gene associated with spinocerebellar ataxias SCAR16 (autosomal recessive) and SCA48 (autosomal dominant). No *STUB1* variant was found in patients carrying $TBP_{47\text{-}54}$ alleles. $TBP_{41\text{-}46}$ expansions and *STUB1* variants cosegregate in all affected family members, whereas the presence of either $TBP_{41\text{-}46}$ expansions or *STUB1* variants individually was never associated with the disease.

Conclusion: Our data reveal an unexpected genetic interaction between *STUB1* and *TBP* in the pathogenesis of SCA17 and raise questions on the existence of SCA48 as a monogenic disease with crucial implications for diagnosis and counseling. They provide a convincing explanation for the incomplete penetrance of intermediate *TBP* alleles and demonstrate a dual inheritance pattern for SCA17, which is a monogenic dominant disorder for $TBP_{\geq 47}$ alleles and a digenic *TBP*/*STUB1* disease (SCA17-DI) for intermediate expansions.

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Introduction

Autosomal dominant spinocerebellar ataxias (SCAs) are rare neurodegenerative genetic diseases characterized by progressive ataxia variably associated with additional noncerebellar symptoms. The estimated prevalence is 1.5 to 4 per 100,000 people.[1,2] Currently, 42 genetic loci have been associated with SCAs (https://neuromuscular.wustl.edu/ataxia/domatax.html). Seven SCAs (SCA1-3, SCA6, SCA7, SCA17, and dentatorubral-pallidoluysian atrophy [DRPLA]) are caused by trinucleotide (CAG or CAG/CAA) repeat expansions encoding polyglutamine, hence the term poly-Q diseases to refer to these forms. SCA17 is caused by CAG/CAA triplet expansions in the TATA-Binding Protein (*TBP*) gene.[3,4] This form is mostly observed as a hereditary trait, but de novo expansions have also been reported. In the numerous (approximately 150) sporadic and familial patients reported thus far, the CAG/CAA expansion ranges from 41 to 66 repeats (Supplemental Table 1). However, *TBP* expanded alleles exhibit variable, size-dependent, penetrance. Alleles with ≥49 CAG/CAA repeats are fully penetrant, with 100% of subjects manifesting the disease within the sixth decade.[4] *TBP* alleles with 41 to 48 CAG/CAA repeats, named intermediate alleles, are associated with reduced penetrance because ~50% of heterozygotes in SCA17 families are healthy at age ≥50.[4-13] However, the pathogenicity of intermediate alleles is puzzling because nearly 2% of individuals in the general population carry a TBP_{41-47} allele.[9,13,14]

The clinical phenotype is characterized by progressive cerebellar ataxia, variably associated with eye movement abnormalities, dystonia, chorea, pyramidal signs, seizures, dementia, and behavioral abnormalities.[8,10,15-17] Unlike other poly-Q disorders, the repeat length and sequence do not clearly influence the age of onset.[4,18,19] Age of onset spans several decades with the vast majority of cases manifesting in adulthood, and clinical heterogeneity can be observed even within the same family.[4] Of note, because the clinical phenotype may overlap with that of Huntington disease, SCA17 is also referred to as Huntington disease-like (HDL) type 4 (HDL4).[20] Recently, a novel form of SCA presenting with an HDL phenotype, SCA48, was associated with heterozygous pathogenic variants in the *STUB1* gene encoding the chaperone-associated E3 ubiquitin ligase CHIP involved in the ubiquitin-mediated proteasomal control of protein homeostasis.[21-24] Currently, ≥30 SCA48 families have been reported, showing a complex phenotype characterized by ataxia and cognitive/behavioral dysfunction variably associated with a spectrum of movement disorders, encompassing both hypokinetic and hyperkinetic features.[25] Interestingly, SCA17 and SCA48 share 2 main features: the clinical picture and the reduced penetrance.[25]

In this study, we investigated a large cohort of patients with an SCA17/HDL phenotype and intermediate *TBP* $(CAG/CAA)_{41-49}$ alleles by next-generation sequencing (NGS) approaches to unravel the genetic bases of the reduced penetrance. Of note, a heterozygous pathogenic or likely pathogenic variant in the SCA48-associated *STUB1* gene was identified in 97% of index cases carrying 41 to 46 CAG/CAA repeats in the *TBP* gene thus demonstrating an unexpected and crucial interplay between *STUB1* and *TBP* in the pathogenesis of SCA17 and unveiling the digenic molecular basis that underlies the incomplete penetrance of intermediate *TBP* alleles.

Materials and Methods

The cohorts of subjects included in the study are described. Cohort 1 included 40 affected index cases presenting with an SCA17/HDL phenotype and carrying a *TBP* allele with ≥41 CAG/CAA repeats and no expansions in the Huntington disease, SCA1, -2, -3, -6, and -7 and dentatorubral-pallidoluysian atrophy genes. For this cohort, we considered the following 2 subgroups: subgroup 1a included 31 affected subjects with intermediate length *TBP* alleles (41-49 CAG/CAA repeats), whereas subgroup 1b included 9 affected subjects with a fully penetrant *TBP* expanded allele (>49 CAG/CAA repeats). Cohort 2 included 292 index cases with cerebellar ataxia for whom *STUB1* had been sequenced previously by NGS analysis. Cohort 3 included 729 unrelated healthy Italian controls previously reported by Mongelli et al.[14] In addition, 55 affected and 51 unaffected relatives of index cases were evaluated for segregation analyses.

Details about methods regarding NGS approaches, *TBP* repeat size analysis, *TBP* repeat sequencing, bioinformatics, and statistical analysis are provided in the Supplemental Materials and Methods.

All variants are described in relation to the reference sequence NM_005861.3.

Results

The study design is illustrated in Figure 1. We studied a cohort of 31 families with a SCA17/HDL phenotype and an intermediate *TBP* allele (41-49 CAG/CAA repeats) (cohort 1a, Figure 1A). In addition, genetic studies were also performed in 9 families carrying fully penetrant *TBP* alleles (TBP_{51-54}) (cohort 1b, Figure 1A) and 10 families with ataxia from cohort 2 (Figure 1B). Pedigrees of all families are shown in Figure 2 and Supplemental Figures 1-5.

NGS analysis of SCA17 families

The 31 affected index cases with a TBP_{41-49} allele (cohort 1a, Figure 1A) underwent genetic analysis using an NGS gene panel (Supplemental Table 2) and/or clinical exome sequencing to exclude a possible differential diagnosis and/or identify additional genetic factors implicated in the observed reduced penetrance.

Interestingly, we identified heterozygous variants in the *STUB1* gene in 27 of 31 (87%) patients (Figure 1A, Table 1,

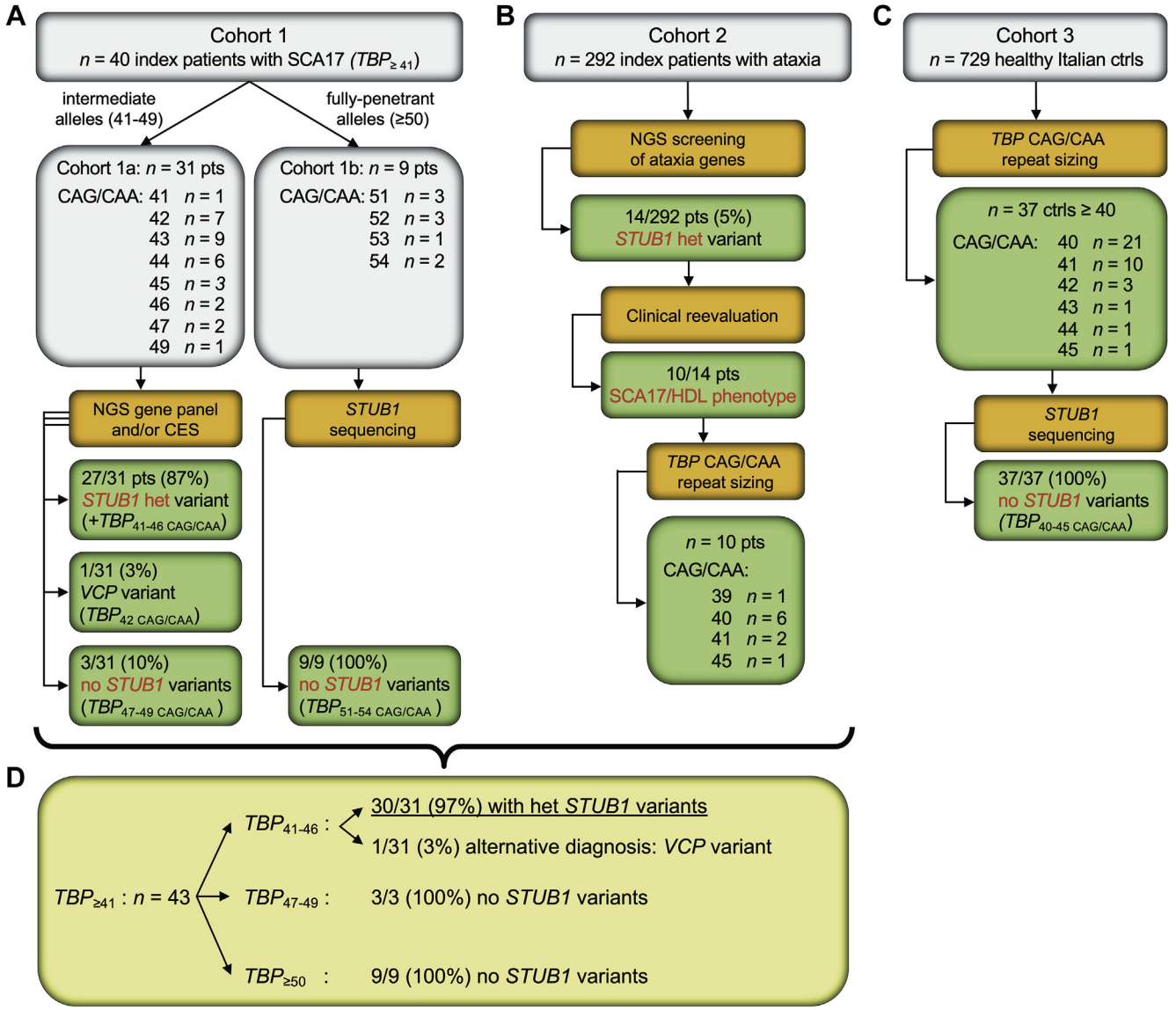

Figure 1 Design of the study and summary of the results. Schematic representation of the study design. A. Genetic analysis of 40 index cases with a diagnosis of SCA17. NGS studies (gene panel and/or CES) performed in 31 probands carrying intermediate *TBP* alleles ($TBP_{41\text{-}49}$), identified a het *STUB1* variant in 27 of 31 patients (87%) carrying $TBP_{41\text{-}46}$. No *STUB1* variant was identified in the 3 patients carrying $TBP_{47\text{-}49}$. An alternative diagnosis (*VCP*, inclusion body myopathy with early-onset Paget disease with or without frontotemporal dementia 1/amyotrophic lateral sclerosis 14) was reached in 1 patient carrying a TBP_{42} allele. *STUB1* gene sequencing revealed no variant in the 9 probands carrying fully penetrant *TBP* alleles ($TBP_{\geq 50}$). B. Genetic and clinical reevaluation of patients with ataxia carrying het *STUB1* variants. Retrospective evaluation of NGS data from 292 ataxia index cases revealed rare het *STUB1* variants in 14 of 292 index cases. A SCA17/Huntington disease-like phenotype was present in 10 of 14 index patients. *TBP* repeat expansion analysis identified TBP_{45} in 1 patient and TBP_{41} in 2 patients, whereas 6 probands carried a TBP_{40} allele and 1 carried a TBP_{39} allele. C. Genetic screening of *STUB1* in healthy ctrls with intermediate $TBP_{40\text{-}45}$ alleles[14] revealed no rare *STUB1* variants. D. Summary of the genetic results in patients with ataxia. Rare *STUB1* variants were identified in 30 of 31 patients carrying $TBP_{41\text{-}46}$ alleles, whereas no variants were identified in the 12 patients carrying $TBP_{47\text{-}54}$ alleles. CES, clinical exome sequencing; ctrls, controls; het, heterozygous; NGS, next-generation sequencing; pts, patients; SCA17, spinocerebellar ataxia 17.

and Supplemental Table 3). All patients carrying *STUB1* variants had a *TBP* expansion ranging from 41 to 46 CAG/CAA repeats (Figures 1A and 2, Supplemental Figure 1). Four patients had no *STUB1* variant: 1 patient with a TBP_{42} allele carried a novel variant (c.1001G>T; p.G334V) in the *VCP* gene (inclusion body myopathy with early-onset Paget disease with or without frontotemporal dementia 1 [IBMPFD1]/amyotrophic lateral sclerosis 14 [ALS14]) (Supplemental Figure 2) and 3 patients with the largest intermediate *TBP* expansions ($TBP_{47\text{-}49}$) did not exhibit additional pathogenic variants in *STUB1* or in other genes related to the SCA17/HDL spectrum phenotype (Supplemental Figure 3). Segregation studies in families with *STUB1* variants demonstrated that subjects carrying

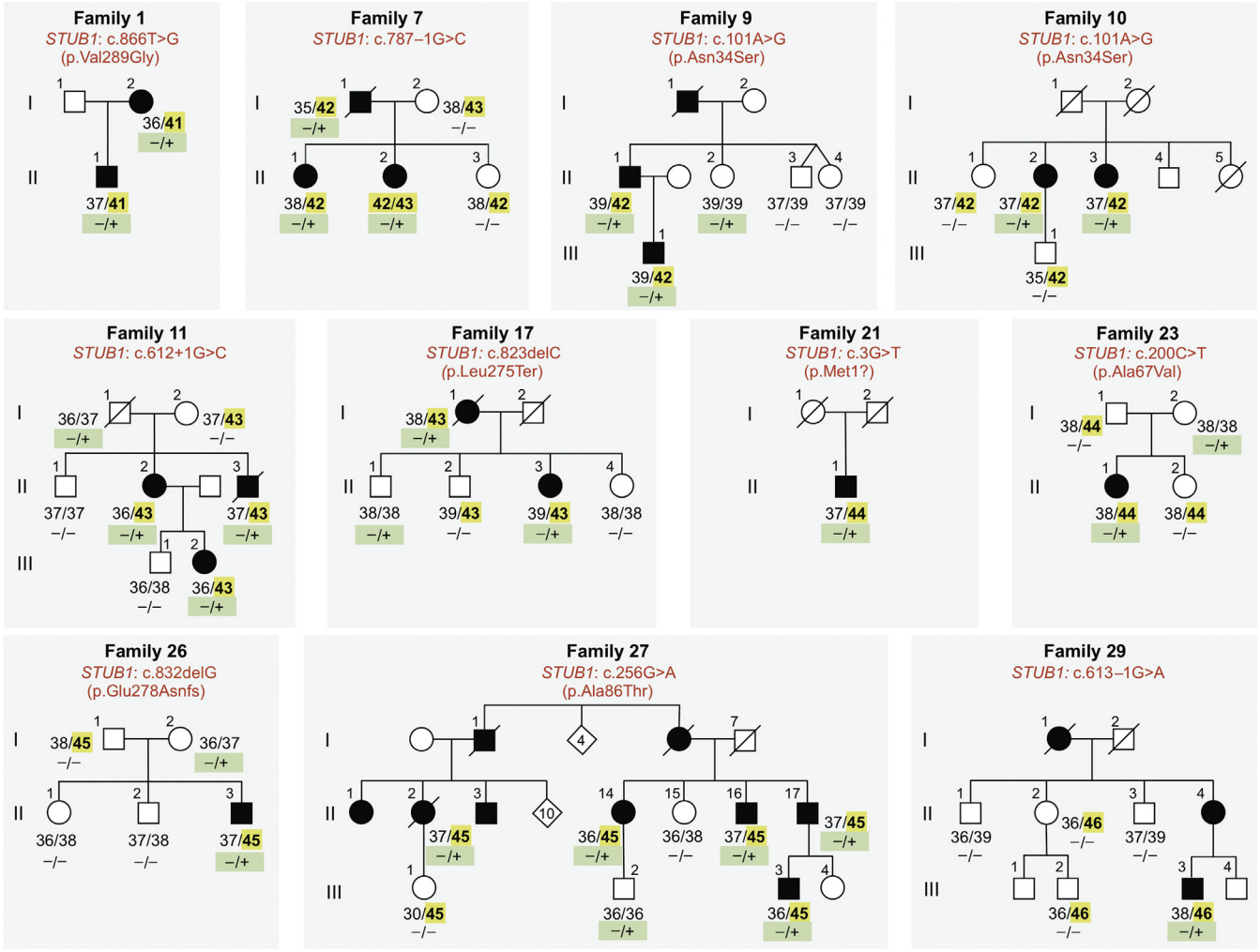

Figure 2 Exemplary families with $TBP_{41\text{-}46}$ and heterozygous variants in *STUB1*. Family pedigrees of 11 index cases with $TBP_{41\text{-}46}$ repeats and heterozygous variants in *STUB1*. Segregation analysis was performed in all available relatives. Pathological *TBP* alleles are in bold highlighted in yellow; *STUB1* heterozygosity (–/+) is highlighted in green; –/–, *STUB1* wild-type genotype.

both a $TBP_{41\text{-}46}$ allele and a heterozygous *STUB1* variant presented with an SCA17/HDL phenotype, whereas subjects heterozygous for either the $TBP_{41\text{-}46}$ allele or a *STUB1* variant were unaffected (Figure 2, Supplemental Figure 1).

STUB1 sequencing performed in the 9 SCA17 index patients (cohort 1b, Figure 1A) carrying fully penetrant *TBP* alleles ($TBP_{51\text{-}54}$) identified no rare or common variants, except for the frequent intronic single-nucleotide variant (formerly single-nucleotide polymorphism) rs3216838 (c.786+8delC, gnomAD_exome AF = 0.511024) (Figure 1A, Supplemental Figure 4).

TBP repeat size in patients with ataxia carrying heterozygous *STUB1* variants

To further investigate the role of digenic *TBP/STUB1* pathogenic expansions/variants in patients with ataxia and an SCA17-like phenotype, we retrospectively evaluated NGS data from 292 ataxia index cases in which *STUB1* had been sequenced (Figure 1B). Of 292 probands, 14 (4.8%) carried a rare heterozygous *STUB1* variant and were clinically reevaluated. Four patients with pure cerebellar ataxia and no cognitive decline were not further considered in the study (Supplemental Table 4). Interestingly, 10 patients presented with an SCA17/HDL phenotype. Subsequent genetic analyses of the *TBP* repeat expansion in these patients revealed that 3 of 10 (30%) carried ≥41 CAG/CAA repeats (1 with TBP_{45} and 2 with TBP_{41} alleles), whereas 6 probands carried a TBP_{40} allele and 1 carried a TBP_{39} allele (Figure 1B). F2-II.1 (TBP_{41}/*STUB1*), F3-II.1 (TBP_{41}/*STUB1*), and F26-II.3 (TBP_{45}/*STUB1*) were sporadic cases (Figure 2, Supplemental Figure 1). Segregation studies in family F26 demonstrated that the 2 unaffected parents carried either the *STUB1* variant or the *TBP* expansion (Figure 2). Segregation analysis was also performed in the 7 families whose index cases carried *STUB1* variants along with *TBP* alleles in the 39 to 40 range (families F43-F49, Supplemental Figure 5). In family F43, both the affected proband (F43-III.1) and the affected

Table 1 Clinical and genetic features of 42 index patients carrying ≥41 CAG/CAA repeats in the *TBP* gene

Family Subject	*TBP* Genotype $(CAG/CAA)_n$	*STUB1* Heterozygous Variant	Family History	Sex/Age	Age at Onset	Signs at Onset	Disability Score	SARA Score	MMSE Score	Motor Abnormalities	Cognitive/ Behavioral Abnormalities
F1-I.2[a]	41-36	c.866T>G p.Val289Gly	AD	F/63	54	CA, BA	4	nd	15	CA	CD, I
F2-II.1	41-37	c.728C>T p.Pro243Leu	AD	F/43	nd	CA	nd	nd	nd	CA	CD, OCD
F3-II.1	41-39	c.393C>G p.Phe131Leu	S	F/70	50	CA	nd	nd	nd	CA	CD
F4-II.2	42-37	c.200C>T p.Ala67Val	AD	F/65	50	CA	3	nd	nd	CA	CD
F5-II.1	42-37	c.689_692delACCT p.Tyr230CysfsTer9	S	F/52	nd	nd	nd	nd	nd	CA	CD
F6-II.3	42-38	c.146A>G p.Tyr49Cys	S	M/50	42	CA, BA	4	20	9	CA	CD, I
F7-II.1	42-38	c.787-1G>C p.?	AD	F/52	33	CA, CD	7	37	7	CA	CD
F8-II.3	42-39	c.524+1G>A p.?	AD	M/56	nd	CA	7	40	nd	CA	CD
F9-II.1	42-39	c.101A>G p.Asn34Ser	AD	M/67	45	BA	2	14	nd	CA, Ch	CD, I
F10-II.2	42-37	c.101A>G p.Asn34Ser	AR	F/76	45	Dys	6	35	8	CA, Ch, Dy	CD
F11-II.2[a]	43-36	c.612+1G>C p.?	AD	F/56	52	CA	2	13	17	CA	CD
F12-II.2	43-36	c.832G>T p.Glu278Ter	AD	F/54	46	CA, CD	7	40	nd	CA	CD
F13-II.4	43-37	c.398 A>T p.Asp133Val	S	F/59	42	CA	2	10	23	CA, Ch, Dy	CD
F14-I.1	43-37	c.400_402delGAC p.Asp134del	AD	M/71	nd	nd	nd	nd	nd	CA	CD
F15-II.1	43-38	c.159+1G>A p.?	AD	F/63	62	CD, BA, Ch	2	nd	nd	CA, Ch, Dy	CD, I
F16-II.1	43-38	c.673C>T p.Arg225Ter	AD	F/44	42	CD, BA	2	nd	nd	CA, Ch	CD, I
F17-II.3	43-39	c.823delC p.Leu275Ter	AD	F/36	34	CA	1	7	16	CA	CD
F18-II.3	43-41	c.728C>T p.Pro243Leu	S	F/42	33	CA	5	22	9	CA, Ch, Dy	CD, OCD
F19-II.1	44-36	c.779_786+5del p.?	AR	F/58	45	CA, CD	2	16	15	CA, Ch	CD
F20-II.1[a]	44-37	c.393C>G p.Phe131Leu	AD	M/63	48	CA, Ch	2	13	20	CA, Ch	CD
F21-I.1	44-37	c.3G>T p.Met1?	nd	nd	nd	nd	nd	nd	nd	CA	CD
F22-II.1	44-37	c.823_824delCT p.Leu275AspfsTer16	AR	F/59	nd	nd	nd	nd	nd	CA	CD
F23-II.1	44-38	c.200C>T p.Ala67Val	S	F/43	34	CA, CD	2	10	23	CA	CD, I
F24-II.1[a]	44-38	c.689_692delACCT p.Tyr230CysfsTer9	AR	F/52	38	CA	2	9.5	nd	CA	CD, I
F25-II.5[a]	45-35	c.400_402delGAC, p.Asp134del	AD	M/44	34	Dys	5	18.5	nd	CA, Ch	CD
F26-II.3	45-37	c.832delG p.Glu278AsnfsTer9	S	M/48	38	CA, Ch	2	12	14	CA, Ch	CD
F27-II.16[a]	45-37	c.256G>A p.Ala86Thr	AD	M/58	55	CA	2	11.5	22	CA, Ch	CD
F28-II.2[a]	45-38	c.338C>T p.Ala113Val	AR	M/49	35	BA	3	23	nd	CA, Ch	CD, I

(continued)

Table 1 Continued

Family Subject	*TBP* Genotype $(CAG/CAA)_n$	*STUB1* Heterozygous Variant	Family History	Sex/Age	Age at Onset	Signs at Onset	Disability Score	SARA Score	MMSE Score	Motor Abnormalities	Cognitive/ Behavioral Abnormalities
F29-III.3	46-38	c.613-1G>A p.?	AD	M/46	30	CA	4	17	7	CA, Ch	CD, I
F30-II.1[a]	46-38	c.862G>T p.Glu288Ter	AD	M/26	19	CA	6	31	nd	CA, Ch	CD
F31-II.1	47-36	no *STUB1* variant	S	F/56	55	CA	5	nd	nd	CA	CD
F32-II.1	47-38		S	F/58	55	CA	nd	nd	nd	CA	CD
F33-II.2	49-43		S	M/66	58	CA, Ch	2	22	19	CA, Ch	CD
F34-I.1[a]	51-38		S	F/68	55	Ch	2	11	nd	CA, Ch	CD
F35-II.1	51-39		AD	F/55	49	CD, Dys	2	12	25	CA	CD
F36-II.2[a]	51-39		AR	M/62	55	BA	2	8.5	nd	CA, Ch	CD, I
F37-II.1	52-38		AD	F/61	45	CA, BA	4	13	23	CA	CD, I
F38-II.5	52-38		AD	M/39	30	CA, CD	2	nd	nd	CA	CD
F39-II.1	52-39		AD	F/69	55	Dys	2	12	24	CA, Ch	CD
F40-II.1[a]	53-38		AD	M/47	40	CA	2	15	19	CA, Ch	CD
F41-II.1	54-35		S	F/43	27	Se	7	40	nd	CA, Ch	CD
F42-II.1[a]	54-37		S	F/42	35	BA	7	40	nd	CA	CD, I

AD, autosomal dominant; *AR*, autosomal recessive; *BA*, behavioral abnormalities; *CA*, cerebellar ataxia; *CD*, cognitive decline; *Ch*, chorea; *Dy*, dystonia; *Dys*, dysarthria; *F*, female; *I*, irritability; *M*, male; *MMSE*, Mini-Mental State Examination; *nd*, not determined; *OCD*, obsessive-compulsive disorder; *S*, sporadic; *SARA*, Scale for Assessment and Rating of Cerebellar Ataxia; *Se*, seizure.

[a]Family previously described: F1;[39] F11;[40] F20, F24, F27, F30, F34, F36, F40, and F42;[8] F25 and F28.[8,41]

maternal aunt (F43-II.2) carried a TBP_{39} allele along with a *STUB1* variant. The affected mother of the proband (F43-II.1) had deceased at age 56 years and was not available for testing. Among the families with 40 repeats, 2 were sporadic cases (families F46-II.1 and F47-II.5), 2 exhibited a recessive pattern (2 affected sibs in families F48 and F45), and 2 exhibited a dominant pattern (families F44 and F49) (Supplemental Figure 5). For families F44 to F47, only the probands could be tested because no other relatives were available for segregation analysis. In family F48, 2 affected sisters (II.4 and II.6) carried TBP_{40} along with a *STUB1* variant, whereas the 3 healthy siblings carried only the TBP_{40} allele. In family F49, 2 of the 3 affected siblings (II.4 and II.7) carried a TBP_{40} allele along with the *STUB1* variant, but notably, the third affected sister (II.1) and 1 healthy brother (II.3) carried the *STUB1* variant along with a TBP_{39} allele. Four healthy siblings carried the TBP_{39} or the TBP_{40} allele with no *STUB1* variant. Because the healthy mother (I.2) carried the TBP_{40} allele with no *STUB1* variant, the deceased affected father (I.1), who could not be tested, should be considered an obligate heterozygote for the *STUB1* variant and the TBP_{39} allele.

Overall, 30 of 31 (97%) patients with an SCA17/HDL phenotype and 41 to 46 CAG/CAA repeats in the *TBP* gene were found to carry a heterozygous *STUB1* variant, whereas an alternative genetic diagnosis (*VCP*, IBMPFD1/ALS14) was reached for 1 of 31 patients. Notably, none of the patients carrying 47 to 54 CAG/CAA repeats in the *TBP* gene had *STUB1* variants (Figure 1D).

Overall, segregation of the *STUB1* variants and the *TBP* expansions was evaluated in 85 subjects (47 affected and 38 healthy relatives) from the 30 families of the index probands with $TBP_{41\text{-}46}$ (Figure 2, Supplemental Figure 1). All the affected subjects (100%) carried both a $TBP_{41\text{-}46}$ allele and a heterozygous *STUB1* variant. By contrast, the healthy relatives carried either the *TBP* expansion (16 of 38) or a heterozygous *STUB1* variant (8 of 38) or neither the expansion nor the *STUB1* variant (14 of 38) (Figure 2, Supplemental Figure 1). Regarding the 40 CAG/CAA repeat allele, co-occurrence of this allele and a *STUB1* variant is associated with the disease in 8 of 8 affected subjects from 6 families (Supplemental Figure 5). With regard to the 39 CAG/CAA repeat allele, the TBP_{39}/*STUB1* genotype was identified in 3 affected subjects from 2 families (F43 and F49, Supplemental Figure 5) and in 2 healthy relatives (F49-II.3 and F9-II.2).

STUB1 sequencing in healthy controls with intermediate SCA17 alleles

In a recent study on a cohort of 729 healthy Italian controls,[14] we found 37 individuals carrying $TBP_{\geq 40}$ alleles: the CAG/CAA repeat length was 40 in 21 subjects; 41 in 10; 42 in 3; and 43, 44, and 45 in 1 subject each (Figure 1C). *STUB1* sequencing performed in these subjects revealed no rare or common variants except for the frequent intronic single-nucleotide variant rs3216838.

STUB1 variants in patients with intermediate SCA17 alleles

We identified 30 extremely rare (minor allele frequency [MAF] $\leq$ 0.0005) *STUB1* variants in 37 families: 20 were

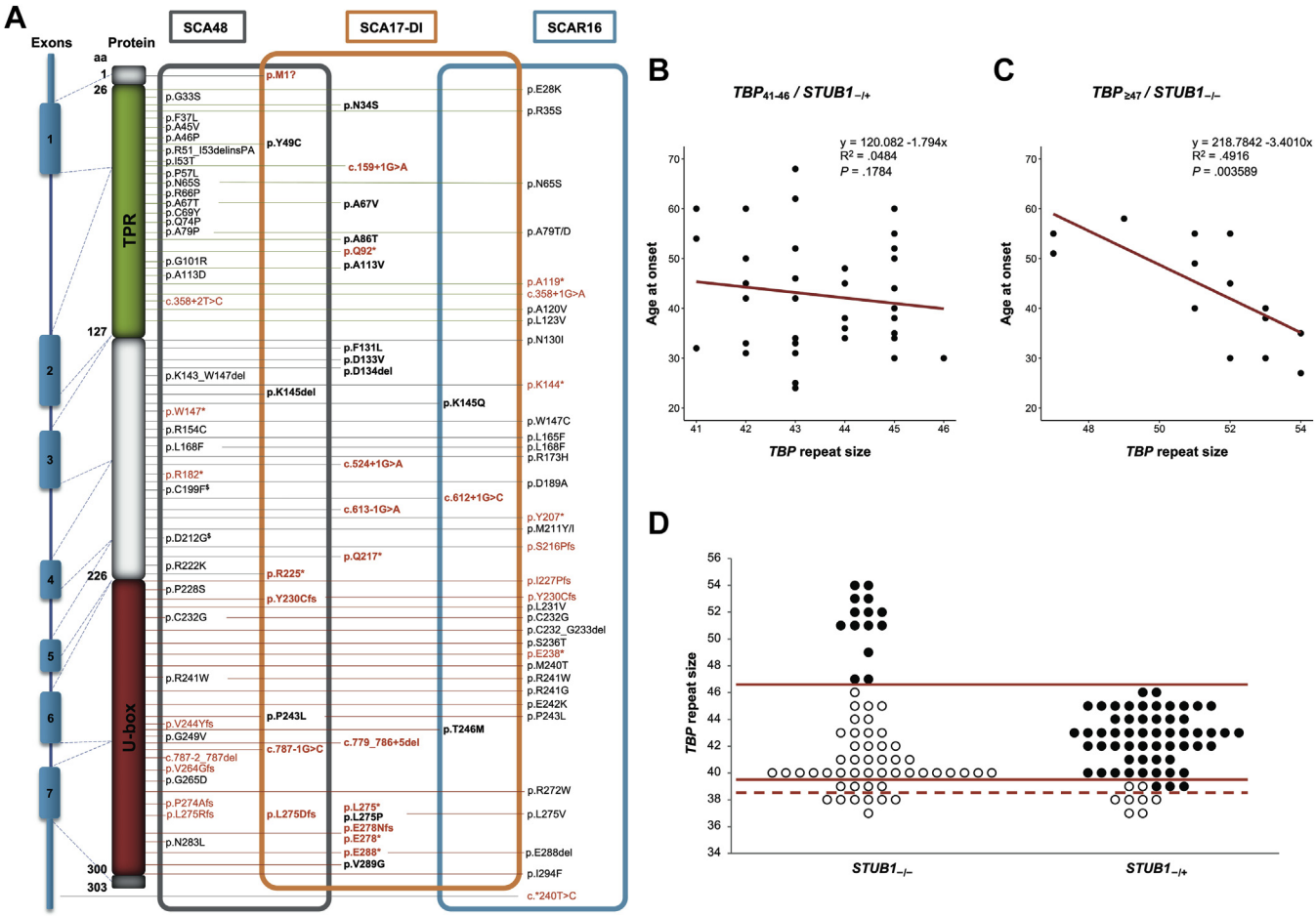

Figure 3 Disease-associated *STUB1* variants and correlation between CAG/CAA repeat length and age at onset. A. Localization along the protein of pathogenic *STUB1* variants associated with SCA17-DI (orange box), SCA48 (gray box), and SCAR16 (blue box). On the left is the exon/intron structure of the *STUB1* gene. Intersection areas show variants associated with >1 form of disease. Loss-of-function variants are in red, and SCA17-DI–associated variants are in bold. B, C. Correlation between age at onset and *TBP* repeat size. SCA17 patients are stratified according to the presence (B) or absence (C) of *STUB1* variants. Linear regression was modeled to test the relationship between age at onset and size of the larger *TBP*. D. A scatter plot showing distribution of affected (black circles) and unaffected (white circles) subjects according to their *TBP*/*STUB1* genotype. Red lines represent the upper and lower thresholds of pathogenic intermediate alleles corresponding to a range (40-47). The red dotted line represents the borderline 39-repeat allele. Affected subjects present either $TBP_{\geq 47}$ alone or $TBP_{40\text{-}46}$ along with a *STUB1* variant. The TBP_{39}/*STUB1* genotype was identified in both affected ($n = 3$) and nonaffected ($n = 2$) subjects: –/+, heterozygosity for *STUB1* variants; –/–, *STUB1* wild-type genotype. SCA48, spinocerebellar ataxia type 48; SCA17-DI, digenic spinocerebellar ataxia type 17; SCAR16, autosomal recessive spinocerebellar ataxia type 16; TPR, tetratricopeptide repeat.

novel, 3 were previously reported in autosomal recessive SCAR16(p.K145Q, c.612+1G>C, p.T246M), 6 were previously reported in SCA48 families (p.Y49C, p.K145del, p.R225*, p.T230Cfs, c.787-1G>C, and p.L275Dfs), and 1 was previously reported in both patients with SCAR16 and patients with SCA48 (p.P243L) (Figure 3A, Supplemental Table 3).

A total of 16 variants were loss-of-function (LoF) variants, 12 were missense variants, and 2 were in-frame deletions (Figure 3A, Supplemental Table 3). Seven variants (5 missense, 1 in-frame deletion, and 1 frameshift) occurred in 2 apparently unrelated families (Table 1). Identified variants are located along the whole gene (Figure 3A): 1 abolishes the translation initiation codon (c.3G>T, p.M1?), 7 variants (5 missense, 1 splicing, and 1 nonsense) are located in the tetratricopeptide repeat (TPR) domain of CHIP essential for ubiquitination mediated by UBE2D1, 10 variants (3 missense, 2 in-frame deletions, 3 splicing, and 2 nonsense) are located in the interdomain part of the protein, and 12 variants (4 missense, 2 splicing, 3 frameshift, and 3 nonsense) are located in the U-box domain required for ubiquitin protein ligase activity.

We compared the variant types and variant localization along the protein of the variants identified in this study ($n = 30$) with those of the rare, nonsynonymous variants (MAF $\leq$ 0.001; $n = 174$) reported in gnomAD (Supplemental Figure 6A). Statistical analysis of variant types revealed a highly significant enrichment of LoF variants in digenic SCA17 (SCA17-DI) (odds ratio [OR] = 9.19, $P = 2.60\text{E}{-}07$) (Supplemental Figure 6B).

By contrast, no significant difference was observed when analyzing variant localization distribution. Moreover, we also compared variant type and variant localization between SCA48 or SCAR16 and gnomAD. Statistical analysis revealed a highly significant enrichment of LoF variants in SCA48 (OR = 3.27, P = 4.03E–03) (Supplemental Figure 6B). Finally, analysis of all disease together revealed a significant enrichment of LoF variants (OR = 10.61; P = 7.66E–08) and variants in both functional domains (TPR: OR = 3.69, P = 2.95E–04; U-box: OR = 2.47, P = 9.51E–03).

Structure of the CAG/CAA repeat

To investigate the correlation between repeat expansion architecture and disease manifestation, SCA17 CAG/CAA repeats were sequenced in 126 subjects (72 affected, 54 unaffected) using a 2-step polymerase chain reaction amplicon-based deep sequencing NGS approach (Supplemental Table 5). In 12.7% (32/252) of alleles, we observed a discrepancy of 1 or 2 triplets between sequencing and fragment size analysis. The error rate was 1.8% (3/165) in the lower-size range (≤40), 26.7% (20/75) in the intermediate range (41-49), and 75% (9/12) in the larger alleles (≥50). The most represented configuration comprising $(CAG)_3(CAA)_3(CAG)_{n1}(CAA)_1(CAG)_1(CAA)_1(CAG)_{n2}$CAACAG triplets divided into 5 domains was observed in 95.7% of alleles (241/252) (Supplemental Table 5). An alternative configuration was observed in 11 subjects from 7 families. The architecture of the repeat region was stable within families among subjects sharing the same allele. Only 1/22 alleles showed intergenerational instability because of a CAG expansion in domain IV when transmitted by the unaffected father (TBP_{45}) to the affected daughter (TBP_{54}) (family F41) (Supplemental Figure 4).

Clinical features of patients with *TBP/STUB1* variants

The clinical features of patients with *TBP* expansions and *STUB1* variants are summarized in Table 1 and Supplemental Table 6. Patients carrying $TBP_{41\text{-}46}$ and heterozygous *STUB1* variants had an adult onset (19-62 years) disease, with 65% subjects manifesting the disease within the fifth decade. The first reported symptoms were cerebellar ataxia (85%), cognitive decline (23%), behavioral abnormalities such as irritability (20%), and chorea (12%). Cognitive decline manifested in 90% of patients. Brain magnetic resonance imaging of patients with SCA17-DI demonstrated the typical features of SCA17 disease consisting of atrophy of the cerebellum, basal ganglia, and cortex (Supplemental Figure 7). The patients carrying $TBP_{39\text{-}40}$ and heterozygous *STUB1* variants had a clinical phenotype indistinguishable from patients carrying $TBP_{>40}$ and heterozygous *STUB1* variants.

We performed a linear regression analysis using the age at onset and *TBP* repeat size as parameters for the analysis (Figures 3B and C). No significant relationship was observed in the SCA17-DI group (y = 120.082 – 1.794x, R^2 = 0.0484, P = .1784). By contrast, a significant correlation was observed in the group of patients with SCA17 carrying $TBP_{\geq 47}$ and no *STUB1* variant (y = 218.7842 – 3.4010x, R^2 = 0.4916, P = .003589).

Estimate of genetic prevalence of SCA17-DI

Genetic prevalence of SCA17-DI disease was calculated as the proportion of people in the population who have a causal genotype composed of a heterozygous *STUB1* variant and an intermediate *TBP* allele, assuming a linkage equilibrium (see Supplemental Results). In the range of 41 to 46 CAG/CAA repeats, the prevalence is estimated to be between 0.4 to 4 per 100,000 people, whereas if the TBP_{39}/*STUB1* genotype was also pathogenic and fully penetrant, it would further increase to 9 to 40 per 100,000 people.

Discussion

SCA17 is caused by a trinucleotide CAG/CAA repeat expansion, encoding poly-Q stretches in the *TBP* gene. *TBP* expanded alleles with more than 49 CAG/CAA repeats are considered fully penetrant.[4] Thus far, *TBP* alleles with 41 to 49 CAG/CAA repeats ($TBP_{41\text{-}49}$) have been associated with reduced penetrance of the phenotype.[4] SCA17 is unique among the poly-Q diseases for the remarkably high number of families with reduced-penetrance alleles, which represent the majority (approximately 70%) of reported index cases (Supplemental Table 1). Furthermore, nearly 2% of subjects in the general population carry *TBP* alleles with 41-47 CAG/CAA repeats.[9,13,14] We studied here the largest cohort of SCA17 families with *TBP* intermediate alleles, clearly showing that approximately 40% of relatives carrying a 41-49 allele were not affected at age ≥50 years (Supplemental Figure 8).

To unravel the genetic factors underlying the missing heritability observed in SCA17, we performed extensive NGS analysis in a cohort of patients presenting with the SCA17/HDL phenotype (Figure 1). Our data revealed an unexpected role of *STUB1* in the pathogenesis of SCA17: (1) all index patients, except 1, (30/31) carrying the $TBP_{41\text{-}46}$ alleles were found to have very rare (MAF ≤ 0.0005) heterozygous variants in the *STUB1* gene; (2) NGS clinical exome sequencing analyses excluded different genetic causes in this group of *STUB1*-positive patients but provided an alternative diagnosis (IBMPFD1/ALS14) for the only *STUB1*-negative patient; (3) segregation analysis in these families clearly showed that *STUB1* variants and $TBP_{41\text{-}46}$ expansions were co-inherited in all the affected patients, whereas the isolated

presence of a $TBP_{41\text{-}46}$ allele or a *STUB1* variant was never associated with the SCA17 phenotype; (4) no *STUB1* variant was found in affected subjects carrying $TBP_{\geq 47}$ alleles; and (5) no *STUB1* variant was detected in healthy controls carrying $TBP_{40\text{-}45}$ alleles.

Altogether, these data strongly indicate that the presence of a *STUB1* heterozygous variant is necessary for disease manifestation in subjects carrying a $TBP_{41\text{-}46}$ intermediate allele, whereas a $TBP_{\geq 47}$ allele appears to be sufficient to cause the disease (Figure 3D).

Notably, *TBP* analysis in a group of 10 ataxia index cases carrying heterozygous *STUB1* variants and presenting with a SCA17/HDL phenotype (Figure 1B) showed 41 to 45 repeats in the larger allele in 3 patients, 40 repeats in 6 patients, and 39 repeats in 1 patient. These data, supported by several lines of evidence, strongly suggest a pathogenic role for the TBP_{40}/*STUB1* genotype, although the data supporting the pathogenic role of the TBP_{39}/*STUB1* genotype are less consistent (Supplemental Table 7). Although the possibility exists that an unknown gene is responsible for the disease in these cases, our hypothesis is that the TBP_{39}/*STUB1* genotype has a variable penetrance mediated by another genetic factor. A study of a larger number of families is needed to clarify this hypothesis.

Altogether, our results indicate that SCA17 has a complex inheritance pattern; although it is a monogenic disease for $TBP_{\geq 47}$, it has to be considered as a digenic *TBP*/*STUB1* disease (SCA17-DI) for the range of intermediate *TBP* alleles (40-46). The involvement of *STUB1* in the pathogenesis of SCA17 is very intriguing because this gene has already been associated with ataxia. Biallelic pathogenic variants were demonstrated to cause SCAR16. Patients with SCAR16 are affected by early-onset cerebellar ataxia and variably associated with hyperkinetic movements, dementia, pyramidal tract damage, epilepsy, myoclonus, and hypogonadism.[26,27] More recently, heterozygous *STUB1* pathogenic variants were also associated with autosomal dominant SCA48, which is clinically characterized by a phenotype highly similar to that of SCA17.[22,23,28]

In this study, we identified 30 *STUB1* variants, 20 of which were novel (Table 1, Supplemental Table 3, and Figure 3A). All variants appear to be pathogenic: (1) they are absent or extremely rare (allele frequency ≤ 0.0005) in the general population; (2) 10 were previously reported in SCAR16 or SCA48 or both; (3) among the novel variants, 11 of 20 are LoF variants; (4) all the novel missense and in-frame variants are predicted to be damaging and affect highly conserved residues (Supplemental Figure 9); and (5) 4 of the 20 novel variants occur in 2 unrelated families. As observed in SCA48, SCA17-DI–associated variants are located along the whole protein. Interestingly, LoF variants are significantly represented in both SCA17-DI and SCA48 (Supplemental Figure 6B), suggesting that the pathogenic mechanism of *STUB1* variants is haploinsufficiency.

The *STUB1* gene encodes CHIP, a cochaperone of the ubiquitin-proteasome system implicated in protein quality control through its E3 ubiquitin ligase activity that mediates the proteasomal degradation of chaperone-bound misfolded proteins.[21] CHIP has the following 3 domains: a TPR domain, which is required for interaction with heat-shock proteins; a U-box domain that confers ubiquitin ligase activity; and a charged interdomain region that mediates CHIP dimerization and activity.[29] CHIP was demonstrated to facilitate the solubility of misfolded proteins involved in several neurodegenerative disorders.[29-31] Notably, CHIP has also been implicated in the pathogenesis of other forms of movement disorders caused by aberrant poly-Q proteins.[32] CHIP and ATXN1 proteins interact and colocalize in the nuclear inclusions of postmortem neurons of patients with SCA1 patients, and CHIP overexpression decreases poly-Q toxicity in a Drosophila SCA1 model.[33] In SCA3 and Huntington disease transgenic mice, suppression of CHIP activity worsened the severity of the phenotype and the poly-Q protein aggregation.[32,34] Altogether, these data make it conceivable that CHIP plays a role in the degradation of TBP. In this scenario, haploinsufficiency of CHIP might favor accumulation of TBP with mildly expanded (40-46) poly-Q tracts. In contrast, larger (≥47) poly-Q tracts would be able to trigger aggregation even in the presence of normal levels of CHIP.

The digenic nature of SCA17-DI raises the interesting question of its genetic prevalence. Considering the frequency of $TBP_{41\text{-}46}$ alleles (0.01-0.005) and that of *STUB1* variants (0.00084-0.0034) in the general population, the $TBP_{41\text{-}46}$/*STUB1* genotype is expected to be carried by 0.4 to 4 individuals per 100,000 individuals, which would imply a disease prevalence that is relatively high when compared with that reported for hereditary ataxias.[1] However, the variability of the first symptom at onset along with the phenotype overlapping with ataxia and chorea might lead to an incorrect classification of patients as occurred in 3 cases in our patient cohort (F15-II.1, F16-II.1, and F26-II.3). Furthermore, *TBP* expansions in the 43 to 46 repeat range have been found in many patients with neurodegenerative disorders ranging from Parkinson disease to multisystem atrophy[9] (Supplemental Table 1), which makes it conceivable that the *TBP*/*STUB1* genotype may underlay a wider spectrum of phenotypes. Our finding of $TBP_{39\text{-}40}$/*STUB1* genotypes is more puzzling because the allele frequencies of TBP_{39} and TBP_{40} are 9 to 40 per 100,000 and 2 to 9 per 100,000, respectively. Although phenotypic variability might still explain the discrepancy between genetic prevalence and disease prevalence, the possibility exists that these genotypes have a reduced penetrance.

Because of the study design and selection criteria, all the patients with $TBP_{41\text{-}46}$/*STUB1* genotype reported here exhibited an SCA17/HDL phenotype similar to that of patients with $TBP_{\geq 47}$ genotype. However, although a significant correlation between CAG/CAA repeat size and age at onset can be observed in this latter group ($R^2 = 0.49$, $P = .004$), no correlation was found in the group of patients with $TBP_{41\text{-}46}$/*STUB1* genotype (Figure 3B and C).

Sequence analysis of the repeat region in all patients showed evidence of intergenerational instability only in 1 of 20 alleles (5%) shared by at least 2 family members. Furthermore, 72 of 73 (98.6%) $TBP_{41\text{-}46}$ alleles showed the classical domain configuration, whereas in the $TBP_{47\text{-}54}$ repeat range, 8 of 15 (53.3%) alleles presented an alternative architecture. In particular, 1 case exhibited loss of CAACAGCAA interruption leading to a stretch of 44 CAG repeats, which makes the allele more susceptible to instability (F38-II.5). Altogether, these results indicate that the size and architecture of the CAG/CAA repeats alone cannot explain the great variability in age at onset observed in patients with $TBP_{41\text{-}46}$/*STUB1* genotype and thus suggest a relevant contribution of the concurrent *STUB1* variant. This genetic interaction may ultimately account for the weaker overall correlation between repeat size and age of onset observed in SCA17 than that observed in other poly-Q disorders.[3,18]

Our data clearly indicate that heterozygous *STUB1* variants alone do not cause the disease, casting doubts on the existence of SCA48 as a monogenic disease. Along with our results, several lines of evidence support this hypothesis. First, none of the obligate heterozygotes of a *STUB1* pathogenic variant were reported to be affected in SCAR16 families.[25] Second, the frequency of potentially pathogenic *STUB1* alleles (allele frequency = 0.003, gnomAD v2.1.1) is too high for a rare autosomal dominant disease. Third, several *STUB1* variants are shared by SCAR16, SCA48, and SCA17-DI, which suggests that there is no disease specificity for the variants.[35] Fourth, although the age of nonaffected *STUB1* heterozygous subjects is not always reported, *STUB1* variants in SCA48 families show evidence of reduced penetrance.[24,25,28,36] Fifth, the clinical phenotype and disease course largely overlap between SCA48 and SCA17-DI. Finally, 2 patients with a *STUB1* variant were reported to carry an intermediate *TBP* allele (41 and 46, respectively) as a second hit.[28] Unfortunately, although most patients with SCA48 are reported to have been screened for poly-Q diseases, none of the numerous papers published thus far provide details on the criteria adopted for SCA17 exclusion nor the *TBP* repeat size observed in the patients carrying *STUB1* variants.[22-24,28,36,37] and Suppl Refs 16,20,23,38,40 Altogether, this evidence strongly suggests that the genetic landscape of SCA48 should ultimately be defined by analyzing the *TBP* repeat size in all patients diagnosed with this disease. Furthermore, *TBP* repeat size should also be investigated in patients with SCAR16 because it might contribute to the variability of the phenotype.

The dual inheritance pattern of SCA17, a monogenic dominant disorder for $TBP_{\geq 47}$ and a true digenic[38] *TBP*/*STUB1* disease for intermediate *TBP* expansions, is relevant for precise diagnostic and counseling approaches. Both *TBP* and *STUB1* genes shall now be considered not only in the diagnostic approach of patients with SCA17/HDL phenotypes (Supplemental Figure 10) but also for an appropriate calculation of the family risk (Supplemental Figure 11). *TBP* repeat analysis is also necessary for appropriate genetic counseling in SCAR16 families for the potential risk of manifesting SCA17-DI. Finally, repeat expansion sequencing results demonstrate that routine fragment size diagnostic analysis underestimates allele length by 1 or 2 triplets with an error rate that increases with allele length up to 75% for $TBP_{\geq 50}$. This discrepancy could be relevant for precise risk assessment particularly for patients carrying border alleles.

Conclusion

Our study demonstrates that digenic *TBP/STUB1* inheritance explains the incomplete penetrance of SCA17 intermediate alleles, which allows more precise and comprehensive genetic diagnosis and counseling and provides novel information on SCA17 pathomechanisms, possibly relevant for precision therapies. Moreover, our data open the possibility that similar mechanisms may occur in other neurodegenerative hereditary diseases characterized by incomplete penetrance and/or phenotypic variability.

Data Availability

Anonymized data from this study are available at https://zenodo.org/communities/besta and will be shared by request from any qualified investigator.

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Ethics Declaration

Individuals were identified in different Italian centers in diagnostic settings approved by the respective institutional review boards: Bioethics Committee of the Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; Bioethics Committee of the IRCCS Ospedale Policlinico San Martino, Genoa, Italy; Bioethics Committee of the IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy; Bioethics Committee of the Città della Salute e della Scienza University Hospital, Turin, Italy. Genetic investigations involving healthy controls from a previous study[14] had been approved by the Bioethics Committee of the Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy. Institutional review board–approved written informed consent was obtained from each participant undergoing clinical and genetic investigations. This study adhered to the World Medical Association Declaration of Helsinki (2013).

Conflict of Interest

The authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2021.08.003) contains supplementary material, which is available to authorized users.

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