



Loss of *KDM1A* in GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome: a multicentre, retrospective, cohort study

Fanny Chasseloup*, Isabelle Bourdeau*, Antoine Tabarin, Daniela Regazzo, Charles Dumontet, Nataly Ladurelle, Lucie Tosca, Larbi Amazit, Alexis Proust, Raphael Scharfmann, Tiphaine Mignot, Frédéric Fiore, Stylianos Tsagarakis, Dimitra Vassiliadi, Dominique Maiter, Jacques Young, Anne-Lise Lecoq, Vianney Deméocq, Sylvie Salenave, Hervé Lefebvre, Lucie Cloix, Philippe Emy, Rachel Dessailoud, Delphine Vezzosi, Carla Scaroni, Mattia Barbot, Wouter de Herder, François Pattou, Martine Tétreault, Gilles Corbeil, Margot Dupeux, Benoit Lambert, Gérard Tachdjian, Anne Guiochon-Mantel, Isabelle Beau, Philippe Chanson, Say Viengchareun, André Lacroix, Jérôme Bouligand, Peter Kamenický

Summary

Background GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome is caused by aberrant expression of the GIP receptor in adrenal lesions. The bilateral nature of this disease suggests germline genetic predisposition. We aimed to identify the genetic driver event responsible for GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome.

Methods We conducted a multicentre, retrospective, cohort study at endocrine hospitals and university hospitals in France, Canada, Italy, Greece, Belgium, and the Netherlands. We collected blood and adrenal samples from patients who had undergone unilateral or bilateral adrenalectomy for GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome. Adrenal samples from patients with primary bilateral macronodular adrenal hyperplasia who had undergone an adrenalectomy for overt or mild Cushing's syndrome without evidence of food-dependent cortisol production and those with GIP-dependent unilateral adrenocortical adenomas were used as control groups. We performed whole genome, whole exome, and targeted next generation sequencing, and copy number analyses of blood and adrenal DNA from patients with familial or sporadic disease. We performed RNA sequencing on adrenal samples and functional analyses of the identified genetic defect in the human adrenocortical cell line H295R.

Findings 17 patients with GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome were studied. The median age of patients was 43.3 (95% CI 38.8–47.8) years and most patients (15 [88%]) were women. We identified germline heterozygous pathogenic or most likely pathogenic variants in the *KDM1A* gene in all 17 patients. We also identified a recurrent deletion in the short p arm of chromosome 1 harboring the *KDM1A* locus in adrenal lesions of these patients. None of the 29 patients in the control groups had *KDM1A* germline or somatic alterations. Concomitant genetic inactivation of both *KDM1A* alleles resulted in loss of *KDM1A* expression in adrenal lesions. Global gene expression analysis showed GIP receptor upregulation with a log₂ fold change of 7.99 (95% CI 7.34–8.66; $p=4.4 \times 10^{-125}$), and differential regulation of several other G protein-coupled receptors in GIP-dependent primary bilateral macronodular hyperplasia samples compared with control samples. In vitro pharmacological inhibition and inactivation of *KDM1A* by CRISPR-Cas9 genome editing resulted in an increase of GIP receptor transcripts and protein in human adrenocortical H295R cells.

Interpretation We propose that GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome results from a two-hit inactivation of *KDM1A*, consistent with the tumour suppressor gene model of tumorigenesis. Genetic testing and counselling should be offered to these patients and their relatives.

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Introduction

Primary bilateral macronodular adrenal hyperplasia is a rare cause of pituitary adrenocorticotrophic hormone-independent cortisol excess with Cushing's syndrome.¹ Germline and secondary somatic inactivating mutations in the *ARMC5* gene are the most frequent underlying genetic cause in around a third of patients

with primary bilateral macronodular adrenal hyperplasia.^{2,3}

In a large proportion of patients with primary bilateral macronodular adrenal hyperplasia and less frequently in those with unilateral adenoma, cortisol excess is also driven by aberrant (ectopic or excessive) expression of several G protein-coupled receptors in adrenal lesions.⁴

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*Co-first authors

Université Paris-Saclay, INSERM, Physiologie et Physiopathologie Endocrinienne, Le Kremlin-Bicêtre, France (F Chasseloup MD, N Ladurelle PhD, L Amazit PhD, T Mignot MSc, Prof J Young PhD, A-L Lecoq PhD, V Deméocq MD, S Salenave MD, Prof A Guiochon-Mantel PhD, I Beau PhD, Prof P Chanson MD, S Viengchareun PhD, J Bouligand PhD, Prof P Kamenický PhD); Division of Endocrinology, Department of Medicine and Research Center (Prof I Bourdeau MD, G Corbeil BSc, Prof A Lacroix MD) and Department of Neurosciences (M Tétreault PhD), Centre hospitalier de l'Université de Montréal, Montréal, QC, Canada; Department of Endocrinology, Diabetes, and Nutrition, Hôpital Haut Lévêque, Centre Hospitalier Universitaire de Bordeaux, Pessac, France (Prof A Tabarin MD); Endocrinology Unit, Department of Medicine (D Regazzo PhD, Prof C Scaroni MD) and Department of Neuroscience (M Barbot PhD), Hospital-University of Padua, Padua, Italy; Université Claude Bernard Lyon 1, UMR INSERM 1052, CNRS 5286, Centre de Recherche de Cancérologie de Lyon, Lyon, France (Prof C Dumontet PhD); Service d'Histologie, Embryologie et Cytogénétique, Assistance

Publique-Hôpitaux de Paris, Hôpital Antoine Bécère, Clamart, France (L Tosca PhD, Prof G Tachdjian PhD); Service d'Anatomie et Cytologie Pathologiques (M Dupeux MD), Service de Chirurgie Digestive et Endocrinienne (B Lambert MD), Service de Génétique Moléculaire et d'Hormonologie (A Proust MSc, Prof A Guiochon-Mantel, J Bouligand), and Service d'Endocrinologie et des Maladies de la Reproduction (Prof J Young, A-L Lecoq, S Salenave MD, Prof P Chanson, Prof P Kamenický), Hôpital Bicêtre, Le Kremlin-Bicêtre, France; UMS 44, Institut Biomédical du Val de Bièvre, Le Kremlin-Bicêtre, France (L Amazit); U1016 INSERM, Institut Cochin, Paris, France (R Scharfmann PhD); US12 Centre d'immunophénomique, Parc Scientifique et Technologique de Luminy, Marseille, France (F Fiore PhD); Department of Endocrinology, Diabetes, and Metabolism, Evangelismos Hospital, Athens, Greece (Prof S Tsagarakis PhD, D Vassiliadi MD); Department of Endocrinology and Nutrition, Université catholique de Louvain, Cliniques Universitaires Saint-Luc, Brussels, Belgium (Prof D Maiter PhD); Department of Endocrinology, Diabetes and Metabolic Diseases, Normandie Univ, Rouen University Hospital, Rouen, France (Prof H Lefebvre PhD); CHR Orleans, Service d'Endocrinologie, Diabète et Nutrition, Orleans, France (L Cloix PhD, P Emy MD); Department of Endocrinology, Diabetes, and Nutrition, and PériTox, UMR-I 01 INERIS, Université de Picardie Jules Verne, Amiens, France (Prof R Dessailoud PhD); Service d'Endocrinologie, Hôpital Larrey, Toulouse, France (Prof D Vezzosi PhD); Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, Netherlands (Prof W de Herder PhD); Service de Chirurgie Générale et Endocrinienne, Univ Lille, Institut Pasteur de Lille, INSERM U1190, Translational Research Laboratory for Diabetes, CHU Lille, Lille, France (F Pattou MD)

Research in context

Evidence before this study

No systematic literature search was done. Relevant articles were cited on the basis of the authors' knowledge of the scientific literature available on PubMed from database inception to June 26, 2021. Since the initial description of sporadic cases of glucose-dependent insulinotropic polypeptide (GIP)-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome nearly 30 years ago, the underlying mechanism leading to ectopic or aberrant GIP receptor expression in the adrenal tissue remained unknown. Several other aberrant G-protein coupled receptors and ligands, such as corticotropin, were found to regulate development and steroidogenesis in primary bilateral macronodular adrenal hyperplasia. The bilateral character of the disease suggested a genetic predisposition, and germline and biallelic pathogenic variants of *ARMC5* were identified in approximately 25% of patients with primary bilateral macronodular adrenal hyperplasia. However, no cases of GIP-dependent primary bilateral macronodular adrenal hyperplasia were found to harbour *ARMC5* mutations. By contrast, in two patients with GIP-dependent unilateral cortisol-secreting adenoma, GIP receptor overexpression resulted from somatic 19q13-32 microduplications containing the *GIP* receptor locus, rearranged with other chromosomal regions.

Added value of this study

In this multicentre, retrospective, cohort study, we performed sequencing of germline and adrenal DNA derived from 17 patients with GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome. We found that familial and sporadic GIP-dependent primary bilateral

ectopic expression of the glucose-dependent insulinotropic polypeptide (GIP) receptor in primary bilateral macronodular adrenal hyperplasia is associated with abnormal circadian cortisol rhythm with low fasting morning plasma cortisol concentrations, which increase after food intake.^{5,6} The postprandial rise of cortisol secretion is induced by GIP, an incretin produced by intestinal K cells following oral lipid, carbohydrate, or protein intake. Activation of the ectopic GIP receptor, functionally coupled to cAMP signalling, triggers adrenal cell proliferation and excessive steroid production. The molecular events leading to ectopic GIP receptor expression in the adrenocortical tissue are not well understood. In 2017, we reported somatic 19q13-32 microduplications containing the *GIP* receptor locus rearranged with other chromosomal regions in cortisol-secreting adenomas from two patients with GIP-dependent Cushing's syndrome.⁷ However, the molecular pathogenesis of ectopic GIP receptor expression in patients with primary bilateral macronodular adrenal hyperplasia remains unknown.

macronodular adrenal hyperplasia is a genetic disease caused by germline inactivating pathogenic variants of the lysine demethylase 1A (*KDM1A*) with loss of heterozygosity of the second *KDM1A* locus in adrenal lesions. This stepwise inactivation of *KDM1A* is suggestive of a tumour suppressor gene model of tumourigenesis. RNA sequencing revealed the global effect of *KDM1A* loss in adrenal tissue on gene transcription and identified differentially regulated genes, including those encoding for GIP receptor and several other G protein-coupled receptors that might be involved in adrenal tumourigenesis and complex regulation of steroidogenesis. Functional *in vitro* studies in human adrenocortical and pancreatic β cells further showed the link between *KDM1A* and ectopic and physiological GIP receptor expression.

Implications of all the available evidence

Uncovering a common genetic mechanism of GIP-dependent primary bilateral macronodular adrenal hyperplasia represents a substantial advancement in the field of adrenal Cushing's syndrome. This finding will enable genetic testing and counselling of patients and earlier detection of the disease, which is important because *KDM1A* pathogenic variants predispose to myelomas or monoclonal gammopathy of undetermined significance. Further, this novel role of *KDM1A* as an epigenetic regulator of GIP receptor expression and that of several other G protein-coupled receptors can have pharmacological implications. Targeting *KDM1A* by inhibitors could possibly be applied beyond the field of adrenal hyperplasia—eg, in the field of endocrine and metabolic diseases—and warrants further investigation.

The bilateral nature of this primary adrenal disease suggests germline genetic predisposition; however, no familial forms of GIP-dependent Cushing's syndrome have been reported to date. The aim of this study was to identify the genetic driver event responsible for GIP-dependent primary bilateral macronodular adrenal hyperplasia and Cushing's syndrome.

Methods

Study design

We conducted a multicentre, retrospective, cohort study at endocrine hospitals and university hospitals in France, Canada, Italy, Greece, Belgium, and the Netherlands.

The patient cohort was constituted by collaborating with several endocrinology experts in adrenal pathologies, who conduct follow-up or reported patients with GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome. All patients gave written informed consent for genetic analyses. The study was approved by local ethics committees. Tissue sample collection was approved by the Comité de Protection des Personnes Ile de France V (number 2020-A02899-30).

Patients

We collected blood and adrenal samples from patients who had undergone unilateral or bilateral adrenalectomy for GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome. The diagnosis was done according to standard criteria showing hypercortisolism¹ in combination with low fasting morning plasma cortisol and adrenocorticotropic hormone concentrations and a meal-induced increase of plasma cortisol concentration of more than 50% from baseline (or following other tests including oral glucose tolerance test and GIP infusion). The histological features of adrenal lesions were examined by an experienced pathologist. Adrenal samples from patients with primary bilateral macronodular adrenal hyperplasia who had undergone an adrenalectomy for overt or mild Cushing's syndrome without evidence of food-dependent cortisol production and those with GIP-dependent unilateral adrenocortical adenomas were used as control groups.

Procedures

Whole genome sequencing was performed by IntegraGen (Evry, France). PCR duplicates were removed from the analysis after alignment using the Sambamba tool. The mean coverage was 45X with 84% of the nucleotide bases being covered at 25X or higher. Variant calling and annotations were made using the Genome Analysis Toolkit (HaplotypeCaller GVCF tool [version 3.7] for germline DNA and MuTect tool [version 2.0] for somatic DNA). We excluded variants with a minor allele frequency greater than 2% using the Genome Aggregation Database (gnomAD) or the 1000 Genomes Project database. The whole genome sequencing data was further filtered to keep protein damaging variants (nonsense, missense, frameshift, indel, and splice variants).

Whole exome sequencing was performed at the Genome Quebec Expertise and Service Center (Montréal, QC, Canada). Quality of the DNA sample and libraries were assessed using a bioanalyser before sequencing. PCR duplicates were removed from analysis after alignment. The mean coverage was 195X with 89% of the bases being covered at 50X or higher. We excluded variants with minor allele frequencies greater than 1% using the 1000 Genomes Project database, 6500 NHLBI Exome Variant Server, and gnomAD, which were observed in more than 30 samples from our in-house database (containing approximately 2000 samples). Whole exome sequencing data were further filtered to retain protein damaging variants.

Targeted next generation sequencing (NGS) was used to sequence somatic DNA with high resolution from all patients and germline DNA with a panel of genes involved in adrenal diseases (including *ARMC5*, *PRKACA*, *PRKACB*, *PRKARIA*, *GNAS*, *MEN1*, and *FH*). The panel also included members of the lysine demethylase family involved in human tumorigenesis (*KDM1A*, *KDM3A*, *JMJD2A*, *JMJD2B*, *JMJD3*, *KDM6A*,

and *KDM5B*).⁸ PCR duplicates were marked with Picardmetrics. The mean coverage was 286X with 95% of the bases being covered at 20X. To investigate genomic copy number alterations on the basis of targeted NGS data, we used Python (version 2.7) to compare the depth of sample coverage between patients and control groups.

Standard quality control analysis for whole genome, whole exome, and targeted NGS were done using FastQC before alignment.

Oligonucleotide array comparative genomic hybridisation (array CGH) analysis was used to analyse genomic imbalances using 180 K or 400 K oligonucleotide arrays (Agilent Technologies, Les Ulis, France). Copy number alterations were considered of importance when they could be defined by three or more oligonucleotides spanning at least 15 kilobases (kb) for 180 K and 35 Kb for 400 K arrays and were not identified in the Database of Genomic Variants. The Genome Browser used to analyse gene content was Hg19, Build37.

RNA sequencing was performed by the iGenSeq platform (Paris Brain Institute, Paris, France). RNA library preparation was done according to manufacturer recommendations (stranded total RNA prep with Ribo-Zero Plus [Illumina, Paris, France]). The final pooled library of samples were sequenced using NovaSeq 6000 with SP Reagent Kit (300 cycles [Illumina, Paris, France]; 2×800 million of 150 base reads; corresponding to 2×30 million reads per sample after demultiplexing). Poor quality sequences were trimmed or removed with the FASTQ tool to retain good quality paired reads. The gencode (version 32.0) annotation GTF file was used for mapping on the Hg38 reference genome and quantification with Illumina DRAGEN Bio-IT Platform (version 3.7.5). More detail on sequencing techniques is provided in the appendix (p 2).

Western Blotting was performed on adrenal protein extracts obtained with a TissueLyser (Qiagen, Les Ulis, France; appendix p 4). Immunohistochemistry was performed on paraffin-embedded sections (4–5 µm thick) with a standardised protocol and counterstained with haematoxylin. Immunohistochemical staining to detect KDM1A was performed using the streptavidin-biotin peroxidase technique. Primary antiserum directed against KDM1A was used at the 1:50 dilution (Abcam, Paris, France; ab129195).

Functional in-vitro studies were performed using the human adrenocortical cell line H295R and the human pancreatic β cell line EndoC-βH1. Cell culture details are described in the appendix (p 3). Cells were incubated for up to 10 days with 0.5 µM GSK-LSD1, an irreversible inhibitor of KDM1A (Sigma-Aldrich, Saint-Quentin-Fallavier, France). Transfection of siRNAs was performed using lipofectamine RNAiMAX reagent (Life Technologies, Villebon-sur-Yvette, France; appendix p 3). Expression of studied genes was measured using quantitative RT-PCR (RT-qPCR). RNA extraction from cell cultures and tissue and RT-qPCR protocols are listed in the appendix (pp 3–4).

Correspondence to:
Prof Peter Kamenický,
INSERM U1185, Faculté de
Médecine Paris-Saclay,
94276 Le Kremlin-Bicêtre, France
peter.kamenicky@universite-paris-saclay.fr

For the **Database of Genomic Variants** see <http://projects.tcag.ca/cgi-bin/variation/gbrowse/hg19>

For the **Genome Browser** see <http://genome.ucsc.edu/>

For the **Genome Aggregation Database** see <https://gnomad.broadinstitute.org>

For the **1000 Genomes Project** see <https://www.internationalgenome.org/>

See **Online** for appendix

For the **6500 NHLBI Exome Variant Server** see <http://evs.gs.washington.edu/EVS>

Indirect immunofluorescence and high throughput automated microscopy was used to quantify protein expression (appendix pp 4–5).

CRISPR-Cas9 genome editing was performed by the Genetic Engineering and Mouse Transgenesis Department US12 Ciphe (Marseille, France). Single or multiple biallelic null mutations were introduced into a bulk or independent clones of H295R cells. Specific oligonucleotide sequences of sgRNA were chosen to minimise the likelihood of off-target cleavage using the publicly available online tool. Details on protocol and sgRNA specific oligonucleotide sequences are provided in the appendix (pp 3, 11).

Statistical analysis

No sample size calculations were done and all available patients with GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome were included. RNA sequencing analyses were conducted with R software (version 4.0.3). Data were normalised with DESeq2 (version 1.26.0) Bioconductor packages before differential analysis was done using a generalised linear model framework likelihood ratio test. To account for the multiple hypotheses being tested, the Benjamin-Hochberg procedure was applied to calculate the false discovery rate. Results are presented in a volcano plot. Differentially expressed genes are also visualised on a heatmap and clustered using complete-linkage hierarchical clustering on the basis of Euclidean distances. Enrichment analysis was done with the clusterProfiler R package (version 3.14.3). In vitro experiments were analysed with Prism (version 9.0.0) GraphPad software. Normal distribution was assessed using the Shapiro–Wilk test. Data are expressed as mean and SEM. A non-parametric Mann-Whitney *U* test or a parametric unpaired *t* test was used to compare two experimental conditions. When more than two conditions were compared, we used ANOVA Kruskal–Wallis test followed by Dunn's multiple comparison test. For repeated experiments we calculated adjusted *p* values using the Bonferroni correction to account for the total number of experiments. *P* values or adjusted *p* values of less than 0.05 were considered statistically significant.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

17 patients diagnosed with GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome were studied (table). The median age of patients was 43.3 (95% CI 38.8–47.8) years and most patients (15 [88%]) were women. Ectopic GIP receptor expression in adrenal lesions was confirmed by RNA sequencing (appendix p 6). Three (18%) patients

belonged to two unrelated families with GIP-dependent Cushing's syndrome diagnosed in two members (figure 1A, B), and 14 (82%) of 17 patients apparently had sporadic disease. Patient 10 (III) from figure 1A is herein referred to as patient 1, and patient 1 (II) and 1 (I) from figure 1B are herein referred to as patients 2 and 3. Two members in family 1 presented with GIP-dependent Cushing's syndrome (figure 1A). Screening of four clinically unaffected *KDM1A* pathogenic variant carriers in family 1 did not detect any biochemical abnormalities at younger ages (age 29–62 years; appendix p 9). Family medical history also included three patients with multiple myeloma, two with monoclonal gammopathy of undetermined significance, one with rectal cancer at age 35 years, and one with a bronchial neuroendocrine tumour diagnosed at age 80 years (figure 1A). Two members of family 2 presented with GIP-dependent Cushing's syndrome (figure 1B). No additional neoplasia was reported in this family. Among 14 patients with apparently sporadic disease, ten were previously reported.^{8–11} Patient 4 (table) had a daughter presenting with craniopharyngioma in infancy and a first-degree woman cousin who underwent unilateral adrenalectomy for an adrenal mass. Patient 10 (table) had breast cancer at age 42 years, patient 11 (table) had a mother and a sister presenting with breast cancer, and patient 12 (table) had a brother with Hodgkin lymphoma. Patient 13 (table) had a daughter who underwent unilateral adrenalectomy for adrenocortical adenoma with Cushing's syndrome, without evidence of food-induced cortisol production. Four patients (patients 1, 3, 9, and 13) had foci of myelolipoma within adrenal lesions defined by the presence of all three haematopoietic lineages. Three patients (patients 2, 4, and 6) presented with myeloid metaplasia (involving one or two haematopoietic lineages) in adrenal glands.

25 patients with primary bilateral macronodular adrenal hyperplasia who underwent unilateral or bilateral adrenalectomy for mild or overt Cushing's syndrome without evidence of food-dependent cortisol production were included as a control group (appendix p 7). Two of 25 patients were members of a family with *ARMC5* mutations and presented with β -adrenergic and vasopressin responsive Cushing's syndrome without food-dependent cortisol secretion.^{12,13} The other 23 patients with primary bilateral macronodular adrenal hyperplasia in the control group were apparently sporadic, including one with luteinising hormone responsive Cushing's syndrome.¹¹ We also studied four patients who underwent unilateral adrenalectomy for GIP-dependent adrenocortical adenomas, including three with Cushing's syndrome and one with primary aldosteronism (appendix p 7). Three of these patients have previously been reported.⁷

Array CGH and targeted NGS were used to map DNA copy number alterations in primary bilateral macronodular adrenal hyperplasia samples derived from

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<http://crispor.tefor.net>

	Sex	Age, years	Adrenal histology	24 h urinary free cortisol, µg per 24 h	Morning plasma corticotropin, pg/mL	Morning plasma cortisol, µg/dL	Post-prandial plasma cortisol, µg/dL	References (patient reported)
Patient 1	Man	54	Primary bilateral macronodular adrenal hyperplasia and myelolipoma	294	<1	9	33	Unpublished
Patient 2	Woman	42	Primary bilateral macronodular adrenal hyperplasia	463	<1	5	35	Lecoq et al ⁷ (patient 13)
Patient 3	Woman	47	Primary bilateral macronodular adrenal hyperplasia and myelolipoma	512	2	7	23	Unpublished
Patient 4	Woman	55	Primary bilateral macronodular adrenal hyperplasia	43	<15	3	28	Lecoq et al ⁷ (patient 7)
Patient 5	Man	35	Primary bilateral macronodular adrenal hyperplasia	372	15	5	27	Lecoq et al ⁷ (patient 8)
Patient 6	Woman	43	Primary bilateral macronodular adrenal hyperplasia	213	<1	5	26	Lecoq et al ⁷ (patient 12)
Patient 7	Woman	43	Primary bilateral macronodular adrenal hyperplasia	230	5	15	40	Lecoq et al ⁷ (patient 9)
Patient 8	Woman	30	Primary bilateral macronodular adrenal hyperplasia	450	5	9	25	Unpublished
Patient 9	Woman	61	Primary bilateral macronodular adrenal hyperplasia and myelolipoma	319	<1	8	38	Larose et al ⁹
Patient 10	Woman	33	Primary bilateral macronodular adrenal hyperplasia	390	1	10	36	Lecoq et al ⁷ (patient 6)
Patient 11	Woman	45	Primary bilateral macronodular adrenal hyperplasia	525	<5	6	35	Lecoq et al ⁷ (patient 10)
Patient 12	Woman	54	Primary bilateral macronodular adrenal hyperplasia	707	<5	16	76	Lecoq et al ⁷ (patient 11)
Patient 13	Woman	42	Primary bilateral macronodular adrenal hyperplasia and myelolipoma	6*	8	3	22	Unpublished
Patient 14	Woman	41	Primary bilateral macronodular adrenal hyperplasia	7*	<5	7	22	Unpublished
Patient 15	Woman	34	Unknown	5*	<5	6	25	Unpublished
Patient 16	Woman	35	Primary bilateral macronodular adrenal hyperplasia	8*	<5	7	20	Albiger et al ¹⁰ (patient 1)
Patient 17	Woman	42	Primary bilateral macronodular adrenal hyperplasia	498	4	6	27	Karapanou et al ¹¹ (patient 1)

Patient 1 is shown as patient 10 (III) in figure 1A and patients 2 and 3 are shown as patients 1 (II) and 1 (I) in figure 1B. *Upper limit of normal. Conversions to International System of Units: µg per 24 h is equivalent to 2.76 nmol/L, pg/mL is equivalent to 0.22 pmol/L; µg/dL is equivalent to 27.6 nmol/L.

Table: Patient characteristics

16 (94%) of 17 patients with GIP-dependent Cushing's syndrome. One somatic sample (from patient 16) was not available for analysis of copy number alterations because of insufficient DNA quality. Low copy number alterations were detected in these samples. However, we identified a recurrent deletion of the short p arm of chromosome 1 in the adrenal specimens of nine patients (figure 1C; appendix p 8). Targeted NGS identified microdeletions of the *KDM1A* locus in three patients. Samples from four patients (figure 1C) did not contain any copy number alterations of the *KDM1A* locus.

Whole genome sequencing from adrenal lesions and germline DNA derived from the index patient of family 1 (figure 1A) showed pathogenic variants affecting the coding sequence of 69 candidate genes common in all three samples (left adrenal, right adrenal, and germline DNA). Cross referencing the whole genome sequencing analysis with the recurrent 1p deletion pointed to three candidate genes. In particular, we identified a

heterozygous germline, frameshift, pathogenic variant leading to a premature stop codon in exon 16 of *KDM1A* (NM_001009999.3; figure 1D), located on chromosome 1. This variant was not reported in gnomAD or in the 1000 Genomes Project databases. In parallel, whole exome sequencing was performed on germline DNA from the apparently sporadic patient 9 and revealed a heterozygous germline nonsense variant in exon 6 of *KDM1A* (figure 1D).

Systematic analysis using targeted NGS of adrenal and germline DNA from patients with GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome identified *KDM1A* pathogenic or most likely pathogenic variants in all 17 patients. These variants included five frameshift, five nonsense, two missense mutations (affecting highly conserved aminoamides located in α helices), one in-frame deletion (located in a β -pleated sheet), and one splicing variant (figure 1C, D; appendix p 9). 11 variants were considered pathogenic and

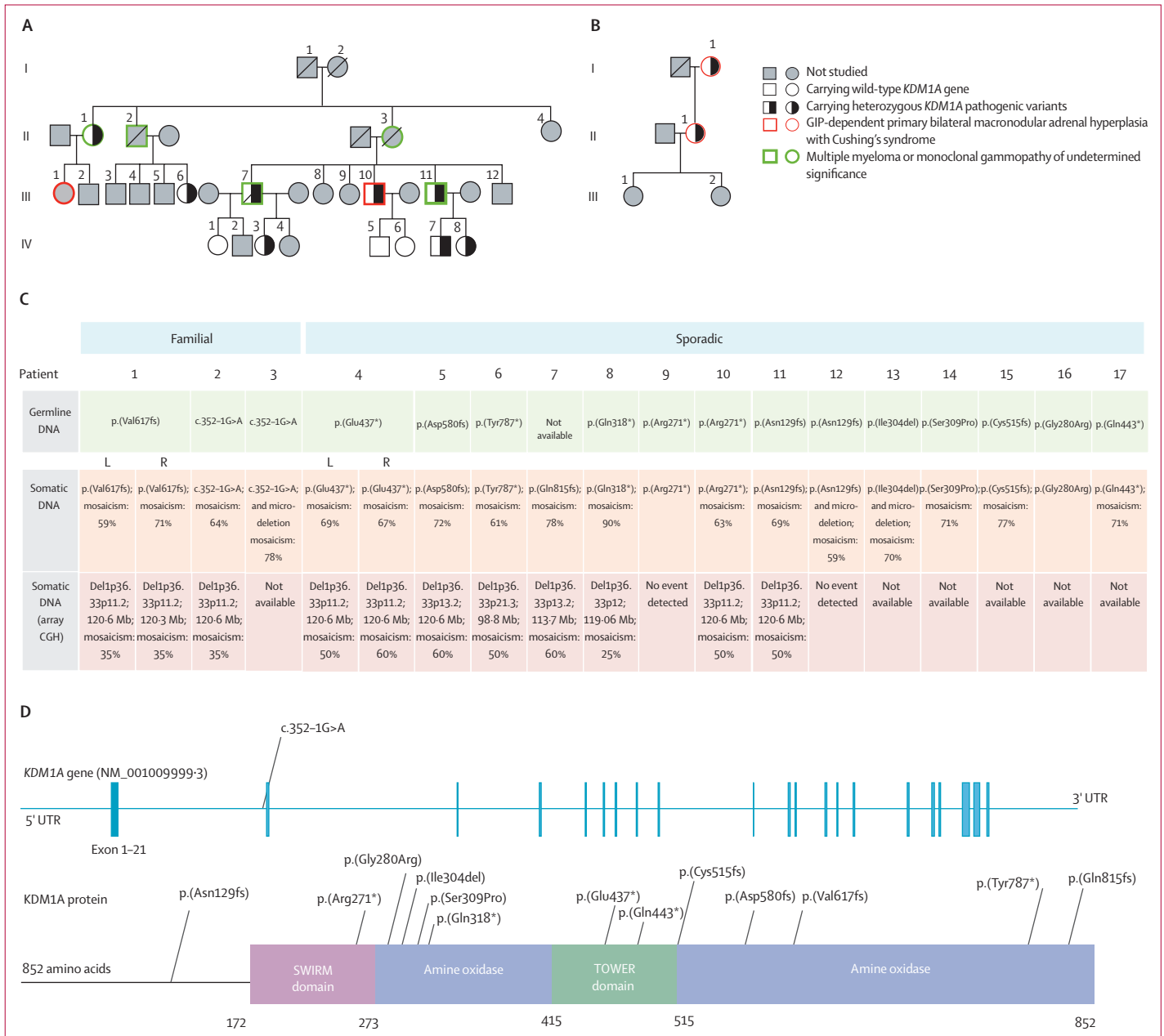


Figure 1: Germline and adrenal genetic *KDM1A* alterations in patients with familial and sporadic GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome Circles represent women and squares represent men. Crossed out symbols represent deceased individuals. (A) Pedigree chart of family 1. Patient 10 (III) from figure 1A is referred to as patient 1 in this Article. (B) Pedigree chart of family 2. Patient 1 (II) and 1 (I) are referred to as patients 2 and 3 in this Article. (C) *KDM1A* genetic alterations detected in germline DNA (green boxes) and somatic DNA from adrenal lesions (orange and red boxes) of 17 patients with GIP-dependent primary bilateral macronodular adrenal hyperplasia. Loss of heterozygosity in chromosome 1p or microdeletion of the *KDM1A* locus, identified by array CGH or targeted NGS. Full information about chromosome position is shown in the appendix (p 9). (D) *KDM1A* gene and protein structure. CGH=comparative genomic hybridisation. GIP=glucose-dependent insulinotropic polypeptide. NGS=next generation sequencing. UTR=untranslated region.

three most likely pathogenic according to the American College of Medical Genetics classification and were not reported in gnomAD or the 1000 Genomes Project databases. Two patients with apparently sporadic disease (patients 11 and 12) from the same region in France carried the same frameshift variant, hence we infer that these people share a common ancestor. Finally, no pathogenic

variants were identified in the *ARMC5* gene, other known genes involved in adrenal tumorigenesis, or other histone demethylases genes in these patients.

Targeted exome sequencing of control samples from patients with non-GIP-dependent primary bilateral macronodular adrenal hyperplasia did not show pathogenic variants in *KDM1A* or copy number alterations of the locus

(appendix p 8). This analysis identified *ARMC5* pathogenic variants in six (24%) of 25 control samples. None of the four patients with GIP-dependent adrenocortical adenomas harboured *KDM1A* pathogenic variants.

Western blot analysis showed loss of expression of *KDM1A* protein in primary bilateral macronodular adrenal hyperplasia samples derived from patients with GIP-dependent Cushing's syndrome (figure 2A), whereas *KDM1A* protein was detected in control primary bilateral macronodular adrenal hyperplasia samples. Immunohistochemical analysis showed the absence of nuclear *KDM1A* staining in primary bilateral macronodular adrenal hyperplasia samples from patients with GIP-dependent Cushing's syndrome in adrenal cells and in the foci of myelolipoma (figure 2B). Thus, germline inactivating variants of *KDM1A* in combination with 1p loss of heterozygosity result in functional loss of *KDM1A* in the adrenal lesions of affected patients.

KDM1A is a transcriptional repressor that regulates gene expression by demethylating histone H3 on lysine 4. To characterise transcriptional consequences of the loss of *KDM1A* in adrenal lesions, we performed a global gene expression analysis using RNA sequencing. 30 adrenal samples were studied, 14 (47%) samples from patients with GIP-dependent primary bilateral macronodular adrenal hyperplasia had *KDM1A* pathogenic variants and 16 (53%) control samples from patients with non-GIP-dependent primary bilateral macronodular adrenal hyperplasia did not have *KDM1A* defects variants, among which six (20%) had *ARMC5* pathogenic variants. Unsupervised hierarchical clustering analysis (figure 3A) showed separate clustering of samples with *KDM1A* inactivation, with *ARMC5* pathogenic variants, and samples without identified pathogenic variants. In samples with *KDM1A* inactivation, 1488 genes were overexpressed, and 1217 genes were downregulated (figure 3B). Among these differentially regulated genes, the GIP receptor was upregulated with a log₂ fold change of 7.99 (95% CI 7.34 to 8.66; $p=4.4 \times 10^{-125}$) and *KDM1A* was downregulated with a log₂ fold change of -1.25 (-1.53 to -0.97 ; $p=1.8 \times 10^{-18}$). *KDM1A* loss in primary bilateral macronodular adrenal hyperplasia samples was further associated with gene overexpression of luteinising hormone and choriogonadotropin receptor (LHCGR; log₂ fold change 2.41, 1.13 to 3.70; $p=0.00024$), angiotensin II type 1 receptor (log₂ fold change 1.08, 0.50 to 1.67; $p=0.00028$), and the KISS1 receptor (log₂ fold change 6.06, 5.10 to 7.01; $p=1.6 \times 10^{-35}$). Several G protein-coupled receptors associated with primary bilateral macronodular adrenal hyperplasia were downregulated, in particular, arginine vasopressin receptor 1A (log₂ fold change -2.66 , -3.51 to -1.82 ; $p=6.1 \times 10^{-10}$), 5-hydroxytryptamine receptor 4 (log₂ fold change -3.44 , -5.23 to -1.66 ; $p=0.00016$), and $\beta 1$ adrenoceptor (log₂ fold change -1.29 , -2.02 to -0.55 ; $p=0.00059$). Expression of melanocortin receptor 2 was

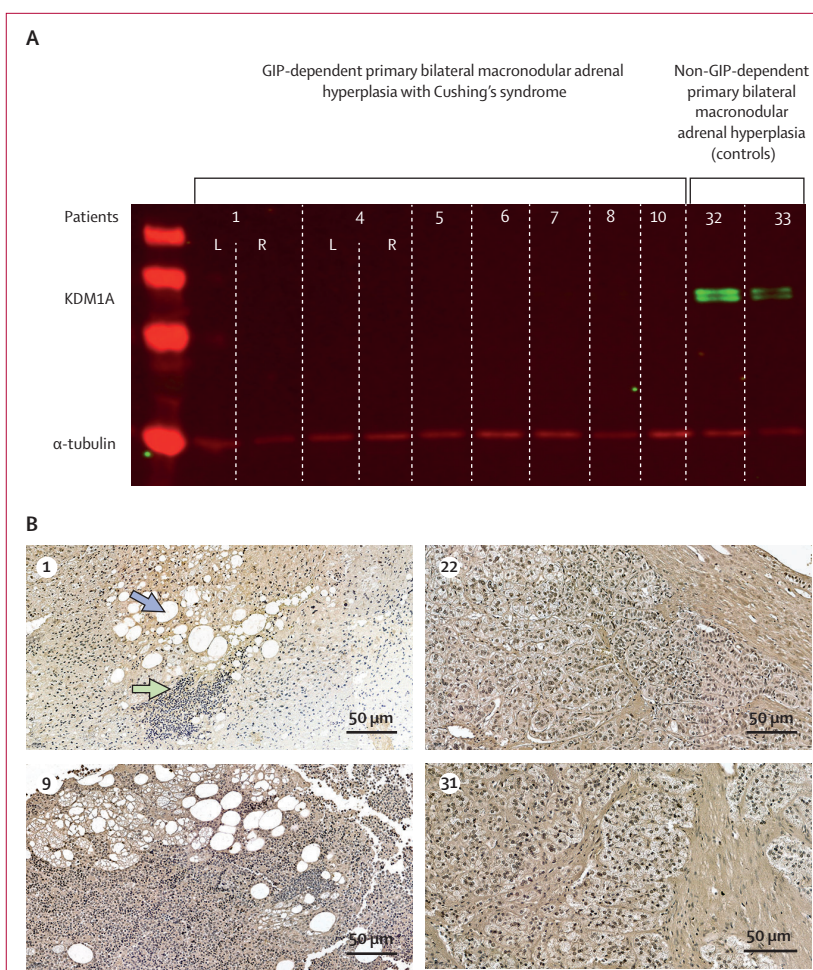


Figure 2: *KDM1A* protein expression in primary bilateral macronodular adrenal hyperplasia
(A) Western blot analysis of protein extract from human adrenal tissue, showing *KDM1A* (green) and α -tubulin control (red). Tissue extracted from left (L) and right (R) adrenal lesions. (B) Immunohistochemistry analysis of *KDM1A* expression in adrenal lesions from patients with GIP-dependent primary bilateral macronodular adrenal hyperplasia (patients 1 and 9; table) and those with non-GIP-dependent primary bilateral macronodular adrenal hyperplasia (patients 22 and 31; control group; appendix p 7). Counterstaining was performed using haematoxylin. Myeloid cells (green arrow) and adipocytes (blue arrow) are shown. GIP=glucose-dependent insulinotropic polypeptide.

also decreased (log fold change -2.79 , -4.06 to -1.52 ; $p=0.000016$; figure 3B; appendix p 6). Gene set enrichment analysis identified differentially regulated pathways in primary bilateral macronodular adrenal hyperplasia samples with loss of *KDM1A* (figure 3C; appendix p 11), including transmembrane transporter activity genes and G protein coupled receptors ($p<0.0001$; $q=0.0287$).

In-vitro experiments in the human adrenocortical cell line H295R confirmed the functional consequence of loss of *KDM1A* on GIP receptor expression (figure 4). At baseline, the relative abundance of GIP receptor transcripts in H295R cells quantified by RT-qPCR was low, estimated at 26 molecules per μg of RNA, which corresponds to levels of GIP receptor expression in healthy adrenal tissue samples.⁷ Treatment of H295R

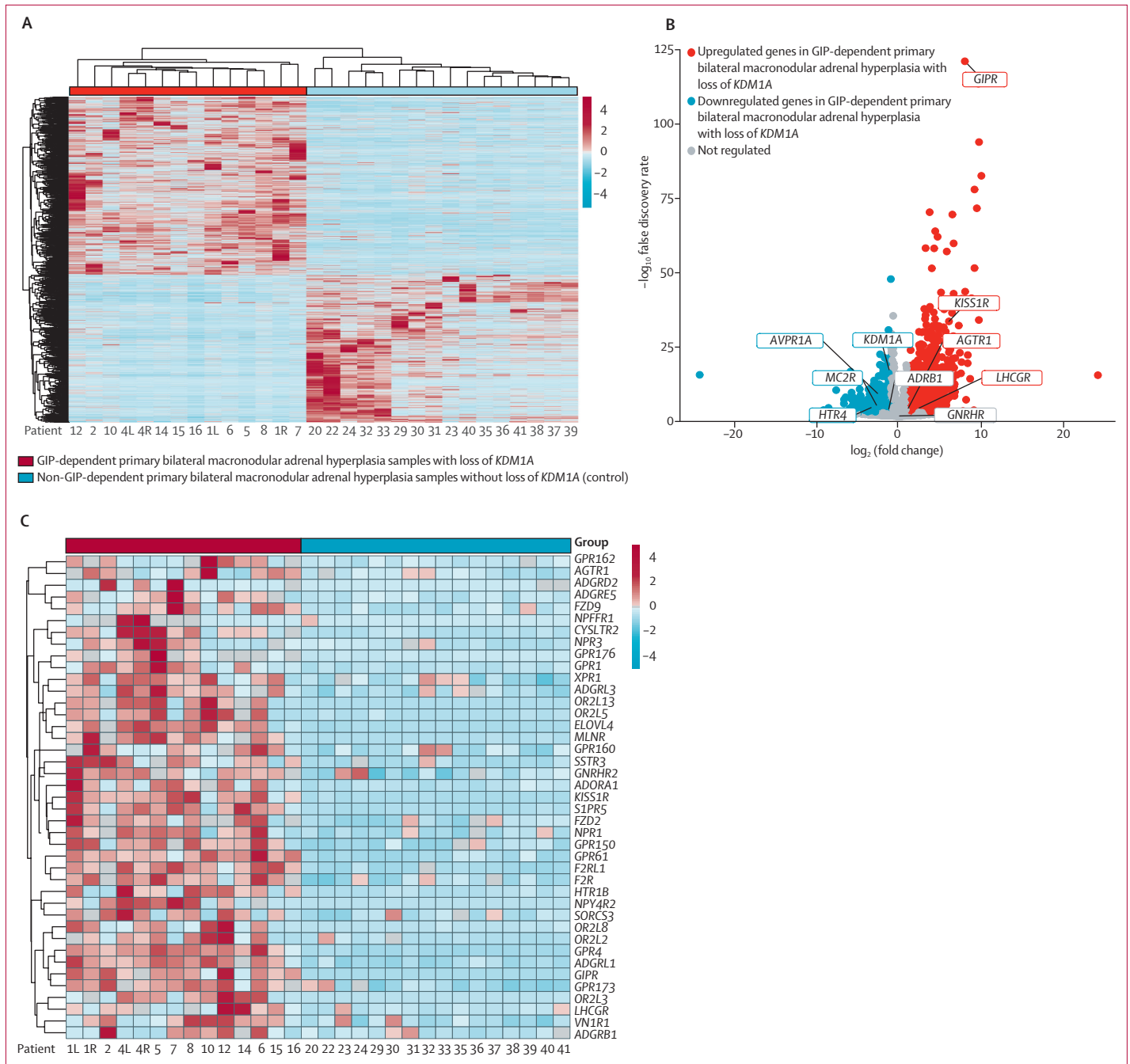


Figure 3: Global gene expression analysis in primary bilateral macronodular adrenal hyperplasia
 Tissue extracted from left (L) and right (R) adrenal lesions. (A) Unsupervised hierarchical clustering analysis of 30 samples from 28 patients; samples harboring *KDM1A* pathogenic variants (n=14) and samples without *KDM1A* variants (n=16) formed two separate clusters. Among control samples, those positive for the *ARMC5* mutation (patients 20, 22, 24, 32, 33, and 41) formed a distinct subgroup. (B) Volcano plot of differentially expressed genes between samples with and without *KDM1A* inactivation. Red dots represent upregulated genes in GIP-dependent primary bilateral macronodular adrenal hyperplasia samples with loss of *KDM1A*. Blue dots are downregulated genes, according to a log₂ fold change threshold of 1 and a false discovery rate threshold of 0.05. (C) Clustering based on differential gene expression of G-protein coupled receptor activity. Primary bilateral macronodular adrenal hyperplasia samples with and without *KDM1A* inactivation formed two separate clusters.

cells with GSK-LSD1, a selective and non-reversible inhibitor of *KDM1A* resulted in increased GIP receptor expression at 3 days (adjusted p=0.0207) and 7 days (adjusted p=0.0246; figure 4A). Furthermore, inactivation

of *KDM1A* by CRISPR-Cas9 genome editing resulted in a 92% reduction of *KDM1A* protein expression, which increased GIP receptor expression, as quantified by RNA sequencing (log₂ fold change of 2.56, 95% CI 0.85–4.27;

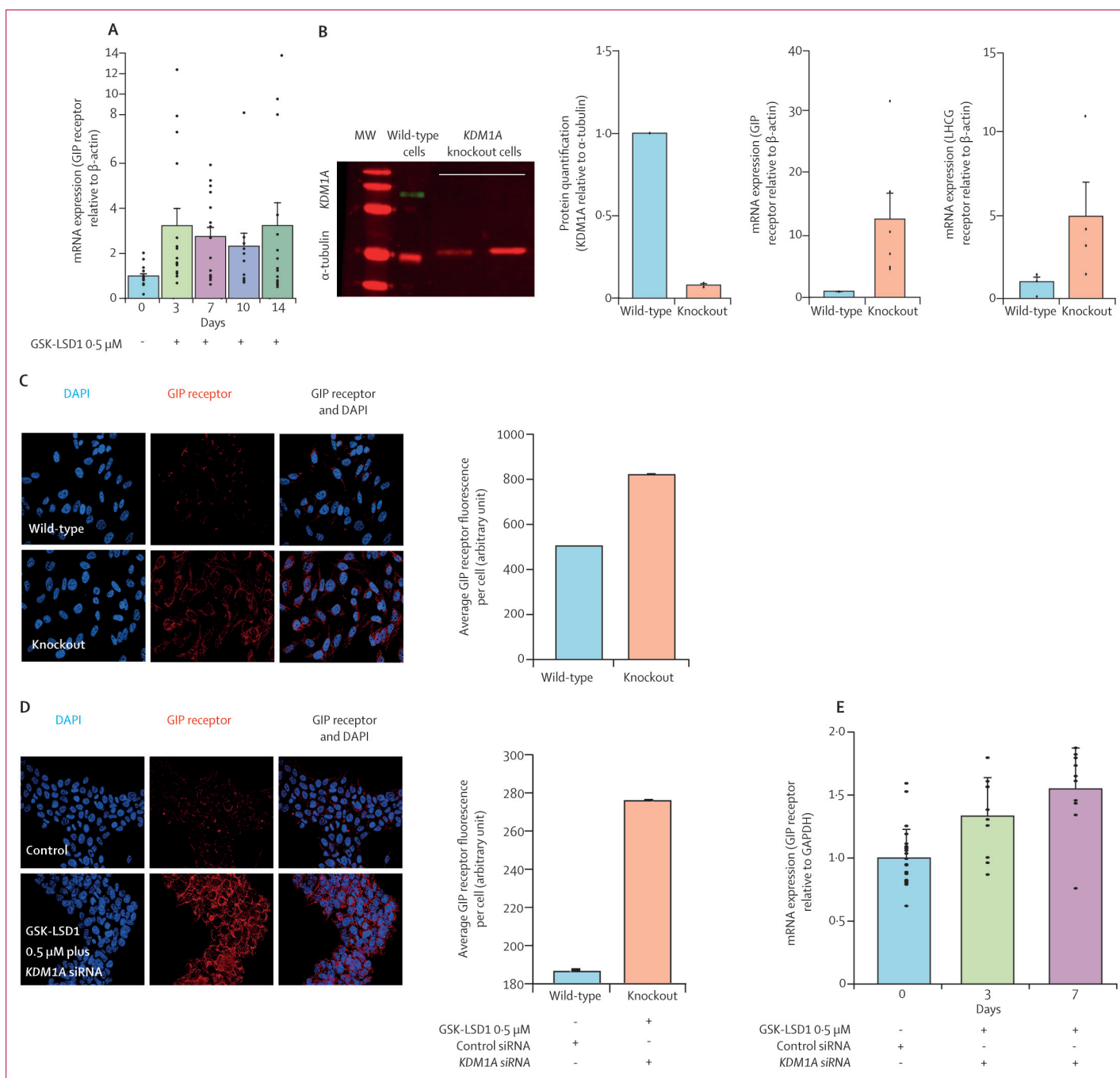


Figure 4: Functional KDM1A inhibition in the adrenocortical cell line H295R and human pancreatic β cell line EndoC- β H1 in vitro

Bars indicate SEM. Dots represent the mRNA quantification of each well of cells. (A) Pharmacological inhibition of KDM1A by GSK-LSD1 at 0.5 μ M in H295R cells, shown as a logarithmic scale. (B) KDM1A knockout by CRISPR-Cas9 genome editing in H295R cells, analysed by western blotting (KDM1A shown in green and α -tubulin shown in red) and quantified by RT-qPCR. Dots represent quantification of western blot signal. (C) GIP receptor protein expression in the cytoplasm of H295R wild-type and knockout cells, quantified by high-throughput microscopy (number of analysed cells >7000). (D) GIP receptor protein expression in the cytoplasm of H295R cells with and without treatment with GSK-LSD1 and KDM1A siRNA, quantified by high-throughput microscopy (number of analysed cells >40 000). (E) KDM1A silencing by siRNA and pharmacological inhibition with GSK-LSD1 on GIP receptor mRNA expression in the human pancreatic β cell line EndoC- β H1. DAPI=diamidino-2-phenylindole. GAPDH=glyceraldehyde 3-phosphate dehydrogenase.

$p=0.0033$). As quantified by RT-qPCR, loss of KDM1A resulted in a significant increase of GIP receptor transcripts ($p=0.0238$; figure 4B) and *LHCGR* transcripts

($p=0.0286$; figure 4B). To further investigate the effect of KDM1A on GIP receptor expression at the protein level, we analysed GIP receptor expression by automated

high-throughput microscopy after CRISPR-Cas9 genome editing or *KDM1A* siRNA and concomitant pharmacological inhibition with GSK-LSD1 (figure 4C, D). The fluorescence signal for the GIP receptor was increased in knock-out cells (62% increase; $p < 0.0001$; figure 4C). A significant decrease of *KDM1A* expression was observed after siRNA transfection (36% decrease; $p < 0.0001$). This transfection with *KDM1A* siRNA and concomitant pharmacological inhibition for 7 days resulted in a significant increase in GIP receptor expression (figure 4D). Finally, to investigate whether *KDM1A* also regulates physiological GIP receptor protein expression, we quantified GIP receptor transcripts in the human pancreatic β cell line EndoC- β H1 by RT-qPCR. *KDM1A* silencing and concomitant pharmacological inhibition with GSK-LSD1 in EndoC- β H1 cells resulted in increased GIP receptor mRNA expression after 3 days (33% increase; adjusted $p = 0.0566$) and 7 days (55% increase; adjusted $p = 0.0006$) of treatment (figure 4E). The pathophysiology of GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome due to *KDM1A* inactivation is shown in figure 5.

Discussion

We identified germline inactivating pathogenic variants in lysine demethylase *KDM1A* in patients from two families with primary bilateral macronodular adrenal hyperplasia and GIP-dependent Cushing's syndrome and in those with apparently sporadic disease, suggesting a common genetic mechanism. These findings were further supported by loss of the second *KDM1A* locus in

adrenal lesions. All *KDM1A* variants were pathogenic or most likely pathogenic and were not reported in gnomAD or in the 1000 Genomes Project databases. Notably, *KDM1A* has a probability of being loss-of-function intolerant score equal to 1, reflecting the low tolerance of the gene to protein truncating variants.¹⁴ Finally, none of the patients with non-GIP-dependent primary bilateral macronodular adrenal hyperplasia or those with GIP-dependent unilateral adrenocortical adenoma in the control groups had *KDM1A* germline or somatic alterations.

We propose a two-hit scenario of *KDM1A* inactivation, consistent with the tumour suppressor gene model of tumorigenesis.¹⁵ This pathogenesis is similar to adrenal tumour formation observed in type 1 multiple endocrine neoplasia^{16,17} and *ARMC5*-related primary bilateral macronodular adrenal hyperplasia.² No *ARMC5* pathogenic variants were detected in patients with GIP-dependent primary bilateral macronodular adrenal hyperplasia in our study or in the literature.¹⁸ Similarly, none of the *ARMC5* positive patients in the control group with primary bilateral macronodular adrenal hyperplasia had *KDM1A* pathogenic variants. Thus, genetic alterations in *ARMC5* and *KDM1A* appear to be mutually exclusive driver events responsible for molecular pathogenic mechanisms of primary bilateral macronodular adrenal hyperplasia. Unilateral cortisol-secreting or aldosterone-producing adrenocortical adenomas with ectopic GIP receptor expression have a different molecular pathogenesis, because none of the samples in our study had *KDM1A* variants. As previously reported,⁷ GIP receptor expressing adrenal adenoma development might

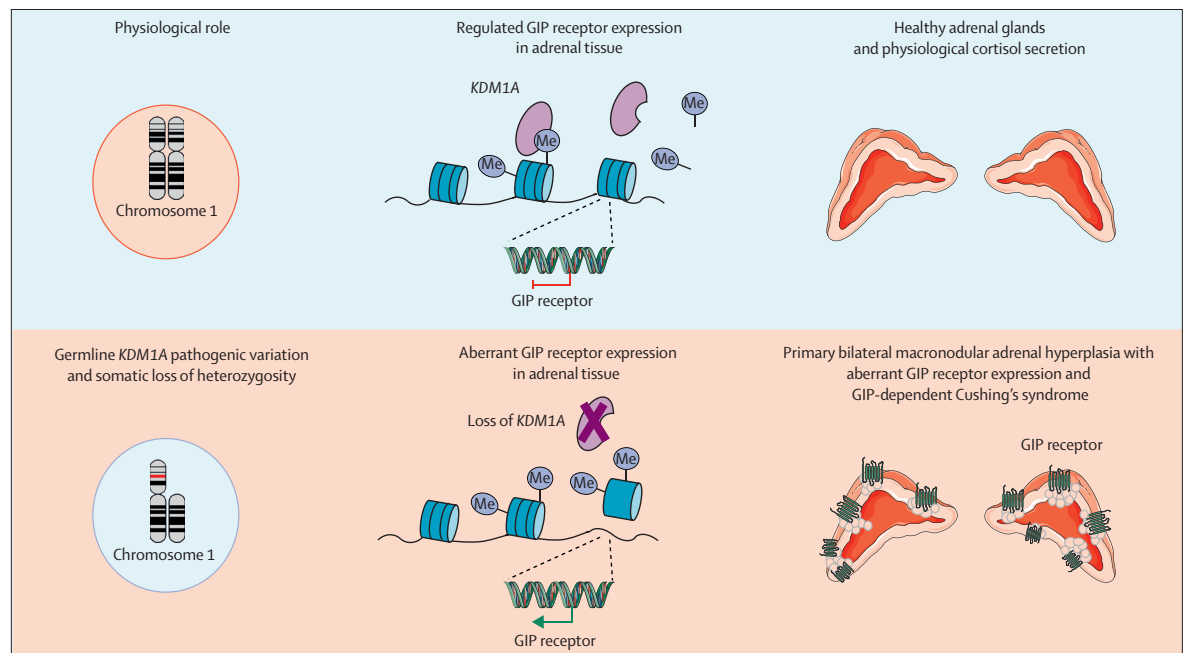


Figure 5: Pathophysiology of GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome
 A free medical art server, Servier Medical Art, was used for some components of the figure. GIP=glucose-dependent insulinotropic polypeptide.

For the Servier Medical Art see <https://smart.servier.com>

result from somatic dup(19)(q13;q32) microduplications with chromosome rearrangements. These chromosome rearrangements generated a novel genomic environment by juxtaposing the GIP receptor gene with cis-acting regulatory sequences, permitting adrenal expression.⁷

Similarly to *ARMC5*-related adrenal disease,² stepwise inactivation of *KDM1A* is associated with development of adrenal masses, and a diagnosis at median age of 43 years of primary bilateral macronodular adrenal hyperplasia and GIP-dependent Cushing's syndrome. Moreover, the occurrence of other neoplasia in several patients suggest possibly a more general implication of *KDM1A* disruption in tumour development. We cannot determine the penetrance of adrenal disease because we did not systematically analyse the apparently sporadic cases.

Thus, we recommend genetic screening of first-degree relatives of patients with GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome. For those who carry *KDM1A* pathogenic variants, we suggest performing clinical examination and biochemical screening including fasting and post-prandial plasma cortisol, urinary free cortisol excretion, and serum protein electrophoresis to detect monoclonal gammopathy.

KDM1A encodes for a histone lysine demethylase, belonging to a larger family of such proteins.⁸ Methylation of lysine residues can be associated with activation or repression of transcription.¹⁹ *KDM1A* promotes a chromatin state refractory to gene expression by demethylating histone H3 on lysine 4, usually linked to active gene transcription.^{19,20} Additionally, *KDM1A* has been shown to affect methylation of non-histone proteins involved in tumorigenesis, such as the cellular tumour antigen p53, RB1, and STAT3.⁸ Both mechanisms might be important in the pathogenesis of GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome. Persistent histone methylation, secondary to loss of *KDM1A* function, can result in aberrant transcriptional activation and absence of *KDM1A* interaction with oncogenic proteins, which can lead to cell cycle dysregulation and consequently adrenal tumorigenesis. Global gene expression analysis revealed the broader effect of *KDM1A* functional loss in adrenal lesions on gene transcription with other differentially regulated pathways including transmembrane cation transporters and G-protein coupled receptors, both involved in adrenal tumorigenesis.¹ Therefore, the loss of *KDM1A* profoundly modifies the epigenetic landscape of adrenal cells and generates a distinct cellular phenotype.

In-vitro pharmacological inhibition, silencing of *KDM1A* with siRNAs or inactivation by CRISPR-Cas9 genome editing resulted in an increase in GIP receptor transcripts and protein in human adrenocortical cells. Targeted exome sequencing did not detect any additional molecular events in genes involved in adrenal tumorigenesis. Loss of *KDM1A* function was sufficient to induce aberrant GIP receptor expression in adrenal cells;

its role and mechanisms in regulating adrenal cell proliferation remain unknown. In several patients, the adrenal hyperplasia included foci of lipomatous tissue with areas of haematopoiesis with a potential role of *KDM1A* inactivation in the development of adrenal lesions with complex histological architecture. *KDM1A* is a key epigenetic regulator of haematopoietic differentiation and is involved in the haematopoietic commitment of haemangioblasts.²¹ Therefore, the presence of myelolipoma or myeloid metaplasia in patients with primary bilateral macronodular adrenal hyperplasia might be a histological trace of *KDM1A* inactivation and should lead to genetic testing.

Wei and colleagues²² reported that germline *KDM1A* variants are a rare cause of familial or early-onset multiple myeloma. Several members of family 1 carrying *KDM1A* pathogenic variants were affected by multiple myeloma or monoclonal gammopathy of undermined significance. This observation supports the role of *KDM1A* as a germline multiple myeloma predisposition gene. Additionally, somatic *KDM1A* alterations have been described in lung, colorectal, and breast cancer, and in acute myeloid leukaemia.^{23–26} Occurrence of such cancers in several patients in our study points to a more general implication of *KDM1A* disruption in tumour development. GIP receptor expression has also been reported in neuroendocrine tumours and somatotroph pituitary adenomas, and linked with DNA hypermethylation.^{27,28} Because DNA methylation and histone methylation are inextricably interlaced,²⁹ *KDM1A* and other demethylase genes appear as new candidates potentially involved in endocrine tumorigenesis. Finally, pharmacological inhibition and silencing of *KDM1A* in human pancreatic β cells increased GIP receptor transcripts, showing a role of *KDM1A* in regulation of GIP receptor expression in tissues that express the receptor. Pharmacological targeting of *KDM1A* could enhance sensitivity of pancreatic β cells to GIP, thus creating new therapeutic avenues for treatment of metabolic disorders with altered insulin or glucagon secretion.

One limitation of our study is that the systematic biological and genetic screening of the relatives of all studied patients with this hereditary form of primary bilateral adrenal hyperplasia has not yet been completed. Another limitation is that in functional in vitro experiments we almost exclusively focused on the link between *KDM1A* inactivation and GIP receptor expression. However, *KDM1A* loss in primary bilateral adrenal hyperplasia results in deregulated expression of several other G protein-coupled receptors and proteins that might contribute to the complex regulation of steroidogenesis and development of adrenal lesions.

In conclusion, we identified germline inactivating *KDM1A* pathogenic variants as a genetic predisposition to primary bilateral macronodular adrenal hyperplasia with GIP-dependent Cushing's syndrome in all patients.

Somatic loss of heterozygosity of the *KDM1A* locus is a prerequisite for adrenal disease development. Hence, primary bilateral macronodular adrenal hyperplasia with aberrant GIP receptor expression is a genetic disease and identification of the syndrome should lead to biochemical and genetic screening of relatives.

Contributors

IBo, AT, DR, CD, ST, DV, DM, JY, A-LL, SS, HL, LC, PE, RD, DV, CS, MB, WdH, FF, BL, PC, AL, and PK obtained the patients samples and collected the data. FC, IBo, IT, LA, AP, RS, TM, FF, A-LL, VD, MT, GC, MD, GT, AG-M, IBe, SV, AL, JB, and PK were involved in the investigation, experimentation, and analysis. FC, IBo, JB, AL, and PK were involved in the writing of the original draft. All authors were involved in writing, reviewing, and editing the manuscript. All authors had full access to all the data in the study and accept responsibility to submit for publication. FC, IBo, JB, and PK have accessed and verified all the data in the study.

Declaration of interests

FC, IBo, JB, AL, and PK are registered inventors of a patent for the diagnosis and treatment of endocrine diseases related to *KDM1A* (#EP21305771.4). All other authors declare no competing interests.

Data sharing

Individual data (clinical and genetic) will be made available after de-identification upon request from academic researchers. Requests need to include a proposal explaining the intended use of data and need to be approved by the corresponding author. Proposals should be directed to Peter Kamenicky (peter.kamenicky@universite-paris-saclay.fr). To gain access, a data access agreement needs to be signed.

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References

- Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. *Lancet* 2015; **386**: 913–27.
- Assié G, Libé R, Espiard S, et al. *ARMC5* mutations in macronodular adrenal hyperplasia with Cushing's syndrome. *N Engl J Med* 2013; **369**: 2105–14.
- Espiard S, Drougat L, Libé R, et al. *ARMC5* mutations in a large cohort of primary macronodular adrenal hyperplasia: clinical and functional consequences. *J Clin Endocrinol Metab* 2015; **100**: E926–35.
- Kamenický P, Lacroix A. Mechanism of ectopic hormone receptors in adrenal tumors and hyperplasia. *Curr Opin Endocr Metab Res* 2019; **8**: 206–12.
- Lacroix A, Bolté E, Tremblay J, et al. Gastric inhibitory polypeptide-dependent cortisol hypersecretion—a new cause of Cushing's syndrome. *N Engl J Med* 1992; **327**: 974–80.
- Reznik Y, Allali-Zerah V, Chayvialle JA, et al. Food-dependent Cushing's syndrome mediated by aberrant adrenal sensitivity to gastric inhibitory polypeptide. *N Engl J Med* 1992; **327**: 981–86.
- Lecoq A-L, Stratakis CA, Viengchareun S, et al. Adrenal GIPR expression and chromosome 19q13 microduplications in GIP-dependent Cushing's syndrome. *JCI Insight* 2017; **2**: e92184.

- Hamamoto R, Saloura V, Nakamura Y. Critical roles of non-histone protein lysine methylation in human tumorigenesis. *Nat Rev Cancer* 2015; **15**: 110–24.
- Larose S, Bondaz L, Mermejo LM, et al. Coexistence of myelolipoma and primary bilateral macronodular adrenal hyperplasia with gip-dependent Cushing's syndrome. *Front Endocrinol (Lausanne)* 2019; **10**: 618.
- Albiger NM, Ceccato F, Zilio M, et al. An analysis of different therapeutic options in patients with Cushing's syndrome due to bilateral macronodular adrenal hyperplasia: a single-centre experience. *Clin Endocrinol (Oxf)* 2015; **82**: 808–15.
- Karapanou O, Vlassopoulou B, Tzanela M, et al. Adrenocorticotrophic hormone independent macronodular adrenal hyperplasia due to aberrant receptor expression: is medical treatment always an option? *Endocr Pract* 2013; **19**: e77–82.
- Lacroix A, Tremblay J, Rousseau G, Bouvier M, Hamet P. Propranolol therapy for ectopic β -adrenergic receptors in adrenal Cushing's syndrome. *N Engl J Med* 1997; **337**: 1429–34.
- Bourdeau I, Oble S, Magne F, et al. *ARMC5* mutations in a large French-Canadian family with cortisol-secreting β -adrenergic/vasopressin responsive bilateral macronodular adrenal hyperplasia. *Eur J Endocrinol* 2016; **174**: 85–96.
- Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016; **536**: 285–91.
- Knudson AG. Antioncogenes and human cancer. *Proc Natl Acad Sci USA* 1993; **90**: 10914–21.
- Larsson C, Skogseid B, Öberg K, Nakamura Y, Nordenskjöld M. Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. *Nature* 1988; **332**: 85–87.
- Chandrasekharappa SC, Guru SC, Manickam P, et al. Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* 1997; **276**: 404–07.
- Gagliardi L, Schreiber AW, Hahn CN, et al. *ARMC5* mutations are common in familial bilateral macronodular adrenal hyperplasia. *J Clin Endocrinol Metab* 2014; **99**: E1784–92.
- Shi Y, Lan F, Matson C, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 2004; **119**: 941–53.
- Liang G, Lin JCY, Wei V, et al. Distinct localization of histone H3 acetylation and H3-K4 methylation to the transcription start sites in the human genome. *Proc Natl Acad Sci USA* 2004; **101**: 7357–62.
- Takeuchi M, Fuse Y, Watanabe M, et al. LSD1/KDM1A promotes hematopoietic commitment of hemangioblasts through downregulation of ETV2. *Proc Natl Acad Sci USA* 2015; **112**: 13922–27.
- Wei X, Calvo-Vidal MN, Chen S, et al. Germline lysine-specific demethylase 1 (*LSD1/KDM1A*) mutations confer susceptibility to multiple myeloma. *Cancer Res* 2018; **78**: 2747–59.
- Takagi S, Ishikawa Y, Mizutani A, et al. LSD1 inhibitor T-3775440 inhibits SCLC cell proliferation by disrupting LSD1 interactions with SNAG domain proteins INSM1 and GFI1B. *Cancer Res* 2017; **77**: 4652–62.
- Jin Y, Ma D, Gramyk T, et al. Kdm1a promotes SCLC progression by transcriptionally silencing the tumor suppressor Rest. *Biochem Biophys Res Commun* 2019; **515**: 214–21.
- Mohammad HP, Smitheman KN, Kamat CD, et al. A DNA hypomethylation signature predicts antitumor activity of LSD1 inhibitors in SCLC. *Cancer Cell* 2015; **28**: 57–69.
- Ramírez-Ramírez R, Gutiérrez-Angulo M, Peregrina-Sandoval J, et al. Somatic deletion of *KDM1A/LSD1* gene is associated to advanced colorectal cancer stages. *J Clin Pathol* 2020; **73**: 107–11.
- Karpathakis A, Dibra H, Pipinikas C, et al. Prognostic impact of novel molecular subtypes of small intestinal neuroendocrine tumor. *Clin Cancer Res* 2016; **22**: 250–58.
- Hage M, Chaligné R, Viengchareun S, et al. Hypermethylator phenotype and ectopic GIP receptor in GNAS mutation-negative somatotropinomas. *J Clin Endocrinol Metab* 2019; **104**: 1777–87.
- Wang J, Hevi S, Kurash JK, et al. The lysine demethylase LSD1 (*KDM1*) is required for maintenance of global DNA methylation. *Nat Genet* 2009; **41**: 125–29.