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An Individual with Blepharophimosis-Ptosis-Epicanthus Inversus Syndrome (BPES) and Additional Features Expands the Phenotype Associated with Mutations in *KAT6B*

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Abstract

Blepharophimosis-Ptosis-Epicanthus Inversus Syndrome (BPES) is an autosomal dominant disorder caused by mutations in *FOXL2*. We identified an individual with BPES and additional phenotypic features who did not have a *FOXL2* mutation. We used whole exome sequencing to identify a de novo mutation in *KAT6B* (lysine acetyltransferase 6B) in this individual. The mutation was a 2 bp insertion leading to a frameshift which resulted in a premature stop codon. The resulting truncated protein does not have the C-terminal serine/methionine transcription activation domain necessary for interaction with other transcriptional and epigenetic regulators. This mutation likely has a dominant-negative or gain-of-function effect, similar to those observed in other genetic disorders resulting from *KAT6B* mutations, including Say-Barber-Biesecker-Young-Simpson (SBBYSS) and Genitopatellar syndrome (GTPTS). Thus, our subject's phenotype broadens the spectrum of clinical findings associated with mutations in *KAT6B*. Furthermore, our results suggest that individuals with BPES without a *FOXL2* mutation should be tested for *KAT6B* mutations. The transcriptional and epigenetic regulation mediated by *KAT6B* appears crucial to early developmental processes, which when perturbed can lead to a wide spectrum of phenotypic outcomes.

Keywords

Blepharophimosis; Ptosis; Epicanthus Inversus; BPES; *KAT6B*; Whole exome sequencing

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INTRODUCTION

Blepharophimosis-Ptosis-Epicanthus Inversus Syndrome (BPES, [OMIM 110100]) is a rare genetic disorder with clinical features including dysplasia of the eyelids and palpebral fissures, low nasal bridge, and ptosis [Johnson et al., 1964; Oley et al., 1988]. It has two forms: Type I with premature ovarian failure, and Type II without ovarian failure [Zlotogora et al., 1983]. Mutations in *FOXL2* (OMIM 605597) have been found in the majority (88%) of individuals with Type I and II BPES. [Crisponi et al., 2001; Beysen et al., 2005]. Causative mutations detected in *FOXL2* range in size from single nucleotide point mutations [Ramírez-Castro et al., 2002; Dollfus et al., 2003; Udar et al., 2003] to deletions and copy number variations (CNV) encompassing *FOXL2* [Crisponi et al., 2001; De Baere et al., 2003; Beysen et al., 2005]. While 81% of mutations were intragenic within *FOXL2*, 12% were microdeletions affecting *FOXL2* and its neighboring genes, the remaining 5% were deletions apparently affecting regulatory regions [Beysen et al., 2009]. Microdeletions encompassing a larger genomic region around *FOXL2* were found in individuals referred to as BPES plus, who in addition to BPES have additional features, such as developmental delay, speech delay and genital anomaly [Costa et al., 1998; D'haene et al., 2009; D'haene et al., 2010; Zahanova et al., 2012]. Further, a subset of BPES plus individuals (33%) was found to have other pathogenic CNVs not affecting *FOXL2* [Gijsbers et al., 2008; D'haene et al., 2010].

Besides *FOXL2*, several genes have also been associated with blepharophimosis-mental retardation (BMR) syndromes [Verloes et al., 2006], such as *UBE3B* (OMIM 608047) in blepharophimosis-ptosis-intellectual disability syndrome (BPIDS, [OMIM 615057]) and *MED12* (OMIM 300188) in X-linked Ohdo syndrome (OHDOX, [OMIM 300895]), demonstrating genetic heterogeneity of this group of disorders [Basel-Vanagaite et al., 2012; Vulto-van Silfhout et al., 2013]. In recent studies, pathogenic mutations in *KAT6B* (OMIM 605880), a histone acetyl transferase gene, have been observed in individuals with phenotypic features that overlap with BPES plus, including an individual initially diagnosed with Noonan syndrome (NS1, [OMIM 163950]) and individuals with Say-Barber-Biesecker-Young-Simpson syndrome (SBBYSS, [OMIM 603736]) and Genitopatellar syndrome (GTPTS, [OMIM 606170]) [Kraft et al., 2011; Clayton-Smith et al., 2011; Simpson et al., 2012; Campeau et al., 2012; Szakszon et al., 2013]. Noonan syndrome is characterized by reduced growth, cardiac defects, and facial dysmorphism, including hypertelorism, ptosis, downslanting palpebral fissures and low-set, posteriorly angulated ears [Noonan, 1994; Shah et al., 1999; Tartaglia et al., 2010]. SBYSS, a variant of Ohdo syndrome, is associated with intellectual disability and has a distinctive facial appearance including severe blepharophimosis, immobile face, bulbous nasal tip and a small mouth with a thin upper lip [Clayton-Smith et al., 2011], while individuals with GTPTS may exhibit microcephaly, broad nose, a small or retracted chin, flexion contractures of lower limbs, abnormal or missing patellae, and urogenital anomalies [Penttinen et al., 2009].

We report an individual with clinical features typically associated with BPES with additional findings including global developmental delay, syndactyly, and cryptorchidism, but who did not have a *FOXL2* mutation. Whole exome sequencing (WES) of the subject and his unaffected parents identified a rare, de novo 2 bp insertion in exon 18 of *KAT6B*.

The mutation resulted in frameshift leading to a premature stop codon. Our subject's clinical presentation broadens the phenotypic spectrum seen in individuals with mutations in *KAT6B*, further suggesting that *FOXL2*-negative BPES individuals with a complex clinical presentation should be tested for *KAT6B* mutations.

MATERIALS AND METHODS

Subject Enrollment and Sample Collection

The patient and his parents were enrolled into an IRB approved research protocol at the Children's Hospital of Philadelphia (CHOP). High quality, unamplified, and unfragmented genomic DNA (A260/A280 ≥ 1.8 and A260/A230 ≥ 1.9) was extracted from whole blood obtained from the subject and his parents using Puregene Blood kit from Qiagen (Valencia, CA).

FOXL2 Mutation Screening

Genomic DNA samples from the patient and his parents (100 ng) were amplified by using two sets of primers specific to *FOXL2* (NM_023067.3). Forward primer 1: 5′-GAGCTTAGGAAAGCGAAAAAGCAC AGAGGG-3′, reverse primer1: 5′-GAAGACATGTTCGAGAAGGGCAACTACCG-3′, forward primer 2: 5′-GTTGAGGAAGCCAGACTGCAGGTACTTGGG-3′, and reverse primer 2: 5′-TCTCCAGAAGTTTGAGACTTGGCCGTAAGC-3′. PCR reaction and conditions were as follows: Promega (Madison, WI) GoTaq Hot Start kit with 1× Master Mix and 400 nM of each primer. PCR began with an initial cycle at 95°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds, 60°C for 30 second and 72°C for 1 minute, finishing with extension at 72°C for 5 minutes. Amplified PCR products were sequenced using the PCR primers as sequencing primers on an ABI (Carlsbad, CA) PRISM *3730xl* at a commercial sequencing facility. High resolution copy number analysis was performed using Affymetrix (Santa Clara, CA) SNP 500K arrays as previously described [Haldeman-Englert et al., 2010].

Exome Sequencing and Data Analysis

Whole exome sequencing was performed on the subject and his parents using Nimblegen (Madison, WI) SeqCap EZ Exome v2.0 followed by sequencing on an Illumina (San Diego, CA) HiSeq 2000. Details of data analysis were similar to the procedure as previously described [Yu et al., 2013]. Approximately 50 million, 90 bp, paired-end reads (>50×) were obtained and mapped to the reference human genome (NCBI build 37) using Burrows-Wheeler Aligner (BWA) [Li et al., 2009a]. Variants were determined by the utilities in the SAMtools [Li et al., 2009b] and further annotated using SeattleSeq (http://snp.gs.washington.edu/SeattleSeqAnnotation134/). Filtering and test of inheritance model were done using tools in Galaxy [Goecks et al., 2010]. Subject's Single Nucleotide Variants (SNVs) were filtered to retain calls that met the following criteria; bases with PHRED-scaled score > 20, SNP and consensus scores > 50, read coverage > 8 with > 25% of the reads containing the variant call. For homozygous/hemizygous variant calls, > 80% reads were required to contain the variant, while for heterozygous variant calls, the number of reads containing the variant call ranged between 25%–80%. Insertions and deletions of <50 bp (InDels) were filtered based on similar criteria except that the SNP and consensus score

was required to be >100. Variants found in segmental duplications, simple- or low-complexity repeats were removed due to the higher likelihood of mapping errors. Sequence data from parental samples were used as an additional filter to confirm variant calls in the subject. The filtering criteria for variant calling in parental data were less stringent than in the subject in order to minimize erroneous classification of variants as unique to the subject. Thus, the criteria for parental data included SNP score >5 for SNVs and SNP score >10 for InDels, and required that at least two reads contain the variant call. Variants were filtered against dbSNP build 135 and 1000 Genomes (November 23, 2010 release version). The sequence data from the family was then used to test for causal variants under different inheritance models, including de novo mutation in a dominant model and compound heterozygous, homozygous and X-linked hemizygous mutations in recessive models.

PCR and Sanger Sequencing Verification

Validation of mutation was by PCR amplification followed by Sanger sequencing using forward primer of 5′-ATACGAGCGAATGGGTCAGAGTGATTTTGG-3′ and reverse primer of 5′-GTTCACAGAGTTGACATTGTAGGCTGGCG-3′. Amplification and sequencing conditions were the same as described above. Mutations detected in *KAT6B* were named using cDNA accession number NM_012330.3.

RESULTS

Clinical Report

The patient was a 7-month-old boy when first evaluated at the Children's Hospital of Philadelphia (CHOP). He was diagnosed with BPES by a pediatric ophthalmologist. In addition to blepharophimosis, ptosis and epicanthus inversus normally associated with BPES, he had cryptorchidism, right hydrocele, wide-spaced nipples, and slight 2–3 syndactyly of toes (Fig. 1). Clinical testing demonstrated a normal karyotype (46, XY), and normal FISH studies for 22q11.2 deletion, Cri-du-Chat syndrome. Thyroid function was normal (thyroxine=4.9 mcg/dL, normal range=4.7–9.9 mcg/dL; TSH (thyroid stimulating hormone=3.58 ulU/mL, normal range=0.5–3.8 ulU/mL). Further, normal 7-dehydrocholesterol level was used to rule out Smith-Lemli-Opitz syndrome. Sanger sequencing and high-resolution CNV analysis with Affymetrix SNP 500K arrays did not identify a *FOXL2* mutation.

Whole Exome Sequencing

Whole exome sequencing analysis was performed on the subject and his unaffected parents to obtain over 50× coverage of targeted exome in each sample. A large number of variants (77,525 variants) were detected in the subject after applying appropriate quality measures (Table I). Our downstream analyses was focused on nonsynonymous coding variants, coding InDels (insertions/deletions <50 bp) and variants affecting splice sites as they are likely to have a functional impact on the gene product, and hence likely pathogenic (9,024 variants). We first filtered out common variants seen in dbSNP and 1000 genomes. This resulted in the identification of 183 rare variants in the subject that were considered for further analysis. Parental WES data was used to detect the pathogenic variant under various inheritance

models including dominant (de novo mutations) and recessive (compound heterozygous, homozygous, and X-linked hemizygous mutations) models.

There was no strong candidate in the recessive models, but under a dominant model we identified a candidate gene with a potentially pathogenic mutation. The candidate, *KAT6B*, carried a de novo heterozygous 2 base-pair insertion/duplication c.5623_5624dupCA in exon 18. The number of variants in each filtering step and the final expected number of rare and de novo mutations (<1 per trio) were in accordance with previous studies [Vissers et al., 2010; O'Roak et al., 2011; Sanders et al., 2012; Neale et al., 2012].

Validation by Sanger Sequencing

The mutation in the subject was verified by Sanger sequencing (Fig. 2A). Both parents had normal alleles, but the subject had a heterozygous 2 bp insertion (or CA duplication) in *KAT6B* (NM_012330.2) located on chromosome 10q22.2. The subjects mutation, c. 5623_5624dupCA, was located in the largest and the last exon (exon 18) of *KAT6B*, and resulted in frameshift and a premature stop codon, p.Gln1875Hisfs*5, interrupting the C-terminal of KAT6B which has a serine/methionine-rich transcription activation domain (Fig. 2B). KAT6B is a histone acetyltransferase believed to be an epigenetic and transcriptional regulator for a broad range of cellular processes [Yang et al., 2007].

DISCUSSION

We used whole exome sequencing in a trio-based (proband and his unaffected, biological parents) approach to identify a de novo mutation in a histone acetyltransferase gene, *KAT6B*, in a patient with BPES and additional features. *KAT6B*, also known as *MYST4*, *MORF*, *MOZ2* or *qkf*, belongs to a 5-membered MYST family of genes also consisting of *Moz*, *Ybf2/Sas3*, *Sas2* and *Tip60* [Champagne et al., 1999]. KAT6B is proposed to function either as a transcription factor or an epigenetic regulator on a broad range of genes and cellular functions [Yang et al., 2007]. KAT6B interacts with other proteins like BRPFs (bromodomain-PHD finger proteins), ING5 (inhibitor of growth 5), and EAF6 (Esa1-associated factor 6 ortholog) [Pelletier et al., 2002; Ullah et al., 2008]. This multi-protein complex then interacts with RUNX transcription factors for transcriptional activation of downstream genes [Champagne et al., 1999; Pelletier et al., 2002; Yang et al., 2007]. The interaction with its protein partners also stimulates the histone acetyltransferase activity of KAT6B allowing it to acetylate nucleosomal histone H3, which is an activating histone mark [Yang et al., 2007; Ullah et al., 2008; Kraft et al., 2011]. Our subject's mutation, c. 5623_5624dupCA is located in exon 18 and truncates the serine/methionine-rich transcription activation domain at the C-terminus. Thus, it is expected that the mutated protein cannot interact with RUNX transcription factors necessary for its function in transcriptional activation.

Mutations in *KAT6B* have been associated with several other disorders associated with a wide variety of phenotypic features. Haploinsufficiency of *KAT6B* resulting from a balanced, de novo translocation t(10;13)(q22.3;q34) interrupting the gene has been observed in an individual with a Noonan syndrome-like phenotype [Kraft et al., 2011]. However, the phenotype observed in our subject appears to be more complex (Table II). Clayton-Smith et

al. [2011] found pathogenic *KAT6B* mutations in 13 individuals with Say-Barber-Biesecker-Young-Simpson syndrome through exome sequencing [Clayton-Smith et al., 2011]. Two additional SBBYSS individuals were later reported by Szakszon et al. [2013]. Whole exome sequencing also identified pathogenic *KAT6B* mutations in 11 individuals with genitopatellar syndrome [Simpson et al., 2012; Campeau et al., 2012]. The presence of genital anomalies in our patient is consistent with similar findings in SBBYSS and genitopatellar syndrome. Thus, based on the available literature [Day et al., 2008; Clayton-Smith et al., 2011], our patient's phenotype most closely resembles SBBYSS.

KAT6B mutations identified in individuals with the above diseases are de novo, nonsense or frameshift mutations resulting in truncated protein (Fig. 2B). Interestingly, most of the mutations in SBBYSS cluster within exon 18 of *KAT6B* lead to a truncated KAT6B with intact N-terminal domains, but missing C-terminal serine/methionine-rich transcription activation domain. These authors concluded that the loss of serine/methionine-rich transcription activation domain has either dominant-negative or gain-of-function effects. Alternatively, in a majority of individuals with GTPTS, the mutations are more centrally located within the acidic region of the protein (Fig.2B).

Although the truncated KAT6B has an intact histone acetyltransferase (HAT) domain, there was a significant reduction of histone H3 and H4 acetylation in the affected individuals, apparently due to its lower histone acetylation activity [Kraft et al., 2011; Simpson et al., 2012]. Yet, the effect of reduced histone acetylation activity of KAT6B on its interaction with RUNX transcription factors remains unclear [Simpson et al., 2012]. Recently, mutations in *MLL2* (OMIM 602113) and *KDM6A* (OMIM 300128) were identified in individuals with Kabuki syndrome 1 (KABUK1, [OMIM 147920]) and Kabuki syndrome 2 (KABUK2, [OMIM 300867]), respectively [Hannibal et al., 2011; Lederer et al., 2012; Miyake et al., 2013]. Kabuki syndrome is a multiple congenital anomaly (MCA) syndrome including distinctive facial features, intellectual disability along with cardiac, skeletal and immunological defects [Niikawa et al., 1981]. Interestingly, MLL2 and KDM6A are histone methyltransferases, involved in histone modification just like KAT6B. This suggests that epigenetic regulation in the form of histone modification plays an important role in early human development, and mutations that perturb these processes can result in genetic disorders leading to MCA.

In summary, the simultaneous exome sequencing of a proband and his unaffected parents has enabled us to identify the causative mutation underlying the MCA. This underscores the efficacy of whole exome and whole genome sequencing technologies in identifying the genetic mutations underlying a majority of Mendelian disorders, especially in one-off cases in which multiple individuals with an overlapping phenotype or syndrome are not available. The candidate gene, *KAT6B*, has been implicated previously in a wide spectrum of disorders with partially overlapping but not characteristic phenotypes. Our findings extend the phenotypic spectrum observed in individuals with *KAT6B* mutations and highlight *KAT6B* as a strong candidate gene in individuals with BPES who present with additional, atypical congenital anomalies.

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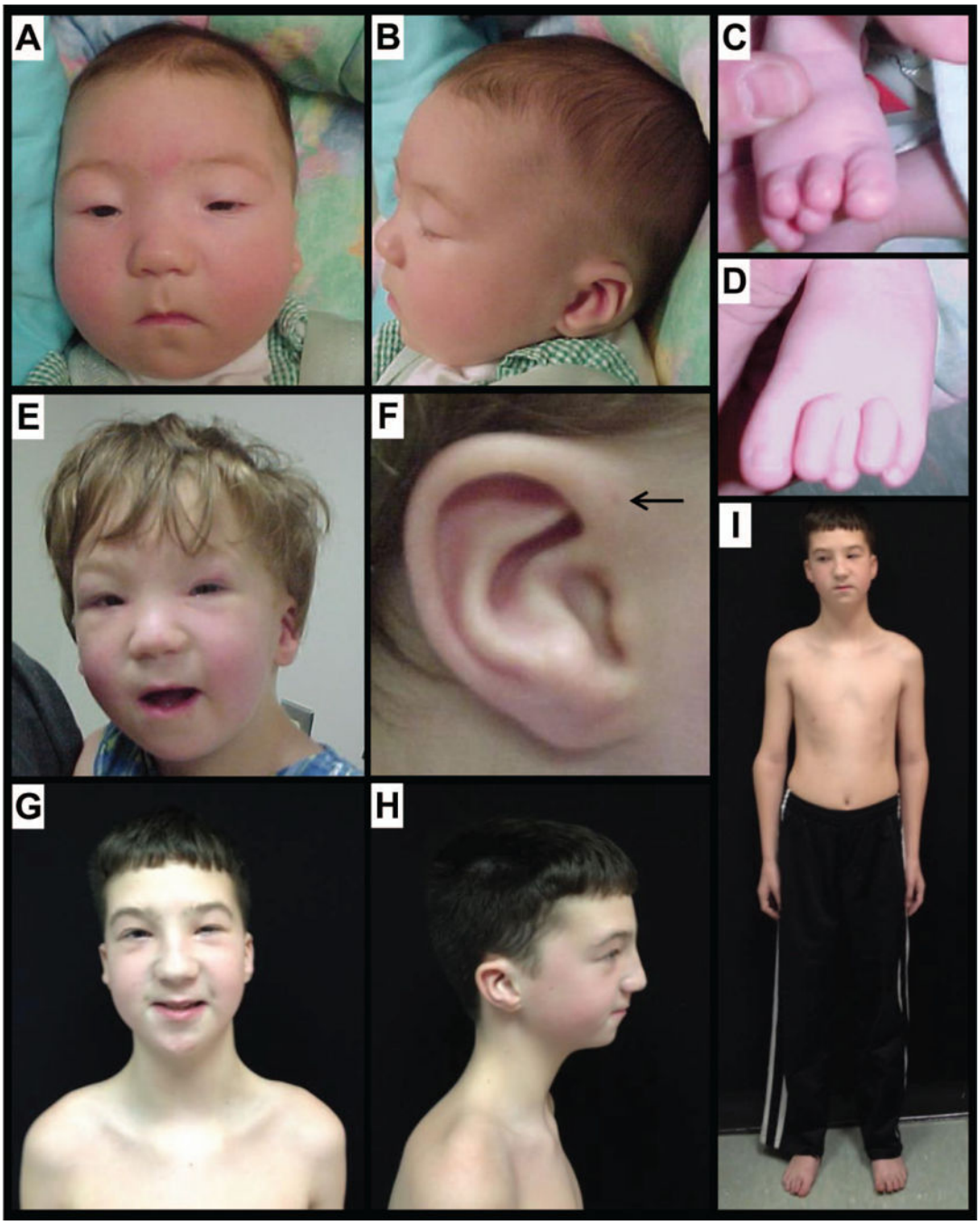

FIG 1.

Clinical features of the subject. A–D: subject at 2 months of age; note blepharophimosis, ptosis, epicanthus inversus (A), posteriorly angulated ears with thickened superior helix and prominent antihelix (B), and slight 2–3 syndactyly of toes in addition to overlapping toes (C, D). E,F: subject at 3.5 years of age following oculoplastic surgery to correct ptosis; note right-sided preauricular ear pit (F, indicated by arrow). G–I: subject at 12 years of age; note the recurrence of ptosis (L>R), arched eyebrows, abnormal ears, thin upper lip vermilion, small pointed chin, downsloping shoulders and wide-spaced and low-set nipples.

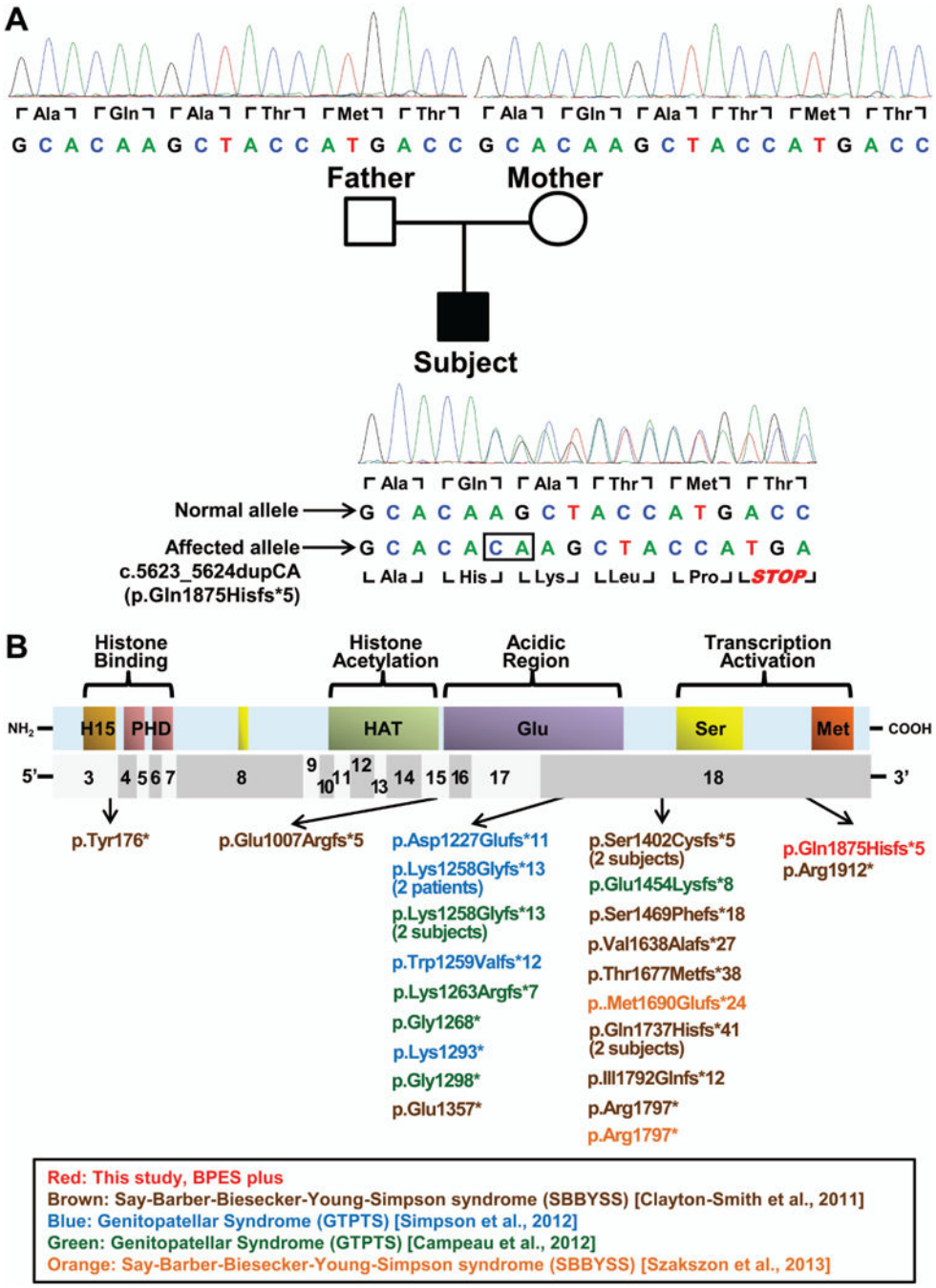

FIG. 2.

Pathogenic mutations in *KAT6B.* A: Chromatograph showing Sanger sequencing results of the subject shows a rare de novo heterozygous c.5623_5624dupCA (p.Gln1875Hisfs*5) frameshift mutation while parents have two normal alleles. The 2 bp CA duplication in the subject results in mixed peaks after the duplication site. B: In the top panel, protein domains of KAT6B are shown. It has a histone binding domain consisting of H15 (linker histones H1- and H5-like module) and PHD (plant homeodomain zink fingers) domains, a histone acetylation HAT (histone acetyltransferase) domain, an acidic glutamate/aspartate-rich

domain, and a transcription activation serine/methionine-rich domain [Champagne et al., 1999; Pelletier et al., 2002]. The second panel shows *KAT6B* coding exons as gray boxes with exon number indicated. Locations of pathogenic mutations identified in our subject and other recently identified genetic disorders are indicated by arrows. Mutations found in our subject (red), SBBYSS (brown and orange), and GTPTS (green and blue) are always nonsense or frameshift truncating mutations.

Table I

Summary of Exome Variants and Test of Inheritance Models

	Subject			
Total variants	77,525			
Coding variants	18,604			
Nonsynonymous, splice-site, InDel variants	9,024			
Rare variants	183			
Test of inheritance model	Dominant model	Recessive models		
	de novo	Compound heterozygous	Homozygous	X-linked hemizygous
	1 (*KAT6B*)	0	0	0

Table II

Comparison of Phenotypic Features

	This study, BPES Plus	Noonan-like[a]	SBBYSS[b,e]	GTPTS[c,d]
Neurological anomalies	Global developmental delay	Microcephaly, ADHD, IQ 75–80, no structural defects	Developmental or intellectual delay, microcephaly in minority, hypotonia, no structural defects	Developmental or intellectual delay, microcephaly in all, agenesis of the corpus callosum, colpocephaly
Facial anomalies	Blepharophimosis, ptosis, epicanthus inversus	Blepharophimosis, ptosis, arched eyebrows, abnormal ears, smooth philtrum, retrognathia, high palate	Blepharophimosis, ptosis, broad and flat nasal bridge, bulbous nose, full cheeks, abnormal ears, small mouth, expressionless facies	Broad or prominent nasal bridge, bulbous nose in minority, full cheeks in minority
Musculo-skeletal anomalies	Widely-spaced nipples, slight 2–3 syndactyly	Short stature, delayed bone age, ligamentous laxity	Long thumbs and toes, patellar anomalies in minority	Absent or hypoplastic patellae in majority, flexion contractures, club feet, costo-vertebral anomalies, pelvic anomalies
Genital anomalies	Cryptorchidism, right hydrocele		Cryptorchidism and hypospadias	Anal anomalies, hypoplastic labia, clitoromegaly, scrotal hypoplasia, cryptorchidism

Adapted from Campeau et al., 2012a.

[a] Kraft et al., 2011.

[b] Clayton-Smith et al., 2011

[c] Simpson et al., 2012

[d] Campeau et al., 2012a

[e] Szakszon et al., 2013