Mutational landscape of marginal zone B-cell lymphomas of various origin: organotypic alterations and diagnostic potential for assignment of organ origin

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Abstract
This meta-analysis aims to concisely summarize the genetic landscape of splenic, nodal and extranodal marginal zone lymphomas (MZL) in the dura mater, salivary glands, thyroid, ocular adnexa, lung, stomach and skin with respect to somatic variants. A systematic PubMed search for sequencing studies of MZL was executed. All somatic mutations of the organs mentioned above were combined, uniformly annotated, and a dataset containing 25 publications comprising 6016 variants from 1663 patients was created. In splenic MZL, KLF2 (18%, 103/567) and NOTCH2 (16%, 118/725) were the most frequently mutated genes. Pulmonary and nodal MZL displayed recurrent mutations in chromatin-modifier-encoding genes, especially KMT2D (25%, 13/51, and 20%, 20/98, respectively). In contrast, ocular adnexal, gastric, and dura mater MZL had mutations in genes encoding for NF-κB pathway compounds, in particular TNFAIP3, with 39% (113/293), 15% (8/55), and 45% (5/11), respectively. Cutaneous MZL frequently had FAS mutations (63%, 24/38), while MZL of the thyroid had a higher prevalence for TET2 variants (61%, 11/18). Finally, TBL1XR1 (24%, 14/58) was the most commonly mutated gene in MZL of the salivary glands. Mutations of distinct genes show origin-preferential distribution among nodal and splenic MZL as well as extranodal MZL at/from different anatomic locations. Recognition of such mutational distribution patterns may help assigning MZL origin in difficult cases and possibly pave the way for novel more tailored treatment concepts.

Keywords Marginal zone lymphoma · Meta-analysis · FAS · KLF2 · NF-κB · TET2

Introduction
Marginal zone lymphomas (MZL) represent 7–8% [1, 2] of all lymphoid neoplasms. The World Health Organization (WHO) [3] subdivides MZL into three distinct entities: splenic MZL (SMZL), nodal MZL (NMZL), and extranodal MZL (EMZL) [2]. The organs most commonly affected by EMZL are the stomach (70%), followed by the lungs (14%), ocular adnexa (12%), thyroid (4%), and the small intestine (1%) [4], while for salivary glands, dura mater, and cutaneous MZL, no incidence data is available [5]. The median age of MZL presentation is 60 years, with a higher proportion of females affected [6]. MZL are mostly indolent with a 5-year overall survival (OS) rate of 85% [6].

There is evidence that some EMZL are associated with and dependent on chronic antigenic stimulation, either by autoantigens or by foreign pathogens, especially bacteria, that lead to accumulation of secondary mucosa-associated lymphoid tissue (MALT) in respective organs due to chronic inflammation, with this MALT serving as soil for neoplastic outgrowth [5]. Infectious agents that have been found to be associated with EMZL are, e.g., Helicobacter pylori and Helicobacter heilmannii in the stomach, Achrombacter xylosoxidans in the lung, Chlamyphila psittaci in the ocular adnexa, and Borrelia burgdorferi in the skin. Moreover, autoimmune diseases such as Sjögren syndrome and Hashimoto thyroiditis predispose to the development of EMZL [7] (Suppl. Table 1). There is a useful, practical aspect in this consideration: since most EMZL retain their dependence on the respective antigenic stimulation, they may regress upon removal of the antigen, e.g., by antibiotics or by modulation of T-/B-cell interactions by immunomodulatory drugs, even in disseminated disease [8–10].
Compared to other mature small B-cell lymphomas, MZL does not display a disease-defining phenotype. Thus, at occasions, the diagnostic borders among each other, i.e., SMZL, NMZL, and EMZL, as well as within EMZL of various organ origin, and to other small B-cell lymphomas without a defined phenotype are blurred [11, 12].

The pathogenesis of EMZL is linked to several recurrent numerical and structural chromosomal aberrations, i.e., trisomies and chromosomal translocations. Trisomies of chromosomes 3, 12, and 18 are found in 20–30% of EMZL [7]. One of the most common translocations in EMZL, t(11;18)(q21;q21), leads to the fusion of BIRC3 to MALT1. It is tightly linked to EMZL of the lung, and occurs in as much as 45% of cases, followed by the stomach (23%) and the intestine (19%) [7]. Further, this BIRC3/MALT1 fusion is specific for EMZL, since it is not reported in SMZL or NMZL [7]. On the other hand, partial deletion of the long arm of chromosome 7, del(7)(q31), is found exclusively in SMZL and may even be a biomarker of more aggressive behavior [13, 14]. Another common chromosomal translocation in MZL is t(3;14)(p14;q32) leading to IGH-FOXP1 rearrangement [7]. Suppl. Table 2 summarizes organotypic chromosomal rearrangements in various MZL.

In the last decade, the genomic landscape of MZL has been extensively studied. With a few exceptions, there seems to be considerable overlap between mutated genes across the various MZL entities and subentities and sites of origin, but this has not yet been integratively analyzed, and being a rare tumor, MZL is still not included in databases such as the International Cancer Genome Consortium (IGGC) and the Cancer Genome Atlas (TCGA).

To address these shortcomings, we performed a meta-analysis of 25 carefully selected PubMed-listed publications reporting on somatic mutations in MZL of various origins, and report here the results of identified variants with consistent and detailed annotation. Whole-genome (WGS), whole exome (WES), targeted high-throughput sequencing (HTS) analysis, and/or Sanger sequencing were read-out methods in these studies.

Materials and methods

Literature search

We performed a literature search in October 2020 using PubMed [15] as the primary source. The keywords used and literature research results are detailed in Supplementary Fig. 1. Only studies explicitly stating that cases included had been reviewed and confirmed by staff pathologists were considered.

Data extraction and annotation

Genomic information was extracted from the supplementary materials of the selected studies and uniformed to the GRCh38-hg38 genome by applying LiftOver - UCSC Genome Browser [15]. The missing information on variants such as genomic location and reference sequence variant effect annotation was obtained with the variant effect predictor (VEP) by Ensemble [15] and Annovar software [16] (Fig. 1).

Meta-analysis of mutated gene frequencies

The number of mutated and unmutated cases was retrieved and the frequencies of mutations per gene was calculated (Suppl. Table 3). Given the main focus of the current study, namely to assess whether somatic nucleotide variants may be of diagnostic importance, a shortlist was generated for mutated genes with a mutational frequency of > 7.5% in at least one entity (Suppl. Table 4).

Due to format incompatibility and insufficient details, the supplementary list of the study by van den Brand et al. [17] was only used for frequency calculation and not further included. Seven patients from the study of Cascione et al. [18] and 14 from the study of Moody et al. [19] were excluded due to unspecified site of origin.

Statistical analysis

All statistical calculations were executed with MS Excel or R statistical packages and Statistical Package of Social Sciences (IBM SPSS version 22.0, Chicago, IL, USA) for Windows. Differences of mutational frequencies between EMZL, NMZL, and SMZL entities, as well as between EMZL subentities, were compared using the two-tailed Fisher’s exact test (Suppl. Table 5, Suppl. Fig. 3). The statistical significance threshold was corrected for multiple testing and was set at \( p < 0.017 \).

Results

Filtering of literature, sequencing techniques, and patient characterization

After removing duplicate entries, 1602 of 3088 manuscripts were considered unique. After selection based on the criteria detailed above, 142 manuscripts remained for further analysis. Next, all manuscripts and their supplementary data were studied to ensure they reported a full list of variants with appropriate sample information and genetic coordinates. At the end, 25 studies were selected; 3 studies implemented WGS comprising 22 cases, 10 studies applied WES in 111 patients, 2 studies applied Sanger sequencing in 185 probands and 23
studies screened 1434 patients utilizing targeted HTS (Suppl. Table 6); several studies utilized a mix of sequencing strategies. Either formalin-fixed paraffin-embedded (FFPE; n = 1327) tissues or/and fresh frozen (FF; n = 478) tissues were examined (Fig. 2, Suppl. Table 7).

**Dataset collation and cohort description**

Six thousand sixteen variants in 2553 genes of 1663 cases (Fig. 2) were extracted (Suppl. Table 8). With 13 studies, SMZL was the most comprehensively investigated entity and encompassed 58% of cases in the total cohort, whereas dural (DMZL) and cutaneous MZL (CMZL) accounted for only 3% each, and data was extracted from one publication each per these two respective sites/organs of origin (Fig. 2, Suppl. Fig. 1). Most MZL studies applied NGS-based techniques, only 2 studies on SMZL investigated cases by Sanger sequencing (Suppl. Fig. 2). Table 1 summarizes mutation frequencies per site and per case. Mutations numbers ranged between 1.8 and 27 per case being highest in NMZL. In all entities, single nucleotide variants (SNV) were the most common mutational type. Mutational frequencies in MZL of different entities are represented in Figs. 3, 4, and 5. The statistical comparison results of mutational frequencies by Fisher’s exact test can be found in the Supplementary Table 5.

**Mutational profile of SMZL**

Thirteen SMZL studies [20–32] consistently showed that KLF2 was the most widely mutated gene (18%, 103/567; rather unique for this sub-entity), followed by NOTCH2 (16%, 118/724) and TP53 (12%, 59/493) (Figs. 3, 4, and 5). SMZL showed a higher prevalence of KLF2 and, to a marginal extent, of NOTCH2 mutations compared to EMZL (4%, 4/90, p = 5.73E–04, and 9%, 16/169, p = 2.33E–02, respectively). TP53 was slightly more often mutated in SMZL compared to NMZL (3%, 2/68, p = 2.15E–02) and considerably to EMZL (4%, 11/279, p = 1.26E–04) (Suppl. Table 5, Suppl. Fig. 3A).

**Mutational profile of NMZL**

In four NMZL studies [17, 20, 21, 33], KMT2D was reportedly the most frequently mutated gene (20%, 20/98). Genes that were second most commonly mutated, with a frequency of 10%, include LRP1B (5/51), TET2 (5/51), and TNFRSF14 (10/98). These were followed by BRAF (4/51), EZH2 (4/51), and HIST1H1E (8/98), with a frequency of 8% each (Figs. 3, 4, and 5). KMT2D was more commonly mutated in NMZL (20%, 20/98) than in SMZL (7%, 28/404, p = 1.80E–04). LRP1B was more frequently mutated in NMZL (10%, 5/51) compared to SMZL (1%, 4/484, p = 6.12E–04). NMZL showed a higher prevalence of TNFRSF14 mutations (10%, 10/98) compared to SMZL (2%, 6/286, p = 1.55E–03). Moreover, we could demonstrate near exclusivity of BRAF (8%, 4/51) mutations in NMZL, which reached statistical significance compared to SMZL (1%, 2/301, p = 4.74E–03).
EZH2 mutations also appeared more frequently in NMZL (8%, 4/51) than in SMZL (1%, 2/265, \(p = 7.12 \times 10^{-3}\)). Lastly, the HIST1H1E mutational rate in NMZL (8%, 8/98) exceeded that in SMZL (2%, 3/188, \(p = 9.35 \times 10^{-3}\)). There was no statistical difference between the mutational profiles of NMZL and EMZL (Suppl. Table 5, Suppl. Fig. 3A).

Mutational profile of EMZL

Due to the differing cohort numbers, mutational rates are more difficult to describe in EMZL. Ten EMZL [18, 19, 33–40] studies extensively looked for TNFAIP3 and TBL1XRI mutations and detected 140/500 and 66/515 mutant cases, respectively. A total of 29 cases with NOTCH1 mutations was found in 324 samples, while 14 cases with KMT2C mutations were identified in 135 studied instances. Only three studies [33–35] explored FAS mutations, which were detected in 26/68 patients. Other genes detected in EMZL studies include PALB2 (2/11), JAK3 (11/122), HIST1H1D (2/23), and PTEN (4/47).

TNFAIP3 mutations were considerably more detectable in EMZL (28%, 140/500) compared to NMZL (14%, 12/88, \(p = 3.63 \times 10^{-3}\)) and SMZL (8%, 52/628, \(p = 2.21 \times 10^{-18}\)). EMZL displayed a high rate of TBLIXRI mutations (13%, 66/515), sequencing (HTS) \(n = 1434\)); twenty-one HTS samples are from an unspecified organ of origin; the different types of tissue source, formalin-fixed paraffin-embedded (FFPE) tissue \(n = 1327\) or fresh frozen (FF) tissue \(n = 478\), are given for each organ/site

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Comparing mutational frequencies of EMZL occurring in different locations, several important differences could be demonstrated:

Two thyroid MZL (TMZL) studies [18, 19] showed a high prevalence of TET2 mutations (61%, 11/18), which statistically significantly exceeded that in salivary gland.
| MZL type | Number of cases | Frequency of cases with mutations | Mean mutations per case* | Mean mutated genes per case* | Types of mutation | Most frequently mutated genes | Most frequently mutated pathways |
|----------|----------------|----------------------------------|--------------------------|----------------------------|------------------|-------------------------------|---------------------------------|
| DMZL     | 11             | 100%                             | 7.2                      | 6.5                        | Missense 72%     | TNFAIP3 45%                   | Chromatin modifiers 73%         |
|          |                |                                  |                          |                            | Frameshift del/ins 10% | NOTCH2 36%                  | NF-κB 63%                       |
|          |                |                                  |                          |                            | Nonsense 8%       | TLX1R1 36%                   | NOTCH 45%                      |
|          |                |                                  |                          |                            | Intronic/Intergenic 6% | EP300 18%                  |                                 |
|          |                |                                  |                          |                            | Splicing site mutation 3% | KLLH6 18%                 |                                 |
|          |                |                                  |                          |                            | Nonframeshift del/ins 1% |                            |                                 |
| OMZL     | 362            | 67%                              | 2.5                      | 1.78                       | Missense 59%     | TNFAIP3 39%                   | NF-κB 64%                       |
|          |                |                                  |                          |                            | Nonsense 18%      | KMT2D 15%                    | Chromatin modifiers 34%         |
|          |                |                                  |                          |                            | Frameshift del/ins 15% | CREBBP 10%                  | NOTCH 25%                      |
|          |                |                                  |                          |                            | Splicing site mutation 4% | LRP1B 10%                  |                                 |
|          |                |                                  |                          |                            | Nonframeshift del/ins 2% | MYD88 10%                  |                                 |
| SAMZL    | 71             | 70%                              | 2.7                      | 2.3                        | Missense 67%     | TBL1XRI 24%                   | NOTCH 44%                       |
|          |                |                                  |                          |                            | Nonframeshift del/ins 17% | GPR34 16%                  | Chromatin modifiers 32%         |
|          |                |                                  |                          |                            | Nonsense 13%      | NOTCH 2 11%                  | NF-κB 28%                      |
|          |                |                                  |                          |                            | Splicing site mutation 2% | SPEN 11%                   |                                 |
|          |                |                                  |                          |                            | Frameshift del/ins 1% | KMT2C 11%                   |                                 |
|          |                |                                  |                          |                            | Intronic/Intergenic 0% |                            |                                 |
| TMZL     | 18             | 83%                              | 4                        | 3.1                        | Missense 60%     | TET2 61%                      | Chromatin modifiers 73%         |
|          |                |                                  |                          |                            | Nonsense 12%      | TNFRSF14 44%                 | NF-κB 20%                       |
|          |                |                                  |                          |                            | Splicing site mutation 12% | PIK3CD 23%                  | NOTCH 20%                      |
|          |                |                                  |                          |                            | Nonframeshift del/ins 1% | SPEN 17%                   |                                 |
|          |                |                                  |                          |                            | Intronic/Intergenic 0% | CREBBP 8%                   |                                 |
| PMZL     | 64             | 70%                              | 2.6                      | 2.6                        | Missense 72%     | KMT2D 25%                     | Chromatin modifiers 74%         |
|          |                |                                  |                          |                            | Nonsense 16%      | TNFAIP3 18%                  | NF-κB 42%                       |
|          |                |                                  |                          |                            | Frameshift del/ins 8% | PRDM1 12%                    | NOTCH 30%                      |
|          |                |                                  |                          |                            | Nonframeshift del/ins 2% | NOTCH1 12%                 |                                 |
|          |                |                                  |                          |                            | Splicing site mutation 2% | EP300 11%                  |                                 |
|          |                |                                  |                          |                            | Intronic/Intergenic 0% |                            |                                 |
| GMZL     | 59             | 64%                              | 5.1                      | 4.4                        | Missense 74%     | NOTCH1 17%                    | NF-κB 61%                       |
|          |                |                                  |                          |                            | Framedel/ins 12%  | NFI 16%                      | Chromatin modifiers 55%         |
|          |                |                                  |                          |                            | Nonsense 8%       | TNFAIP3 15%                  | NOTCH 42%                      |
|          |                |                                  |                          |                            | Splicing site mutation 5% | TRAF3 13%                  |                                 |
|          |                |                                  |                          |                            | Nonframeshift del/ins 1% | ATM 13%                    |                                 |
|          |                |                                  |                          |                            | Intronic/Intergenic 0% |                            |                                 |
| NMZL     | 118            | 75%                              | 29                       | 27                         | Missense 75%     | KMT2D 20%                     | Chromatin modifiers 70%         |
|          |                |                                  |                          |                            | Nonsense 8%       | TNFAIP3 14%                  | NOTCH 53%                      |
|          |                |                                  |                          |                            | Frameshift del/ins 7% | CREBBP 12%                  | NF-κB 45%                      |
|          |                |                                  |                          |                            | Nonframeshift del/ins 5% | FAS 12%                    |                                 |
|          |                |                                  |                          |                            | Splicing site mutation 4% | KLF2 12%                   |                                 |
|          |                |                                  |                          |                            | Intronic/Intergenic 1% |                            |                                 |
| SMZL     | 922            | 53%                              | 5.8                      | 5.9                        | Missense 77%     | KLF2 18%                      | NOTCH 53%                       |
|          |                |                                  |                          |                            | Frameshift del/ins 10% | NOTCH2 16%                 | Chromatin modifiers 43%         |
MZL (SAMZL), gastric MZL (GMZL), pulmonary MZL (PMZL), and ocular adnexal MZL (OMZL).

In the two studies with available information on sub-localization of the OMZL (conjunctival versus periorbital) [37, 38], total numbers of mutations in conjunctival OMZL were higher than in periorbital OMZL (median 2 versus 1; mean 2.38 versus 1.56, range 0–9 versus 0–5; \( p = 0.028 \)).

**TBL1XR1** mutations were enriched in conjunctival OMZL (8/27 versus 1/17, \( p = 4.63 \times 10^{-2} \) [37]; 7/22 versus 0/12, \( p = 0.095 \) [38]).

Compared to other MZL, **FAS** (63%, 24/38) was the most frequently mutated gene in CMZL [35] (Figs. 3, 4, and 5). These characteristic FAS mutations were substantially linked to CMZL compared to GMZL and DMZL, displaying such mutations in 5% (1/19, \( p = 3.58 \times 10^{-5} \)) and 9% (1/11, \( p = 1.92 \times 10^{-3} \)) of cases, respectively. Compared to all other MZL, CMZL also showed the highest proportion of splice-site mutations.

A detailed comparison of mutations of EMZL of various sites can be found in the supplementary files.

**Preferred activation of the NOTCH pathway and NF-κB pathway by mutations across different MZL entities**

Mutations related to the NOTCH pathway, NF-κB signaling pathway and in genes encoding for chromatin modifiers were grouped and analyzed regarding their role in different MZL. We could observe that mutations related to the NOTCH pathway were rather mutually exclusive to mutations of genes playing a role in the NF-κB pathway and to chromatin modifier-encoding genes. In MZL containing sufficient information density (adequate coverage of genes related to these pathways) to address this issue, 140 cases displayed mutations in both the NF-κB and NOTCH pathway, while 553 cases bore mutations exclusively of genes affecting either pathway, and 242 cases were unmutated, suggesting a nonrandom mutual exclusivity (\( p = 1 \times 10^{-9} \)). Analyzing the different entities separately, statistically significant differences in that consideration were observable in SMZL (\( p = 4 \times 10^{-8} \)) and OMZL (\( p = 8 \times 10^{-3} \)), and as a trend in GMZL. Regarding chromatin modifiers, 207 cases displayed mutual mutations in the NOTCH pathway, while 407 cases bore mutations exclusively of genes affecting either cellular process (\( p = 1 \times 10^{-3} \)). This applied to SMZL (\( p = 1 \times 10^{-3} \)) and OMZL (\( p = 7 \times 10^{-3} \)), and as a trend to SAMZL.

**Concordance between three NMZL WES studies**

An additional aim of our study was to perform an unbiased analysis of the genomic landscape of MZL derived from WES as well as targeted HTS to provide an estimation of the overlap of various mutational frequencies of different protein-coding
genes. To examine the concordance between studies, we compared WES data of three NMZL studies (Suppl. Fig. 5) [20, 21, 41]. A total of 34 samples sequenced by WES, accounting for 1593 variants, were included in the final list. Similar to a previous report [42] that addressed this concordance in SMZL, our analysis showed a very limited concordance across all three NMZL studies, with only 11 overlapping genes in all three studies.

**Discussion**

Our knowledge about the genetic landscape of MZL has increased with the application of new sequencing techniques. However, separate study cohorts, usually derived from archives of one institution, are still limited in size and mutational profiles have been obtained applying different methods. As a result, a general overview of the mutational landscape across all MZL subtypes is lacking. We aimed to perform a comparative meta-analysis of reported genetic variants in various MZL subtypes to address the question of site/organ-of-origin-specific differences.

Some entities displayed similar mutational profiles. These comprise OMZL, PMZL, GMZL, and DMZL, which all showed recurrent TNFAIP3 mutations and high concordant mutational rates in genes encoding for other compounds of the NF-κB pathway; TNFAIP3 inhibits NF-κB activation by exerting dual ubiquitin-editing functions [43], thus inactivating mutations of TNFAIP3 provide an advantage to the cells via activating NF-κB-related signaling.

In contrast, some genes were predominantly mutated in distinct MZL of specific organs/sites, including TMZL that showed a high prevalence of TET2 mutations and CMZL, which demonstrated a predominance of FAS mutations. TET2 is involved in epigenetic regulation; like in TNFAIP3, TET2 mutations are generally loss-of-function mutations that result in an inactive protein and, thus, a net general hypermethylated state of the cells [44]. TET2 mutations are commonly seen in myeloid neoplasms, ranging from myelodysplastic and overlap syndromes to acute myeloid leukemias as well as in T-cell lymphomas [45]. In B-cell lymphomas in general, they are rather uncommon. Therefore, it is notable that TET2 mutations occurred in 61% of TMZL, in contrast to all other MZL with TET2 mutation frequencies <
15% (Fig. 5). Thus, \textit{TET2} mutations can be regarded as rather specific for TMZL and might be of diagnostic help in distinguishing TMZL from other EMZL types of the head and neck.

Another gene primarily mutated in TMZL was \textit{TNFRSF14}. TNFRSF14 is a member of the tumor necrosis factor receptor superfamily and has been described in both follicular lymphomas [46] and diffuse large B-cell lymphomas [47]. It is involved in lymphomagenesis since its inactivating mutations lead to increased B-cell receptor dependent signaling and, via its ligand BTLA, to disrupted interaction of lymphoma B-cells with modulatory T-helper cells [48], thus linking lymphomagenesis to disrupted immune cell crosstalk.

\textit{FAS} was most frequently mutated in CMZL (63%) (Fig. 5), with predominantly splice-site mutations. \textit{FAS} belongs to the tumor necrosis factor receptor family and its mutations affect the death domain fostering anti-apoptotic properties leading to disrupted protein function and empowering cancer cells with survival advantages [35, 49]. Indeed, Maurus and colleagues reported that all CMZL patients bearing \textit{FAS} mutations showed at least one cutaneous relapse during 84.5 months, while 50% of patients without \textit{FAS} mutations remained free of disease after therapy [35]. \textit{FAS} splice site mutation render cells insensitive to FAS-mediated apoptotic stimuli [50]. \textit{FAS} mutations were, though rarely, also observed in NMZL and SMZL [20, 21, 32]. Thus, \textit{FAS} mutations can be regarded as rather specific for CMZL and might be of diagnostic help in distinguishing primary CMZL from other EMZL types, and pseudolymphoma of the skin.

There were also some other mutations, which tended to be rather organ/site-specific such as \textit{KLF2} and \textit{TP53} in SMZL, \textit{BRAF} and \textit{PTPRD} in NMZL, \textit{NOTCH1} and \textit{NF1} in GMZL, as well as \textit{TBL1XR1} in MZL of the head and neck region. These mutations could also help to provide a tailored diagnostic and may play a role in distinguishing between entities.

In OMZL, the mutational profile of conjunctival and periorbital cases differs, raising the question whether OMZL of different anatomic sub-sites are, e.g., linked to different etiologies and should generally be further subdivided.

Besides single gene comparisons, we also performed analyses of pathways in order to see whether different types of MZL rely on different intracellular signaling conduits. In the majority of cases, we could show that mutations related to the NOTCH pathway were rather mutually exclusive to mutations in the NF-κB pathway and in chromatin modifier-encoding genes, while the two latter showed overlap. This mutual exclusivity was most prominently seen in SMZL and OMZL,
and to a lesser extent in SAMZL and GMZL. This again underlines the heterogeneity of MZL and might pave the way towards considerations on tailored targeted treatment approaches for distinct subentities.

The comparably low mutation rates in e.g. GMZL or PMZL might be explained by higher rates of translocations in these entities, which activate the NF-κB pathway. Notably, chromosomal translocations may thus play a more important role in molecular differentiation of MZL entities/subentities than nucleotide-level mutations (Suppl. Table 2). Due to methodological restrictions of the last years, mainly the necessity to perform studies based on FISH, which are both labor- and material-intensive, translocations have not been investigated and compared at large scale between different MZL so far, yet older data suggest certain diagnostic potential linked to distinct rearrangements in MZL [51]. The advent of RNA-based sequencing techniques has the potential to overcome these issues in near future [52].

Limited numbers of patients for some entities/subentities and the heterogeneity of the investigated cohorts without consistent clinical data are potential limitations of the present study, along with differences in sequencing strategies and bioinformatic work-up. Also, the nature of the material employed—either FF or FFPE tissue—may have affected the results. Indeed, discrepancies between the results of single observations, especially when comparing WES-based studies, became obvious, as shown in the Venn diagram for NMZL, which revealed a very small overlap (0.7%) of mutated genes found, although considering the large amount of different genes bearing mutations, this was not surprising (Suppl. Fig. 5). In order to tackle these issues, we homogenized the published data using the algorithms provided and normalized data based on reference genome hg38. Regarding the limitations based on the type of material (FFPE vs FF), Pillonel et al. showed for NMZL an excellent linear correlation between results obtained on either material type as it has been also shown for DLBCL [20, 53], suggesting that at least this might not represent a major confounding factor.

Unfortunately, information regarding infectious agents such as Helicobacter pylori (GMZL), Borrelia burgdorferi (CMZL), or Chlamydia psittaci (OMZL) has not been consistently provided to address the interrelations between mutational profiles and infectious etiology with exception of three studies on OMZL, in which all cases were tested negative for Chlamydia psittaci. As the authors of these studies stated in their discussions, infection of OMZL by Chlamydia psittaci seems to have a very distinct geographic distribution. Similarly, no information on autoimmune diseases, especially in SAMZL and TMZL, had been provided in the studies included to address mutational differences in instances arising in an autoimmune background.

To conclude, our meta-analysis was able to identify some unique characteristics of organ/site-specific MZL subtypes. FAS mutations were found to be restricted to CMZL, while TET2 and TNFRSF14 mutations were predominantly found in TMZL. In addition, mutations of KLF2 and TP53 (SMZL), BRAF and PTPRD (NMZL), NOTCH1 and NF1 (GMZL), and TBL1XR1 (MZL of the head and neck region) might help in equivocal instances. Furthermore, TNFAIP3 mutations and mutations affecting the NF-κB pathway in general are commonly found in OMZL, PMZL, GMZL and DMZL. Recognition of such mutational distribution patterns may be of additional help assigning MZL origin in difficult cases and might possibly pave the way for novel tailored treatment concepts.

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**Author contribution** AT, VV and DJ designed the study. VV, DJ, SD, TM, and AT accrued and analyzed the data. VV and TM wrote the manuscript. All authors critically reviewed the manuscript.

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**Data availability** All raw data is supplied in the supplementary files.

**Code availability** Not applicable.

**Declarations**

Ethics approval was obtained from the local ethics committee (applicable to the previously published own studies on NMZL, OMZL and PMZL). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

**Conflict of interest** The authors declare no competing interests.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

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