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Mutation Update for Kabuki syndrome genes KMT2D and KDM6A and further delineation of X-linked Kabuki syndrome subtype 2

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Complete List of Authors:	Bögershausen, Nina; University Medical Center Goettingen, Institute of Human Genetics; University of Cologne, Institute of Human Genetics Gatinois, Vincent; CHU Montpellier, Laboratory of Rare and Autoinflammatory Diseases; University of Montpellier; INSERM UMR1183 Riehmer, Vera; University of Cologne, Institute of Human Genetics Kayserili, Hülya; Koç University School of Medicine (KUSOM) , Medical Genetics Department Becker, Jutta; University of Cologne, Institute of Human Genetics Thoenes, Michaela; University of Cologne, Institute of Human Genetics Simsek-Kiper, Pelin; Hacettepe University, Ihsan Dogramaci Children's Hospital BARAT-HOUARI, MOUNA; CHRU de Montpellier, Laboratoire de génétique des maladies rares et autoinflammatoires ; INSERM UMR1183, Elcioglu, Nursel; Marmara University Medical Faculty, Department of Pediatric Genetics Wieczorek, Dagmar; Heinrich-Heine-Universitat Dusseldorf Medizinische Fakultat, Institute of Human Genetics Tinschert, Sigrid; Medical University Innsbruck, Division of Human Genetics; Technische Universität Dresden, Institute for Clinical Genetics Sarrabay, Guillaume; Centre Hospitalier Regional Universitaire de Montpellier, Laboratory of Rare and Autoinflammatory Diseases; University of Montpellier; INSERM UMR1183 Strom, Tim; Helmholtz Zentrum München, Institute of Human Genetics; Technische Universität München, Institute of Human Genetics FABRE, Aurélie; CHRU de Montpellier, GENETIC Baynam, Gareth; University of Western Australia, Genetic Services of Western Australia; Western Australian Register of Developmental Anomalies; Telethon Kids Institute; University of Western Australia, School of Paediatrics and Child Health Sanchez, Elodie; INSERM UMR1183 Nürnberg, Gudrun; University of Cologne, Cologne Center for Genomics Altunoglu, Umut; Istanbul Medical Faculty, Istanbul University, Department of Medical Genetics Capri, Yline; APHP-Robert DEBRE University Hospital, Paris VII University, Department of Genetics Isidor, Bertrand; Nantes University Hospital, Department of Genetics

Lacombe, Didier; Centre Hospitalier Universitaire de Bordeaux, INSERM U1211, Bordeaux University, Department of Medical Genetics
Corsini, Carole; University of Montpellier; INSERM UMR1183; CHU Montpellier, Department of Medical Genetics, Reference Center for Developmental Abnormalities
Cormier-Daire, Valerie; Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, INSERM U1163, Paris Descartes-Sorbonne Paris Cité University; Hopital universitaire Necker-Enfants malades, Service de Génétique, Assistance Publique - Hôpitaux de Paris
Sanlaville, Damien; Hospices Civils Lyon, HCL Genetic department, INSERM U1028 CNRS UMR 5292, UCBL1, CRNL, GENDEV Team
Giuliano, Fabienne; CHU Hôpital l'Archet 2, Service de Génétique Médicale
Le Quan Sang, Kim-Hanh; Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, INSERM U1163, Hôpital Necker-Enfants Malades
Kayirangwa, Honorine; Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, INSERM U1163, Hôpital Necker-Enfants Malades
Nürnberg, Peter; Cologne Center for Genomics (CCG), University of Cologne
Meitinger, Thomas; Helmholtz Zentrum München, Institute of Human Genetics; Technische Universität München, Institute of Human Genetics
Boduroğlu, Koray; Hacettepe University School of Medicine Ihsan Dogramaci Children's Hospital, Department of Pediatrics Clinical Genetics Section
Zoll, Barbara; University Medical Center Goettingen, Institut of Human Genetics
LYONNET, Stanislas; Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, INSERM U1163; Hopital universitaire Necker-Enfants malades, Service de Génétique, Assistance Publique - Hôpitaux de Paris
Tzschach, Andreas; Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Institute for Clinical Genetics
Verloes, Alain; APHP-Robert DEBRE University Hospital, Paris VII University, Department of Genetics
DiDonato, Nataliya; Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Institute for Clinical Genetics
Touitou, Isabelle; Centre Hospitalier Regional Universitaire de Montpellier, Laboratory of Rare and Autoinflammatory Diseases; University of Montpellier; INSERM UMR1183
Netzer, Christian; University of Cologne, Institute of Human Genetics;
Li, Yun; University of Cologne, Institute of Human Genetics; University Medical Center Goettingen, Institute of Human Genetics
Geneviève, David; University of Montpellier; INSERM UMR1183; CHU de Montpellier, Département de Génétique médicale, Reference Center for Developmental Abnormalities
Yigit, Gokhan; University Medical Center Goettingen, Institute of Human Genetics; University of Cologne, Institute of Human Genetics
Wollnik, Bernd; University Medical Center Goettingen, Institute of Medical Genetics; University of Cologne, Institute of Human Genetics

Key Words: Kabuki Syndrome, KMT2D, MLL2, KDM6A, UTX, UTY, Mutation

Mutation Update for Kabuki syndrome genes *KMT2D* and *KDM6A* and further delineation of X-linked Kabuki syndrome subtype 2

Nina Bögershausen[1], Vincent Gatinois[2,3,4], Vera Riehmer[5], Hülya Kayserili[6], Jutta Becker[5], Michaela Thoenes[5], Pelin Özlem Simsek-Kiper[7], Mouna Barat-Houari [2,4], Nursel H. Elcioglu[8], Dagmar Wieczorek[9], Sigrid Tinschert[10,11], Guillaume Sarrabay[2,3,4], Tim M. Strom[12,13], Aurélie Fabre[2], Gareth Baynam[14,15,16,17], Elodie Sanchez[4], Gudrun Nürnberg[18], Umut Altunoglu[19], Yline Capri[20], Bertrand Isidor[21], Didier Lacombe[22], Carole Corsini[3,4,23] Valérie Cormier-Daire[24,25], Damien Sanlaville[26], Fabienne Giuliano[27], Kim-Hanh Le Quan Sang[24], Honorine Kayirangwa[24], Peter Nürnberg[18], Thomas Meitinger[12,13], Koray Boduroglu[7], Barbara Zoll[1], Stanislas Lyonnet[24,25], Andreas Tzschach[10], Alain Verloes[20], Nataliya Di Donato[10], Isabelle Touitou[2,3,4], Christian Netzer[5], Yun Li[1], David Geneviève[3,4,23], Gökhan Yigit[1], and Bernd Wollnik[1]

[1]Institute of Human Genetics, University Medical Center Goettingen, Goettingen, Germany; [2]Laboratory of Rare and Autoinflammatory Diseases, CHU Montpellier, Montpellier, France; [3]University of Montpellier, Montpellier, France; [4]INSERM UMR1183, Montpellier, France; [5]Institute of Human Genetics, University of Cologne, Cologne, Germany; [6]Medical Genetics Department, Koç University School of Medicine (KUSOM) İstanbul, Turkey; [7]Pediatric Genetics Unit, Department of Pediatrics, Hacettepe University Medical Faculty, Ankara, Turkey; [8]Department of Pediatric Genetics, Marmara University Medical Faculty, Istanbul, Turkey; [9]Institute of Human Genetics, University of Duesseldorf, Duesseldorf, Germany; [10]Institute for Clinical Genetics, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Germany; [11]Zentrum für Humangenetik, Medizinische Universität Innsbruck, Austria; [12]Institute of Human Genetics, Technische Universität München, Munich, Germany; [13]Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany; [14]Genetic Services of Western Australia, Princess Margaret and King Edward Memorial Hospitals, Perth, Australia; [15]Western Australian Register of Developmental Anomalies, Perth Australia; [16]Telethon Kids Institute, Perth Australia; [17]School of Paediatrics and Child Health, University of Western Australia, Perth, Australia; [18]Cologne Center for Genomics, University of Cologne, Cologne, Germany; [19]Department of Medical Genetics, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey; [20]Department of Genetics, APHP-Robert DEBRE University Hospital, Paris VII University, Denis Diderot Medical School, Paris, France; [21]Department of Genetics, Nantes University Hospital, Nantes, France; [22]CHU Bordeaux, INSERM U1211, Bordeaux University, Department of Medical Genetics, Bordeaux, France; [23]Department of Medical Genetics, Reference Center for Developmental Abnormalities, CHU, Montpellier, France; [24]Institut Imagine, INSERM U1163, Paris Descartes-Sorbonne Paris Cité University, Paris, France; [25]Service de Génétique, Hôpital Universitaire Necker-Enfants Malades, Assistance Publique - Hôpitaux de Paris, Paris, France; [26]HCL Genetic department, INSERM U1028 CNRS UMR 5292, UCBL1, CRNL, GENDEV Team, Lyon, France; [27]Department of Medical Genetics, l'Archet II Hospital, Nice, France.

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Corresponding author: Prof. Bernd Wollnik, MD
Institute of Human Genetics
University Medical Center Göttingen
Heinrich-Düker-Weg 12, 37073 Göttingen, Germany
Tel: +49-551-39-7590 Fax: +49-551-39-9303
EMail: bernd.wollnik@med.uni-goettingen.de

ABSTRACT

Kabuki syndrome (KS) is a rare but recognizable condition that consists of a characteristic face, short stature, various organ malformations and a variable degree of intellectual disability. Mutations in *KMT2D* have been identified as the main cause for KS, while mutations in *KDM6A* are a much less frequent cause. Here, we report a mutation screening in a case series of 347 unpublished patients, in which we identified 12 novel *KDM6A* mutations (KS type 2) and 208 mutations in *KMT2D* (KS type 1), 132 of them novel. Two of the *KDM6A* mutations were maternally inherited and 9 were shown to be *de novo*. We give an up-to-date overview of all published mutations for the two Kabuki syndrome genes and point out possible mutation hot spots and strategies for molecular genetic testing. We also report the clinical details for 11 patients with KS type 2, summarize the published clinical information, specifically with a focus on the less well defined X-linked KS type 2, and comment on phenotype-genotype correlations as well as sex-specific phenotypic differences. Finally, we also discuss a possible role of *KDM6A* in Kabuki-like Turner syndrome and report a mutation screening of *KDM6C* (*UTY*) in male KS patients.

Key words: Kabuki syndrome, *KDM6A, MLL2, KMT2D, UTY, KDM6C*

BACKGROUND

Kabuki syndrome (KS) is a rare genetic syndrome that is characterized by postnatal growth retardation, mild to moderate intellectual disability, organ malformation, endocrinological and hematological abnormalities in combination with very recognizable facial features. It is mainly caused by heterozygous mutations in lysine (K)-specific methyltransferase 2D (*KMT2D;* formerly *MLL2*; MIM 602113; NM_003482.3*)* Approximately 56% to 75% of Kabuki syndrome cases are caused by mutations in *KMT2D* [Ng et al., 2010; Hannibal et al., 2011; Li et al., 2011; Bögershausen and Wollnik, 2013]. *KMT2D* encodes a methyltransferase responsible for histone 3 lysine 4 (H3K4) di- and trimethylation, which is an epigenetic mark for euchromatin and active transcription [Issaeva et al., 2007; Smith et al., 2011]. The H3K4 methyltransferases (KMT2 group, also called trithorax group) act in multi-protein complexes that contain various shared and some distinct components that contribute to the specific function of each complex [Smith et al., 2011]. One important component of the KMT2D containing complex (called ASCOM) is KDM6A, a H3K27 demethylase responsible for removal of repressive polycomb-derived methylation marks [Agger et al., 2007; Hong et al., 2007]. Whole-gene and intragenic deletions as well as point mutations in lysine (K)-specific demethylase 6A (*KDM6A;* formerly *UTX*; MIM 300128; NM_021140.3) have been identified in patients with KS, which led to the definition of two subtypes of KS: *KMT2D*-associated, autosomal-dominant Kabuki syndrome type 1 (KS1) and *KDM6A*-associated, X-linked-dominant Kabuki syndrome type 2 (KS2). Several mutation screening studies have revealed that mutations in *KDM6A* account for approximately 5 to 8% of Kabuki syndrome cases [Banka et al., 2015; Cheon et al., 2014; Dentici et al., 2015; Micale et al., 2014; Miyake et al., 2013b]. Very recently, we reported mutations in the genes *RAP1A* (MIM 179520) and *RAP1B* (MIM 179530) as novel rare causes of Kabuki and Kabuki-like syndromes [Bögershausen et al., 2015]. Furthermore, a homologue of *KDM6A* called *KDM6C* (*UTY*; MIM 400009; NM_182660.1), another H3K27 demethylase, is located on the Y-chromosome

[Walport et al., 2014] and constitutes a possible candidate gene for Kabuki syndrome in male individuals.

In this study, we collected a cohort of 347 unpublished patients with a clinical diagnosis of Kabuki syndrome and screened them for mutations in *KMT2D* and subsequently in *KDM6A*. 208 patients in our cohort harbored mutations in *KMT2D*. Of the *KMT2D* negative patients, one received whole exome sequencing and 88 received Sanger sequencing of *KDM6A,* by which we identified twelve novel *KDM6A* mutations. We discuss the molecular and clinical findings and compare them to the literature with a focus on the rare X-linked KS2. We also report a mutation screening of *KDM6C* (*UTY*) in male patients, which did not identify any mutations, and discuss Kabuki-like Turner syndrome as an important differential diagnosis for female patients.

METHODS

Patients

We obtained written informed consent from all patients or their legal guardians for the molecular genetic analyses and for publication of the results. We obtained written informed consent for publication of photographs from the concerned parties. The study was performed according to the Declaration of Helsinki protocol. Blood samples were collected from the patients and their parents and DNA was extracted from peripheral blood lymphocytes by standard extraction procedures. Patient IDs presented in this publication were assigned arbitrarily by order of mutations and do not relate to the identity of the patients.

Whole-exome sequencing

Exonic and adjacent intronic regions were enriched from genomic DNA of one patient (P1) and her parents using the 50 Mb SureSelect XT Human All Exon enrichment kit from Agilent Technologies (Santa Clara, USA) and sequencing was performed on a GAIIx sequencer from Illumina (Illumina, San Diego, USA). Alignment against the GRCh37 human reference was performed with Burrows-Wheeler Aligner (BWA, version 0.6.2), PCR-duplicates marking with Picard (version 1.84), indel realignment, base quality recalibration and variant calling with the Genome Analysis Toolkit (GATK, version 2.3-4), and annotation with Annovar (version 2013Feb21). The resulting variants were filtered to exclude variants present in dbSNP 135, the Exome Variant Server, the 1000 Genomes Project, or our in-house database and variants that were not predicted to affect protein sequence or exon splicing (please see prediction programs and databases for URLs). For *de novo* analysis, all variant loci in the patient's dataset were compared to the parental datasets. Only variants covered in all three samples and present in less than 5% of the reads in the parental datasets were considered.

Mutation screening and Sanger sequencing

Mutation screenings were performed using standard methods for PCR amplification and Sanger sequencing. Primer sequences for *KDM6A and KMT2D* were designed with the primer 3 software, available at the UCSC genome browser, or the primer 3 webtool (http://primer3.ut.ee/). Specific primers for *KDM6C* (*UTY*) were custom-designed using the Oligo® software (Molecular Biology Insights, Cascade, USA) in order to avoid amplification of the highly homologous *KDM6A* gene. Primer sequences are available on request. The entire coding sequence of the respective genes was analyzed and mutations were confirmed by a second PCR on an independent DNA solution.

Identified mutations were classified as disease causing if they were 1.) either truncating or predicted to be deleterious (see below), or 2.) proven to be *de novo* or already published as *de novo* in another patient with Kabuki syndrome, and 3.) absent from the current databases of normal genetic variation (EVS, ExAC, dbSNP). Variants of unknown significance were defined as variants that were 1.) non-truncating, 2.) predicted to be deleterious, and 3.) absent from the current databases of normal genetic variation (EVS, ExAC, dbSNP) but for which *de novo* occurrence could not be proven. Non-disease-causing variants were defined as variants that were 1.) inherited from a healthy parent and/or 2.) annotated in a database of normal genetic variation (EVS, ExAC, dbSNP). Non-disease-causing variants (polymorphisms) identified in our cohort are not reported in this study.

De novo occurrence of the *KDM6A* mutation identified by whole-exome sequencing in patient P1 was confirmed by Sanger sequencing of the specific exon according to standard methods.

Current HGVS standard was employed for mutation nomenclature. Nucleotide numbering referring to cDNA uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1. Mutation nomenclature was double checked with the Mutalyzer software: https://mutalyzer.nl/.

Novel variants were submitted to the locus specific databases at LOVD: www.lovd.nl/KDM6A www.lovd.nl/KMT2D.

SNP array

SNP arrays were performed in three patients with cytogenetically diagnosed Turner syndrome who presented with a Kabuki-like phenotype: one patient with a 45,X, one patient with a 45,X/46,X,i(Xq), and one patient with a 45,X/46,X,r(X) karyotype. We employed the Affymetrix genome-wide Human SNP Array 6.0 utilizing more than 906,600 SNPs and more than 946,000 probes for the detection of copy number variations. Quantitative data analysis was performed with GTC 4.1 (Affymetrix Genotyping Console) using a reference file of ATLAS Biolabs GmbH (100 samples). We used the Segment Reporting Tool (SRT) to locate segments with copy number changes in the copy number data with the assumption of a minimum of 10 kb per segment and minimum genomic size of five markers of a segment.

Prediction programs

Prediction of the mutation effect was performed for missense mutations and in-frame deletions with the programs PROVEAN (http://provean.jcvi.org/index.php), SIFT (http://sift.jcvi.org/), and Mutation Taster (http://www.mutationtaster.org/). The effect of splice site mutations was analyzed with Human Splicing Finder version 3 (http://www.umd.be/HSF3/) and Mutation Taster. Please see Supp. Table 3 and Supp. Table 4 for in-silico prediction output.

Databases

The following databases were used for this study: The Exome Aggregation Consortium (ExAC): http://exac.broadinstitute.org/; The Exome Variant Server (EVS): http://evs.gs.washington.edu/EVS/; Database of human single nucleotide Polymorphisms (dbSNP): http://www.ncbi.nlm.nih.gov/projects/SNP/; The 1000 Genomes:

http://www.1000genomes.org/; HGMD: http://www.biobase-international.com/product/hgmd; The UCSC browser: http://genome.ucsc.edu/; The human protein reference database: http://www.hprd.org/; COSMIC: http://cancer.sanger.ac.uk/cosmic; DECIPHER: https://decipher.sanger.ac.uk/; PubMed: http://www.ncbi.nlm.nih.gov/pubmed/.

Literature review

We searched the HGMD database for mutations in *KMT2D* and *KDM6A* and, additionally, conducted a search for further mutations described in original articles in PubMed using the terms “Kabuki syndrome”, “*MLL2* mutation”, and “*KMT2D* mutation” in different combinations. We examined the clinical and molecular information available from the retrieved 20 mutation screening studies [Banka et al., 2012; Cheon et al., 2014; Courcet et al., 2013; Dentici et al., 2015; Hannibal et al., 2011; Li et al., 2011; Lin et al., 2015; Lindgren et al., 2013; Lindsley et al., 2015; Liu et al., 2015; Makrythanasis et al., 2013; Micale et al., 2011; Micale et al., 2014; Miyake et al., 2013; Morgan et al., 2015; Ng et al., 2010; Paderová et al., 2016; Paulussen et al., 2011; Subbarayan et al., 2014; Van Laarhoven et al., 2015] and 18 molecularly proven case reports [Brackmann et al., 2013; Cappuccio et al., 2014; Gohda et al., 2015; Karagianni et al., 2016; Kim et al., 2013; 2016; Kokitsu-Nakata et al., 2012; McVeigh et al., 2015; Ratbi et al., 2013; Riess et al., 2012; Roma et al., 2015; Schulz et al., 2014; Soden et al., 2014; Takagi et al., 2014; Tanaka et al., 2012; Verhagen et al., 2014; Yuen et al., 2015; Zaidi et al., 2013; Zarate et al., 2012]. Only articles that were fully available online were included in the analysis. However, to ensure a consistent genotype-phenotype analysis, we did not consider any case reports from before the identification of *KMT2D* as the first causative gene. We evaluated all published mutations in *KMT2D* (Supp. Table 1) and *KDM6A* (Supp. Table 2) and assigned them to three variant classes: disease-causing variant (DC), variant of unknown significance (VUS), or non-disease-causing variant (NDC). According to our classification, a disease-causing (DC) variant must fulfil the following criteria: It is either a truncating variant or a non-truncating variant

that was proven to be *de novo* or has been described as *de novo* in another patient with a comparable phenotype and it is not listed in any public database of normal genetic variation. A variant of unknown significance (VUS) is a non-truncating sequence alteration with unknown inheritance, which is not present in any public database of normal genetic variation (such as the ExAC browser, the dbSNP database, the 1000 Genomes, or the Exome variant server, see databases) and preferably predicted to be disease causing by at least one prediction algorithm (see Supp. Table 3, Supp. Table 4), however the last criterion is not requisite if a variant is absent from all databases. Finally, a variant will be classified as a non-disease-causing (NDC) variant if it is a non-truncating variant, the inheritance of which is unknown or which was inherited from an unaffected parent, and/or which is listed in public databases (see above), and/or if the same patient additionally carries a separate variant that is judged as disease causing.

Mutation load score

To evaluate the mutation load of a single exon as a function of its size, we established a mutation load score (MLS), calculated as the number of mutations (n) divided by the number of basepairs (bp) of an exon, multiplied by 100 (MLS = $\frac{n}{bp} \cdot 100$). The score was calculated for disease-causing variants identified by literature review and our own study, and the numbers include recurrent mutations. Mutations affecting more than one exon, i.e. large deletions/duplications, were excluded from the calculation. Mutations affecting splice sites were allocated to the corresponding exon (i.e. intron 2 = exon 2). A score of 1 equals 1 mutation per 100 bp. For *KMT2D* we retrieved an average MLS of 3.74, with a standard deviation (SD) of 3.80. According to the expected normal distribution, a MLS > mean + 2 SD (= 11.33) was regarded as the cut-off for an unexpectedly high mutation load. For *KDM6A* we obtained an average MLS 0.82 +/- a standard deviation of 1.08, and a cut-off of 2.98. However, the small

number of known mutations in this gene impedes the interpretation of this result, which is therefore only exemplary.

PATIENT COHORT

The present cohort consists of 347 patients with a tentative diagnosis of Kabuki syndrome, established by external clinicians, from different referral centers. It includes patients from Germany, France, Turkey, and Australia. The DNAs were sent to our laboratories in Cologne and Montpellier with a request for molecular genetic analysis of the Kabuki syndrome genes *KMT2D* and *KDM6A*. The patients reported here have not been previously reported elsewhere. The only patient who had already been included in our first mutation screening study [Li et al., 2011] is Patient 1 (P212); she was then negative for a mutation in *KMT2D* and we now performed whole-exome sequencing. Four of the patients with *KDM6A* mutations were referred from Turkish centers (P212, P214, P216, P220) and two came from German centers (P209 and P211), P211 being of Turkish descent, and the other six came from France. Five patients with Kabuki-like Turner syndrome originated from Turkey and one from Australia. They had already been cytogenetically diagnosed and were referred due to their striking clinical overlap with Kabuki syndrome. Of the *KMT2D* negative patients, one received whole exome sequencing and 88 received Sanger sequencing of *KDM6A.* Clinical details were available for 11 patients with KS2, unfortunately we were unable to obtain clinical details for patient P215, as well as the mothers of patients P214 and P215.

IDENTIFIED *KMT2D* MUTATIONS

Sanger sequencing of all coding exons and exon-intron boundaries of *KMT2D* in 347 patients with a tentative diagnosis of Kabuki syndrome identified 208 mutations (Table 1), 132 of which have not been reported before. We identified 76 nonsense mutations, 69 small deletions/duplications, 45 missense variants, 15 splice site mutations, and 3 in-frame deletions.

De novo occurrence was proven if parental DNA was available (n = 103). Three patients had inherited the mutation from an affected parent.

The mutations c.166C>T, p.(Gln56*); c.6295C>T, p.(Arg2099*); c.7903C>T, p.(Arg2635*); c.8200C>T, p.(Arg2734*); c.11944C>T, p.(Arg3982*); c.12592C>T, p.(Arg4198*); c.13450C>T, p.(Arg4484*); c.14710C>T, p.(Arg4904*); c.14946G>A, p.(Trp4982*); c.15079C>T, p.(Arg5027*); c.16501C>T, p.(Arg5501*); c.4135_4136delAT, p.(Met1379Valfs*52); c.5627_5630delACAG, p.(Asp1876Glyfs*38); c.16489_16491delATC, p.(Ile5497del); c.4267C>T, p.(Arg1423Cys); c.15142C>T, p.(Arg5048Cys); c.15143G>A, p.(Arg5048His); c.15461G>A, p.(Arg5154Gln); c.15536G>A, p.(Arg5179His); c.15536G>T, p.(Arg5179Leu); c.15640C>T, p.(Arg5214Cys); c.16273G>A, p.(Glu5425Lys) were found in two or more patients (Table 1). The most frequent mutation was c.15142C>T, p.(Arg5048Cys) in exon 48 which was identified in 5 patients, followed by c.6295C>T, p.(Arg2099*) and c.15079C>T, p.(Arg5027*), which were found in 4 patients each.

192 mutations identified in this study could be classified as disease causing (DC). 16 mutations were classified as variants of unknown significance (VUS) due to lack of parental samples for segregation analysis. These were mostly novel, non-truncating mutations, which were predicted to be damaging and absent from the queried databases of human genetic variations (for details on in-silico prediction for *KMT2D* missense mutations and in-frame deletions please refer to Supp. Table 3). Non-disease causing variants (polymorphisms) identified in our patients are not reported.

PUBLISHED *KMT2D* MUTATIONS

To date, 424 variants in the *KMT2D* gene have been reported. Except for one patient with autism spectrum disorder and one patient with congenital heart disease, all reported patients with *KMT2D* variants had been diagnosed with Kabuki syndrome (Supp. Table 1). Among these 424 variants were 121 nonsense mutations, 106 small deletions, 55 small insertions or

duplications, 93 missense variants, and 36 splice site variants. Additionally, five indels, six large deletions (>20 bp), and two large insertions have been published (Supp. Table 1, Figure 1A).

When we evaluated the reported variants against the above described pathogenicity criteria (mutation type, segregation, prediction, annotation in public databases of normal genetic variation), we assessed 33 of these variants as non-disease-causing (NDC) (Supp. Table 1). 32 variants were judged as VUS (Supp. Table 1), consisting of 24 missense variants, two non-frameshifting small deletions, one non-frameshifting small insertion, one non-frameshifting large deletion, and four splice site variants. Segregation analysis would be needed in order to confirm pathogenicity of these variants. We judged 359 of the reported mutations as disease causing, 42 of which are recurrent mutations (reported 2 to 7 times; Supp. Table 1). The mutation types from our study and the literature are depicted in Figure 1A. We counted each mutation by number of published records (= number of patients) to analyze the exon distribution in detail, and together with the newly identified mutations in this study, we were able to analyze the distribution of 621 disease-causing variants (NDC and VUS excluded) (Figure 1C).

IDENTIFIED *KDM6A* MUTATIONS

Trio whole-exome sequencing (WES) in a *KMT2D* mutation-negative patient (P212) identified the novel one-basepair duplication c.171dupT in exon 2 of *KDM6A*. This mutation leads to a frameshift and a premature stop codon at amino acid position 64: p.(Gly58Trpfs*7). *De novo* occurrence was observed in the WES data sets and subsequently confirmed by Sanger sequencing (Supplementary Figure 1). Sanger sequencing in 88 additional patients who were negative for mutations in *KMT2D* identified 11 additional variants in *KDM6A* (Figure 2; Table 2, Supplementary Figure 1), including two nonsense mutations, two small insertions, three missense variants, and four splice site mutations. Of the 12 patients with KS2, seven are female and five are male (Table 2). Nine of the mutations were shown to be *de novo*, while two were inherited. One male patient (P214) had inherited the c.2729A>G, p.(Asn910Ser) variant from his

mother (Supplementary Figure 1), whose phenotype could not be ascertained, and another (P215) had inherited the c.3073A>G, p.(Ser1025Gly) mutation from his clinically affected mother. While the boy showed a recognizable Kabuki phenotype, the mother's phenotype was reported to be mild. However, clinical details on this family are unavailable. The mutation in P214 affects a conserved asparagine residue at position 910 and was predicted to be damaging by the prediction programs Mutation Taster and PROVEAN. Most importantly, it is not annotated in the current databases of normal genetic variation (EVS, ExAC, dbSNP), and it was therefore considered to be most likely disease causing with reduced penetrance. However, according to our classification system, the variant was classified as VUS. The mutation in P215 is also predicted to affect protein function and was absent from the above mentioned databases. Because of the mild Kabuki syndrome phenotype visible in the carrier parent, the mutation was classified as disease causing (for details on in-silico prediction for inherited and *de novo KDM6A* missense mutations please refer to Supp. Table 3).

The mutation detection rate for *KDM6A* among the *KMT2D* negative group was 13.5%.

PUBLISHED *KDM6A* MUTATIONS

To date, 33 germline mutations in *KDM6A* have been published. The 18 published point mutations consist of five nonsense mutations, five small deletions, two missense variants, and six splice site mutations. Additionally, seven large deletions, seven large duplications/insertions, and one complex genomic rearrangement, have been published (Supp. Table 2). Most of the published *KDM6A* mutations were judged as disease causing according to our classification system. Only the missense variant c.2939A>T, p.(Asp980Val) published by Micale et al. [2014] and four large duplications published by Lindgren et al. [2013] were judged as VUS because proper segregation had not been proven (Supp. Table 2). The mutation types of the disease-causing mutations from the literature (n = 29, including one recurrent mutation) and this study

(n = 11) are depicted in Figure 1B. The exon distribution of all point mutations from the literature and our own study (n = 29, including one recurrent mutation) is depicted in Figure 1D.

MUTATION SCREENING OF *KDM6C*

We also investigated the hypothesis of the *KDM6A* homologue *KDM6C* (*UTY*) as a candidate gene for Kabuki syndrome in male patients. Mutation screening of 15 male KS patients negative for *KMT2D* mutations did not identify any causative mutation in *KDM6C* (*UTY*).

FINDINGS IN KABUKI-LIKE TURNER SYNDROME

The patients with Kabuki-like Turner syndrome all had long palpebral fissures, arched eye-brows, dense eye-lashes, and a short columella. The typical eversion of the lower eye-lid was seen in two patients. A remarkable similarity was seen in the form of the nose: a round, fleshy, sometimes bulbous nasal tip was seen in most patients. The eyebrows, although arched were also bushy and not laterally sparse as it is frequently seen in KS. They all had short stature with normal head circumference. One had a bicuspid aortic valve and aortic coarctation, as well as hydronephrosis. A second patient had a horseshoe kidney with double collecting system. Another had congenital hip dislocation.

For three of the six patients with Kabuki-like Turner syndrome, we confirmed the respective karyotypes by SNP arrays, but did not detect any additional chromosomal aberrations that might explain the Kabuki-like phenotype. In the patients with the 45,X and the 45,X/46,X,i(Xq) karyotypes, one copy of *KDM6A*, which is located on chromosome Xp11.3, is missing. In the patients with the 45,X/46,X,r(X) karyotype, the exact breakpoint of the ring chromosome could not be defined, thus, it is unknown whether *KDM6A* is present within the ring or not. Interestingly, many literature reports of patients with Kabuki-like Turner syndrome state that *KDM6A* was included in the ring, meaning that two copies should be present. However it is

possible, that the ring structure of the chromosome impedes correct transcription of this copy or, that enhancer elements/long range regulators are missing from the ring chromosome.

KDM6A mutation screening of all six Kabuki-like Turner syndrome patients with either a 45,X, a 45,X/46,X,i(X), or a 45,X/46,X,r(X) karyotype did not reveal any sequence variant that might be considered causative of the Kabuki-like phenotype in these patients.

DIAGNOSTIC RELEVANCE OF THE MOLECULAR RESULTS FOR *KMT2D*

In our case series mutations in *KMT2D* were identified in 208 patients (60%). The identified mutations were mainly truncating (76 nonsense and 69 frameshifting mutations). Exon 39 seems to be prone to nonsense mutations, while missense mutations occurred most frequently in exon 48. Overall, exon 48 showed the highest number of mutations in our study (46), closely followed by exon 39 (45 mutations). Taken together, the largest exons (10, 11, 31, 34, 39, and 48) account for 69.71% of all mutations identified in this study (Figure 1C) and 63.37% of all mutations analyzed (this study and literature), which is an expected result.

To further analyze the exon distribution of the published and novel mutations and to establish mutation hot spots independent of exon size, we established a mutation load score (MLS), which images the number of mutations relative to the number of basepairs of an exon. For this calculation, we used the location of all disease-causing variants retrieved from the literature or identified in our study (including recurrent mutations) and we found that in most of the largest exons the number of mutations does not exceed the expected mutation load (cut-off 11.33). Thus, the apparent clustering of mutations in these exons is mainly attributable to their size. Only exons 14, 52 and 53 hold an unexpectedly high number of mutations, with MLS of 12.36, 21.62 and 15.60, respectively. Exon 48 is the only large exon with a MLS close to the cut-off of 9.47, and it would probably exceed the cut-off if all missense variants classified as VUS were

included in the calculation. Together with the high MLS of exons 52 and 53 this might indicate a potential clustering of mutations at the 3' end of the *KMT2D* gene (Figure 1C).

Based upon these observations, two-step diagnostic approaches, for example starting with exons 27 to 54 or starting with the large exons and exons 51-53, could be useful and economic diagnostic testing strategies if Sanger sequencing is to be applied (see clinical relevance).

A further aspect about *KMT2D* mutations is that they are mostly private mutations, reported in only a single patient (Supp. Table 1): only 58 of the 621 disease-causing mutations have been found in more than one patient. The most frequently identified mutations are c.15142C>T, p.(Arg5048Cys) in exon 48 (9 patients) and c.6595delT, p.(Tyr2199Ilefs*65) in exon 31 (8 patients).

While most patients harbor only a single disease causing *KMT2D* mutation, the studies by Makrythanasis et al. [2013], Micale et al. [2014], and Liu et al. [2015] each described a patient who carried two disease-causing, *de novo* missense variants in *KMT2D* (Supp. Table 1, mutations marked with asterisks). Due to the rareness of *de novo* mutations, *de novo* occurrence of a mutation in the gene that is known to cause the phenotype diagnosed in a patient is usually considered a strong indicator of pathogenicity. The mutations in the patients mentioned above were both judged disease causing according to our criteria. However, in a vital developmental gene like *KMT2D* we would expect biallelic mutations with deleterious functional consequences to be lethal at the embryonic stage. Thus, it appears most likely that these mutations are located in-cis, a phenomenon that has already been described in Rett syndrome [Bunyan and Robinson, 2008]. Another possibility is false paternity.

Finally, large genomic aberrations of the *KMT2D* locus seem to be very rare: Banka et al. [2013] identified intragenic or whole-gene deletions/duplications of *KMT2D* in 3 out of 64 patients by

MLPA analysis. However, deletions or duplications of the *KMT2D* locus have been reported in only 10 patients in the DECIPHER database, and >80 MLPA analyses in patients with Kabuki syndrome in our own laboratory have not identified a single aberration. Priolo et al. [2012] did not find any deletions/duplications *KMT2D* in a cohort of 120 patients with Kabuki syndrome, indicating that large deletions of *KMT2D* are relatively rare events, compared to point mutations,.

DIAGNOSTIC RELEVANCE OF THE MOLECULAR RESULTS FOR *KDM6A*

In our case series, we identified twelve novel *KDM6A* mutations (Figure 2, Table 2, Supplementary Figure 1) in a cohort of 89 patients (= 13.5%). Nine of the mutations could be shown to be *de novo,* while two were inherited (Table 2, Supplementary Figure 1). Parental samples were unavailable for patient P213. The mutations c.171dupT and c.190G>T identified in patients P1 and P2 represent the most N-terminal mutations yet described and are located before the first TPR motif of the KDM6A protein (Figure 2).

Apart from these 5' mutations, the identified and the published mutations in *KDM6A* show a clustering towards the 3' end of the gene (Figure 1D). We also calculated mutation load scores (MLS) for *KDM6A.* However, the result is not representative due to the small number of *KDM6A* point mutations yet described. Overall, 69% of all disease causing point mutations were located in exons 16 – 29 (Figure 1D). Therefore, it may be advisable to divide this large gene into two sets for diagnostic Sanger sequencing approaches, starting with exons 16 - 29, followed by exons 1 – 15.

In terms of mutation type, *KMT2D* and *KDM6A* show different profiles with regard to point mutations. Both genes show a large proportion of nonsense mutations and small deletions/insertions (Figure 1A,B), but splice site mutations are the most frequent mutation type for *KDM6A* as opposed to *KMT2D* where splice site mutations play a minor role (27.5% vs. 7.9%, Figure 1A,B).

Genomic aberrations of the *KDM6A* locus appear to be much more frequent than genomic aberrations of the *KMT2D* locus: 67 patients with deletions, duplications, triplications or complex genomic rearrangements of the *KDM6A* locus have been annotated in DECIPHER. Additionally, *KDM6A* was initially identified as a causative gene for Kabuki syndrome by the identification of whole-gene or intragenic deletions in three patients by Lederer et al. [2012]. However, Priolo et al. [2012] did not find any deletions/duplications of *KDM6A* or *KMT2D* in a cohort of 120 patients with Kabuki syndrome, indicating that such aberrations seem to be relatively rare compared to the other known genetic causes of the disease.

Interestingly, the *KDM6A* missense mutation c.3763C>T, p.(Arg1255Trp), identified in a patient in this study, which has never been described in Kabuki syndrome before, has been found as a somatic mutation in stomach carcinoma (COSMIC ID: COSM4109565). Somatic mutations in *KMT2D* and *KDM6A* are frequently found in cancer [Huether et al., 2014]; however, an increased cancer risk has not yet been described for patients with germline mutations. Long-term follow up of these patients will be needed to confirm or exclude an associated cancer risk in Kabuki syndrome.

Since *KDM6A* is located on the X-chromosome, we wondered about a potential connection to Kabuki-like Turner syndrome. A small proportion of patients with Turner syndrome, and especially of those with a derivative X-chromosome, have been described in the literature to present with facial features reminiscent of Kabuki syndrome [Bögershausen and Wollnik, 2013 and references therein], and also the patients described by Lederer et al. [2012], carrying larger deletions of *KDM6A*, have overlapping features with Kabuki-like Turner syndrome. We asked whether patients with Kabuki-like Turner syndrome might have modifying variants within *KDM6A* or a submicroscopic chromosomal aberration in addition to the missing X-chromosome.

However, screening of six unrelated Turner syndrome patients with Kabuki-like features did not identify any sequence variants of *KDM6A* that might account for the peculiar phenotype. Neither did the SNP array analyses in three patients reveal any additional chromosomal aberrations or a shared X-chromosomal abnormality. Thus, the cause of the Kabuki-like features in these patients with Turner syndrome remains unclear. Clinically, both syndromes constitute important differential diagnoses in girls with Kabuki-like facial features and short stature, which may be hard to distinguish. We noted earlier that the facial features in Kabuki-like Turner syndrome tend to be coarser than in true KS [Bögershausen and Wollnik, 2013]. Multiple lentigines may also point towards Kabuki-like Turner syndrome and warrant karyotyping before the initiation of the molecular analysis of the KS genes.

KDM6A escapes X-inactivation [Greenfield et al., 1998; Miyake et al., 2013b]. It has been hypothesized that *KDM6C* (*UTY*), the Y-chromosome homologue of *KDM6A*, may compensate for the loss of the single *KDM6A* copy in male patients with X-linked KS2. A recent study could now show that, contrary to prior reports [Agger et al., 2007; Hong et al., 2007], KDM6C does indeed catalyze demethylation of histone 3 lysine 27 [Walport et al., 2014], a finding that supports the assumed functional redundancy of KDM6A and KDM6C, making *KMD6C* an interesting candidate gene for KS in male patients. Lederer et al. [2012] previously reported a mutation screening of *KDM6C* in 15 *KMT2D* mutation-negative patients, which did not identify any disease-causing mutations. Neither did our screening of 15 unrelated male KS patients reveal a causative mutation. X-Inactivation in female patients seems to be independent of *KDM6A* mutation status, as shown by Miyake et al. [2013b]. X-Inactivation was determined in one of our patients (P5) and, in reference to an assumed cut-off of 90%:10%, did not appear skewed with 78%:22%.

CLINICAL RELEVANCE

The identification of the second Kabuki syndrome gene, *KDM6A,* has allowed defining two subgroups of the disorder by molecular genetic criteria. The question remains whether the two subtypes can also be distinguished by clinical criteria. For this study, the clinical details of eleven patients with KS2 were analyzed and compared with the literature (Table 3; Figure 3, Figure 4): Renal abnormalities have been reported to appear in approximately 40% of patients with KS1 [Bögershausen and Wollnik, 2013]. In this study we observed a renal malformation in three patients (= 27%): P210 has ureteral duplication and hydronephrosis and P210 has a horseshoe kidney, the exact type of malformation was not documented in P219. Miyake et al. [2013b] reported that all of their patients with KS2, but only half of their patients with KS1 showed short stature. We have reported short stature to be present in 58% and microcephaly to appear in 29% to 56% of patients with KS1 [Bögershausen and Wollnik, 2013]. Interestingly, four of our patients with KS2 were of short stature (36%) and five had microcephaly (45%), indicating that postnatal growth retardation appears at comparable frequencies in both KS subtypes. Miyake et al. [2013b] also noted that arched eyebrows, fifth finger brachydactyly, and hypotonia in infancy were more frequent in individuals with KS1 than in individuals with KS2. However, 9/11 patients with KS2 in this study had a combination of at least seven typical facial features (Table 3). 8/11 had arched eyebrows, and we noted the eyebrows to be rather bushy in most of them (Figure 3). 8/11 even had the typical eversion of the lower eyelid. Thus, in our study the facial phenotype of KS2 appeared quite classical. Hypotonia in infancy and feeding difficulties were each observed in 9/11 patients. Fifth finger brachydactyly and fifth finger clinodactyly were seen in 7/11 and 6/11 patients, respectively. The rate of congenital heart disease (CHD) in this cohort was similar to the reported frequency in KS1 (40-50%) [Bögershausen and Wollnik, 2013]. We observed CHD in 4 out of 11 patients: Septal defects in three, and coarctation of the aorta in one patient. One patient had a bicuspid aortic valve and one had left ventricular hypertrophy in addition (Table 3).

Interestingly, not all of our patients presented with intellectual disability (10/11 patients), whereas all of the mutation-positive patients in the studies of Miyake et al. [2013b] and Banka et al. [2015] had some degree of intellectual disability. The finding of an intellectually normal female patient with KS2 is in line with the observation of Lederer et al. [2012], who described two mentally normal females, whose male offspring presented with intellectual disability.

Banka et al. [2015] suggested that neonatal hypoglycemia may be more frequent among the KS2 patient group, and indeed, this complication was observed in 5/10 patients in this cohort.

Long incisors and long great toes have been proposed as hallmark features of KS2 [Banka et al., 2015; Lederer et al., 2012], but neither could be observed in our patients (Table 3). The former may, however, still develop with secondary dentition. A long first toe was also seen in the patient reported by Yang et al. [2016], who had a 227 kb deletion of chromosome X including exons 1 and 2 of *KDM6A*. Thus, a long great toe, initially described by Lederer et al [2012], may be an indicator of a *KDM6A* exonic deletion.

The most consistent features observed among our patients with KS2 (long palpebral fissures, large, prominent ears, persistent fetal finger pads, and intellectual disability (Figure 3, Table 3)) are also among the key clinical features that mark KS1. Summing up, we could identify no clinical features specific for KS2 or KS1, which would allow distinguishing the two subtypes clinically. Consequently, the classical diagnostic approach should be based on the frequency of detected mutations and should thus entail Sanger sequencing of *KMT2D*, followed by Sanger sequencing of *KDM6A*, followed by MLPA for both genes and/or high resolution array-CGH. While MLPA may be more sensitive and detect small gains or losses of genetic material, array-CGH would allow the simultaneous detection of differential diagnoses. In view of the large number of exons (54 + 29 = 83), a next-generation-sequencing (NGS) panel or exome sequencing, in combination with array-CGH or MLPA represents a more up-to-date and cost-effective approach. However, an NGS strategy might not yet be possible for routine diagnostics

in some countries, because the NGS techniques may presently not be reimbursed by health insurances.

GENOTYPE-PHENOTYPE CORRELATIONS

The small number of published patients with *KDM6A* mutations does not yet allow establishing solid genotype-phenotype correlations with regard to mutation type or location. Reviews of the published patient cohorts and our own clinical experience have taught us that no valid genotype-phenotype correlations yet exist for *KMT2D*-associated Kabuki syndrome subtype 1. Miyake et al. [2013b] proposed that the facial phenotype might be less pronounced in patients with non-truncating versus truncating *KMT2D* mutations. However, of the patients whose pictures are shown, the two patients with the least typical facial phenotype (namely KMS-02 and KMS-91) carry sequence variants of *KMT2D* that we judged to be either non-disease-causing or of unknown significance according to our classification system. These patients might thus have been misdiagnosed. The other three patients with non-truncating mutations (KMS-42, KMS-56, and KMS-58) carry disease-causing *de novo* missense mutations and they show a rather typical facial phenotype. In our initial study [Li et al., 2011], we also observed that the facial phenotype can even be quite unremarkable in patients with truncating *KMT2D* mutations. Thus, the impression that the facial phenotype is less typical in patients with non-truncating mutations is not necessarily true. In general, the recognition of the typical facial features may also depend on the age at clinical presentation. We and others [Banka et al., 2012; Bögershausen and Wollnik, 2013] noted that the facial features may be hard to distinguish in the neonatal period and in adulthood, while they are most striking in toddlers and children in the school age (Figure 4).

Furthermore, sex-specific phenotypic differences between male and female patients with pathogenic *KDM6A* mutations have been proposed. The only female patient in the study of Miyake et al. [2013a] showed a much milder phenotype than the two male patients; however,

she had a 3-bp in-frame deletion, while the male patients carried truncating mutations. Banka et al. [2015] observed in their study that the intellectual disability was more profound in male patients. We can confirm this finding, but would like to add that the mutation type might also play a role for expressivity: We identified the frameshifting mutation c.2226_2227dupCA, p.(Ser743Thrfs*13) in exon 17 of *KDM6A* in a male patient (P213) with a convincing facial phenotype, and severe intellectual disability, muscular hypotonia and feeding problems. At age 10 years he could neither walk nor speak and was severely cachectic in spite of hypercaloric feeding (Table 3). Our female KS2 patients on the other hand showed a rather mild phenotype with mild to moderate intellectual disability and a low frequency of organ malformations. Only patient P212, carrying an N-terminal truncating mutation, showed cortical atrophy and white matter anomalies on cranial MRI in addition to seizures and intellectual disability, i.e., a severe manifestation. On the other hand, patient P216, who carries a *de novo* missense mutation in exon 26, shows normal cognitive capacities and development, except for a mild motor delay in the second year of life. This also indicates that, apart from sex, the functional effect of the respective mutations might be a modulator of disease severity.

Another male patient (P214), who carried the hemizygous *KDM6A* missense mutation c.2729A>G, p.(Asn910Ser), presented with some, but not all of the classic KS facial features. He had intellectual disability and bilateral cleft lip/palate, but no heart or renal malformations. His mother carries the mutation in the heterozygous state. At presentation she appeared unaffected. Unfortunately, she was not available for clinical reevaluation. Lederer et al. [2014] reported a three-generation family with two affected boys whose mother and maternal grandmother were both carriers of a truncating *KDM6A* mutation and showed only few features reminiscent of KS but not the typical KS phenotype. Lederer et al. [2014] argued in the direction of a more pronounced phenotype in male patients, especially with regard to facial features and cognitive achievements, an observation also made by Banka et al. [2015]. The fact that patient P214 inherited the *KDM6A* mutation from his seemingly unaffected mother also argues in favor

of reduced expressivity or even reduced penetrance of the KS2 phenotype in females. In consequence, female mutation carriers with mild phenotypes might be undetected until they give birth to an affected son. Further studies are needed to confirm this hypothesis.

ANIMAL MODELS FOR KDM6A

According to Welstead et al. [2012], *Kdm6a* knock-out (KO) mice show a reduced number of somites, neural tube defects and heart malformations that cause midgestation lethality. Interestingly, female homozygous KO embryos were more severely affected than hemizygous males, indicating a partial compensation of *Kdm6a* loss by *Kdm6c* (*UTY*). Thieme et al. [2013] recently generated a conditional KO mouse model and showed that *Kdm6a* is responsible for stem cell migration and hematopoiesis. Adult conditional KO female mice showed myelodysplasia, while males did not, supporting the mentioned role of *Kdm6c.* Wang et al. [2012] also observed notochord, cardiac and hematopoietic abnormalities in *Kdm6a* KO mice with survival until birth in males and midgestation lethality in females. Lee et al. [2012] could show that Kdm6a promotes a developmental program that is essential for heart development by inducing chromatin changes at cardiac-specific enhancers. They could show that *Kdm6a* KO mice exhibit heart defects and embryonic lethality. Work on *Kdm6a* KO embryonic stem cells (ESCs) has shown that KDM6A has functions related and unrelated to H3K27 demethylase activity and is required for the induction of ecto- and mesoderm during differentiation as well as epigenetic reprogramming [Mansour et al., 2012; Morales Torres et al., 2013]. In the zebrafish, loss of *kdm6a* leads to craniofacial and brain defects [Lindgren et al., 2013; Van Laarhoven et al., 2015; Bögershausen et al., 2015]. Interestingly, morpholino knock-down (MO) of the established Kabuki syndrome genes *kmt2d* and *kdm6a* as well as of the novel causative genes *rap1a* and *rap1b* cause similar craniofacial abnormalities, and zebrafish morphants for *kmt2d* and *rap1*, as well as *Kmt2d* knock-out mice show aberrations of the MAPK signaling pathway [Bögershausen et al., 2015].

CONCLUSIONS AND PROSPECTS

In summary, we expand the known clinical and molecular spectrum of the new Kabuki syndrome subtype KS2 and add to the mutation spectrum of KS1. We were able to confirm that female patients with KS2 may have a rather mild manifestation of Kabuki syndrome and may even develop normally with regard to cognitive function. Phenotypic features that might allow distinguishing between the Kabuki syndrome subtypes could not be defined. Therefore, molecular genetic testing should be performed by order of frequency in case of a Sanger sequencing approach or, if possible, by next generation sequencing. We hypothesize that screening of larger cohorts might still identify very rare mutations in *KDM6C*. Future studies applying modern sequencing technologies in large cohorts will most likely identify additional causative genes for Kabuki syndrome, as we have recently demonstrated by the identification of *RAP1A* and *RAP1B* [Bögershausen et al., 2015].

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DISCLOSURE STATEMENT

The authors have no conflict of interest to declare.

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LEGENDS

Figure 1. Overview of mutation type and exon distribution of *KMT2D* and *KDM6A* mutations. **A,** Mutation types of previously published and newly identified disease-causing mutations in *KMT2D*. Recurrent mutations were counted by times of reports, thus n corresponds to the number of patients with the reported mutation type. **B,** Mutation types of all previously published and newly identified disease-causing mutations in *KDM6A*. Recurrent mutations were counted by times of reports, thus n corresponds to the number of patients with the reported mutation type. **C,** Exon distribution of the previously published and newly identified disease-causing point mutations in *KMT2D*, including recurrent mutations. Mutations that affect more than one exon, i.e. large deletions/duplications, were excluded. N = number of mutations, MLS = mutation load score. The red line indicates the MLS cut-off. **D,** Exon distribution of the previously published and newly identified disease-causing mutations in *KDM6A* including recurrent mutations. Mutations that affect more than one exon, i.e. large deletions/duplications, were excluded. N = number of mutations, MLS = mutation load score.

Figure 2. Overview of identified *KDM6A* mutations relative to a schematic representation of the *KDM6A* gene and KDM6A protein structure.

Figure 3. Clinical characteristics of patients with KS type 2. **A,** Facial features of patients P209, P210, P214, P216, P219 and P220: Note the typical facial features with long palpebral fissures, arched and nicked eyebrows, prominent ears, a depressed nasal tip, and downslanting corners of the mouth. Note repaired cleft lip/palate in P3. **B,** Lateral views of patients P209, P210, P214, and P219. Characteristic features such as large or dysplastic ears, long palpebral fissures and a depressed nasal tip, might be more readily appreciable from the side. **C,** Hands of patients P209, P210, P214, P211, P216, and P219: Note persistent fetal finger pads. P209 shows a

simian crease on the left and 5th finger clinodactyly (pictures are from newborn period). P210 shows 5th finger brachy- and clinodactyly. P214 shows a distally placed thumb on the left hand and 5th finger clinodactyly on both. Patients P210, P211, and P219 show relatively thick thumbs.

Figure 4. Facial features of patient P211 over the time span of 6 years: as a newborn, at 2.5 and at 6 years of age (y = years). Note how the typical facial features are hardly visible in the newborn period but become more pronounced with increasing age.

Mutation Update for Kabuki syndrome genes *KMT2D* and *KDM6A* and further delineation of X-linked Kabuki syndrome subtype 2

Nina Bögershausen[1,2], Vincent Gatinois[2,3,4], Gökhan Yigit[1,2], Vera Riehmer[2]Riehmer[5], Hülya Kayserili[3]Kayserili[6], Jutta Becker[2]Becker[5], Michaela Thoenes[2]Thoenes[5], Pelin Özlem Simsek-Kiper[4]Kiper[7], Mouna Barat-Houari[2,4], Nursel H. Elcioglu[5]Elcioglu[8], Dagmar Wieczorek[6]Wieczorek[9], Sigrid Tinschert[7]Tinschert[10,8,11], Guillaume Sarrabay[2,3,4], Tim M. Strom[9]Strom[12,10,13], Aurélie Fabre[2], Gareth Baynam[11]Baynam[14,12,15,13,16,14,17], Elodie Sanchez[4], Gudrun Nürnberg[15]Nürnberg[18], Umut Altunoglu[16]Altunoglu[19], Yline Capri[20], Bertrand Isidor[21], Didier Lacombe[22], Carole Corsini[3,4,23], Valérie Cormier-Daire[24,25], Damien Sanlaville[26], Fabienne Giuliano[27], Kim-Hanh Le Quan Sang[24], Honorine Kayirangwa[24], Peter Nürnberg[15]Nürnberg[18], Thomas Meitinger[9]Meitinger[12,10,13], Alain Verloes[17], Koray Boduroglu[4]Boduroglu[7], Barbara Zoll[1], Stanislas Lyonnet[24,25], Andreas Tzschach[7]Tzschach[10], Alain Verloes[20], Nataliya Di Donato[7]Donato[10], Isabelle Touitou[2,3,4], Christian Netzer[2]Netzer[5], Yun Li[1], David Geneviève[3,4,23], Yun Li[1,2], Gökhan Yigit[1], and Bernd Wollnik[1,2]

[1]Institute of Human Genetics, University Medical Center Goettingen, Goettingen, Germany; [2]Institute of Human Genetics, University of Cologne, Cologne, Germany; [2]Laboratory of Rare and Autoinflammatory Diseases, CHU Montpellier, Montpellier, France; [3]University of Montpellier, Montpellier, France; [4]INSERM UMR1183, Montpellier, France; [5]Institute of Human Genetics, University of Cologne, Cologne, Germany; [3]Medical [6]Medical Genetics Department, Koç University School of Medicine (KUSOM) İstanbul, Turkey; [4]Pediatric [7]Pediatric Genetics Unit, Department of Pediatrics, Hacettepe University Medical Faculty, Ankara, Turkey; [5]Department [8]Department of Pediatric Genetics, Marmara University Medical Faculty, Istanbul, Turkey; [6]Institute [9]Institute of Human Genetics, University of Duesseldorf, Duesseldorf, Germany; [7]Institute [10]Institute for Clinical Genetics, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Germany; [8]Zentrum [11]Zentrum für Humangenetik, Medizinische Universität Innsbruck, Austria; [9]Institute [12]Institute of Human Genetics, Technische Universität München, Munich, Germany; [10]Institute [13]Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany; [11]Genetic [14]Genetic Services of Western Australia, Princess Margaret and King Edward Memorial Hospitals, Perth, Australia; [12]Western [15]Western Australian Register of Developmental Anomalies, Perth Australia; [13]Telethon [16]Telethon Kids Institute, Perth Australia; [14]School [17]School of Paediatrics and Child Health, University of Western Australia, Perth, Australia; [15]Cologne [18]Cologne Center for Genomics, University of Cologne, Cologne, Germany; [16]Department [19]Department of Medical Genetics, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey; [17]Department [20]Department of Genetics, APHP-Robert DEBRE University Hospital, Paris VII University, Denis Diderot Medical School, Paris, France; [21]Department of Genetics, Nantes University Hospital, Nantes, France; [22]CHU Bordeaux, INSERM U1211, Bordeaux University, Department of Medical Genetics, Bordeaux, France; [23]Department of

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Medical Genetics, Reference Center for Developmental Abnormalities, CHU, Montpellier, France; [24]Institut Imagine, INSERM U1163, Paris Descartes-Sorbonne Paris Cité University, Paris, France; [25]Service de Génétique, Hôpital Universitaire Necker-Enfants Malades, Assistance Publique - Hôpitaux de Paris, Paris, France; [26]HCL Genetic department, INSERM U1028 CNRS UMR 5292, UCBL1, CRNL, GENDEV Team, Lyon, France; [27]Department of Medical Genetics, l'Archet II Hospital, Nice, France.

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Corresponding author: Prof. Bernd Wollnik, MD
Institute of Human Genetics
University Medical Center Göttingen
Heinrich-Düker-Weg 12,
37073 Göttingen, Germany
Tel: +49-551-39-7590 Fax: +49-551-39-9303
EMail: bernd.wollnik@med.uni-goettingen.de

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ABSTRACT

Kabuki syndrome (KS) is a rare but recognizable condition that consists of a characteristic face, short stature, various organ malformations and a variable degree of intellectual disability. Mutations in *KMT2D* ~~has~~ have been identified as the main caus~~ativ~~e ~~gene~~ for KS, while mutations in *KDM6A* are a much less frequent cause ~~of KS~~. Here, we report a mutation screening in a case series of 347 unpublished patients, in which we identified ~~we report six~~ 12 novel ~~-~~ *KDM6A* mutations (KS type 2) and ~~in *KDM6A* (KS type 2) and~~ ~~44~~ 208 mutations in *KMT2D* (KS type 1), 132 of them novel ~~in a case series of 98 unpublished patients~~. Two of the *KDM6A* mutations were maternally inherited and 9 were shown to be *de novo*. ~~We also review all published mutations in both genes and point out possible mutation hot spots and strategies for molecular genetic testing.~~ We give an up-to-date overview of all published mutations for the two Kabuki syndrome genes and point out possible mutation hot spots and strategies for molecular genetic testing. We also report the clinical details for 11 patients with KS type 2. ~~We~~ summarize the published clinical information, specifically with a focus on the less well defined X-linked KS type 2, and comment on phenotype-genotype correlations as well as sex-specific phenotypic differences. ~~Moreover, we present the second instance of a maternally inherited *KDM6A* mutation with probable reduced penetrance in the mother.~~ Finally, we also discuss a possible role of *KDM6A* in Kabuki-like Turner syndrome and report a mutation screening of *KDM6C* (*UTY*) in male KS patients.

Key words: Kabuki syndrome, *KDM6A, MLL2, KMT2D, UTY, KDM6C*

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BACKGROUND

Kabuki syndrome (KS) is a rare ~~intellectual disability/multiple malformation~~genetic syndrome that is characterized by postnatal growth retardation, mild to moderate intellectual disability, organ malformation, endocrinological and hematological abnormalities in combination with very recognizable facial features. It is mainly caused by heterozygous mutations in lysine (K)-specific methyltransferase 2D (*KMT2D;* formerly *MLL2*; MIM 602113; NM_003482.3) . ~~–~~Approximately 56% to 75% of Kabuki syndrome cases are caused by mutations in *KMT2D* [Ng et al., 2010; Hannibal et al., 2011; Li et al., 2011; Bögershausen and Wollnik, 2013]. *KMT2D* encodes a methyltransferase responsible for histone 3 lysine 4 (H3K4) di- and trimethylation, which is an epigenetic mark for euchromatin and active transcription [Issaeva et al., 2007; Smith et al., 2011]. The H3K4 methyltransferases (KMT2 group, also called trithorax group) act in multi-protein complexes that contain various shared and some distinct components that contribute to the specific function of each complex [Smith et al., 2011]. One important component of the KMT2D containing complex (called ASCOM) is KDM6A, a H3K27 demethylase responsible for removal of repressive polycomb-derived methylation marks [Agger et al., 2007; Hong et al., 2007]. Whole-gene and intragenic deletions as well as point mutations in lysine (K)-specific demethylase 6A (*KDM6A;* formerly *UTX*; MIM 300128; NM_021140.3) have been identified in patients with KS, which led to the definition of two subtypes of KS: *KMT2D*-associated, autosomal-dominant Kabuki syndrome type 1 (KS1) and *KDM6A*-associated, X-linked-dominant Kabuki syndrome type 2 (KS2). Several mutation screening studies have revealed that mutations in *KDM6A* account for approximately 5 to 8% of Kabuki syndrome cases [Banka et al., 2015; Cheon et al., 2014; Dentici et al., 2015; Micale et al., 2014; Miyake et al., 2013b]. Very recently, we reported mutations in the genes *RAP1A* (MIM 179520) and *RAP1B* (MIM 179530) as novel rare causes of Kabuki and Kabuki-like syndromes [Bögershausen et al., 2015]. Furthermore, a homologue of *KDM6A* called *KDM6C* (*UTY*; MIM 400009; NM_182660.1),

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another H3K27 demethylase, is located on the Y-chromosome [Walport et al., 2014] and constitutes a possible candidate gene for Kabuki syndrome in male individuals.

In this study, we collected a cohort of ~~98~~ 347 unpublished patients with a clinical diagnosis of Kabuki syndrome and screened them for mutations in *KMT2D* and subsequently in *KDM6A*. ~~44~~ 208 patients in our cohort harbored mutations in *KMT2D*. Of the *KMT2D* negative patients, ~~and in~~ one received whole exome sequencing and ~~the~~ 88 ~~patients negative for *KMT2D*~~ received Sanger sequencing of *KDM6A*, ~~mutations~~ by which we identified ~~six~~ twelve novel *KDM6A* mutations. We discuss the molecular and clinical findings and compare them to the literature with a focus on the rare X-linked KS2. We also report a mutation screening of *KDM6C* (*UTY*) in male patients, which did not identify any mutations, and discuss Kabuki-like Turner syndrome as an important differential diagnosis for female patients.

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METHODS

Patients

We obtained written informed consent from all patients or their legal guardians for the molecular genetic analyses and for publication of the results. We obtained written informed consent for publication of photographs from the concerned parties. The study was performed according to the Declaration of Helsinki protocol. Blood samples were collected from the patients and their parents and DNA was extracted from peripheral blood lymphocytes by standard extraction procedures. Patient IDs presented in this publication were assigned arbitrarily by order of mutations and do not relate to the identity of the patients.

Whole-exome sequencing

Exonic and adjacent intronic regions were enriched from genomic DNA of one patient (P1) and her parents using the 50 Mb SureSelect XT Human All Exon enrichment kit from Agilent Technologies (Santa Clara, USA) and sequencing was performed on a GAIIx sequencer from Illumina (Illumina, San Diego, USA). Alignment against the GRCh37 human reference was performed with Burrows-Wheeler Aligner (BWA, version 0.6.2), PCR-duplicates marking with Picard (version 1.84), indel realignment, base quality recalibration and variant calling with the Genome Analysis Toolkit (GATK, version 2.3-4), and annotation with Annovar (version 2013Feb21). The resulting variants were filtered to exclude variants present in dbSNP 135, the Exome Variant Server, the 1000 Genomes Project, or our in-house database and variants that were not predicted to affect protein sequence or exon splicing (please see prediction programs and databases for URLs). For *de novo* analysis, all variant loci in the patient's dataset were compared to the parental datasets. Only variants covered in all three samples and present in less than 5% of the reads in the parental datasets were considered.

Mutation screening and Sanger sequencing

Mutation screenings were performed using standard methods for PCR amplification and Sanger sequencing. Primer sequences for *KDM6A and KMT2D* were designed with the primer 3 software, available at the UCSC genome browser, or the primer 3 webtool (http://primer3.ut.ee/). Specific primers for *KDM6C* (*UTY*) were custom-designed using the Oligo® software (Molecular Biology Insights, Cascade, USA) in order to avoid amplification of the highly homologous *KDM6A* gene. Primer sequences are available on request. The entire coding sequence of the respective genes was analyzed and mutations were confirmed by a second PCR on an independent DNA solution.

Identified mutations were classified as disease causing if they were 1.) either truncating or predicted to be deleterious (see below), or 2.) proven to be *de novo* or already published as *de novo* in another patient with Kabuki syndrome, and 3.) absent from the current databases of normal genetic variation (EVS, ExAC, dbSNP). Variants of unknown significance were defined as variants that were 1.) non-truncating, 2.) predicted to be deleterious, and 3.) absent from the current databases of normal genetic variation (EVS, ExAC, dbSNP) but for which *de novo* occurrence could not be proven. Non-disease-causing variants were defined as variants that were 1.) inherited from a healthy parent and/or 2.) annotated in a database of normal genetic variation (EVS, ExAC, dbSNP). Non-disease-causing variants (polymorphisms) identified in our cohort are not reported in this study.

De novo occurrence of the *KDM6A* mutation identified by whole-exome sequencing in patient P1 was confirmed by Sanger sequencing of the specific exon according to standard methods.

Current HGVS standard was employed for mutation nomenclature. Nucleotide numbering referring to cDNA uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1. Mutation nomenclature was double checked with the Mutalyzer software: https://mutalyzer.nl/.

Novel variants were submitted to the locus specific databases at LOVD: www.lovd.nl/KDM6A www.lovd.nl/KMT2D.

SNP array

SNP arrays were performed in three patients with cytogenetically diagnosed Turner syndrome who presented with a Kabuki-like phenotype: one patient with a 45,X, one patient with a 45,X/46,X,i(Xq), and one patient with a 45,X/46,X,r(X) karyotype. We employed the Affymetrix genome-wide Human SNP Array 6.0 utilizing more than 906,600 SNPs and more than 946,000 probes for the detection of copy number variations. Quantitative data analysis was performed with GTC 4.1 (Affymetrix Genotyping Console) using a reference file of ATLAS Biolabs GmbH (100 samples). We used the Segment Reporting Tool (SRT) to locate segments with copy number changes in the copy number data with the assumption of a minimum of 10 kb per segment and minimum genomic size of five markers of a segment.

Prediction programs

Prediction of the mutation effect was performed for missense mutations and in-frame deletions with the programs ~~PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/),~~ PROVEAN (http://provean.jcvi.org/index.php), SIFT (http://sift.jcvi.org/), and Mutation Taster (http://www.mutationtaster.org/). The effect of splice site mutations was analyzed with Human Splicing Finder version 3 (http://www.umd.be/HSF3/) and ~~BDGP splice site prediction (http://www.fruitfly.org/seq_tools/splice.html)~~Mutation Taster. Please see Supp. Table 3 and Supp. Table 4 for in-silico prediction output.

Databases

The following databases were used for this study: The Exome Aggregation Consortium (ExAC): http://exac.broadinstitute.org/; The Exome Variant Server (EVS):

http://evs.gs.washington.edu/EVS/; Database of human single nucleotide Polymorphisms (dbSNP): http://www.ncbi.nlm.nih.gov/projects/SNP/; The 1000 Genomes: http://www.1000genomes.org/; HGMD: http://www.biobase-international.com/product/hgmd; The UCSC browser: http://genome.ucsc.edu/; The human protein reference database: http://www.hprd.org/; COSMIC: http://cancer.sanger.ac.uk/cosmic; DECIPHER: https://decipher.sanger.ac.uk/; PubMed: http://www.ncbi.nlm.nih.gov/pubmed/.

Literature review

We searched the HGMD database for mutations in *KMT2D* and *KDM6A* and, additionally, conducted a search for further mutations described in original articles in PubMed using the terms "Kabuki syndrome", "*MLL2* mutation", and "*KMT2D* mutation" in different combinations. We examined the clinical and molecular information available from the retrieved 20 mutation screening studies [Banka et al., 2012; Cheon et al., 2014; Courcet et al., 2013; Dentici et al., 2015; Hannibal et al., 2011; Li et al., 2011; Lin et al., 2015; Lindgren et al., 2013; Lindsley et al., 2015; Liu et al., 2015; Makrythanasis et al., 2013; Micale et al., 2011; Micale et al., 2014; Miyake et al., 2013; Morgan et al., 2015; Ng et al., 2010; Paderová et al., 2016; Paulussen et al., 2011; Subbarayan et al., 2014; Van Laarhoven et al., 2015] and 18 molecularly proven case reports [Brackmann et al., 2013; Cappuccio et al., 2014; Gohda et al., 2015; Karagianni et al., 2016; Kim et al., 2013; 2016; Kokitsu-Nakata et al., 2012; McVeigh et al., 2015; Ratbi et al., 2013; Riess et al., 2012; Roma et al., 2015; Schulz et al., 2014; Soden et al., 2014; Takagi et al., 2014; Tanaka et al., 2012; Verhagen et al., 2014; Yuen et al., 2015; Zaidi et al., 2013; Zarate et al., 2012]. Only articles that were fully available online were included in the analysis. However, to ensure a consistent genotype-phenotype analysis, we did not consider any case reports from before the identification of *KMT2D* as the first causative gene. We evaluated all published mutations in *KMT2D* (~~Supplementary~~Supp. Table 1) and *KDM6A*

(~~Supplementary~~Supp. Table 2) and assigned them to three variant classes: disease-causing variant (DC), variant of unknown significance (VUS), or non-disease-causing variant (NDC). According to our classification, a disease-causing (DC) variant must fulfil the following criteria: It is either a truncating variant or a non-truncating variant that was proven to be *de novo* or has been described as *de novo* in another patient with a comparable phenotype and it is not listed in any public database of normal genetic variation. A variant of unknown significance (VUS) is a non-truncating sequence alteration with unknown inheritance, which is not present in any public database of normal genetic variation (such as the ExAC browser, the dbSNP database, the 1000 Genomes, or the Exome variant server, see ~~above~~databases) and ~~which is~~preferably predicted to be disease causing by ~~the~~ at least one prediction ~~programs~~ algorithm (see ~~above~~Supp. Table 3, Supp. Table 4), however the last criterion is not requisite if a variant is absent from all databases. Finally, a variant will be classified as a non-disease-causing (NDC) variant if it is a non-truncating variant, the inheritance of which is unknown or which was inherited from an unaffected parent, and/or which is listed in public databases (see above), and/or if the same patient additionally carries a separate variant that is judged as disease causing.

Mutation load score

To evaluate the mutation load of a single exon as a function of its size, we established a mutation load score (MLS), calculated as the number of mutations (n) divided by the number of basepairs (bp) of an exon, multiplied by 100 (MLS = $\frac{n}{bp} \cdot 100$). The score was calculated for disease-causing variants identified by literature review and our own study, and the numbers include recurrent mutations. Mutations affecting more than one exon, i.e. large deletions/duplications, were excluded from the calculation. Mutations affecting splice sites were allocated to the ~~closest~~ corresponding exon (i.e. intron 2 = exon 2). A score of 1 equals 1

mutation per 100 bp. For *KMT2D* we retrieved an average MLS of ~~2.94~~3.74, with a standard deviation (SD) of ~~2.49~~3.80. According to the expected normal distribution, a ~~score~~ MLS > ~~MLS~~ mean + 2 SD (= ~~7.92~~11.33) was regarded as the cut-off for an unexpectedly high mutation load. For *KDM6A* we obtained an average MLS 0.~~62~~82~~,~~ +/- a standard deviation of 1.~~07~~08, and a cut-off of 2.~~76~~98. However, the small number of known mutations in this gene impedes the interpretation of this result, which is therefore only exemplary.

PATIENT COHORT

The present cohort consists of ~~98~~ 347 patients with a tentative diagnosis of Kabuki syndrome, established by external clinicians, from different referral centers. It includes patients from Germany, France, Turkey, and Australia. The DNAs were sent to our ~~laboratory~~ laboratories in Cologne and Montpellier with a request for molecular genetic analysis of the Kabuki syndrome genes *KMT2D* and *KDM6A*. ~~We started the study in 2012, after we had completed our pilot study~~The patients reported here have not been previously reported elsewhere ~~[Li et al., 2011].~~ . The only patient who had already been included in ~~the~~ our first mutation screening study [Li et al., 2011] is Patient 1 (~~P1~~P212); she was then negative for a mutation in *KMT2D* and we now performed whole-exome sequencing. Four of the patients with *KDM6A* mutations were referred from ~~-~~Turkish centers (P21~~1~~2, ~~P3~~P214, ~~P4~~P216, ~~P6~~P220) and two came from German centers (P209 and ~~P5~~P211), ~~with one (P5~~P211~~)~~ being of Turkish descent, and the other six came from France. ~~Patients with *KDM6A* mutations were not preselected according to clinical criteria and did not obviously differ from the overall cohort.~~ Five patients with Kabuki-like Turner syndrome originated from Turkey and one from Australia. They had already been cytogenetically diagnosed and were referred due to their striking clinical overlap with Kabuki syndrome. Of the *KMT2D* negative patients, one received whole exome sequencing and 88 received Sanger sequencing of *KDM6A*. Clinical details were available for 11 patients with KS2, unfortunately we

were unable to obtain clinical details for patient P215, as well as the mothers of patients P214 and P215.

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IDENTIFIED *KMT2D* MUTATIONS

Sanger sequencing of all coding exons and exon-intron boundaries of *KMT2D* in ~~98~~ 347 patients with a tentative diagnosis of Kabuki syndrome identified ~~44~~ 208 mutations (Table 1), ~~24~~ 132 of which have not been reported before ~~(Table 1), while 20 were recurrent (Table 2)~~. We identified ~~16~~ 76 nonsense mutations, ~~14~~ 69 small deletions/duplications, ~~8~~ 45 missense variants, 15 splice site mutations, and ~~one~~ 3 in-frame deletions. *De novo* occurrence was proven if parental DNA was available (n = ~~28~~103). Three patients had inherited the mutation from an affected parent.

The mutations c.166C>T, p.(Gln56*); c.6295C>T, p.(Arg2099*); c.7903C>T, p.(Arg2635*); c.8200C>T, p.(Arg2734*); c.11944C>T, p.(Arg3982*); c.12592C>T, p.(Arg4198*); c.13450C>T, p.(Arg4484*); c.14710C>T, p.(Arg4904*); c.14946G>A, p.(Trp4982*); c.15079C>T, p.(Arg5027*); c.16501C>T, p.(Arg5501*); c.4135_4136delAT, p.(Met1379Valfs*52); c.5627_5630delACAG, p.(Asp1876Glyfs*38); c.16489_16491delATC, p.(Ile5497del); c.4267C>T, p.(Arg1423Cys); c.15142C>T, p.(Arg5048Cys); c.15143G>A, p.(Arg5048His); c.15461G>A, p.(Arg5154Gln); c.15536G>A, p.(Arg5179His); c.15536G>T, p.(Arg5179Leu); c.15640C>T, p.(Arg5214Cys); c.16273G>A, p.(Glu5425Lys) were found in two or more patients (Table 1). The most frequent mutation was c.15142C>T, p.(Arg5048Cys) in exon 48 which was identified in 5 patients, followed by c.6295C>T, p.(Arg2099*) and c.15079C>T, p.(Arg5027*), which were found in 4 patients each.

192 mutations identified in this study could be classified as disease causing (DC). 16 mutations were classified as variants of unknown significance (VUS) due to lack of parental samples for segregation analysis. These were mostly novel, non-truncating mutations, which were predicted

to be damaging and absent from the queried databases of human genetic variations (for details on in-silico prediction for *KMT2D* missense mutations and in-frame deletions please refer to Supp. Table 3). Non-disease causing variants (polymorphisms) identified in our patients are not reported.

~~Non truncating mutations were located in the important domain coding exons 48 to 53, which encode the FYRN, FYRC, and SET domains of KMT2D, except for one missense mutation in exon 28 (c.6109G>C, p.(Asp2037His)). This mutation is not listed in the current databases of normal genetic variation (EVS, ExAC, dbSNP), is annotated as an oncogenic mutation in the COSMIC database (COSM4109565), and was predicted to be damaging by four prediction programs (Mutation Taster, PolyPhen 2, SIFT, PROVEAN). *De novo* occurrence could not be proven for this mutation due to lack of parental DNA. This is thus the only variant identified in this study that we classified as a variant of unknown significance (VUS). Known non disease-causing variants identified in our cohort are not reported.~~

PUBLISHED *KMT2D* MUTATIONS

To date, ~~415~~ 424 ~~mutations~~ variants in the *KMT2D* gene have been reported. Except for one patient with autism spectrum disorder and one patient with congenital heart disease, all reported patients with ~~reported~~ *KMT2D* variants ~~mutations~~ had been diagnosed with Kabuki syndrome (~~Supplementary~~Supp. Table 1). Among these ~~415~~ 424 variants ~~mutations~~ were ~~117~~ 121 nonsense mutations, ~~98~~ 106 small deletions, 55 small insertions or duplications, ~~96~~ 93 missense variants~~mutations~~, and ~~37~~ 36 splice site variants~~mutations~~. Additionally, ~~four~~ five indels, six ~~gross~~large deletions (>20 bp), and two ~~gross~~large insertions have been published (~~Supplementary~~Supp. Table 1, Figure 1A).

When we evaluated the reported variants ~~mutations~~ against the above described pathogenicity criteria (mutation type, segregation, prediction, annotation in public databases of normal genetic variation), we assessed ~~39~~ 33 of these variants as non-disease-causing (NDC)

(~~Supplementary~~Supp. Table 1). ~~31~~ 32 variants were judged as VUS (~~Supplementary~~Supp. Table 1), consisting of 24 missense variants, ~~one~~ two non-frameshifting small deletions, one non-frameshifting small insertion, one non-frameshifting ~~gross~~large deletion, and four splice site variants. Segregation analysis would be needed in order to confirm pathogenicity of these variants. We judged ~~345~~ 359 of the reported mutations as disease causing, ~~35~~ 42 of which are recurrent mutations (reported 2 to ~~5~~ 7 times; ~~Supplementary~~Supp. Table 1). The mutation types from our study and the literature are depicted in Figure 1A. We counted each mutation by number of published records (= number of patients) to analyze the exon distribution in detail, and together with the newly identified mutations in this study, we were able to analyze the ~~mutation types and d~~distribution of ~~420~~ 621 disease-causing variants (NDC and VUS excluded) (Figure ~~1A~~1C).

IDENTIFIED *KDM6A* MUTATIONS

Trio whole-exome sequencing (WES) in a *KMT2D* mutation-negative patient (~~P1~~P212) identified the novel one-basepair duplication c.171dupT in exon 2 of *KDM6A*. This mutation leads to a frameshift and a premature stop codon at amino acid position 64: p.(Gly58Trpfs*7). *De novo* occurrence was observed in the WES data sets and subsequently confirmed by Sanger sequencing (~~Figure 2A~~Supplementary Figure 1). Sanger sequencing in ~~43~~ 88 additional patients who were ~~also~~ negative for mutations in *KMT2D* identified ~~five~~ 11 additional ~~mutations~~ variants in *KDM6A* (~~Figure 2A, B~~Figure 2; Table ~~3~~2, Supplementary Figure 1), including ~~two~~ two nonsense mutations, ~~one~~ two small insertions, ~~two~~ three missense variants, and ~~one~~ four splice site mutations. Of the 12 patients with KS2, seven~~affected patients, five~~ are female and ~~one~~ five ~~is~~ are male (Table 2~~P3~~). ~~The~~ Nine ~~five female patients were shown to have~~of the mutations were shown to be ~~*de novo* mutations~~, while two were inherited. ~~the~~One male ~~male~~ patient (P214) had inherited the c.2729A>G, p.(Asn910Ser) ~~mutation~~ variant from his mother (Supplementary Figure 1~~Figure 2A~~), whose phenotype could not be ascertained, and another

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(P215) had inherited the c.3073A>G, p.(Ser1025Gly) mutation from his clinically affected mother. While the boy showed a recognizable Kabuki phenotype, the mother's phenotype was reported to be mild. However, clinical details on this family are unavailable. ~~A KS phenotype of the mother was not remarked at the presentation of her son. The family was lost to follow up, and the mother could not be clinically reevaluated.~~ The mutation in ~~P3~~ P214 affects a ~~highly~~ conserved asparagine residue at position 910 and was predicted to be damaging by the prediction programs Mutation Taster and PROVEAN. Most importantly, it is not annotated in the current databases of normal genetic variation (EVS, ExAC, dbSNP), and it was therefore considered to be most likely disease causing with reduced penetrance. However, according to our classification system, the variant was classified as VUS. The mutation in P215 is also predicted to affect protein function and was absent from the above mentioned databases. Because of the mild Kabuki syndrome phenotype visible in the carrier parent, the mutation was classified as disease causing (for details on in-silico prediction for inherited and *de novo KDM6A* missense mutations please refer to Supp. Table 3). ~~*KDM6A* could not be tested in 10 of our patients, either because we did not receive their consent for *KDM6A* testing or because we did not have sufficient DNA.~~

~~T~~The mutation detection rate for *KDM6A* among the *KMT2D* negative group was ~~was 6.1% in the overall cohort and~~ 13.65% ~~among the *KMT2D* negative patients.~~ .

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PUBLISHED *KDM6A* MUTATIONS

To date, ~~30~~ 33 germline mutations in *KDM6A* have been published. The ~~16~~ 18 published point mutations consist of ~~four~~five nonsense mutations, five small deletions, two missense variants, and ~~five~~ six splice site mutations. Additionally, ~~six~~ seven ~~gross~~large deletions, seven ~~gross~~large duplications/insertions, and one complex genomic rearrangement, have been published (~~Supplementary~~Supp. Table 2). Most of the published *KDM6A* mutations were judged as disease causing according to our classification system. Only the missense variant c.2939A>T,

p.(Asp980Val) published by Micale et al. [2014] and four ~~gross~~large duplications published by Lindgren et al. [2013] were judged as VUS because proper segregation had not been proven (~~Supplementary~~Supp. Table 2). The mutation types of the disease-causing mutations from the literature (n = ~~26~~29, including one recurrent mutation) and this study (n = ~~5~~11) are depicted in Figure 1B ~~(n = 31)~~. The exon distribution of all point mutations from the literature and our own study (n = 29, including one recurrent mutation) is depicted in Figure 1D.

~~Except for the large imbalanced inversion, all of the large genomic rearrangements published by Lindgren et al. [2013], were retrieved from CNV databases, including DECIPHER (https://decipher.sanger.ac.uk). An up-to-date overview of all patients with genomic imbalances including the *KMT2D* or the *KDM6A* gene annotated in the DECIPHER database is given in Supplementary Table 3.~~

MUTATION SCREENING OF *KDM6C*

We also investigated the hypothesis of the *KDM6A* homologue *KDM6C* (*UTY*) as a candidate gene for Kabuki syndrome in male patients. Mutation screening of 15 male KS patients negative for *KMT2D* mutations did not identify any causative mutation in *KDM6C* (*UTY*).

FINDINGS IN KABUKI-LIKE TURNER SYNDROME

The patients with Kabuki-like Turner syndrome all had long palpebral fissures, arched eye-brows, dense eye-lashes, and a short columella. The typical eversion of the lower eye-lid was seen in two patients. A remarkable similarity was seen in the form of the nose: a round, fleshy, sometimes bulbous nasal tip was seen in most patients. The eyebrows, although arched were also bushy and not laterally sparse as it is frequently seen in KS. They all had short stature with normal head circumference. One had a bicuspid aortic valve and aortic coarctation, as well as

hydronephrosis. A second patient had a horseshoe kidney with double collecting system. Another had congenital hip dislocation.

For three of the six patients with Kabuki-like Turner syndrome, we confirmed the respective karyotypes by SNP arrays, but did not detect any additional chromosomal aberrations that might explain the Kabuki-like phenotype. In the patients with the 45,X and the 45,X/46,X,i(Xq) karyotypes, one copy of *KDM6A*, which is located on chromosome Xp11.3, is missing. In the patients with the 45,X/46,X,r(X) karyotype, the exact breakpoint of the ring chromosome could not be defined, thus, it is unknown whether *KDM6A* is present within the ring or not. Interestingly, many literature reports of patients with Kabuki-like Turner syndrome state that *KDM6A* was included in the ring, meaning that two copies should be present. However it is possible, that the ring structure of the chromosome impedes correct transcription of this copy or, that enhancer elements/long range regulators are missing from the ring chromosome.

KDM6A mutation screening of all six Kabuki-like Turner syndrome patients with either a 45,X, a 45,X/46,X,i(X), or a 45,X/46,X,r(X) karyotype did not reveal any sequence variant that might be considered causative of the Kabuki-like phenotype in these patients.

DIAGNOSTIC RELEVANCE OF THE MOLECULAR RESULTS FOR *KMT2D*

In our case series mutations in *KMT2D* were identified in 44 208 patients (4560%). ~~24 of these mutations have not been reported before (Table 1), while 20 were recurrent mutations (Table 2).~~ The identified mutations were mainly truncating (16 76 nonsense and 14 69 frameshifting mutations). Exon 39 seems to be prone to nonsense mutations, while ~~frameshifting mutations were predominantly located in exon 31. M~~missense mutations occurred most frequently in exon 48. Overall, exon 31 48 showed the highest number of mutations in our study (946), closely followed by exon 48 39 (458 mutations). Taken together, the largest exons (10, 11, 31, 34, 39,

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and 48) account for 6369.71% of all mutations identified in this study. (Figure 1C) and 63.37% of all mutations analyzed (this study and literature), which is an expected result.

The distribution of the *KMT2D* mutations identified in our study is similar to previously published results: the highest number of mutations can be found in the largest exons (10, 11, 31, 34, 39, and 48), which is an obvious result. To further analyze the exon distribution of the published and novel mutations and to establish mutation hot spots independent of exon size, we established a mutation load score (MLS), which images the number of mutations relative to the number of basepairs of an exon. For this calculation, we used the location of all disease-causing variants retrieved from the literature or identified in our study (including recurrent mutations) and we found that in most of the largest exons the number of mutations does not exceed the expected mutation load (cut-off 7.9211.33). Thus, the apparent clustering of mutations in these exons is mainly attributable to their size. Only exons 14, 52 and 53 hold an unexpectedly high number of mutations, with MLS of 12.36, 9.1721.62 and 13.5115.60, respectively, . Exon 48 is the only large exon with a MLS close to the cut-off of 9.47, and it would probably exceed the cut-off if all missense variants classified as VUS were included in the calculation. Together with the high MLS of exons 52 and 53 this might indicateindicating a potential clustering of mutations at the 3' end of the *KMT2D* gene (Figure 1C).

Based upon these observations, two-step diagnostic approaches to Sanger sequencing, for example starting with exons 27 to 54 or starting with the large exons + and exons 51-53, could be useful and economic diagnostic testing strategies if Sanger sequencing is to be applied (see clinical relevance).

A further aspect about *KMT2D* mutations is that they are mostly private mutations, reported in only a single patient (SupplementarySupp. Table 1): only 35 58 of the 420 621 disease-causing mutations have been found in more than one patient. Interestingly, 19 (54%) of these recurrent

~~mutations, are located in exons 48 to 53. Thus, exons 48 to 53 may be regarded as a hot spot for recurrent mutations. However, t~~The most frequently ~~reported~~ identified mutations are c.15142C>T, p.(Arg5048Cys) in exon 48 (9 patients) and ~~(~~c.6595delT, p.(Tyr2199Ilefs*65)~~),~~ in exon 31 (8 patients) ~~which has been found in five patients so far, is located in exon 31.~~ .

While most patients harbor only a single disease causing *KMT2D* mutation, the studies by Makrythanasis et al. [2013], Micale et al. [2014], and Liu et al. [2015] each described a patient who carried two disease-causing, *de novo* missense variants in *KMT2D* (~~Supplementary~~Supp. Table 1, mutations marked with asterisks). Due to the rareness of *de novo* mutations, *de novo* occurrence of a mutation in the gene that is known to cause the phenotype diagnosed in a patient is usually considered a strong indicator of pathogenicity. The mutations in the patients mentioned above were both judged disease causing according to our criteria. However, in a vital developmental gene like *KMT2D* we would expect biallelic mutations with deleterious functional consequences to be lethal at the embryonic stage. Thus, it appears most likely that these mutations are located in-cis, a phenomenon that has already been described in Rett syndrome [Bunyan and Robinson, 2008]. Another possibility is false paternity.

Finally, large genomic aberrations of the *KMT2D* locus seem to be very rare: Banka et al. [~~2012~~2013] identified intragenic or whole-gene deletions/duplications of *KMT2D* in 3 out of 64 patients by MLPA analysis. However, deletions or duplications of the *KMT2D* locus have been reported in only 10 patients in the DECIPHER database ~~(Supplementary Table 3)~~, and >80 MLPA analyses in patients with Kabuki syndrome in our own laboratory have not identified a single aberration. Priolo et al. [2012] did not find any deletions/duplications *KMT2D* in a cohort of 120 patients with Kabuki syndrome, indicating that large deletions of *KMT2D* are relatively rare events, compared to point mutations,.

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DIAGNOSTIC RELEVANCE OF THE MOLECULAR RESULTS FOR *KDM6A*

~~Our study recapitulates the published mutation detection rate for *KDM6A*: i~~In our case series, we identified ~~six~~ twelve novel *KDM6A* mutations (~~Figure 2A, B,~~Figure 2, Table ~~3~~2, Supplementary Figure 1) ~~in five female and one male patient out of~~in a cohort of ~~44~~89 patients~~. This equals 6.1% of the entire cohort and~~ (= 13.~~6~~5%~~) of the analyzed *KMT2D* mutation-negative group~~. ~~Five~~ Nine of the mutations could be shown to be *de novo,* ~~and four of them were truncating~~while two were inherited (Table ~~3~~2, Supplementary Figure 1). Parental samples were unavailable for patient P213. The mutations c.171dupT and c.190G>T identified in patients P1 and P2 represent the most N-terminal mutations yet described and are located before the first TPR motif of the KDM6A protein (Figure ~~22~~B~~, 2A~~).

Apart from these 5' mutations, the identified and the published mutations in *KDM6A* show a clustering towards the 3' end of the gene (Figure 1D). We also calculated mutation load scores (MLS) for *KDM6A*. However, the result is not representative due to the small number of *KDM6A* point mutations yet described. Overall, ~~78.26~~69% of all disease causing point mutations were located in exons 16 – 29 (Figure 1D). ~~Thus, the distribution of mutations in *KDM6A* appears to be shifted towards the 3' end.~~ Therefore, it may be advisable to divide this large gene into two sets for diagnostic Sanger sequencing approaches, starting with exons 16 - 29, followed by exons 1 – 15.

In terms of mutation type, *KMT2D* and *KDM6A* show ~~a similar~~different profiles with regard to point mutations. Both genes show a large proportion of nonsense mutations and small deletions/insertions (Figure 1A,B), but ~~. The only striking difference is a relatively high number of~~ splice site mutations are the most frequent mutation type for ~~in~~ *KDM6A* ~~compared with~~as opposed to *KMT2D* where splice site mutations play a minor role ~~*KMT2D*~~ (~~29~~27.5% vs. 7.9%, Figure 1A,B).

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Genomic aberrations of the *KDM6A* locus appear to be much more frequent than genomic aberrations of the *KMT2D* locus: 67 patients with deletions, duplications, triplications or complex genomic rearrangements of the *KDM6A* locus have been annotated in DECIPHER ~~(Supplementary Table 3)~~. Additionally, *KDM6A* was initially identified as a causative gene for Kabuki syndrome by the identification of whole-gene or intragenic deletions in three patients by Lederer et al. [2012]. However, Priolo et al. [2012] did not find any deletions/duplications of *KDM6A* or *KMT2D* in a cohort of 120 patients with Kabuki syndrome, indicating that such aberrations seem to be relatively rare compared to the other known genetic causes of the disease.

Interestingly, the *KDM6A* missense mutation c.3763C>T, p.(Arg1255Trp), identified in a patient in this study, which has never been described in Kabuki syndrome before, has been found as a somatic mutation in stomach carcinoma (COSMIC ID: COSM4109565). Somatic mutations in *KMT2D* and *KDM6A* are frequently found in cancer [Huether et al., 2014]; however, an increased cancer risk has not yet been described for patients with germline mutations. Long-term follow up of these patients will be needed to confirm or exclude an associated cancer risk in Kabuki syndrome.

Since *KDM6A* is located on the X-chromosome, we wondered about a potential connection to Kabuki-like-Turner syndrome. A small proportion of patients with Turner syndrome, and especially of those with a derivative X-chromosome, have been described in the literature to present with facial features reminiscent of Kabuki syndrome [Bögershausen and Wollnik, 2013 and references therein], and also the patients described by Lederer et al. [2012], carrying larger deletions of *KDM6A*, have overlapping features with Kabuki-like-Turner syndrome. We asked whether patients with Kabuki-like-Turner syndrome might have modifying variants within *KDM6A* or a submicroscopic chromosomal aberration in addition to the missing X-chromosome.

However, screening of six unrelated Turner syndrome patients with Kabuki-like features did not identify any sequence variants of *KDM6A* that might account for the peculiar phenotype. Neither did the SNP array analyses in three patients reveal any additional chromosomal aberrations or a shared X-chromosomal abnormality. Thus, the cause of the Kabuki-like features in these patients with Turner syndrome remains unclear. Clinically, both syndromes constitute important differential diagnoses in girls with Kabuki-like facial features and short stature, which may be hard to distinguish. We noted earlier that the facial features in Kabuki-like Turner syndrome tend to be coarser than in true KS [Bögershausen and Wollnik, 2013]. Multiple lentigines may also point towards Kabuki-like Turner syndrome and warrant karyotyping before the initiation of the molecular analysis of the KS genes.

KDM6A escapes X-inactivation [Greenfield et al., 1998; Miyake et al., 2013b]. It has been hypothesized that *KDM6C* (*UTY*), the Y-chromosome homologue of *KDM6A*, may compensate for the loss of the single *KDM6A* copy in male patients with X-linked KS2. A recent study could now show that, contrary to prior reports [Agger et al., 2007; Hong et al., 2007], KDM6C does indeed catalyze demethylation of histone 3 lysine 27 [Walport et al., 2014], a finding that supports the assumed functional redundancy of KDM6A and KDM6C, making *KMD6C* an interesting candidate gene for KS in male patients. Lederer et al. [2012] previously reported a mutation screening of *KDM6C* in 15 *KMT2D* mutation-negative patients, which did not identify any disease-causing mutations. Neither did our screening of 15 unrelated male KS patients reveal a causative mutation. X-Inactivation in female patients seems to be independent of *KDM6A* mutation status, as shown by Miyake et al. [2013b]. X-Inactivation was determined in one of our patients (P5) and, in reference to an assumed cut-off of 90%:10%, did not appear skewed with 78%:22%.

CLINICAL RELEVANCE

The identification of the second Kabuki syndrome gene, *KDM6A,* has allowed defining two subgroups of the disorder by molecular genetic criteria. The question remains whether the two subtypes can also be distinguished by clinical criteria~~. At this stage, it appears that the clinical features of patients with both KS types are essentially the same~~. For this study, the clinical details of eleven patients with KS2 were analyzed and compared with the literature (Table 3; Figure 3, Figure 4)~~:~~ Renal abnormalities have been reported to appear in approximately 40% of patients with KS1 [Bögershausen and Wollnik, 2013]. In this study ~~seem to be less frequent in KS2 than in KS1 [Lederer et al., 2014]. In our cohort,~~ we ~~also~~ observed a renal malformation in three patients (= 27%)~~only in a single patient~~: P210 ~~had~~ has ureteral duplication and hydronephrosis and P210 has a horseshoe kidney, the exact type of malformation was not documented in P219~~. (Table 4).~~ Miyake et al. [2013b] reported that all of their patients with KS2, but only half of their patients with KS1 showed short stature. We have reported short stature to be present in 58% and microcephaly to appear in 29% to 56% of patients with KS1 [Bögershausen and Wollnik, 2013]. Interestingly, ~~none~~ four of our patients with KS2 ~~was~~ were of short stature (36%) and ~~only three~~ five had microcephaly (45%), indicating that postnatal growth retardation appears at comparable frequencies in both KS subtypes.~~.~~ Miyake et al. [2013b] also noted that arched eyebrows, fifth finger brachydactyly, and hypotonia in infancy were more frequent in individuals with KS1 than in individuals with KS2. ~~All~~ However, 9/11 patients~~of our patients~~ with KS2 in this study had a combination of at least seven typical facial features (Table ~~4~~3)~~,~~. 8/11 ~~and all of them~~ had arched eyebrows, and we noted the eyebrows to be rather bushy in most of them (Figure 3). ~~long palpebral fissures, and a depressed nasal tip.~~ 8/11 even had the typical eversion of the lower eyelid. Thus, in our study the facial phenotype of KS2 appeared quite classical. Hypotonia in infancy and feeding difficulties were each observed in 9/11 patients~~5/6 of our patients with KS2~~. Fifth finger brachydactyly and fifth finger clinodactyly were seen in ~~3/5~~7/11 and 6/11 ~~and 4/ 5~~ patients, respectively~~, respectively~~. The rate of congenital

heart disease (CHD) in this cohort was similar to the reported frequency in KS1 (40-50%) [Bögershausen and Wollnik, 2013]. We observed CHD in 4 out of 11 patients: Septal defects in three, and coarctation of the aorta in one patient. One patient had a bicuspid aortic valve and one had left ventricular hypertrophy in addition (Table 3).~~Dental anomalies have been frequently reported in *KMT2D* mutation-positive patients, but were not observed among our KS2 patients.~~

Interestingly, not all of our patients presented with intellectual disability (~~5/6~~10/11 patients), whereas all of the mutation-positive patients in the studies of Miyake et al. [2013b] and Banka et al. [2015] had some degree of intellectual disability. The finding of an intellectually normal female patient with KS2 is in line with the observation of Lederer et al. [2012], who described two mentally normal females, whose male offspring presented with intellectual disability. ~~In our cohort, the most consistent features were long palpebral fissures, arched eyebrows, large, prominent ears, a depressed nasal tip due to a short columella, as well as joint hyperlaxity and persistent fetal finger pads (Figure 3A, B); all of these features are also present in the majority of patients with KS1.~~

Banka et al. [2015] suggested that neonatal hypoglycemia may be more frequent among the KS2 patient group, and indeed, this complication was observed in 5/10 patients in this cohort.~~; however, this complication was only observed in one of our patients.~~

Long incisors and long great toes have been proposed as hallmark features of KS2 [Banka et al., 2015; Lederer et al., 2012], but neither could be observed in our patients (Table ~~4~~3). The former may, however, still develop with secondary dentition. A long first toe was also seen in the patient reported by Yang et al. [2016], who had a 227 kb deletion of chromosome X including exons 1 and 2 of *KDM6A*. Thus, a long great toe, initially described by Lederer et al [2012], may be an indicator of a *KDM6A* exonic deletion.

The most consistent features observed among our patients with KS2 (long palpebral fissures, large, prominent ears, persistent fetal finger pads, and intellectual disability (Figure 3, Table 3))

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are also among the key clinical features that mark KS1. ~~The phenotypes annotated for the patients with large genomic aberrations of *KDM6A* and *KMT2D* in DECIPHER include a variety of symptoms that also occur in Kabuki syndrome, and some of the patients may very well have a Kabuki-like phenotype, while others may show unspecific syndromic features. The phenotype may be modulated by the presence of more than one genomic aberration, or very large genomic aberrations that span numerous genes in some patients (Supplementary Table 3). All in all, the phenotype and family information is too limited and not standardized enough to draw meaningful conclusions.~~

~~Presently~~Summing up, ~~it seems that there are~~we could identify no clinical features specific for KS2 or KS1, which would allow distinguishing the two subtypes clinically. Consequently, ~~in~~ the classical diagnostic approach should be based on the frequency of detected mutations and should thus entail Sanger sequencing of *KMT2D*, followed by Sanger sequencing of *KDM6A*, followed by MLPA for both genes and/or high resolution array-CGH. While MLPA may be more sensitive and detect small gains or losses of genetic material, array-CGH would allow the simultaneous detection of differential diagnoses. In view of the large number of exons (54 + 29 = 83), a next-generation-sequencing (NGS) panel or exome sequencing, in combination with ~~Array~~array-CGH or MLPA represents a more up-to-date and cost-effective approach. However, an NGS strategy might not yet be possible for routine diagnostics in some countries, because the NGS techniques may presently not be reimbursed by health insurances.

GENOTYPE-PHENOTYPE CORRELATIONS

The small number of published patients with *KDM6A* mutations does not yet allow establishing solid genotype-phenotype correlations with regard to mutation type or location. Reviews of the published patient cohorts and our own clinical experience have taught us that no valid genotype-phenotype correlations yet exist for *KMT2D*-associated Kabuki syndrome subtype 1.

Miyake et al. [2013b] proposed that the facial phenotype might be less pronounced in patients with non-truncating versus truncating *KMT2D* mutations. However, of the patients whose pictures are shown, the two patients with the least typical facial phenotype (namely KMS-02 and KMS-91) carry sequence variants of *KMT2D* that we judged to be either non-disease-causing or of unknown significance according to our classification system. These patients might thus have been misdiagnosed. The other three patients with non-truncating mutations (KMS-42, KMS-56, and KMS-58) carry disease-causing *de novo* missense mutations and they show a rather typical facial phenotype. In our initial study [Li et al., 2011], we also observed that the facial phenotype can even be quite unremarkable in patients with truncating *KMT2D* mutations. Thus, the impression that the facial phenotype is less typical in patients with non-truncating mutations is not necessarily true. In general, the recognition of the typical facial features may also depend on the age at clinical presentation. We and others [Banka et al., 2012; Bögershausen and Wollnik, 2013] noted that the facial features may be hard to distinguish in the neonatal period and in adulthood, while they are most striking in toddlers and children in the school age (Figure 4).

Furthermore, sex-specific phenotypic differences between male and female patients with pathogenic *KDM6A* mutations have been proposed. The only female patient in the study of Miyake et al. [2013a] showed a much milder phenotype than the two male patients; however, she had a 3-bp in-frame deletion, while the male patients carried truncating mutations. Banka et al. [2015] observed in their study that the intellectual disability was more profound in male patients. We can confirm this finding, but would like to add that the mutation type might also play a role for expressivity: We identified the frameshifting mutation c.2226_2227dupCA, p.(Ser743Thrfs*13) in exon 17 of *KDM6A* in a male patient (P213) with a convincing facial phenotype, and severe intellectual disability, muscular hypotonia and feeding problems. At age 10 years he could neither walk nor speak and was severely cachectic in spite of hypercaloric feeding (Table 3). Our female KS2 patients ~~with~~ on the other hand ~~KS2 (Table 4)~~ showed a

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rather mild phenotype with mild to moderate intellectual disability and a low frequency of organ malformations. Only patient P~~1~~212, carrying an N-terminal truncating mutation, showed cortical atrophy and white matter anomalies on cranial MRI in addition to seizures and intellectual disability, i.e., a severe manifestation. On the other hand, patient P216, who carries a *de novo* missense mutation in exon 26, shows normal cognitive capacities and development, except for a mild motor delay in the second year of life. This also indicates that, apart from sex, the functional effect of the respective mutations might be a modulator of disease severity.

~~The~~ Another male patient ~~in this study~~ (~~P3~~P214), who carried the hemizygous *KDM6A* missense mutation c.2729A>G, p.(Asn910Ser), presented with some, but not all of the classic KS facial features. He had intellectual disability and bilateral cleft lip/palate, but no heart or renal malformations. His mother carries the mutation in the heterozygous state. At presentation she appeared unaffected. Unfortunately, she was not available for clinical reevaluation. Lederer et al. [2014] reported a three-generation family with two affected boys whose mother and maternal grandmother were both carriers of a truncating *KDM6A* mutation and showed only few features reminiscent of KS but not the typical KS phenotype. Lederer et al. [2014] argued in the direction of a more pronounced phenotype in male patients, especially with regard to facial features and cognitive achievements, an observation also made by Banka et al. [2015]. The fact that patient ~~P3~~ P214 inherited the *KDM6A* mutation from his seemingly unaffected mother also argues in favor of reduced expressivity or even reduced penetrance of the KS2 phenotype in females. In consequence, female mutation carriers with mild phenotypes might be undetected until they give birth to an affected son. Further studies are needed to confirm this hypothesis.

ANIMAL MODELS FOR KDM6A

According to Welstead et al. [2012], *Kdm6a* knock-out (KO) mice show a reduced number of somites, neural tube defects and heart malformations that cause midgestation lethality. Interestingly, female homozygous KO embryos were more severely affected than hemizygous

males, indicating a partial compensation of *Kdm6a* loss by *Kdm6c* (*UTY*). Thieme et al. [2013] recently generated a conditional KO mouse model and showed that *Kdm6a* is responsible for stem cell migration and hematopoiesis. Adult conditional KO female mice showed myelodysplasia, while males did not, supporting the mentioned role of *Kdm6c*. Wang et al. [2012] also observed notochord, cardiac and hematopoietic abnormalities in *Kdm6a* KO mice with survival until birth in males and midgestation lethality in females. Lee et al. [2012] could show that Kdm6a promotes a developmental program that is essential for heart development by inducing chromatin changes at cardiac-specific enhancers. They could show that *Kdm6a* KO mice exhibit heart defects and embryonic lethality. Work on *Kdm6a* KO embryonic stem cells (ESCs) has shown that KDM6A has functions related and unrelated to H3K27 demethylase activity and is required for the induction of ecto- and mesoderm during differentiation as well as epigenetic reprogramming [Mansour et al., 2012; Morales Torres et al., 2013]. In the zebrafish, loss of *kdm6a* leads to craniofacial and brain defects [Lindgren et al., 2013; Van Laarhoven et al., 2015; Bögershausen et al., 2015]. Interestingly, morpholino knock-down (MO) of the established Kabuki syndrome genes *kmt2d* and *kdm6a* as well as of the novel causative genes *rap1a* and *rap1b* cause similar craniofacial abnormalities, and zebrafish morphants for *kmt2d* and *rap1*, as well as *Kmt2d* knock-out mice show aberrations of the MAPK signaling pathway [Bögershausen et al., 2015].

CONCLUSIONS AND PROSPECTS

In summary, we expand the known clinical and molecular spectrum of the new Kabuki syndrome subtype KS2 and add to the mutation spectrum of KS1. We were able to confirm that female patients with KS2 may have a rather mild manifestation of Kabuki syndrome and may even develop normally with regard to cognitive function. Phenotypic features that might allow distinguishing between the Kabuki syndrome subtypes could not be defined. Therefore, molecular genetic testing should be performed by order of frequency in case of a Sanger

sequencing approach or, if possible, by next generation sequencing. We hypothesize that screening of larger cohorts might still identify very rare mutations in *KDM6C*. Future studies applying modern sequencing technologies in large cohorts will most likely identify additional causative genes for Kabuki syndrome, as we have recently demonstrated by the identification of *RAP1A* and *RAP1B* [Bögershausen et al., 2015].

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~~**ACCESSION NUMBERS**~~

~~*KMT2D* (*MLL2*; MIM 602113; NM_003482.3); *KDM6A* (*UTX*; MIM 300128; NM_021140.3), *KDM6C* (*UTY*; MIM 400009; NM_182660.1)~~

DISCLOSURE STATEMENT

The authors have no conflict of interest to declare.

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LEGENDS

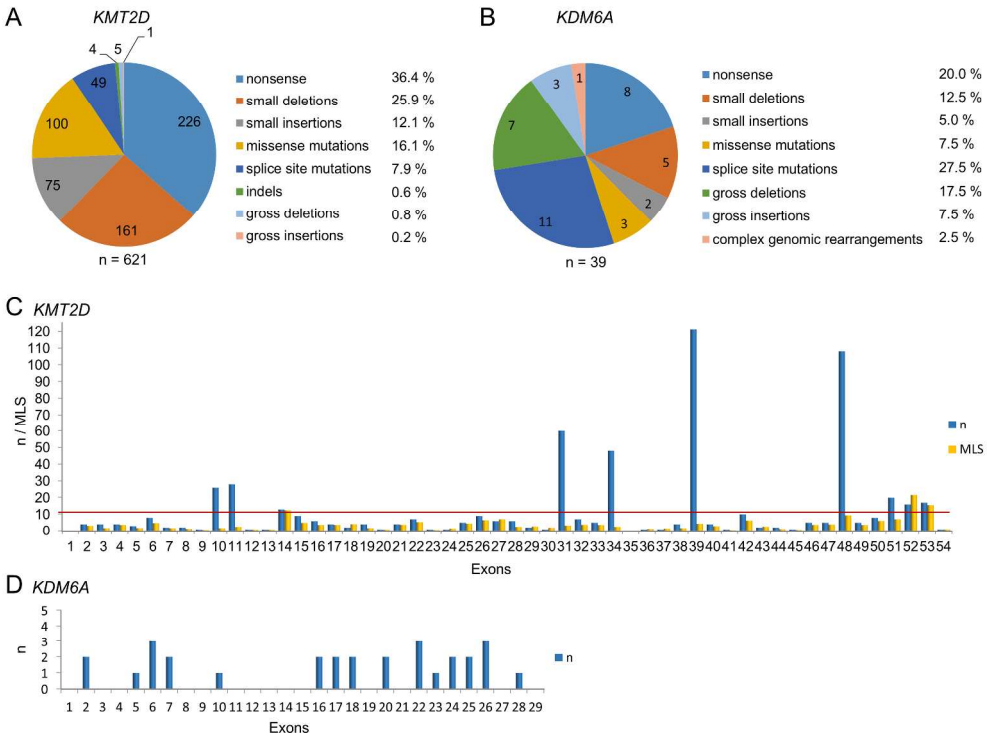
Figure 1. Overview of mutation type and exon distribution of *KMT2D* and *KDM6A* mutations. **A,** Mutation types of previously published and newly identified disease-causing mutations in *KMT2D*. Recurrent mutations were counted by times of reports, thus n corresponds to the number of patients with the reported mutation type. **B,** Mutation types of all previously published and newly identified disease-causing mutations in *KDM6A*. Recurrent mutations were counted by times of reports, thus n corresponds to the number of patients with the reported mutation type. **C,** Exon distribution of the previously published and newly identified disease-causing point mutations in *KMT2D*, including recurrent mutations. Mutations that affect more than one exon, i.e. large deletions/duplications, were excluded. N = number of mutations, MLS = mutation load score. The red line indicates the MLS cut-off. **D,** Exon distribution of the previously published and newly identified disease-causing mutations in *KDM6A* including recurrent mutations. Mutations that affect more than one exon, i.e. large deletions/duplications, were excluded. N = number of mutations, MLS = mutation load score.

Figure 2. ~~Identified *KDM6A* mutations. **A**, Electropherograms of the identified mutations in patients P1-6. **B,**~~ Overview of identified *KDM6A* mutations relative to a schematic representation of the *KDM6A* gene and KDM6A protein structure.

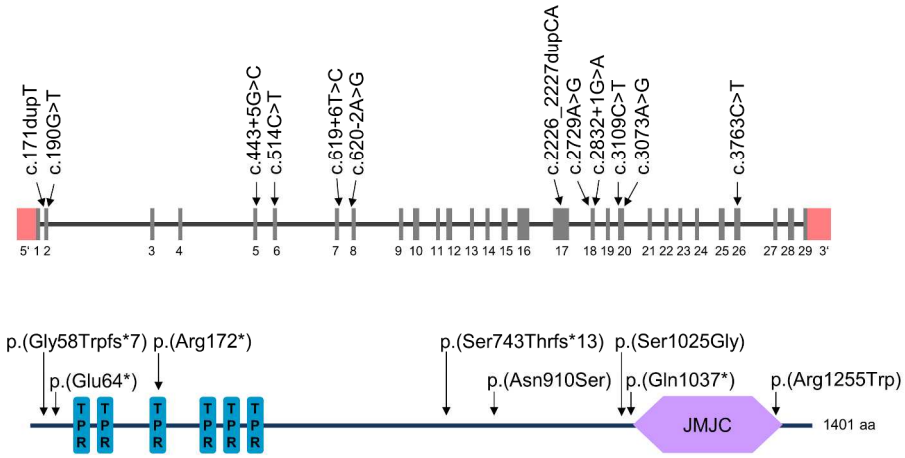
Figure 3. Clinical characteristics of patients with KS type 2. **A,** Facial features of patients P209, P210, ~~P3~~P214, ~~P4~~P216, P219 and ~~P6~~P220: Note the typical facial features with long palpebral fissures, arched and nicked eyebrows, prominent ears, a depressed nasal tip, and downslanting corners of the mouth. Note repaired cleft lip/palate in P3. **B,** Lateral views of patients P209, P210, ~~and~~ ~~P3~~P214, and P219. Characteristic features such as large or dysplastic ears, long palpebral fissures and a depressed nasal tip, might be more readily appreciable from the side.

C, Hands of patients P209, P210, ~~P3~~P214, ~~P4~~P211, ~~P5~~P216, and P219: Note persistent fetal finger pads. P209 ~~additionally~~ shows ~~aberrant with~~ a simian crease on the left and 5th finger clinodactyly (pictures are from newborn period). P210 shows 5th finger brachy- and clinodactyly. ~~P3~~ P214 shows a distally placed thumb on the left hand and 5th finger clinodactyly on both. Patients P210, P211, and P219 show relatively thick thumbs.

Figure 4. Facial features of patient ~~P5~~ P211 over the time span of 6 years: as a newborn, at 2.5 and at 6 years of age (y = years). Note how the typical facial features are hardly visible in the newborn period but become more pronounced with increasing age.



Overview of mutation type and exon distribution of KMT2D and KDM6A mutations.
254x190mm (300 x 300 DPI)



Overview of identified KDM6A mutations relative to a schematic representation of the KDM6A gene and KDM6A protein structure.
254x190mm (300 x 300 DPI)

Clinical characteristics of patients with KS type 2.
190x254mm (300 x 300 DPI)

Facial features of patient P211 over the time span of 6 years.
190x254mm (300 x 300 DPI)

Table 1. Identified point mutations in *KMT2D*.

Case	Mutation	Protein change	Exon/Intron	Segregation	Variant class	Published record
	***KMT2D* nonsense**					
P1	c.166C>T	p.(Gln56*)	2	Inherited*	DC	novel
P2	c.166C>T	p.(Gln56*)	2	n.a.	DC	novel
P3	c.741T>A	p.(Cys247*)	6	*de novo*	DC	novel
P4	c.2398C>T	p.(Gln800*)	10	*de novo*	DC	novel
P5	c.2819C>G	p.(Ser940*)	11	n.a.	DC	novel
P6	c.3178A>T	p.(Lys1060*)	11	n.a.	DC	novel
P7	c.4521C>A	p.(Cys1507*)	16	*de novo*	DC	novel
P8	c.5707C>T	p.(Arg1903*)	26	n.a.	DC	Miyake 2013
P9	c.5764C>T	p.(Gln1922*)	26	*de novo*	DC	novel
P10	c.6622C>T	p.(Gln2208*)	30	n.a.	DC	novel
P11	c.6295C>T	p.(Arg2099*)	31	n.a.	DC	Ng 2010, Micale 2011
P12	c.6295C>T	p.(Arg2099*)	31	n.a.	DC	Ng 2010, Micale 2011
P13	c.6295C>T	p.(Arg2099*)	31	n.a.	DC	Ng 2010, Micale 2011
P14	c.6295C>T	p.(Arg2099*)	31	n.a.	DC	Ng 2010, Micale 2011
P15	c.6325C>T	p.(Gln2109*)	31	n.a.	DC	novel
P16	c.6962T>G	p.(Leu2321*)	31	n.a.	DC	novel
P17	c.7411C>T	p.(Arg2471*)	31	*de novo*	DC	novel
P18	c.7726C>T	p.(Gln2576*)	31	n.a.	DC	novel
P19	c.7903C>T	p.(Arg2635*)	31	*de novo*	DC	Micale 2011
P20	c.7903C>T	p.(Arg2635*)	31	n.a.	DC	Micale 2011
P21	c.8200C>T	p.(Arg2734*)	32	*de novo*	DC	Paulussen 2011
P22	c.8200C>T	p.(Arg2734*)	32	*de novo*	DC	Paulussen 2011
P23	c.8488C>T	p.(Arg2830*)	34	n.a.	DC	Ng 2010, Hannibal 2011
P24	c.8743C>T	p.(Arg2915*)	34	*de novo*	DC	Li 2011
P25	c.9022G>T	p.(Glu3008*)	34	*de novo*	DC	novel
P26	c.9820C>T	p.(Gln3274*)	34	n.a.	DC	novel
P27	c.9829C>T	p.(Gln3277*)	34	n.a.	DC	Courcet 2013
P28	c.11203C>T	p.(Gln3735*)	39	n.a.	DC	novel
P29	c.11269C>T	p.(Gln3757*)	39	*de novo*	DC	Micale 2011
P30	c.11377C>T	p.(Gln3793*)	39	*de novo*	DC	novel
P31	c.11524C>T	p.(Gln3842*)	39	*de novo*	DC	novel
P32	c.11632C>T	p.(Gln3878*)	39	*de novo*	DC	novel
P33	c.11645C>G	p.(Ser3882*)	39	*de novo*	DC	novel

P34	c.11728C>T	p.(Gln3910*)	39	n.a.	DC	novel
P35	c.11851C>T	p.(Gln3951*)	39	*de novo*	DC	novel
P36	c.11899C>T	p.(Gln3967*)	39	*de novo*	DC	novel
P37	c.11944C>T	p.(Arg3982*)	39	*de novo*	DC	Paulussen 2011, Miyake 2013
P38	c.11944C>T	p.(Arg3982*)	39	n.a.	DC	Paulussen 2011, Miyake 2013
P39	c.11977C>T	p.(Gln3993*)	39	n.a.	DC	novel
P40	c.12301C>T	p.(Gln4101*)	39	*de novo*	DC	novel
P41	c.12469C>T	p.(Gln4157*)	39	n.a.	DC	novel
P42	c.12592C>T	p.(Arg4198*)	39	n.a.	DC	Banka 2012, Makrythanasis 2013, Cheon 2014
P43	c.12592C>T	p.(Arg4198*)	39	n.a.	DC	Banka 2012, Makrythanasis 2013, Cheon 2014
P44	c.12598C>T	p.(Gln4200*)	39	*de novo*	DC	novel
P45	c.12655C>T	p.(Gln4219*)	39	*de novo*	DC	novel
P46	c.12667C>T	p.(Gln4223*)	39	n.a.	DC	novel
P47	c.12688C>T	p.(Gln4230*)	39	n.a.	DC	Hannibal 2011, Miyake 2013 , Van Laarhoven 2015
P48	c.12760C>T	p.(Gln4254*)	39	n.a.	DC	novel
P49	c.12943C>T	p.(Gln4315*)	39	*de novo*	DC	novel
P50	c.12955A>T	p.(Arg4319*)	39	n.a.	DC	Micale 2014
P51	c.12964C>T	p.(Gln4322*)	39	*de novo*	DC	Subbarayan 2014
P52	c.13285C>T	p.(Gln4429*)	39	n.a.	DC	Hannibal 2011
P53	c.13450C>T	p.(Arg4484*)	39	*de novo*	DC	Paulussen 2011, Makrythanasis 2013, Dentici 2015
P54	c.13450C>T	p.(Arg4484*)	39	*de novo*	DC	Paulussen 2011, Makrythanasis 2013, Dentici 2015
P55	c.13606C>T	p.(Arg4536*)	40	n.a.	DC	Ng 2010
P56	c.14189G>A	p.(Trp4730*)	44	*de novo*	DC	novel
P57	c.14710C>T	p.(Arg4904*)	48	*de novo*	DC	Ng 2010
P58	c.14710C>T	p.(Arg4904*)	48	n.a.	DC	Ng 2010
P59	c.14720C>A	p.(Ser4907*)	48	*de novo*	DC	novel
P60	c.14803G>T	p.(Glu4935*)	48	n.a.	DC	novel
P61	c.14873C>G	p.(Ser4958*)	48	n.a.	DC	novel
P62	c.14945G>A	p.(Trp4982*)	48	*de novo*	DC	novel
P63	c.14946G>A	p.(Trp4982*)	48	*de novo*	DC	Hannibal 2011
P64	c.14946G>A	p.(Try4982*)	48	n.a.	DC	Hannibal 2011
P65	c.15079C>T	p.(Arg5027*)	48	*de novo*	DC	Paulussen 2011, Micale 2011
P66	c.15079C>T	p.(Arg5027*)	48	*de novo*	DC	Paulussen 2011, Micale 2011
P67	c.15079C>T	p.(Arg5027*)	48	n.a.	DC	Paulussen 2011, Micale 2011
P68	c.15079C>T	p.(Arg5027*)	48	n.a.	DC	Paulussen 2011
P69	c.15256C>T	p.(Arg5086*)	48	n.a.	DC	Banka 2012
P70	c.15730A>T	p.(Lys5244*)	48	*de novo*	DC	novel
P71	c.15781C>T	p.(Gln5261*)	48	*de novo*	DC	novel
P72	c.15920C>G	p.(Ser5307*)	49	*de novo*	DC	novel

P73	c.16342C>T	p.(Arg5448*)	52	*de novo*	DC	Hannibal 2011
P74	c.16360C>T	p.(Arg5454*)	52	n.a.	DC	Ng 2010, Paulussen 2011, Hannibal 2011
P75	c.16501C>T	p.(Arg5501*)	53	*de novo*	DC	Ng 2010
P76	c.16501C>T	p.(Arg5501*)	53	*de novo*	DC	Ng 2010
	***KMT2D* small deletion**					
P77	c.1363del	p.(Glu455Asnfs*475)	10	n.a.	DC	novel
P78	c.1425del	p.(Ala476Hisfs*454)	10	*de novo*	DC	novel
P79	c.1576_1577del	p.(Ser526Thrfs*7)	10	n.a.	DC	novel
P80	c.2164del	p.(Glu722Serfs*208)	10	*de novo*	DC	novel
P81	c.2345del	p.(Val782Glyfs*148)	10	*de novo*	DC	novel
P82	c.3251_3255del	p.(Pro1084Leufs*29)	11	n.a.	DC	novel
P83	c.3326_3336del	p.(Ala1109Glyfs*2)	11	*de novo*	DC	novel
P84	c.3540del	p.(Pro1181Hisfs*31)	11	n.a.	DC	novel
P85	c.3626_3627del	p.(Ser1209*)	11	*de novo*	DC	novel
P86	c.4135_4136del	p.(Met1379Valfs*52)	14	n.a.	DC	Micale 2011
P87	c.4135_4136del	p.(Met1379Valfs*52)	14	*de novo*	DC	Micale 2014, Cheon 2014
P88	c.4799del	p.(Leu1600Argfs*4)	19	*de novo*	DC	novel
P89	c.5090del	p.(Gly1697Valfs*25)	21	*de novo*	DC	novel
P90	c.5627_5630del	p.(Asp1876Glyfs*38)	25	n.a.	DC	Banka 2012
P91	c.5627_5630del	p.(Asp1876Glyfs*38)	25	*de novo*	DC	Banka 2012
P92	c.5819del	p.(Pro1940Glnfs*107)	27	*de novo*	DC	novel
P93	c.6278_6279del	p.(Ile2093Serfs*3)	31	*de novo*	DC	novel
P94	c.6480_6483del	p.(Phe2160Leufs*103)	31	*de novo*	DC	novel
P95	c.6595del	p.(Tyr2199Ilefs*65)	31	*de novo*	DC	Ng 2010, Li 2011, Micale 2011, Banka 2012, Morgan 2015,
P96	c.6629del	p.(Pro2210Argfs*54)	31	n.a.	DC	novel
P97	c.6794del	p.(Gly2265Glufs*21)	31	*de novo*	DC	Micale 2014
P98	c.7282del	p.(Arg2428Glyfs*57)	31	n.a.	DC	novel
P99	c.8027_8028del	p.(Glu2676Alafs*47)	31	n.a.	DC	novel
P100	c.8410del	p.(Tyr2804Ilefs*47)	34	*de novo*	DC	novel
P101	c.9164del	p.(Pro3055Leufs*16)	34	*de novo*	DC	Cheon 2014
P102	c.9579_9597del	p.(Leu3195*)	34	*de novo*	DC	novel
P103	c.10694del	p.(Lys3565Serfs*93)	38	n.a.	DC	novel
P104	c.11679del	p.(Met3894Trpfs*85)	39	n.a.	DC	novel
P105	c.12116_12117del	p.(Glu4039Glyfs*17)	39	n.a.	DC	novel
P106	c.12183del	p.(Glu4061Aspfs*5)	39	*de novo*	DC	novel
P107	c.12413_12414del	p.(Ser4138Cysfs*29)	39	*de novo*	DC	novel
P108	c.12442_12455del	p.(Met4148Serfs*15)	39	n.a.	DC	novel
P109	c.12700_12701del	p.(Gln4235Glyfs*98)	39	n.a.	DC	novel
P110	c.12811_12814del	p.(Thr4271Alafs*6)	39	n.a.	DC	novel

P111	c.12835del	p.(Ala4279Glnfs*105)	39	n.a.	DC	novel
P112	c.13040_13041del	p.(Gln4347Argfs*24)	39	*de novo*	DC	novel
P113	c.13446del	p.(Leu4483Cysfs*36)	39	n.a.	DC	novel
P114	c.13780del	p.(Ala4594Profs*23)	41	*de novo*	DC	novel
P115	c.13904del	p.(Gln4635Argfs*5)	42	*de novo*	DC	novel
P116	c.13948del	p.(Glu4650Serfs*16)	42	n.a.	DC	novel
P117	c.14879_14889del	p.(Arg4960Profs*6)	48	*de novo*	DC	novel
P118	c.14975del	p.(Leu4992Argfs*3)	48	n.a.	DC	novel
P119	c.15163_15168del	p.(Asp5055_Leu5056del)	48	n.a.	VUS	novel
P120	c.15330del	p.(Asn5111Metfs*36)	48	n.a.	DC	novel
P121	c.15842del	p.(Leu5281Argfs*8)	49	n.a.	DC	novel
P122	c.16489_16491del	p.(Ile5497del)	53	n.a.	DC	Micale 2011, Hannibal 2011
P123	c.16489_16491del	p.(Ile5497del)	53	n.a.	DC	Micale 2011, Hannibal 2011
P124	c.16489_16491del	p.(Ile5497del)	53	n.a.	DC	Micale 2014, Banka 2012
	***KMT2D* small insertion/duplication**					
P125	c.751dup	p.(Tyr251Leufs*22)	6	n.a.	DC	novel
P126	c.1142_1143insACCC	p.(Thr382Profs*3)	9	*de novo*	DC	novel
P127	c.1966dup	p.(Leu656Profs*12)	10	n.a.	DC	novel
P128	c.2506dup	p.(Gln836Profs*3)	10	n.a.	DC	novel
P129	c.3669dup	p.(Glu1224Argfs*26)	11	*de novo*	DC	novel
P130	c.3859dup	p.(Glu1287Glyfs*38)	11	n.a.	DC	novel
P131	c.3903dup	p.(Gln1302Thrfs*23)	11	*de novo*	DC	novel
P132	c.5395_5398dup	p.(Gly1800Valfs*27)	23	*de novo*	DC	novel
P133	c.6987_6988insT	p.(Pro2330Serfs*47)	31	*de novo*	DC	novel
P134	c.7061dup	p.(Ala2355Cysfs*22)	31	*de novo*	DC	novel
P135	c.7199dup	p.(Arg2401Serfs*33)	31	*de novo*	DC	novel
P136	c.7378dup	p.(Arg2460Profs*2)	31	*de novo*	DC	novel
P137	c.8709dup	p.(Pro2904Thrfs*8)	34	n.a.	DC	novel
P138	c.8903dup	p.(Ser2969Valfs*4)	34	n.a.	DC	novel
P139	c.11223_11225dup	p.(Gln3745dup)	39	n.a.	VUS	novel
P140	c.11473dup	p.(Arg3825Lysfs*187)	39	n.a.	DC	novel
P141	c.11770dup	p.(Gln3924Profs*88)	39	n.a.	DC	novel
P142	c.12600_12604dup	p.(Gln4202Argfs*15)	39	n.a.	DC	novel
P143	c.12986_13010dup	p.(Pro4338Alafs*4)	39	*de novo*	DC	novel
P144	c.13297dup	p.(Arg4433Lysfs*54)	39	*de novo*	DC	novel
P145	c.15337dup	p.(Tyr5113Leufs*25)	48	n.a.	DC	novel
P146	c.15545dup	p.(Leu5183Profs*16)	48	n.a.	DC	novel
P147	c.15546_15547insG	p.(Leu5183Alafs*16)	48	*de novo*	DC	novel
P148	c.16116dup	p.(Asn5373Glnfs*86)	51	n.a.	DC	novel

	***KMT2D* missense**					
P149	c.3622A>C	p.(Ile1208Leu)	11	n.a.	VUS	novel
P150	c.4093G>T	p.(Val1365Phe)	13	n.a.	VUS	novel
P151	c.4171G>A	p.(Glu1391Lys)	14	*de novo*	DC	Micale 2011
P152	c.4214A>T	p.(His1405Leu)	14	*de novo*	DC	novel
P153	c.4267C>G	p.(Arg1423Gly)	15	n.a.	VUS	novel
P154	c.4267C>T	p.(Arg1423Cys)	15	Inherited*	VUS	Miyake 2013
P155	c.4267C>T	p.(Arg1423Cys)	15	Inherited*	VUS	Miyake 2013
P156	c.4267C>T	p.(Arg1423Cys)	15	n.a.	VUS	Miyake 2013
P157	c.4359C>A	p.(His1453Gln)	15	n.a.	VUS	novel
P158	c.4413C>G	p.(Cys1471Trp)	15	*de novo*	DC	novel
P159	c.6109G>C	p.(Asp2037His)	31	n.a.	VUS	novel
P160	c.6544G>A	p.(Ala2182Thr)	31	n.a.	VUS	novel
P161	c.9145C>G	p.(Leu3049Val)	34	*de novo*	DC	novel
P162	c.11791C>T	p.(Leu3931Phe)	39	n.a.	VUS	novel
P163	c.14055C>G	p.(His4685Gln)	43	*de novo*	DC	novel
P164	c.15142C>T	p.(Arg5048Cys)	48	*de novo*	DC	Banka 2012,Makrythanasis 2013, Van Laarhoven 2015
P165	c.15142C>T	p.(Arg5048Cys)	48	*de novo*	DC	Banka 2012,Makrythanasis 2013, Van Laarhoven 2015
P166	c.15142C>T	p.(Arg5048Cys)	48	*de novo*	DC	Banka 2012,Makrythanasis 2013, Van Laarhoven 2015
P167	c.15142C>T	p.(Arg5048Cys)	48	*de novo*	DC	Banka 2012,Makrythanasis 2013, Van Laarhoven 2015
P168	c.15142C>T	p.(Arg5048Cys)	48	n.a.	DC	Banka 2012,Makrythanasis 2013, Van Laarhoven 2015
P169	c.15143G>A	p.(Arg5048His)	48	n.a.	VUS	Miyake 2013, Makrythanasis 2013
P170	c.15143G>A	p.(Arg5048His)	48	n.a.	VUS	Miyake 2013, Makrythanasis 2013
P171	c.15176A>G	p.(His5059Arg)	48	n.a.	VUS	novel
P172	c.15206T>A	p.(Val5069Glu)	48	*de novo*	DC	novel
P173	c.15349T>G	p.(Cys5117Gly)	48	*de novo*	DC	novel
P174	c.15397T>C	p.(Cys5133Arg)	48	n.a.	VUS	novel
P175	c.15461G>A	p.(Arg5154Gln)	48	*de novo*	DC	Li 2011, Miyake 2013, Morgan 2015
P176	c.15461G>A	p.(Arg5154Gln)	48	n.a.	DC	Li 2011, Miyake 2013, Morgan 2015
P177	c.15535C>T	p.(Arg5179Cys)	48	*de novo*	DC	Dentici 2014
P178	c.15536G>A	p.(Arg5179His)	48	*de novo*	DC	Ng 2010, Hannibal 2011, Miyake 2013, Morgan 2015
P179	c.15536G>A	p.(Arg5179His)	48	n.a.	DC	Ng 2010, Hannibal 2011, Miyake 2013, Morgan 2015
P180	c.15536G>T	p.(Arg5179Leu)	48	*de novo*	DC	novel
P181	c.15536G>T	p.(Arg5179Leu)	48	n.a.	DC	novel
P182	c.15565G>A	p.(Gln5189Arg)	48	*de novo*	DC	Miyake 2013
P183	c.15634G>C	p.(Ala5212Pro)	48	*de novo*	DC	novel
P184	c.15640C>T	p.(Arg5214Cys)	48	*de novo*	DC	Hannibal 2011
P185	c.15640C>T	p.(Arg5214Cys)	48	n.a.	DC	Hannibal 2011, Makrythanasis 2013
P186	c.15673C>T	p.(Arg5225Cys)	48	*de novo*	DC	novel

P187	c.16019G>A	p.(Arg5340Gln)	50	*de novo*	DC	Micale 2011
P188	c.16052G>A	p.(Arg5351Gln)	50	n.a.	DC	Miyake 2013
P189	c.16273G>A	p.(Glu5425Lys)	51	n.a.	DC	Micale 2011, Lin 2015
P190	c.16273G>A	p.(Glu5425Lys)	51	n.a.	DC	Micale 2011, Lin 2015
P191	c.16295G>A	p.(Arg5432Gln)	51	n.a.	DC	Kokitsu-Nakata 2012
P192	c.16315C>G	p.(Arg5439Gly)	51	*de novo*	DC	novel
P193	c.16442G>A	p.(Cys5481Tyr)	53	*de novo*	DC	Banka 2012
	***KMT2D* splice site**					
P194	c.177-2A>G	n.a.	2	n.a.	DC	novel
P195	c.400+2T>C	n.a.	3	n.a.	DC	novel
P196	c.839+2T>A	n.a.	6	*de novo*	DC	novel
P197	c.2797+1G>C	n.a.	10	*de novo*	DC	novel
P198	c.3906+1G>T	n.a.	11	*de novo*	DC	novel
P199	c.3906+2T>C	n.a.	11	n.a.	DC	novel
P200	c.8366+2T>C	n.a.	33	n.a.	DC	novel
P201	c.13531-2A>C	n.a.	39	*de novo*	DC	novel
P202	c.14076-1G>A	n.a.	43	*de novo*	DC	novel
P203	c.14515+1del	n.a.	46	n.a.	DC	novel
P204	c.14516-1G>C	n.a.	46	*de novo*	DC	Paulussen 2011
P205	c.14643+1G>T	n.a.	47	*de novo*	DC	novel
P206	c.15784+5G>A	n.a.	48	*de novo*	DC	novel
P207	c.16412+4A>G	n.a.	52	*de novo*	DC	Banka 2012
P208	c.16412+5G>C	n.a.	52	n.a.	DC	novel

Abbreviations: DC = Disease-causing variant, definitely or very likely pathogenic (truncating variant, or non-truncating and *de novo*, or described *de novo* in another patient, prediction disease causing), VUS = variant of unknown significance (non-truncating, inheritance unknown, not present in any public database of normal genetic variation, prediction disease causing), n.a. = not applicable. * = Inherited from an affected parent. RefSeq: NM_003482.3. Mutation nomenclature according to HGVS. Nucleotide numbering referring to cDNA uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1.

Table 2. Point mutations in *KDM6A* identified in our cohort.

Case	Sex	Mutation	Protein change	Exon/Intron	Segregation	Variant class	Published /novel
		***KDM6A* nonsense**					
P209	f	c.190G>T	p.(Glu64*)	2	de novo	DC	novel
P210	f	c.514C>T	p.(Arg172*)	6	de novo	DC	novel
P211	f	c.3109C>T	p.(Gln1037*)	20	de novo	DC	novel
		***KDM6A* small insertion**					
P212	f	c.171dupT	p.(Gly58Trpfs*7)	2	de novo	DC	novel
P213	m	c.2226_2227dupCA	p.(Ser743Thrfs*13)	17	n.a.	DC	novel
		***KDM6A* missense**					
P214	m	c.2729A>G	p.(Asn910Ser)	18	Inherited*	VUS	novel
P215	m	c.3073A>G	p.(Ser1025Gly)	20	Inherited**	DC	novel
P216	f	c.3763C>T	p.(Arg1255Trp)	26	de novo	DC	novel
		***KDM6A* splice site**					
P217	f	c.443+5G>C	n.a.	5	de novo	DC	novel
P218	m	c.619+6T>C	n.a.	7	de novo	DC	novel
P219	m	c.620-2A>G	n.a.	7	de novo	DC	novel
P220	f	c.2832+1G>A	n.a.	18	de novo	DC	novel

Abbreviations: DC = Disease-causing variant, definitely or very likely pathogenic (truncating variant, or non-truncating and *de novo*, or described *de novo* in another patient, prediction disease causing), VUS = variant of unknown significance (non-truncating, inheritance unknown, not present in any public database of normal genetic variation, prediction disease causing), n.a. = not applicable. * Maternally inherited, maternal phenotype unknown. ** Inherited from affected mother. RefSeq: NM_021140.3. Mutation nomenclature according to HGVS. Nucleotide numbering referring to cDNA uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1.

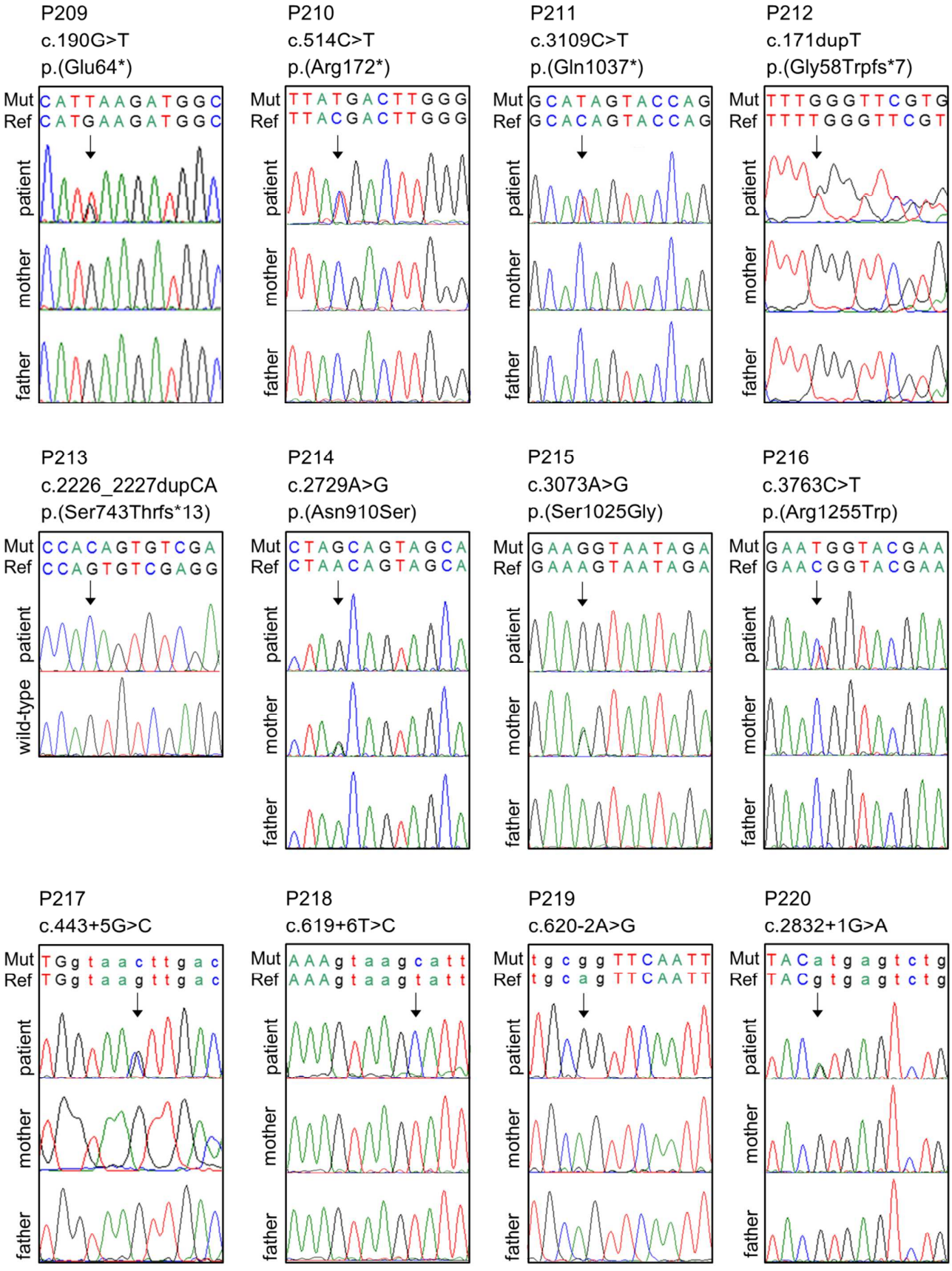
Table 3. Clinical findings in patients with *KDM6A* mutations.

Patient ID	P209	P210	P211	P212	P213	P214	P216	P217	P218	P219	P220	
Sex	f	f	f	f	m	m	f	f	m	m	f	
Growth anomalies												
Small for gestational age	-	+	-	-	+	-	-	-	-	+	+	4/11
Short stature	-	+	-	-	+	-	-	-	-	+	+	4/11
Microcephaly	-	+	-	+	-	+	-	-	-	+	+	5/11
Facial features												
Large / dysplastic ears	+	-	+	+	+	+	+	+	+	+	+	10/11
Long palpebral fissures	+	+	+	+	+	+	+	+	+	+	+	11/11
Eversion of the lower eye-lid	-	-	+	+	+	-	+	+	+	+	+	8/11
Long, thick eyelashes	-	-	+	+	+	+	+	+	-	-	+	7/11
Blue sclerae	-	-	+	-	-	-	+	+	+	+	+	6/11
Arched eyebrows	+	+	+	+	+	+	+	-	+	-	-	8/11
Lateral sparseness of eyebrows	+	+	+	+	-	-	+	+	+	+	-	8/11
Depressed nasal tip	+	-	+	+	+	+	+	-	-	-	+	7/11
Short columella	+	-	+	+	-	+	+	+	+	+	+	9/11
Downslanting corners of mouth	+	-	-	+	+	-	+	+	+	+	-	7/11
Eyes												
Cataracts	-	-	-	-	-	-	-	n.a.	n.a.	n.a.	-	0/8
Strabismus	-	-	-	-	+	-	-	-	-	+	+	3/7
Mouth												
Cleft palate	-	-	-	-	-	+	-	-	-	-	-	1/11
High arched palate	+	+	+	-	n.a.	-	+	+	-	+	+	7/10
Micrognathia	+	-	-	+	+	-	-	-	-	+	+	5/11
Dental anomalies												
Selective tooth agenesis	n.a.	+	-	n.a.	n.a.	-	-	n.a.	-	-	-	1/6
Oligodontia	n.a.	n.a.	-	n.a.	n.a.	-	-	n.a.	n.a.	n.a.	-	0/4
Supernumerary teeth	n.a.	-	-	n.a.	n.a.	-	-	n.a.	n.a.	n.a.	-	0/5
Dental crowding	n.a.	n.a.	-	n.a.	n.a.	-	-	n.a.	n.a.	n.a.	-	0/4
Malocclusion	n.a.	+	-	n.a.	n.a.	+	-	n.a.	n.a.	n.a.	-	2/5
Dental caries	n.a.	-	-	n.a.	n.a.	-	-	n.a.	n.a.	n.a.	-	0/5
Prominent upper incisors	n.a.	+	-	n.a.	-	-	-	n.a.	n.a.	n.a.	-	1/6
Skeletal findings												
Brachydactyly of the 5th finger	+	+	-	+	+	+	+	-	-	+	-	7/11
Clinodactyly of the 5th finger	+	+	+	-	+	+	-	-	-	-	+	6/11
Scoliosis	-	-	-	-	-	-	-	n.a.	-	-	-	0/10

Hip dysplasia	-	-	+	+	-	-	-	+	-	-	-	3/11
Joint laxity	+	-	-	+	+	+	+	-	n.a.	+	+	7/10
Foot deformity	-	-	+	-	-	-	-	n.a.	-	n.a.	+	2/9
Ectodermal findings												
Nail dystrophy	-	-	-	-	-	-	-	-	+	-	-	1/11
Thin temporal hair (infancy)	+	-	-	+	n.a.	-	+	+	-	-	-	4/10
Hypertrichosis	-	-	-	-	-	-	-	-	-	-	+	1/11
Persistent fetal finger pads	n.a.	+	+	+	+	+	+	+	+	+	+	10/10
Neurological findings												
Intellectual disability	+	+	+	+	++	+	-	+	+	+	+	10/11
Muscular hypotonia	+	-	+	+	++	-	+	+	+	+	+	9/11
Feeding difficulties	+	+	+	+	++	-	-	+	+	+	+	9/11
Seizures	-	-	-	+	-	-	-	-	-	-	-	1/11
Structural brain anomaly	-	-	n.a.	+	-	n.a.	n.a.	+	n.a.	n.a.	-	2/6
Hearing loss	-	-	-	-	-	-	-	-	n.a.	-	-	0/10
Congenital heart defects												
ASD/VSD	+	-	-	-	-	-	-	+	+	-	-	3/11
Coarctation of Aorta	-	-	-	-	-	-	+	-	-	-	-	1/11
Tetralogy of Fallot	-	-	-	-	-	-	-	-	-	-	-	0/11
Other	-	-	-	-	-	-	+	+	+	-	-	3/11
Kidneys												
Renal malformation	+	+	-	-	-	-	-	-	-	+	-	3/11
Renal malfunction	-	-	-	-	-	-	-	-	-	-	-	011
Hematological findings												
Anemia	-	-	-	-	-	-	-	-	-	-	-	0/11
Thrombocytopenia	-	-	-	-	-	-	-	-	-	-	-	0/11
Pancytopenia	-	-	-	-	-	-	-	-	-	-	-	0/11
Autoimmunity	-	-	-	-	n.a.	-	-	n.a.	-	+	-	1/9
Immunology												
Pulmonary infections	n.a.	-	-	+	-	-	-	-	-	-	-	1/10
Frequent upper airway infections	n.a.	-	-	+	-	-	+	-	-	-	-	2/10
Recurrent otitis media in infancy	n.a.	-	+	+	n.a.	-	-	-	-	-	+	3/9
Immunodeficiency	n.a.	-	-	-	-	-	-	n.a.	-	-	-	0/9
Oncology												
Tumor	n.a.	-	-	-	-	-	-	-	-	-	-	0/10
Leukemia	n.a.	-	-	-	-	-	-	-	-	-	-	0/10
Endocrinological findings												
Neonatal hypoglycemia	-	+	+	-	n.a.	-	-	+	+	+	-	5/10

Obesity	n.a.	-	-	-	-	-	-	n.a.	n.a.	-	-	0/8
Precocious puberty	n.a.	-	-	-	n.a.	-	-	n.a.	n.a.	n.a.	-	0/6
Premature thelarche	n.a.	-	-	-	n.a.	n.a.	+	n.a.	n.a.	n.a.	-	1/5
Other findings												
Additional findings:	Sacral dimple, simian crease, widely spaced nipples	Horseshoe kidney	Juvenile idiopathic osteoarthritis	-	Cachexia, no walking, no speech at age 10	Thorax asymmetry	Bicuspid aortic valve, accessory spleen	Left ventricular hypertrophy	Isolated persistent left superior vena cava, Hyperinsulinism	Autoimmunity suspected due to Vitiligo	Mild unilateral ptosis, bilateral simian crease, hyperactivity, hand-flapping, bruxism	

Abbreviations: f = female, m= male, n.a. = not applicable, ASD/VSD = atrial/ventricular septal defect.



Supplementary Figure 1. Electropherograms of the identified mutations in patients P209-220. Mut = mutated sequence, Ref = reference sequence.

Supplementary Table 1: Published mutations in *KMT2D*.

Exon / Intron	Nucleotide change	Amino acid change	Phenotype	Published record	Variant class[a]
	***KMT2D* nonsense**				
5	c.669T>G	p.(Tyr223*)	Kabuki syndrome	Micale 2011	DC
6	c.697G>T	p.(Glu233*)	Kabuki syndrome	Banka 2012	DC
8	c.1012G>T	p.(Glu338*)	Kabuki syndrome	Makrythanasis 2013	DC
10	c.1921G>T	p.(Glu641*)	Kabuki syndrome	Micale 2011	DC
10	c.2488G>T	p.(Glu830*)	Kabuki syndrome	Paderová 2016	DC
10	c.2608G>T	p.(Glu870*)	Kabuki syndrome	Miyake 2013	DC
11	c.2877C>A	p.(Tyr959*)	Kabuki syndrome	Morgan 2015	DC
11	c.3511G>T	p.(Glu1171*)	Kabuki syndrome	Miyake 2013	DC
11	c.3532C>T	p.(Gln1178*)	Kabuki syndrome	Dentici 2014	DC
11	c.3754C>T	p.(Arg1252*)	Kabuki syndrome	Lindsley 2015, Lin 2015	DC
12	c.3958G>T	p.(Gly1320*)	Kabuki syndrome	Li 2011	DC
14	c.4140T>A	p.(Cys1380*)	Kabuki syndrome	Liu 2015	DC
14	c.4171G>T	p.(Glu1391*)	Kabuki syndrome	Banka 2012	DC
16	c.4419G>A	p.(Trp1473*)	Kabuki syndrome	Micale 2011	DC
17	c.4633C>T	p.(Gln1545*)	Kabuki syndrome	Miyake 2013	DC
18	c.4843C>T	p.(Arg1615*)	Kabuki syndrome	Ng 2010	DC
22	c.5212G>T	p.(Glu1738*)	Kabuki syndrome	Micale 2014	DC
22	c.5263C>T	p.(Gln1755*)	Kabuki syndrome	Schulz 2014	DC
22	c.5269C>T	p.(Arg1757*)	Kabuki syndrome	Miyake 2013, Lin 2015	DC
26	c.5674C>T	p.(Gln1892*)	Kabuki syndrome	Micale 2014	DC
26	c.5707C>T	p.(Arg1903*)	Kabuki syndrome	Miyake 2013	DC
27	c.5832C>A	p.(Tyr1944*)	Kabuki syndrome	Hannibal 2011	DC
27	c.5845C>T	p.(Gln1949*)	Kabuki syndrome	Subbarayan 2014	DC
28	c.6010C>T	p.(Gln2004*)	Kabuki syndrome	Ng 2010	DC
29	c.6130C>T	p.(Gln2044*)	Kabuki syndrome	Makrythanasis 2013	DC
31	c.6295C>T	p.(Arg2099*)	Kabuki syndrome	Ng 2010, Micale 2011	DC
31	c.7228C>T	p.(Arg2410*)	Kabuki syndrome	Hannibal 2011	DC
31	c.7246C>T	p.(Gln2416*)	Kabuki syndrome	Micale 2011	DC
31	c.7426G>T	p.(Glu2476*)	Kabuki syndrome	Lindsley 2015	DC
31	c.7903C>T	p.(Arg2635*)	Kabuki syndrome	Micale 2011	DC
31	c.7933C>T	p.(Arg2645*)	Kabuki syndrome	Paulussen 2011	DC
31	c.7936G>T	p.(Glu2646*)	Kabuki syndrome	Micale 2014	DC
31	c.8032G>T	p.(Glu2678*)	Kabuki syndrome	Makrythanasis 2013	DC
32	c.8059C>T	p.(Arg2687*)	Kabuki syndrome	Banka 2012	DC

32	c.8107G>T	p.(Glu2703*)	Kabuki syndrome	Cheon 2014	DC
32	c.8160G>A	p.(Trp2720*)	Kabuki syndrome	Dentici 2014	DC
32	c.8200C>T	p.(Arg2734*)	Kabuki syndrome	Paulussen 2011	DC
33	c.8311C>T	p.(Arg2771*)	Kabuki syndrome	Paulussen 2011, Banka 2012	DC
34	c.8401C>T	p.(Arg2801*)	Kabuki syndrome	Miyake 2013	DC
34	c.8431C>T	p.(Gln2811*)	Kabuki syndrome	Li 2011	DC
34	c.8488C>T	p.(Arg2830*)	Kabuki syndrome	Ng 2010, Hannibal 2011	DC
34	c.8665G>T	p.(Gly2889*)	Kabuki syndrome	Hannibal 2011	DC
34	c.8721C>G	p.(Tyr2907*)	Kabuki syndrome	Hannibal 2011	DC
34	c.8743C>T	p.(Arg2915*)	Kabuki syndrome	Li 2011, Lin 2015, Van Laarhoven 2015, Paderová 2016	DC
34	c.9805C>T	p.(Gln3269*)	Kabuki syndrome	Banka 2012	DC
34	c.9829C>T	p.(Gln3277*)	Kabuki syndrome	Courcet 2013	DC
34	c.9931C>T	p.(Gln3311*)	Kabuki syndrome	Zarate 2012	DC
34	c.9961C>T	p.(Arg3321*)	Kabuki syndrome	Ng 2010, Hannibal 2011, Banka 2012, Van Laarhoven 2015	DC
34	c.10090C>T	p.(Gln3364*)	Kabuki syndrome	Miyake 2013	DC
34	c.10135C>T	p.(Gln3379*)	Kabuki syndrome	Micale 2011	DC
38	c.10738C>T	p.(Gln3580*)	Kabuki syndrome	Ng 2010	DC
39	c.10750C>T	p.(Gln3584*)	Kabuki syndrome	Micale 2014	DC
39	c.10841C>G	p.(Ser3614*)	Kabuki syndrome	Micale 2011	DC
39	c.11047C>T	p.(Gln3683*)	Kabuki syndrome	Hannibal 2011	DC
39	c.11119C>T	p.(Arg3707*)	Kabuki syndrome	Micale 2011	DC
39	c.11149C>T	p.(Gln3717*)	Kabuki syndrome	Ng 2010, Hannibal 2011	DC
39	c.11269C>T	p.(Gln3757*)	Kabuki syndrome	Micale 2011	DC
39	c.11290C>T	p.(Gln3764*)	Kabuki syndrome	Makrythanasis 2013	DC
39	c.11434C>T	p.(Gln3812*)	Kabuki syndrome	Micale 2011	DC
39	c.11515C>T	p.(Gln3839*)	Kabuki syndrome	Cheon 2014	DC
39	c.11527C>T	p.(Gln3843*)	Kabuki syndrome	Banka 2012	DC
39	c.11674C>T	p.(Gln3892*)	Kabuki syndrome	Banka 2012	DC
39	c.11704C>T	p.(Gln3902*)	Kabuki syndrome	Micale 2014	DC
39	c.11707C>T	p.(Gln3903*)	Kabuki syndrome	Paulussen 2011	DC
39	c.11722C>T	p.(Gln3908*)	Kabuki syndrome	Paulussen 2011	DC
39	c.11743C>T	p.(Gln3915*)	Kabuki syndrome	Makrythanasis 2013	DC
39	c.11761C>T	p.(Gln3921*)	Kabuki syndrome	Miyake 2013	DC
39	c.11764C>T	p.(Gln3922*)	Kabuki syndrome	Hannibal 2011	DC
39	c.11821C>T	p.(Gln3941*)	Kabuki syndrome	Hannibal 2011	DC
39	c.11833C>T	p.(Gln3945*)	Kabuki syndrome	Cheon 2014	DC
39	c.11869C>T	p.(Gln3957*)	Kabuki syndrome	Micale 2014	DC
39	c.11887C>T	p.(Gln3963*)	Kabuki syndrome	Banka 2012	DC
39	c.11917C>T	p.(Gln3973*)	Kabuki syndrome	Miyake 2013	DC

39	c.11944C>T	p.(Arg3982*)	Kabuki syndrome	Paulussen 2011, Miyake 2013	DC
39	c.11962C>T	p.(Gln3988*)	Kabuki syndrome	Miyake 2013	DC
39	c.12076C>T	p.(Gln4026*)	Kabuki syndrome	Micale 2011	DC
39	c.12220C>T	p.(Gln4074*)	Kabuki syndrome	Miyake 2013	DC
39	c.12241C>T	p.(Gln4081*)	Kabuki syndrome	Ng 2010, Banka 2012	DC
39	c.12274C>T	p.(Gln4092*)	Kabuki syndrome	Micale 2011	DC
39	c.12307C>T	p.(Gln4013*)	Kabuki syndrome	Lin 2015	DC
39	c.12511C>T	p.(Gln4171*)	Kabuki syndrome	Makrythanasis 2013	DC
39	c.12592C>T	p.(Arg4198*)	Kabuki syndrome	Banka 2012, Makrythanasis 2013	DC
39	c.12688C>T	p.(Gln4230*)	Kabuki syndrome	Hannibal 2011, Miyake 2013, Van Laarhoven 2015	DC
39	c.12697C>T	p.(Gln4233*)	Kabuki syndrome	Ng 2010	DC
39	c.12703C>T	p.(Gln4235*)	Kabuki syndrome	Ng 2010	DC
39	c.12808C>T	p.(Gln4270*)	Kabuki syndrome	Makrythanasis 2013	DC
39	c.12823C>T	p.(Gln4275*)	Kabuki syndrome	Morgan 2015	DC
39	c.12844C>T	p.(Arg4282*)	Kabuki syndrome	Micale 2014	DC
39	c.12955A>T	p.(Arg4319*)	Kabuki syndrome	Micale 2014	DC
39	c.12964C>T	p.(Gln4322*)	Kabuki syndrome	Subbarayan 2014	DC
39	c.13159C>T	p.(Gln4387*)	Kabuki syndrome	Morgan 2015	DC
39	c.13201C>T	p.(Gln4401*)	Kabuki syndrome	Hannibal 2011, Makrythanasis 2013	DC
39	c.13285C>T	p.(Gln4429*)	Kabuki syndrome	Hannibal 2011	DC
39	c.13390C>T	p.(Gln4464*)	Kabuki syndrome	Ng 2010, Banka 2012	DC
39	c.13450C>T	p.(Arg4484*)	Kabuki syndrome	Paulussen 2011, Makrythanasis 2013, Dentici 2015	DC
39	c.13507C>T	p.(Gln4503*)	Kabuki syndrome	Micale 2014	DC
40	c.13579A>T	p.(Lys4527*)	Kabuki syndrome	Ng 2010	DC
40	c.13606C>T	p.(Arg4536*)	Kabuki syndrome	Ng 2010	DC
40	c.13666A>T	p.(Lys4556*)	Kabuki syndrome	Micale 2011	DC
42	c.13903C>T	p.(Gln4635*)	Kabuki syndrome	Miyake 2013	DC
42	c.13906C>T	p.(Gln4636*)	Kabuki syndrome	Banka 2012	DC
48	c.14659G>T	p.(Glu4887*)	Kabuki syndrome	Van Laarhoven 2015	DC
48	c.14710C>T	p.(Arg4904*)	Kabuki syndrome	Ng 2010, Hannibal 2011, Makrythanasis 2013	DC
48	c.14861C>A	p.(Ser4954*)	Kabuki syndrome	Miyake 2013	DC
48	c.14878C>T	p.(Arg4960*)	Kabuki syndrome	Paulussen 2011, Banka 2012 (2 patients)	DC
48	c.14946G>A	p.(Trp4982*)	Kabuki syndrome	Hannibal 2011	DC
48	c.15022G>T	p.(Glu5008*)	Kabuki syndrome	Micale 2014	DC
48	c.15061C>T	p.(Arg5021*)	Kabuki syndrome	Banka 2012	DC
48	c.15079C>T	p.(Arg5027*)	Kabuki syndrome	Paulussen 2011, Micale 2011	DC
48	c.15195G>A	p.(Trp5065*)	Kabuki syndrome	Ng 2010 (2 patients)	DC
48	c.15217C>T	p.(Gln5073*)	Kabuki syndrome	Ng 2010	DC
48	c.15256C>T	p.(Arg5086*)	Kabuki syndrome	Banka 2012	DC

48	c.15339C>A	p.(Tyr5113*)	Kabuki syndrome	Hannibal 2011	DC
48	c.15351T>A	p.(Cys5117*)	Kabuki syndrome	Banka 2012	DC
48	c.15618T>G	p.(Tyr5206*)	Kabuki syndrome	Ng 2010	DC
49	c.15844C>T	p.(Arg5282*)	Kabuki syndrome	Tanaka 2012	DC
50	c.16018C>T	p.(Arg5340*)	Kabuki syndrome	Paulussen 2011	DC
50	c.16135C>T	p.(Gln5379*)	Kabuki syndrome	Lin 2015	DC
52	c.16342C>T	p.(Arg5448*)	Kabuki syndrome	Hannibal 2011	DC
52	c.16360C>T	p.(Arg5454*)	Kabuki syndrome	Ng 2010, Hannibal 2011, Paulusen 2011	DC
53	c.16501C>T	p.(Arg5501*)	Kabuki syndrome	Ng 2010	DC
Exon	***KMT2D* small deletions**[b]				
3	c.303delG	p.(Ser102Alafs*28)	Kabuki syndrome	Lindsley 2015	DC
4	c.472delT	p.(Cys158Valfs*50)	Kabuki syndrome	Micale 2011	DC
5	c.588delC	p.(Cys197Alafs*11)	Kabuki syndrome	Makrythanasis 2013	DC
5	c.589delT	p.(Cys197Alafs*11)	Kabuki syndrome	Makrythanasis 2013	DC
6	c.702delG	p.(Pro235Glnfs*26)	Kabuki syndrome	Hannibal 2011	DC
6	c.705delA	p.(Glu237Serfs*24)	Kabuki syndrome	Micale 2011	DC
6	c.721delC	p.(Leu241Cysfs*20)	Kabuki syndrome	Dentici 2014	DC
8	c.1035_1036delCT	p.(Cys346Serfs*17)	Kabuki syndrome	Micale 2011	DC
10	c.1300delC	p.(Leu434*)	Kabuki syndrome	Miyake 2013	DC
10	c.1301delT	p.(Leu434Glnfs*496)	Kabuki syndrome	Paulussen 2011	DC
10	c.1328delC	p.(Pro443Hisfs*487)	Kabuki syndrome	Ng 2010	DC
10	c.1345_1346delCT	p.(Leu449Valfs*5)	Kabuki syndrome	Micale 2011	DC
10	c.1483_1486delTCTC	p.(Ser495Argfs*434)	Kabuki syndrome	Li 2011	DC
10	c.1512_1513delTC	p.(Pro506Thrfs*2)	Kabuki syndrome	Li 2011	DC
10	c.1634delT	p.(Leu545Argfs*385)	Kabuki syndrome	Banka 2012	DC
10	c.2110delG	p.(Asp704Thrfs*226)	Kabuki syndrome	Paulussen 2011	DC
10	c.2272delG	p.(Glu758Serfs*172)	Kabuki syndrome	Paulussen 2011	DC
10	c.2558_2559delCT	p.(Pro853Argfs*3)	Kabuki syndrome	Paulussen 2011	DC
11	c.3095delT	p.(Leu1032Argfs*24)	Kabuki syndrome	Liu 2015	DC
11	c.3161_3171del11	p.(Pro1054Hisfs*10)	Kabuki syndrome	Cappuccio 2014	DC
11	c.3281_3282delTC	p.(Leu1094Profs*20)	Kabuki syndrome	Miyake 2013	DC
11	c.3354delA	p.(Glu1120Lysfs*44)	Kabuki syndrome	Banka 2012	DC
11	c.3730delG	p.(Val1244Serfs*86)	Kabuki syndrome	Micale 2014	DC
11	c.3889delC	p.(Arg1297Valfs*33)	Kabuki syndrome	Paulussen 2011	DC
13	c.4021delG	p.(Val1341Leufs*35)	Kabuki syndrome	Micale 2014	DC
14	c.4135_4136delAT	p.(Met1379Valfs*52)	Kabuki syndrome	Micale 2014, Cheon 2014	DC
14	c.4219_4222delTACT	p.(Tyr1407Valfs*9)	Kabuki syndrome	Paulussen 2011	DC
15	c.4292_4300delAGGTGTGTG	p.(Glu1431_Cys1433del)	Kabuki syndrome	Morgan 2015	DC

16	c.4454delC	p.(Pro1485Leufs*21)	Kabuki syndrome	Micale 2014	DC
16	c.4490_4491delAC	p.(His1497Leufs*30)	Kabuki syndrome	Miyake 2013	DC
16	c.4549delG	p.(Glu1517Argfs*4)	Kabuki syndrome	Paderová 2016	DC
18	c.4736_4737delAG	p.(Glu1579Alafs*23)	Kabuki syndrome	Miyake 2013	DC
19	c.4895delC	p.(Ser1632*)	Kabuki syndrome	Micale 2011	DC
19	c.4896_4905del10	p.(Asp1633Alafs*86)	Kabuki syndrome	Micale 2014	DC
21	c.5124_5125delAC	p.(Arg1709Hisfs*25)	Kabuki syndrome	Banka 2012	DC
21	c.5135_5136delAG	p.(Lys1712Argfs*22)	Kabuki syndrome	Makrythanasis 2013	DC
21	c.5166delT	p.(Ser1722Argfs*9)	Congenital heart disease	Zaidi 2013	DC
22	c.5256_5257delGA	p.(Lys1753Alafs*34)	Kabuki syndrome	Kim 2013	DC
25	c.5585delA	p.(Lys1862Serfs*14)	Kabuki syndrome	Paulussen 2011	DC
25	c.5627_5630delACAG	p.(Asp1876Glyfs*38)	Kabuki syndrome	Banka 2012, Paderová 2016	DC
26	c.5779delC	p.(Gln1927Lysfs*120)	Kabuki syndrome	Micale 2011	DC
27	c.5857delC	p.(Leu1953Trpfs*94)	Kabuki syndrome	Micale 2014	DC
28	c.5908_5915delGACAGCCC	p.(Asp1970Leufs*20)	Kabuki syndrome	Van Laarhoven 2015	DC
28	c.5912delG	p.(Ser1971Thrfs*76)	Kabuki syndrome	Paulussen 2011	DC
28	c.5954delC	p.(Thr1985Lysfs*62)	Kabuki syndrome	Micale 2014	DC
29	c.6149_6150delGA	p.(Arg2050Lysfs*6)	Kabuki syndrome	Micale 2014	DC
31	c.6297_6298delAC	p.(Pro2100Glyfs*54)	Kabuki syndrome	Miyake 2013	DC
31	c.6334delG	p.(Ala2112Hisfs*32)	Kabuki syndrome	Hannibal 2011	DC
31	c.6583delA	p.(Thr2195Profs*69)	Kabuki syndrome	Micale 2014	DC
31	c.6594delC	p.(Tyr2199Ilefs*65)	Kabuki syndrome	Hannibal 2011	DC
31	c.6595delT	p.(Tyr2199Ilefs*65)	Kabuki syndrome	Ng 2010, Li 2011, Micale 2011, Banka 2012, Morgan 2015, Makrythanasis2013, Van Laarhoven 2015	DC
31	c.6638_6641delGCGC	p.(Gly2213Alafs*50)	Kabuki syndrome	Micale 2011	DC
31	c.6738delA	p.(Lys2246Asnfs*18)	Kabuki syndrome	Micale 2014	DC
31	c.6794delG	p.(Gly2265Glufs*21)	Kabuki syndrome	Micale 2014	DC
31	c.6844delC	p.(Arg2282Glyfs*4)	Kabuki syndrome	Lindsley 2015	DC
31	c.6991delC	p.(Leu2331*)	Kabuki syndrome	Makrythanasis 2013	DC
31	c.7297delG	p.(Glu2433Lysfs*52)	Kabuki syndrome	Makrythanasis 2013	DC
31	c.7479delG	p.(Phe2494Serfs*49)	Kabuki syndrome	Makrythanasis 2013	DC
31	c.7649_7650delCT	p.(Pro2550Argfs*104)	Kabuki syndrome	Lin 2015	DC
31	c.7650delT	p.(Val2551Serfs*32)	Kabuki syndrome	Hannibal 2011	DC
31	c.7822delT	p.(Ser2608Profs*83)	Kabuki syndrome	Banka 2012	DC
32	c.8196delG	p.(Ser2733Valfs*24)	Kabuki syndrome	Micale 2014	DC
33	c.8273delG	p.(Gly2758Alafs*29)	Kabuki syndrome	Micale 2011	DC
33	c.8307_8308delTG	p.(Asp2769Glufs*75)	Kabuki syndrome	Banka 2012	DC
34	c.8463_8475del13	p.(Ala2823Profs*24)	Kabuki syndrome	Banka 2013	DC

34	c.8727_8730delAAGT	p.(Ser2910Argfs*32)	Kabuki syndrome	Hannibal 2011	DC
34	c.8952delG	p.(Lys2985Serfs*19)	Kabuki syndrome	Hannibal 2011	DC
34	c.9164delC	p.(Pro3055Leufs*16)	Kabuki syndrome	Cheon 2014	DC
34	c.9203delA	p.(Glu3068Glyfs*3)	Kabuki syndrome	Micale 2014	DC
34	c.9329delG	p.(Arg3110Profs*9)	Kabuki syndrome	Paulussen 2011	DC
34	c.9460delC	p.(Leu3154*)	Kabuki syndrome	Li 2011	DC
34	c.9494delA	p.(Asp3165Valfs*32)	Kabuki syndrome	Banka 2012	DC
34	c.9581delA	p.(His3194Profs*3)	Kabuki syndrome	Hannibal 2011	DC
34	c.10114_10126del13	p.(Ser3372Cysfs*16)	Kabuki syndrome	Paulussen 2011	DC
36	c.10395delA	p.(Pro3466Leufs*36)	Kabuki syndrome	Hannibal 2011	DC
38	c.10606delC	p.(Arg3536Alafs*122)	Kabuki syndrome	Micale 2011	DC
39	c.11066_11078del13	p.(Ala3689Valfs*56)	Kabuki syndrome	Micale 2011	DC
39	c.11102delC	p.(Pro3701Leufs*48)	Kabuki syndrome	Banka 2012	DC
39	c.11456delG	p.(Gly3819Alafs*11)	Kabuki syndrome	Morgan 2015	DC
39	c.11497delC	p.(Arg3833Glyfs*48)	Kabuki syndrome	Paulussen 2011	DC
39	c.11729_11734delAGCAAC	p.(Gln3910_Gln3911del)	Kabuki syndrome	Liu 2015	VUS
39	c.11794_11797delCAAC	p.(Gln3932Serfs*46)	Kabuki syndrome	Ng 2010	DC
39	c.11796_11813del	p.(Gln3934_Gln3939del)	Kabuki syndrome	Van Laarhoven 2015	VUS
39	c.11843_11860del	p.(Leu3948_Gln3953del)	Kabuki syndrome	Micale 2014	NDC
39	c.12151delA	p.(Ile4051*)	Kabuki syndrome	Miyake 2013	DC
39	c.12164_12165delCT	p.(Pro4055Argfs*6)	Kabuki syndrome	Paulussen 2011	DC
39	c.12179_12182delCTGA	p.(Thr4060Asnfs*5)	Kabuki syndrome	Banka 2012	DC
39	c.12441delC	p.(Met4148*)	Kabuki syndrome	Banka 2012	DC
39	c.12647delC	p.(Pro4216Leufs*62)	Kabuki syndrome	Micale 2014	DC
39	c.12753_12754delTC	p.(Leu4253Profs*80)	Kabuki syndrome	Banka 2012	DC
39	c.12966delA	p.(Gln4322Hisfs*62)	Kabuki syndrome	Micale 2014	DC
42	c.13895delC	p.(Pro4632Hisfs*8)	Kabuki syndrome	Hannibal 2011, Banka 2012	DC
46	c.14404delG	p.(Ala4802Glnfs*6)	Kabuki syndrome	Cheon 2014	DC
48	c.15031delG	p.(Glu5011Serfs*40)	Kabuki syndrome	Micale 2014	DC
48	c.15446_15447delTT	p.(Phe5149Cysfs*9)	Kabuki syndrome	Ng 2010	DC
48	c.15452delT	p.(Val5151Alafs*12)	Kabuki syndrome	Morgan 2015	DC
48	c.15235_15238delAATG	p.(Asn5079Trpfs*10)	Kabuki syndrome	Van Laarhoven 2015	DC
51	c.16085_16086delAG	p.(Lys5362Serfs*96)	Kabuki syndrome	Roma 2015	DC
51	c.16101delC	p.(Phe5368Serfs*50)	Kabuki syndrome	Banka 2012	DC
	c.16327delT	p.(Tyr5443Thrfs*13)	Kabuki syndrome	Gohda 2015	DC
52	c.16371_16374delTGAA	p.(Glu5458Metfs*2)	Kabuki syndrome	Banka 2012, Paderová 2016	DC
53	c.16437delT	p.(Asn5480Thrfs*7)	Kabuki syndrome	Hannibal 2011	DC
53	c.16428delC	p.(Cys5477Valfs*10)	Kabuki syndrome	McVeigh 2015	DC

53	c.16438_16441delAACT	p.(Asn5480Valfs*6)	Kabuki syndrome	Hannibal 2011	DC
53	c.16469_16470delAA	p.(Lys5490Argfs*21)	Kabuki syndrome	Micale 2011	DC
53	c.16489_16491delATC	p.(Ile5497del)	Kabuki syndrome	Hannibal 2011 (2 patients), Micale 2011	DC
Exon	***KMT2D* small insertions/duplications**[b]				
7	c.859_860insT	p.(Lys287Ilefs*6)	Kabuki syndrome	Makrythanasis 2013	DC
10	c.1448dupT	p.(Leu483Phefs*17)	Kabuki syndrome	Banka 2012	DC
10	c.1503dupT	p.(Pro502Serfs*7)	Kabuki syndrome	Micale 2014	DC
10	c.2008_2009insT	p.(Pro670Leufs*7)	Kabuki syndrome	Lindsley 2015	DC
10	c.2433_2434insCA	p.(Glu812Glnfs*119)	Kabuki syndrome	Miyake 2013	DC
11	c.2993dupC	p.(Met999Tyrfs*69)	Kabuki syndrome	Micale 2011	DC
11	c.3318dupC	p.(Ser1107Glnfs*8)	Kabuki syndrome	Dentici 2014	DC
11	c.3326_3336dup11	p.(Asp1113Profs*10)	Kabuki syndrome	Miyake 2013	DC
11	c.3585dupA	p.(Pro1196Thrfs*11)	Kabuki syndrome	Ng 2010	DC
14	c.4162_4163insCG	p.(Arg1388Profs*30)	Kabuki syndrome	Makrythanasis 2013	DC
14	c.4168dupG	p.(Ala1390Glyfs*42)	Kabuki syndrome	Makrythanasis 2013	DC
15	c.4366dupT	p.(Cys1456Leufs*35)	Kabuki syndrome	Soden 2014	DC
15	c.4395dupC	p.(Lys1466Glnfs*25)	Kabuki syndrome	Liu 2015	DC
19	c.4958dupG	p.(Glu1654*)	Kabuki syndrome	Ng 2010	DC
20	c.5058dupA	p.(Arg1687Thrfs*4)	Kabuki syndrome	Banka 2012	DC
22	c.5268dupG	p.(Arg1757Alafs*31)	Kabuki syndrome	Li 2011	DC
24	c.5527dupA	p.(Thr1843Asnfs*5)	Kabuki syndrome	Banka 2012	DC
26	c.5652dupC	p.(Lys1885Glnfs*18)	Kabuki syndrome	Makrythanasis 2013	DC
26	c.5775dupT	p.(Leu1926Serfs*31)	Kabuki syndrome	Cheon 2014	DC
28	c.5877_5893dup17	p.(Glu1965Glyfs*88)	Kabuki syndrome	Ng 2010	DC
31	c.6613dupG	p.(Ala2205Glyfs*38)	Kabuki syndrome	Takagi 2013	DC
31	c.6729dupA	p.(Phe2244Ilefs*11)	Kabuki syndrome	Makrythanasis 2013	DC
31	c.6971dupC	p.(Asp2325*)	Kabuki syndrome	Subbarayan 2014	DC
31	c.7289dupT	p.(Ser2431Valfs*3)	Kabuki syndrome	Li 2011	DC
31	c.7307_7308insT	p.(Ser2438Ilefs*11)	Kabuki syndrome	Makrythanasis 2013, Karagianni 2016	DC
31	c.7481dupT	p.(Ala2496Serfs*10)	Kabuki syndrome	Micale 2014, Van Laarhoven 2015	DC
34	c.8430_8431insAA	p.(Gln2811Asnfs*41)	Kabuki syndrome	Micale 2014	DC
34	c.8740dupC	p.(His2914Profs*6)	Kabuki syndrome	Makrythanasis 2013	DC
34	c.9109dupC	p.(His3037Profs*4)	Kabuki syndrome	Brackmann 2012	DC
34	c.9223dupT	p.(Ser3075Phefs*3)	Kabuki syndrome	Paulussen 2011	DC
34	c.9770dupA	p.(Lys3258Glufs*43)	Kabuki syndrome	Paulussen 2011	DC
34	c.9831_9833dupGCA	p.(Gln3282dup)	Kabuki syndrome	Hannibal 2011	NDC
34	c.9831_9848dup18	p.(Gln3277_Gln3282dup)	Kabuki syndrome	Miyake 2013	NDC
39	c.10772dupT	p.(Met3592Hisfs*83)	Kabuki syndrome	Makrythanasis 2013	DC

39	c.11515dupC	p.(Gln3839Profs*173)	Kabuki syndrome	Banka 2012	DC
39	c.11714_11716dupAGC	p.(Gln3905dup)	Kabuki syndrome	Micale 2014	NDC
39	c.11806_11807dupCA	p.(Gln3936Hisfs*44)	Kabuki syndrome	Miyake 2013	DC
39	c.11819_11836dup18	p.(Leu3940_Gln3945dup)	Kabuki syndrome	Micale 2011	NDC
39	c.12414dupT	p.(Val4139Cysfs*29)	Kabuki syndrome	Makrythanasis 2013	DC
39	c.12969dupA	p.(Pro4324Thrfs*10)	Kabuki syndrome	Paulussen 2011	DC
39	c.13102dupA	p.(Thr4368Asnfs*4)	Kabuki syndrome	Banka 2012	DC
39	c.13129dupT	p.(Trp4377Leufs*33)	Kabuki syndrome	Micale 2011	DC
39	c.13277dupT	p.(Ala4428Serfs*59)	Kabuki syndrome	Micale 2014	DC
42	c.13884dupC	p.(Thr4629Hisfs*18)	Kabuki syndrome	Micale 2014	DC
46	c.14485dupG	p.(Glu4829Glyfs*8)	Kabuki syndrome	Banka 2012	DC
47	c.14592dupG	p.(Pro4865Alafs*48)	Kabuki syndrome	Micale 2014	DC
48	c.14760_14761insA	p.(Leu4921Ilefs*11)	Kabuki syndrome	Banka 2012	DC
48	c.14845_14848dupCCTC	p.(Leu4950Profs*9)	Kabuki syndrome	Paulussen 2011	DC
48	c.15073_15080dupGTACCGCG	p.(Asp5028Tyrfs*26)	Kabuki syndrome	Hannibal 2011	DC
48	c.15163_15168dupGACCTG	p.(Asp5055_Leu5056dup)	Kabuki syndrome	Micale 2011	VUS
48	c.15522_15525dupGCTG	p.(His5176Alafs*24)	Kabuki syndrome	Morgan 2015	DC
48	c.15374dupT	p.(Phe5126Leufs*12)	Kabuki syndrome	Makrythanasis 2013	DC
50	c.15947dupA	p.(Asn5316Lysfs*29)	Kabuki syndrome	Makrythanasis 2013	DC
51	c.16156dupT	p.(Ser5386Phefs*73)	Kabuki syndrome	Banka 2012	DC
51	c.16204dupG	p.(Ala5402Glyfs*57)	Kabuki syndrome	Banka 2012	DC
Exon	***KMT2D* indels**				
15	c.4249_4252delATGCinsGTGA	p.(Met1417_Leu1418delinsValMet)	Kabuki syndrome	Micale 2011	NDC
27	c.5865_5867delTAGinsCCCCC	p.(Arg1956Profs*92)	Kabuki syndrome	Hannibal 2011	DC
31	c.6349_6350delCCinsA	p.(Pro2117Thrfs*27)	Kabuki syndrome	Paderová 2016	DC
34	c.8641_8646delins23	p.(Arg2881Aspfs*35)	Kabuki syndrome	Paulussen 2011	DC
34	c.8859_8861delGGGinsCA	p.(Lys2953Asnfs*51)	Kabuki syndrome	Hannibal 2011	DC
Exon	***KMT2D* missense**				
2	c.96C>G	p.(Asp32Glu)	Kabuki syndrome	Liu 2015	VUS
3	c.346T>C	p.(Ser116Pro)	Kabuki syndrome	Micale 2014	VUS
5	c.626C>T	p.(Thr209Ile)	Kabuki syndrome	Micale 2014	NDC
8	c.1010C>T	p.(Ser337Leu)	Kabuki syndrome	Banka 2012	NDC
10	c.1628C>T	p.(Ser543Leu)	Kabuki syndrome	Li 2011	NDC
10	c.1940C>A	p.(Pro647Gln)	Kabuki syndrome	Li 2011, Makrythanasis 2013	NDC‡
11	c.2992C>G	p.(Pro998Ala)	Kabuki syndrome	Subbarayan 2014	NDC
11	c.3103C>A	p.(Gln1035Lys)	Autism spectrum disorder	Yuen 2015	DC
11	c.3392C>T	p.(Pro1131Leu)	Kabuki syndrome	Micale 2014	NDC
11	c.3524C>T	p.(Thr1175Ile)	Kabuki syndrome	Micale 2014	NDC

11	c.3572C>T	p.(Pro1191Leu)	Kabuki syndrome	Micale 2014	NDC
11	c.3574G>A	p.(Val1192Met)	Kabuki syndrome	Li 2011	DC
11	c.3773G>A	p.(Arg1258Gln)	Kabuki syndrome	Micale 2011	NDC
13	c.4127T>G	p.(Met1376Arg)	Kabuki syndrome	Miyake 2013	VUS
14	c.4138T>C	p.(Cys1380Arg)	Kabuki syndrome	Makrythanasis 2013	VUS
14	c.4160G>A	p.(Gly1387Asp)	Kabuki syndrome	Morgan 2015	DC
14	c.4163G>T	p.(Arg1388Leu)	Kabuki syndrome	Hannibal 2011	NDC
14	c.4171G>A	p.(Glu1391Lys)	Kabuki syndrome	Micale 2014	DC
15	c.4267C>T	p.(Arg1423Cys)	Kabuki syndrome	Miyake 2013	VUS
15	c.4271G>T	p.(Cys1424Phe)	Kabuki syndrome	Cheon 2014	DC
15	c.4283T>C	p.(Ile1428Thr)	Kabuki syndrome	Micale 2014	NDC
15	c.4288T>C	p.(Cys1430Arg)	Kabuki syndrome	Hannibal 2011	VUS
15	c.4333T>G	p.(Cys1445Gly)	Kabuki syndrome	Miyake 2013	DC
15	c.4358A>G	p.(His1453Arg)	Kabuki syndrome	Li 2011	DC
15	c.4411T>C	p.(Cys1471Arg)	Kabuki syndrome	Makrythanasis 2013	DC
15	c.4412G>A	p.(Cys1471Tyr)	Kabuki syndrome	Hannibal 2011	VUS
16	c.4427C>G	p.(Ser1476Cys)	Kabuki syndrome	Micale 2014	VUS
16	c.4565A>G	p.(Gln1522Arg)	Kabuki syndrome	Micale 2011	NDC
16	c.4577G>T	p.(Cys1526Phe)	Kabuki syndrome	Miyake 2013	DC
17	c.4664C>T	p.(Ser1555Phe)	Kabuki syndrome	Liu 2015	DC
21	c.5153C>T	p.(Ala1718Val)	Kabuki syndrome	Li 2011	VUS
22	c.5226G>C	p.(Glu1742Asp)	Kabuki syndrome	Micale 2014	VUS
28	c.5993A>G	p.(Tyr1998Cys)	Kabuki syndrome	Lin 2015	DC
31	c.6638G>A	p.(Gly2213Asp)	Kabuki syndrome	Micale 2014	NDC
31	c.6811C>T	p.(Pro2271Ser)	Kabuki syndrome	Micale 2014	NDC
31	c.6970C>A	p.(Pro2324Thr)	Kabuki syndrome	Micale 2014	VUS
31	c.7378C>T	p.(Arg2460Cys)	Kabuki syndrome	Paulussen 2011	NDC
31	c.7829T>C	p.(Leu2610Pro)	Kabuki syndrome	Micale 2011	NDC
34	c.8521C>A	p.(Pro2841Thr)	Kabuki syndrome	Micale 2011	VUS
34	c.8639T>C	p.(Leu2880Pro)	Kabuki syndrome	Liu 2015	DC
34	c.10192A>G	p.(Met3398Val)	Kabuki syndrome	Micale 2014	NDC
37	c.10499G>T	p.(Gly3500Val)	Kabuki syndrome	Micale 2014	DC
39	c.10966C>T	p.(Arg3656Cys)	Kabuki syndrome	Micale 2014	NDC
39	c.11638C>A	p.(Leu3880Met)	Kabuki syndrome	Liu 2015	VUS
39	c.11794C>G	p.(Gln3932Glu)	Kabuki syndrome	Micale 2014	VUS
39	c.12070A>G	p.(Lys4024Glu)	Kabuki syndrome	Micale 2014	NDC
39	c.12199C>T	p.(Pro4067Ser)	Kabuki syndrome	Liu 2015	DC*
39	c.12485G>A	p.(Arg4162Gln)	Kabuki syndrome	Micale 2014	NDC
39	c.12488C>T	p.(Pro4163Leu)	Kabuki syndrome	Micale 2014	VUS

39	c.13058C>T	p.(Pro4353Leu)	Kabuki syndrome	Banka 2012	NDC
39	c.13256C>T	p.(Pro4419Leu)	Kabuki syndrome	Micale 2014	NDC
39	c.13259G>A	p.(Arg4420Gln)	Kabuki syndrome	Cheon 2014	NDC
48	c.14732C>T	p.(Pro4911Leu)	Kabuki syndrome	Van Laarhofen 2015	VUS
48	c.14893G>A	p.(Ala4965Thr)	Kabuki syndrome	Micale 2014	NDC
48	c.14896C>T	p.(Arg4966Try)	Kabuki syndrome	Banka 2012	NDC
48	c.15084C>G	p.(Asp5028Glu)	Kabuki syndrome	Micale 2011	DC**
48	c.15088C>T	p.(Arg5030Cys)	Kabuki syndrome	Makrythanasis 2013	DC***
48	c.15100T>G	p.(Phe5034Val)	Kabuki syndrome	Micale 2011	DC**
48	c.15104G>C	p.(Cys5035Ser)	Kabuki syndrome	Lindsley 2015	VUS
48	c.15119A>G	p.(Asp5040Gly)	Kabuki syndrome	Miyake 2013	DC
48	c.15140C>T	p.(Ala5047Val)	Kabuki syndrome	Banka 2012	VUS
48	c.15142C>T	p.(Arg5048Cys)	Kabuki syndrome	Hannibal 2011, Banka 2012 (familial), Makrythanasis 2013, Van Laarhofen 2015	DC
48	c.15143G>A	p.(Arg5048His)	Kabuki syndrome	Makrythanasis 2013, Miyake 2013	DC
48	c.15176A>C	p.(His5059Pro)	Kabuki syndrome	Micale 2011	DC
48	c.15185G>A	p.(Cys5062Tyr)	Kabuki syndrome	Morgan 2015	DC
48	c.15275G>A	p.(Cys5092Tyr)	Kabuki syndrome	Dentici 2014	VUS
48	c.15292A>C	p.(Thr5098Pro)	Kabuki syndrome	Micale 2014	VUS
48	c.15326G>T	p.(Cys5109Phe)	Kabuki syndrome	Ng 2010, Lin 2015	DC
48	c.15461G>A	p.(Arg5154Gln)	Kabuki syndrome	Li 2011 (2 patients), Miyake 2013, Morgan 2015, Lindsley 2015	DC
48	c.15535C>T	p.(Arg5179Cys)	Kabuki syndrome	Dentici 2014	DC
48	c.15536G>A	p.(Arg5179His)	Kabuki syndrome	Ng 2010 (2 patients), Hannibal 2011, Miyake 2013, Morgan 2015	DC
48	c.15548T>C	p.(Leu5183Pro)	Kabuki syndrome	Morgan 2015	DC
48	c.15562A>G	p.(Ile5188Val)	Kabuki syndrome	Makrythanasis 2013	NDC
48	c.15565G>A	p.(Gly5189Arg)	Kabuki syndrome	Micale 2011, Miyake 2013	DC†
48	c.15629A>G	p.(Tyr5210Cys)	Kabuki syndrome	Paulussen 2011	DC
48	c.15640C>T	p.(Arg5214Cys)	Kabuki syndrome	Hannibal 2011, Banka 2012, Makrythanasis 2013	DC***
48	c.15641G>A	p.(Arg5214His)	Kabuki syndrome	Ng 2010, Hannibal 2011 (2 patients)	DC
48	c.15649T>C	p.(Trp5217Arg)	Kabuki syndrome	Micale 2014	DC
50	c.16019G>A	p.(Arg5340Gln)	Kabuki syndrome	Micale 2011	DC
50	c.16019G>T	p.(Arg5340Leu)	Kabuki syndrome	Ng 2010	VUS
50	c.16052G>A	p.(Arg5351Gln)	Kabuki syndrome	Miyake 2013	DC
51	c.16273G>A	p.(Glu5425Lys)	Kabuki syndrome	Micale 2014, Lin 2015	DC
51	c.16283G>A	p.(Gly5428Asp)	Kabuki syndrome	Paulussen 2011	DC
51	c.16295G>A	p.(Arg5432Gln)	Kabuki syndrome	Kokitsu-Nakata 2012 (familial), Liu 2015	DC*
51	c.16294C>T	p.(Arg5432Trp)	Kabuki syndrome	Tanaka 2012, Makrythanasis 2013, Lindsley 2015	DC
52	c.16384G>C	p.(Asp5462His)	Kabuki syndrome	Giordano 2014	VUS

52	c.16391C>T	p.(Thr5464Met)	Kabuki syndrome	Ng 2010 (2 patients, 1 familial), Lin 2015 (familial)	DC
52	c.16412G>T	p.(Arg5471Met)	Kabuki syndrome	Micale 2014	VUS
52	c.16412G>C	p.(Arg5471Thr)	Kabuki syndrome	Hannibal 2011	VUS
52	c.16442G>A	p.(Cys5481Tyr)	Kabuki syndrome	Banka 2012	DC
53	c.16493C>T	p.(Ser5498Phe)	Kabuki syndrome	Li 2011, Makrythanasis 2013	DC
53	c.16498C>T	p.(Arg5500Trp)	Kabuki syndrome	Lin 2015	DC
54	c.16528T>G	p.(Tyr5510Asp)	Kabuki syndrome	Micale 2014	DC
Intron	***KMT2D* splice site deletions/insertions/indels**				
26	c.5783-1_5784delGGTinsA	n.a.	Kabuki syndrome	Banka 2012	DC
27	c.5867+1delG	n.a.	Kabuki syndrome	Makrythanasis 2013	DC
45	c.14252-6_14252-5insGAAA	n.a.	Kabuki syndrome	Micale 2014	DC
49	c.15919_15921+8del11	n.a.	Kabuki syndrome	Banka 2012	DC
Intron	***KMT2D* splice site point mutations**				
2	c.177-2A>C	n.a.	Kabuki syndrome	Micale 2014	DC
3	c.400+1G>A	n.a.	Kabuki syndrome	Micale 2011	DC
3	c.401-3A>G	n.a.	Kabuki syndrome	Micale 2011	DC
Ex. 4	c.509A>T	n.a.	Kabuki syndrome	Makrythanasis 2013	VUS
Ex. 4	c.510G>A	n.a.	Kabuki syndrome	Makrythanasis 2013 (familial)	DC
Ex. 4	c.510G>C	n.a.	Kabuki syndrome	Makrythanasis 2013	DC[L]
4	c.510+1G>A	n.a.	Kabuki syndrome	Miyake 2013	DC
6	c.840-1G>A	n.a.	Kabuki syndrome	Hannibal 2011	DC
7	c.954+1G>T	n.a.	Kabuki syndrome	Li 2011	DC
15	c.4419-1G>T	n.a.	Kabuki syndrome	Miyake 2013	DC
17	c.4693+1G>T	n.a.	Kabuki syndrome	Miyake 2013, Ratbi 2013	DC
22	c.5320-2A>G	n.a.	Kabuki syndrome	Paulussen 2011	DC
26	c.5783-1G>A	n.a.	Kabuki syndrome	Lindsley 2015	DC
29	c.6183+3G>T	n.a.	Kabuki syndrome	Lindsley 2015	VUS
33	c.8366+5G>C	n.a.	Kabuki syndrome	Banka 2012	VUS
35	c.10356-9G>A	n.a.	Kabuki syndrome	Banka 2012	VUS
39	c.13531-1G>T	n.a.	Kabuki syndrome	Li 2011	DC
42	c.13999+1G>C	n.a.	Kabuki syndrome	Banka 2012	DC
42	c.13999+5G>A	n.a.	Kabuki syndrome	Paulussen 2011, Micale 2014	DC
44	c.14251+1G>A	n.a.	Kabuki syndrome	Hannibal 2011	DC
46	c.14516-1G>C	n.a.	Kabuki syndrome	Paulussen 2011	DC
47	c.14643+1G>A	n.a.	Kabuki syndrome	Micale 2014	DC
47	c.14644-3C>G	n.a.	Kabuki syndrome	Micale 2014	DC
47	c.14644-2A>G	n.a.	Kabuki syndrome	Paulussen 2011	DC
48	c.15784+1G>A	n.a.	Kabuki syndrome	Banka 2012	DC

48	c.15785-1G>C	n.a.	Kabuki syndrome	Hannibal 2011	DC
49	c.15921+2T>G	n.a.	Kabuki syndrome	Banka 2012	DC
50	c.16052+1G>C	n.a.	Kabuki syndrome	Miyake 2013	DC
51	c.16338+1G>T	n.a.	Kabuki syndrome	Miyake 2013	DC
51	c.16339-2A>G	n.a.	Kabuki syndrome	Banka 2012	DC
52	c.16412+1G>C	n.a.	Kabuki syndrome	Banka 2012	DC
52	c.16413-1G>C	n.a.	Kabuki syndrome	Van Laarhoven 2015	DC
Exon	***KMT2D* gross deletions**[c]				
10	c.2532_2591del60	p.(Arg845_Pro864del)	Kabuki syndrome	Micale 2014	VUS
38	c.10599_10630del32	p.(Val3534Glnfs*11)	Kabuki syndrome	Ng 2010	DC
39	c.12986_13010del25	p.(Gln4329Leufs*47)	Kabuki syndrome	Verhagen 2014	DC
All	entire gene	n.a.	Kabuki syndrome	Banka 2013	DC
43-54	ex. 43-54	n.a.	Kabuki syndrome	Banka 2013	DC
14-15	incl ex. 14-15	n.a.	Kabuki syndrome	Riess 2012 (twins)	DC
Exon	***KMT2D* gross duplications**[c]				
39	c.11854_11874dup21	p.Gln3952_Gln3958dup	Kabuki syndrome	Micale 2014	NDC
15-34	ex. 15-34	n.a.	Kabuki syndrome	Banka 2013	DC

a) DC = Disease-causing variant, definitely or very likely pathogenic (truncating variant, or non-truncating and *de novo*, or described *de novo* in another patient, prediction disease causing), VUS = variant of unknown significance (non-truncating, inheritance unknown, not present in any public database of normal genetic variation, prediction disease causing), NDC = unlikely pathogenic or definitely not pathogenic (non-truncating, inheritance unknown, or inherited from normal parent, present in public databases of normal genetic variation, or patient carries a separate, disease causing variant). b) Lesions affecting less than 20 bp. c) Lesions affecting more than 20 bp. ǂ = patient in Li et al. (2011) also carries a truncating pathogenic variant, which was found after publication; the variant is annotated 47 times in the ExAC browser; found *de novo* by Makrythanasis et al (2013). ł Maternally inherited in the study by Micale et al. (2014) with maternal phenotype unknown, proven *de novo* in this study. Ł = Affects last base of the exon, predicted to disrupt the donor splice site. *, **, *** = two variants identified in a single patient. N.a. = not applicable. RefSeq: NM_003482.3. Mutation nomenclature according to HGVS. Nucleotide numbering referring to cDNA uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1.

Supplementary Table 2: Published mutations in *KDM6A*.

Exon / Intron	Nucleotide change	Amino acid change	Phenotype	Published record	Variant class[a]
	***KDM6A* nonsense**				
6	c.514C>T	p.(Arg172*)	Kabuki syndrome	Banka 2014	DC
10	c.752G>A	p.(Trp251*)	Kabuki syndrome	Van Laarhoven 2015	DC
16	c.1555C>T	p.(Arg519*)	Kabuki syndrome	Miyake 2013a	DC
25	c.3717G>A	p.(Try1239*)	Kabuki syndrome	Miyake 2013a	DC
28	c.4051C>T	p.(Arg1351*)	Kabuki syndrome	Miyake 2013b	DC
Exon	***KDM6A* small deletions[b]**				
16	c.1846_1849delACTC	p.(Thr616Tyrfs*8)	Kabuki syndrome	Micale 2014	DC
18	c.1909_1912delTCTA	p.(Ser637Thrfs*53)	Kabuki syndrome	Miyake 2013b	DC
17	c.2515_2518delAACA	p.(Asn839Valfs*27)	Kabuki syndrome	Lederer 2014	DC
23	c.3354_3356delTCT	p.(Leu1119del)	Kabuki syndrome	Miyake 2013a	DC
24	c.3501delT	p.(Phe1167Leufs*11)	Kabuki syndrome	Banka 2014	DC
Exon	***KDM6A* missense**				
6	c.563A>G	p.(Lys188Arg)	Kabuki syndrome	Banka 2014	DC
19	c.2939A>T	p.(Asp980Val)	Kabuki syndrome	Micale 2014	VUS
Intron	***KDM6A* splice site deletions/insertions/indels**				
22	c.3284+3_3284+6delAAGT	n.a.	Kabuki syndrome	Micale 2014	DC
26	c.3876_3878+1delTAAG	n.a.	Kabuki syndrome	Cheon 2014	DC
26	c.3878+3_3878+6delAAGT	n.a.	Kabuki syndrome	Banka 2014	DC
Intron	***KDM6A* splice site point mutations**				
22	c.3284+1G>T	n.a.	Kabuki syndrome	Banka 2014, Morgan 2015	DC
24	c.3548+2T>C	n.a.	Kabuki syndrome	Banka 2014	DC
25	c.3736+2T>C	n.a.	Kabuki syndrome	Van Laarhoven 2015	DC
Exon	***KDM6A* gross deletions[c]**				
1-2	227 kb	n.a.	Kabuki syndrome	Yang 2016	DC
6	ex. 6, c.444-?_564+?del	n.a.	Kabuki syndrome	Banka 2014	DC
5-9	45.4 kb, ex. 5-9	n.a.	Kabuki syndrome	Lederer 2012	DC
21-29	283.5 kb, ex. 21-29 + CXorf36	n.a.	Kabuki syndrome	Lederer 2012	DC
all	3.52 Mb incl. entire gene + part *CASK*	n.a.	SS, microcephaly, CP, ID, seizures	Lindgren 2013	DC
all	3.72 Mb incl. entire gene	n.a.	SS, SGA, hypoglycinemia	Lindgren 2013	DC
all	815.7 kb, entire gene + *CXorf36*, *DUSP21* and *FUNDC1*	n.a.	Kabuki syndrome	Lederer 2012	DC
Exon	***KDM6A* gross duplications[c]**				
n.a.	210 kb incl. partial gene	n.a.	Autism spectrum disorder	Lindgren 2013	VUS
n.a.	6.03 Mb incl. partial gene + *CASK, DDX3X*	n.a.	ID, DD and obesity	Lindgren 2013	VUS

all	6.4 Mb incl. entire gene + *WAS, ARAF, ELK1, PIM2*	n.a.	DD, macrocephaly, seizures	Lindgren 2013	DC
all	7.2 Mb incl. entire gene + *CASK, DX3X*	n.a.	Encephalopathy, epilepsy, DD	Lindgren 2013	VUS
all	7.6 Mb incl. entire gene + *CASK, WAS, ARAF, ELK1, PIM2*	n.a.	DD and dysmorphic features	Lindgren 2013	DC
all	7.9 Mb incl. entire gene + *CASK, DDX3X, ARAF, ELK1*	n.a.	DD and dysmorphic features	Lindgren 2013	DC
all	713 kb incl. entire gene	n.a.	Autism spectrum disorder	Lindgren 2013	VUS
	***KDM6A* complex genomic rearrangement**				
n.a.	t(X;5)(p11.3;q35.3)inv(5)(q35.3q35.1)dn	n.a.	ID, SS, CP, seizures	Lindgren 2013	DC

a) DC = Disease-causing variant, definitely or very likely pathogenic (truncating variant, or non-truncating and de novo, or described de novo in another patient, prediction disease causing), VUS = variant of unknown significance (non-truncating, inheritance unknown, not present in any public database of normal genetic variation, prediction disease causing), NDC = unlikely pathogenic or definitely not pathogenic (non-truncating, inheritance unknown, or inherited from normal parent, present in public databases of normal genetic variation, or patient carries a separate, disease causing variant). b) Lesions affecting less than 20 bp. c) Lesions affecting more than 20 bp. Abbreviations: CP = cleft palate, DD = developmental delay, ID = intellectual disability, n.a. = not applicable, SGA = small for gestational age, SS = short stature. RefSeq: NM_021140.3. Mutation nomenclature according to HGVS. Nucleotide numbering referring to cDNA uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1.

Supplementary Table 3: In-silico prediction for all missense variants and non-frameshifting deletions / duplications in *KDM6A* and *KMT2D* identified in this study.

Gene	Variation		Protein sequence change					PROVEAN		SIFT		Mutation Taster	Annotation			
	Name	PROVEAN input	ENSP	Codon	AA pos	Ref	Alt	Score	Prediction (cutoff=-2.5)	Score	Prediction (cutoff=0.05)	Prediction	dbSNP	ExAC	1000G	EVS
KDM6A	c.2729A>G	X,44935968,A,G	ENSP00000372355	A[A/G]C	917	N	S	-3.46	Deleterious	0.120	Tolerated	Disease causing	0	0	0	0
KDM6A	c.3073A>G	X,44938525,A,G	ENSP00000372355	[A/G]GT	1032	S	G	-3.18	Deleterious	0.001	Damaging	Disease causing	0	0	0	0
KDM6A	c.3763C>T	X,44949994,C,T	ENSP00000372355	[C/T]GG	1262	R	W	-5.30	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.11223_11225dup	12,49427263,T,TTGT	ENSP00000301067	C[-/ACA]AG	3742	Q	HK	-0.80	Neutral	NA	NA	Polymorphism	0	0	0	0
KMT2D	c.15163_15168del	12,49420581,CAGGTC,.	ENSP00000301067	[GACCTG/-]	5054	LD	.	-19.42	Deleterious	NA	NA	Polymorphism	0	0	0	0
KMT2D	c.16489_16491del	12,49415856,GAT,.	ENSP00000301067	[ATC/-]	5496	I	.	-8.83	Deleterious	NA	NA	Disease causing	0	0	0	0
KMT2D	c.3622A>C	12,49443749,T,G	ENSP00000301067	[A/C]TC	1208	I	L	-0.50	Neutral	0.013	Damaging	Polymorphism	0	0	0	0
KMT2D	c.4093G>T	12,49442480,C,A	ENSP00000301067	[G/T]TT	1365	V	F	-4.27	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.4171G>A	12,49441813,C,T	ENSP00000301067	[G/A]AG	1391	E	K	-3.29	Deleterious	0.001	Damaging	Disease causing	0	0	0	0
KMT2D	c.4214A>T	12,49441770,T,A	ENSP00000301067	C[A/T]C	1405	H	L	-9.03	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.4267C>G	12,49440543,G,C	ENSP00000301067	[C/G]GT	1423	R	G	-6.00	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.4267C>T	12,49440543,G,A	ENSP00000301067	[C/T]GT	1423	R	C	-6.86	Deleterious	0.053	Tolerated	Disease causing	0	0	0	0
KMT2D	c.4359C>A	12,49440451,G,T	ENSP00000301067	CA[C/A]	1453	H	Q	-6.86	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.4413C>G	12,49440397,G,C	ENSP00000301067	TG[C/G]	1471	C	W	-9.43	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.6109G>C	12,49435872,C,G	ENSP00000301067	[G/C]AC	2037	D	H	-6.47	Deleterious	0.001	Damaging	Disease causing	0	0	0	0
KMT2D	c.6544G>A	12,49435009,C,T	ENSP00000301067	[G/A]CC	2182	A	T	-1.19	Neutral	0.126	Tolerated	Disease causing	0	1	0	0

KMT2D	c.9145C>G	12,49431994,G,C	ENSP00000301067	[C/G]TG	3049	L	V	-0.78	Neutral	0.003	Damaging	Disease causing	0	0	0	0
KMT2D	c.11791C>T	12,49426697,G,A	ENSP00000301067	[C/T]TT	3931	L	F	-1.17	Neutral	0.071	Tolerated	Polymorphism	0	0	0	0
KMT2D	c.14055C>G	12,49423204,G,C	ENSP00000301067	CA[C/G]	4685	H	Q	-6.48	Deleterious	0.001	Damaging	Disease causing	0	0	0	0
KMT2D	c.15142C>T	12,49420607,G,A	ENSP00000301067	[C/T]GT	5048	R	C	-7.68	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.15143G>A	12,49420606,C,T	ENSP00000301067	C[G/A]T	5048	R	H	-4.80	Deleterious	0.011	Damaging	Disease causing	0	0	0	0
KMT2D	c.15176A>G	12,49420573,T,C	ENSP00000301067	C[A/G]C	5059	H	R	-7.68	Deleterious	0.001	Damaging	Disease causing	0	0	0	0
KMT2D	c.15206T>A	12,49420543,A,T	ENSP00000301067	G[T/A]G	5069	V	E	-5.76	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.15349T>G	12,49420400,A,C	ENSP00000301067	[T/G]GT	5117	C	G	-11.51	Deleterious	0.002	Damaging	Disease causing	0	0	0	0
KMT2D	c.15397T>C	12,49420352,A,G	ENSP00000301067	[T/C]GT	5133	C	R	-11.51	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.15461G>A	12,49420288,C,T	ENSP00000301067	C[G/A]G	5154	R	Q	-3.84	Deleterious	0.002	Damaging	Disease causing	0	0	0	0
KMT2D	c.15535C>T	12,49420214,G,A	ENSP00000301067	[C/T]GT	5179	R	C	-7.68	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.15536G>A	12,49420213,C,T	ENSP00000301067	C[G/A]T	5179	R	H	-4.80	Deleterious	0.010	Damaging	Disease causing	0	0	0	0
KMT2D	c.15565G>A	12,49420184,C,T	ENSP00000301067	[G/A]GA	5189	G	R	-7.68	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.15634G>C	12,49420115,C,G	ENSP00000301067	[G/C]CC	5212	A	P	-4.36	Deleterious	0.002	Damaging	Disease causing	0	0	0	0
KMT2D	c.15640C>T	12,49420109,G,A	ENSP00000301067	[C/T]GC	5214	R	C	-7.68	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.15673C>T	12,49420076,G,A	ENSP00000301067	[C/T]GC	5225	R	C	-7.68	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.16019G>A	12,49418394,C,T	ENSP00000301067	C[G/A]A	5340	R	Q	-3.84	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.16052G>A	12,49418361,C,T	ENSP00000301067	C[G/A]G	5351	R	Q	-3.84	Deleterious	0.082	Tolerated	Disease causing	0	0	0	0
KMT2D	c.16273G>A	12,49416438,C,T	ENSP00000301067	[G/A]AG	5425	E	K	-3.70	Deleterious	0.000	Damaging	Disease causing	0	0	0	0

KMT2D	c.16295G>A	12,49416416,C,T	ENSP00000301067	C[G/A]G	5432	R	Q	-3.70	Deleterious	0.000	Damaging	Disease causing	0	0	0	0
KMT2D	c.16315C>G	12,49416396,G,C	ENSP00000301067	[C/G]GG	5439	R	G	-4.73	Deleterious	0.001	Damaging	Disease causing	0	0	0	0
KMT2D	c.16442G>A	12,49415905,C,T	ENSP00000301067	T[G/A]T	5481	C	Y	-10.50	Deleterious	0.000	Damaging	Disease causing	0	0	0	0

Abbreviations: AA pos = amino acid position, Ref = reference amino acid, Alt = alternative amino acid, dbSNP = database of single nucleotide polymorphisms, ExAC = Exome Accession Consortium, 1000G = 1000 Genomes, EVS = Exome Variant Server. Mutation nomenclature according to HGVS. Nucleotide numbering referring to cDNA uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1. URLs for databases and prediction programs can be found in the methods section.

Supplementary Table 4: In-silico prediction for all splice-site variants in *KDM6A* and *KMT2D* identified in this study.

Gene	Variation			HSF3		Mutation Taster	Annotation			
	Name	Intron	ENST	Prediction	(%)Variation*	Prediction	dbSNP	ExAC	1000G	EVS
KDM6A	c.443+5G>C	5	ENST00000377967	Broken WT Donor Site	-12.85	Disease causing	0	0	0	0
KDM6A	c.619+6T>C	7	ENST00000377967	No significant splicing motif alteration detected	-2.46	Disease causing	0	0	0	0
KDM6A	c.620-2A>G	7	ENST00000377967	Broken WT Acceptor Site	-33.02	Disease causing	0	0	0	0
KDM6A	c.2832+1G>A	18	ENST00000377967	Broken WT Donor Site	-31.26	Disease causing	0	0	0	0
KMT2D	c.177-2A>G	2	ENST00000301067	Broken WT Acceptor Site	-33.51	Disease causing	0	0	0	0
KMT2D	c.400+2T>C	3	ENST00000301067	Broken WT Donor Site	-27.64	Disease causing	0	0	0	0
KMT2D	c.839+2T>A	6	ENST00000301067	Broken WT Donor Site	-30.04	Disease causing	0	0	0	0
KMT2D	c.2797+1G>C	10	ENST00000301067	Broken WT Donor Site	-27.37	Disease causing	0	0	0	0
KMT2D	c.3906+1G>T	11	ENST00000301067	Broken WT Donor Site	-27.7	Disease causing	0	0	0	0
KMT2D	c.3906+2T>C	11	ENST00000301067	Broken WT Donor Site	-27.7	Disease causing	0	0	0	0
KMT2D	c.8366+2T>C	33	ENST00000301067	Broken WT Donor Site	-29.12	Disease causing	0	0	0	0
KMT2D	c.13531-2A>C	39	ENST00000301067	Broken WT Acceptor Site	-36.47	Disease causing	0	0	0	0
KMT2D	c.14076-1G>A	43	ENST00000301067	Broken WT Acceptor Site	-32.79	Disease causing	0	0	0	0
KMT2D	c.14515+1del	46	ENST00000301067	Broken WT Donor Site / New Donor Site	-79.82 / +478.95	Disease causing	0	0	0	0
KMT2D	c.14516-1G>C	46	ENST00000301067	Broken WT Acceptor Site	-30.21	Disease causing	0	0	0	0
KMT2D	c.14643+1G>T	47	ENST00000301067	Broken WT Donor Site / New Donor Site	-29.28 / +53.74	Disease causing	0	0	0	0
KMT2D	c.15784+5G>A	48	ENST00000301067	Broken WT Donor Site	-13.39	Disease causing	0	0	0	0
KMT2D	c.16412+4A>G	52	ENST00000301067	Broken WT Donor Site	-8.64	Disease causing	0	0	0	0
KMT2D	c.16412+5G>C	52	ENST00000301067	Broken WT Donor Site	-12.45	Disease causing	0	0	0	0

Abbreviations: HSF3 = Human Splicing Finder Version 3, ENST = Transcript ID, WT = wild-type, dbSNP = database of single nucleotide polymorphisms, ExAC = Exome Accession Consortium, 1000G = 1000 Genomes, EVS = Exome Variant Server. Mutation nomenclature according to HGVS. Nucleotide numbering referring to cDNA uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1. URLs for databases and prediction programs can be found in the methods section. *Threshold: +/-10%.