Histone Lysine Methylases and Demethylases in the Landscape of Human Developmental Disorders

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Histone lysine methyltransferases (KMTs) and demethylases (KDMs) underpin gene regulation. Here we demonstrate that variants causing haploinsufficiency of KMTs and KDMs are frequently encountered in individuals with developmental disorders. Using a combination of human variation databases and existing animal models, we determine 22 KMTs and KDMs as additional candidates for dominantly inherited developmental disorders. We show that KMTs and KDMs that are associated with, or are candidates for, dominant developmental disorders tend to have a higher level of transcription, longer canonical transcripts, more interactors, and a higher number and more types of post-translational modifications than other KMT and KDMs. We provide evidence to firmly associate *KMT2C*, *ASH1L*, and *KMT5B* haploinsufficiency with dominant developmental disorders. Whereas *KMT2C* or *ASH1L* haploinsufficiency results in a predominantly neurodevelopmental phenotype with occasional physical anomalies, *KMT5B* mutations cause an overgrowth syndrome with intellectual disability. We further expand the phenotypic spectrum of *KMT2B*-related disorders and show that some individuals can have severe developmental delay without dystonia at least until mid-childhood. Additionally, we describe a recessive histone lysine-methylation defect caused by homozygous or compound heterozygous *KDM5B* variants and resulting in a recognizable syndrome with developmental delay, facial dysmorphism, and camptodactyly. Collectively, these results emphasize the significance of histone lysine methylation in normal human development and the importance of this process in human developmental disorders. Our results demonstrate that systematic clinically oriented pathway-based analysis of genomic data can accelerate the discovery of rare genetic disorders.

Post-translational methylation and demethylation of lysine residues on histone tails is a key dynamic chromatin modification that is mediated by specific methyltransferases (KMTs) and demethylases (KDMs) and underpins gene regulation and several cellular processes.[1,2] Twenty-seven KMT- and 24 KDM-encoding genes, classified into eight groups each, are known (Table S1).[3] Of these, heterozygous variants in seven KMT and four KDM genes are associated with autosomal and X-linked dominant inherited human developmental disorders (DDs) in the Online Mendelian Inheritance in Man database (OMIM) (Table S1).[4–18]

We reviewed published disease-causing variants in KMTs and KDMs in the Human Gene Mutation Database[19] and deduced that ~75% of these were predicted to be heterozygous protein-truncating variants (PTVs), suggesting that haploinsufficiency is the predominant mechanism for the associated diseases (Figure 1A) (Table S2). This is consistent with previous studies that have shown a high prevalence of *de novo* (DN) PTVs in dominant DDs.[20–22] We reviewed phenotypes of the available mouse models for KMT and KDM orthologs (Table S1)[23] and found that heterozygous mouse models for six of the 11 known dominant DD-associated KMTs and KDMs and 12 of the 40 of remaining KMTs and KDMs demonstrate anomalies. We reviewed phenotypes of the available zebrafish knockdown (KD) models for KMT and KDM orthologs (Table S1).[24] Anomalies were observed in KD of seven of the 11 known dominant DD-associated KMTs and KDMs and 18 of the 40 of remaining KMTs and KDMs. The human mutational landscape of KMTs and KDMs and the information from animal models led us to hypothesize that germline

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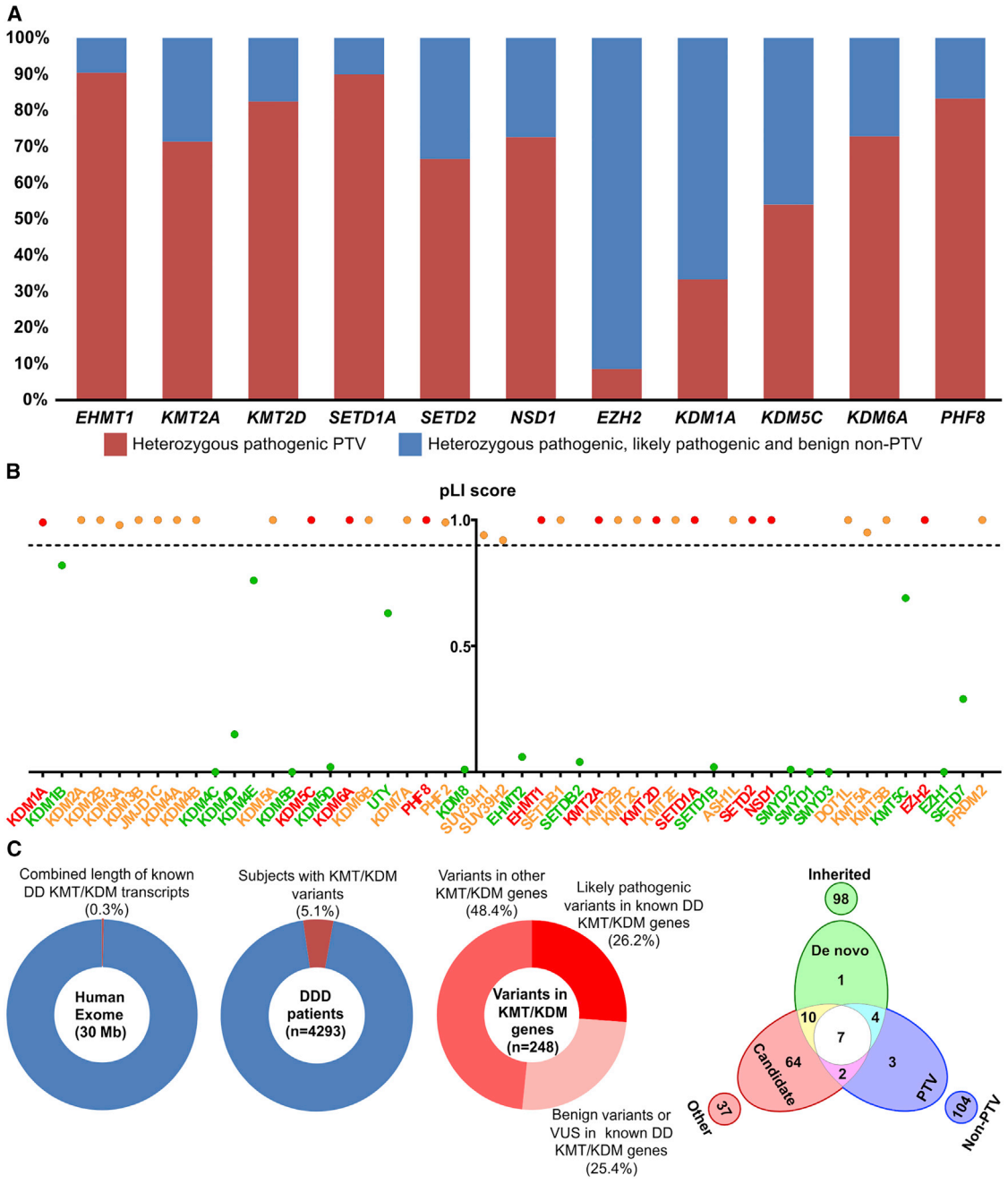

Figure 1. Variants in Histone Lysine Methyltransferases and Demethylases Are Frequent in Developmental Disorders and Haploinsufficiency Is Their Predominant Mechanism

(A) The bar graph shows the proportions of postulated disease causing published heterozygous protein-truncating variants (PTVs) (in red) and protein altering variants (PAVs) (in blue) in known dominant developmental disorder (DD)-associated KMTs and KDMs.

(B) A plot of the probability of being LoF intolerant (pLI) for all KMTs and KDMs. Red dots represent the pLI scores for known dominant DD-associated KMTs and KDMs, orange dots depict these scores from KMTs and KDMs that are candidates for involvement in dominant DDs, and green dots display the pLI scores for non-candidate KMT and KDM genes. The dotted line depicts the cut-off for defining the candidate genes (pLI > 0.9).

(C) Proportion of canonical transcripts of known DD KMTs and KDMs from the total human exome (left donut graph), proportion of individuals with pathogenic variants in known KMT and KDM genes from the Deciphering Developmental Disorders (DDD) Study cohort (central donut graph), proportion of pathogenic, benign variants or variants of uncertain significance (VUS) in known KMT and KDM genes, and the percentage of variants in other KMTs and KDMs from the total number of KMT and KDM variants seen in the DDD cohort (right donut graph). The Venn diagram shows the distribution of the 120 rare high-quality variants that were detected in the DDD cohort in KMTs and KDMs not yet firmly associated with DDs.

The green circle and the ellipse represent the number of variants according to their inheritance, the blue circle and the ellipse represent the number of variants according to their predicted protein effect, and the red circle and the ellipse represent the number of variants detected in candidate genes for dominant DDs and the other genes.

heterozygous PTVs in additional KMTs and KDMs might underlie as-yet-unknown DDs.

For each of the 51 KMTs and KDMs, we compiled selected indices of predicted intolerance to loss-of-function (LoF) pathogenic variants (Table S1). The pLI (probability of being LoF intolerant) scores obtained from the ExAC Browser[25] were found to be within a narrow range of 0.99–1.0 for KMTs and KDMs already linked with dominant human DDs, suggesting a high reliability. The ranges of the residual variation intolerance score (0.06–51.92) and haploinsufficiency index (3.06–62.96) scores for these genes were broad.[26,27] We used a pLI score[25] cut-off of >0.9 to determine an additional 11 KMTs and 11 KDMs as candidates for as-yet-unknown dominant human DDs (Figure 1B).

We examined the data from 4,293 trios who underwent exome sequencing as part of the Deciphering Developmental Disorders (DDD) study.[22] All these procedures were in accordance with the ethical standards (Multi-Centre Research Ethics Committee approval 10/H0305/83 and GEN/284/12), and all participants provided informed consent. The previously described pipeline was used for identifying rare high-quality and possibly deleterious variants in our list of 51 KMTs and KDMs. Rare variants were defined as those with minor allele frequencies of <0.001 (for *de novo*, X-linked, and dominant heterozygous inheritances) or <0.01 (for compound heterozygous and recessive homozygous inheritances) in the Exome Aggregation Consortium[25] (ExAC, Version 0.3.1), the 1000 Genomes Project (1K-G),[28] Ensembl version 80-GRCh37, the NHLBI-GO Exome Sequencing Project (ESP),[29] and UK10K.[30] High-quality variants were defined as those with a read depth of >20 and a genotype quality score of >20. Truncating or missense variants in canonical transcripts were defined as possibly deleterious. In total, we identified 218 probands with high-quality rare variants in the 51 KMTs and KDMs (Figure S1 and Tables S3, S4, and S5). Of these, 65 affected individuals (~1.5% of all the probands) had likely causal monoallelic LoF variants (Figure 1C and Table S3) in the 11 KMTs and KDMs already associated with dominant DDs. Of note, the combined coding size of the canonical transcripts of these 11 genes is ~0.3% out of the total human exome size (0.092/30 Mb)[31](Figure 1C). Hence, this is an important group of genes in rare undiagnosed developmental disorders. Of these 218 affected individuals, 52 had likely benign variants or variants of uncertain significance in these 11 KMTs and KDMs (Figure 1C) (Table S4).[22,31]

One hundred and two of 218 probands had 120 rare high-quality-call genetic variants in KMTs and KDMs not yet firmly associated with DDs (Table S5). Of these, 83 variants, including nine PTVs and 16 DN protein-altering variants, were in our 22 candidates for dominant DDs (PAV) (Table S5). A chi-square test revealed a 1.87-fold enrichment (95% confidence interval [CI] = 0.93–3.76; p = 0.072) in the frequency of PTVs in these 22 genes in our cohort against the data from the ExAC Browser[25] (Table S6). Similarly, a 4.85-fold enrichment of DN PAVs (95% CI = 1.78–13.26; p = 0.00065) was observed in these 22 genes in our cohort against the entries marked as "controls" in "denovo-db"[32] (Table S6).This observation supported our hypothesis that germline heterozygous PTVs in additional KMTs and KDMs might underlie as-yet-unknown dominant DDs.

We then focused on DN PTVs in our curated list of candidate KMTs for dominant DD because these variants are highly likely to be causal (equivalent to category 1 in the American College of Medical Genetics and Genomics guidelines[33]). We interrogated the v*cf.* files of each trio through VarSeq version 1.3.4 (Golden Helix) to ensure that the probands did not carry additional causal pathogenic variants in other genes. Collectively, we identified seven variants that fulfilled these criteria. (Table 1) (Table S5). Specifically, these included two DN PTVs each in *ASH1L* (MIM: 607999), *KMT2C* (MIM: 606833), and *KMT5B* (formerly known as *SUV420H1*) (MIM: 610881) and one in *KMT2B* (MIM: 606834) (Figure 2). A chi-square test revealed a 5.51-fold enrichment (95% confidence interval [CI] = 2.3–13.2; p = 0.0000165) in the frequency of PTVs in these four genes in our cohort against the data from ExAC.[25] Fisher's exact test revealed a 34.87-fold enrichment of DN PAVs (95% CI = 2.0545–591.9943; p = 0.000039) in these four genes in our cohort against the entries marked as "controls" in "denovo-db"[32], further supporting a high likelihood of causality. Where possible, variants were confirmed by Sanger sequencing (Table S8 and Figure S2). Importantly, rare variants in these genes have been previously reported in several case-control cohorts of individuals with autism, ID, bipolar disorder, and congenital heart anomalies, but their causality has not been confirmed, and the associated phenotypes have not been fully described.[20,22,34–40] Detailed phenotype information of the affected individuals was, therefore, collected (Table 1) (Figure 3) (Supplemental Note: case reports).

Of note, we also detected non-truncating DN PAVs in other candidate KMTs and KDMs for dominant DDs (*DOT1L*, *KDM3A*, *PRDM2, SETDB1*); there is insufficient evidence for causality of PAVs in these genes at present. Additionally, we detected DN PTVs in non-candidate KMTs and KDMs for dominant DDs (*KDM5B* and *SETD1B*). PTVs in these genes could be coincidental, or they could be phenotype modifiers in some affected individuals, or they could be non-penetrant in some unaffected individuals in the general population. Finally, we detected PTVs in other candidate KMTs and KDMs for dominant DDs (*KDM3A* and *PRDM2*) inherited from a parent who did not share the proband's phenotype. This observation suggests that these PTVs might have incomplete penetrance or that these genes are not haploinsufficiency intolerant, in contrast to the predictions of their pLI scores (Figure 2). Overall, further studies are needed if we are to determine the pathogenicity of heterozygous PAVs and PTVs in *DOT1L*, *KDM3A*, *KDM5B, PRDM2, SETDB1*, and *SETD1B*.

Table 1. Clinical and Genetic Characteristics of Affected Individuals with Candidate Variants in Lysine Methyltransferases and Demethylases

Gene	Sex (Age at Study)	Individual Number	Genomic Position (hg19)	cDNA[a] (Protein Consequence) or Deletion Size	Inheritance or Zygosity	Perinatal History	DD or ID	Neuropsychiatric Disorders and CNS Anomalies	Malformations and Anomalies	Height (SD)/ Weight (SD)/ OFC (SD)	CD	Other Medical Issues
KMT2B	F (11 y)	1	19:36212057	c.1808dupC p.(Leu604Profs*72)	DN Het	IUGR and feeding difficulties	severe	abnormal gait and behavioral problems	PDA, long & narrow hands, broad halluces	SS (−2.7)/ LW (−2.9) Mi (−3.34)	yes	nystagmus, gastrostomy, urinary incontinence, constipation and growth hormone deficiency
KMT2C	F (17 y)	2	7:151884849	c.4744G>T p.(Gly1582*)	DN Het	no	severe	elective mutism	duplicated right thumb and left preauricular tag	SS (−2.1) LW (−2.74) Mi (−2.42)	yes	hearing loss and delayed puberty
	F (4 y)	3	7:151873688-151873689	c.8849_8850delAT p.(His2950Argfs*17)	DN Het	hydrocephalus and Dandy-Walker anomaly	severe	hydrocephalus and hypoplasia of cerebellar vermis	no	SS (−2) LW (−2) Mi (−1.97)	yes	no
	F (5 y)	4	7:151836279	c.14526dupG p.(Pro4843Alafs*12)	DN Het	no	severe (motor delay was mild)	autistic traits, developmental regression, insensitivity to pain and abnormal gait	no	N (0.4) N (0.18) N (−1)	yes	constipation
ASH1L	F (13 y)	5	1:155449628	c.3033delA p.(Val1014Cysfs*24)	DN Het	feeding difficulties	mild	behavioral problems	bicuspid AV, VSD and PFO	N (0.2) N (0.82) N (1.16)	yes	hypermetropia, precocious puberty and hypermobility
	M (9 y)	6	1:155322602	c.7276C>T p.(Arg2426*)	DN Het	feeding difficulties and hydronephrosis	severe	seizures, autistic traits and hypotonia.	cryptorchidism and inguinal hernia	N (1.6) O (2.33) N (1.36)	yes	hypermetropia, hyperacusis and hypermobility
	M (7 y)	7	1:155271366-155804269	532.9 Kb	DN	no	severe	behavioral problems	cryptorchidism and blocked nasolacrimal duct	N (−0.59 SD) N (−0.44 SD) Mi (1.72SD)	yes	constipation
KMT5B	F (13 y)	8	11:67953337	c.219delC p.(Ala74Profs*10)	DN Het	no	moderate	autistic traits	no	TS (2.91) N (0.9) Ma (4.43)	yes	hypermobility
	M (19 y)	9	11:67941365	c.559C>T p.(Arg187*)	DN Het	no	severe	seizures,hypotonia and autistic traits	no	N (0.74) N (0.9) N (1.93)	yes	no
	M (14 y)	10	11:67888021-68287033	399.01 Kb	DN	no	mild	seizures, enlarged right ventricle and white matter signal alterations	no	N (0.63) Ma (2)	yes	strabismus and scoliosis
	M (16 y)	11	11:67550395-68389391	839 Kb	DN	no	mild to moderate	no	cryptorchidism, pectus excavatum, and overlapping 2nd and 3rd toes	N (1.68) N (0.24) N (1.87)	yes	strabismus, diabetes mellitus and hypermobility

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Table 1. *Continued*

Gene	Sex (Age at Study)	Individual Number	Genomic Position (hg19)	cDNA[a] (Protein Consequence) or Deletion Size	Inheritance or Zygosity	Perinatal History	DD or ID	Neuropsychiatric Disorders and CNS Anomalies	Malformations and Anomalies	Height (SD)/ Weight (SD)/ OFC (SD)	CD	Other Medical Issues
KDM5B	M (18 y)	12	1:202700104	c.4109T>G p.(Leu1370*)	Mat & Pat Hom	feeding difficulties	severe	abnormal gait and agenesis of corpus callosum	inguinal hernia and camptodactyly of 4th and 5th fingers	N (−0.23) LW (−1.52) N (−1.66)	yes	myopia and astigmatism
	M (10 y)	13	1:202711635 1:202731850	c.2475-2A>G; c.895C>T (p.Arg299Ter)	Mat & Pat CoHet	no	moderate	no	dolichocephaly and supernumerary nipple	N (0.98) N (0.51) N (0.26)	no	no
	M (11 y)	14	1:202702532 1:202736143	c.3906delC, (p.Asn1302Lysfs*45) c.622dupT (p.Tyr208Leufs*5)	Mat & Pat Het	feeding difficulties,	moderate	no	atrial septal defect, cryptorchidism, hypospadias and camptodactyly of 4th and 5th fingers	N (−0.09 SD) N (−1.05)	yes	myopia and strabismus

Abbreviations: AV = aortic valve; CNS = central nervous system; CD = craniofacial dysmorphisms; CoHet = compound heterozygous; DD = developmental delay; DN = *de novo*; F = female; Het = heterozygous; Hom = homozygous; ID = intellectual disability; IUGR = intra-uterine growth retardation; LW = low weight; M = male; Ma = macrocephaly; mat = maternal; Mi = microcephaly; N = normal/not present; O = overweight; PDA = patent ductus arteriosus; OFC = occipitofrontal circumference; PFO = patent foramen ovale; SD = standard deviation; SS = short stature; TS = tall stature; VSD = ventricular septal defect; y = years.
[a]The transcript IDs are *KMT2B* NM_014727.2; *KMT2C* NM_170606.2; *ASH1L* ENST00000368346.7; *KMT5B* NM_017635.4; and *KDM5B* NM_001314042.1.

Next, we interrogated the data from >200 individuals from the CAUSES study of children with developmental disorders (see Web Resources) for potentially pathogenic variants in *KMT2B*, *KMT2C*, and *KMT5B* and identified one additional individual with a DN PTV in *KMT2C* (Table 1; Figure 2).

Copy-number variants (CNVs) can be informative for dissecting the molecular basis of genetic disorders.[41–43] We therefore examined the DECIPHER database,[44] with >41,800 individuals with CNVs, and identified 71 deletions encompassing one of the four genes—*ASH1L*, *KMT2B*, *KMT2C*, or *KMT5B* (Table S7). Where possible, we collected additional detailed phenotype information about the affected individuals (Table 1 and Figure 3) (Supplemental Note: case reports). Of note, we considered only individuals whose deletions did not include other possibly causal DD-related gene(s) for further analysis.

Collectively, we identified three individuals with DN *KMT2C* PTV and 41 deletions encompassing this gene (Table 1, Table S5, Table S7, and Figures 2 and 3). All affected individuals for whom detailed clinical information was available had severe developmental delay and ID (Table 1). KMT2C is a H3K4 methyltransferase[45] that is highly expressed in the developing and adult human brain, specifically in the cerebellum.[46,47] It is interesting to note that individual 3 has hypoplasia of the cerebellar vermis. In mice, a homozygous *Kmt2c* in-frame deletion of exons 25 and 26 results in prenatal and postnatal growth retardation and lethality in some embryos.[48]

We identified two individuals with *ASH1L* PTVs and five deletions encompassing this gene (Table 1, Tables S5 and S7, and Figure 2). All affected individuals for whom detailed clinical information was available, displayed variable degrees of ID with or without global developmental delay; seizures; hypotonia; and aberrant behavior (Table 1). ASH1L is a methyltransferase that catalyzes mono- and dimethylation of H3K36.[49] *ASH1L* is highly expressed in both embryonic and adult human brains.[46,47] Injection of *ash1a* morpholinos in zebrafish led to a reduction in the number of neurons produced in the epiphysis.[50] Heterozygous and homozygous knock-in mice expressing mutant *Ash1l* containing a short in-frame deletion within the catalytic SET domain display a range of skeletal anomalies.[51] Hypomorphic mice, with an exon 1 *Ash1l* gene trap outside the catalytic SET domain, have reduced levels of normal protein and display impaired fertility.[52] The heterozygous mice for a reporter-tagged deletion allele show impaired pupillary reflex and abnormal coat appearance.[23]

We identified two individuals with DN *KMT5B* PTVs and seven deletions encompassing this gene (Table 1, Tables S5 and S7, and Figures 2 and 3). All affected individuals for whom detailed clinical information was available had mild to moderate global developmental delay and ID; macrocephaly; tall stature; and similar facial dysmorphisms (Table 1). KMT5B is a H4K20 di- and tri-methyltransferase

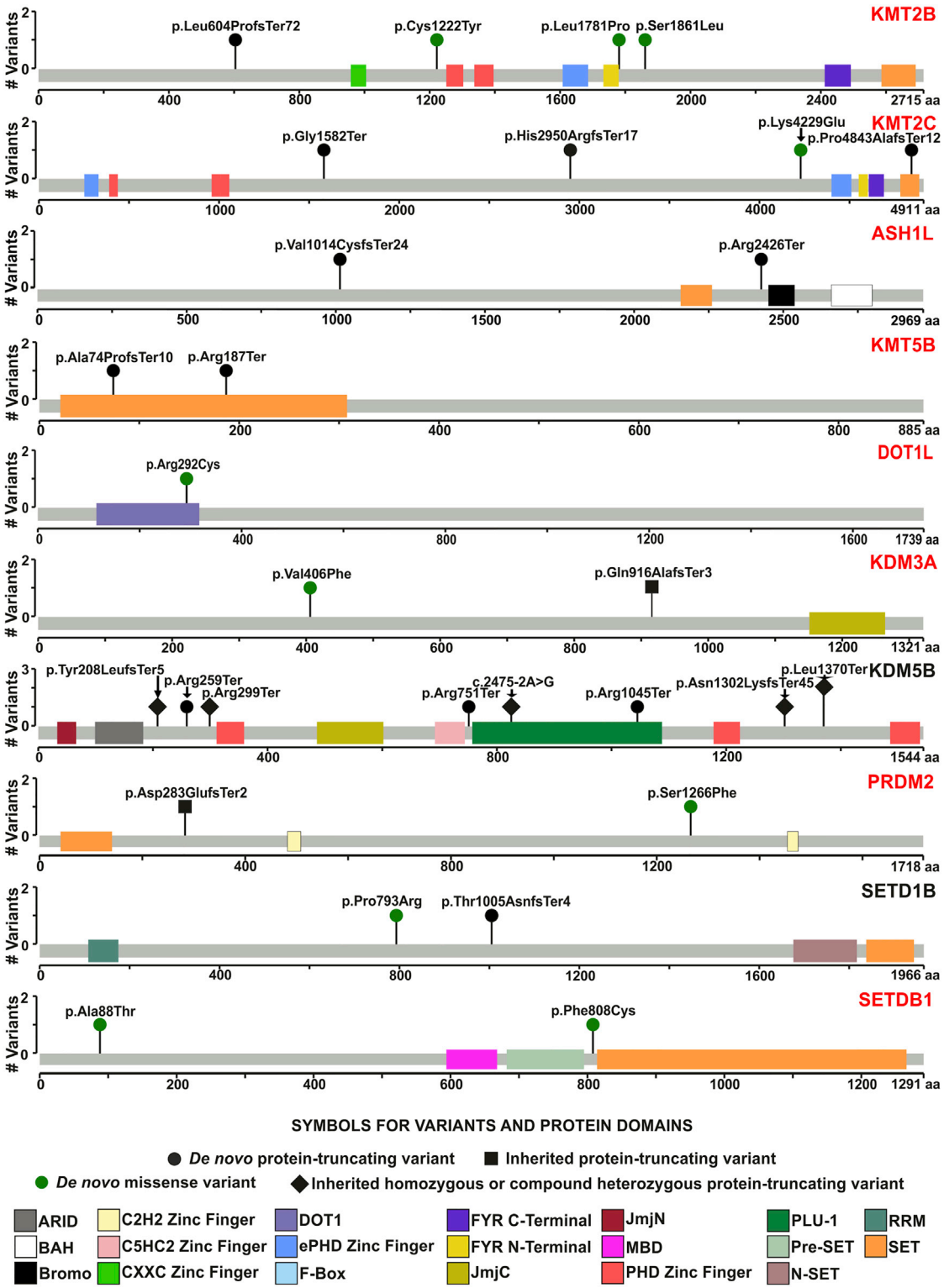

Figure 2. Variants of Interest Identified in This Study

Locations of selected plausible candidate variants identified in this study are shown. Candidate genes for dominant DDs with DN PTVs are indicated in red font, and the other genes are in black font. The *de novo* PTVs in candidate KMTs and KDMs for dominant DD genes (*KMT2B*, *KMT2C*, *ASH1L* and *KMT5B*) (n = 8) are highly likely to be causal. We have also shown *de novo* protein-altering variants (PAVs) in candidate KMTs and KDMs for dominant DD genes (*KMT2B*, *KMT2C*, *DOT1L*, *KDM3A*, *PRDM2*, *SETDB1*) (n = 9) with limited evidence for causality at present (apart from those in *KMT2B*, which have been shown to cause early-onset dystonia). Inherited PTVs in candidate KMTs and KDMs (*KDM3A* and *PRDM2*) (n = 2) are shown. PTVs in these genes might cause non-penetrant phenotypes, or this might

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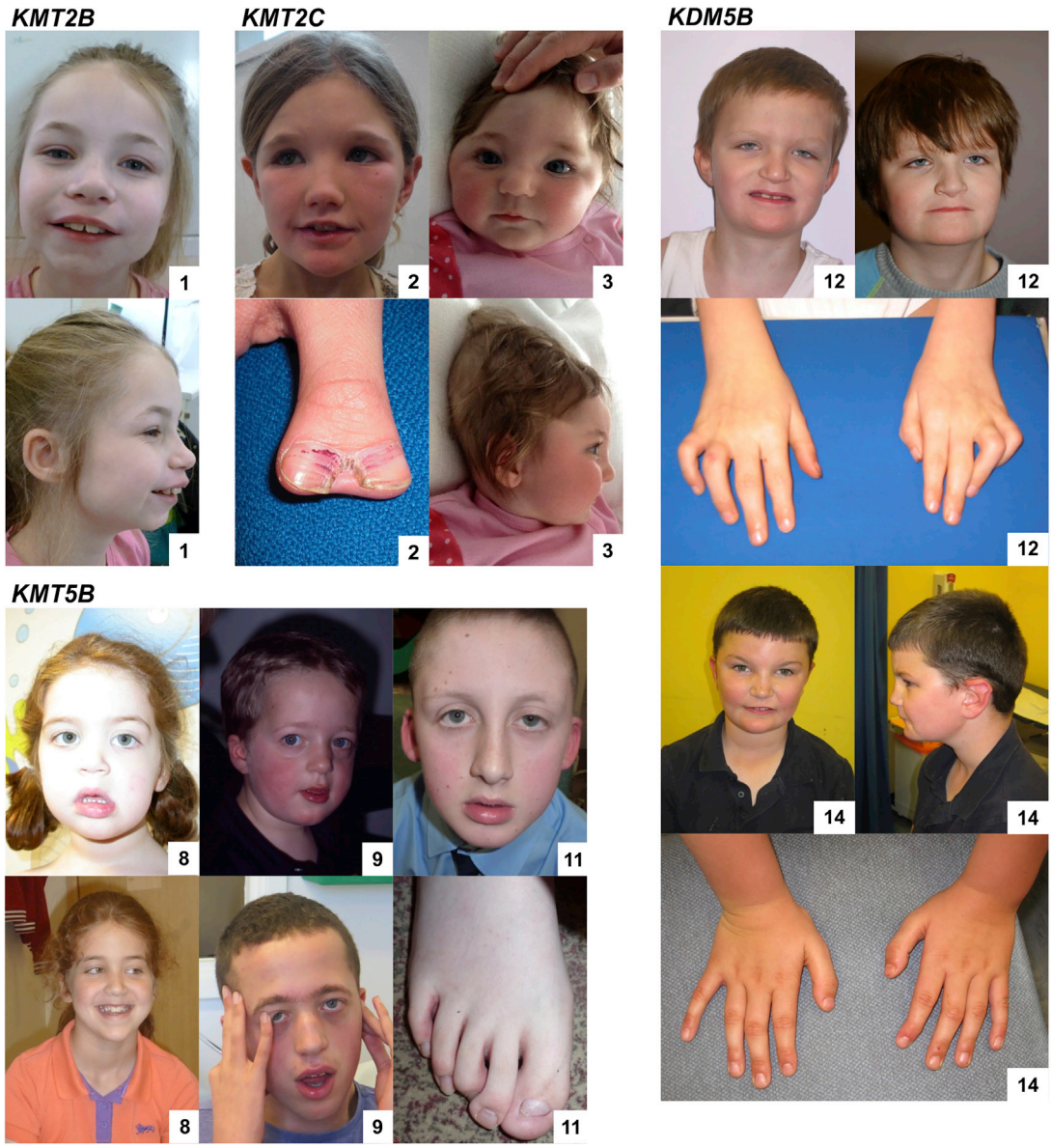

Figure 3. Photographs from Individuals with Truncating Variants or Deletions of *KMT2B*, *KMT2C*, *KMT5B*, and *KDM5B*
The numbers on each picture denote the corresponding individual in Table 1. Individual 1, with a *KMT2B de novo* PTV, has spare scalp hair, a large mouth, and no ear lobes; individual 2, with a *KMT2C de novo* PTV, has marked infra-orbital creases, down-slanting palpebral fissures, and a duplicated right thumb. Individual 3, with a *KMT2C de novo* PTV, has marked plagiocephaly and bilateral marked bulging just below the temporal region. Individual 8, with a *KMT5B de novo* PTV, has a broad and large forehead that has persisted over time. Individual 9, with a *KMT5B de novo* PTV, has a prominent forehead, thick ear lobes,a broad philtrum, an open mouth appearance, and synophrys, which is more noticeable in the more recent photograph. Individual 11, with a de novo *KMT5B* deletion, has a long and oval face, ptosis, prominent eyes, protruded ears, an open mouth, thick lips, and overlapping third and second toes. Individual 12, with a homozygous *KDM5B* PTV, has down-slanting palpebral fissures, a slightly bulbous nasal tip, low-hanging columella, a smooth philtrum, and thin upper and lower lips. He has bilateral camptodactyly of the fourth and fifth fingers. Individual 14, with a compound heterozygous *KDM5B* PTV, has a prominent metopic region, a high nasal bridge, a bulbous nasal tip, a smooth philtrum, thin lips, and a triangular ear with an absent superior crux of helix. He has also mild camptodactyly of the fourth and fifth fingers.

that promotes transcriptional repression.[53] *KMT5B* is highly expressed in both embryonic and adult human brains.[46,47] The *Kmt5b*-null mice die at embryonic stages and have decreased body length and weight,[54] whereas the heterozygous mice have decreased body weight and fat, as well as vertebral anomalies.[23]

indicate that these genes tolerate haploinsufficiency, unlike what is suggested by their pLI scores. *De novo* PTVs in non-candidate KMTs and KDMs for dominant DDs (*KDM5B* and *SETD1B*) (n = 4) are also shown. These PTVs could be coincidental or might be acting as phenotype modifiers, or they could be non-penetrant in some individuals in the general population. Homozygous and compound heterozygous PTVs in *KDM5B* (n = 5) show that recessive histone-tail lysine-methylation disorders also exist.

We identified one DN heterozygous frameshift, three missense *KMT2B* variants, and 18 deletions encompassing this gene (Table 1, Table S5, Table S7, and Figure 2). All but one of these individuals were recently reported in a study identifying PTVs and deletions in this gene associated with childhood-onset dystonia 28 (MIM: 617284).[55,56] The only previously unreported individual in this cohort is a girl (Table 1) with a *de novo* p.Leu604Profs*72 frameshift variant and severe global developmental delay and additional features (Figure 3). Importantly, in contrast with the previously described individuals, this girl did not show any evidence of dystonia by the age of 11 years, even with careful reverse phenotyping.[57] Interestingly, some of the previously reported individuals had normal development.[55,56] Our findings broaden the phenotype of *KMT2B* variants and show that any combination of developmental delay and dystonia can result from heterozygous PTVs in this gene. *KMT2B* is highly expressed in both embryonic and adult human brains.[46,47] In adults, it is specifically highly expressed in pituitary, cerebellum, and bladder.[46] Of note, the affected individual that we describe has growth hormone deficiency, abnormal gait, nystagmus, and urinary incontinence. The *Kmt2b* KO mice die before stage E11.5 and display growth retardation, neural-tube defects, pericardial effusion, abnormal heart looping, and head abnormalities, whereas the heterozygous mice exhibit fasting hyperinsulinemia, glucose intolerance, and fatty-liver disease.[58,59]

Next, we used the UniProtKB, GTEx and BrainSpan databases[46,47,60] to systematically explore the differences between the 33 KMTs and KDMs that are known to be involved in or are candidates for involvement in dominant DDs and the other 18 KMTs and KDMs in terms of their gene or protein attributes and expression patterns. Mann-Whitney tests were performed with an exact p value $<$ 0.05 considered as significant. Candidate or known dominant DD KMTs and KDMs had longer canonical transcripts, a greater number of interactors, and a significantly higher number and types of post-translational modifications (adjusted for protein length) (Figure 4 and Tables S9 and S10).[60] These distinctions are maintained independently for both KMTs and KDMs. This observation is consistent with the general properties of genes that are considered to be haploinsufficient (HI)[27] and suggests that candidate or known dominant DD KMTs and KDMs are likely to be key players performing multiple roles in embryogenesis. Similarly, the expression of candidate or known dominant DD KDMs was found to be significantly higher in almost all fetal brain structures and adult human tissues than that of other KDMs (Table S12 and Table S14) (Figure 4F),[46] which agrees with previous observations regarding HI genes.[27] However, surprisingly we did not find a significant difference between the expression of the candidate or known dominant DD KMTs and other KMTs in most human tissues. Exceptions were certain brain areas where the candidate or known dominant DD KMTs are significantly highly expressed before the 10th week after conception (Table S11, Table S13, and Figure 4F).[47] Further studies will be needed to confirm these unexpected findings. One possibility is that the KMTs that were classified in this study as not being candidates for dominant DDs may be candidates for adult-onset phenotypes. Alternatively, these results might reflect technical limitations, such as lack of cell-type level resolution, of large-scale gene expression experiments.

Lastly, we turned our focus to testing the hypothesis that recessive disorders associated with bi-allelic variants in some KMTs and KDMs might exist. This hypothesis was based on our observation that five KMT and two KDM homozygous-knockout mice are viable but show multiple anomalies (Table S1). In the cohort of 4,293 subjects from the DDD study, we identified 27/102 probands with bi-allelic variants in KMTs and KDMs. On subsequent analyses, most of these were considered likely non-deleterious. However, one individual had bi-allelic homozygous *KDM5B* (MIM: 605393) PTVs (Table S5) (Figures 2 and 3) and severe global developmental delay (Table 1 and Supplemental Note: case reports). Fisher's exact test revealed a 96.89-fold enrichment of homozygous PTVs (95% CI = 3.95–2,378.87; p = 0.03) in *KDM5B* in our cohort against the data from gnomAD.[25] Additionally, no homozygous *KDM5B*-knockout genotype was seen in 3,222 adults with high parental relatedness,[61] and the *Kdm5b*-knockout mice die prematurely from respiratory failure and display disorganized cranial nerves, defects in eye development, increased incidences of exencephaly, and skeletal anomalies.[62] Next, we examined exome data from 5,332 additional individuals from the DDD study and identified two further individuals with bi-allelic *KDM5B* PTVs and striking overlapping phenotypes of severe global developmental delay, camptodactyly, and overlapping facial dysmorphism (Table 1 and Figures 2 and 3). Hence, bi-allelic *KDM5B* LoF variants cause a recessive DD. KDM5B is a H3K4 demethylase that modulates RNA polymerase II initiation and elongation rates and alternative splicing in embryonic stem cells.[63]

Overall, our results demonstrate the importance of defects in histone lysine methylation in human DDs. In particular, variants in six of eight KMT2 methyltransferases can now be considered to result in dominant DDs.[5,8,14,34,40,55,56,64–67] KMT2 genes encode enzymes that monomethylate, dimethylate, and/or trimethylate the H3K4[1,68] and mark active promoters and enhancers.[69] Our observation emphasizes the significance of the correct dosage of KMT2 genes in normal development, despite their apparently redundant enzymatic function. Distinct phenotypes associated with variants in each of the KMT2 genes support their unique biological roles. Furthermore, the possibility of treating some of these conditions makes them highly relevant for future research.[70–73] Our findings enable the grouping of phenotypes on the basis of broad transcriptional consequences of defects in histone lysine methylation. For example, variants in genes promoting transcriptional

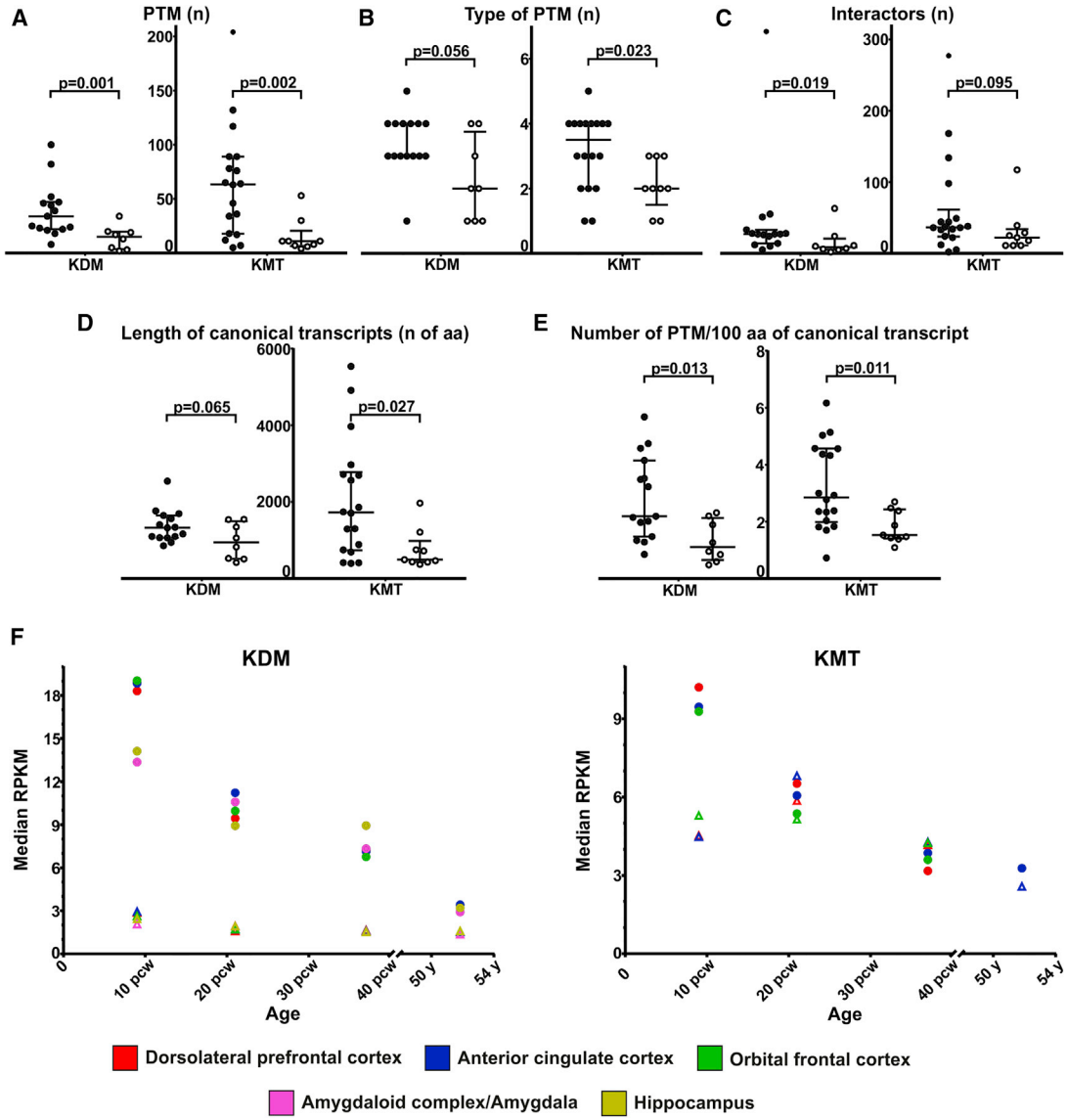

Figure 4. Comparison of Gene and Protein Properties between KMTs and KDMs That Are Known to Be Dominant for DDs or Are Candidate Genes for DDs and other KMTs and KDMs

The comparisons were made with the data from UniProtKB, and a Mann-Whitney test in which an exact p value < 0.05 was considered to be significant was performed. The results are represented in dot plots as follows: (A) number of post-translational modifications (PTMs); (B) number of types of PTMs; (C) number of interactors; (D) length of canonical transcripts; (E) number of PTMs per 100 amino acids of canonical transcripts in KMTs and KDMs; and (F) the median reads per kilobase per million (RPKM) for candidate and non-candidate KMTs and KDMs in brain structures; significant differences across several stages are evident. Black (A–E) or colored (F) dots denote KMT and KDM genes that are known or are predicted to be candidates for dominant DDs. Unfilled dots (A–E) or colored triangles (F) denote KMT and KDM genes that are predicted not to be candidates for dominant DDs. The colors in (F) correspond to the legend for the brain structures provided in the figure. The longer horizontal lines in all the graphs represent the respective medians, the shorter horizontal lines indicate the inter-quartile ranges, and the p values are given at the top of each graph, where relevant.

activity (e.g., H3K4 methyltransferases) appear to cause growth retardation, whereas variants in transcriptional suppressors predominantly result in overgrowth (e.g., *NSD1* [MIM: 606681], *EZH2* [MIM: 601573], and now *KMT5B*). Finally, these results demonstrate that a systematic clinically oriented pathway-based approach (e.g., histone lysine methylation in this study) for analysis of large-scale exome or genome sequencing studies can help to reduce the statistical noise and further accelerate the discovery of rare genetic disorders.

Supplemental Data

Supplemental Data include a Supplemental Note, two figures, and 14 tables and can be found with this article online at https://doi.org/10.1016/j.ajhg.2017.11.013.

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Web Resources

BrainSpan, http://www.brainspan.org/
CAUSES Study, www.causes.clinic
DECIPHER, https://decipher.sanger.ac.uk
Denovo-db, http://denovo-db.gs.washington.edu/denovo-db/
Ensembl GRCh37, http://grch37.ensembl.org
ExAC Browser, http://exac.broadinstitute.org/
Exome Variant Server, http://evs.gs.washington.edu/EVS/
gnomAD, http://gnomad.broadinstitute.org/
GTEx Portal, https://www.gtexportal.org/home
HGMD® Professional Version, https://www.qiagenbioinformatics.com/products/human-gene-mutation-database/
HUGO Gene Nomenclature Committee, http://www.genenames.org/

IMPC, http://www.mousephenotype.org/
MutationMapper, http://www.cbioportal.org/mutation_mapper.jsp
MutationTaster2, http://www.mutationtaster.org/
OMIM, https://www.omim.org/
RVIS, http://genic-intolerance.org/Search?query=kncn
The 1000 Genomes Project, http://phase3browser.1000genomes.org/index.html
UK10K Project, https://www.uk10k.org/
UniProtKB, http://www.uniprot.org/
ZFIN, http://zfin.org/

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