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REVIEW

A common classification framework for histone sequence alterations in tumours: an expert consensus proposal

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Abstract

The description of genetic alterations in tumours is of increasing importance. In human genetics, and in pathology reports, sequence alterations are given using the human genome variation society (HGVS) guidelines for the description of such variants. However, there is less adherence to these guidelines for sequence variations in histone genes. Due to early cleavage of the N-terminal methionine in most histones, the description of histone sequence alterations follows their own nomenclature and differs from the HGVS-compliant numbering by omitting this first amino acid. Next generation sequencing reports, however, follow the HGVS guidelines and as a result, an unambiguous description of sequence variants in histones cannot be provided. The coexistence of these two nomenclatures leads to confusions for pathologists, oncologists, and researchers. This review provides an overview of tumour entities with sequence alterations of the *H3-3A* gene (HGNC ID = HGNC:4764), highlights the problems associated with the coexistence of these two nomenclatures, and proposes a standard for the reporting of histone sequence variants that allows an unambiguous description of these variants according to HGVS principles. We hope that scientific journals will adopt the new notation, and that both geneticists and pathologists will include it in their reports.
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Introduction

The diagnosis and management of tumours increasingly depends on the demonstration of sequence variants (colloquially known as mutations and other genetic alterations) in genes related to their causation. The discovery of such tumour-associated sequence variants not only facilitates the diagnosis of tumour types that are difficult to classify based on histomorphology and immunohistochemistry alone but can also be of prognostic and therapeutic value. To unify the description of such variants and to make new discoveries available for clinicians and pathologists, the Human Genome Variation Society (HGVS) has developed and maintained a nomenclature for the standardised and unambiguous description of sequence variants [1–4]. This has become the universally accepted nomenclature for all next generation sequencing (NGS) and whole genome sequencing reports and provides the basis for consensus recommendations for molecular pathology [5] and clinical oncology [6].

Biologists and clinicians around the world have applied these guidelines to report new discoveries in DNA sequence variation, with the apparent exception of those working on histones, which present unique challenges.

Histones are a family of basic proteins that associate with DNA and allow condensation to form chromatin. The four canonical histones H2A, H2B, H3, and H4 form dimers between H2A and H2B (H2A–H2B) and between H3 and H4 (H3–H4). When pairs of these dimers are bound to DNA, they form a histone octamer, which corresponds to a nucleosome. A chain of nucleosomes is wrapped in a spiral called a solenoid, in which the nucleosomes are further stabilised by the linker histone H1 to support the chromatin structure [7,8].

Histone post-translational modifications such as acetylation, phosphorylation, methylation, ubiquitylation, and sumoylation, as well as histone exchange, affect

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DNA transcription by regulating RNA polymerase II's access to DNA. Consequently, complex cell processes can be influenced by histone modifications and its related alterations in gene expression. There are isoforms for the core histones H3, H2A, and H2B, as well as for the H1 linker histone, which show alterations of either a few amino acids or larger domains [7]. These histone isoforms are further subdivided into replicative or replacement types. Whereas replicative types are only highly expressed during the S-phase, replacement histone types are typically synthesised in a cell-cycle-independent manner and incorporated into chromatin. Since replacement histone types localise to specific chromatin domains, they can lead to differential expression in different tissue compartments. Therefore, a single histone isoform can fulfil different functions. Replacement types H3.3 and H2A.Z are involved in the regulation of transcription, whereas the replicative isoforms H3.1 and H3.2 are incorporated during replication and the replicative H3.5 contributes to DNA condensation during spermatogenesis [9,10].

Well-documented discrepancies in histone sequence notation reflect the historical roots of this scientific field. The amino acid (AA) sequence of histones was first deciphered in the 1960s and 1970s [11–13], using chromatographic separation methods, enzymatic and chemical cleavage, followed by Edman degradation to determine the sequences of the functional histone proteins. After histone genes had been sequenced, it became apparent that the AA encoded by the first codon of histone genes, a methionine, is post-translationally cleaved from most proteins and could therefore not be detected with previous protein-based methods. However, the previously established numbering of the AA sequences remained the standard in this field. Therefore, the sites of histone sequence variants are typically reported lacking one AA, relative to the DNA-based AA numbering [11,14]. This discrepancy is particularly problematic at sites where two identical AAs follow each other in the protein sequence. In human H3.3 histone A (encoded by the *H3-3A* gene; HGNC ID = HGNC:4764), such DNA-based numbering sites are at positions 13/14, 25/26, 34/35, 37/38, 53/54, 61/62, 88/89, and 129/130. Unfortunately, one of the crucial sites widely accepted as an oncogenic driver is the H3.3 histone A sequence variant at AA 34/35, depending on the numbering scheme being used. Consequently, it is unclear which AA is meant, because in the HGVS-compliant protein reference sequence (NCBI RefSeq NP_002098.1) there is a glycine at both AA 34 and AA 35. Thus, the histone legacy AA variant H3.3 G34 corresponds to a DNA-based H3.3 G35 variant using current variant reporting standards, as would be reported by current NGS data pipelines [15].

While in some instances the use of legacy AA numbering may have little significance, it is not sufficient for use in tumour classification. For histone sequence variants, the coexistence of two numbering systems (nomenclatures) can lead to confusion for pathologists, researchers, and clinicians alike. The International Agency for Research on Cancer (IARC) noted this issue during production of the 5th edition of the WHO Classification of Tumours, published as the widely used WHO Blue Books (http://whobluebooks.iarc.fr), and decided to explore the matter further in the hope of providing precise and unequivocal description of tumour-associated sequence variants in histone genes to avoid the potential for errors based on the coexistence of two different nomenclatures.

We therefore propose the use of the following notation: in line with existing guidance for reporting genetic alterations, histone sequence variants should be reported using the HGVS recommended format. First, reference sequences must be specified for the transcript (mRNA) and the corresponding encoded protein. Once that has been done, the gene symbol may be used to indicate the gene that harbours the DNA sequence. For example, the transcript and protein reference sequences for the *H3-3A* gene are RefSeq [16] entries NM_002107.7 and NP_002098.1, respectively. Based on these reference sequences, a variant in *H3-3A* at the transcript and protein level would be described as *H3-3A*:c.103G>A p.Gly35Arg. This variant description is standards-compliant and corresponds to the variant commonly known as H3.3 G34R, in which the AA number is based on the legacy protein sequence and the amino acids are demonstrated in a single letter code. This legacy designation is also used to label antibodies raised against specific protein isoforms and variants. It should be noted that there is currently no agreed HGVS nomenclature syntax for the reporting of legacy/common variant descriptions. For now, the reporting format from DNA sequencing should be *H3-3A*:c.103G>A p.Gly35Arg (G34R), noting that there is a space, but no punctuation, between the DNA, protein, and the legacy protein variant descriptions, often identified by immunohistochemistry. The parentheses are used to indicate the legacy protein sequence description.

The WHO Classification of Tumours will adopt this nomenclature within its publications both online and in print. Molecular pathology reports should also cite the version of the human genome used, e.g. GRCh37 or GRCh38, when a variant is described in terms of a genome position following genome or exome sequencing, as well as the method used to obtain the results [17,18].

We established the extent of the nomenclature problem for sequence variants of histones using these conditions, parameters, and assumptions:

- Nucleotide and AA sequence variants are described in accordance with the current HGVS recommendations (Version 20.05) for the description of sequence variants [1]. In line with these recommendations, the term 'sequence variant' is used instead of 'mutation'. In agreement with current recommendations, the three-letter AA code is used to reduce reporting errors. To simplify the presentation of HGVS-compliant protein sequence variant descriptions, an assumption is made that all such variants have been confirmed by independent methods and that none are purely predictions from variants at the nucleotide level.

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- Gene symbols, names, and gene IDs are used in accordance with the recommendations of the HUGO Gene Nomenclature Committee (HGNC) [19].
- Nucleotide and protein reference sequences for the reporting of sequence variants are from the NCBI RefSeq Reference Sequence Database [16].
- Sequence variant descriptions are validated using VariantValidator (https://variantvalidator.org/) [20].
- The WHO Classification of Tumours online database (https://tumourclassification.iarc.who.int/home) and PubMed were searched as a rapid mapping review for references to 'histone', 'K27', 'G34', 'K28', and 'G35' to identify those tumour types whose classification, and therefore potentially diagnosis, is supported by and sometimes depends on histone AA positions.
- Searches in PubMed (last performed July 2020):
  Search (('2015/05/01'[Date - Publication]: '3000'[Date - Publication])) AND ((((Mutation) NOT methylation)) AND (histone OR H3 OR H3F3A OR H3-3A)) AND (((tumor OR tumour OR neoplasm)))
  Search (((K27 OR K28 OR G34 OR G35)) AND (histone OR H3 OR H3F3A)) AND (((tumor OR tumour OR neoplasm)))
- Further literature searches were conducted in PubMed, for each tumour type and the reported histone AA sequence variant, to identify potential issues of nomenclature to obtain examples with potential for confusion.
- The papers were reviewed for relevance in abstract form by two authors (HL and IAC) and included as shown in the PRISMA flow diagram (Figure 1) [21].
- Additional searches were made in COSMIC, the Catalogue Of Somatic Mutations In Cancer (https://cancer.sanger.ac.uk/cosmic), for *H3F3A* sequence variants and the results tabulated. The use of *H3F3A* rather than *H3-3A* is due to the use of the old gene symbol in COSMIC. Tabulated data from tumours listed in COSMIC for *H3F3A* sequence variants are given in supplementary material, Table S1. A list of relevant website resources is provided in Table 1.

Tumour entities that harbour histone-related changes

The discovery of histone sequence variants as oncogenic drivers in neoplasms has opened a new chapter in tumour diagnostics. Indeed, there are an increasing number of tumour entities that harbour histone-related changes (Table 2). Such entities include tumours of the central nervous system, bone and soft tissue neoplasms, head and neck squamous cell carcinoma, malignant melanoma, bladder and colorectal cancer, ovarian cancer, and haematological neoplasms. These are described here and summarised in Table 3.

Digestive system tumours

Few *H3-3A* sequence variants have been reported in COSMIC (*H3F3A*) for digestive system tumours, and there is as yet little consistency, with fewer than five reports of the same tumour type. Though these are small numbers of reports, the few anatomic tumour sites that carry alterations in more than one case comprise *H3-3A* sequence variants encoding p.Ala115Gly, p.Ala88Ser, p.Ala88Thr, p.Ile90Val, p.Lys28Arg, p.Arg50His, or p.Arg50Cys missense variants. Such changes have been detected in intestinal adenocarcinoma, tumours involving the oesophagus and pancreas, as well as hepatocellular cholangiocarcinoma [34]. The biological significance of these sequence alterations is uncertain.

Breast tumours

There is a high diversity of *H3-3A* sequence variants described for breast tumours in the COSMIC database, most being invasive breast carcinomas (IBCs) of no special type (NST). However, COSMIC lists two ductal carcinomas *in situ* in combination with Paget's disease, which both show a p.Arg73Gln substitution.

Soft tissue and bone tumours

There is an established and consistent pattern of sequence alteration in giant cell tumour of bone [23,26,35], with the *H3-3A*:c.103G>T p.Gly35Trp sequence variant being reported in the vast majority of cases. Additionally, chondroblastoma, chondrosarcoma, clear cell chondrosarcoma, and osteosarcoma have been reported to harbour *H3-3A*:c.110A>T p.Lys37Met sequence variants [23,36].

Female genital tumours

There are few reports of *H3-3A* gene sequence alterations in tumours of the female genital tract, with most reported in endometrial carcinomas (18/24 in COSMIC; supplementary material, Table S1) [37].

Thoracic tumours

There are few reports of sequence variants in thoracic tumours. Two small cell lung carcinomas have been reported to harbour sequence variants leading to a stop at p.Gly34Ter [38]. Squamous cell carcinoma cases of the lung have been described with the introduction of stop codons at p.Gln6Ter or p.Tyr100Ter (CGP study 418).

Central nervous system tumours

The majority of *H3-3A* sequence variants have been reported in tumours of the CNS, some of which are now named accordingly:

Diffuse hemispheric glioma, H3 G34-mutant

The incidence of diffuse hemispheric glioma, H3 G34-mutant, ranges from 8% to 16% of paediatric high-grade gliomas with hemispheric location [25,29,39]. So far, *H3-3A* sequence alterations resulting in a p.Gly35Arg (G34R) or a p.Gly35Val (G34V) substitution have been described for this tumour type. The vast majority of tumours show a glycine-to-arginine substitution (p.Gly35Arg; 94%),

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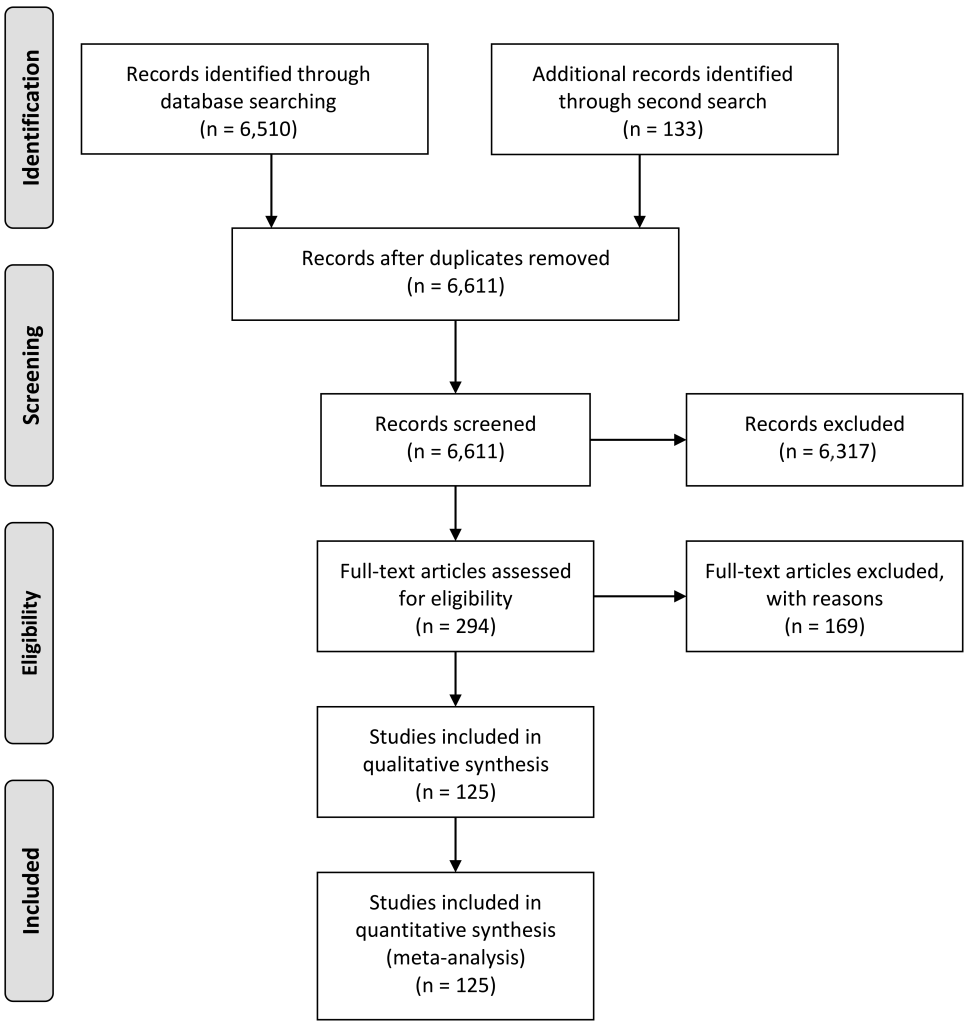

Figure 1. PRISMA diagram from the literature search according to the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement' [21] (for more information visit http://www.prisma-statement.org).

Table 1. Website resources.

Resource	URL
COSMIC	https://cancer.sanger.ac.uk/cosmic
HGVS Sequence Variant Nomenclature	https://varnomen.hgvs.org/
HUGO Gene Nomenclature Committee	https://www.genenames.org/
NCBI RefSeq	https://www.ncbi.nlm.nih.gov/refseq/
PubMed	https://pubmed.ncbi.nlm.nih.gov/
VariantValidator	https://variantvalidator.org/
WHO Classification of Tumours Online	http://tumourclassification.iarc.who.int/welcome/

whereas the glycine-to-valine substitution is rare (p.Gly35Val; 6%).

Diffuse midline glioma, H3 K27-altered

In children, diffuse midline gliomas, H3 K27-altered, are preferentially located in the brainstem, particularly in the pons, also known as diffuse intrinsic pontine glioma (DIPG), whereas diffuse midline gliomas, H3 K27-altered, in adults are more commonly seen in the thalamus or spinal cord [40,41]. The vast majority of H3 K27-altered diffuse midline gliomas contain the p.Lys28Met (K27M) missense change in one of the histone H3 isoforms [25,42], while individual cases of DIPG may carry a p.Lys28Ile (K27I) substitution [24]. There have been several histone genes reported that may contain analogous sequence variants, with *H3-3A* being most commonly affected, while *H3-3B*, *H3C2*, *H3C3*, and *H3C14* variants are less common and mostly restricted to DIPG [24,25,42].

Pilocytic astrocytoma (with histone H3 gene alteration)

Pilocytic astrocytoma carrying a histone H3 sequence alteration that results in a p.Lys28Met (K27M) substitution is extremely rare. Some of the reported cases showed fast tumour progression and hence are more likely to resemble diffuse midline gliomas, H3 K27-altered. However, there are individual cases with a prolonged overall survival of about 10 years [43], which are biologically more in accordance with pilocytic astrocytomas.

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Table 2. Tumour-associated sequence variants of histones.

HGVS gene symbol (HGNC ID)	Previous gene symbol	NCBI RefSeq reference transcript sequence	Transcript-level variant description	NCBI RefSeq reference protein sequence	Protein-level variant description	Legacy protein-level variant description*	PMID
H1-6 (HGNC:4720)	*HIST1H1T*	NM_005323.4	c.426G>C	NP_005314.2	p.Lys142Asn	K141N	[22]
H3C10 (HGNC:4775)	*HIST1H3H*	NM_003536.3	c.37G>C	NP_003527.1	p.Gly13Arg	G12R	[23]
H3C2 (HGNC:4776)	*HIST1H3B*	NM_003537.4	c.83A>T	NP_003528.1	p.Lys28Met	K27M	[24]
H3C3 (HGNC:4768)	*HIST1H3C*	NM_003531.3	c.83A>T	NP_003522.1	p.Lys28Met	K27M	[24]
H3C14 (HGNC:20503)	*HIST2H3C*	NM_021059.3	c.83A>T	NP_066403.2	p.Lys28Met	K27M	[24]
H3-3A (HGNC:4764)	*H3F3A*	NM_002107.7	c.83A>T	NP_002098.1	p.Lys28Met	K27M	[25]
H3-3A (HGNC:4764)	*H3F3A*	NM_002107.7	c.83_84delinsTT	NP_002098.1	p.Lys28Ile	K27I	[24]
H3-3A (HGNC:4764)	*H3F3A*	NM_002107.7	c.104G>A	NP_002098.1	p.Gly35Glu	G34E	[26]
H3-3A (HGNC:4764)	*H3F3A*	NM_002107.7	c.103_104delinsCT	NP_002098.1	p.Gly35Leu	G34L	[26]
H3-3A (HGNC:4764)	*H3F3A*	NM_002107.7	c.103_104delinsAT	NP_002098.1	p.Gly35Met	G34M	[26]
H3-3A (HGNC:4764)	*H3F3A*	NM_002107.7	c.103G>A	NP_002098.1	p.Gly35Arg	G34R	[25]
H3-3A (HGNC:4764)	*H3F3A*	NM_002107.7	c.103G>C	NP_002098.1	p.Gly35Arg	G34R	[27]
H3-3A (HGNC:4764)	*H3F3A*	NM_002107.7	c.104G>T	NP_002098.1	p.Gly35Val	G34V	[25]
H3-3A (HGNC:4764)	*H3F3A*	NM_002107.7	c.103G>T	NP_002098.1	p.Gly35Trp	G34W	[23]
H3-3B (HGNC:4765)	*H3F3B*	NM_005324.5	c.103G>A	NP_005315.1	p.Gly35Arg	G34R	[23]
H3-3A (HGNC:4764)	*H3F3A*	NM_002107.7	c.110A>T	NP_002098.1	p.Lys37Met	K36M	[23]
H3-3B (HGNC:4765)	*H3F3B*	NM_005324.5	c.110A>T	NP_005315.1	p.Lys37Met	K36M	[23]
H4-16 (HGNC:20510)	*HIST4H4*	NM_175054.2	c.167G>A	NP_778224.1	p.Arg56Gln	R55Q	[22]

*The legacy protein-level variant descriptions are based upon amino acid numbering of the mature histone protein following post-translational removal of the initiating methionine, as used in COSMIC v91.

Ganglioglioma (with histone H3 gene alteration)

Histone sequence variants in gangliogliomas are rare. Reported cases with histone H3 p.Lys28Met (K27M) alteration are, so far, associated with a midline location of the tumour. Such sequence variants have been more frequently described in anaplastic gangliogliomas (WHO CNS grade 3) rather than in classic gangliogliomas (WHO CNS grade 1) [44]. The outcome for these patients has been reported to be poor, even in the absence of signs of anaplasia, although there are patients with prolonged overall survival [44,45]. There are single reports showing that diffuse hemispheric gliomas with the *H3-3A*:c.103G>A p.Gly35Arg (G34R) variant may contain a dysplastic neuronal component, as typically seen in gangliogliomas. Whether these resemble a morphological variant of diffuse hemispheric glioma, H3 G34-mutant, or whether they are true gangliogliomas is, to date, unclear and requires further evaluation [46].

Posterior fossa ependymoma group PFA (with histone H3 gene alteration)

Posterior fossa ependymomas group A (PFA) exhibit EZH inhibitory protein (EZHIP) overexpression that may mimic the biological effects of the histone H3 p.Lys28Met (K27M) variant [47]. Only exceptional cases of posterior fossa ependymomas group A show an *H3-3A*:c.83A>T p.Lys28Met (K27M) sequence alteration [48,49]. The progression-free survival of PFA cases is worse compared with its counterpart, the posterior fossa ependymoma group B, which lacks sequence alterations of one of the histone H3 isoforms [50].

Subependymoma (with histone H3 gene alteration)

A single study reported on individual brainstem subependymomas with *H3-3A*:c.83A>T p.Lys28Met (K27M) alteration and long overall survival [51].

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Table 3. Individual tumour types with histone H3.3 sequence variants described in COSMIC, and selected references. Tumours with fewer than five entries containing the same *H3-3A* sequence variants in COSMIC (supplementary material, Table S1) are excluded.

Tumour type	Histone sequence variants	Reference
1. Digestive system tumours	Fewer than five entries	
2. Breast tumours	Fewer than five entries	
3. Soft tissue and bone tumours	*H3-3A*:c.103G>T p.Gly35Trp (H3.3 G34W)	[28]
	H3-3A:c.110A>T p.Lys37Met (H3.3 K36M)	[23]
4. Female genital tumours	Fewer than five entries	
5. Tumours of the lung, pleura, thymus and heart	Fewer than five entries	
6. Tumours of the central nervous system	*H3-3A*:c.83A>T p.Lys28Met (H3.3 K27M)	[29]
	H3-3A:c.103G>A p.Gly35Arg (H3.3 G34R)	[30]
	H3-3A:c.103G>C p.Gly35Arg (H3.3 G34R)	[31]
	H3-3A:c.104G>T p.Gly35Val (H3.3 G34V)	[32]
7. Tumours of the urinary system and male genital organs	Fewer than five entries	
8. Head and neck tumours	*H3-3A*:c.344C>G p.Ala115Gly (H3.3 A114G)	[33]
9. Tumours of endocrine organs	*H3-3A*:c.103G>T p.Gly35Trp (H3.3 G34W)	[22]
10. Tumours of haematopoietic and lymphoid tissues	Fewer than five entries	
11. Skin tumours	Fewer than five entries	
12. Tumours of the eye	Fewer than five entries	

Meningiomas

Two intronic *H3-3A* variants have been described in rare cases of intraventricular meningiomas, *H3-3A*:c.128 +52C>T and *H3-3A*:c.129-41G>T [52]. The biological impact of these alterations remains elusive.

Urinary and male genital tumours

There are relatively few reports of *H3-3A* sequence variants in prostate, bladder, and renal carcinomas [34], with H3.3 histone A p.Ala48Val, p.Lys5Met, p.Arg50His or p.Arg50Leu protein alterations each being listed at least twice (supplementary material, Table S1).

Head and neck tumours

Squamous cell carcinomas of the upper respiratory tract [34,53] have been reported to carry a recurrent *H3-3A*: c.344C>G p.Ala115Gly alteration, though numbers remain small. So far, these alterations have only been found in HPV-independent tumours.

Endocrine and neuroendocrine tumours

Histone sequence variants have been studied in relatively small numbers of cases, but there is a consistent *H3-3A*: c.103G>T p.Gly35Trp variant in four phaeochromocytomas listed in COSMIC, and in four paragangliomas studied [22]. This variant also occurs in thyroid carcinomas (2/4 cases, type not specified), whereas the other two cases of *H3-3A*-mutant thyroid cancer displayed an *H3-3A*: c.86G>C p.Ser29Thr variant (CGP study 676).

Haematolymphoid tumours

Histone sequence variants occur in a variety of myeloid and lymphocytic haematogenous malignancies, with T-cell lymphomas showing a p.Ala115Gly, p.Lys28Glu, p.Lys28Met or p.Lys28Asn substitution in H3.3 histone A in more than one case. A single case of a diffuse large B-cell-lymphoma with an *H3-3A*:c.45A>C p.Lys15Asn substitution has been described (CGP 632).

Lohr *et al* [54] reported an accumulation of variants in the linker histone H1 family in diffuse large B-cell lymphomas. The functional significance of these sequence alterations remains to be explored, although the authors report that hotspot variants of *H3C2* and potentially other histone core proteins might be related to activation-induced cytidine deaminase (AID)-mediated somatic hypermutation [54].

Additionally, single leukaemia cases including AML [34] and T-ALL [55,56], as well as follicular lymphomas [57], have been reported to show alterations in histone genes.

Skin tumours

There are rare cases of melanomas with *H3-3A* sequence variants [34].

Advantages of using the proposed reporting system for histone sequence alterations

Our analysis of the published literature and the COSMIC database suggests that *H3-3A* sequence variants occur in tumours from various sites of the body, though overall relatively rarely, with CNS and bone tumours accounting for the majority of reported cases.

The use of a standardised nomenclature for reporting genetic alterations, as described in the HGVS Sequence Variant Nomenclature, allows the unambiguous description of sequence variants, independent of the analytical method, tumour type or field of research. The HGVS recommendations have thus become the almost universally accepted guidelines for the description of genetic alterations.

The benefit of the recommended HGVS-compliant reporting scheme is that it includes the sequence alteration at the DNA level, as well as the predicted resulting change at the protein level. This is particularly relevant for histones for the following reasons. Histone H3.3 is encoded by two genes, *H3-3A* and *H3-3B*, with each

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gene encoding identical protein products despite the genes themselves having distinct and different nucleotide sequences. Sequence variants can occur in either of these genes, resulting in the same altered protein product. For example, in chondroblastomas [58], the potential p.Lys37Met substitution of histone H3.3 results from sequence variants in both the *H3-3A* and the *H3-3B* genes, with the majority of these cases showing the sequence variation in *H3-3B* [23] (supplementary material, Figure S1). An 'H3.3 K37M' description would thus not be sufficient to unequivocally report the differences at the site of genomic sequence alteration. The report of the specific mutated gene or alteration may also have prognostic impact, as observed in paediatric patients with H3 K27-altered diffuse midline glioma located in the pons, i.e. diffuse intrinsic pontine glioma (DIPG). DIPG patients with an *H3-3A*:c.83A>T p.Lys28Met (K27M) sequence variant have a median overall survival of 11 months. In contrast, patients with the same AA substitution resulting from sequence variation in the *H3C2*, *H3C3* or *H3C14* genes have a median overall survival of 15 months [59–61]. Since this information can be useful for clinicians and patients, the recommended HGVS-compliant nomenclature encourages pathologists to correctly report alterations that are associated with different outcomes. Such information is important for future studies to unequivocally report genetic information that is known to be of prognostic relevance. Additionally, it enables better-defined patient stratification that might result in the identification of new prognostically or therapeutically relevant sequence alterations in the future.

Despite the clearly defined site of sequence variation and report of potentially different outcome in patients, the so far-used AA-based description of the site of histone sequence alteration is potentially inaccurate. The legacy description (H3 K27) in 'diffuse midline glioma, H3 K27-altered' is based on the processed histone protein, after cleavage of the initiating methionine, which is assumed to be the tumour driver protein. However, histones contain several N-terminal cleavage sites. For histone H3 variants, several cathepsin L cleavage sites have been reported [62] in murine and human embryonic stem cells (ESCs), such as after p.Ala22, p.Thr23, p.Lys24, p.Ala25, p.Arg27 and p.Lys28. Mass spectrometry analysis has demonstrated that such post-translational cleavage occurs. Even though the role of such cleavage has not been entirely resolved, Zhou *et al* [62] reported that it may influence the epigenetic signature upon differentiation and that it could play a role in apoptosis as well as in immune cell recruitment. Alterations of these hypothesised functions are tightly associated with tumour development [63]. In addition, there has been a serine protease activity detected in human ESCs with an additional cleavage site after p.Ala32 in H3.1 and/or H3.2 isoforms [64]. To our knowledge, this serine protease has not been fully characterised and thus we lack further information regarding the biological impact. The use of an AA-based nomenclature, as is the current standard in the histone field, is not only potentially ill-defined but also potentially erroneous regarding the tumour driver protein, with implications for both diagnostics and research.

Another aspect is that there is at least one histone (H2B type W-T) in which the initiating methionine is not cleaved. Therefore, the histone nomenclature description of sequence variants necessitates awareness of whether cleavage of the initiating methionine occurs [7].

It becomes even more complicated in the case of histone 3-like centromeric protein A (CENP-A). Even though this protein shows cleavage of the initiating methionine after nucleosome deposition, Bailey *et al* found that approximately 10% of pre-nucleosomal CENP-A still retains the initiating methionine [65]. Thus, the notation of the AA-sequence changes for CENP-A depends on its biological state. Since CENP-A H4 heterodimers form a pre-nucleosomal complex with Holliday junction recognition protein (HJURP), which is active in G2 and during mitosis, it is speculated that the variant with the retained initiating methionine might be involved in tumour development [65].

Due to the coexistence of the two reporting systems, namely the legacy AA-based nomenclature of the mature histone protein as well as the DNA-based nomenclature, as used in genetic testing reports, there are many ambiguous reports in the literature. However, combined use of gene symbols, DNA sequence variants (based on reference transcript sequences), and the predicted resulting substitutions of the protein unequivocally defines the site of the sequence alteration. Furthermore, it enables a DNA-based description of histone sequence variants, which is regarded as the gold standard for the reporting of variants according to the HGVS sequence variant recommendations, and allows unambiguous description of these alterations.

Application of the proposed nomenclature on histone gene alterations found in brain tumours

The most common CNS tumour harbouring *H3-3A* sequence variants is the 'diffuse midline glioma, H3 K27-altered'. In this entity, a c.83A>T sequence variant within *H3-3A* leads to the replacement of the AA lysine (Lys) with methionine (Met) (p.Lys28Met). Such alterations should be reported as *H3-3A*:c.83A>T p.Lys28Met (K27M) [15].

The second most frequent glial tumour harbouring an *H3-3A* sequence variant is the diffuse hemispheric glioma, H3 G34-mutant, which is characterised by the substitution of glycine (Gly) by either arginine (Arg) or valine (Val) (p.Gly35Arg or p.Gly35Val). Following the notation proposed here, the sequence alteration should be reported as *H3-3A*:c.103G>A p.Gly35Arg (G34R), or *H3-3A*:c.103G>C p.Gly35Arg (G34R), or *H3-3A*:c.104G>T p.Gly35Val (G34V) [15].

The same reporting system would apply for the single cases of gangliogliomas, pilocytic astrocytomas,

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ependymomas [48], and subependymomas [51] that were reported to contain *H3-3A* sequence variants. It needs to be acknowledged that there is a debate about whether cases with histological features of a ganglioglioma and presence of a p.Gly35 substitution in the *H3-3A*-encoded protein are part of the spectrum of diffuse hemispheric glioma, previously reported as H3.3 G34-mutant, or whether they resemble real gangliogliomas with rare *H3-3A* sequence variation and are therefore not listed as such in COSMIC.

Application of the proposed nomenclature on histone gene alterations found in bone and soft tissue tumours

In the bone and soft tissue field, there are two bone tumour types that account for most of the soft tissue and bone tumours associated with recurrent histone sequence alterations: there is an established and consistent pattern of sequence variants in giant cell tumour of bone [23,26,35] with an *H3-3A*:c.103G>T p.Gly35Trp (G34W) substitution in the vast majority of those reported that supports the diagnosis. It should be noted that p.Lys37Met alterations predominantly encoded in *H3-3B* are seen in about 95% of chondroblastomas [23]. A few cases of chondrosarcoma, clear cell chondrosarcoma, and osteosarcoma [23,36] have also been reported to contain H3.3 histone A p.Lys37Met substitutions. The vast majority of chondroblastomas harbour p.Lys37Met encoded by *H3-3B* as opposed to *H3-3A*. Such sequence variants should be reported, for example, as *H3-3A*:c.110A>T p.Lys37Met (K36M) or *H3-3B*:c.110A>T p.Lys37Met (K36M).

Techniques for the detection of sequence alterations

Various techniques are available for analysing tissue samples for sequence variants using methods based on DNA sequencing or PCR. Some individual methods are capable of determining the number of variant DNA molecules relative to the normal reference sequence and hence are recognised as being quantitative or semi-quantitative. The choice of method and interpretation of results should depend upon the experience of the institution performing the analysis [66,67]. Also, the use of immunohistochemistry has become a routine in tumour diagnostics, as it is a fast and inexpensive method to report the presence or absence of tumour-related AA sequence alterations in neoplastic tissue. In addition, it also enables pathologists to clearly define the distribution and the cellular components that bear such sequence alterations. This not only allows for a better understanding of tumour types but also defines areas of tumour tissue where molecular analyses would be most promising for revealing conclusive results (by avoiding cellular components that are negative for these alterations). In addition to the sequence variant-specific antibodies against IDH1 R132H and BRAF VE1 (BRAF V600E) that are commonly used in tumour diagnostics, there are also antibodies available that detect H3 isoform-

Table 4. Summary of commonly used histone antibodies and clones for immunohistochemical analysis of tumours.

Antibody	Suggested antibody name	Clone	Target	Host/isotype	Reactivity	Websites [Accessed 20 September 2020]
H3.3 K27M	H3.3 K28M	Recombinant monoclonal (RM192)	H3.3 p.K28M	Rabbit/IgG	Human	https://www.thermofisher.com/antibody/product/H3-3-K27M-oncohistone-mutant-Antibody-clone-RM192-Recombinant-Monoclonal/MA5-27916
H3K27me3	H3 K28me3	Monoclonal (G.299.10)	H3 K28me3	Rabbit/IgG	Human, mouse, non-human primate, rat, *Xenopus*, zebrafish	https://www.thermofisher.com/antibody/product/H3K27me3-Antibody-clone-G-299-10-Monoclonal/MA5-11198
H3.3 G34R	H3.3 G35R	RM240	H3.3 p.G35R	Rabbit/IgG	All	https://www.revmab.com/index.php/product/anti-histone-h3-3-g34r-rabbit-monoclonal-antibody-clone-rm240-histone-h3-3-g34r-mutant/
H3.3 G34V	H3.3 G35V	329E5; RM307	H3.3 p.G35V	Rabbit/IgG	All	https://www.revmab.com/index.php/product/anti-histone-h3-3-g34v-rabbit-monoclonal-antibody-clone-rm307-histone-h3-3-g34v-mutant/
H3.3 G34W	H3.3 G35W	RM263	H3.3 p.G35W	Rabbit/IgG	All	https://www.revmab.com/index.php/product/anti-histone-h3-3-g34w-rabbit-monoclonal-antibody-clone-rm263-histone-h3-3-g34w-mutant/
H3 K36M	H3 K37M	RM193	H3.3 p.K37M	Rabbit/IgG	All	https://www.revmab.com/index.php/product/anti-histone-h3-k36m-rabbit-monoclonal-antibody-clone-rm193-histone-h3-k36m-mutant/

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related alterations, such as H3.3 p.Lys28Met (K27M), H3.3 p.Gly35Arg (G34R) or H3.3 p.Gly35Val (G34V), as well as H3.3 p.Gly35Trp (G34W) and H3.3 p.Lys37Met (K36M) (Table 4).

Even though a positive immunohistochemical result is considered to be sufficient to define the tumour entity, further elucidation of a sequence variant's prognostic value, e.g. in H3 K27-altered diffuse midline glioma located in the pons (DIPG), may depend on the specific gene in which the sequence is changed (see above), and hence requires additional molecular testing [59–61] (Table 5). Combined use of molecular analysis and immunohistochemistry also further controls for false-positive or false-negative results, which have been reported for both sequencing and immunohistochemistry [68]. Histone sequence variant-specific antibodies have been developed and named according to legacy histone AA numbering. To avoid confusion with the labelling of these antibodies, we suggest for the time being that these legacy descriptions be added in brackets following the HGVS-compliant standard notation (see above).

Practical handling of the proposed new nomenclature

Knowledge of the subject as summarised in this present paper has several important limitations. Ascertainment bias is a known issue in databases such as COSMIC, leading to a greater number of rare sequence variants being listed which then appear to be more common than they are. Reporting bias in publications is also likely, as many are case reports or small series rather than comprehensive studies, presenting an incomplete picture that requires more research. Sequencing of histone genes on a population basis in multiple geographical areas to account for any ethnic differences would be very helpful. Studies using tumour banks may fulfil this need, at least for the more common tumour types. Nevertheless, we have been able to identify some tumour types in which the reports are frequent enough to suggest that histone sequence variation may be important in their biology.

Since sequencing is usually more time-consuming than histomorphological and immunohistochemical assessment, the proposed nomenclature of entities with a histone sequence variant encourages a two-stage diagnosis. With the help of variant-specific antibodies and histone sequence variant-defining immunohistochemical features, a diagnosis can be given which includes the histomorphology and the suspected histone sequence variant based on the immunohistochemical findings, such as diffuse midline glioma with immunohistochemical detection of a p.Lys28Met (H3 K27M) sequence variant in one of the histone 3 isoforms. In some circumstances, such as in H3 K27-altered diffuse midline glioma located in the pons (DIPG), sequencing may not only be helpful to confirm the diagnosis but may also be of prognostic value, as indicated above. Following the proposed nomenclature, cases of diffuse hemispheric glioma, H3 G34-mutant, with, for example, immunohistochemical detection of H3.3 G34R positivity, might also be genetically evaluated to confirm changes in the nucleotide sequences, which have so far been described to be either *H3-3A*:c.103G>A p.Gly35Arg (G34R) or *H3-3A*:c.103G>C p.Gly35Arg (G34R).

Conclusion

This overview of tumour entities with sequence alterations of the *H3-3A* gene (HGNC ID = HGNC:4764) highlights the problems associated with the coexistence

Table 5. Advantages and disadvantages of different methods for assessing histone alterations.

Method	Advantage	Disadvantage
Immunohistochemistry using sequence variant-specific antibodies	1. Fast 2. Inexpensive 3. Information provided on spatial distribution and cell-type-associated presence of sequence alteration 4. Single cells with sequence alterations can be detected 5. Presence of the protein product allows diagnostic statements in small samples, where the amount or quality of the material could potentially be insufficient for genetic testing, particularly with multiple genes to be involved	1. Only protein products with similar core amino acid sequence can be detected, whereas unknown sequence alterations are missed 2. No information on the specific gene that is altered, in cases where the same protein is encoded by more than a single gene, which might be prognostically relevant 3. The tissue can only be investigated for a limited number of sequence alterations per section 4. The ability to detect sequence variants by immunohistochemistry might be compromised in poor-quality tissue samples, leading to false-negative results
Genetic analyses	1. Information on specific sequence alterations and gene involvement that might be prognostically relevant 2. Rare or even unknown sequence alterations can be detected, which potentially provides the basis for the development of new tumour entity-specific antibodies 3. All potential alterations in the analysed genes can be detected 4. Direct information whether DNA/RNA quality is sufficient/insufficient, limiting false results based on tissue quality	1. Time-consuming 2. More expensive than immunohistochemistry 3. Only limited spatial resolution of sequence alterations is possible 4. A sufficient number of cells harbouring the sequence alteration are needed 5. Only genes specifically analysed by the method are investigated

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of two distinct nomenclatures and proposes a standard for the reporting of histone sequence variants that allows an unambiguous description of these variants according to HGVS principles. Here, the gene, DNA, derived protein sequence, and legacy (antibody) sequence are given to account for the inconsistency in numbering resulting from the assumed post-translational modification of the protein. We hope that scientific journals will adopt the new notation, and that both geneticists and pathologists will find it helpful to include it in their reports.

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Author contributions statement

HL and IC conceived the manuscript based on discussions during publication of the 5th Edition of the WHO Classification of Tumours. HL and RD wrote the manuscript with input from IC, AL and GR.

References

1. den Dunnen JT, Dalgleish R, Maglott DR, *et al*. HGVS recommendations for the description of sequence variants: 2016 update. *Hum Mutat* 2016; **37:** 564–569.
2. Deans ZC, Fairley JA, den Dunnen JT, *et al*. HGVS nomenclature in practice: an example from the United Kingdom National External Quality Assessment Scheme. *Hum Mutat* 2016; **37:** 576–578.
3. Ogino S, Gulley ML, den Dunnen JT, *et al*. Standard mutation nomenclature in molecular diagnostics: practical and educational challenges. *J Mol Diagn* 2007; **9:** 1–6.
4. den Dunnen JT, Paalman MH. Standardizing mutation nomenclature: why bother? *Hum Mutat* 2003; **22:** 181–182.
5. Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17:** 405–424.
6. Li MM, Datto M, Duncavage EJ, *et al*. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017; **19:** 4–23.
7. Maze I, Noh KM, Soshnev AA, *et al*. Every amino acid matters: essential contributions of histone variants to mammalian development and disease. *Nat Rev Genet* 2014; **15:** 259–271.
8. Scott WA, Campos EI. Interactions with histone H3 & tools to study them. *Front Cell Dev Biol* 2020; **8:** 701.
9. Venkatesh S, Workman JL. Histone exchange, chromatin structure and the regulation of transcription. *Nat Rev Mol Cell Biol* 2015; **16:** 178–189.
10. Urahama T, Harada A, Maehara K, *et al*. Histone H3.5 forms an unstable nucleosome and accumulates around transcription start sites in human testis. *Epigenetics Chromatin* 2016; **9:** 2.
11. DeLange RJ, Hooper JA, Smith EL. Complete amino-acid sequence of calf-thymus histone 3. *Proc Natl Acad Sci U S A* 1972; **69:** 882–884.
12. DeLange RJ, Fambrough DM, Smith EL, *et al*. Calf and pea histone IV. 3. Complete amino acid sequence of pea seedling histone IV; comparison with the homologous calf thymus histone. *J Biol Chem* 1969; **244:** 5669–5679.
13. Iwai K, Ishikawa K, Hayashi H. Amino-acid sequence of slightly lysine-rich histone. *Nature* 1970; **226:** 1056–1058.
14. Turner BM. Reading signals on the nucleosome with a new nomenclature for modified histones. *Nat Struct Mol Biol* 2005; **12:** 110–112.
15. Leske H, Rushing E, Budka H, *et al*. K27/G34 versus K28/G35 in histone H3-mutant gliomas: a note of caution. *Acta Neuropathol* 2018; **136:** 175–176.
16. Sayers EW, Beck J, Brister JR, *et al*. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 2020; **48:** D9–D16.
17. Cree IA, Deans Z, Ligtenberg MJ, *et al*. Guidance for laboratories performing molecular pathology for cancer patients. *J Clin Pathol* 2014; **67:** 923–931.
18. Deans ZC, Costa JL, Cree I, *et al*. Integration of next-generation sequencing in clinical diagnostic molecular pathology laboratories for analysis of solid tumours; an expert opinion on behalf of IQN Path ASBL. *Virchows Arch* 2017; **470:** 5–20.
19. Bruford EA, Braschi B, Denny P, *et al*. Guidelines for human gene nomenclature. *Nat Genet* 2020; **52:** 754–758.
20. Freeman PJ, Hart RK, Gretton LJ, *et al*. VariantValidator: accurate validation, mapping, and formatting of sequence variation descriptions. *Hum Mutat* 2018; **39:** 61–68.
21. Moher D, Liberati A, Tetzlaff J, *et al*. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6:** e1000097.
22. Toledo RA, Qin Y, Cheng ZM, *et al*. Recurrent mutations of chromatin-remodeling genes and kinase receptors in pheochromocytomas and paragangliomas. *Clin Cancer Res* 2016; **22:** 2301–2310.
23. Behjati S, Tarpey PS, Presneau N, *et al*. Distinct *H3F3A* and *H3F3B* driver mutations define chondroblastoma and giant cell tumor of bone. *Nat Genet* 2013; **45:** 1479–1482.
24. Castel D, Philippe C, Calmon R, *et al*. Histone *H3F3A* and *HIST1H3B* K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathol* 2015; **130:** 815–827.
25. Schwartzentruber J, Korshunov A, Liu XY, *et al*. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* 2012; **482:** 226–231.
26. Righi A, Mancini I, Gambarotti M, *et al*. Histone 3.3 mutations in giant cell tumor and giant cell-rich sarcomas of bone. *Hum Pathol* 2017; **68:** 128–135.
27. Mackay A, Burford A, Molinari V, *et al*. Molecular, pathological, radiological, and immune profiling of non-brainstem pediatric high-grade glioma from the HERBY phase II randomized trial. *Cancer Cell* 2018; **33:** 829–842.e5.
28. Yoshida KI, Nakano Y, Honda-Kitahara M, *et al*. Absence of *H3F3A* mutation in a subset of malignant giant cell tumor of bone. *Mod Pathol* 2019; **32:** 1751–1761.

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29. Mackay A, Burford A, Carvalho D, *et al*. Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. *Cancer Cell* 2017; **32:** 520–537.e5.
30. Yoshimoto K, Hatae R, Sangatsuda Y, *et al*. Prevalence and clinicopathological features of H3.3 G34-mutant high-grade gliomas: a retrospective study of 411 consecutive glioma cases in a single institution. *Brain Tumor Pathol* 2017; **34:** 103–112.
31. Pathak P, Jha P, Purkait S, *et al*. Altered global histone-trimethylation code and H3F3A-ATRX mutation in pediatric GBM. *J Neurooncol* 2015; **121:** 489–497.
32. Zehir A, Benayed R, Shah RH, *et al*. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 2017; **23:** 703–713.
33. Martin D, Abba MC, Molinolo AA, *et al*. The head and neck cancer cell oncogenome: a platform for the development of precision molecular therapies. *Oncotarget* 2014; **5:** 8906–8923.
34. Nacev BA, Feng L, Bagert JD, *et al*. The expanding landscape of 'oncohistone' mutations in human cancers. *Nature* 2019; **567:** 473–478.
35. Lim J, Park JH, Baude A, *et al*. The histone variant H3.3 G34W substitution in giant cell tumor of the bone link chromatin and RNA processing. *Sci Rep* 2017; **7:** 13459.
36. Koelsche C, Schrimpf D, Tharun L, *et al*. Histone 3.3 hotspot mutations in conventional osteosarcomas: a comprehensive clinical and molecular characterization of six H3F3A mutated cases. *Clin Sarcoma Res* 2017; **7:** 9.
37. Mehnert JM, Panda A, Zhong H, *et al*. Immune activation and response to pembrolizumab in *POLE*-mutant endometrial cancer. *J Clin Invest* 2016; **126:** 2334–2340.
38. Wagner AH, Devarakonda S, Skidmore ZL, *et al*. Recurrent WNT pathway alterations are frequent in relapsed small cell lung cancer. *Nat Commun* 2018; **9:** 3787.
39. Kasper LH, Baker SJ. Invited review: emerging functions of histone H3 mutations in paediatric diffuse high-grade gliomas. *Neuropathol Appl Neurobiol* 2020; **46:** 73–85.
40. Roux A, Pallud J, Saffroy R, *et al*. High-grade gliomas in adolescents and young adults highlight histomolecular differences with their adult and paediatric counterparts. *Neuro Oncol* 2020; **22:** 1190–1202.
41. Meyronet D, Esteban-Mader M, Bonnet C, *et al*. Characteristics of H3 K27M-mutant gliomas in adults. *Neuro Oncol* 2017; **19:** 1127–1134.
42. Wu G, Broniscer A, McEachron TA, *et al*. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet* 2012; **44:** 251–253.
43. Hochart A, Escande F, Rocourt N, *et al*. Long survival in a child with a mutated K27M-H3.3 pilocytic astrocytoma. *Ann Clin Transl Neurol* 2015; **2:** 439–443.
44. Kleinschmidt-DeMasters BK, Donson A, Foreman NK, *et al*. H3 K27M mutation in gangliogliomas can be associated with poor prognosis. *Brain Pathol* 2017; **27:** 846–850.
45. Pagès M, Beccaria K, Boddaert N, *et al*. Co-occurrence of histone H3 K27M and BRAF V600E mutations in paediatric midline grade I ganglioglioma. *Brain Pathol* 2018; **28:** 103–111.
46. Andreiuolo F, Lisner T, Zlocha J, *et al*. H3F3A-G34R mutant high grade neuroepithelial neoplasms with glial and dysplastic ganglion cell components. *Acta Neuropathol Commun* 2019; **7:** 78.
47. Hübner JM, Müller T, Papageorgiou DN, *et al*. EZHIP/CXorf67 mimics K27M mutated oncohistones and functions as an intrinsic inhibitor of PRC2 function in aggressive posterior fossa ependymoma. *Neuro Oncol* 2019; **21:** 878–889.
48. Ryall S, Guzman M, Elbabaa SK, *et al*. H3 K27M mutations are extremely rare in posterior fossa group A ependymoma. *Childs Nerv Syst* 2017; **33:** 1047–1051.
49. Pratt D, Natarajan SK, Banda A, *et al*. Circumscribed/non-diffuse histology confers a better prognosis in H3K27M-mutant gliomas. *Acta Neuropathol* 2018; **135:** 299–301.
50. Pajtler KW, Witt H, Sill M, *et al*. Molecular classification of ependymal tumors across all CNS compartments, histopathological grades, and age groups. *Cancer Cell* 2015; **27:** 728–743.
51. Yao K, Duan Z, Wang Y, *et al*. Detection of H3K27M mutation in cases of brain stem subependymoma. *Hum Pathol* 2019; **84:** 262–269.
52. Jungwirth G, Warta R, Beynon C, *et al*. Intraventricular meningiomas frequently harbor NF2 mutations but lack common genetic alterations in TRAF7, AKT1, SMO, KLF4, PIK3CA, and TERT. *Acta Neuropathol Commun* 2019; **7:** 140.
53. Papillon-Cavanagh S, Lu C, Gayden T, *et al*. Impaired H3K36 methylation defines a subset of head and neck squamous cell carcinomas. *Nat Genet* 2017; **49:** 180–185.
54. Lohr JG, Stojanov P, Lawrence MS, *et al*. Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. *Proc Natl Acad Sci U S A* 2012; **109:** 3879–3884.
55. Huether R, Dong L, Chen X, *et al*. The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. *Nat Commun* 2014; **5:** 3630.
56. Collord G, Martincorena I, Young MD, *et al*. Recurrent histone mutations in T-cell acute lymphoblastic leukaemia. *Br J Haematol* 2019; **184:** 676–679.
57. Okosun J, Bödör C, Wang J, *et al*. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. *Nat Genet* 2014; **46:** 176–181.
58. Presneau N, Baumhoer D, Behjati S, *et al*. Diagnostic value of *H3F3A* mutations in giant cell tumour of bone compared to osteoclast-rich mimics. *J Pathol Clin Res* 2015; **1:** 113–123.
59. Castel D, Kergrohen T, Tauziède-Espariat A, *et al*. Histone H3 wild-type DIPG/DMG overexpressing *EZHIP* extend the spectrum diffuse midline gliomas with PRC2 inhibition beyond H3-K27M mutation. *Acta Neuropathol* 2020; **139:** 1109–1113.
60. Castel D, Philippe C, Kergrohen T, *et al*. Transcriptomic and epigenetic profiling of 'diffuse midline gliomas, H3 K27M-mutant' discriminate two subgroups based on the type of histone H3 mutated and not supratentorial or infratentorial location. *Acta Neuropathol Commun* 2018; **6:** 117.
61. Hoffman LM, Veldhuijzen van Zanten SEM, Colditz N, *et al*. Clinical, radiologic, pathologic, and molecular characteristics of long-term survivors of diffuse intrinsic pontine glioma (DIPG): a collaborative report from the International and European Society for Pediatric Oncology DIPG Registries. *J Clin Oncol* 2018; **36:** 1963–1972.
62. Zhou P, Wu E, Alam HB, *et al*. Histone cleavage as a mechanism for epigenetic regulation: current insights and perspectives. *Curr Mol Med* 2014; **14:** 1164–1172.
63. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144:** 646–674.
64. Vossaert L, Meert P, Scheerlinck E, *et al*. Identification of histone H3 clipping activity in human embryonic stem cells. *Stem Cell Res* 2014; **13:** 123–134.
65. Bailey AO, Panchenko T, Sathyan KM, *et al*. Posttranslational modification of CENP-A influences the conformation of centromeric chromatin. *Proc Natl Acad Sci U S A* 2013; **110:** 11827–11832.
66. Matsuda K. PCR-based detection methods for single-nucleotide polymorphism or mutation: real-time PCR and its substantial contribution toward technological refinement. *Adv Clin Chem* 2017; **80:** 45–72.
67. Tanzima Nuhat S, Sakata-Yanagimoto M, Komori D, *et al*. Droplet digital polymerase chain reaction assay and peptide nucleic acid-locked nucleic acid clamp method for *RHOA* mutation detection in angioimmunoblastic T-cell lymphoma. *Cancer Sci* 2018; **109:** 1682–1689.
68. Schaefer IM, Fletcher JA, Nielsen GP, *et al*. Immunohistochemistry for histone H3G34W and H3K36M is highly specific for giant cell tumor of bone and chondroblastoma, respectively, in FNA and core needle biopsy. *Cancer Cytopathol* 2018; **126:** 552–566.

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SUPPLEMENTARY MATERIAL ONLINE

Figure S1. Alignment of the coding sequence (CDS) of the reference transcript sequences NM_002107.7 and NM_005324.5 for the histone genes *H3-3A* and *H3-3B*, respectively, with the respective identical amino acid reference sequences NP_002098.1 and NP_005315.1 of histone protein H3.3

Table S1. Tabulated results of tumours listed in COSMIC for *H3F3A* sequence variants

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