Defects in B-Cell Development and Function: Agammaglobulinemia, Hyper Immunoglobulin M Syndrome, and Common Variable Immunodeficiency
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Agammaglobulinemia, Hyper Immunoglobulin M Syndrome, and Common Variable Immunodeficiency

Frequently used abbreviations: **AID** – activation-induced cytidine deaminase; **APRIL** – a proliferation-inducing ligand; **BAFF** – B-cell activating factor; **BAFF-R** – B-cell activating factor receptor; **BCMA** – B-cell maturation protein A; **BCR** – B-cell receptor; **C** – constant; **CSR** – class-switch recombination; **CVID** – Combined Variable Immunodeficiency; **HIGM** – Hyper Immunoglobulin M Syndrome; **Ig** – immunoglobulin; **IVIG** – intravenous immunoglobulin; **MHC** – major histocompatibility complex; **S** – switch; **SHM** – somatic hypermutation; **TACI** – transmembrane activator and CAML interactor; **TNF** – tumor necrosis factor; **TNFR** – tumor necrosis factor receptor; **UNG** – uracil-DNA-glycosylase; **V** – variable; **XLA** – X-linked Agammaglobulinemia

Introduction

Agammaglobulinemia, Hyper Immunoglobulin M (HIGM), and Common Variable Immunodeficiency (CVID) syndromes are rare disorders caused by defects in B-cell development. Loss-of-function mutations in at least eleven different genes have been implicated in these syndromes, which are broadly characterized by increased susceptibility to bacterial infections. While the clinical presentation of these syndromes may be similar, severity of disease varies depending on the genetic mutation. Intravenous immunoglobulin (IVIG) therapy and aggressive use of antibiotics have proven successful in controlling infections in individuals suffering from agammaglobulinemia, HIGM, or CVID, particularly when diagnosed early. Bone marrow transplantation may also be recommended to address the more severe immune dysfunction associated with certain forms of HIGM.

Genetic testing for agammaglobulinemia, HIGM, and CVID can help to distinguish between these syndromes. Knowledge of the specific defect in B-cell development may have implications for treatment, prognosis, and genetic counseling. In addition, genetic testing can identify asymptomatic carriers and facilitate timely initiation of treatment in descendants of carriers.

Types and Causes of Defects in B-Cell Development

Agammaglobulinemia, HIGM, and CVID have been linked to loss-of-function mutations in at least eleven different genes.

<table>
<thead>
<tr>
<th>Type of B-Cell Deficiency</th>
<th>Mutated Gene</th>
<th>Affected Protein</th>
<th>Inheritance</th>
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<tr>
<td>agammaglobulinemia, X-linked (XLA)</td>
<td><strong>BTK</strong></td>
<td>Bruton’s tyrosine kinase</td>
<td>X-linked</td>
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<td>IgM heavy chain</td>
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<td></td>
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<td>IgA</td>
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<td></td>
<td><strong>CD79A</strong></td>
<td>B-cell linker protein</td>
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<td><strong>BLNK</strong></td>
<td>LRRC8</td>
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<td></td>
<td><strong>LRRC8</strong></td>
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<tr>
<td>HIGM1</td>
<td><strong>CD40LG</strong></td>
<td>CD40 ligand (CD154)</td>
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<tr>
<td>HIGM2</td>
<td><strong>AICDA</strong></td>
<td>activation-induced cytidine deaminase (AID)</td>
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<tr>
<td>HIGM3</td>
<td><strong>CD40</strong></td>
<td>CD40</td>
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<td>HIGM5</td>
<td><strong>UNG</strong></td>
<td>uracil-DNA-glycosylase (UNG)</td>
<td>autosomal recessive</td>
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<td>CVID</td>
<td><strong>TNFRSF13B</strong></td>
<td>transmembrane activator and CAML interactor (TACI)</td>
<td>see section on CVID below</td>
</tr>
</tbody>
</table>
X-linked (Bruton's) Agammaglobulinemia (XLA)

Mutations in Bruton's tyrosine kinase (Btk) have been implicated in X-linked agammaglobulinemia (XLA).1, 2 Btk is a member of the Tec family of protein tyrosine kinases. Signaling by Btk plays an important role during multiple stages of the B-cell life cycle, including proliferation, development, differentiation, survival and apoptosis.29-31 Btk first becomes involved in B-cell maturation during the pro-B-cell to pre-B-cell transition, which is characterized by expression of the pre-B-cell receptor.1 When the pre-B-cell receptor is cross-linked by antigen, a signaling pathway is initiated that leads to activation and phosphorylation of Btk.8, 11 Activated Btk then serves as an intermediary in a signaling pathway that ultimately leads to B-cell proliferation and differentiation. Loss-of-function mutations in BTK disrupt this signaling pathway, arresting B-cell development at the pro-B-cell stage. Patients with XLA typically have normal numbers of pro-B cells in the bone marrow, but these cells are unable to mature further in the absence of Btk.22 The disruption of B-cell development due to mutations in BTK results in a virtual absence of mature B lymphocytes8, 8 and an inability to produce immunoglobulins of any class.3, 10

For a brief overview of B-Cell Maturation, please refer to Appendix 1 (p. 8).

Other Causes of Agammaglobulinemia

The autosomal form of agammaglobulinemia has been linked to mutations in five genes, namely IGHM,11 IGLL1,12 CD79A,13 BLNK,14 and LRRC8.15 CD79A, IGLL1, and IGHM encode the CD40 receptor (CD40L, also called CD154) is a type II integral membrane protein of the tumor necrosis factor (TNF) family. It is transiently expressed on activated helper T cells and interacts with the CD40 receptor (CD40), which is a member of the TNF receptor family of cytokine receptors that is expressed on all antigen-presenting cells, including B cells, monocyes/macrophages, and dendritic cells.23

Interaction of CD40L with CD40 expressed on antigen-presenting B cells initiates a signaling pathway necessary for B-cell proliferation, germinal-center formation, class-switch recombination (CSR – gives rise to the different antibody isotypes), somatic hypermutation (SHM – the process underlying affinity maturation), and generation of plasma cells.24 Defects in CD40L or CD40 lead to a failure of T-cell–B-cell communication, resulting in formation of defective germinal centers and impairment of both CSR and SHM.22, 25 Lack of CSR leads to low levels or complete absence of IgG, IgA, and IgE, resulting in increased susceptibility to certain bacterial infections. IgM is still produced at normal or elevated levels in response to T-cell independent antigens such as certain bacterial polysaccharides, polymeric proteins, and lipopolysaccharides.

CD40–CD40L interaction also mediates T-cell activation of dendritic cells and macrophages as part of the cellular immune response. Defects in CD40L or CD40 therefore affect the T-cell-macrophage mediated immune response, resulting in susceptibility to opportunistic pathogens.20

For a brief overview of CSR and SHM, please refer to Appendix 2 (p. 9).

HIGM2 and HIGM3

HIGM1, also known as CD40L deficiency, is an X-linked disorder caused by mutations in CD40LG, the gene encoding CD40 ligand.17-21 HIGM3 (CD40 deficiency) results from autosomal recessive mutations in the gene that encodes the CD40 receptor.22 CD40 ligand (CD40L, also called CD154) is a type II integral membrane protein of the tumor necrosis factor (TNF) family. It is transiently expressed on activated helper T cells and interacts with the CD40 receptor (CD40), which is a member of the TNF receptor family of cytokine receptors that is expressed on all antigen-presenting cells, including B cells, monocyes/macrophages, and dendritic cells.23

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CVID
CVID is a heterogeneous disorder, often appearing sporadically. 5-10% of patients with CVID harbor germline mutations in the gene TNFRSF13B (also called TACI), which are believed to confer a strong predisposition for CVID. The effect of individual variants ranges from a virtually monogenic dominant association with CVID for some variants, although with a varying degree of penetrance, to a weaker contributory effect for other variants. Notably, variants that constitute risk factors for the development of CVID are unlikely to play a role in the development of IgA deficiency.

The TNFRSF13B gene product, transmembrane activator and CAML interactor (TACI), is a member of the tumor necrosis factor receptor (TNFR) family. TACI is expressed on peripheral B cells and forms a homotrimeric complex in response to ligand binding. TACI and two other TNFR family members, B-cell activating factor receptor (BAFF-R) and B-cell maturation protein A (BCMA), function as receptors for the TNF-like ligands, BAFF (also called BLys, THANK, TALL-1, zTNF4) and a proliferation-inducing ligand (APRIL; also called TALL-2, TRLD-1). Interactions between TACI and its ligands mediate the communication between B cells and macrophages and B cells and dendritic cells necessary for T-cell independent induction of CSR in B cells, which gives rise to the different antibody isotypes. The interaction between TACI and BAFF results in production of IgG and IgE, while interaction between TACI and APRIL is necessary for production of IgA as well as IgG and IgE. Defects in TACI cause impaired T-cell independent CSR, and are associated with hypogammaglobulinemia.

Clinical Presentation of Agammaglobulinemia, HIGM, and CVID
Agammaglobulinemia, HIGM, and CVID are characterized by recurrent infection with extracellular bacteria, in particular pyogenic bacteria such as Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes, and Staphylococcus aureus, leading to recurrent respiratory tract infections, otitis, or sinusitis. The frequent recurrence of bacterial infections can eventually cause anatomical damage, particularly to the airways of the lungs. However, agammaglobulinemia, the different HIGM subtypes, and CVID differ in the degree of susceptibility to infections as well as in other aspects of their clinical presentation.

X-linked Agammaglobulinemia (XLA)
Most patients with XLA are diagnosed at less than 5 years of age. They have few or no B cells and consequently lack secondary lymphoid organs, such as lymph nodes and tonsils. Patients exhibit a normal response to infections with opportunistic intracellular bacteria and are able to successfully resolve most viral infections. However, enteroviruses such as echo, coxsackie, or poliovirus may cause a fatal, slowly progressing disease affecting the central nervous system.

Agammaglobulinemia (AR or minor)
The autosomal recessive form of agammaglobulinemia presents with clinical symptoms similar to those observed for XLA, but is often diagnosed at a younger age and tends to lead to more severe complications. Skin infections, neutropenia, and pseudomonas or staphylococcal sepsis have been reported in patients with mutations in the μ heavy chain, Igα, or BLNK.

HIGM1 and HIGM3
HIGM1 and HIGM3 are classified as combined B-cell and T-cell immunodeficiencies, and lead to more severe disease. Patients with all types of HIGM typically present with normal B-cell levels and excess IgM, while levels of IgG, IgA, and IgE are low or undetectable. In some cases, patients with HIGM1 may exhibit normal IgA levels, likely resulting from CD40L–CD40-independent CSR attributable to B-cell activation by B-cell activating factor (BAFF) and a proliferation inducing ligand (APRIL) as well as TGF-β signaling. HIGM1 and HIGM3 are distinguished by small lymph nodes, lacking germinal centers, and increased susceptibility to opportunistic infections, due to the inability of T cells to activate monocytes/macrophages and dendritic cells via the CD40–CD40L interaction. This failure of cellular immunity can lead to infection by Pneumocystis carinii, causing pneumonia, and Cryptosporidium, leading to chronic inflammation of the bile ducts (cholangitis). Neutropenia, hemolytic anemia, and thrombocytopenia are also frequently observed.

HIGM2 and HIGM5
HIGM2 and HIGM5 are strictly associated with B-cell defects and result in milder symptoms. Patients with HIGM2 and HIGM5 often show an enlarged spleen and enlarged lymph nodes and tonsils with giant germinal centers. These patients are not susceptible to infection by opportunistic pathogens. Autoimmunity is frequently observed (25% of patients), manifesting as hemolytic anemia, thrombocytopenia, or autoimmune hepatitis.

CVID
CVID is usually diagnosed in patients between the ages of 13 and 30, although symptoms can appear at any age. Although B-cell numbers are typically normal, serum levels of IgA and, sometimes, IgG and/or IgM are low or undetectable. Inflammatory bowel disease and chronic diarrhea due to infection by *Giardia lamblia*, *Salmonella*, *Shigella* or *Campylobacter* are also frequently observed. Approximately one-third of CVID patients suffer from lymphoproliferative disorders, resulting in enlarged spleen and lymph nodes, and in some cases, malignant lymphomas. Autoimmune diseases are diagnosed in ~20% of patients, commonly manifesting as idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, or neutropenia.

**Diagnosis of Agammaglobulinemia, HIGM, and CVID**

Agammaglobulinemia, HIGM, and CVID are suspected in infants and young children presenting with recurrent, persistent, or severe infections by pyogenic bacteria that ultimately result in hospitalization. Diagnosis of these disorders currently relies on detection of reduced serum immunoglobulin levels, immunoglobulin isotyping, and flow-cytometric detection of B-cell levels, and is supported by a family history of recurrent or persistent bacterial infections, or a family history of agammaglobulinemia, HIGM, or CVID.

**Agammaglobulinemia** can be distinguished from other primary immunodeficiencies by the reduction in the number of circulating B cells and all classes of immunoglobulins in the presence of normal T-cell levels. Patients with agammaglobulinemia are also characterized by the lack of secondary lymphoid organs, due to the absence of B cells.

Patients with **HIGM** have normal numbers of both B and T cells. However, their B cells exclusively express IgM and IgD, but not IgG, IgA or IgE. **HIGM1** and **HIGM3** are further characterized by a defect in T-cell activation, resulting in susceptibility to opportunistic infections such as *Pneumocystis carinii* or *Cryptosporidium*. In the case of HIGM1, the defect in T-cell activation can be demonstrated by the inability of activated T cells to bind soluble CD40. A lymph-node biopsy will reveal a lack of germinal centers in patients with HIGM1 and HIGM3. Patients with **HIGM2** and **HIGM5**, in contrast, usually present with enlarged secondary lymphoid organs with giant germinal centers.

Patients with **CVID** usually have normal B-cell numbers, while serum levels of IgA, IgG, and/or IgM are low or undetectable. Diagnosis of CVID also requires the exclusion of other causes of antibody deficiency, including XLA and HIGM. In young male children with reduced B-cell numbers, CVID may be difficult to distinguish from XLA. Similarly, in children with normal B-cell numbers and normal IgM levels, CVID may be confused with HIGM.

Genetic testing can confirm or establish a diagnosis of agammaglobulinemia or HIGM or indicate a predisposition for CVID and may facilitate differential diagnosis, based on a single blood sample. Importantly, it can detect carriers, allowing early diagnosis and preventative therapy of any affected descendants.

**Treatment of Agammaglobulinemia and HIGM**

The primary form of treatment for all forms of agammaglobulinemia, HIGM, and CVID is intravenous immunoglobulin replacement therapy (IVIG) accompanied by aggressive use of antibiotics. Regular IVIG therapy is
often successful in keeping patients free of infection, particularly when early detection allows patients to begin treatment prior to contracting potentially life-threatening infections.\textsuperscript{55,57,61,62}

Patients with HIGM1 and HIGM3 suffer from the most severe symptoms. These patients frequently develop neutropenia, which can be treated with granulocyte-macrophage cell-stimulating factor (GM-CSF). Bone marrow transplantation is recommended to address the defect in T cell-mediated immunity.\textsuperscript{26,55}

**Genetics of Agammaglobulinemia and HIGM**

XLA is a fully penetrant X-linked recessive disorder and exclusively affects males.\textsuperscript{9,63} B cells of females carrying a mutant form of BTK exhibit a non-random pattern of X-inactivation.\textsuperscript{64} Mutations associated with XLA have been found throughout the entire Btk gene, including the non-coding sequences,\textsuperscript{65} and no single mutation accounts for more than 3% of patients.\textsuperscript{61} The nature of the specific mutation appears to affect the severity of the disease, although there is no strong genotype-phenotype correlation.\textsuperscript{61}

Autosomal agammaglobulinemia is caused by autosomal recessive loss-of-function mutations in the genes encoding the IgM heavy chain, \( \lambda_5 \), Ig\( \alpha \), or BLNK. Mutations in LRRC8 may be autosomal dominant. The autosomal form of agammaglobulinemia affects both males and females.\textsuperscript{66}

HIGM1 is caused by X-linked recessive loss-of-function mutations in CD40LG, which codes for CD40L, and almost exclusively affects males.\textsuperscript{25} In very rare cases, HIGM1 has been observed in females as a result of skewed X inactivation or a chromosomal translocation.\textsuperscript{67,68} HIGM3 is due to autosomal recessive loss-of-function mutations in CD40, the gene encoding the CD40 receptor, and is seen in both males and females.\textsuperscript{22}

HIGM2 is caused by autosomal recessive loss-of-function mutations in AICDA, which codes for AID,\textsuperscript{27,28} while HIGM5, which is closely related to HIGM2, results from autosomal recessive loss-of-function mutations in UNG, the gene encoding UNG.\textsuperscript{29} Both HIGM2 and HIGM5 are observed in males and females. Mutations in AICDA that cause HIGM2 have been identified throughout the gene.\textsuperscript{55} Of note, a rare autosomal dominant mutation in AICDA appears to affect only CSR, leading to a milder form of HIGM2.\textsuperscript{69-71}

Many cases of CVID are sporadic; however, recent reports suggest that mutations in TNFRSF13B are present in \( \sim10-15\% \) of CVID cases.\textsuperscript{38,39} The penetrance of TNFRSF13B mutations seems to be highly variable.\textsuperscript{40,41}

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Affected Gene</th>
<th>Relative Frequency</th>
<th>Affects</th>
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<tr>
<td>agammaglobulinemia, X-linked</td>
<td>BTK</td>
<td>85%</td>
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<tr>
<td>agammaglobulinemia, autosomal recessive</td>
<td>IGHM, IGLL1, CD79A, BLNK, LRRC8</td>
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Table 2

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### Table 3

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<th>Syndrome</th>
<th>Affected Gene</th>
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<td>HIGM5</td>
<td>UNG</td>
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<td>CVID</td>
<td>TNFRSF13B</td>
<td>~10-15% of CVID</td>
<td>males and females</td>
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</table>

### Defect in B-Cell Function Late in B-cell Development

Defect in B-cell function late in B-cell development is associated with increased levels of IgM and low numbers of other Ig isotypes, suggesting a potential defect in the late stages of B-cell development. This is characteristic of defects in B-cell function and may be associated with conditions such as XLA, HIGM, or CVID.

### Genetic Testing for Defects in B-Cell Development

Genetic testing can confirm or establish a differential diagnosis of agammaglobulinemia, HIGM, or CVID. Genetic testing should be considered for infants and children who suffer from recurrent, persistent, or severe bacterial infections and show low serum immunoglobulin levels. A family history of unusual susceptibility to infection also indicates an immunodeficiency. Absence of B cells is suggestive of XLA, while elevated levels of IgM and low numbers of the other Ig isotypes point to HIGM. Reduced levels of IgA accompanied by variable IgG and IgM levels may indicate CVID, which should be considered only after other causes of immunoglobulin deficiency have been excluded.

Genetic testing also allows detection of asymptomatic carriers of immunodeficiency-related mutations. This is especially important in the case of X-linked diseases such as XLA and HIGM1, since female carriers are asymptomatic, while their sons are at a 50% risk of being affected. Affected descendants of known carriers can then be diagnosed early, allowing timely initiation of treatment.

### How Is Genetic Testing for Defects in B-Cell Development Performed?

DNA for sequencing is obtained from leukocytes present in a small blood sample. The coding sequences of the genes in question are amplified in a highly specific manner through a polymerase chain reaction (PCR), and all PCR products are fully sequenced. Sequencing results are interpreted, and a detailed result report is sent to the patient’s physician.
References

Appendix 1

B-Cell Maturation

The exquisite specificity of immunoglobulins is based on variation in the amino acid sequence of the antigen binding site, encoded by the variable (V) regions of the heavy and light chain genes. Much of this variability is achieved through modular assembly of the immunoglobulin genes. The heavy chain V region is assembled from three segments, the variable (V<sub>H</sub>), diversity (D<sub>H</sub>), and joining (J<sub>H</sub>) segment, while the light chain V region is constructed from only a V<sub>L</sub> and a J<sub>L</sub> segment. Each segment exists in multiple copies, each with a unique sequence, allowing numerous combinations as the segments are joined during heavy chain [V(D)J] or light chain (V-J) rearrangement. Every rearranged heavy chain variable region can then be linked to different constant regions, which define the function of the different isotypes.

The development of primary B cells is characterized by the ordered rearrangement and expression of the heavy and light chain immunoglobulin genes. B-cell development is initiated when hematopoietic stem cells in the bone marrow become committed to the B-cell lineage. Successful V(D)J rearrangement in pro-B cells results in transient expression of the heavy chain variable region linked to a μ constant region and leads to the next stage in B-cell development, the pre-B cell. The newly rearranged μ heavy chain combines with a surrogate light chain, comprised of the λ5 and V<sub>preB</sub> proteins, and two invariant accessory proteins, Igα and Igβ, to form the pre-B cell receptor. Expression of the pre-B cell receptor initiates a signaling pathway causing pre-B cells to divide, expanding the population of cells with successfully rearranged μ heavy chains. The next stage of B-cell development involves V-J rearrangement of the light chain. Upon successful light-chain rearrangement, the light chain is expressed and combines with the μ heavy chain to form a complete IgM molecule. Expression of the IgM molecule on the cell surface, as the B-cell receptor, defines the immature B cell. Immature B cells then undergo selection for self-tolerance and begin circulating through the peripheral lymphoid tissue. B cells that survive this selection process may undergo further differentiation to produce cell surface. These cells are considered mature, or naïve, B cells.

Mature, circulating B cells undergo proliferation and further differentiation in response to antigen and T-cell binding. Activated B cells migrate to germinal centers where somatic hypermutation and class switch recombination take place. Somatic hypermutation generates further diversity and higher affinity by introducing point mutations within the variable regions of immunoglobulin genes. Class-switch recombination links a V region to a constant (C) region other than μ or δ, resulting in expression of antibodies with identical specificity, but different functions. Subsequently, B cells undergo further differentiation into memory cells or plasma cells. Memory cells, which are long-lived, express antibodies on their surface, while plasma cells secrete soluble antibody.
Appendix 2

Class-Switch Recombination and Somatic Hypermutation

When a mature, circulating B cell encounters an antigen that is recognized by its B-cell receptor, the antigen is internalized and degraded. Fragments of the antigen are then displayed on the B-cell surface as peptides bound to MHC class II molecules. When a helper T cell recognizes this peptide:MHC class II complex, the CD40 ligand (CD40L, also called CD154) is expressed on the surface of the helper T cell. In response to CD40 receptor binding by CD40L, the T cell releases interleukin-4, stimulating the B cell to proliferate, ultimately inducing formation of germinal centers. B cells undergo several important modifications within the germinal center, including somatic hypermutation (SHM) and class-switch recombination (CSR).

The process of SHM introduces point mutations into the variable (V) region of rearranged heavy and light chains at a very high rate, thereby generating further diversity. Many of the mutations will disrupt antibody structure and will therefore be selected against. Some mutations will result in improved antigen binding, and B cells expressing these mutant immunoglobulin molecules will be selected to mature into antibody-secreting cells (affinity maturation). SHM requires transcription of the immunoglobulin V genes. During transcription, single-stranded DNA is recognized by activation-induced cytidine deaminase (AID), which deaminates cytosine nucleotides, generating deoxyuracil residues (dUs). Repair of these lesions often leads to a permanent base change at the deamination site. It is thought that endonucleases cleave the DNA at this abasic site, and that repair of the DNA nick by error-prone polymerases then introduces a mutation.

The heavy chain constant region defines the function, and the isotype, of an antibody. Initially, all rearranged heavy-chain variable regions are associated with the μ or the δ constant regions, which are encoded immediately downstream of the variable region. Class-switch recombination (CSR), also called isotype switching, allows a heavy-chain variable (V) region to become associated with the γ, α, or ε constant (C) region, thus changing the antibody’s function while preserving its antigen specificity. CSR involves irreversible DNA recombination, guided by stretches of repetitive DNA called switch (S) regions that are located in the introns between the different C genes. The mechanism of CSR bears certain similarities to SHM. CSR is initiated by transcription of S regions, allowing AID to access single stranded DNA and deaminate cytosine to uracil within these regions. Deoxyuracil residues are subsequently removed by UNG, generating abasic sites. The presence of abasic sites induces the recruitment of generic DNA repair enzymes that catalyze the nonhomologous recombination between the switch regions.