De Novo Mutations of the Gene Encoding the Histone Acetyltransferase *KAT6B* Cause Genitopatellar Syndrome

Michael A. Simpson,[1,11] Charu Deshpande,[2,11] Dimitra Dafou,[1,11] Lisenka E.L.M. Vissers,[3] Wesley J. Woollard,[1] Susan E. Holder,[4] Gabriele Gillessen-Kaesbach,[5] Ronny Derks,[3] Susan M. White,[6] Ruthy Cohen-Snuijf,[7] Sarina G. Kant,[8] Lies H. Hoefsloot,[3] Willie Reardon,[9] Han G. Brunner,[3] Ernie M.H.F. Bongers,[3,12,*] and Richard C. Trembath[1,10,12,*]

Genitopatellar syndrome (GPS) is a rare disorder in which patellar aplasia or hypoplasia is associated with external genital anomalies and severe intellectual disability. Using an exome-sequencing approach, we identified de novo mutations of *KAT6B* in five individuals with GPS; a single nonsense variant and three frameshift indels, including a 4 bp deletion observed in two cases. All identified mutations are located within the terminal exon of the gene and are predicted to generate a truncated protein product lacking evolutionarily conserved domains. *KAT6B* encodes a member of the MYST family of histone acetyltranferases. We demonstrate a reduced level of both histone H3 and H4 acetylation in patient-derived cells suggesting that dysregulation of histone acetylation is a direct functional consequence of GPS alleles. These findings define the genetic basis of GPS and illustrate the complex role of the regulation of histone acetylation during development.

Genitopatellar syndrome (GPS [MIM 606170]) is an uncommon disorder in which patellar aplasia or hypoplasia is associated with external genital and renal anomalies, congenital flexion deformities of the limbs, distinctive facial features, corpus callosum agenesis, and severe intellectual disability. This reported spectrum of ancillary phenotypic features is consistent with a fundamental defect in developmental processes.[1–3] Congenital patellar malformations comprise a clinically diverse and genetically heterogeneous group, including nail patella syndrome (MIM 161200) and small patella syndrome (MIM 147891), caused by mutations in the transcription factors *LMX1B* and *TBX4*, respectively, and Meier-Gorlin syndrome (ear, patella, short-stature syndrome [MIM 224690]) caused by mutations in five different prereplication complex genes.[4–7] In contrast to the latter syndromes, GPS is accompanied by severe intellectual disability and cerebral anomalies. Recent studies have demonstrated the effectiveness of exome sequencing in the elucidation of the genetic basis of recessive and dominant monogenic traits, including those caused by de novo mutations.[8,9]

To search for disease alleles in GPS we undertook whole exome sequencing of six unrelated affected individuals. All had characteristic clinical features of GPS (Figures 1A-1C and Table 1). Ethical approval for this study was obtained from the Guy's and St Thomas' NHS Foundation Trust local research ethics committee (08/H0802/84 "Systematic Characterisation of Genes in Inherited Disorders") and the Regional Committee on Research Involving Human Subjects Nijmegen-Arnhem (0006-0119) and written informed consent was obtained from all participating families, including publication of photographs. DNA was extracted from blood and whole-exome capture was performed by in-solution hybridization using the Sure Select system (Agilent) followed by massively parallel sequencing (Illumina GAIIx [samples from individuals GPS-01, GPS-02, GPS-03, and GPS-06] and Life Technologies SOLiD 4 [GPS-04 and GPS-05]). Over 4.7 gigabases of mappable sequence data were generated for each subject, such that >70% of the coding bases of the exome defined by the GENCODE Project were represented by at least 20 reads (Tables S1 and S2). Variant profiles were generated with our respective in-house variant-calling pipelines (Table S3).[8,9] Reads generated on the Illumina GAIIx platform were aligned to the reference human genome with the Novoalign software package (Novocraft Technologies Sdn Bhd). Duplicate reads, resulting from PCR clonality or optical duplicates, and reads mapping to multiple locations were excluded from downstream analysis. Depth and breadth of sequence coverage was calculated with custom scripts and the BedTools package.[10] Single nucleotide substitutions and small insertion deletions were identified and quality filtered within the SamTools software package[11] and in-house software tools. Variants

[1]Division of Genetics and Molecular Medicine, King's College London School of Medicine, Guy's Hospital, London SE1 9RT, UK; [2]Clinical Genetics, Guy's and St Thomas' National Health Service (NHS) Foundation Trust, Guy's Hospital, London SE1 9RT, UK; [3]Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen 6500 HB, The Netherlands; [4]North West Thames Regional Genetics Service, North West London Hospitals NHS Trust, Harrow HA1 3UJ, UK; [5]Institut für Humangenetik, Universität zu Lübeck, Lübeck 23538, Germany; [6]Genetic Health Services Victoria, Murdoch Childrens Research Institute, Melbourne 3052 Australia; [7]Ipse de Bruggen, Centre for People with Intellectual Disability, Nieuwveen 2441 CP, The Netherlands; [8]Centre for Human and Clinical Genetics, Department of Clinical Genetics, Leiden University Medical Center, Leiden 2300 RC, The Netherlands; [9]National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland; [10]Queen Mary University of London, Barts and The London School of Medicine and Dentistry, London E1 2AD, UK
[11]These authors contributed equally to this work
[12]These authors contributed equally to this work
*Correspondence: e.bongers@antrg.umcn.nl (E.M.H.F.B.), richard.trembath@kcl.ac.uk (R.C.T.)
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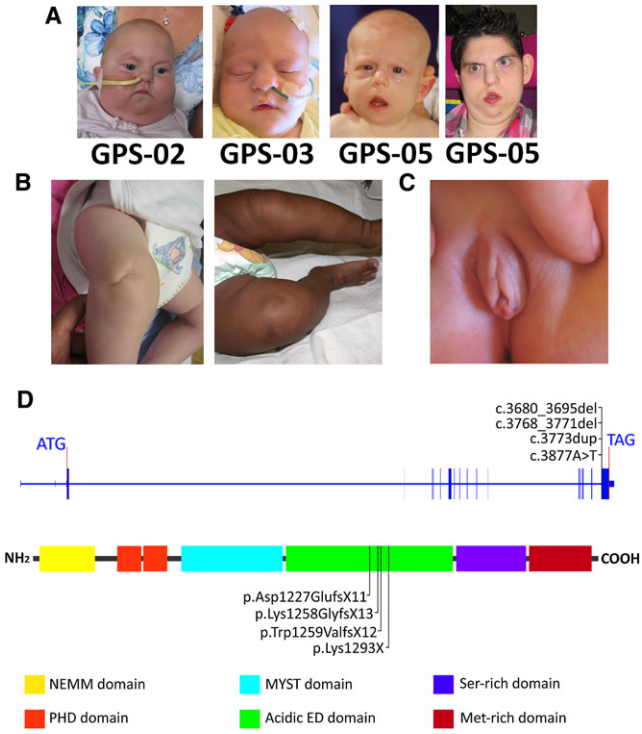

Figure 1. Clinical and Genetic Findings of Individuals with Genitopatellar Syndrome
(A) Facial features, including a coarse face, a broad bulbous nose, downturned corners of the mouth, and micrognathia (individuals GPS-02 [age 5 months], GPS-03 [age 3 months], and GPS-05 [age 5 months and 25 years, respectively]).
(B) Flexion contractures of the knees with skin dimples suggestive of absent patella.
(C) Scrotal hypoplasia and cryptorchidism.
(D) Observed mutations with respect to the genomic organization of KAT6B and the domain structure of *KAT6B*.

were annotated with respect to genes and transcripts with the Annovar tool.[12] Reads generated on the Life Technologies SOLiD platform were mapped to the reference genome with the SOLiD bioscope software (Life Technologies). Single nucleotide variants were subsequently called by the diBayes algorithm (Life Technologies). Small insertions and deletions were detected using the SOLiD Small InDel Tool (Life Technologies). All called variants and indels were combined and annotated with custom analysis scripts. As sibling recurrence of GPS has previously been observed,[1] we initially compared the six exome profiles under a model of a rare autosomal-recessive inheritance, but did not identify any gene harboring novel homozygous or compound heterozygous protein-altering variants in more than one individual (data not shown). Subsequently, we interrogated these data under the assumption that each of the causative alleles is dominant and had arisen de novo; requiring at least one previously unobserved heterozygous nonsynonymous or splice-site substitution or a coding insertion or deletion in the same gene in all six individuals. This process did not reveal a single gene matching the criteria in all six individuals (Table S4). However, evaluation of these data with a prior expectation of genetic heterogeneity highlighted *KAT6B* (MIM 605880) as the only candidate gene harboring previously unobserved heterozygous variants in five of the six individuals (Table S4); a single nonsense variant (c.3877A>T [p.Lys1293X]) and three frameshift indels, including a 4 bp deletion observed in two cases (c.3768_3771del [p.Lys1258GlyfsX13], c.3680_3695del [p.Asp1227GlufsX11], and c.3773dup [p.Trp1259ValfsX12]) (Table 1).

The variants are all located within a 66 bp region of exon 18, the terminal exon, of *KAT6B*, which is located on chromosome 10 and encodes an 8.5 kb transcript (NM_012330.2). Each variant is predicted to lead to the premature termination of the protein product that has a high likelihood of functional impact (Figure 1D). None of the observed mutations is present in dbSNP, has been observed by the 1000 Genomes Project, or was detected in 600 unrelated control exome profiles. The variants were confirmed by Sanger sequencing in the respective probands. Moreover, none of the mutations was present in unaffected parental DNA from leukocytes, consistent with the notion that the disease causing alleles have arisen de novo. Of note, in the affected individual in whom exome sequencing had not revealed any novel variation (GPS-06), Sanger sequencing confirmed the wild-type coding sequence of all 16 coding exons and their associated splice sites of *KAT6B*. Further to this, high-resolution comparative genomic hybridization microarray analysis did not identify any CNVs affecting *KAT6B* or any other genomic abnormalities that could potentially explain the phenotype (data not shown). This individual (GPS-06) has very similar clinical features to the five individuals in whom we identified mutations (Table 1). This case may therefore represent a phenocopy of GPS, be explained by locus heterogeneity in GPS or may arise through an GPS allele comprising a non-coding or undetected coding variant in *KAT6B*.

KAT6B (K(lysine) acetyltransferase 6B, previously known as MORF, QKF, and MYST4) is a member of the MYST family of proteins that are defined by a conserved acetyltransferase domain.[13] Individual members of the MYST family contain additional functional domains, in this regard KAT6B shares structural similarity with KAT6A (previously known as MOZ and MYST3). Both comprise a conserved amino terminus containing an NEMM domain and two consecutive PHD domains. The central portion of these proteins is acidic and contains the MYST domain. The carboxy terminus consists of a serine-rich and methionine-rich domains that are unique to these proteins. Interestingly, the four GPS alleles identified in this study are all predicted to lead to truncation of the *KAT6B* before translation of the serine-rich and methionine-rich domains (Figure 1). To establish if the mutant *KAT6B* alleles generate a stable transcript we Sanger sequenced the *KAT6B* transcript from individual GPS-02, heterozygous for the c.3768_3771del allele, which confirmed the transcription of both mutant and wild-type

Table 1. Major Clinical and Genetic Findings in Six Individuals with Genitopatellar Syndrome

	Individual					
	GPS-01[a]	**GPS-02**	**GPS-03**	**GPS-04**	**GPS-05**	**GPS-06**[a]
KAT6B nucleotide alteration	c.3680_3695del	c.3768_3771del	c.3768_3771del	c.3877A>T	c.3773dup	–
KAT6B amino acid alternation	p.Asp1227Glu*fs*X11	p.Lys1258Gly*fs*X13	p.Lys1258Gly*fs*X13	p.Lys1293X	p.Trp1259ValfsX12	–
Gender	female	female	male	male	female	male
Age at last examination	16 months	7 months	3 months	2 years	25 years	6 months
Genital Anomalies						
Scrotal hypoplasia	NA	NA	+	+	NA	+
Cryptorchidism	NA	NA	+	+	NA	–
Clitoral hypertrophy	+	+	NA	NA	+	NA
Small clitoris	–	–	NA	NA	–	NA
Hypoplastic labia minora	+	–	NA	NA	+	NA
Patellar Malformations						
Absent patellae	+ (right)	+	+	+	+	+
Hypoplastic patellae	+ (left)	–	–	–	–	–
Lower Limb Flexion Deformities						
Flexion contractures of the hips	+	+	–	–	+	+
Flexion contractures of the knees	+	+	+	+	+	+
Club foot	+	+	+	+	–	+
Neurological						
Aplasia of corpus callosum	+	+	+	+	+	+
Severe intellectual disability	+	+	+	+	+	NA
Delayed motor milestones	+	+	+	+	+	+
Craniofacial Features						
Microcephaly	+	+	+	–	+	+
Coarse face	+	+	+	+	+	+
Broad/bulbous nose	+	+	+	+	+	+
Micrognathia	+	+	+	–	+	+
Renal Anomalies						
Hydronephrosis	+	+	+	+	–	+
Multicystic kidneys	–	–	–	–	–	–
Other Anomalies						
Pelvic anomaly	–	+	U	+	+	–
Rib anomaly	–	–	U	–	–	–
Pulmonary hypoplasia	U	U	U	–	–	–
Heart defect	+ (ASD, VSD, PDA)	–	+ (ASD)	+ (PDA)	–	–

The following symbols and abbreviations are used: +, feature present; –, feature absent, NA, not applicable; ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; U, unknown.
[a] Case reports from individuals GPS-01 and GPS-06 presented in Reardon[2] and Brugha et al.,[3] respectively.

alleles (data not shown). We then used a quantitative RT-PCR approach (Kapa Biosystems) performed on a *7900HT* Fast Real-Time PCR System (Life Technologies) to demonstrate that total abundance of *KAT6B* transcript is equivalent to wild-type controls (Figure 2A). We interpreted these findings to be in keeping with the established notion that nonsense mutations in the terminal exon of many genes have reduced capacity to activate the process of nonsense mediated decay.[14] Indeed, we were able to detect a protein of the size of the predicted

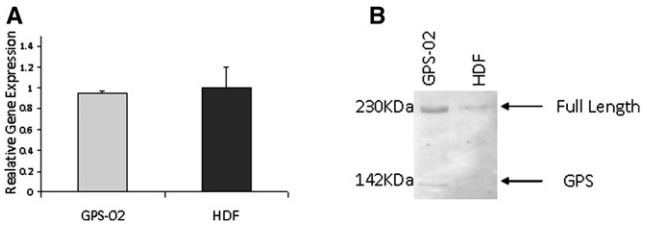

Figure 2. Evaluation of Mutant *KAT6B* Transcript Abundance and Detection of Mutant KAT6B Protein

(A) Assessment of abundance of total KAT6B transcript in primary skin fibroblasts from individual GPS-02 and the average of two unrelated healthy controls (HDF). Error bars represent standard error of the mean of three independent experiments.

(B) Detection of full length and truncated endogenous KAT6B in individual GPS-02 heterozygous for the KAT6B alteration p.Lys1258GlyfsX13.

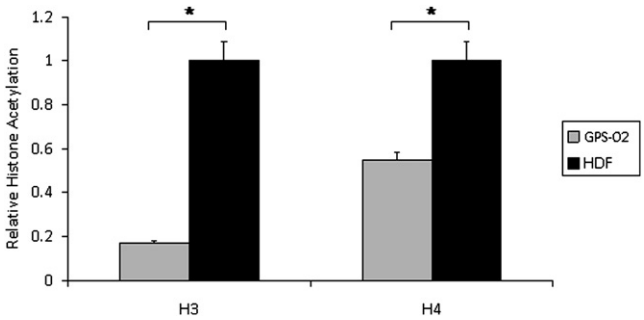

Figure 3. Effect of KAT6B Mutation p.Lys1258GlyfsX13 on H3 and H4 Acetylation

Significant decrease of H3 and H4 acetylation in fibroblasts from individual GPS-02 compared to the average of two HDF (*$p < 0.05$; t test). Error bars represent standard deviation of triplicate measurements.

truncated KAT6B in primary skin fibroblasts from individual GPS-02 heterozygous for the mutation p.Lys1258GlyfsX13 using the ab58823 polyclonal rabbit antibody (1:1,000) (Abcam) (Figure 2B). Taken together, these findings suggest that GPS alleles generate a mature truncated KAT6B protein.

KAT6B is the catalytic subunit of a tetrameric complex that also comprises ING5, EAF6, and one of the members of the bromodomain-PHD finger protein family BRPF.[15] The formation of this complex stimulates the acetylation of E-amino groups on lysine residues which has been principally studied in the context of chromatin modeling. A series of KAT6B deletion constructs have illustrated a direct interaction between the BN domains of the BRPF family with the MYST domain of KAT6B within the molecular complex.[15] The truncated forms of KAT6B observed in this study are predicted to contain the full length MYST domain and are therefore sufficient for complex formation. However, quantitative assessment of global H3/H4 acetylation of extracted histones demonstrated a significant reduction in H3 and H4 acetylation in primary skin fibroblasts from individual GPS-02, heterozygous for the mutation Lys1258GlyfsX13, compared to primary skin fibroblasts from healthy volunteers (EpiQuick Global H3/H4 acetylation assay, performed according to the manufacturer's protocol) (Figure 3). This reduction suggests that histone acetylation activity in affected individuals is compromised. GPS alleles are predicted to encode a protein product that lacks the carboxy-terminal serine-rich and methionine-rich domains. Taken together, these data provide the basis to speculate that the absent domains in the truncated proteins observed in GPS may contribute to the regulation of the acetylation activity of the tetrameric complex. Interestingly, the serine-rich and methionine-rich domains have potent transcriptional activation potential[13] and have been demonstrated to directly interact with the runt domain transcription factor Runx2.[16] The interaction negatively regulates the transcriptional activation potential of these domains. However, the effect of the interaction with RUNX2 on the acetyltransferase activity of KAT6B has not yet been investigated.

Mice homozygous for a hypomorphic genetrap allele of *Myst4* (the mouse ortholog of *KAT6B*) are known as Querkopf mice and have expression levels of *Myst4* that are 5% of the levels in wild-type mice, which leads to severe defects in the cerebral cortex and bone growth development and exhibit facial dysmorphisms.[17] In humans, a chromosomal breakpoint disrupting *KAT6B* has recently been reported in a single individual with short stature, ptosis, and low set ears that are reminiscent of Noonan syndrome although no cardiac anomaly was reported.[18] The disruption of the gene in this individual leads to a null allele resulting in 50% reduction in *KAT6B* expression in the affected individual. The phenotypic features of both the Querkopf mouse and the Noonan-like phenotype have been ascribed to disrupted MAPK signaling via a global dysregulation of histone acetylation through haploinsufficiency of KAT6B.[18] GPS is clearly discrete from the phenotype that results from KAT6B haploinsufficiency and likely results from distinct functional consequences of the GPS alleles that lack the serine-rich and methionine-rich domains. Of additional note, Clayton-Smith and colleagues have very recently identified mutations in *KAT6B* in a series of individuals with the Say-Barber-Biesecker-Young-Simpson variant of Ohdo syndrome, a multisystem developmental disorder associated with severe intellectual disability and a characteristic facial appearance.[19] This form of Ohdo syndrome is phenotypically distinct from GPS and further expands the range of phenotypic expression associated with mutations of this histone acetyltransferase.

In conclusion, we have used whole-exome sequencing and identified de novo mutations of *KAT6B as* the molecular genetic basis of GPS. Our data demonstrate that GPS alleles generate a truncated protein lacking evolutionarily conserved domains and functionally are associated with disruption of histone acetylation. These findings illustrate the critical and complex role of the regulation of histone acetylation during development.

Supplemental Data

Supplemental Data include four tables and can be found with this article online at http://www.cell.com/AJHG/.

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Web Resources

The URLs for data presented herein are as follows:

1000 Genomes Project, http://www.1000genomes.org
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/
Gencode Project, http://www.gencodegenes.org/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org

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