Chapter 2

Review of Literature

2.1 Virus:

Viruses are unique group of infectious agents with a simple, acellular organization and pattern of reproduction. A virus replicates only inside living cells of an organism. Viruses can infect all types of life, from animals and plants to microorganisms and are found in almost all of the Earth's ecosystems. Viruses are known to have either RNA or DNA as their genetic material and the nucleic acid can be either single- or double-stranded. The entire particle of the virus, called the virion, is composed of the nucleic acid and an outer layer of protein coat called 'capsid'. It may also contain an additional outer-layer derived from host cell-membranes. A virion thus, is either naked or an enveloped nucleocapsid. The structure containing only the nucleic acid and the capsid is called 'nucleocapsid' and without the outer layer is considered 'naked'. The capsid is composed of several copies of a single type of protein or few different types of proteins each being encoded by a single viral gene. Due to this, a virus is able to encode all the information required to make a relatively large capsid in very small number of genes. The envelope may contain carbohydrates, lipids, and proteins of viral origin.

Viruses are known to infect a wide range of hosts. Virus that infects only bacteria is called a bacteriophage; viruses that infect animal or plant cells are generally called animal or plant viruses. Some viruses can proliferate in both plants and insects that feed on them. Animal viruses do not normally cross phyla, however, in some cases, they are found to infect only closely related species such as the primates. The host-cell range of some animal viruses is further restricted to a limited number of cell types since only these cells have suitable surface receptors to which virions can attach and have permissive mileu where viruses can proliferate (Lodish *et al*., 2000).

Most viruses cause disease conditions in humans. It can be either benign infection or some severe conditions like cancer, polio and AIDS. At the cellular level, an infection is the introduction of an entity into cell and its ability to reproduce itself. A viral infection in the host cell is initiated by interactions between viral capsid or envelope proteins and usually specific cell-surface receptors. The virion then enters either by fusion of the envelope and the plasma membrane or via endocytic pathways (Smith and

Helenius, 2004; Marsh and Helenius, 2006). Attachment to cellular receptors usually triggers conformational changes in virus surface structures or can activate specific signaling pathways that facilitate entry of virus into the cell. Virus uses host-cell machinery for its genome replication, protein synthesis, assembly of viral particles and release of virions from the cell. The release of the virion occurs via budding followed by exocytosis or cell lysis (Marsh and Helenius, 2006).

Classification of viruses consists of naming viruses and placing them in a taxonomic category. Viruses are primarily classified based on their phenotypic characteristics, such as morphology, type of nucleic acid, mode of replication, host organisms and the type of disease they cause. The formal taxonomic classification of viruses is done by International Committee on Taxonomy of Viruses (ICTV) which is responsible for designing and implementing rules for naming and classification of viruses. The viral classification starts at the level of realm and continues as follows: Realm, Sub-realm, Kingdom, Sub-kingdom, Phylum, Sub-phylum, Class, Subclass, Order, Suborder, Family, Subfamily, Genus, Subgenus and Species. More than 30,000 different virus isolates are known today and are grouped into more than 6590 species, 1421 genera and 168 families (Virus Taxonomy 2019 release). Baltimore classification system, named after David Baltimore (first defined in 1971) can also be used to group viruses into one of seven groups based on the type of nucleic acid (DNA or RNA), strandedness (single or double) and on the method of replication. The seven groups under the Baltimore classification are: dsDNA viruses, ssDNA viruses, dsRNA viruses, (+)ssRNA viruses, (-)RNA viruses, ssRNA-RT viruses, dsDNA-RT viruses.

2.2 DNA Virus:

DNA viruses have DNA as their genetic material that is replicated using either host or virally encoded DNA polymerase. Nucleic acid is usually double stranded DNA (dsDNA) but can also be single stranded DNA (ssDNA). The genomes of DNA viruses that infect animals vary in size from less than 2 kb of single-stranded DNA to more than 375 kb of double-stranded DNA. Some larger DNA viruses are known that infect eukaryotic microorganisms (Payne, 2017). The genome can either be circular or linear.

Some of the larger DNA viruses encode their own DNA polymerases, whereas the smaller ones depend on host cell machinery for replication. DNA viruses contain genes that are expressed separately in early and late phases. Early transcription occurs before DNA replication to provide for the protein products needed during the synthesis and are termed as “early genes”. After DNA replication, gene expression of structural proteins is initiated needed to package DNA and form virions. These genes are referred to as "late genes" (Payne, 2017).

Infections with DNA virus can either be acute, which leads to life-threatening diseases or chronic, that results in the persistent infection through manipulation of host immune responses. DNA viruses are known to cause a wide variety of diseases including cancer in Humans. Human papillomavirus (HPV) leads to the development of cervical cancer and head and neck cancers (Gillison *et al*., 2015). Chronic hepatitis B virus (HBV) infection leads to liver cancer (Masrour-Roudsari and Ebrahimpour, 2017). Infection with Varicella-zoster virus (VZV) leads to chicken pox and in some cases reactivation of the virus may cause shingles (Gershon and Gershon, 2013). Epstein-Barr virus (EBV) is associated with various types of cancers that include Burkitt lymphoma, Hodgkin lymphoma, post-transplant lymphomas, gastric cancers, and almost all endemic nasopharyngeal carcinomas (Taylor *et al*., 2015).

2.3 Polyomavirus:

Polyomaviruses (PyVs) belong to the *Polyomaviridae* family. They are small, non-enveloped, icosahedral, double-stranded small DNA viruses having a genome size of about 5000 base pairs. Characteristics of the members of the *Polyomaviridae* family are described in **Table 1**. The *Polyomaviridae* family is classified as a Group I virus (dsDNA virus) as per Baltimore classification. Polyomaviruses and papillomaviruses used to be clubbed together under the *Papovaviridae* family as they shared similarities in structural features but have different genomic organizations. In October 2010, the International Committee on Taxonomy of Viruses (ICTV) recommended dividing the Polyomaviridae family into three genera. Genus Orthopolyomavirus and genus Wukipolyomavirus thus contained the mammalian species and genus Avipolyomavirus contained the avian

species. As of 2019, the ICTV classification has recognized four genera and 102 species, out of which nine could not be assigned a genus. The four genera are *Alphapolyomavirus*, *Betapolyomavirus*, *Gammapolyomavirus* and *Deltapolyomavirus*. Members of these genera can infect mammals and birds, and recently been found in fish also (Moens *et al*., 2017). Each member of the family has a restricted host range. Fourteen (14) out of these are known to infect humans. This system maintains the distinction between avian and mammalian viruses, grouping the avian subset in the genus Gammapolyomavirus (Virus Taxonomy 2019 release).

Table 1: Characteristics of the members of the family *Polyomaviridae*

Characteristics	**Description**
Virion	Non-enveloped; 40–45 nm; icosahedral
Genome	Approximately 5 kbp; circular dsDNA
Host Range	Mammals, birds and fish
Taxonomy	Realm *Monodnaviria,* Kingdom *Shotokuvirae*, Phylum *Cossaviricota*, Class *Papovaviricetes*, Order *Sepolyvirales*; four Genera containing 102 species

Note: Adapted from Moens *et al.,* 2017.

Murine polyomavirus (mPyV) was the first in the group to be discovered by Ludwig Gross in the year 1953, during a study done on the transmission of mouse leukemias (Gross, 1953). In a follow up study done by Stewart and Eddy, they found that the virus was capable of inducing different types of tumours when inoculated artificially into the mice and hence was named '*Polyoma'* (Stewart *et al*., 1958). The name "polyoma" is derived from Greek in which *poly* means many and *oma*, means tumour. SV40 was discovered in the year 1960 by Sweet and Hilleman as the contaminating agent in the Rhesus monkey kidney cells (Sweet and Hilleman, 1960). Human polyomavirus was suspected to be present in the organs in the year 1965 (Zu Rhein and Chou, 1965; Silverman and Rubeinstein, 1965) and were isolated later in the year 1972.

2.3.1 Structure and Genome:

Polyomavirus contain a circular dsDNA molecule having a genome size of about 5000 bp. The DNA molecules associated with histones are packaged into the chromatin which is present inside the non-enveloped icosahedral capsid of the virus, having a diameter of approximately 500Å and a sedimentation coefficient of 240S (**Fig.1**).

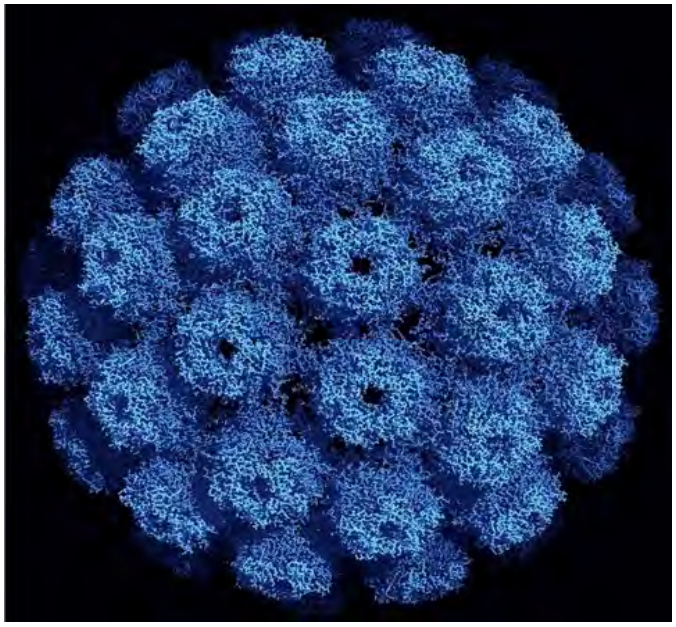


Figure 1: Three-dimensional structure of an SV40 particle at 3.1 angstroms (Å) resolution obtained using X-ray diffraction. The pentameric VP1 subunits are tied together by extended C-terminal arms. The diameter of this particle is about 500 Å or 50 nm (Moens *et al*., 2017).

The capsid is constituted by 360 copies of major capsid protein VP1, that are arranged into 72 pentamers where each one of them is connected to a single copy minor capsid protein VP2 or VP3. The genome is divided into an “early” and a “late” region. And these two regions are separated by a Non-Coding Control Region (NCCR) that contains the origin of viral DNA replication. The early region is transcribed prior to the initiation of viral DNA replication i.e., during entry and uncoating of the virus. They encode three to four viral tumour antigens (LTAg, STAg and MTAg). The late region is expressed after the onset of viral DNA replication and encodes viral capsid proteins (VP1, VP2, VP3 and VP4). VP4 has only been detected in SV40 and has been found to

interact with experimental biological membranes and facilitate their disruption (Daniels *et al*., 2007; Raghava *et al*., 2011). A small non-structural protein called Agnoprotein or leader protein 1 is also encoded from the late leader region in a few Polyomaviruses such as in BKPyV and JCPyV and are involved in viral gene expression, replication, and virus release (Gerits and Moens, 2012). The non-coding regulatory region or non-coding control region (NCCR) is the most variable region that contains regulatory elements and usually undergoes sequence rearrangements during reactivation, thus largely influencing the outcome of the infection. Diverse deletions or duplications within the NCCRs from immunocompromised patients have been shown to augment early gene expression and the rate of replication in BKPyV and JCPyV (Gosert *et al.*, 2008, Gosert *et al.*, 2010). The major capsid protein VP1 (~45 kDa) is responsible for the capsid assembly and the minor capsid proteins VP2 (~38 kDa) and VP3 (~27 kDa) help in the uncoating process during entry into host cells. Polyomavirus infection in permissive cells is initiated by the binding of the virion to a receptor on the outer cell membrane. The tumour antigens (large or T and small or t antigens) are found to have roles in alteration of cellular control systems, replication of the viral DNA, transcription of late genes of the virus and their assembly of virions (Brodsky and Pipas, 1998). Both T- and t- antigens are encoded from the same mRNA via alternative splicing. Some of the polyomaviruses SV40, MPyV, JCPyV and BKPyV are known to express miRNAs from the late transcripts (Sullivan *et al*., 2005; Seo *et al*., 2008). These miRNAs have been found to autoregulate early gene expression during infection.

2.4 Human Polyomavirus:

The first two human polyomavirus species to be characterized are JC polyomavirus (JCPyV) and BK polyomavirus (BKPyV). Both of them were identified in the year 1971 and were named after the initials of the patients from whom the viruses were cultured and subsequently isolated (Gardner *et al.*, 1971; Padgett *et al.*, 1971). BKPyV was isolated from the urine of renal transplant patient and JCPyV from the brain tissue of a patient suffering from Hodgkin's disease. Reactivation of BKPyV in immunocompromised hosts has been associated with haemorrhagic cystitis, BKV-nephropathy, and ureteral stenosis (Hirsch, 2002; Trofe *et al*., 2002). Persistent infection

of the virus in allograft recipients is the main cause for the graft dysfunction and loss. JCPyV is a human neurotropic virus that is associated with a neurological disease called Progressive Multifocal Leucoencephalopathy (PML). PML is a rare fatal demyelinating disease of the central nervous system that occurs in individuals with suppressed immune system involving both humoral and T-cell-mediated immune response and is principally observed in HIV-infected individuals (Major, 2010). It has also been found in patients suffering from chronic lymphocytic leukemia, Hodgkin's disease, lymphosarcoma, and sarcoidosis (Gardner *et al*., 1984; Greenlee, 1997). The role of JCPyV in malignancy is controversial.

After 30 years of their discovery, two more human polyomaviruses Karolinska Institute polyomavirus (KIPyV) and Washington University polyomavirus (WUPyV) were identified in the year 2007 and were named after the institution where they were identified (Allander *et al*., 2007; Gaynor *et al*., 2007). Both of them were isolated from respiratory samples of pediatric patients. Both KIPyV and WUPyV have been detected worldwide suggesting a widespread infection of the virus. But their involvement in human disease or tumour development is still not clear (Giraud *et al.*, 2008; Giraud *et al.*, 2009).

Merkel cell polyomavirus (MCPyV) was discovered in the year 2008 and was named so because of its association with skin cancer called Merkel Cell Carcinoma or MCC (Feng *et al.,* 2008). MCC is a rare but aggressive form of skin cancer that affects elderly and immune suppressed individuals. MCPyV is considered the only human Polyomavirus to cause tumours in natural host (Arora *et al*., 2012; Spurgeon and Lambert, 2013). It has also been detected in non-melanoma skin cancers, such as in squamous cell carcinoma, basal cell carcinoma, and Bowen's disease in immunosuppressed individuals (Kassem *et al*. 2009). Trichodysplasia spinulosa-associated polyomavirus (TSPyV) was also named after the disease it is associated with i.e., Trichodysplasia spinulosa and was discovered in the year 2010 (Meijden *et al*., 2010). Trichodysplasia spinulosa is a rare skin disease characterised by the development of facial follicular papules and keratotic protrusions (spicules or spines) with alopecia of

the eyelashes and brows. The presence and high seroprevalence of TSPyV in healthy individuals suggest a subclinical latent infection.

Human polyomavirus 6 (HPyV6), human polyomavirus 7 (HPyV7) and human polyomavirus 9 (HPyV9) were named according to the order of their discovery (Schowalter *et al.,* 2010; Scuda *et al.,* 2011). Human polyomaviruses 6 and 7 (HPyV6 and HPyV7) were detected in human skin (Schowalter *et al*., 2010) and Human Polyomavirus 9 (HPyV9) was first detected in the serum of a kidney transplant patient (Scuda *et al*., 2011) and has not been associated with any disease. Both HPyV6 and HPyV7 have been detected in urine, feces, and nasopharyngeal swabs in transplant recipients (Siebrasse *et al*., 2012).

Malawi polyomavirus (MWPyV) (Siebrasse *et al*., 2012), also known as HPyV10 (Buck *et al*., 2012) or Mexico polyomavirus (Yu *et al.,* 2012), were detected in stool and skin samples roughly at the same time. The association of the virus with infection in the gastrointestinal system or any other disease remains to be investigated. St Louis polyomavirus (STLPyV) was detected in clinical stool specimen in Malawi but its association with diarrhea was not understood (Lim *et al*., 2013). Human polyomavirus 12 (HPyV12) was detected in the liver, colon, rectum, and feces but its relation with disease remains to be investigated (Korup *et al*., 2013). New Jersey polyomavirus (NJPyV) was isolated from the muscle biopsy of a 33-year-old pancreatic transplant recipient and was named after the place from where the patient belonged (Mishra *et al*., 2014). The last in the group to be identified is Lyon IARC polyomavirus (LIPyV) which was isolated from human skin of patients with several diseases but its relation with any disease is not yet known (Gheit *et al*., 2017). The list of all the Human polyomaviruses and the disease they are associated with are given in **Table 2.**

Table 2: List of Human Polyomavirus and the associated disease						
Sl. No.	**Virus Name**	**Abbreviation**	**Genus**	**Year of Discovery**	**Disease associations**	**References**
1	BK polyomavirus	BKPyV	Beta	1971	Hemorrhagic cystitis and nephropathy	Gardner *et al.*, 1971
2	JC polyomavirus	JCPyV	Beta	1971	Progressive multifocal leucoencephalopathy	Padgett *et al.*, 1971
3	KI polyomavirus	KIPyV	Beta	2007	Respiratory symptoms (?)	Allander *et al*., 2007
4	WU polyomavirus	WUPyV	Beta	2007	Respiratory symptoms (?)	Gaynor *et al*., 2007
5	Merkel cell polyomavirus	MCPyV	Alpha	2008	Merkel cell carcinoma	Feng *et al.*, 2008).
6	Human polyomavirus 6	HPyV6	Delta	2010	Squamous cell carcinoma (?); keratoacanthoma (?)	Schowalter *et al*., 2010
7	Human polyomavirus 7	HPyV7	Delta	2010	Pruritic rash (?); thymoma (?)	Schowalter *et al*., 2010
8	Trichodysplasia spinulosa associated polyomavirus	TSPyV	Alpha	2010	Trichodysplasia spinulosa	Meijden *et al*., 2010
9	Human polyomavirus 9	HPyV9	Alpha	2011	Not known	Scuda *et al.*, 2011
10	MW polyomavirus	MWPyV	Delta	2012	Not known	Siebrasse *et al*., 2012
11	STL polyomavirus	STLPyV	Delta	2013	Not known	Lim *et al*., 2013
12	Human polyomavirus 12	HPyV12	Alpha	2013	Not known	Korup *et al.*, 2013
13	New Jersey polyomavirus	NJPyV	Alpha	2014	Vascular myopathy (?)	Mishra *et al*., 2014
14	Lyon IARC polyomavirus	LIPyV	Alpha	2017	Not known	Gheit *et al*., 2017

2.4.1 Infection in Host Cell:

Viral infection in the host cell is initiated by interactions between viral capsid or envelope proteins and cell surface receptors. Internalization occurs either by fusion of the envelope and the plasma membrane or via endocytic pathways that lead to escape of the virion from an endocytic or other vesicular compartment into the cytosol (Smith and Helenius, 2004; Marsh and Helenius, 2006). Attachment to the cellular receptors can directly trigger conformational changes in virus surface structures or can activate specific signaling pathways that facilitate viral entry. A number of cell surface receptors have been identified, some of which act as single molecular species and some as a set of multiple receptors for viruses. Virus–host cell receptor interactions are specific and use distinct receptors and entry mechanisms to infect cells.

Glycoproteins are proteins containing oligosaccharide chains covalently attached to polypeptide side-chains, and glycolipids are lipids attached to the carbohydrate by a glycosidic bond. Gangliosides are glycolipids, i.e., glycosphingolipids, with 1 or more sialic acids (e.g., *N*-acetylneuraminic acid). More than 60 gangliosides are known and they differ by their position and number of NANA residues. Sialic acid is an N- or O-substituted derivative of neuraminic acid found mostly in glycoproteins and gangliosides. Glycosaminoglycans (GAGs) or mucopolysaccharides are long unbranched polysaccharides that consist of a repeating disaccharide unit consisting of an amino sugar along with uronic sugar or galactose, except for the GAG keratin. List of receptors/co-receptors associated with different types of Human polyomaviruses are shown in the **Table 3.**

Table 3: Human polyomaviruses, their receptor/co-receptor and entry mechanism				
S.No.	**Virus Name**	**Abbreviations**	**Receptor(s)/Co-receptor(s) (entry mechanism)**	**References**
1	BK polyomavirus	BKPyV	Gangliosides GD1b, GT1b (caveolin-mediated endocytosis)	Dugan *et al*., 2005; Low *et al*., 2006
2	JC polyomavirus	JCPyV	LSTc/5-HT_2 (clathrin-mediated endocytosis)	Liu *et al*., 1998; Assetta *et al*., 2013
3	KI polyomavirus	KIPyV	Not Known	-
4	WU polyomavirus	WUPyV	Not Known	-
5	Merkel cell polyomavirus	MCPyV	Heparan sulphate/Sialyted glycan (Not Known)	Schowalter *et al*., 2011; Neu *et al*., 2012
6	Human polyomavirus 6	HPyV6	Not Known	-
7	Human polyomavirus 7	HPyV7	Not Known	-
8	Trichodysplasia Spinulosa-associated polyomavirus	TSPyV	Not Known	-
9	Human polyomavirus 9	HPyV9	Not Known	-
10	MW polyomavirus	MWPyV	Not Known	-
11	STL polyomavirus	STLPyV	Not Known	-
12	Human polyomavirus 12	HPyV12	Not Known	-
13	New Jersey polyomavirus	NJPyV	Not Known	-
14	Lyon IARC polyomavirus	LIPyV	Not Known	-

JCPyV enters into the target cells via clathrin-mediated endocytosis (**Fig.2**) and is dependent on actin-polymerization and proper assembly of clathrin-coated pits (Pho *et al*., 2000). It requires sialic acids to bind to host cells, specifically an N-linked glycoprotein with α2,6-linked sialic acid. A structural homology model of JCPyV, based on X-ray crystal structure of mPyV, revealed a sialic acid binding pocket in the BC-, DE- and HI- loops of JCPyV VP1 region (Gee *et al*., 2004). JCPyV has also been found to interact with 5HT2R before clathrin-mediated endocytosis into the cells (Maginnis *et al*., 2010). After its entry into the cells, the virus localizes to the early and late endosomes and is hydrolyzed within the lysosome. A low pH environment within the lysosome is critical for productive JCPyV infection. JCPyV uses microtubules and microfilaments to traffic inside the host cell (Ashok and Atwood, 2003). To accomplish efficient trafficking through ER and cytoplasm JCPyV exploit ERAD pathway (Nelson *et al*., 2012).

Replication of the viral genome, viral protein synthesis, assembly of the viral particles, and their release from the cells use the host cellular machinery. Assembly of virions occurs either in the cytosol or in the nucleus of a host cell and involves polymerization of the capsid protein-like viral mini-chromosomes (Garber *et al*., 1980). The release of the virion occurs via budding followed by exocytosis or cell lysis (Marsh and Helenius, 2006). The release of non-enveloped viruses usually occurs through cell lysis, but in some cases, they may escape either by some secretary mechanisms (Altenburg *et al*., 1980) or may use cellular autophagy pathways for exit (Jackson *et al*., 2005).

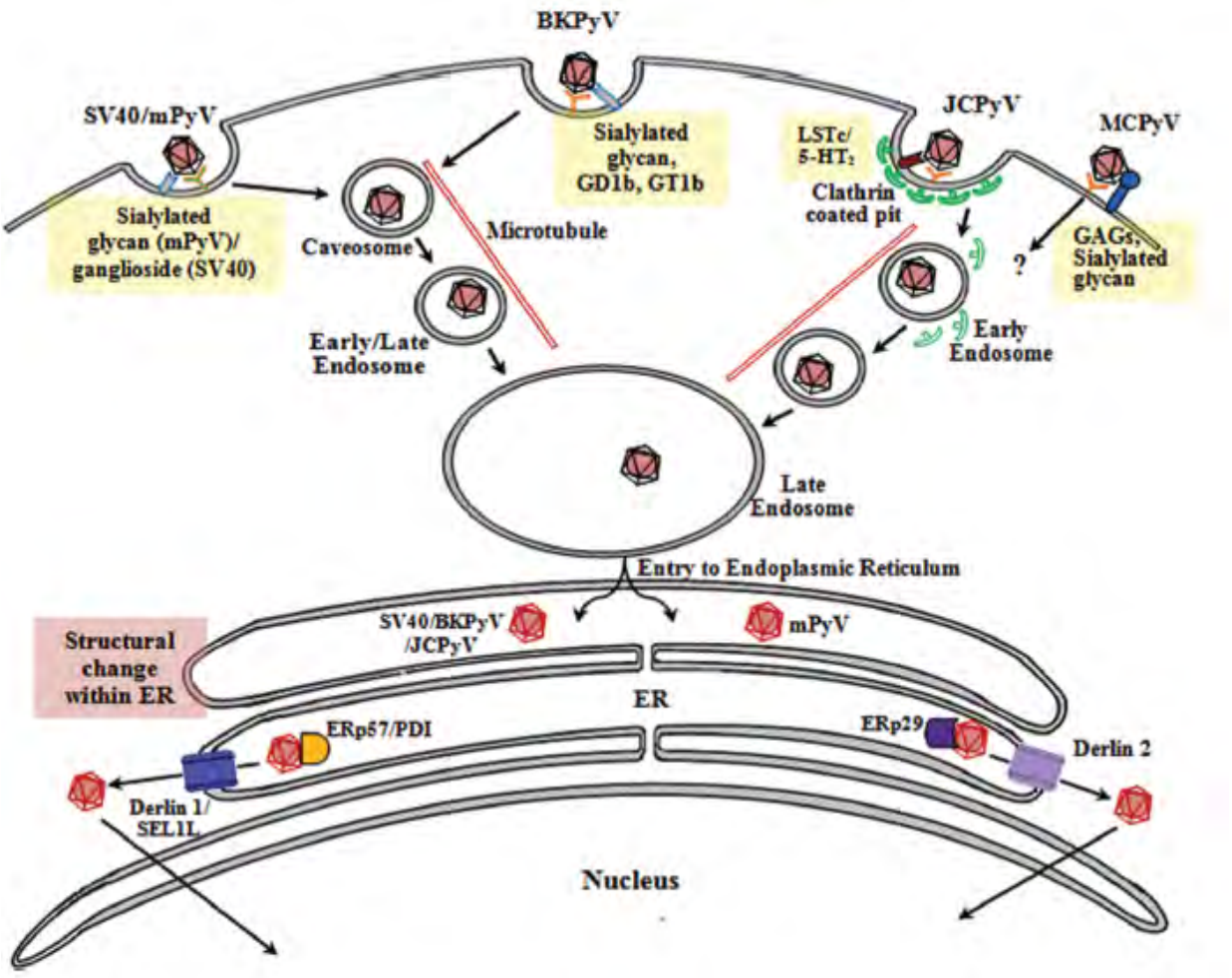


Figure 2: Receptor-mediated endocytosis and intracellular trafficking to endoplasmic reticulum (ER) in polyomaviruses. JCPyV require LSTc and 5-HT2 for its attachment and entry, respectively. JCPyV is internalized through clathrin-coated vesicles. Within the ER lumen, JCPyV interact with ERp57 and PDI, a process required for the partial disassembly of viral capsids, and are then retro-translocated to the cytoplasm via the ER transmembrane proteins Derlin-1 and SelL1.

2.4.2 Epidemiology:

Seroepidemiological surveys conducted so far throughout the world show that the seroprevalence of BKPyV, JCPyV, KIPyV, WUPyV, and MCPyV is high in populations (Brown *et al.*, 1975; Stolt *et al.*, 2003; Kean *et al.*, 2009). Antibodies to JCPyV are present in 70%–80% of the adult population. Seroprevalence rate for JCPyV is 39%

(Kean *et al*., 2009) and 10 to 30% adults have been found to excrete JCPyV in their urine (Ferenczy, 2012). The virus appears to be ubiquitous, with almost no region of the world being free, except in some remote populations (Brown *et al*. 1975). Primary infections with JCPyV virus probably occur in childhood and are presumed to be largely subclinical (Padgett and Walker 1973; Shah *et al*. 1973). Early seroepidemiological studies relied on hemagglutination, which was later replaced by enzyme-linked immuno-absorbent assays. PCR detection for viral DNA allows for accurate and sensitive detection of the virus. Age-specific prevalence studies indicate that JCPyV seroprevalence steadily increases from childhood to late adulthood (Knowles *et al*., 2003; Egli *et al*., 2009). In USA, about 40% of the JCPyV serpositive individuals excrete the virus, whereas in Taiwan the rate was found to increase, from less than 5% in children to 80% in individuals above 70 years of age (Arthur, 1992; Chang *et al*., 2002). Several studies have also reported a higher excretion rate of JCPyV in males than in females (Agostini *et al*., 1996; Stoner *et al*., 1996; Ling *et al*., 2003). The virus presumably harbours peripheral blood lymphocytes or can remain latent in the kidney (Salzman, 1986; Dorries *et al*., 1994; Greenlee, 1997). It has been hypothesized that the JCPyV can probably persist indefinitely in the kidney and is reactivated and excreted in the urine during immunological impairment such as in case of pregnancy or advanced age (Coleman *et al*., 1977; Markowitz *et al*., 1991). This model of infection may be true for all human polyomaviruses (Heritage *et al*., 1981; Arthur *et al*., 1986). Different phenotypes of JCPyV have been found in urine of patients suffering from autoimmune diseases such as systemic lupus erythematosus, Sjogren's syndrome, rheumatoid arthritis, and dermatomyositis (Chang *et al*., 1996; Sundsfjord *et al*., 1999; Bendiksen *et al*., 2000). Faecal-oral, oral and respiratory transmission routes have been suggested for different types of human polyomaviruses (Rockett *et al*., 2013). Studies of urban waste water samples suggest that JCPyV could be acquired through water, food and fomites contaminated with feces (Bofill-Mas *et al*., 2000; McQuaig *et al*., 2009). Transmission of virus from parent to child can also occur (Boldorini *et al*., 2011). Transmission of JCPyV within the family or in the community is supported by the presence of certain genotypes of JCPyV in population groups which may have occurred due to cohabitation in the same group for many years (Agostini *et al*., 1998; Suzuki *et al*., 2002).

Two types of molecular variation are observed in JCPyV: i) deletion, duplication in the regulatory region of the genome and ii) nucleotide substitutions in the coding region that may lead to amino acids sequence changes (Yogo and Sugimoto, 2001). Nucleotide changes in the coding region have so far allowed identification of 8 major genotypes differing by only 1-2.6% (Sundsfjord *et al*., 1994). Types 1 and 4 are mostly found in Europe and North America, Types 2, 5 and 7 in Asia, Types 3 and 6 in Africa, and Type 8 in Papua New Guinea.

2.5 JC Polyomavirus:

JC polyomavirus or John Cunningham virus (JCPyV) is a member of the *Polyomaviridae* family. JCPyV was detected first as icosahedral shaped virus particles in ultrathin sections of brain tissue of a PML patient (Zu Rhein and Chou, 1965). It was then isolated in the year 1971 from the brain tissue of a patient suffering from Hodgkin's lymphoma and was named after the initials of that patient (Padgett *et al*., 1971).

JCPyV causes a neurological disease called progressive multifocal leukoencephalopathy (PML) in immunocompromised hosts which involves demyelination of white matter. Immune suppression due to leukemia, HIV infection and organ transplantation may lead to the activation of JCPyV and ultimately to PML (Laghi *et al*., 1999). Symptoms of PML include cognitive deterioration, coordination abnormalities, limb paresis and seizure activity.

Initial infection with the virus occurs through either oral or respiratory route during childhood and remains latent in organs like kidney, B-lymphocytes etc until its reactivation (Knowles, 2006). JCPyV is very common in human population infecting about 70% to 90% of the individuals (Agostini *et al.*, 1997; Shackelton *et al*., 2006). JCPyV genome has been detected in various cell types and tissues such as in tonsillar stromal cells, B lymphoid cells, kidney epithelial cells, and upper and lower parts of the gastrointestinal tract, including the mucosa of the colon (Laghi *et al*., 1999). JCPyV nucleic acid have been detected in urine, brain autopsy and CNS tissues; serum/peripheral blood leukocytes (PBL), bone marrow aspirates of normal or non-

immunocompromized human subjects of different age groups from many regions of the world (Gu *et al*., 2003; Delbue *et al*., 2008; Tan *et al*., 2009; Husseiny *et al*., 2010). It is latent in the kidney and is excreted in the urine (Arthur and Shah, 1989). Incidences of JCPyV viruria in different tribal populations showed variations with respect to the groups studied: 56 to 66% in Native Americans (Agostini *et al*., 1997), 20 to 22% in African tribals (Chima *et al*., 1998), 47 to 55% in Bunun tribes of Taiwan (Chang *et al*., 1999) and 48 to 67% in Myanmar tribals (Saruwatari *et al*., 2002). JCPyV strains are likely to be transmitted horizontally either within families or outside of it (Kitamura *et al*., 1994).

Infection of the cell with the virus requires binding of the VP1 protein to the N-linked glycoprotein with sialic acid in permissive cells. JCPyV can also bind to the serotonin receptor 5HT2A found in brain and kidney cells and also to the ganglioside GT1b. After entering the host cell via clathrin-dependent endocytosis, it moves to the nucleus where it is uncoated and transcription of the early region begins. The early product T-Ag then binds to the viral origin of replication and allows replication of viral DNA. As replication progresses, late genes VP1, VP2 and VP3 are expressed. The expressed late products then assemble with the viral DNA to form the complete virion which is released by lysis of host cell (Delbue *et al*., 2017).

Cells such as oligodendrocyte support viral DNA replication resulting in a lytic infection and in non-permissive cells JCPyV is responsible for silent infection or may be involved in cell transformation (Haggerty *et al*., 1989).

Minor genetic variations have been found in strains of JCPyV of different geographical areas. Thus, genetic analysis of the virus can be used to trace the history of human migration (Pavesi, 2005).

2.5.1 Genome and Structure:

JCPyV virus is a non-enveloped virus having a diameter of 42 nm with a circular double-stranded DNA genome of about 5100 base pairs which is packaged as mini-chromosomes with cellular histones H2A, H2B, H3 and H4. The viral genome is divided into three regions: early and late coding regions separated by a non-coding control region

(NCCR). The early region encodes large tumour antigen (T-Ag) and small tumour (t-Ag) antigen and various splice variants via alternative splicing of one major pre-mRNA transcript known as T'135, T'136 and T'165. These genes have a role in viral DNA replication and late region gene transcription. The late region encodes three capsid proteins VP1, VP2 and VP3 and a small multifunctional protein (Agnoprotein). VP1 represents 70% of the total viral protein and VP2 and VP3 together make for the 30% of the total protein in the virion. The viral capsid DNA is composed 360 copies of major capsid protein VP1 molecules assembled into of 72 pentamers and each one of them is associated with one unit of the minor VP2 and VP3 proteins (Jiang *et al*., 2009). Exposed residues on surface of VP1 monomer make specific contacts with sialic acid containing receptors for entry into the host cell and facilitate infection (Neu *et al*., 2010). Agnoprotein binds to many host-cell and viral proteins and cooperates with them to sustain the lytic cycle (Saribas *et al.,* 2012). The non-coding control region (NCCR) present between the early and late coding region contains the origin for viral DNA replication, TATA-box and sequences responsible for early and late gene transcription. It is the most variable region in the viral genome. It has a role in the regulation of early and late gene expression. The archetype NCCR strain consists of six blocks: A, B, C, D, E and F. These blocks are the sites for binding of host transcriptional factors. JCPyV has been found to encode miRNAs: JC-miRNA-3p and JC-miRNA-5p. They are encoded by the late transcript and target the mRNA region shared by large T-Ag and small t-Ag.They are responsible for down regulating the T-Ag protein (Seo *et al*., 2008).

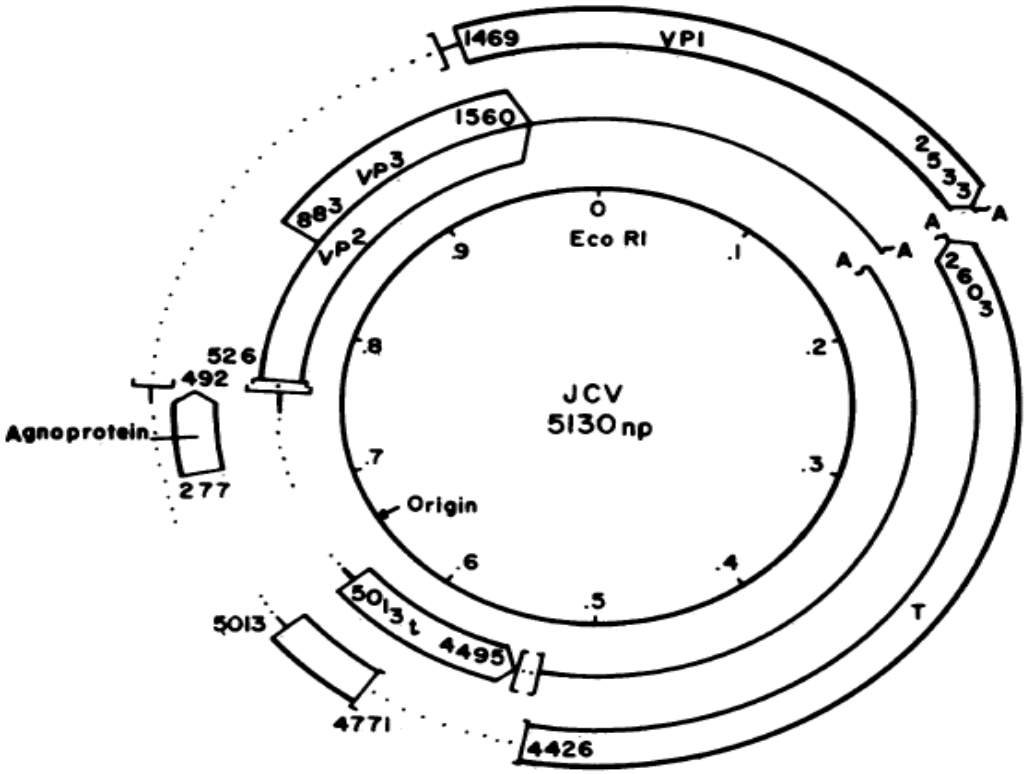


Figure 3: Circular map of the JCPyV genome (Mad l strain) (Frisque *et al.*, 1984).

2.5.2 Types of JC virus based on NCCR architecture:

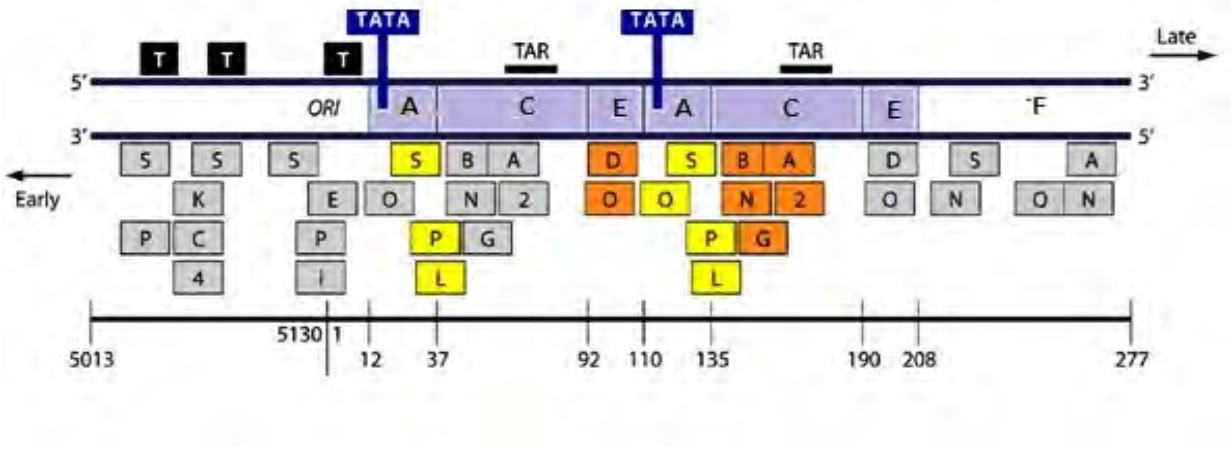
A naturally occurring variant of the NCCR found in the CY strain of JCPyV is termed as 'Archetype'. Archetype strain is rarely been associated with PML and is mostly found in kidney and urine. NCCR sequence variants of archetype strains have been detected in brain, CSF and blood of PML patients. These variants are derived from archetype virus strain during reactivation via rearrangement, insertion or deletion of sequences and are referred to as 'Prototype' (Sabath and Major, 2002).

The archetypal NCCR strain is a sequence of six blocks, denoted A, B, C, D, E and F. Each block harbors binding sites for host transcriptional factors, essential for the regulation of viral transcription. Archetype strain consists of only a single copy of 98 bp repeat of A, C and E with 23 bp (B) and 66 bp (D) sequence between the A, C and E blocks that yield a structure with A, B, C, D, and E blocks (**Fig. 4**). The prototype strain Mad-1 is characterized by a 98 bp tandem repeat resulting in duplication of TATA-Box

and increase in number of transcription factor binding sites. It has been postulated that the rearrangement in the NCCR may change the biological properties of the virus in due of a persistent infection. Multiple studies have reported different deletions of segment 'D' as the most commonly affected part in prototype JCPyV, isolated from PML-patients (Gosert *et al*., 2010; Reid *et al.*, 2011). *In vitro* deletion of block D in the archetype NCCR displayed strongly elevated expression of the viral early genes (Gosert *et al*., 2010).

Several transcription factors are implicated in the regulation of JCPyV gene expression which include NF-κB (Ranganathan and Khalili, 1993), NFAT4 (Wollebo *et al*., 2012), upstream Target or up-TAR (Chowdhury *et al*., 1993), Tst-1 (Wegner *et al*., 1993), Sp-1 (Henson *et al*., 1992), Spi-B (Marshall *et al*., 2010), GBP-i (Raj and Khalili, 1994), Y-box binding protein 1 (YB-1) and Purα (Chen and Khalili, 1995), Nuclear factor 1 or NF-1 (Amemiya *et al*. 1989), CREB/ATF-1 (Lonze and Guinty, 2002), Activator Protein 1 (AP-1) family members (Sadowska *et al*., 2003), p53 (Ariza *et al*., 1994), Early growth response-1 protein or Egr-1 (Romagnoli *et al*., 2008), Bcl-2-associated athano gene-1 or BAG-1 (Devireddy *et al*., 2000) and CAAT/enhancer binding protein beta or C/EBPβ (Romagnoli *et al*., 2009).

Prototype (Mad1):



Archetype:

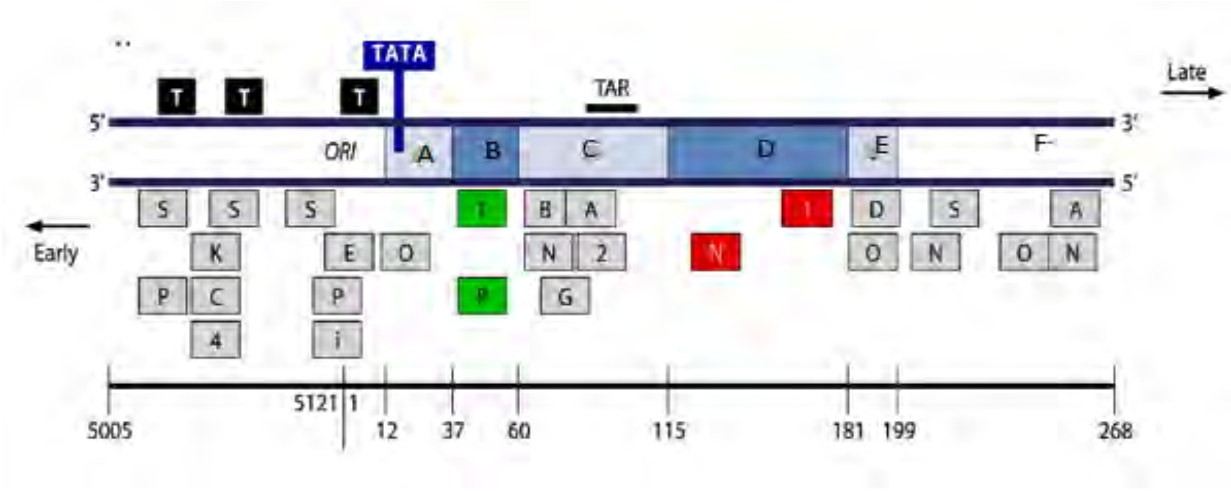


Figure 4: DNA sequence block representation of NCCRs of prototype variant Mad-1 and the archetype variant CY. The numbering scheme is that of Frisque *et al.*, 1984. The origin of replication is denoted "*ORI*." The letters "A," "B," "C," "D," "E," and "F" indicate sequence blocks. The locations of TATA boxes, as well as binding sites for JCPyV large T antigen and sequences are similar to the HIV tat-responsive element, known as the transactivation response element (TAR), are above the DNA sequence. Transcription factor bindings sites are represented by boxes under the regulatory control region and letters indicate abbreviations which are as follows:1:Sp1; 2:SF2/ASF; 4:NFAT4; A:AP-1(c-jun); B:Bag-1; C:C/EBPβ; D:DDX-1; G:GF-1/SμBP-2; H:HIF-1α;i:GBP-i; K:NF-κB; L:LCP-1; N:NFI; O:Tst-1/Oct-6/SCIP; P:Purα and YB-1; S:Spi-B. (Adapted from Ferenczy *et al.*, 2012).

2.5.3 JC Virus Genotypes:

Previously, JCPyV isolates were classified depending on the rearrangement of the NCCR architecture of the virus. Several viral typing systems have been developed since then. Yogo and co-workers proposed a classification system for the virus based on nucleotide sequences of the viral genome (Yogo *et al.*, 1991). Full length JCPyV DNA was isolated from different regions of Asia, Africa and Europe and was analyzed by RFLP that divided them into three groups: Type A, Type B and Type C. Type A was prevalent in the population of Europe, Type B in Asia and Africa with a minor subtype in Europe and Type C was mostly found in the population of West Africa (Yogo *et al.*, 1991; Guo *et al.*, 1996). With the use of DNA sequencing techniques, types were defined according to their genetic sequence. Sugimoto and colleagues classified the JCPyV genome based on variation in the 610 bp Intergenic region (Sugimoto *et al.*, 1997). For this, about 200 sequences were obtained from all over the world, amplified, sequenced and analyzed by creating a phylogenetic tree. The tree identified 12 genotypes: EU, Af1, Af2, Af3, B1-a, B1-b, B1-c, B1-d, B2, CY, MY and SC (Sugimoto *et al.*, 1997; Guo *et al.*, 1998). Some genotypes were further subdivided, for example EU was divided into three genotypes EU-a, EU-b and EU-c (Sugimoto *et al.*, 2002). Eu-a and Eu-b are mainly spread over Europe and Mediterranean areas and a minor genotype B1-C is also found in that region. Af2 is spread throughout Africa and in West and South Asia. Af1 and Af3 are localized in West and Central Africa, respectively. B1-a, -b, -d, B2, CY, MY and SC are distributed throughout East Asia. Multiple genotypes have been found in areas where multiple human populations have mixed. In a more recent study, sequencing of 100 full-length JCPyV sequences using predicted amino acid sequences of all the coding regions was done to define types. Eight (8) JCPyV types were identified, numbered 1 through 8 each with multiple subtypes (Cubitt *et al.*, 2001). Type 5 was found to be a minor member of type 3 (Agostini *et al.*, 1997). Different types of JCPyV have been associated with different populations and have been used to study migration of human population. All the JCPyV subtypes are listed in **Table 4** along with the regions they are mostly prevalent. It was hypothesized that the Type 6 was the original JCPyV type and JCPyV split as humans migrated out of Africa, with one type moving toward Eurasia and the

other type only to Europe (Pavesi, 2003). Type 1 and type 4 are generally found in Europeans and European-Americans, while type 2A in Asians and Native American populations. Types 3 and 6 are associated with Africans and African-Americans. Types 2D and 7C are found in both Asians and South Asians (Yanagihara *et al*., 2002; Cui *et al*., 2004). Types 2E, 8A, and 8B are found in Western Pacific populations (Yanagihara *et al*., 2002). Type 8A is detected only in Papua New Guinea populations (Jobes *et al*., 2001).

Table 4: JCPyV subtypes

Sl. No.	**Subtypes according to**		**Major domains**
	Sugimoto *et al*., 1997 and Guo *et al*., 1998	**Jobes *et al*., 1998**	
1	EU	Type 1, Type 4	Europe, Mediterranean areas
2	Af1	Type 6	Central and West Africa
3	Af2	Type 3	Africa, West Asia
4	Af3	-	Central Africa
5	B1-a	-	China
6	B1-b	Type 2D	Central and West Asia
7	B1-c	Type 2B	Europe
8	B1-d	-	Saudi Arabia, Greece
9	B2	-	India, Mauritius
10	MY	Type 2A, Type 2C	Japan, South Korea
11	CY	-	Northeast Asia
12	SC	Type 7	Southeast Asia, South China

2.5.4 Progressive Multifocal Leucoencephalopathy or PML:

JCPyV causes a rare and fatal disease of the central nervous system called Progressive multifocal leucoencephalopathy that usually affects adults. It is a demyelinating disease of the central nervous system and is highly prevalent in individuals

with suppressed immune system such as in patients with HIV infections (Berger *et al.*, 1987), organ transplant patients (Crowder *et al*., 2005) or in case of hematological malignancies (Brook and Walker, 1984; Koralnik, 2004). PML has also been associated with the use of immunosuppressive therapies such as in patients treated with monoclonal antibodies. Among the monoclonal antibody associated with PML, the most common are natalizumab (Langer-Gould *et al*., 2005; Van Assche *et al.*, 2005) and rituximab (Carson *et al*., 2009).

PML was first described in the year 1958 in two patients. The patients showed progressive dementia, motor dysfunction, vision loss followed by death. Histopathological studies showed severe demyelination, abnormal oligodendrocytes and giant astrocytes (Astrom *et al*., 1958). In 1965, Zu Rhein and Chou suggested Papovavirus (i.e., Polyomavirus) to be the causative agent of PML after observation by electron microscopy. JCPyV was isolated in the year 1971 by Padgett and colleagues and was named after the initials of the patient from whose brain it was isolated (Padgett *et al*., 1971).

PML was considered a rare disease earlier. In a study done by Brooks and Walker in 1984 out of the 230 cases of PML examined, the underlying cause of the disease were lymphoproliferative diseases (62.2%), myeloproliferative diseases (6.5%), carcinoma (2.2%), tuberculosis and sarcoidosis (7.4%), and other immune impairments (16.1%). AIDS was included later in the category that comprised of only 2.1% of the cases (Brooks and Walker, 1984). However, with the increase in the rate of occurrence of HIV infection and AIDS, the incidence rate of PML has also increased having an incidence rate of about 3 to 5% (Major, 2010). Implementation of antiretroviral therapy (HAART) for the treatment of HIV infected patients resulted in an overall decrease in the incidence rate of PML in patients with HIV infection. Despite this, PML still remains a significant complication in individuals with HIV infection.

Patients with PML show subacute, progressive neurologic deficits. Hemiparesis, visual impairment, and altered mentation are the most common and frequent signs in AIDS-related PML. Some patients showed signs and symptoms such as, ataxia,

dysmetria, and dysarthria, that indicates involvement of the cerebellum and brain stem. Other signs and symptoms include headache, vertigo, seizures, sensory deficits, parkinsonism, aphasia, and neglect syndromes.

JCPyV infects both oligodendrocytes and astrocytes (Sabath and Major, 2002). Histologically PML is characterized by subcortical demyelinated plaque, enlarged oligodendrocytes containing intranuclear inclusion bodies and bizarre astrocytes. Oligodendrocytes support the lytic cycle of JCPyV that results in demyelination. The affected oligodendrocytes are enlarged due to assembly of mature virion particles and contain intranuclear inclusion bodies. Astrocytes contain one or more irregular nuclei with condensed chromatin and a prominent nucleolus. They also contain small inclusion bodies both in nucleus and cytoplasm (Dell vale and Pina-Ovieodo, 2006).

Infection with the virus is suggested to occur in tonsillar tissue or through ingestion of contaminated food or water (Bofill-mas and Girones, 2003). Tonsillar lymphocytes infected with JCPyV carry virions to the kidney and bone marrow, which are the primary sites of virus latency. JCPyV have been observed in tonsils, bone marrow, peripheral blood cells and plasma (Dorries, 1999; Jensen and Major, 1999). Higher levels of JCPyV DNA were found in peripheral blood mononuclear cells (PBMCs) in HIV-1–infected patients, than in immunocompetent individuals.

Rearrangement and/or formation of tandem repeats in the NCCR of the JCPyV are required for the infection of the virus in the glial cells (Berger, 2007). Rearrangement in the NCCR region results in the increased number of binding sites for nuclear factor, which may be the reason for the increased pathogenecity of the virus (Tan and Koralnik, 2010).

PML have also been associated in individuals with autoimmune diseases such as multiple sclerosis (MS) being treated with monoclonal antibody natalizumab that binds to α4 subunit of α4β1 and α4β7 integrins expressed on surface of all leukocytes except neutrophils. α4 integrin is required for the movement of white blood cells into organs. Natalizumab prevents crossing of immune cells from blood vessel walls to the affected

organs (Yoursry *et al*., 2006). The incidence rate of PML due to natalizumab use is 1 in 1000 patients (Rudick and Sandrock, 2004). The risk of developing PML in these patients depends on several factors, depending on whether the patient is seropositive or seronegative for JCPyV, whether the patient has previously been treated with immunosuppressants, and also on the duration of the patient's exposure to natalizumab (Williamson and Berger, 2015).

2.5.4.1 Diagnosis:

Neuroimaging is useful in the diagnosis of PML in a patient with a progressive course of disease. In patients with PML, the brain biopsy results have a sensitivity of 64-96% and a specificity of 100% (Tan and Koralnik, 2009). Histologically, demyelination and gliosis can be observed in abnormal astrocytes and macrophages (Moll *et al.*, 2007). Due to the risk associated with the isolation of brain tissue, CSF is used for the test. Serological studies are not very helpful because JCPyV antibody levels tend not to increase during illness and viral antibodies are not detected in the cerebrospinal fluid. The sensitivity of detectionof JCPyV by PCR is 72-92% with a specificity of 92-100% (Cinque *et al.,* 1997). But in HIV infected patients receiving anti-retroviral therapy (HAART), the sensitivity of PCR detection in CSF decreased to 58% (Marzochetti *et al*., 2005).

2.5.4.2 Treatment:

Antiviral drugs such as cidofovir have been used in combination with antiretroviral therapy to treat PML in HIV patients but have not been shown to be effective. Mefloquine, a drug used to treat malaria has been found to have an effect on the activity of JCPyV (Brickelmaier *et al*., 2009). Mirtazapine, an inhibitor of 5-HT2A receptor has also been shown to inhibit the infection of a human astroglial cell line (Verma *et al*., 2007). Cytrabine, a chemotherapeutic agent that interferes with the DNA synthesis has shown an effect on JCPyV replication in *in vitro* studies (Marzochetti *et al*., 2009). Discontinuing the immunosuppressive drug is the first step in the management of patients who developed PML, which in turn may lead to recovery of the immune system

and development of the Immune Reconstitution Inflammatory Syndrome (IRIS) (Wenning *et al*., 2009). In HIV-PML patients, IRIS has been reported to arise 1 week to 26 months after the initiation of HAART (Tan and Koralnik, 2010). IRIS is triggered during the restoration of immune competence after its suppression that may be due to HIV/AIDS, hematological malignancies and lymphoproliferative disorders, chemotherapy, immuno-modulating drug therapy, organ transplantation and auto-immune diseases (Walker *et al*., 2015). It may occur during the post-partum immunological changes after pregnancy (Singh and Perfect, 2007). IRIS is common in HIV/AIDS patient receiving HAART, and can exhibit a variety of clinical conditions such as: Tuberculous (TB)-IRIS, Cryptococcal-IRIS, PML-IRIS, *Toxoplasma*-IRIS, *Cytomegalovirus* (CMV)-IRIS, and *Pneumocystis*-IRIS, amongst others (Tan *et al*., 2009; Perez-Rueza *et al*., 2017). IRIS has also been associated with auto-immune conditions (like sarcoidosis), or inflammatory reactions associated with malignancies such as Kaposi's sarcoma, and non-Hodgkin's lymphoma (Lai *et al*., 2013). The incidence of IRIS varies considerably from 3–54%, depending on the degree of immune suppression and prevalence of some opportunistic infections in that particular region in which the patient resides (Narendran *et al*., 2013; Perez-Rueza *et al*., 2017). In HIV positive patients with PML, optimization of HAART is the best treatment option and in HIV-negative patients, elimination of sources of immunosuppression, such as steroids or calcineurin inhibitors in transplant recipients (Antinori *et al*., 2003; Tan and Koralnik, 2010).

2.5.4.3 Prognosis:

One-third to one-half of people with PML dies within the first few months of diagnosis, depending on the severity of their underlying disease. Survivors can end up with varying degrees of neurological disability. With the introduction of anti-retroviral therapy in 1996, HIV positive patients with PML are living longer.

2.6 JCPyV and its association with Cancer:

JCPyV DNA and proteins have been detected in a wide range of human tumours such as gliomas, ependymomas and medulloblastomas, colorectal carcinoma but their

role in human malignancies is still unclear (Burnett-Hartman *et al*., 2008). Like other polyomaviruses, JCPyV has also shown oncogenic potential in experimental animal models. Inoculation of newborn Golden Syrian hamsters has been shown to lead to the development of tumours such as medulloblastoma, astrocytoma, glioblastoma multiforme, primitive neuroectodernal tumours and peripheral neuroblastoma (Walker *et al.*, 1973; Zu Rhein and Varakis, 1979; Zu Rhein, 1983). The type of the tumours induced by the virus depends upon the type of animal, its age, and the site of viral inoculation, for example, intracerebral, intraperitoneal or subcutaneous. Intracerebral inoculation of JCPyV into owl and squirrel monkeys resulted in the development of astrocytoma, glioblastoma and neuroblastoma (Del Valle *et al*., 2001), whereas intraocular inoculation in neonatal hamsters leads to the development of abdominal neuroblastomas (Varakis *et al*, 1978; Gordon and Khalili, 1998). The mechanism by which JCPyV induces tumours in experimental animals is not clear at present.

The first evidence of presence of JCPyV in an elderly PML patient with chronic lymphocytic leukemia was reported by Richardson (1961). Further investigations were carried out to see association of brain tumours and JCPyV and at least ten cases of CNS neoplasia in patients with PML were reported (White and Khalili, 2005; Brasseco *et al*., 2013). Association of JCPyV with a variety of human tumours in patients without PML has also been reported such as in oligoastrocytoma (Rencic *et al.*, 1996), colorectal cancer (Laghi *et al.*, 1999) and medulloblastomas (Krynska *et al.*, 1999). Medulloblastoma is among the most common grade IV brain tumour with highest number of cases in children. JCPyV T-Ag was observed in 11 out of 23 pediatric medulloblastoma tissues (Krynska *et al.*, 1999). In a study involving CNS lymphoma, JCPyV DNA was detected in 81% of cases but expression of LTAg was observed only in 18.5% of the cases (Del Valle *et al.,* 2004). Presence of JCPyV DNA and expression of T-Ag have also been reported in gastrointestinal tumours such as esophageal carcinoma (Del valle *et al.*, 2005), gastric carcinoma (Murai *et al*., 2007), sporadic adenomatous polyps (Jung *et al*., 2008) and colorectal carcinomas (Laghi *et al*., 1999).

Like SV40 T-antigen, JCPyV T-antigen has a modular structure with multifunctional activities including ATPase, helicase, DNA binding, and α polymerase

that are essential in the process of DNA replication (Pipas, 1992; Sullivan *et al.*, 2000). T-Ag is the main regulator of the infectious process and is responsible for the cell transformation and tumour development. It is a multifunctional protein that is divided into several domains, the DNaJ domain that links to the cellular factor HSc70, the LxCxE motif that binds and inactivates the Rb family members, the origin binding domain (OBD) that binds to the viral origin of replication, the NLS domain necessary for the nuclear localization of the protein, the Helicase domain containing the Zinc and nucleotide binding domains and p53 binding domain (Moens *et al.*, 2007; Moens *et al.*, 2014). All of the domains are responsible for binding and inactivating cellular proteins that prevent the transition to S-phase. The progression is mainly due to the binding between T-Ag LxCxE motif and the members of the Rb tumour suppressor family (Bollag *et al.*, 1989; Vogelstein *et al.,* 2000). T-Ag sequestration of hypophosphorylated form of pRb activates transcription factors such as E2F1 which in turn activates transcription of some genes such as c-fos, c-myc needed to enter the S-phase of cell cycle (White and Khalili, 2006; Moens *et al.*, 2007). Disruption of pRB/E2Fs complex is mediated by J domain of T-Ag that binds to Hsc70, a chaperon. It increases its ATPase activity when associated with T-Ag and the energy produced during ATP hydrolysis is used for the separation of pRb and E2Fs (Sullivan and Pipas, 2002; Craig *et al.,* 2006). C-terminal region of T-Ag contains p53 binding domain (Sharma and Kumar, 1991). p53 is a tumour suppressor gene. Binding of T-Ag inactivates p53 resulting in the progression of cell cycle in presence of DNA damage (Bollag *et al.*, 1989; Vogelstein *et al.,* 2000).

Cellular proteins such as insulin receptor substrate 1 (IRS-1), β-catenin, neurofibromatosis type 2 gene product and anti-apoptotic protein Survivin are also involved in binding to the JCPyV T-Ag. IRS-1, a membrane associated tyrosine kinase is responsible for triggering cell proliferation and sending antiapoptotic signals. T-Ag is able to bind to it directly and causes its translocation into the nucleus and has an important role in the homologous-recombinant directed DNA repair mechanism. B-catenin is part of Wnt pathway and is involved in cell proliferation, survival and transcription processes. Mutations in the proteins of this pathway have been associated with the development of tumours (Reya and Clevers, 2005; Moon and Gough, 2016).

Binding of T-Ag to the β-catenin induces stabilization of cellular proteins and transcription of c-myc and cyclin D1 (Enam *et al*., 2002). The neurofibromatosis type 2 (NF2) interacts with the T-Ag but the consequences of this interaction is not clear (Beltrami *et al*., 2013). The interaction between T-Ag and the anti-apoptotic protein Survivin leads to significant decrement in the apoptotic process (Pina-oviedo *et al*., 2007).

JCPyV small t-Ag contains binding site for Protein phosphatase 2A (PP2A), a serine/threonine- specific protein phosphatase, which is involved in the mitogen-activated protein kinase i.e., MAPK pathway. JCPyV agnoprotein mediates this binding which results in the interference with the phosphatase activity and activation of pathways inducing cell proliferation (Sariyer *et al*., 2008). Small t-Ag also binds to the members of the Rb family i.e., pRb, p107 and p130 which may have role in cell cycle progression (Bollag *et al*., 2010).

The JCPyV late region encodes a regulatory protein call Agnoprotein of 71 amino acid length. It is produced late in the cycle. Phosphorylation of the agnoprotein is necessary for the functionality of the protein and replication of the virus (Sariyer *et al*., 2006). JCPyV agnoprotein have been shown to bind to both viral as well as host cellular proteins. It also plays a role in viral transcription, translation, assembly and also in cell cycle progression. Agnoprotein directly binds to the p53 causing cell cycle arrest (Darbiyan *et al*., 2002).

2.7 Detection of JCPyV:

The original methods of cytology and electron microscopy (EM) were not sensitive enough and virus isolation was a slow and laborious process. There are a number of ways to measure serum antibodies to JCPyV. Hemagglutination inhibition (HI) was the first to be developed. In HI assay, sialic acid receptors present on the surface of RBCs binds to the hemagglutinin glycoprotein found on the surface of virus that creates a network or a lattice structure. Antigen detection by ELISA is the more recent one to be used since it is more rapid and have greater sensitivity and precision relative to

HI (Arthur *et al*., 1983; Arthur *et al*., 1985; Gibson *et al*., 1985; Cobb *et al*., 1987; Kitamura *et al*., 1990; Kitamura *et al*., 1994). In ELISA, antigens to be tested are attached to a surface and then the matching antibody linked with an enzyme is applied over it to allow binding. Then substrate is added to the reaction mixture that produces a detectable signal.

For identification of human polyomaviruses, *in situ* nucleic acid hybridization, immunocytochemistry and Polymerase Chain Reaction (PCR) are mainly employed these days. *In situ* hybridization uses a complementary labeled DNA or RNA to localize a specific nucleotide sequence in a section of tissue or the whole tissue. In similar way, immunohistochemistry is used to localize proteins in tissue sections. Antibody used for the detection can either be conjugated to an enzyme or be tagged to a fluorophore. By immunohistochemistry, viral proteins can be detected in both oligodendrocytes and astrocytes. The early gene product T-Antigen is found in the nucleus and the capsid protein VP1 is found in both the cytoplasm and the nuclei of infected cells. Agnoprotein is located in the cytoplasm of both cell types with a characteristic prominence in the perinuclear region and a very little amount present in the nucleus (Cinque *et al*., 1997; Okada *et al*., 2002; Khalili *et al*., 2005).

Conventional PCR has been used to detect JCPyV DNA in urine of different patient groups, in urine of pregnant women and also from different types of human tissues (Coleman *et al.,* 1980; Arthur *et al.,* 1989; Markowitz *et al.,* 1991). Regulatory regions of JCPyV have been amplified from urine of Bone Marrow Transplant and renal transplant patients, from both HIV positive or negative individuals and in patients with various autoimmune diseases. JCPyV DNA has also been isolated in immunocompetent older individuals and peripheral blood leucocytes of immunocompetent individuals (Kitamura *et al*., 1990; Elsner and Dorries, 1992; Dorries *et al*., 1994; Baksh *et al*., 2001).

Quantitative PCR assay is being employed to determine viral DNA copies in body fluids or tissues of the patients to measure the viral load (Randhawa *et al.,* 2002; Priftakis *et al.,* 2003). Development of quantitative polymerase chain reaction (qPCR) assays

provides a specific, sensitive and quantitative method to measure JC viral load. Quantitative PCR assays have the capacity to identify small copy numbers of viral DNA (Pal *et al*., 2006). Biopsy of the brain is a hazardous procedure; hence, there is an increase in reliance on PCR from CSF samples and MRI.

MRI is much more sensitive than computed tomography for the detection of demyelinated lesions in PML patients (Berger, 2011). Comparative studies showed that there are no significant differences between AIDS and non-AIDS associated PML but perhaps a slightly more severe demyelination and a higher rate of infected cells was observed in the AIDS-related cases (Aksamit *et al*., 1990).