Germline mutations predisposing to diffuse large B-cell lymphoma

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Genetic studies of diffuse large B-cell lymphomas (DLBCLs) in humans have revealed numerous targets of somatic mutations and an increasing number of potentially relevant germline alterations. The latter often affect genes involved in DNA repair and/or immune function. In general, defects in these genes also predispose to other conditions. Knowledge of these mutations can lead to disease-preventing measures in the patient and relatives thereof. Conceivably, these germline mutations will be taken into account in future therapy of the lymphoma. In other hematological malignancies, mutations originally found as somatic aberrations have also been shown to confer predisposition to these diseases, when occurring in the germline. Further interrogations of the genome in DLBCL patients are therefore expected to reveal additional hereditary predisposition genes. Our review shows that germline mutations have already been described in over one-third of the genes that are somatically mutated in DLBCL. Whether such germline mutations predispose carriers to DLBCL is an open question. Symptoms of the inherited syndromes associated with these genes range from anatomical malformations to intellectual disability, immunodeficiencies and malignancies other than DLBCL. Inherited or de novo alterations in protein-coding and non-coding genes are envisioned to underlie this lymphoma.

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INTRODUCTION

With an age-adjusted incidence of 7 per 100,000 individuals in the United States and 3.8 per 100,000 individuals in Europe, diffuse large B-cell lymphoma (DLBCL) has the highest incidence of any hematological malignancy. Although the prognosis of patients diagnosed with DLBCL has improved considerably by the introduction of immunochemotherapy, still 30–40% of the affected patients will eventually die from this disease.

As holds true for cancer in general, a combination of inherited and environmental factors can lead to the development of DLBCL. These B-cell lymphomas differ from many other cancers because of the role of their cells of origin in the immune system. The malignant transformation of these cells largely relies on the same genetic mechanisms that physiologically optimize their immunoglobulin antigen receptor (V(D)J) gene recombination, class switch recombination and somatic hypermutation. These very dynamic processes require a high-fidelity DNA repair system. No wonder inherited defects in genes involved in DNA damage responses were shown to predispose to this disease (often via incapacitation of appropriate anti-viral, in particular Epstein Barr virus (EBV), responses by simultaneously affecting T cells, which use an identical enzymatic machinery to generate antigen specific T-cell receptors). Presumably together with an underlying defect in the non-homologous end joining (NHEJ) repair pathway, enzymatic activity of RAG1, RAG2 and activation-induced cytidine deaminase (AID) needed for antibody diversification and B-cell antigen receptor refinement can erroneously juxtapose oncogenes (such as BCL2 or MYC) or immune checkpoint genes (such as PD-L1) to immunoglobulin genes. These immunoglobulin gene translocation partners then use the immunoglobulin heavy-chain gene promoter for their overexpression to induce survival and growth or immune escape. AID can, in addition, induce off-target mutations by aberrant somatic hypermutation. The enzyme deaminates cytosine into uracil in single-stranded DNA. Uracil: Guanine mismatches either result in double-strand breaks or mutations. How AID is targeted to DNA outside of the Ig-loci is not completely resolved. The enzyme may be misguided to super-enhancer sequences and non-coding RNAs appear to be essential for the recognition of target DNA motifs. Chronic antigenic stimulation can also promote genomic instability in rapidly dividing B cells and AID-dependent lymphoma. Antibodies, but perhaps also other antigen receptors, may diversify under these circumstances beyond the classical V(D)J recombination via interchromosomal DNA insertions encoding antigen-recognizing protein sequences. Finally, infections with the human immunodeficiency virus can cause an immunosurveillance failure facilitating other infections and DLBCL.

Next-generation sequencing of DNA and RNA has been primarily used to identify potentially targetable somatic mutations in DLBCL to improve treatment. These studies have yielded a wealth of information on the genetic landscape of DLBCL. Most studies compared lymphoma sequences with peripheral blood sequences to discard germline variants. On the basis of the number of somatic mutations detected, DLBCL is a very heterogeneous disease, although the functional consequence of many of these mutations still needs to be resolved. In the majority of these studies, whole-exome sequencing was performed and a limited number of germline variations were actually reported. A whole-genome study by Morin et al. revealed that DLBCL is not necessarily the consequence of a gradual accumulation of...
chromosomal translocations and somatic mutations.\textsuperscript{18} It may also result as shown previously in Chronic Lymphocytic Leukemia and other cancers from chromothripsis, a single genomic catastrophe in which chromosomes are shattered into pieces and chaotically repaired.\textsuperscript{14}

Knowledge on inherited mutations predisposing to DLBCL largely comes from studies of patients with immunodeficiencies. A better understanding of the mechanisms underlying these immune disorders has led to the identification of several genes, in which germline mutations promote the development of DLBCL and other cancers.

As has been observed in other malignancies, an increase in genetic screenings will reveal that mutations originally found somatically in DLBCL may also occur in the germline of patients. We will first review the literature, including supplementary files not readily retrievable by PubMed searches, on inherited or de novo pathogenic germline alterations in DLBCL patients. Second, our analysis of somatically mutated genes will illustrate that it is conceivable that many of the somatic mutations in DLBCL also predispose to this disease when occurring in the germ line.

GERMLINE MUTATIONS IN DLBCL

Forty-nine genes with a (presumed) causative role in lymphoma-genesis, in which germline variants in humans with DLBCL have been identified by positional cloning, targeted sequencing or whole-exome/genome sequencing are depicted in Table 1. These variants are highly enriched in DLBCL patients in comparison with the control population from the ExAC database. The functional effects of missense mutations in these genes are not always understood. Of note, synonymous mutations can also be functionally relevant by affecting gene splicing.\textsuperscript{15}

DNA REPAIR

Thirty-five genes of which germline variants have been observed in DLBCL are involved in DNA repair. Systematic studies of these germline mutations have thus far been very limited. A study using targeted capture sequencing of 73 key DNA repair genes reported novel and/or rare germline variants in these genes in 20 out of 22 DLBCL patients with an average of two but up to four variants (in four different genes) per patient.\textsuperscript{16} Although many of these missense mutations were unknown variants requiring functional validation, the authors showed that potentially functional germline mutations in mismatch repair genes were present in 27% of their DLBCL patients. These MMR mutations were associated with higher numbers of somatic mutations, which may serve as targets to the immune system and contribute to the sensitivity of such lymphomas to anti-PD1 immune checkpoint blockade.\textsuperscript{8}

A DLBCL with microsatellite instability was reported in a colorectal cancer patient with a germline MLH1 mutation.\textsuperscript{17} Although DLBCL may not be prominent in Lynch syndrome,\textsuperscript{18} hematological malignancies including DLBCL have a prevalence of 15%\textsuperscript{19} to 21%\textsuperscript{20} in constitutional mismatch repair deficiency resulting from bi-allelic germline mutations in one of the four MMR genes MLH1, MSH2, MSH6 or PMS2.\textsuperscript{21}

Several DNA repair genes are associated with hereditary cancer syndromes of which DLBCL is not considered a recurrent feature. DLBCL is not among the classical tumors in the Li–Fraumeni syndrome (LFS) caused by germline TP53 mutations.\textsuperscript{22} LFS families do harbor lymphomas, and a possible LFS patient with a germline TP53 mutation with a brain tumor and a subsequent DLBCL was reported from Japan.\textsuperscript{22} A single case report was published on the occurrence of breast cancer, ovarian cancer and DLBCL in a patient with a BRCA1 mutation.\textsuperscript{23}

Some of the germline mutations in the DNA damage response gene CHEK2 observed in an analysis of 235 DLBCL patients\textsuperscript{24} are identical to those that were shown to confer an increased risk for solid tumors,\textsuperscript{24} and possibly essential thrombocytemia\textsuperscript{25} and polycythemia vera.\textsuperscript{26} Mechanistically, the CHEK2 c.444+1G>A mutation leads to a truncated protein lacking the kinase activation domain and part of the functionally relevant forkhead homology-associated domain. The 1157T mutation disturbs the interaction of the CHEK2 protein with p53 and BRCA1 in a dominant-negative manner.\textsuperscript{27}

Targeted CHEK2 gene sequence analysis in another group of 340 patients from the Czech republic with different types of B-cell lymphoma revealed many germline variants of the CHEK2 gene including the well-known c.1100delC founder mutation. Nonsense- and missense-inherited CHEK2 mutations were detected in 6.1% of 180 DLBCL cases.\textsuperscript{28} These additional mutations were not included in Table 1, because detailed information on the distribution of the variants was not provided specifically for DLBCL.

In general, the coincidence of DLBCL in patients with solid tumors is in all likelihood underreported, and the incidence of underlying germline DNA repair mutations is underestimated due to a lack of systematic DNA analyses.

IMMUNODEFICIENCY

For mutations in LI4 (DNA ligase IV),\textsuperscript{29–31} NHEJ1 (XLF),\textsuperscript{32} DCLRE1C (Artemis),\textsuperscript{33} NBN (Nibrin),\textsuperscript{34} WAS,\textsuperscript{35} PIK3CD,\textsuperscript{36} PIK3R1,\textsuperscript{37} SH2D1A (SAP),\textsuperscript{38,39} IFNGR1,\textsuperscript{40} STAT3,\textsuperscript{41} perforin-encoding PRF1\textsuperscript{42–44} and FAS,\textsuperscript{45,46} an association with DLBCL has been reported. Severe combined immunodeficiencies due to homozygous or compound heterozygous mutations in LI4, NHEJ1 and DCLRE1C, genes involved in NHEJ, are known to cause EBV-associated DLBCLs, that become manifest in childhood. Heterozygous mutations, in for instance LI4, are probably more prevalent than previously thought. Patients of over 40 years of age have been diagnosed with compound heterozygosity for a LI4 mutation, which can cause myelodysplasia.\textsuperscript{47} An adult patient with a homozygous congenital DNA ligase 4 mutation and non-EBV-associated DLBCL has also been reported.\textsuperscript{48} A 27-year-old woman with bilateral breast cancer and myelodysplasia was compound heterozygous for both an intronic mutation and a functional polymorphism of DCLRE1C.\textsuperscript{49} This illustrates that patients with inherited mutations in LI4 and DCLRE1C are not solely encountered by pediatric hematologists. In addition to immunodeficiency and increased propensity to develop lymphomas and other malignancies, patients with defects in NHEJ can exhibit developmental delay (short stature, microcephaly).\textsuperscript{37} A germline heterozygous splice site mutation in PIK3R1 leads, like PIK3CD mutations, to an activated PI3Kδ6 syndrome (APDS) designated PASLI, which stands for p110δ-activating mutations causing senescent T cells, lymphadenopathy and immunodeficiency.\textsuperscript{50,51} Depending on the underlying genetic defect, PASLI-CD/APDS1 and PASLI-R1/APDS2 are discerned. Both cause a combined immunodeficiency with phenotypic variability. Apart from infectious complications, lymphoproliferation, splenomegaly and autoimmunity, growth retardation and mild neurodevelopmental delay are among the potential clinical manifestations of inherited mutations in the PIK3R1 gene. Age of onset of lymphoma (DLBCL, Hodgkin lymphoma, marginal zone lymphoma and Chronic Lymphocytic Leukemia) for APDS1 can range from 6 to 40 years.\textsuperscript{37} Size constraints prevented us from including in Table 1 all germline SH2D1A mutations that underlie X-linked lymphoproliferative syndrome type-I and can give rise to EBV-dependent and -independent DLBCL and Burkitt lymphomas in children and also in adults.\textsuperscript{38,39} EBV-associated DLBCL has been reported in an adult with an inherited homozygous mutation in the IFNGR1 gene.\textsuperscript{40} A second EBV-negative DLBCL (E van de Vosse, personal communication) was observed in a Dutch adult with a different homozygous mutation.\textsuperscript{51} Both mutations lead to a complete deficiency of the interferon gamma receptor 1 protein.\textsuperscript{40,51}
| Gene                        | Sequence mutation | Reference | Allele frequency |
|-----------------------------|-------------------|-----------|------------------|
| **TP53**                    |                   |           |                  |
| V31I                        | 17: 7579705C>T    | 16        | 0.05             |
| C242Y                       | 17: 7577555G>A    | 22        | ND               |
| **TP53BP1**                 |                   |           |                  |
| T519A                       | 15: 108139269G>A  | 16        | 0.05             |
| A1714S                      | 15: 9390591T>C    | 16        | 0.05             |
| **ATM**                     |                   |           |                  |
| R924W                       | 11: 89842191G>A   | 16        | 0.05             |
| L1125F                      | 11: 89805934C>T   | 16        | 0.05             |
| **FANCA**                   |                   |           |                  |
| T620I                       | 16: 35075603A>C   | 16        | 0.05             |
| **FANCG**                   |                   |           |                  |
| R1321H                      | 13: 29130703G>    | 16        | 0.05             |
| I357V                       | 13: 2914277A>G    | 16        | 0.05             |
| **MLH3**                    |                   |           |                  |
| I1929V                      | 13: 7551620C>T    | 16        | 0.05             |
| **MLH1**                    |                   |           |                  |
| C40Y                        | 5: 35075603A>C    | 16        | 0.05             |
| **MLH3**                    |                   |           |                  |
| I988M                       | 5: 29130703G>    | 16        | 0.05             |
| **BRCA2**                   |                   |           |                  |
| R519A                       | 17: 29130703G>    | 16        | 0.05             |
| **RAD51B**                  |                   |           |                  |
| I357V                       | 13: 29130703G>    | 16        | 0.05             |
| **RAD51**                   |                   |           |                  |
| C242Y                       | 17: 7577555G>A    | 22        | ND               |
| A1714S                      | 15: 9390591T>C    | 16        | 0.05             |
| **CHEK2**                   |                   |           |                  |
| R3W                         | 17: 108139269G>A  | 16        | 0.05             |
| I517T                       | 17: 9390591T>C    | 16        | 0.05             |
| **CHEK1**                   |                   |           |                  |
| H352L                       | 5: 35075603A>C    | 16        | 0.05             |
| **BRCA2**                   |                   |           |                  |
| S776G                       | 17: 108139269G>A  | 16        | 0.05             |
| E528K                       | 17: 9390591T>C    | 16        | 0.05             |
| **PARP1**                   |                   |           |                  |
| S776G                       | 17: 108139269G>A  | 16        | 0.05             |
| E149Kfs*12                  | 17: 9390591T>C    | 16        | 0.05             |
| **MLH1**                    |                   |           |                  |
| C40Y                        | 5: 35075603A>C    | 16        | 0.05             |
| **MLH3**                    |                   |           |                  |
| I988M                       | 5: 29130703G>    | 16        | 0.05             |
| **MSH3**                    |                   |           |                  |
| T636N                       | 5: 35075603A>C    | 16        | 0.05             |
| **MSH6**                    |                   |           |                  |
| T636N                       | 5: 35075603A>C    | 16        | 0.05             |
| **RPA1**                    |                   |           |                  |
| G160R                       | 17: 108139269G>A  | 16        | 0.05             |
| **TNFAIP3**                 |                   |           |                  |
| P7145                       | 17: 108139269G>A  | 16        | 0.05             |
| **UNG**                     |                   |           |                  |
| E121Q                       | 17: 108139269G>A  | 16        | 0.05             |
| **LIG1**                    |                   |           |                  |
| T311M                       | 17: 108139269G>A  | 16        | 0.05             |
| **LIG3**                    |                   |           |                  |
| R343Q                       | 17: 108139269G>A  | 16        | 0.05             |
| **D2-2R**                   |                   |           |                  |
| M249V                       | 13: 108139269G>A  | 16        | 0.05             |
| **NHEJ1**                   |                   |           |                  |
| R178X                       | 13: 108139269G>A  | 16        | 0.05             |
| **DCLRE1C**                 |                   |           |                  |
| D451fs                      | 13: 108139269G>A  | 16        | 0.05             |
| **NBN**                     |                   |           |                  |
| I237N                       | 13: 108139269G>A  | 16        | 0.05             |
| **WAS**                     |                   |           |                  |
| X: 41delG                   | 13: 108139269G>A  | 16        | 0.05             |
| **PIK3CD3**                 |                   |           |                  |
| D911E:D935I                 | 13: 108139269G>A  | 16        | 0.05             |
Like what has been observed with germline FAS mutations with or without a concomitant PRF1 mutation, patients with the autoimmune lymphoproliferative syndrome caused by germline STAT3-activating mutations are expected to be at risk for developing DLBCL. So far, however, DLBCL has only been described as a consequence of a hyper IgE syndrome caused by a germline STAT3-inactivating mutation.

**TRANSCRIPTION/CHROMATIN REMODELING**

A single putative germline TET2 mutation was seen in human DLBCL. The importance of a KMT2A alteration in DLBCL pathogenesis was underpinned by its segregation with the disease in a family. A germline heterozygous mutation in TP63 was detected in a Japanese girl with ECC (ectrodactyly, ectodermal dysplasia, clefting) syndrome type 3, who subsequently developed DLBCL.

**SIGNAL TRANSDUCTION**

Of the remaining genes presented in Table 1 two (RNF31 and TNFRSF13C) may be classed as involved in signal transduction. Germline variants of the linear ubiquitin chain assembly complex subunit RNF31 provide an example how novel possibilities for therapeutic interventions may arise from the mechanistic understanding of the role of these proteins in a physiological and malignant setting. These variants are characterized as the gain-of-function polymorphisms that enhance the activity of the nuclear factor kappa B (NFκB) pathway. The germline H159Y mutation in TNFRSF13C may also lead, via TRAF3- and TRAF6-mediated signal transduction, to increased NFκB activity and appears to be associated with various lymphoma types including DLBCL.

**AUTOPHAGY**

Autophagy-associated ULK4 gene contained a rare mutation in germline DNA from a DLBCL patient. Another variant of ULK4 was shown to predispose to multiple myeloma and monoclonal gammopathy of undetermined significance. Whether Unc-51-like kinase 4 mutations also promote the development of DLBCL is by no means established yet.

**CHROMOSOMAL ABERRATIONS**

Apart from single-nucleotide variants, and small insertions and deletions, germline alterations also encompass relatively rare fusion genes generated by gene duplication or inversion. Furthermore, germline copy-number neutral loss of heterozygosity involving chromosomes 3, 6, 7, 8, 9 and 20 was recently reported. Such germline loss of heterozygosity involved 29 regions of the genome with each loss occurring in at least 5% of DLBCL cases (n = 40) versus 0–0.8% of normal controls (n = 500). The functional oncogenic consequences of these chromosomal alterations remain to be elucidated.

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**Table 1. (Continued)**

| Gene       | Sequence mutation | chr | g.DNA | Reference | Allele frequency |
|------------|-------------------|-----|-------|-----------|-----------------|
| PIK3R1     | del434-475        |     |       |           |                 |
| STAT3      | K340T             |     |       |           |                 |
| STAT3      | E690P699del        | A3A |       |           |                 |
| PRF1       | N25S2             |     |       |           |                 |
| FAS        | T225P             |     |       |           |                 |
| TET2       | H184SN            |     |       |           |                 |
| RNF31      | Q584H             |     |       |           |                 |
| TNFRSF13C  | H159Y             |     |       |           |                 |
| ULK4       | S770R             |     |       |           |                 |

Abbreviations: DLBCL, diffuse large B-cell lymphoma; ND, not determined; NR, not reported; NA, not applicable. Allele frequency in DLBCL calculated from literature. Data of controls are from the Exome Aggregation Consortium (ExAC), Cambridge, MA, USA (http://exac.broadinstitute.org). *Exon 1 of the IFNGR1 gene harbors four cytosines; c.del25 and 22delC refer to the same mutation. **Mutation of which germline nature is likely.
**GERMLINE MUTATIONS OF SOMATICALLY MUTATED GENES IN DLBCL**

On the basis of gene expression analysis, DLBCL can be classified in two major subtypes of germinal center type and activated B-cell type. Although different somatic mutations are associated with these two DLBCL subtypes, these mutations are not strictly subtype-specific, and intra-tumoral heterogeneity may occur. Germinal center-type DLBCL are associated with mutations in EZH2, GNA13, S1PR2, P2RY8, PTEN and ARHGEF1, primarily affecting histone modification and cellular homing, respectively. Activated B-cell type DLBCL may carry mutations in CARD11 also known as CARMA1, BCL10, MALT1, CD79A, CD79B, MYD88, TNAIP3, SYK, P13K, BTK and PKCB. These mutations mainly involve B-cell receptor signaling and the NFκB pathway.

In addition to the 42 most frequently mutated genes, data were compiled from five studies to obtain a list of 626 genes in which somatic mutations have been found in human DLBCL (Supplementary Table S1). Of these 626 genes, 118 (depicted bold) are significantly mutated in DLBCL as compared to other tumors. In 24 (depicted in red) of the 626 genes, potential pathogenic mutations have also been observed in the germline of patients with DLBCL. These genes are presented in Table 1. Among the remaining 602 genes with somatic mutations in DLBCL, we identified 211 genes (depicted in blue) with mutations in germline DNA that may underlie other diseases, metabolic problems or developmental defects (Supplementary Information). These germline mutations, if not associated with embryonic lethality, represent candidate predisposing genes for the development of DLBCL.

**GENES ASSOCIATED WITH MALIGNANCY**

By and large, the list of 211 genes somatically mutated in DLBCL, of which an inherited or a de novo mutation not known to predispose to this type of lymphoma was found, contains 39 genes of which germline alterations are known to predispose to cancer. Germline mutations in genes such as POLE, WIFI1 and PTEN have thus far been associated primarily with solid tumors. Inherited MSH2 mutations also predominantly predispose for solid tumors, but a germline MSH2 mutation was observed in a patient with follicular lymphoma. Heterozygous germline mutations in the histone methyltransferase EZH2 gene cause the so-called Weaver syndrome with increased body height as one of its salient features. This syndrome may underlie lymphoblastic lymphoma, Acute Lymphoblastic Leukemia and Acute Myeloid Leukemia. It is associated with intellectual disability of highly variable severity. These congenital mutations overlap with the somatic mutations in EZH2 observed in hematological malignancies.

The finding of somatic mutations in ETV6 in DLBCL is noteworthy as germline alterations of this transcription factor were shown to cause autosomal dominant transmission of thrombocytopenia, and predisposition to diverse hematological malignancies, colon cancer, melanoma, myopathy and gastrointestinal dysmotility. Germline mutations in ETV6 occur in ~1% of children with Acute Lymphoblastic Leukemia. A recent study of somatic mutations in relapsed and refractory DLBCL added NFKBIZ as a new mutation target. Similar to some of the genes already discussed here, an inherited mutation in this gene may predispose to colorectal cancer.

**GENES AFFECTING IMMUNE FUNCTION**

Congenitally mutated CARD11 has been associated with polyclonal B-cell lymphocytosis. In this rare disorder, an identical mutation in CARD11 as observed somatically in DLBCL leads to the so-called BENTA disease (B-cell expansion with NFκB and T-cell anergy), a congenital syndrome of lymphocytosis, splenomegaly, lymphadenopathy and T-cell anergy, which may evolve into a B-cell malignancy. In contrast to these heterozygous gain-of-function mutations, homozygous loss-of-function mutations in CARD11 compromise canonical NFκB signaling causing a combined immunodeficiency of variable severity with normal T- and B-cell numbers. The T-cell defect in this disorder may even be alleviated by an acquired somatic CARD11 mutation.

Loss-of-function mutations of the NFκB inhibitor NFKBIA will enhance NFκB activity. A heterozygous gain-of-function mutation in NFKBIA was shown to impair NFκB activation and associate with X-linked anhidrotic ectodermal dysplasia, T-cell immunodeficiency and polyclonal lymphocytosis. Congenital CIXCR4 and BTK mutations underlie primary immunodeficiency states WHIM (warts, hypogammaglobulinemia, infections and myelokathexis syndrome) and Bruton’s type agammaglobulinemia.

Homozygosity or compound heterozygous mutations in CERC1, encoding adenosine deaminase 2, may cause hypogammaglobulinemia and polyarteritis nodosa, an autoimmune disorder associated with an increased incidence of lymphoma. Germline BCL10, DOCK2 and RHOQ mutations cause a form of combined immunodeficiency by affecting B and T cells as well as other cells. By impairing anti-viral immunity, they can promote the development of lymphoma. A patient born with a RHOQ deficiency indeed developed a Burkitt lymphoma. Inherited mutations in CD79A or CD79B can manifest themselves as hypogammaglobulinemia. IRF8 mutations cause a dendritic cell deficiency in either an autosomal recessive or (phenotypically milder) autosomal dominant inheritance pattern. CITA is a relatively frequent target of somatic mutations in DLBCL. Germline mutation of this gene in DLBCL was thus far only reported in canine DLBCL.

**GENES WITH LESS OBVIOUS CAUSALITY**

One can readily imagine that alterations in genes that predispose to cancer in general or control immune function can play a causal role in DLBCL pathogenesis. Numerous genes that are recurrently mutated in DLBCL lack such intuitive function, and the possible contribution of their congenitally observed mutations to lymphomagenesis is much less obvious. Some of these genes may be solely somatically mutated in DLBCL as a consequence of their presence in late replicating regions, but this hypothesis awaits appropriate functional testing. When occurring in the germ line, some mutations can give rise to developmental, neurological, cardiac or metabolic disturbances. The precise nature of these mutations may differ from those observed somatically in DLBCL. The fact that mutations in these genes can occur in germline DNA does not automatically turn them into predisposition genes for DLBCL. In addition, phenotypic manifestations of these mutations can depend on homozygous or compound heterozygous alterations, which may or may not occur somatically. Versatility in terms of function of these genes can be developmental stage, cellular context, dosage, isoforms or alternative transcripts, and post-translational modifications dependent. Finally, germline variants of somatically mutated genes in DLBCL may constitute only weak predisposition genes on their own. As one variant can interact with a variant in another gene, a combination of variants could lead to a higher susceptibility for DLBCL. WIFI1 and HNRRP40 provide an example of such a combinatorial effect in solid tumors. For lymphomas, such an epistatic interaction was suggested for variants in the DNA repair genes MRE11A and NBS1.

Large-scale whole-genome sequencing of germline DNA from patients diagnosed with DLBCL across different ethnic groups and geographic regions should reveal the true incidence and recurrent nature of underlying germline mutations. With only six matched tumor and normal DNA pairs, Pasqualucci et al. already identified by whole-exome sequencing 106 rare germline variants...
among which the SERPINA6 gene. This gene is involved in corticosteroid bioavailability by encoding corticosteroid-binding globulin\(^9\) and it is also targeted by somatic mutations in DLBCL (Supplementary Table s1). Many of the germline variants described by Pasqualucci et al. belong to the same functional classes of genes that are somatically mutated in this disease and could well be relevant for its development. MTMR15 now known as FAN1 (Fanconi anemia-associated nuclease 1) of which germline mutations cause hereditary colorectal cancer and other solid tumors\(^9\) is an example of such a germline variant. Recognition of genes as bona fide DLBCL predisposing genes will require functional studies, animal models and segregation with disease (not exclusively DLBCL) in families.

PHENOTYPIC CATEGORIZATION OF GERMLINE MUTATIONS OF SOMATICALLY MUTATED GENES IN DLBCL

Recurrent somatic mutations in DLBCL have been functionally categorized.\(^4\) As clinical symptoms caused by germline mutations in a gene can vary depending on the mutation and the patient, a strict phenotypic categorization is virtually impossible. In addition to the recurrent themes of tumor development and immune function as specified above, the clinical phenotypic spectrum of a given germline mutation frequently comprises complex syndromes that are difficult to assign to a single category. NFI, KRAS, BRAF and A2ML1 mutations, for instance, underlie the so-called RASopathies and may give rise to several syndromes, that is, neurofibromatosis type I, cardiofaciocutaneous syndrome and Noonan syndrome.\(^{94}\) The large phenotypic spectrum of mutated genes is explained for some by their functional role as transcription factors. For example, HNF1B can predispose to solid tumors as well as maturity onset diabetes of the young.\(^{95}\)

Through compilation of the phenotypic consequences of germline mutations in genes not known to cause DLBCL of which somatic mutations have been observed in this lymphoma, certain prevalent phenotypes became evident. Bearing the limitations outlined above in mind, genes were assigned to a single phenotypic category. Phenotypes to which at least two genes could be linked are graphically displayed in Figure 1 and comprise 193 of the 211 genes somatically mutated in DLBCL of which a germline variant with a phenotype is known (Supplementary Information). In general, these variants can be considered pathogenic mutations and involve any part of the body. Perhaps most striking is the high number of genes of which inherited mutations are associated with intellectual disability.

NON-CODING GENES

In addition to inherited or somatically acquired mutations in protein-coding genes, somatic mutations have been observed in DLBCL in genes encoding microRNAs\(^9\) and long non-coding RNAs.\(^9\) The B-Raf pseudogene BRAFP1 represents an example of a non-coding transcript that can act as a competitive endogenous RNA to promote the development of DLBCL or other malignancies via the sequestration of microRNAs.\(^9\) Germline mutations in the long non-coding RNA RMRP cause cartilage–hair hypoplasia, immunodeficiency and an increased risk of malignancies including lymphomas (to the best of our knowledge no DLBCL has yet been reported). Two microRNAs derived from this long non-coding RNA cause gene silencing, which explains at least part of the effects of these RMRP mutations. The carrier frequency of these mutations is particularly high in the Amish and the Finnish populations.\(^{99}\) Aberrant somatic hypermutation of non-immunoglobulin genes by AID is an important mechanism for somatic oncogenic mutations in DLBCL. As RNAs guide AID to its site of activity,\(^{10}\) it is conceivable that germline mutations in these RNAs can misguide this enzyme and as such contribute to lymphomagenesis.

Epistatic interactions in DLBCL may occur (just like in solid tumors)\(^{100}\) not only between coding genes but also between coding and non-coding genes as well.

CONCLUDING REMARKS

The rapidly expanding knowledge on the somatic mutations in DLBCL has reinforced the notion of the heterogeneity of this disease. Molecularly targeted treatment strategies are currently...
being developed and may replace or be added to the established therapy backbone of R-CHOP immunotheraphy. The activ-
vated B-cell subtype of DLBCL is the forerunner to explore pathway-directed therapeutic benefit.

Germline mutations add another layer of complexity. With this review, we aim at heighten physicians’ awareness to the fact that inherited mutations that contribute to the pathogenesis of DLBCL can also predispose to other malignancies, various degrees of immunodeficiencies and a wide spectrum of organic disturbances of various severities.

A better understanding of DLBCL predisposition genes is clinically relevant, as their detection can lead to preventive measures in both patients and their relatives. Underlying defects in mismatch repair for instance can predispose to other cancers, which can be prevented by active surveillance, for example, colonoscopy. Increasing knowledge on the mode of action of these genes can also create new therapeutic options or influence the choice among established therapies. Inherited mutations in DNA double-strand break repair genes can be exploited to the benefit of the patient by selecting platinum-containing che-
motherapy or the use of Poly ADP ribose polymerase inhibitors to induce synthetic lethality. Depending on the severity of the phenotype associated with the mutation, pre-implantation or prenatal diagnosis can be discussed, and upon further refinement of gene-editing technology even gene therapy can be envisioned.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Studies have shown that germline mutations in the DNA damage response pathway are associated with non-Hodgkin lymphoma. For instance, mutations in the DNA repair gene FAN1 have been linked to colorectal cancer. Additionally, mutations in the RHOH gene have been found to cause a complete block in B-cell development.

Inherited DOCK2 deficiency in patients with early-onset invasive infections has also been investigated. This genetic variant impairs hematopoietic and non-hematopoietic immunity, leading to susceptibility to EV-HPV infections.

Furthermore, germline mutations in RHOH have been implicated in the development of autoimmune diseases. Mutations in the antibody production pathways have been associated with B-cell lymphoma.

The BRAF pseudogene functions as a competitive endogenous RNA and induces lymphoma in vivo.

MicroRNA-142 is implicated in the pathogenesis of diffuse large B-cell lymphoma.

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