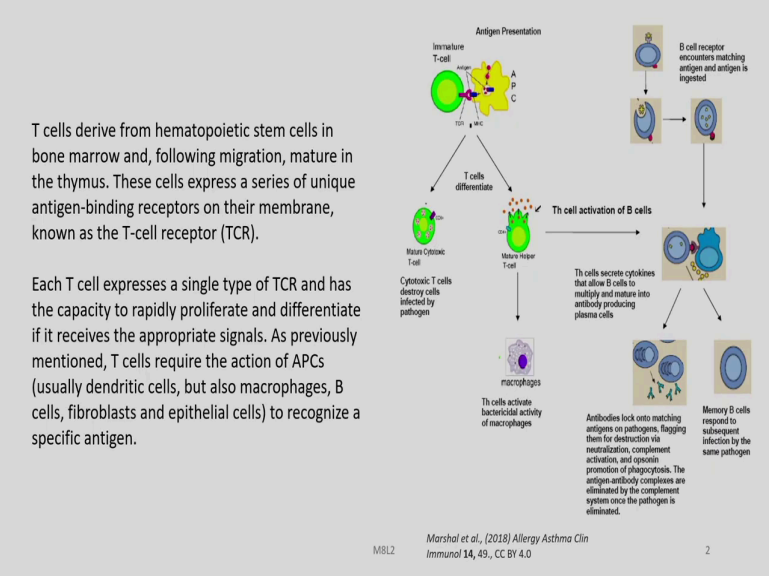
Genome Editing and Engineering
Prof. Utpal Bora
Department of Bioscience and Bioengineering
Indian Institute of Technology, Guwahati

Module - 08
Applications of genome editing in treating human diseases
Lecture - 30
Human cell engineering in diseases: Severe combined immunodeficiency (SCID) - Part A

Welcome to my course on Genome Editing and Engineering. We are discussing module 8 which is Applications of genome editing in treating human diseases. In the last lecture, we discussed about thalassemia. In this lecture, we are going to discuss about Severe combined immunodeficiency or SCID. Let us have some basic concepts of T cells and B cells and natural killer cells.

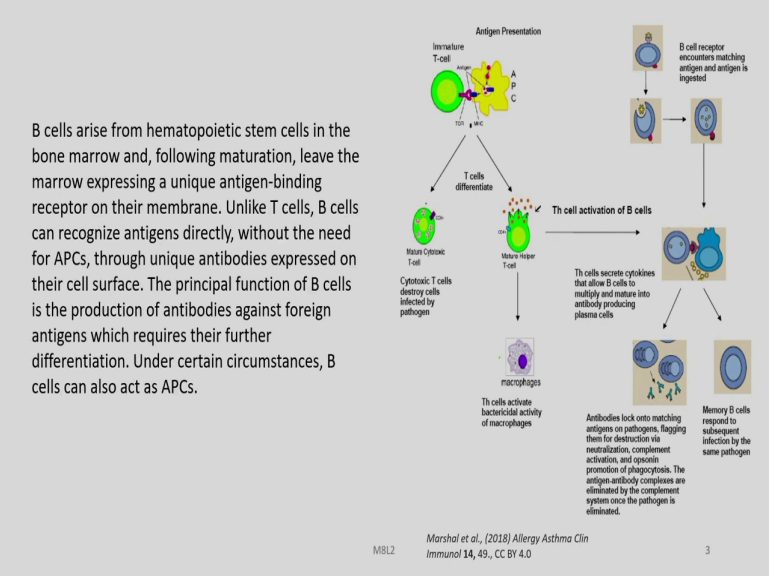
(Refer Slide Time: 00:59)



So, we know that T cells derive from hematopoietic stem cells in bone marrow and following migration mature in the thymus. These cells express a series of unique antigen binding receptors on their membrane known as the T cell receptor or TCRs. Each T cell express a single type of TCR and has the capacity to rapidly proliferate and differentiate if it receives the appropriate signal.

And, T cells require the action of APCs usually dendritic cells, but also macrophages to recognize a specific antigen. This figure and text has been taken from Marshal et al and you in this figure you can see the immature T cell interacting with a APCs. And, then they are differentiating to mature cytotoxic T cell and mature helper T cell. And, then they are also involved in the activation of the B cells and the T cells secret cytokines that allow B cells to multiply and mature into antibody producing plasma cells.

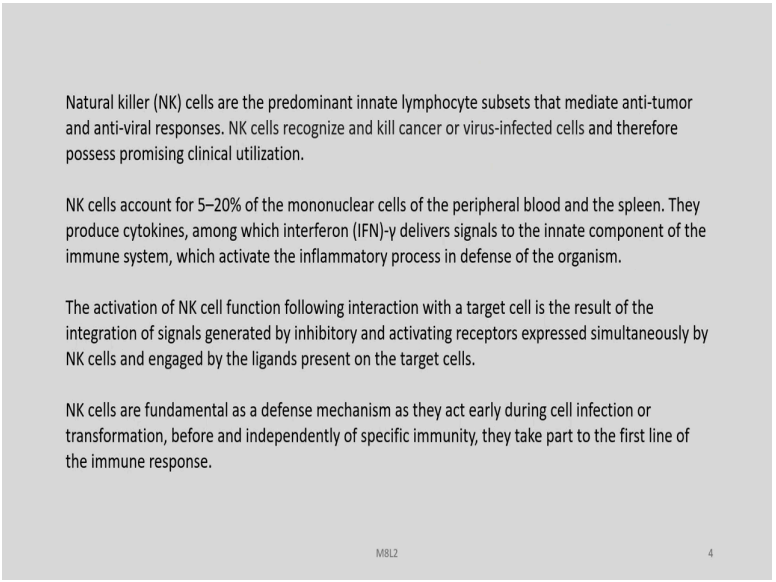
(Refer Slide Time: 02:22)



So, let us discuss about B cells which arise from hematopoietic stem cells in the bone marrow and following maturation leave the marrow expressing a unique antigen binding receptor on the membrane. However, unlike the T cells, B cells can recognize antigens directly without the need for antigen presenting cells through unique antibodies expressed on their cell surface.

The principal function of B cells is the production of antibodies against foreign antigens which require their further differentiation. Under certain circumstances, B cells can also act as antigen presenting cells.

(Refer Slide Time: 03:03)

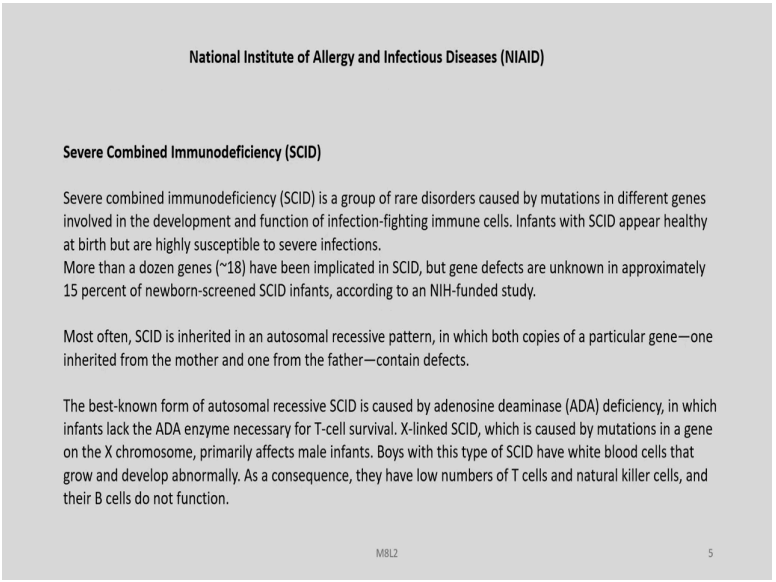


This is the another type of cell in the immune system which are known as the Natural killer cells or NK cells are the predominant innate lymphocyte subsets that mediate anti tumor and antiviral responses. NK cells recognize and kill cancer or virus infected cells and therefore, possess promising clinical utilization. They account for around 5 to 20 percent of the mononuclear cells of the peripheral blood and the spleen.

And, they produce cytokines among which interferon gamma, delivers signals to the innate component of the immune system which activate the inflammatory process in defense of the organism. The activation of NK cell function following interaction with a target cell is the result of the integration of signals generated by inhibitory and activating receptors express simultaneously by NK cells and engage with the ligands present on the target cells.

NK cells are basic and fundamental to the defense mechanism as they act early during cell infection or transformation before and independently of specific immunity, they take part to the first line of the immune response.

(Refer Slide Time: 04:23)

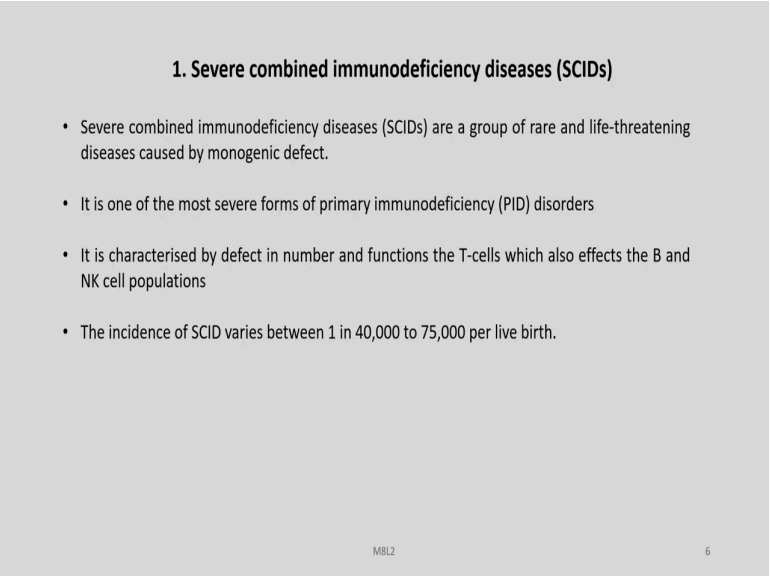


With this basic idea and function of the various cells like T cell, B cell and NK cells; let us now move on to the severe combined immuno deficiency disease and as defined by the NIAID, National Institute of Allergy and Infectious Disease. Severe combined immunodeficiency is a group of rare disorders, it is not a single disease. And, it is caused by mutations in not one, but different genes involved in the development and function of infection fighting immune cells which we just discussed prior to this.

Infants with SCID appear healthy at birth, but they are highly susceptible to severe infections. More than a dozen genes roughly around 18 have been implicated in SCID, but gene defects are known in approximately 15 percent of newborn screened SCID infants, as reported by a NIH study. Often, SCID is inherited in an autosomal recessive pattern in which both copies of a particular gene one from the mother and one from the father contain the defect which we will also see through illustrative examples in the next slide.

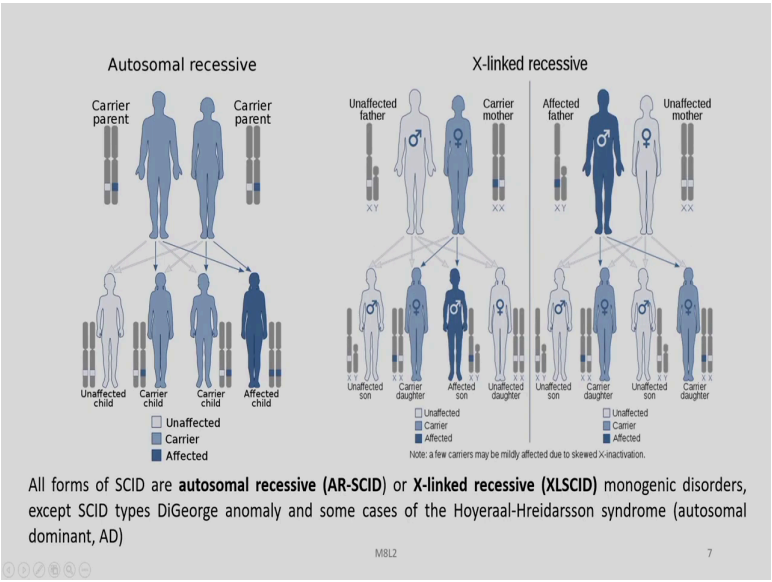
The best known form of autosomal recessive SCID is caused by adenosine deaminase or ADA deficiency in which infants lack the ADA enzyme necessary for T cell survival. X-linked SCID which is caused by mutations in a gene on the X chromosome, primarily affects male infants. Boys with this type of SCID have white blood cells that grow and develop abnormally. As a consequence, they have low numbers of T cells and natural killer cells and their B cells do not function.

(Refer Slide Time: 06:36)



So, we now know that SCID are a rare group of genetic diseases and they are life threatening and mostly caused by a monogenic defects. It is one of the most severe forms of primary immuno immunodeficiency disorder or called PID. It is characterized by defect in number and functions of the T cells which also affect the B and NK cell population. The incidence of SCID varies between 1 in 40,000 to 75,000 per live birth.

(Refer Slide Time: 07:09)



So, here are the autosomal recessive and X-linked SCID illustrated with example and you can see in this case both the parents are a carriers with one mutation in each chromosome

respectively. And, when the normal chromosomes are inherited by a child, he she remains unaffected. And, if one of the chromosome inherited is normal, while the other is having the mutation; these child act as a carrier cell or a male or a female.

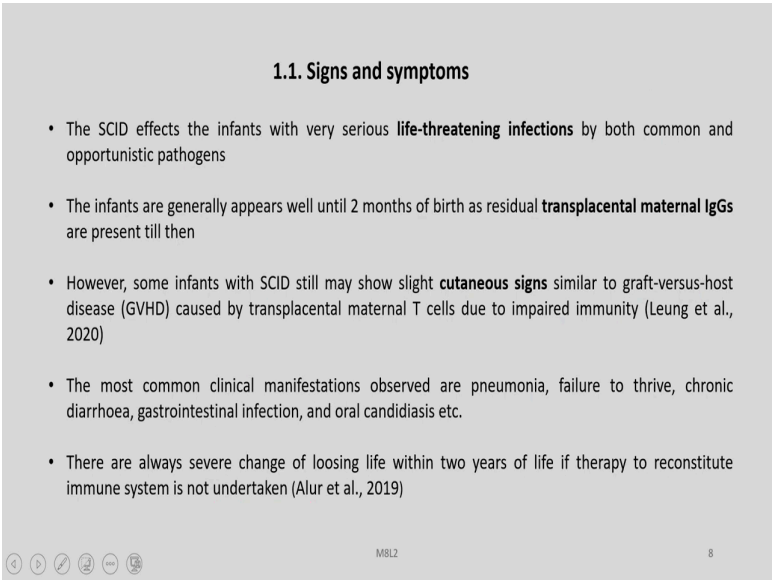
How the unfortunate ones carrying or inheriting both the copies of the mutation lying in two different chromosomes gets affected and diseased. In the X-linked recessive monogenetic disorder, you can see here the unaffected a father, but the mother is a carrier in this case. And, then here the father is affected only due to one copy, while this is the mother who is unaffected. And, if they their progeny will have this type of inheritance and phenotype as you can see.

If the boy gets a normal X and then a copy of the Y chromosome from the father he is unaffected. And, while a girl who will inherit one of the normal chromosomes X chromosome from the father. But, the chromosome having the mutation from the mother will be a carrier. In the case of again male, the inheritance of the defective chromosomal mutation from the mother and, the normal Y chromosome for the from the father he is affected or diseased.

And, in case of inheritance of both copies of the normal X chromosome from the two parents, the daughter remains unaffected. So, here we have two unaffected one is to one male female and one carrier daughter and one affected male. In the other case, you can see one unaffected son and then one carrier daughter and then another unaffected son and one more carrier daughter, due to the inheritance pattern of the mutation lying in the X chromosome of the affected father or AR-SCID or X-linked recessive.

In brief, all forms of SCID are autosomal recessive or AR-SCID or X-linked recessive, XLSCID and monogenic disorders, except SCID types DiGeorge anomaly and some cases of Hoyeraal-Hreidarsson syndrome.

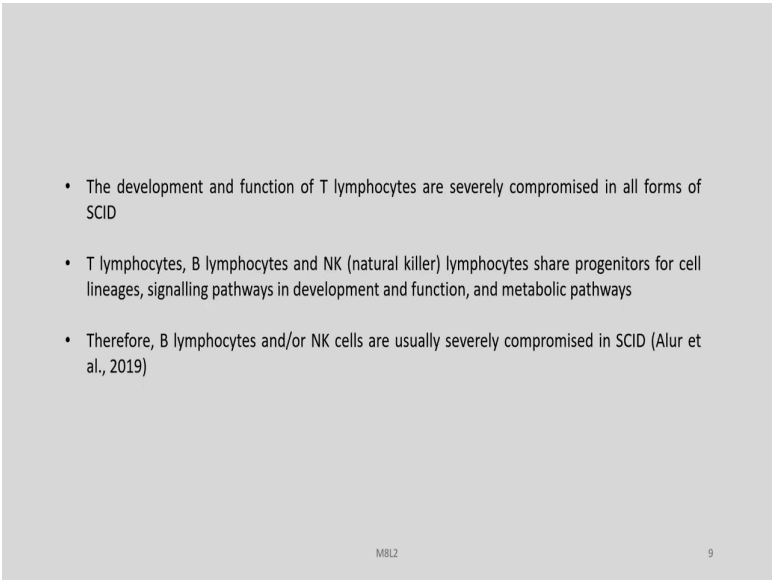
(Refer Slide Time: 11:03)



What are the signs and symptoms of a SCID? The SCID effects the infants with very serious life threatening infections by both common and opportunistic pathogens because, we know that the immune system cells are involved and hampered. The infants are generally well until 2 months of birth as residual transplacental maternal immunoglobin Gs are present in them. However, some infants with SCID still may show slight cutaneous signs similar to graft versus host disease caused by transplacental maternal T cells due to impaired immunity.

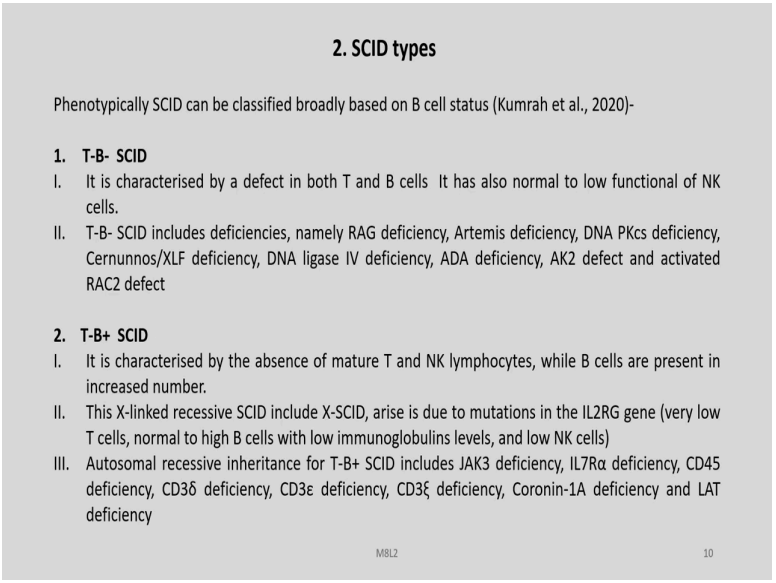
The most common clinical manifestations observed are pneumonia, failure to thrive, chronic diarrhoea, a gastrointestinal infection and oral candidiasis. There are always severe change of losing life within two years, sorry retake. Let us start. There are always severe chance of losing life within two years of life if therapy to reconstitute immune system is not undertaken.

(Refer Slide Time: 12:10)



The development and function of T lymphocytes are severely compromised in all forms of a SCID. T lymphocytes, B lymphocytes and NK killer cells share progenitors for cell lineages, signalling pathways in development and function and metabolic pathways. Therefore, B lymphocytes and or NK cells are usually severely compromised in a SCID as already discussed.

(Refer Slide Time: 12:34)

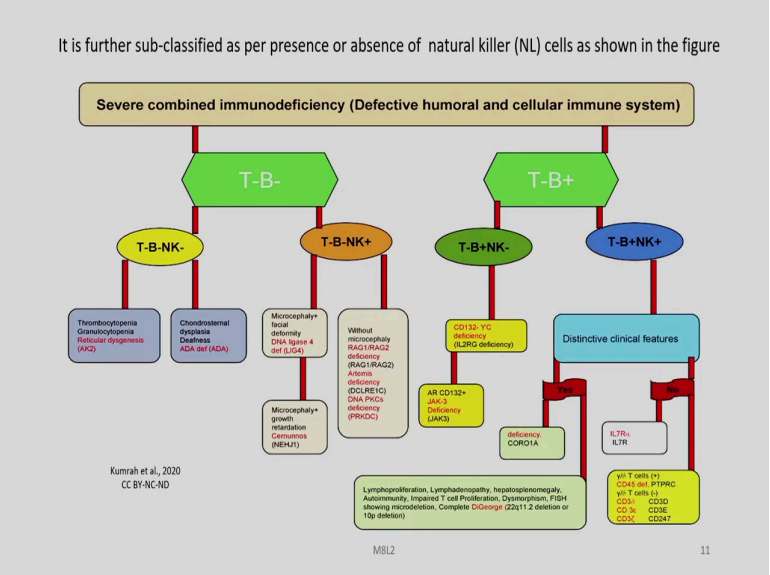


What are the various types of SCID? Phenotypically, it can be classified broadly based on B cell status T dash or T minus B minus SCID. It is characterized by a defect in both T and B

cells. It is also normal to low functional levels of NK cells. T minus B minus SCID includes deficiencies namely RAG deficiency, Artemis deficiency, DNA PKCs deficiency, Cernunnos XLF deficiency, DNA ligase IV deficiency, ADA deficiency, AK2 defect and activated RAC2 defect.

The second type is T minus B plus SCID. It is characterized by the absence of T and NK lymphocytes, while B cells are present in increased numbers. This X-linked recessive SCID includes X-SCID and arise due to mutations in the IL-2RG gene. Autosomal recessive inheritance of T minus B plus SCID includes JAK3 deficiency, IL7RA deficiency, CD45 deficiency, CD3 delta deficiency, CD3 epsilon deficiency etcetera.

(Refer Slide Time: 13:56)



It is further classified or sub classified as per the presence or absence of natural killer cells, as can be seen in this figure. T dash T minus B minus, it can be T minus B minus NK minus or it can be T minus B minus NK plus. In the case of T minus B plus, it can be T minus B plus NK minus and T minus B plus NK plus.

So, in the T minus B minus NK minus, you can see thrombocytopenia, granulocytopenia and reticular dysgenesis AK2 or chondrosternal dysplasia, a deafness, other deficiency. While, in the T minus B minus NK plus we can see microcephaly plus facial deformity, DNA ligase 4 deficiency, then microcephaly growth retardation. And, then without microcephaly RAG1, RAG2 deficiency, Artemis deficiency, DNA PKCs deficiency.

And, on the other side where we have the T minus B plus type and in the case of NK minus there is CD132 deficiency, IL-2RG deficiency and then JAK3 deficiency. While, in the case of NK plus, we have a CORO1A and IL7R alpha. Then there is gamma delta T cells or CD45 deficiency, CD epsilon and so on.

(Refer Slide Time: 15:57)

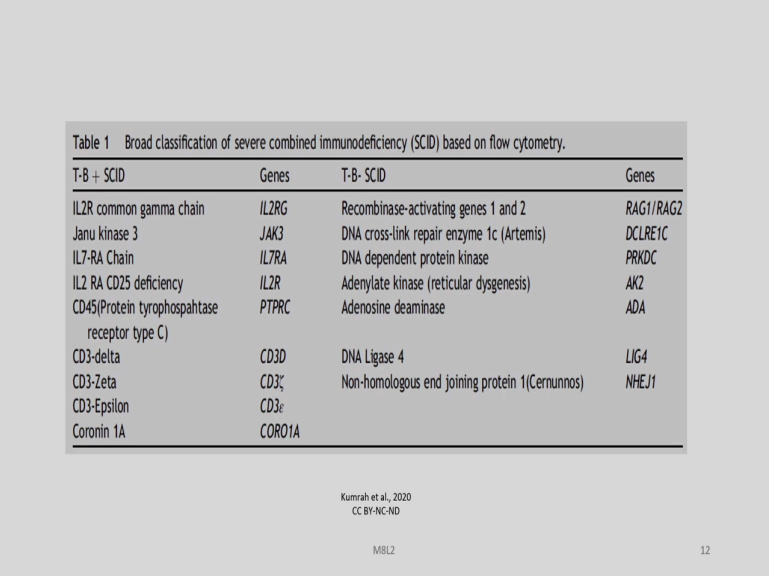


Table 1 Broad classification of severe combined immunodeficiency (SCID) based on flow cytometry.

T-B + SCID	Genes	T-B- SCID	Genes
IL2R common gamma chain	*IL2RG*	Recombinase-activating genes 1 and 2	*RAG1/RAG2*
Janu kinase 3	*JAK3*	DNA cross-link repair enzyme 1c (Artemis)	*DCLRE1C*
IL7-RA Chain	*IL7RA*	DNA dependent protein kinase	*PRKDC*
IL2 RA CD25 deficiency	*IL2R*	Adenylate kinase (reticular dysgenesis)	*AK2*
CD45(Protein tyrophospahtase receptor type C)	*PTPRC*	Adenosine deaminase	*ADA*
CD3-delta	*CD3D*	DNA Ligase 4	*LIG4*
CD3-Zeta	*CD3ζ*	Non-homologous end joining protein 1(Cernunnos)	*NHEJ1*
CD3-Epsilon	*CD3ε*		
Coronin 1A	*CORO1A*		

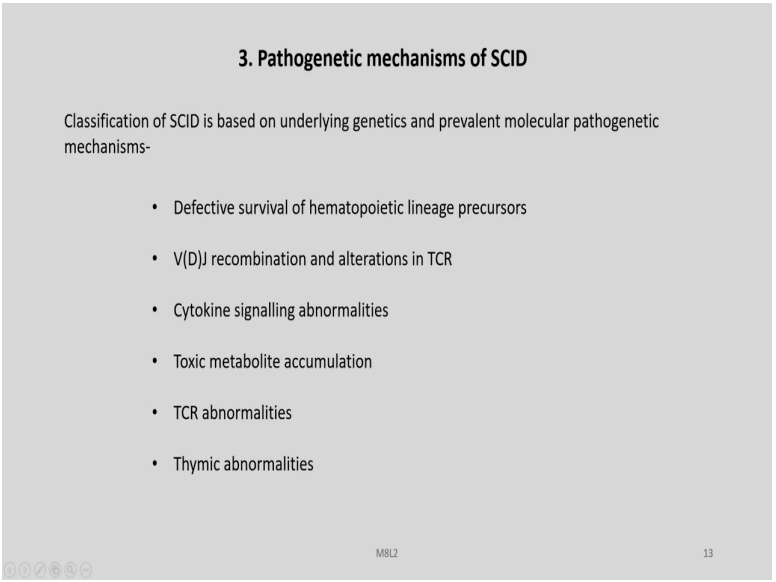
Kumrah et al., 2020
CC BY-NC-ND

M8L2 12

This table gives us a broad classification of severe combined immunodeficiency based on flow cytometry. So, this is the T minus B plus SCID and the genes involve in the particular class. And, this is here we can see the T minus B minus SCID and the respective genes which are involved.

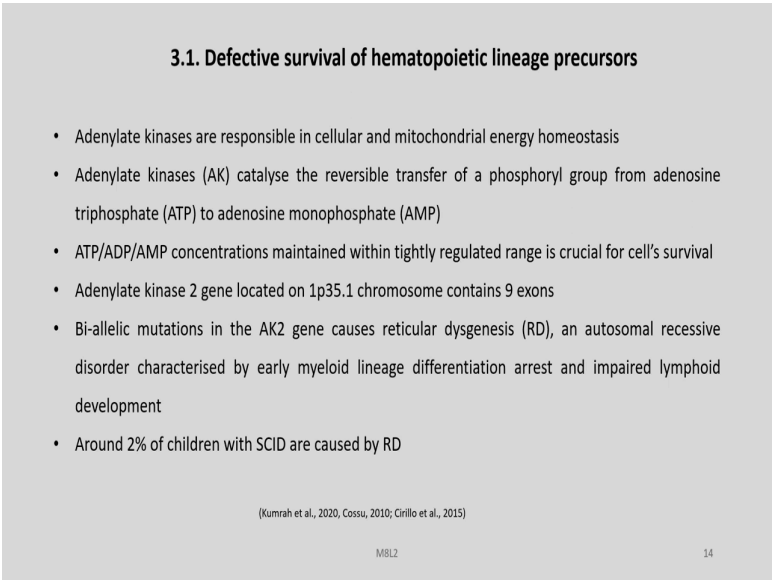
So, IL2R common gamma chain type and the Janu kinase 3 or JAK3. And, then similarly we have CD3 epsilon, CD3 zeta, CD3 delta and so on. Similarly, on the other side we have RAG1 or recombinase activating genes 1 and 2, DNA cross link repair enzyme 1c or Artemis, DNA dependent protein kinase PRKDC, adenosine deaminase ADA and so on.

(Refer Slide Time: 17:05)



What are the pathogenic mechanisms involved in the development of SCID? So, we now know that classification of SCID is based on underlying genetics and prevalent molecular pathogenetic mechanisms. Defective survival of the hematopoietic lineage precursors, VDJ recombination and alterations in T cell receptors, cytokine signalling abnormalities, toxic metabolite accumulation, TCR abnormalities, thymic abnormalities.

(Refer Slide Time: 17:38)

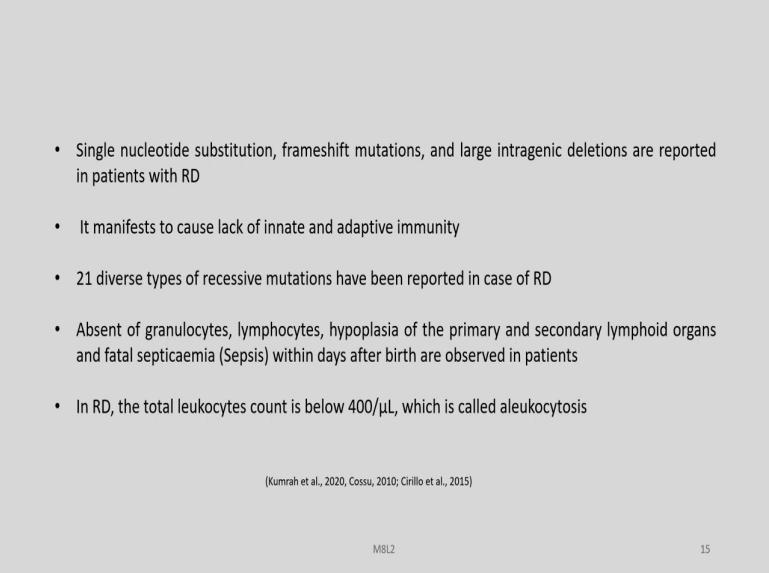


Let us discuss about the defective survival of hematopoietic lineage precursors. Adenylate kinases are responsible in cellular and mitochondrial energy homeostasis. Adenylate kinase

catalyses the reversible transfer of a phosphoryl group from adenosine triphosphate to adenosine monophosphate. ATP, ADP, AMP concentrations maintained within tightly regulated range is crucial for cells survival.

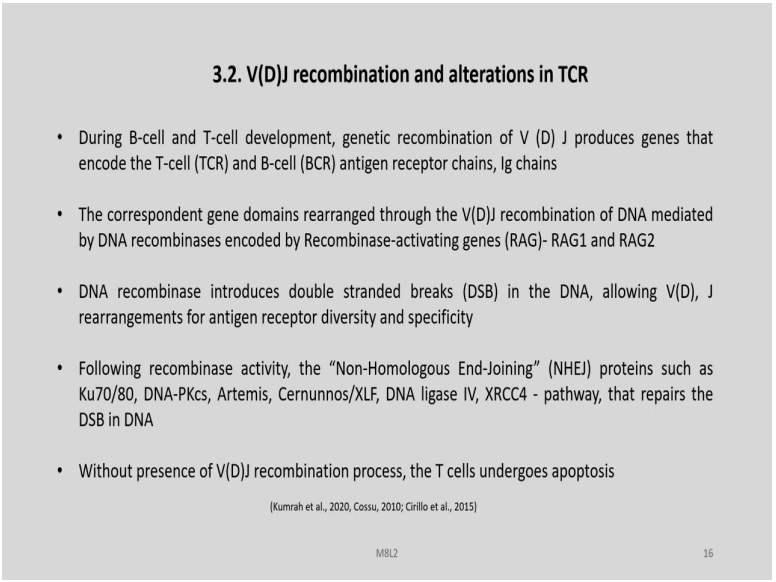
Adenylate kinase 2 gene located on 1p35.1 chromosome contains 9 exons. Biallelic mutations in the AK2 gene causes reticular dysgenesis, an autosomal recessive disorder characterized by early myeloid lineage differentiation arrest and impaired lymphoid development. Around 2 percent of children with SCID are caused by reticular dysgenesis.

(Refer Slide Time: 18:44)



Single nucleotide substitution, frame shift mutations and large intragenic deletions are reported in patients with RD. It manifest to cause lack of innate and adaptive immunity. 21 diverse types of recessive mutations have been reported in the case of RD. Absence of granulocytes or lymphocytes, hyperplasia of the primary and secondary lymphoid organs and fatal septicaemia within days after birth are observed in patients. In RD, the total leukocyte count is below 400 per micro litre which is called as aleukocytosis.

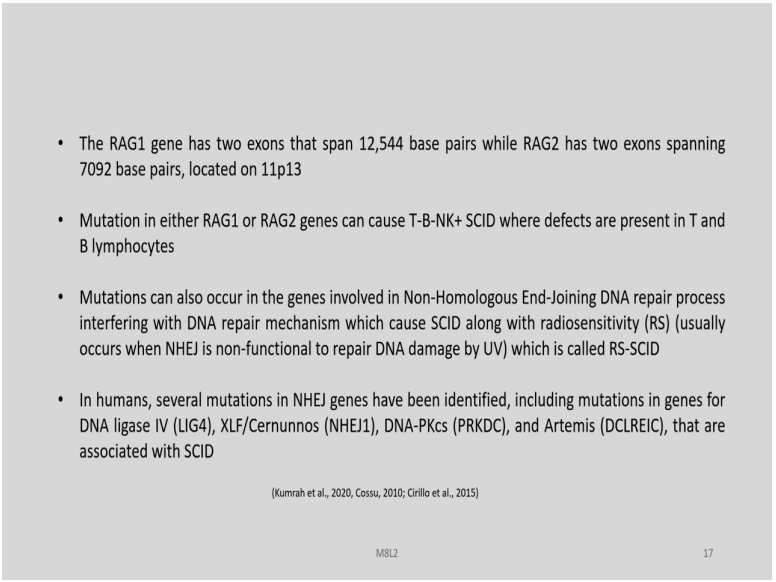
(Refer Slide Time: 19:30)



V(D)J recombination and alterations in TCR. During B cell and T cell development, genetic recombination of V(D)J produces genes that encode the T cell and B cell antigen receptor chains and Ig chains. The correspondent gene domains rearranged through the V(D)J recombination of DNA mediated by DNA recombinases encoded by recombinase activating genes RAG1 and RAG2.

DNA recombinase introduces double stranded breaks in the DNA, allowing V(D)J rearrangement for antigen receptor diversity and specificity. Following the recombinase activity, the non-homologous end joining proteins such as Ku70 oblique 80 DNA-PKcs, Artemis, Cernunnos XLF, DNA ligase IV, XRCC 4 pathways that repairs the DSB in DNA. Without presence of V(D)J recombination process, the T cells undergoes apoptosis.

(Refer Slide Time: 20:47)

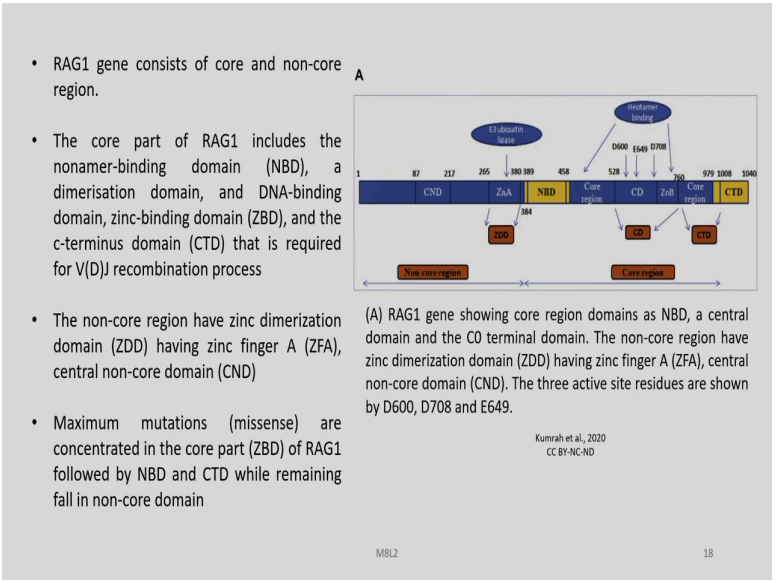


The RAG1 gene has two exons that span 12,544 base pairs while RAG2 has two exons spanning 7092 base pairs, located on 11p13. Mutations in either RAG1 or RAG2 genes cause a T minus B minus NK plus SCID whereas, defects are present in T and B lymphocytes.

Mutations can also occur in the genes involved in non-homologous end joining DNA repair process interfering with DNA repair mechanisms which causes SCID along with radio sensitivity which usually occurs when a non-homologous end joining is non-functional to repair DNA damage by UV which is called RS-SCID.

In humans, several mutations in NHEJ genes have been identified, including mutations in genes for DNA ligase IV, XLF Cernunnos, DNA PKCs and Artemis, that are associated with a SCID.

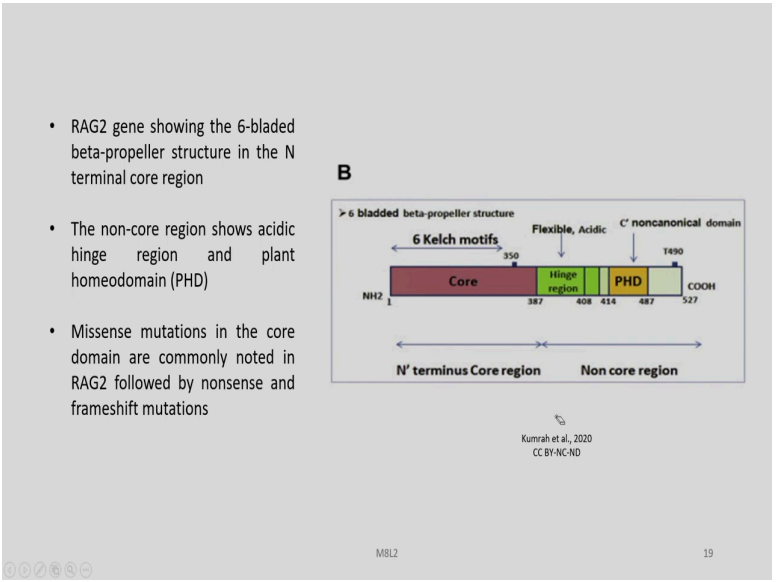
(Refer Slide Time: 21:55)



So, in this picture you can see RAG1 gene showing core region domains as NBD, a central domain and C0 domain. The non-core region have a zinc dimerization domain or ZDD having a zinc finger A, central non-core domain CND. The three active site residues are shown by D600, D708 and E649.

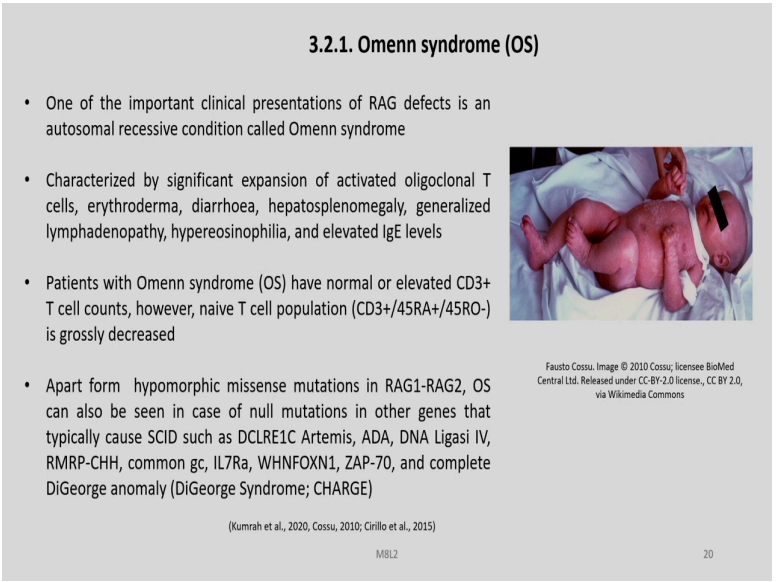
So, we have already shown in the picture RAG1 gene consists of core and non-core regions and the NBD, then ZBD and CTD which are required for V(D)J a recombination process. The non-core region have zinc dimerization domain and central non-core domain. Maximum mutations or missense are concentrated in the core part ZBD of RAG1 followed by NBD and CTD while remaining fall in non-core domain.

(Refer Slide Time: 23:23)



RAG2 gene showing the 6-bladed beta propeller structure in the N terminal core region. The non-core region shows acidic hinge region and plant homeodomain, the PHD. Missense mutations in the core domain are commonly noted in RAG2 followed by nonsense and a frameshift mutations.

(Refer Slide Time: 23:55)

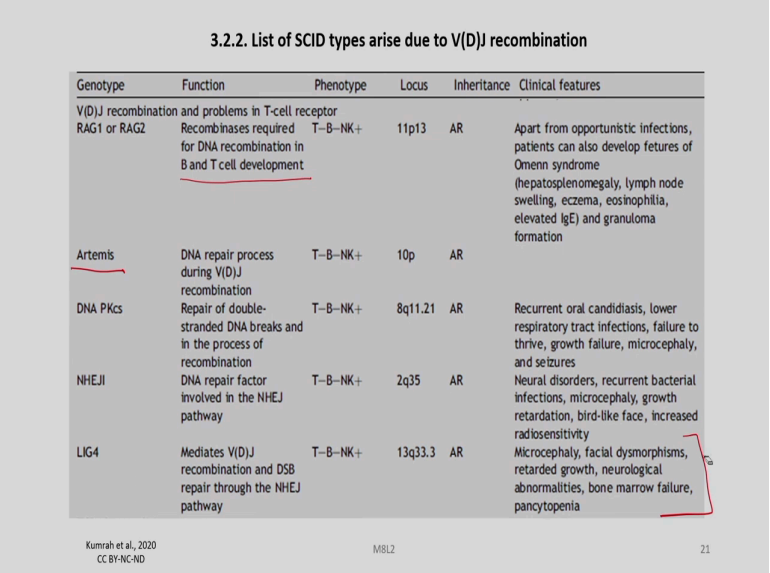


Let us discuss about Omenn syndrome or OS which is one of the important clinical presentations of RAG defect and it is a autosomal recessive condition which is characterized by significant expansion of activated oligoclonal T cells, erythroderma, diarrhoea,

hepatosplenomegaly, generalized lymphadenopathy, hypereosinophilla and elevated IgE levels.

Patients with Omenn syndrome have normal or elevated CD3 plus T cell counts, however naive T cell population is grossly decreased. Apart from hypomorphic missense mutations in RAG1 RAG2, OS can also be seen in case of null mutations in other genes that typically cause SCID such as DCLRE1C Artemis, ADA, DNA ligase IV, RMRP-CHH, common gc, IL7RA etcetera and complete DiGeorge anomaly.

(Refer Slide Time: 25:10)



This table shows a list of SCID types which arise due to V(D)J recombination. So, we have the genotype in column 1, phenotype in column 3 and the locust in column 4 and the in clinical features in the last column. So, for every genotype there are certain functions associated. In V(D)J recombination and problems in T cell receptor, RAG1 or RAG2 genotype is represented by T minus B minus NK plus.

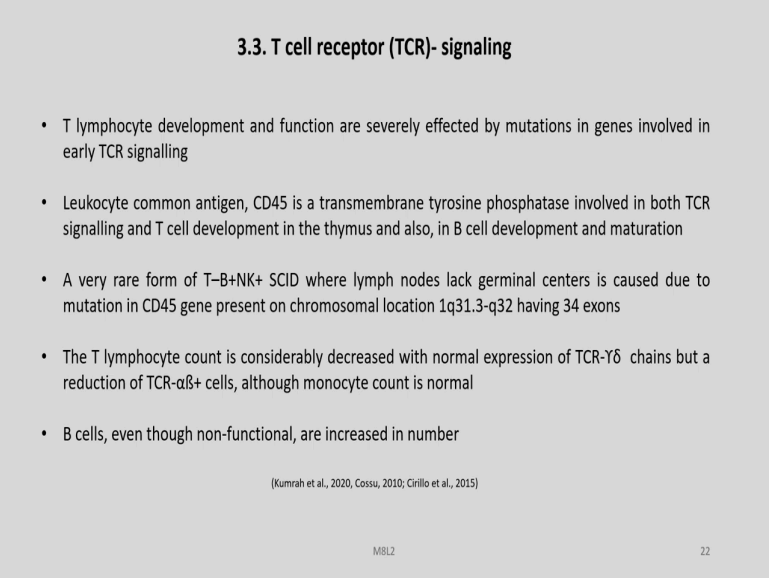
And, its function is the recombination is required for DNA recombination in B and T cell development. And, the clinical features of this type of a phenotype is the opportunistic infections are there in the patient and patient also develop features of the Omenn syndrome.

In Artemis genotype, DNA repair process during V(D)J recombination has the phenotype T minus B minus NK plus, lies in the locus 10p. And, similarly in the case of DNA PKCs which is involved in the function repair of double stranded DNA breaks and in the process of

recombination. There will be recurrent oral candidiasis, a lower respiratory tract infections, failure to thrive, growth failure, a microcephaly and even seizures.

Other genotypes like NHEJI, DNA repair a factor involved in the NHEJ pathway. Here neural disorders, recurrent bacterial infections, microcephaly, growth retardation, bird like face, increased radio sensitivity are the marked clinical features. In the case of LIG4, the function is the mediation of V(D)J recombination and DSB repair through the NHEJ pathway. The clinical features are microcephaly, facial dysmorphisms, retarded growth, neurological abnormalities, a bone marrow failure, pancytopenia.

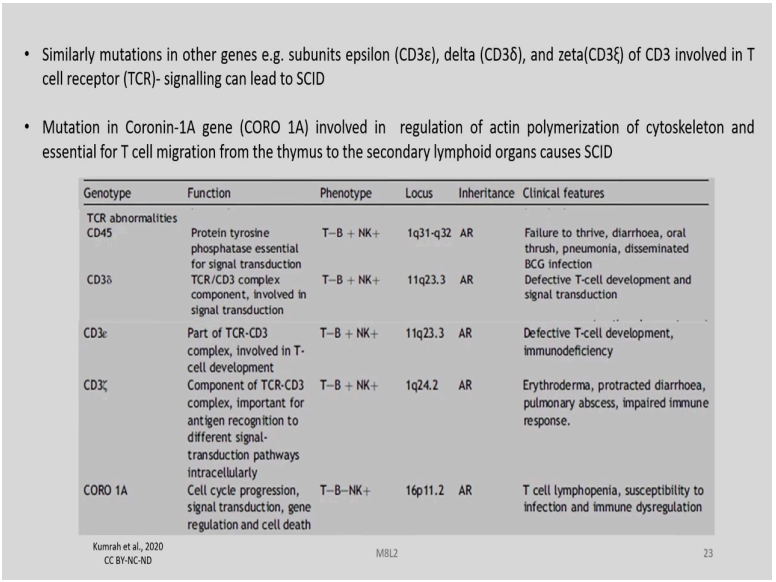
(Refer Slide Time: 27:54)



T cell receptor signalling. T lymphocyte development and functions are severely affected by mutations in genes involved in early TCR signalling. A leukocyte common antigen, CD45 is a transmembrane tyrosine phosphatase involved in both TCR signalling and T cell development in the thymus and also in B cell development and a maturation.

A very rare form of T minus B plus NK plus SCID where lymph nodes lack germinal centers is caused due to mutation in CD45 gene present on chromosomal locations 1q31.3-q32 having 34 exons. The T lymphocyte count is considerably decreased with normal expression of TCR gamma delta chains, but the reduction of TCR alpha beta plus cells, although monocyte count is normal. B cells even though non-functional, are increased in number in this case.

(Refer Slide Time: 28:59)

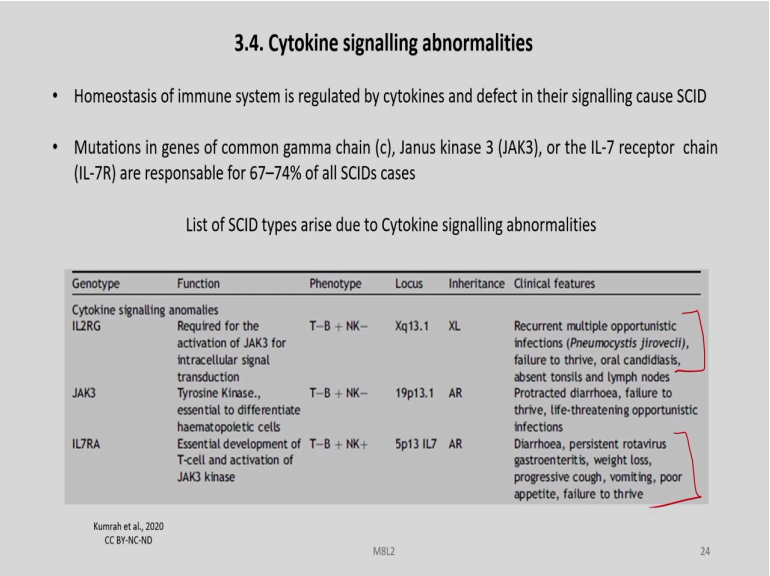


Similarly, mutations in other genes example subunits epsilon delta and zeta of CD3 involved in T cell receptor signalling can lead to the SCID. Mutation in Coronin 1A gene involved in regulation of actin polymerization of cytoskeleton and essential for T cell migration from the thymus to the secondary lymphoid organs also causes a SCID.

In this table, we have the typical genotype and the corresponding phenotype and locus and the inheritance and the clinical features described. For example, in the genotype CD45, the function is the protein tyrosine phosphatase essential for signal transduction. And, due to the phenotype T minus B plus NK plus, there is failure to thrive, diarrhoea, oral thrush, pneumonia, disseminated BCG infection and so on.

So, with a different genotypic representation, we have varied and certain common clinical features. For example, here we have both defective T cell development and signal transduction in this case and immunodeficiency. And, then in the next case, we have the erythroderma, protracted diarrhoea, pulmonary abscess, impaired immune response. In the last case, a CORO1 a we have T cell lymphopenia, susceptibility to infection and immune dysregulation.

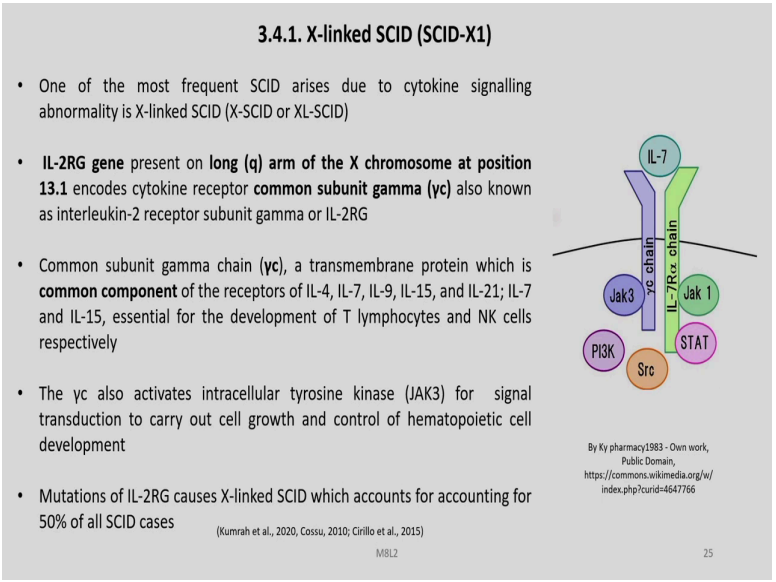
(Refer Slide Time: 31:04)



Let us discuss about the cytokine signalling abnormalities. Homeostasis of immune system is regulated by a cytokines and defect in their signalling causes a SCID. Mutations in genes of common gamma chain, Janus kinase 3 or the interleukin 7 receptor chain are responsible for as high as 67 to 74 percent of all SCID cases. In the table, we can see the list of a SCID types which arise due to cytokine signalling abnormalities involving the genotypes; IL-2RG, JAK3, IL7RA.

And, the corresponding clinical features like recurrent multiple opportunistic infections, a failure to thrive, oral candidiasis, then absent tonsils and lymph nodes. In the second case of JAK3, we have protracted diarrhoea, failure to thrive, life threatening opportunistic infections. And, IL7RA there is diarrhoea, persistent rotavirus gastroenteritis, weight loss, progressive cough, vomiting, poor appetite and failure to thrive.

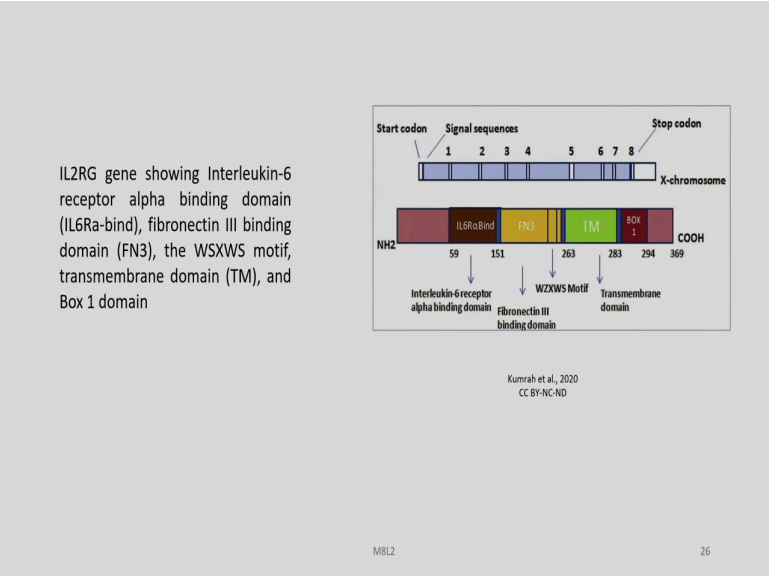
(Refer Slide Time: 32:25)



Let us discuss about the X-linked SCID or SCID X1. So, one of the most frequent SCID arise due to cytokine signalling abnormalities is X-linked SCID. IL-2RG gene present on a long q arm of the X chromosome at position 13.1 encodes a cytokine receptor common unit gamma or gamma c also known as interleukin-2 receptor subunit gamma or IL-2RG.

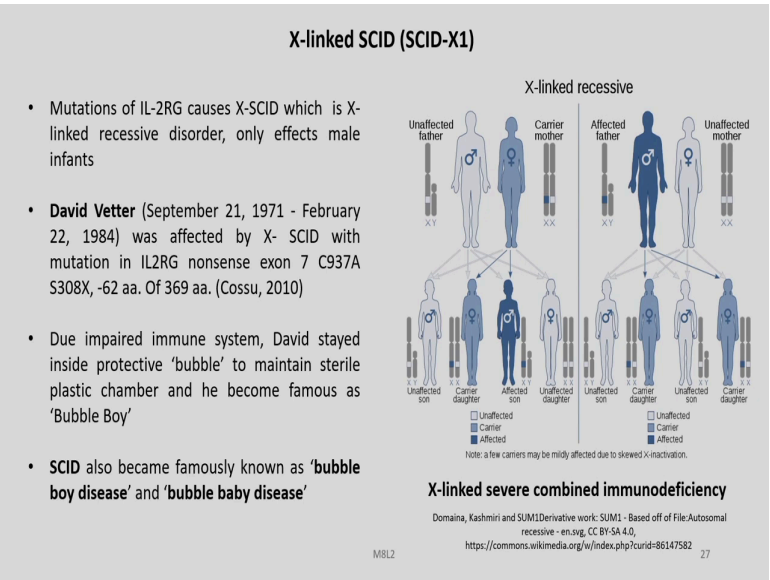
Common subunit gamma chain, a transmembrane protein which is component of the receptors of IL4, 7, 9, 15, 21; IL7 and IL15 essential for the development of T lymphocytes and a NK cells respectively. The gamma c also activates intracellular tyrosine kinase JAK3 for signal transduction to carry out cell growth and control of hematopoietic cell development. Mutations of IL-2RG causes X-linked SCID which accounts for 50 percent of all SCID cases.

(Refer Slide Time: 33:42)



IL2RG gene showing interleukin 6 receptor alpha binding domain IL6R alpha bind here and then fibronectin III binding domain FN3 and WSXWS motif, transmembrane domain TM and the Box 1 domain as shown in this picture.

(Refer Slide Time: 34:15)



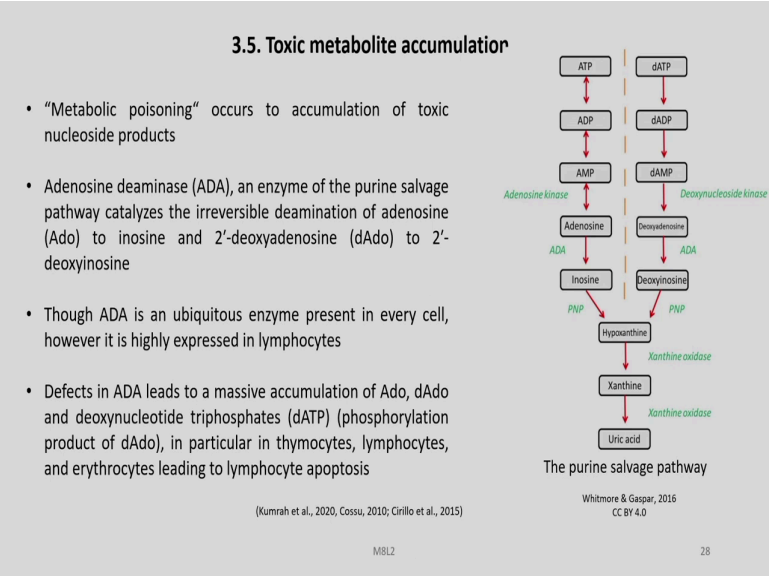
So, here we see the X-linked recessive inheritance which we have discussed earlier. When an unaffected father and a carrier mother is there, the inheritance pattern is one unaffected son, one carrier daughter, one affected son and one unaffected daughter. While, in the case of an

affected father and unaffected mother, we have unaffected son two and then we have a carrier daughters two. So, this is 1 is to 1 unaffected son is to a carrier daughter.

The mutations of IL-2RG causes X SCID which is X-linked recessive disorder and it affects male infants only as you can see over here. David Vetter was affected by X-SCID with mutation in IL2RG nonsense exon C937A S308X minus 62 amino acid of 369 amino acid, as reported by Cossu and, and somewhere around September 21, 1971 to 22, 1974.

Due to the impaired immune system, David stayed inside protective bubble to maintain sterile plastic chamber and he became famous as the 'Bubble Boy'. A SCID also became famously known as bubble boy disease due to these and bubble baby disease.

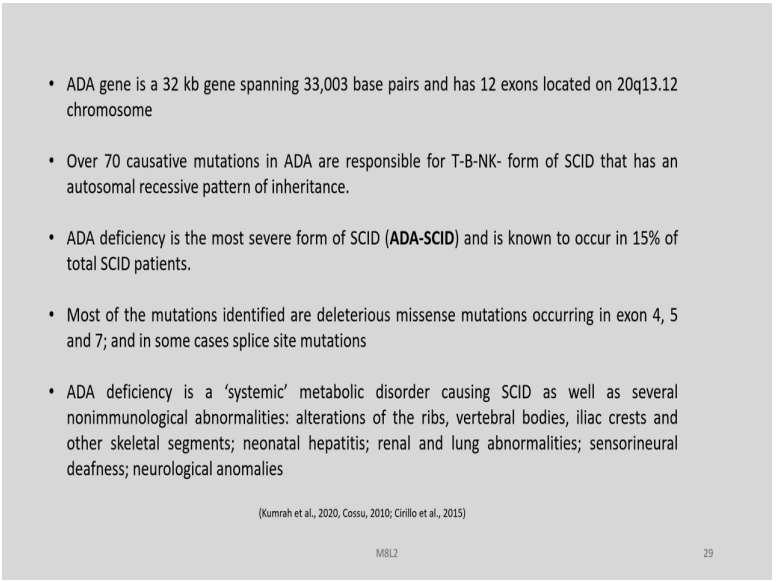
(Refer Slide Time: 36:19)



Accumulation of toxin toxic metabolites or metabolite poisoning occurs due to these accumulation of toxic nucleoside products. Adenosine deaminase ADA is an enzyme of the purine salvage pathway which catalyzes the irreversible deamination of adenosine to inosine and 2 deoxyadenosine to 2 deoxy inosine as shown in this figure.

Though ADA is an unique enzyme present in every cell; however, it is highly expressed in lymphocytes. Defects in ADA leads to massive accumulation of Ado or adenosine, dAdo and deoxynucleotide a triphosphates dATP, in particular in thymocytes, lymphocytes and erythrocytes leading to lymphocyte apoptosis.

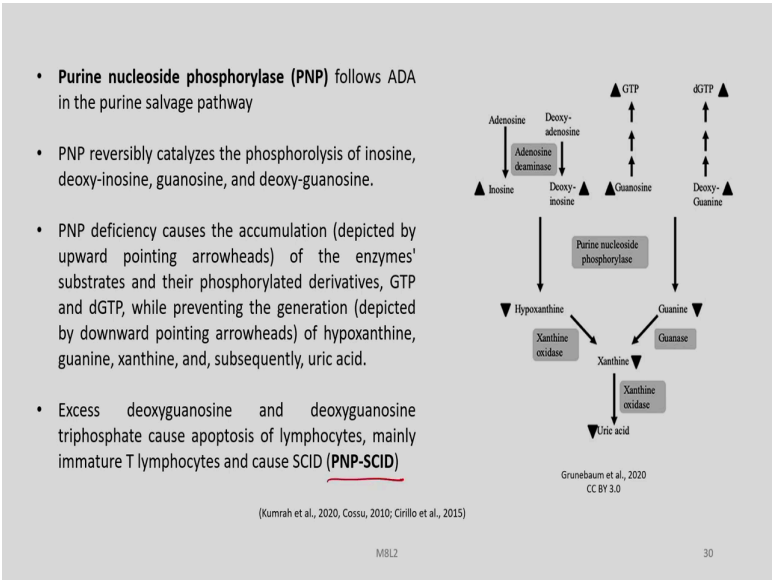
(Refer Slide Time: 37:33)



ADA gene is a 32 kb gene spanning 33,003 base pairs and has 12 exons located on 20q13.12 chromosome. Over 70 causative mutations in ADA are responsible for T minus B minus NK minus form of SCID that has an autosomal recessive pattern of inheritance. ADA deficiency is the most severe form of a SCID, ADA-SCID and is known to occur in 15 percent of total SCID patients.

Most of the mutations identified are deleterious missense mutations occurring in exon 4, 5 and 7; in some cases splice site mutations. ADA deficiency is a systemic metabolic disorder causing SCID as well as several non-immunological abnormalities: alterations of the ribs, vertebral bodies, iliac crests and other skeletal segments, neonatal hepatitis, renal and lung abnormalities, sensorineural deafness, a neurological anomalies are also common.

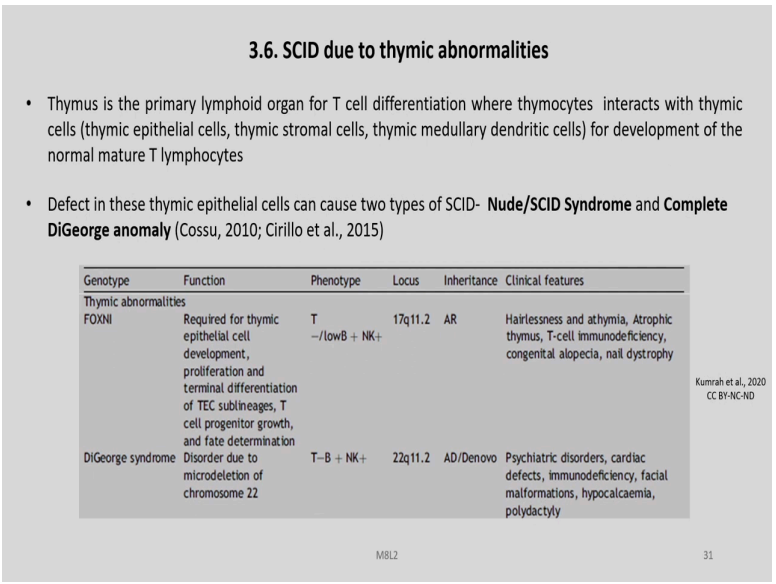
(Refer Slide Time: 38:42)



Purine nucleoside phosphorylase PNP follows ADA in the purine salvage pathway. PNP reversibly catalyzes the phosphorolysis of inosine, deoxy-inosine, guanosine and deoxy guanosine. And, its deficiency causes the accumulation depicted by upward pointing arrows here of the enzymes, substrates and their phosphorylated derivatives, GTP and dGTP.

While, preventing the generation of hypoxanthine, guanine, xanthine and subsequent uric acid. Excess deoxy guanosine and deoxyguanosine triphosphate causes apoptosis of lymphocytes, mainly immature T lymphocytes and cause SCID or PNP-SCID.

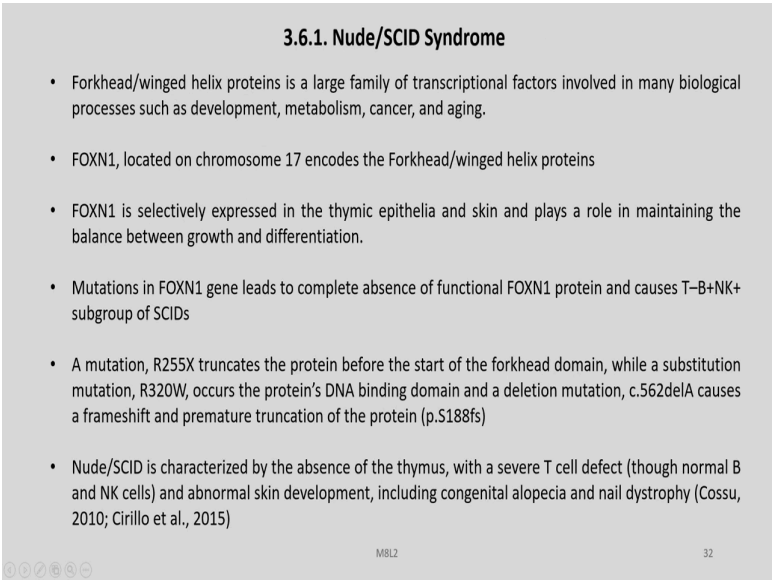
(Refer Slide Time: 40:06)



SCID due to thymic abnormalities. Thymus is the primary lymphoid organ of T cell differentiation where thymocytes interact with thymic cells for development of the normal mature T lymphocytes. Defects in these thymic epithelial cells can cause two types of SCID, a nude SCID syndrome and complete DiGeorge anomaly.

So, in this table we can see the genotypes of thymic abnormalities, FOXNI or DiGeorge's syndrome which are having the corresponding phenotype like T minus low B plus NK plus or T minus B plus NK plus. And, corresponding clinical features like hairlessness and athymia, atrophic thymus, T cell immunodeficiency, congenital alopecia, nail dystrophy. And, in the case of DiGeorge syndromes, we have psychiatric disorders, cardiac defects, immunodeficiency, facial malformations, hypocalcaemia, polydactyly.

(Refer Slide Time: 41:24)

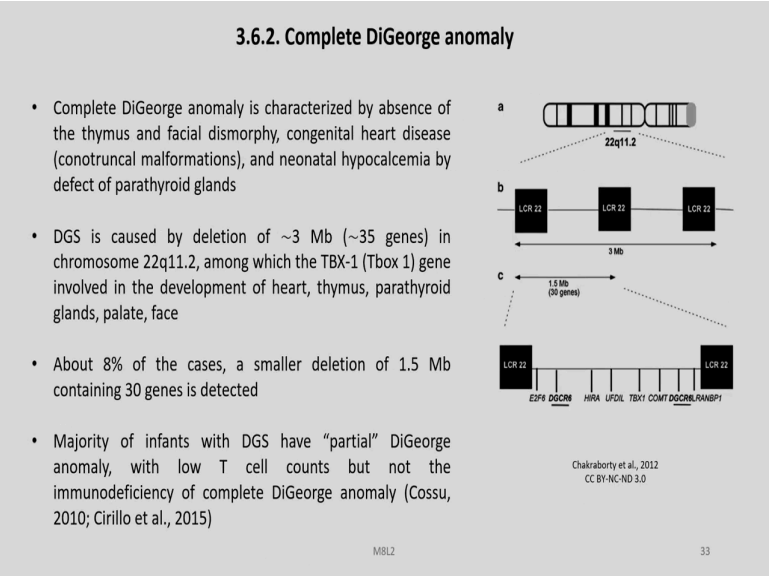


Nude SCID syndromes: forkhead, winged helix proteins is a large family of transcriptional factors involved in many biological processes such as development, metabolism, cancer and aging. FOXN1 located on chromosome 17 encodes the forkhead winged helix proteins.

FOXN1 is selectively expressed in the thymic epithelia and skin and plays a role in maintaining the balance between growth and differentiation. Mutations in FOXN1 gene leads to complete absence of functional FOXN1 protein and causes T minus B plus NK plus subgroups of SCIDs.

A mutation R255X truncates the protein before the start of the forkhead domain, while a substitution mutation R320W, occurs in the protein's DNA binding domain and a deletion mutation, c.562delA causes a frameshift and premature truncation of the protein. Nude SCID is characterized by the absence of the thymus, with a severe T cell defect and abnormal skin development, including congenital alopecia and nail dystrophy.

(Refer Slide Time: 42:44)



The complete DiGeorge anomaly is characterized by absence of the thymus and facial dismorphy, congenital heart diseases and neonatal hypocalcemia by defect of parathyroid glands. DGS is caused by deletion of around 3 Mb corresponding to around 35 genes in chromosome 22q11.2, among which the TBX-1 Tbox1 gene involved in the development of heart, thymus, parathyroid thyroid glands, palate and a face.

About 8 percent of the cases, a smaller deletion of 1.5 Mb containing 30 genes is detected. Majority of infants with DGS have partial DiGeorge anomaly, with low T cell counts, but not the immunodeficiency of complete DiGeorge anomaly. With this, we come to end of part A of the SCID disease. We will continue this discussion in part B of this lecture.

Thank you.