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Molecular Aspects of Loss of Y-linked Proteins and Their Potential Applications in Cell Biology

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Abstract
Loss of proteins linked to Y chromosome (LOY) is a biomarker in human that is typically found in male blood samples. In healthy males, its frequency often rises with age.Additionally, it has lately been linked to numerous illnesses, including cancer withsignificantly high prevalence. A healthy male development depends on the Y chromosome and associated proteins. The deletion of this chromosome's proteins or even its LOY variant may have effects on the male body. These proteins serve purposes beyond those of the male reproductive system.Paternity testing, ancestry research, and sexual assault investigations are just a few of the forensic situations where Y-linked protein analyses are frequently used. Due to its connection to the aging process, LOY detection has the benefit of being a biological age biomarker. The possibility of using LOY as a biomarker brings to light the need to define the molecular process underlying its occurrence and its potential applications in both health and forensic studies.
Humans frequently experience LOY, a non-physiological post-zygotic molecular change that mostly affects male blood cells[Forsberg, 2017; Forsberg *et al.*, 2017]. It is a natural part of aging and has been related to a number of illnesses, including as Alzheimer's, Autoimmune disorders, Schizophrenia, Cardiovascular problems, and different malignancies[Holmes *et al.*, 1985;Persani*etal.*, 2012; Dumanski*et al*., 2016; Forsberg, 2017; Forsberg *et al*., 2017; Haitjema*et al*., 2017;]. It is possible to think about LOY as a biological age marker and a biomarker that predicts male age-related disorders [Dumanski*et al*., 2016].However, LOY analysis might obstruct the forensic examination of male samples while also being helpful in forensic investigations by offering important details.

Keywords: Y-linked proteins, Biomarker, Health and forensic studies, Microdeletion, Infertility, Aging process, Haplotype, Haplogroups.

Introduction

One of the two sex chromosomes present in males is the Y chromosome.Nettie Stevens discovered it to be a sex-determining chromosome in 1905.It is one of the smallest chromosomes of the human karyotype. This chromosome contains the sex-determining region Y (SRY), a protein that causes the male phenotype throughout embryonic development, was found in 1990 by Andrew Sinclair and his team.*Pseudoautosomal regions (PAR)* and the *male-specific region of the Y chromosome (MSY)* were

two more Y chromosome regions that were discovered in 1985[Figure 1].The pseudoautosomal regions (PAR) are divided into two parts: ***PAR1*** is found at the terminal region of the short arm (Yp), while ***PAR2*** is found at the tip of the long arm (Yq)[Figure 1]. The "Non-Recombining Y" (***NRY***) or"male-specific region of the Y chromosome" (MSY)makes up the majority (95%) of the length of the Y chromosome, while PAR1 and PAR2 make up only 5% of the overall chromosome. Three classes of MSY proteins exist: ***X-transposed, X-degenerate***, and ***ampliconic***.MSY includes the euchromatic and heterochromatic regions of the chromosome. The euchromatic zone has many highly repeated sequences as well as some proteins involved in crucial biological functions.The Y chromosome's 30 Mb heterochromatin is rich in Alu and LINE repetitive sequences that create a distinctive profile transmitted down through generations.

Y-linked proteins are a significant subset of proteins that are encoded by the Y chromosome. These proteins are essential for the development of sexuality, sperm production, and male fertility. Numerous diseases and illnesses, such as infertility and problems in sexual development, can result from loss or changes in these Y-linked proteins.The LOYhas garnered significant attention not only in the fields of cell biology and disease research but also in forensic science. However, LOY has been observed in a considerable proportion of aging males, raising concerns about its potential applications in forensic science.This stems from the fact that LOY is not limited to certain cell types; instead, it impacts all cells throughout an individual's body. Consequently, LOY can serve as a biomarker, providing insights into the identity and biological characteristics of an individual.

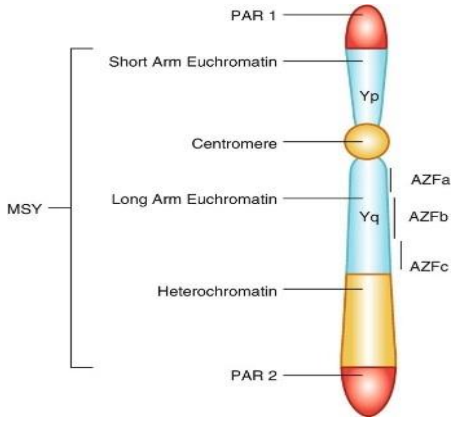


Figure 1: Structure of Y chromosome.

Y-Associated Proteins

A number of proteins is associated with theY linked genetic elements[Figure 2].Some of them are described below:

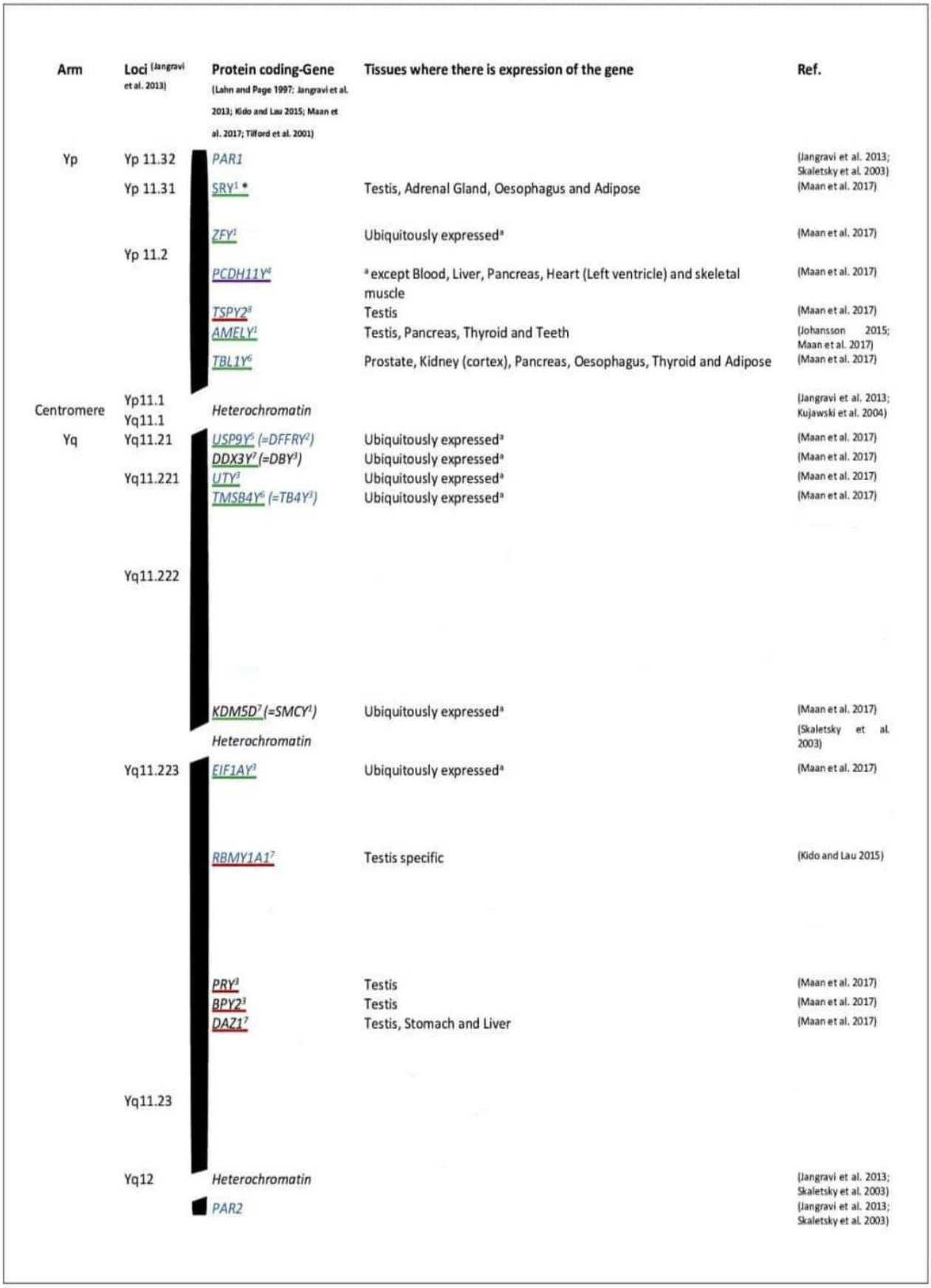
- **SRY**: Sex-determining region Y protein (SRY) is a DNA-binding protein that belongs to the SOX (SRY-like box) family. It is an intronless sex-determining protein on the Y chromosome. It is crucial for starting testis development and differentiating the bipotential gonad into Sertoli cells, which enable the development and differentiation of the male germline. Consequently, it has been suggested that this protein is the master protein controlling the process of testis determination. It is expressed in the testis as well as in somatic tissues such as oesophagus, adipose tissue, and the adrenal gland.

- **ZFY**:Zinc finger Y-chromosomal protein (ZFY), a protein encoded by the ZFY gene, is assumed to play a role in spermatogenesis, specifically in encouraging meiotic division and sperm production, and is expressed in all somatic tissues.
- **PCDH11Y**:Protocadherin 11, Y linked (PCDH11Y), a Homo male gene encoding Protocadherin 11Yprotein, is believed to contribute to the emergence of cerebral asymmetries and cell-cell recognition during brain development.It is expressed in multiple tissues including the testis and brain.
- **TSPY2**: Testis-specific protein Y linked 2 (TSPY2), a protein encoded by the TSPY2 gene,regulates the cell cycle and acts as a proto-oncogene and tumor suppressor, respectively.It is expressed in the testis.
- **AMELY**: Amelogenin, Y isoform (AMELY),encodes an extracellular matrix protein that is a member of the amelogenin family and is involved in biomineralization during the formation of tooth enamel.It is expressed in the testis, pancreas, thyroid, and teeth.
- **TBL1Y**:Transducin Beta-like 1Y (TBL1Y) is a Y-linked homologue of TBL1X. Recent research has shown thatTBL1Y is expressed differently during the cardiac development of human embryonic stem cells.It is expressed in the prostate, kidney (cortex), pancreas, oesophagus, thyroid, and adipose.
- **USP9Y**: The first protein discovered in the AZFa sub-region was Ubiquitin specific peptidase 9, Y-linked(USP9Y). It controls protein turnover, is involved in male germ cell development, and is required for sperm production.USP9Y is ubiquitously expressed in adult and embryonic tissues.
- **DDX3Y**: DDX3Y, an ATP-dependent RNA helicase, is expressed in pre-meiotic male germ cells. It regulates the beginning of cyclin E1's translation, which is necessary for cell cycle progression from the G1 to the S phase.Its expression in human testicular germ cells begins at 17 weeks of gestation, suggesting its potential role in early spermatogonial proliferation.
- **UTY:**Ubiquitously transcribed tetratricopeptide repeat containing,Y linked (UTY),aprotein found in various human tissues, may act as a chaperone and is involved in protein-protein interactions. Itparticipates in a transcriptional regulatory network that is crucial for prostate differentiation.
- **TB4Y:**Thymosin beta 4 Y linked (TB4Y) is a protein that, in humans,is encoded by TB4Y gene. It is one of the main activators of natural killer cell cytotoxicity. It is expressed in various tissues.
- **KDM5D**: Lysine Demethylase 5D (KDM5D), an enzyme,forms a protein complex withMutS protein homolog 5 [MSH5]DNA repair factor during spermatogenesis. This protein complex isinvolved inmale germ cell chromatin remodeling during leptotene/zygotene stage.By interacting with androgen receptor signaling, KDM5D plays a crucial role in determining docetaxel sensitivity, which is used to treat prostate cancer.
- **EIF1AY**: Eukaryotic Translation Initiation Factor 1A, Y linked (EIF1AY)is a Y-linked member of the EIF-1A family involved in translation initiation. The 43S complex's binding to the end of capped RNA during protein synthesis is stabilized by the EIF-1A proteins. It is widely expressed in multiple tissues.
- **PRY**: PRY is only found in the testis.It is believed that the PRY proteins play a role in controlling apoptosis, which is responsible for eliminating aberrant sperm.
- **RBMY1A1**: RNA-binding motif protein, Y chromosome, family 1 member A1/C(RBMY1A1) is a protein that, in humans, is encoded by the RBMY1A1 gene.By forming several protein-protein and

protein-RNA complexes, RBMY1A1 participates in a number of meiotic and pre-meiotic regulation-related processes.

- **BPY2**: Basic protein Y-linked 2 (BPY2), aproteinencoded by the BPY2 gene, is expressed only in the testis and is essential for the maturation of male germ cells.It is involved in the regulation of the cytoskeleton during spermatogenesis.
- **DAZ1**: Deleted in azoospermia 1 (DAZ1) is a protein that, in humans, is encoded by the DAZ1 gene. It only manifests in pre-meiotic germ cells, especially in spermatogonia. It produces an RNA-binding protein vital to spermatogenesis. It is expressed in testis, stomach, and liver.

Protein name	Cellular/ tissue specificexpression
SRY	Testis, Adrenal gland, Oesophagus,and Adipose
ZFY	Ubiquitously expressed
PCDH11Y	Testis and Brain
TSPY2	Testis
AMELY	Testis, Pancreas, Thyroid, and Teeth
TBL1Y	Prostate, Kidney (cortex), Pancreas, Oesophagus, Thyroid, and Adipose
USP9Y	Ubiquitously expressed
DDX3Y	Ubiquitously expressed
UTY	Ubiquitously expressed
TB4Y	Ubiquitously expressed
KDM5D	Ubiquitously expressed
EIF1AY	Ubiquitously expressed
PRY	Testis
RBMY1A1	Testis specific
BPY2	Testis
DAZ1	Testis, Stomach, and Liver



Arm	Loci (Jangravi et al. 2013)	Protein coding-Gene (Lahn and Page 1997; Jangravi et al. 2013; Kido and Lau 2015; Maan et al. 2017; Tilford et al. 2001)	Tissues where there is expression of the gene	Ref.
Yp	Yp 11.32	*PAR1*		(Jangravi et al. 2013; Skaletsky et al. 2003)
	Yp 11.31	*SRY*[1] *	Testis, Adrenal Gland, Oesophagus and Adipose	(Maan et al. 2017)
		ZFY[1]	Ubiquitously expressed[a]	(Maan et al. 2017)
	Yp 11.2	*PCDH11Y*[4]	[a] except Blood, Liver, Pancreas, Heart (Left ventricle) and skeletal muscle	(Maan et al. 2017)
		TSPY2[8]	Testis	(Maan et al. 2017)
		AMELY[1]	Testis, Pancreas, Thyroid and Teeth	(Johansson 2015; Maan et al. 2017)
		TBL1Y[6]	Prostate, Kidney (cortex), Pancreas, Oesophagus, Thyroid and Adipose	(Maan et al. 2017)
Centromere	Yp11.1 Yq11.1	*Heterochromatin*		(Jangravi et al. 2013; Kujawski et al. 2004)
Yq	Yq11.21	*USP9Y*[5] *(=DFFRY*[2]*)*	Ubiquitously expressed[a]	(Maan et al. 2017)
		DDX3Y[7] *(=DBY*[3]*)*	Ubiquitously expressed[a]	(Maan et al. 2017)
	Yq11.221	*UTY*[3]	Ubiquitously expressed[a]	(Maan et al. 2017)
		TMSB4Y[6] *(=TB4Y*[3]*)*	Ubiquitously expressed[a]	(Maan et al. 2017)
	Yq11.222			
		KDM5D[7] *(=SMCY*[1]*)*	Ubiquitously expressed[a]	(Maan et al. 2017)
		Heterochromatin		(Skaletsky et al. 2003)
	Yq11.223	*EIF1AY*[3]	Ubiquitously expressed[a]	(Maan et al. 2017)
		RBMY1A1[7]	Testis specific	(Kido and Lau 2015)
		PRY[3]	Testis	(Maan et al. 2017)
		BPY2[3]	Testis	(Maan et al. 2017)
		DAZ1[7]	Testis, Stomach and Liver	(Maan et al. 2017)
	Yq11.23			
	Yq12	*Heterochromatin*		(Jangravi et al. 2013; Skaletsky et al. 2003)
		PAR2		(Jangravi et al. 2013; Skaletsky et al. 2003)

Figure 2: An outline of the Y-encoded MSY proteins.

Role of the MSY ProteinsinAnalysis of Diseases

The Y chromosome plays crucial role in the formation of male gonad during embryogenesis, fertility maintenance, and Y-linked phenotypic male features as well as skeletal growth, tooth size, handedness, and brain asymmetry [Singh *et al.*, 2011]. According to a research by Jangravi*et al.*, 2013, MSY proteins have been linked to several biological functions, such as transcription, sex differentiation, cell proliferation, cell adhesion, metabolic processes, tissue development, chromatin modification, protein translation, and cell differentiation [Figure 3]. For example, TSPY (an MSY protein) having proto-oncogenic properties plays a role in spermatogonia renewal and meiotic cycle regulation[Lau *et al.*, 2019], whereas SRY inhibits the activation of androgen receptors by interacting with them [Yuan *et al.*, 2001].

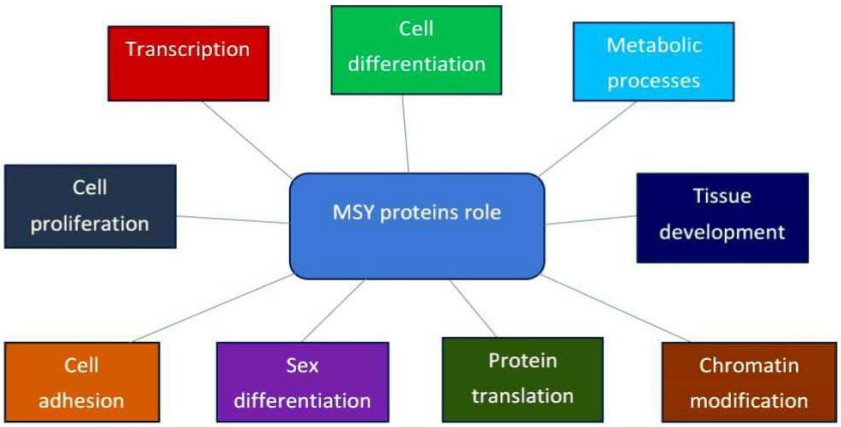


Figure 3: A word diagram showing role of MSY proteins.

Numerous research on Y-linked disorders have focused on the MSY proteins. These are important for male spermatogenesis because male infertility is usually accompanied by microdeletions in the azoospermia factor(AZF)regions(AZFa, AZFb, and AZFc)located in the long arm (Yq) of Y[Figure 4] [Batiha*et al.*, 2018].The sexual development disorder,Y chromosome gonadal dysgenesis (Y-GD) is characterized by testicular underdevelopment and frequently manifests as gonadoblastoma, a benign tumor that has been linked to a region close to the Y chromosome centromere [Berberoglu*et al.*, 2018]. Y-GD patients are phenotypically divided into two groups – complete and partial, and their karyotypic description is either 46,XY GD or 45,X/46,XY GD [Figure 5] [Berberoglu*et al.*, 2018].

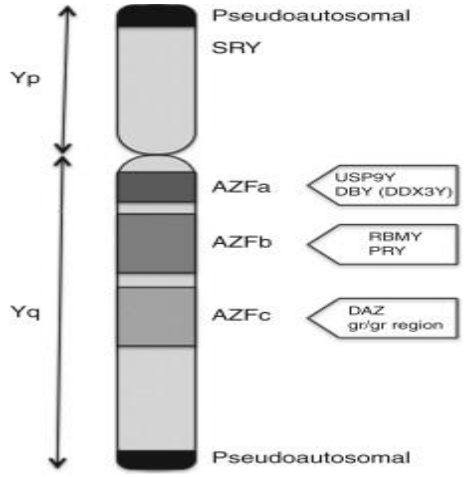


Figure4: Regions of the Y chromosome required for male fertility.

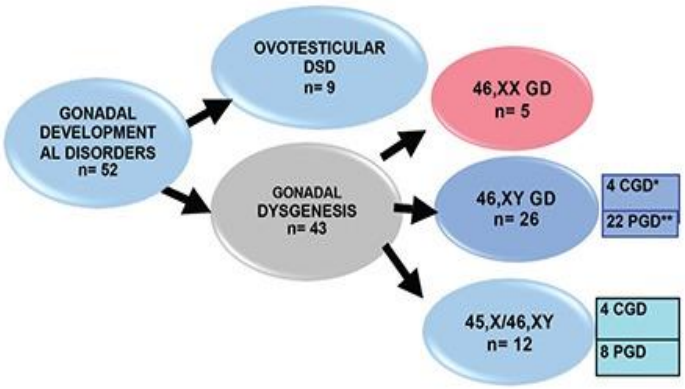


Figure 5: Distribution of patients with gonadal developmental disorders.

The MSY has been linked to viral infections and cardiovascular diseases like hypertension, coronary artery disease, myocardial infarction, and stroke [Case and Teuscher, 2015; Johansson, 2015]. In spontaneously hypertensive rats (SHR) and stroke-prone spontaneously hypertensive rats (SHRSP), high blood pressure has been related to this chromosome [Ely and Turner, 1990].In their 1990 study, Ely and Turner demonstrated the Y chromosome's function in controlling blood pressure by transplanting the Y chromosome of SHR to WKY (normotensive Wistar Kyoto) rats, which caused a 12 mmHg rise in systolic blood pressure. Sry is a gene complex found in SHR rats that consists of seven distinct Sry copies (Sry1, Sry2, Sry3, Sry3A, Sry3B, Sry3B1, and Sry3C), each of which produces a functional protein with varied degrees of expression in various tissues and all have the capacity to create an SRY protein [Ely *et al.*, 2011].SRY1 influences tyrosine hydroxylase and norepinephrine levels to raise blood pressure in rats via boosting sympathetic nervous system activity [Milsted*et al.*, 2010].The Sry3A gene was inserted into WKY rats, and this resulted in a 50% increase in renal sodium reabsorption, probably as a result of a rise in renal angiotensin II (Ang-II) [Ely *et al.*, 2011]. Co-transfecting Sry1, Sry2, and Sry3 expression vectors into cultured hamster cells was observed to cause SRY3 to downregulate ACE2 promoter activity and to upregulate the angiotensin, renin, and ACE gene promoters activity [Figure 6] [Milsted*et al.*, 2010].These genes encode proteins that are components of the renin-angiotensin system (RAS), which is primarily responsible for controlling blood pressure through the actions of Ang-II (which elevates blood pressure) and Ang-(1–7) (which lowers blood pressure) [Figure 7] [Milsted*et al*., 2010].Angiotensin, renin, and ACE gene overexpression (which raises Ang-II levels) and ACE2 genedownregulation(which raises Ang-(1–7) levels) are the main causes of elevated blood pressure [Milsted *et al.*, 2010].

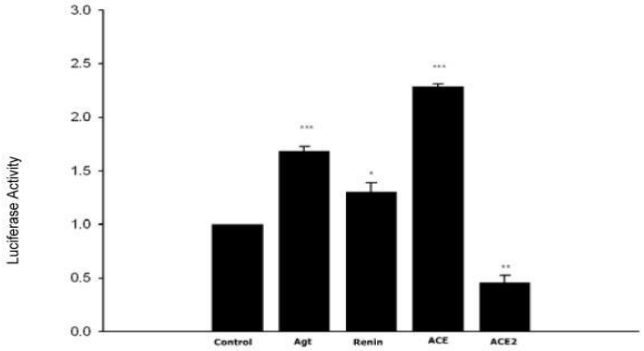


Figure 6: Sry3 increases activity of renin, angiotensinogen and ACE promoters while decreasing activity of ACE2.

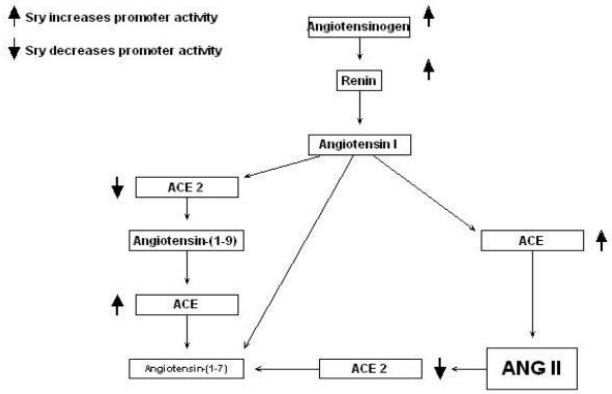


Figure 7: The classical renin-angiotensin system, with arrows indicating promoter responses to Sry. The combined effects of Sry on genes of the renin angiotensin system would favor increased levels of Ang II and decreased levels of Ang-(1-7).

Different variants of the Y-linked protein elements, which is only passed down through paternal lineages, are identified by distinctive molecular alterations in the MSY region. A&B, CT, C, D, E, F, G, H, I, J, K, L&T, K2, K2a, K2a1, K2b1, NO & NO1, N, O, M&S, P, Q, and R are important Y variants. Numerous research works have previously assessed the correlation between various Y variants andillnesses. One-fifth of the European population belongs to Y variant I, which is linked to coronary artery disease(CAD), increased HAART (highly active antiretroviral therapy) resistance, and faster HIV progression. By analyzing monocyte and macrophage transcriptomes of males with Y elements, the study explored the molecular mechanisms behind the association between variant I and CAD. It discovered variations in the expression of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways between men with variant I and carriers of other variants [Figure 8].Nineteen pathways were associated with immune signaling cascades or inflammation, characterized by activation of inflammatory pathways and downregulation of proteins involved in autoimmune and adaptive immunity [Charchar*et al.*, 2012; Maan*et al.*, 2017]. A 2-fold higher risk of developing atherosclerotic plaque has been associated with variant K [Hiura *et al.*, 2008].Young men with a history of myocardial infarction have greater LDL levels when they have the Y chromosome HindIII polymorphism[Figure 9] [Charchar *et al.*, 2004].

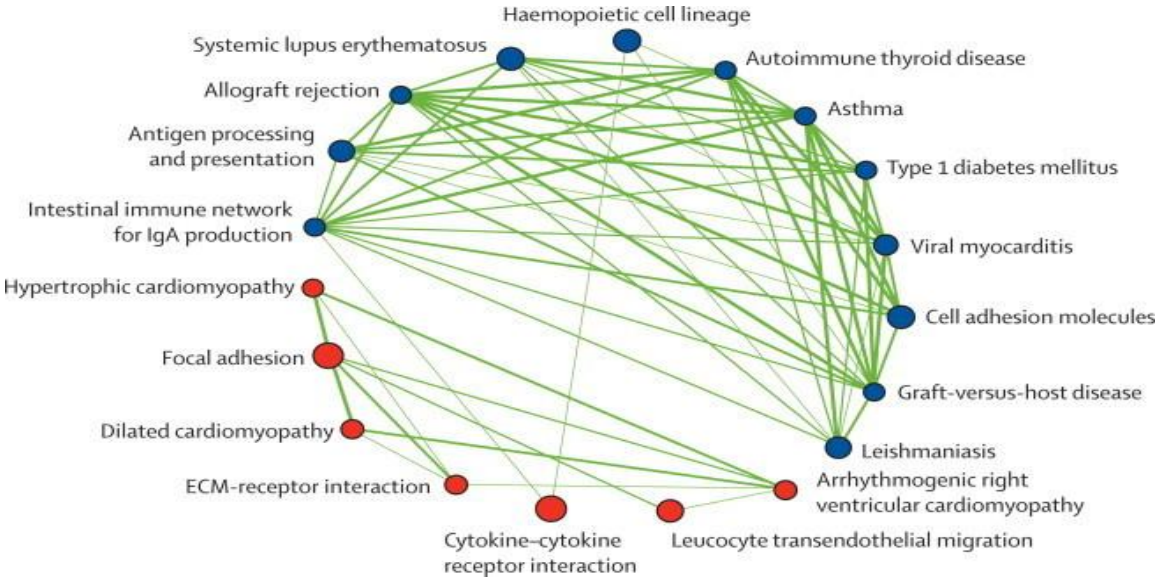


Figure 8: Immune pathways showing significant differential expression in macrophages from men with Y variant I compared with carriers of all other variants. Red nodes show upregulated pathways and blue nodes show downregulated pathways in men with variant I (Adapted from the Kyoto Encyclopedia of Genes and Genomes).

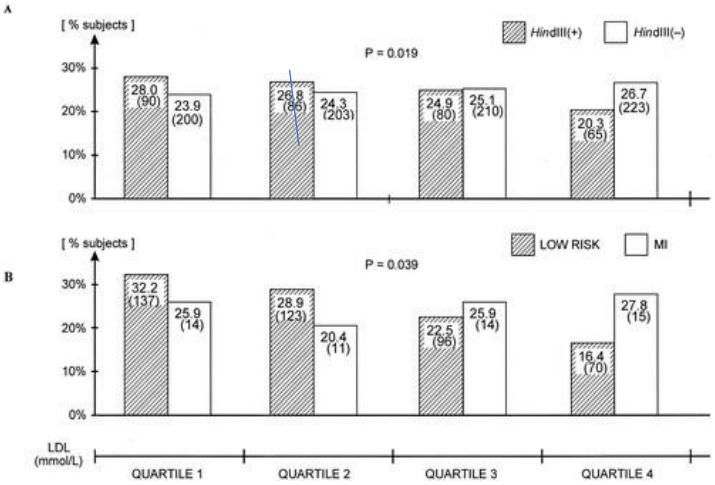


Figure 9: A,HindIII(+/−) genotypes across increasing quartiles of LDL in Young Men Cardiovascular Association (YMCA) study. B, Paternal history of cardiovascular risk (MI, fathers with history of myocardial infarction; LOW RISK, fathers with low cardiovascular risk, without a history of hypertension, CHD and diabetes) across increasing quartiles of LDL in YMCA study. [Adapted from Charchar*et al.*, 2004]

Studies have suggested a connection between the Y chromosome and prostate cancer (PC). Research indicates thatY variant O3in the Japanese population has a higher propensity to develop PC whileY variant DE is more likely to experience PC [Paracchini*et al.*, 2003; Ewis*et al.*, 2006]. The low incidence of PC in the Japanese population may be explained by these facts. But Y variant R1a has a higher PC frequency than other variants, probably because SRY expression is different [PlaseskaKaranfilska*et al.*, 2009]. Loss of the Y chromosome, the most prevalent aberration in this kind of cancer, is one of the Y chromosome-related changes in PC [Johansson, 2015].

Research suggests that the Y chromosome, which has been associated with a number of disorders, may be a factor in the reported disparities in lifespan between men and women [Forsberg, 2017; Forsberg *et al.*, 2017].

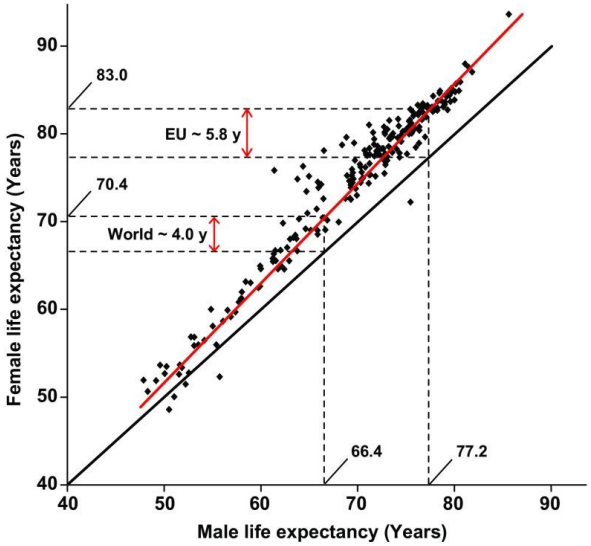


Figure 10: Men live on average shorter lives compared to women. Each dot shows data from one nation or human population. The red line represents the observed difference in lifespan and the black line represents a null hypothesis with no difference in longevity between the sexes. The dotted lines mark the male and female life expectancies globally and in the European Union (EU). Data from The World Factbook 2013 on male and female life expectancy at birth in different human populations in 2013.

Uses of MSY Proteins in Forensics

In forensic sciences, the Y linked proteinsare widely employed for research on human migration patterns, anthropology, paternity testing, evidence analysis and genealogy analysis [Butler, 2012]. Short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs) are two common variants that are examined in forensic applications of the Y chromosome [Kayser, 2017]. Each individual has a unique set of autosomal STRs and SNPs that together form their unique fingerprint, enabling human differentiation and individualization [Kayser, 2017].In situations where autosomal polymorphisms are insufficient, forensic commercial kits sometimes incorporate Y chromosome-specific polymorphisms, such as Y-STRs and Y-SNPs. There are already 27 Y-STRs that have been validated, making it possible to characterize paternal lineage with a high level of certainty [Gopinath*et al*., 2016]. Additionally, the present amplification of more than 40 Y-STR sequences is made possible by the combination of various commercial kits and additional multiplexes [Roewer, 2019].

Forensic science relies heavily on the MSY proteins to distinguish males from other genders in DNA sample combinations [Prinz and Sansone, 2001]. It can be used in forensic cases as a haplotype because of its uniparental transmission from father to son, with 95% of it not recombinating, making it a very helpful tool[Butler, 2012].

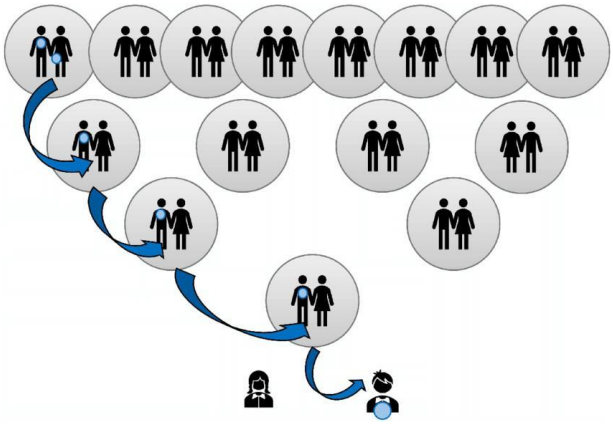


Figure 11: Illustration of Y chromosome inheritance over four generations indicates ancestral males that share the same Y chromosome haplotype as the child at the base.

When analyzing the Y chromosome, biallelicand multiallelicgroupings based on polymorphisms emerge, designating haplogroups and haplotypes accordingly [Butler, 2012]. Haplotypes are distinguished by Y-STRs and minisatellites, which have elevatedmutation rates than Y-SNPs [de Knijff, 2000], whereas haplogroupsare identified by Y-SNPs and Alu elements, which have reduced mutation rates than Y-STRs [Hammer, 1994]. When it comes to paternity testing, Y-haplotypes are more like a “family fingerprint” than Y-haplogroups, which are utilized to research human migration and biogeographic ancestry [Phillips, 2015; Kayser, 2017]. When the father is absent or unavailable for autosomal STR testing, Y-STRs might be used to test a male relative in his place, assuming the child is related to the missing father if the Y haplotypes match [Butler, 2012]. However, the bulk of the Y chromosome is non-

recombinant, which reduces the discrimination ability between members of the same family, rendering Y haplotypes insufficient to differentiate between male members of the same family linage [Butler, 2012]. As a result,as was previously noted, Y-STRs are only investigated when autosomal STRs alone are unable to provide answers to legal proceedings [Butler, 2012].

Y-linkedproteins are also employed for forensic sample sex typing [Butler and Li, 2014]. For further DNA profiling, the majority of commercial kits employ the amelogenin (AMEL) protein and a large assortment of autosomal STRs[Butler and Li, 2014].The AMEL locus enables chromosomal sex determination because it contains two homologous proteins on both the X and Y chromosomesthat differ by 6 bp from each other. [Mannucci*et al.*, 1994].Even though AMEL fragments are used all over the world, multiple incidents of failure have been documented due to AMELY deletions.Amelogenin sex test failure rates have been found to range from as low as 0.018% to as high as 8% depending on the population [Butler and Li, 2014]. SRY and TSPY proteins, which are involved in gonadal genesis and spermatogenesis, respectively, are employed to circumvent this. These markers are essential for preventing inaccurate chromosomal sex determination brought on by the deletion of the AMELY protein because they provide more precise chromosomal sex determination [Morikawa*et al.*, 2011]. These markers must be included in industrial forensic kits.

Loss of Y-linked(LOY) Proteins

Leukocytes in older men are affected by the condition LOY [Jacobs *et al.*, 1963]. In 1995, in situ hybridization was used to assess the lymphocyte nuclei of 138 male probands, ranging in age from 1 week to 93 years, for Y chromosome loss. Age-dependent loss of Y chromosome was seen in donors older than 16 years.As people aged, Y hypoploidy became more common.Men's Y hypoploidy was extremely low (0.05%) till the age of 15 but steadily grew to 1.34% in the 76–80 age range[Guttenbach*et al.*, 1995]. Recent sequencing data revealed decreased amplification of all Y chromosome-specific loci in LOY samples, albeit the exact mechanism behind LOY is yet unknown [Arseneault*etal.*, 2017]. Some theories suggest that environmental factors that cause missegregation during mitosis are the cause of LOY, which happens as a neutral event [Dumanski*etal.*, 2015].Telomeric shortening raises chromosomal instability, which encourages chromosomal decay and eventually results in Y chromosome linked protein loss in elderly guys [Guttenbach*et al.*, 1995].Due to Y-chromosomal replication's propensity to take place at a late stage of the S phase, anaphase shortening favours unintentional losses [Persani*et al.*, 2012].

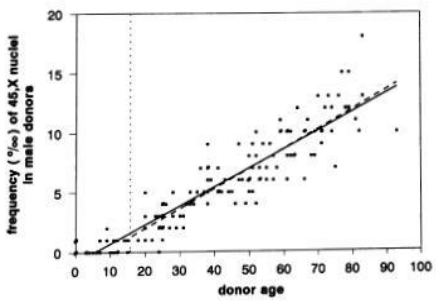


Figure 12: Frequency of Y chromosome loss in lymphocyte nuclei of male probands plotted against donor age.

In 1985, a study that showed patients with acute myeloid leukemia had Y chromosome hypoploidy in their bone marrow became the first to correlate LOY with illness [Holmes *et al.*, 1985]. Since then,

numerous publications have suggested a possible connection between LOY and a number of illnesses, including Alzheimer's, cardiovascular problems,autoimmune disorders and malignancies[Holmes *et al*., 1985; Persani*et al*., 2012; Dumanski*et al*., 2016; Forsberg, 2017; Forsberg *et al*., 2017; Haitjema*et al*., 2017;]. Additionally, male samples of normal and clear cell renal cell carcinoma were used by Arsenault*et al*., 2017 to acquire a gene expression RNA-Seq dataset. The results were associated with 11 Y-linked proteins that express less, including KDM5D, an epigenetic regulator whose deficit contributes to the emergence of clear cell renal cell carcinoma [Arseneault*et al*., 2017].By promoting cell proliferation and interacting with apoptosis and cancer-related signaling pathways, the LOY chromosomal content may aid in the emergence of illness [Forsberg, 2017; Forsberg *et al*., 2017; Wright *et al*., 2017]. Error repairing and cell cycle progression have been shown to be adversely affected by molecular variations related with LOY [Wright *et al*., 2017]. Higher genomic instability and possible detrimental consequences on leukocyteimmunological function are additional mechanisms connecting LOY to higher illness risk [Loftfield*et al*., 2019]. To validate LOY's impact on immunological function, more functional investigations are required.

Biological Causes of LOY

The factors responsible for LOY are as follows:

(i) Age is the single biggest risk factor for LOY in the somatic cells of aging males, a phenomenon that has been documented for over 50 years. Recent research revealed that in male lymphocytes, LOY is extremely low till the age of 15, but in men 76–80 years old, it rises to 1.34%. Significant population-based GWAS (genome-wide association studies) revealed that the prevalence of LOY in blood samples is less than 2% in men under 60, 15–40% in those between 70 and 85 [Forsberg *et al.* 2014; Dumanski*et al.* 2015;], and 57% in those over 93. In the dorsolateral prefrontal cortex and buccal mcosa cells, age-related rise in LOY have also been reported. According to these facts, LOY develops dramatically with age and tends towards inevitability [Zhou et al. 2016; Forsberg et al. 2019].

(ii) Despite being extremely polygenic, LOY is mostly related to age, and its molecular cause is unknown. In order to comprehend the risk of LOY, it is crucial to identify the genetic variant that is influencing LOY. TCL1A is the initial susceptibility variant for LOY, and there are eighteen other genetic loci associated with cancer susceptibility, genomic instability, and cell cycle regulation after that [Thompson *et al.*, 2019]. In more recent times, 31 new LOY-associated genetic loci have been described in a Japanese population, and 137 novel autosomal genetic determinants of LOY have been discovered. It's interesting to note that different demographics and ethnic groups exhibit different levels of LOY; males with African heritage exhibit lower levels of LOY than men with European ancestry. To elucidate the causative variations in beginning and modifying LOY, more research is required.

(iii) Y chromosome structural abnormalities have the potential to cause LOY because the long arm of Y is prone to intra-chromosomal recombination due to the presence of several ampliconic and palindromic sequences. As a result, Y is vulnerable to intra-chromosomal deletions and copy number variation, but it also permits gene conversion. LOY in lymphocytes and sperm has been linked to Y chromosome microdeletion.Y chromosome recombination between sister chromatids may result in isodicentricChrY (idicY), which leads to the loss of the chromosome during segregation. LOY is also triggered by other anomalies related toY, such as rings and derivates. It is yet unclear to what degree these abnormalities contribute to LOY.

(iv) Environmental stimuli from the outside as well as the inside can cause LOY. Smoking has a substantial correlation with LOY; those who smoke currently have a higher degree of LOY than people who do not smoke or who have smoked in the past. Moreover, LOY can be brought on by insecticides, outdoor air pollution, and polycyclic aromatic hydrocarbons (PAHs). Men who are obese and heavy drinkers also show greater prevalence of LOY. It's intriguing to see if LOY could act as a mediator in the relationship between environmental stresses and the detrimental health effects they cause. Nevertheless, the correlation between LOY and alcohol, pollution, and obesity is not as developed, and additional research is required to validate and reproduce these results.

Overall, aging,genetic variations, Y chromosome structural abnormalities, and environmental stresses are all recognized risk factors for LOY, suggesting that multiple processes contribute to the emergence of LOY.

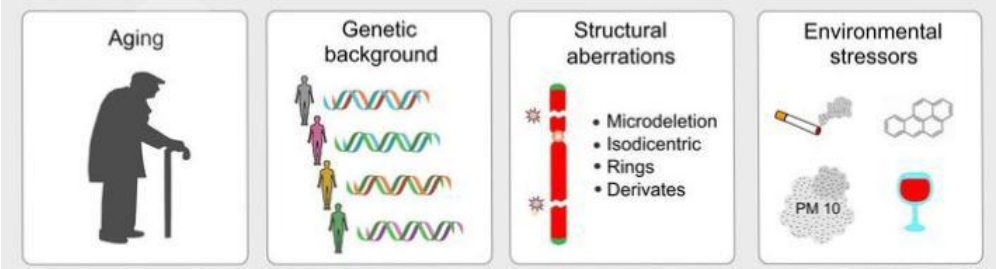


Figure 13: Diagram of the causes of loss of Y chromosome (LOY).

Common Diseases Associated with LOY

Recent studies show LOY in blood increases susceptibility to common aging-related diseases like Alzheimer's disease(AD), cancer, cardiovascular diseases, and diabetes.

- **Alzheimer's disease(AD):** Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disease of the central nervous system that accounts for about 70% of dementia cases. AD can be classified into two categories: early-onset familial AD and late-onset sporadic AD.The most common causes of familial AD are mutations in PSEN1, PSEN2, and APP. Many genes have been found to be significant risk factors for sporadicAD. Of these, the apolipoprotein E4 (ApoE4) allele is the most significant genetic risk factor. Patients who are heterozygous for ApoE4 have a threefold greater chance of developing AD, whereas ApoE4 homozygotes are 14 times more likely to develop AD than non-carriers. It is noteworthy that LOY, a genetic variant that differs fundamentally from ApoE4, has a 6.8-fold increased risk of sporadic AD diagnosis in blood (Dumanski*et al.,* 2016). Likewise, there is a slight correlation between LOY in the dorsolateral prefrontal cortex and the risk of sporadic AD as well as cognitive disorders.

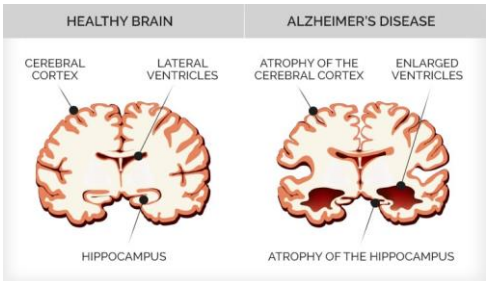


Figure 14: The structure of the healthy brain and Alzheimer's disease (AD) brain.

- **Acute myeloid leukemia (AML):**Acute myeloid leukemia (AML) is a cancerous condition that originates in the bone marrow, the organ that producesblood cells.It is typified by therapid development of aberrant white blood cells, which build up in the bone marrow and obstruct the formation of healthy blood cells. Mutations that activate oncogenes or inhibit tumor suppressor genes can be the cause of cancers, including AML. For example, mutations in genes like FLT3, c-KIT, and RAS are frequently observed in AML cells. These kinds of alterations may prevent bone marrow cells from developing normally or may encourage uncontrollably high cell growth.Larger chromosomal alterations, most likely caused by modifications to a small number of genes on that particular chromosome, can also cause AML.The risk of AML rises with age, primarily affecting elderly persons. A higher incidence of AML has been associated with LOY. According to a research in the Journal of the National Cancer Institute, men with LOY had a more than 6-fold higher chance of getting AML than men without LOY.

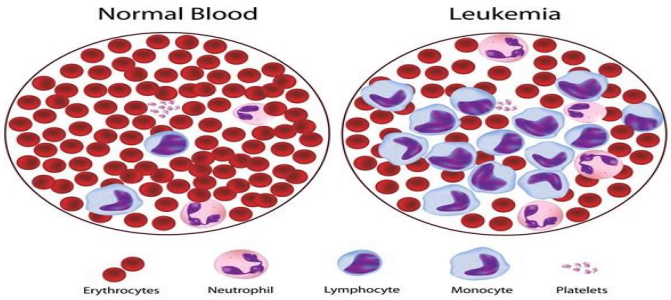


Figure 15: Figure showing normal blood and leukemia blood cells.

LOY as a Biomarker

The possibility of LOYas a biomarker for elevated mortality and illness risk in aging men has been raised [Forsberg, 2017; Forsberg*et al*., 2017a; Loftfield*et al*., 2018].The term “biomarker” refers to a molecule that can be easily detected within the body and that has the ability to affect or predict the occurrence or course of a disease[Strimbu and Tavel, 2010].It has been discovered that exposure to smoking and outdoor pollution raises the frequency of LOY, which raises the risk of mortality [Dumanski*et al*., 2015; Wong*et al*., 2018]. The onset of pathogenic processes like colorectal cancer and PC may also be detected by LOY [Noveski*et al*., 2016]. According to conventional wisdom, primordial germ cells that are prevented from maturing are the source of familial TGCT(testicular germ cell tumor). Environmental factors and genetic susceptibility factorsare assumed to be responsible for this process, which is believed to start during fetal development. In patients with familial TGCT, LOY was considerably higher than in cancer-free people [Machiela*et al*., 2017]. Healthy people under 50 exhibited lower LOY frequencies than TGCT patients, which suggests that LOY could be used as a biomarker of carcinogenesis and cancer aggressiveness [Forsberg *et al*., 2014].According to a study, LOY is a risk factor for head and neck cancer that may not be a good indicator of prognosis and may perhaps make patients more resistant to treatment[Hollows *et al.*, 2019].As the amount of LOY differs among disorders, a more individualized diagnosis and/or course of treatment could be based on LOY frequency[Silva Veiga*et al*., 2012; Dumanski*et al*., 2016, 2017; Arseneault*et al*., 2017; Haitjema*et al*., 2017]. Recent studies have shed additional light on the heredity of LOY, showing that myeloid lineage cells exhibit LOY more frequently than lymphoid lineage cells, while Thompson*et al*., 2019 discovered

that the LOY-associated gene had the greatest impact on haematopoietic stem progenitor cells [Dumanski*et al*., 2019; Thompson *et al*., 2019].Haematopoietic stem cells, multipotent progenitor cells, and common myeloid progenitor cells are three distinct temporal modes in the haematopoiesis tree that are all impacted by molecular variations linked to LOY [Thompson *et al*., 2019]. As a result, LOY may represent geneticinstability that occurs in other cells and tissues, potentially enhancing disease prevention, detection, monitoring and prognosis [Thompson *et al*., 2019; Grassmann*et al*., 2020].

A person's biological age—a measure of their aging process and overall health—can be ascertained using LOY [Kang *et al*., 2018]. More so than age in years, it is a longevity predictor [Jylhävä*et al*., 2017]. The frequency of LOY increases with age, with lower frequencies in males under 50 and higher frequencies in those over 80 [Loftfield*et al*., 2019]. Particularly in the evaluation of work-related damage, LOY can enhance lifespan assessment and health status [Kang *et al*., 2018].LOY may be useful in demonstrating the detrimental impacts of exposure to a particular work-related or professional aspect that may shorten an employee's life expectancy. Insurance companies can also benefit from it, since life insurance premiums are determined by the client's longevity [Chang, 2009; Kang*et al*., 2018]. To safeguard human rights, more government controls on the use of biological age are necessary, as this presents privacy concerns. By offering a resource to access biological age, LOY may be useful to the forensic and medical industries.

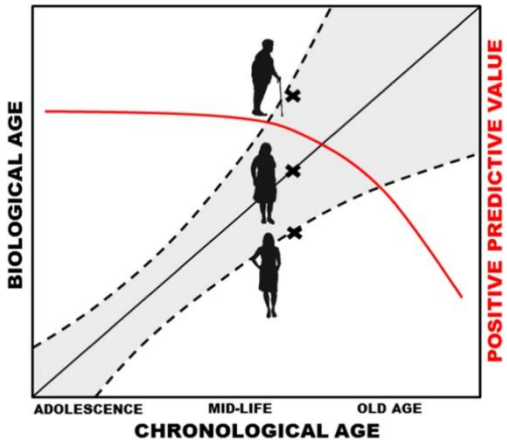


Figure 16: The concept of biological age predictors. A biological age predictor could be defined as a biomarker correlated with chronological age (black line), which brings additive information in the risk assessments for age-related conditions on top of chronological age. Hence, adult individuals of the same chronological age could possess different risks for age-associated diseases as judged from their biological ages (Xs infigure).Usually, the positive predictive value (red line) of a biological age predictor decreases from mid-life and onwards due to the increased biological heterogeneity at old age (confidence interval described by dashed lines increases at old age).

According to Kimura *et al*., 2018 there is a correlation between LOY and a greater completion rate ofsuicidein men. Additionally, post-mortem blood and brain samples from both controls and suicidecompleters showed abnormal LOY rates, indicating that LOY may have applications in forensic psychology[Kimura*et al*., 2018].

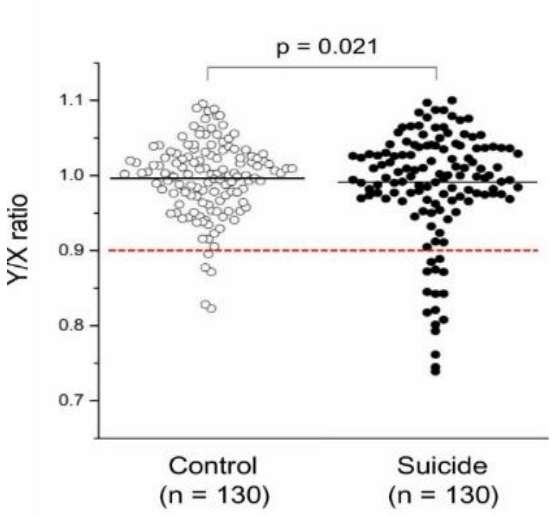


Figure 17: Dot plot of LOY in blood of suicide completers and healthy controls.

Lack of Y-STRs can result in fewer Y-STR or Y-SNP content detection, while the ramifications of LOY in forensics are not yet obvious. Analyzed signal strength may change as a result of this occurrence, which is called Y-STR allelic dropout[Andersen *et al*., 2013]. By tampering with the accuracy of sample analysis, LOY might jeopardize forensic investigations. The association between LOY and Y-STR or Y-SNP allelic dropout has not been studied, however research is being done on other aberrations such as microdeletions[Wang *et al*., 2019].

In forensic investigations, blood and buccal samples are commonly utilized as information sources, and several studies have documented LOY [Jacobs*et al*., 2012; Shewale and Liu, 2016; Zhou *et al*., 2016; Forsberg *et al*., 2019]. But in normal processes like DNA analysis of biological materials and Y-STR analysis by capillary electrophoresis, LOY might introduce skewed outcomes.It could be challenging to establish a male individual's involvement in the crime scene if LOY affects their buccal or blood cells, as this could result in missing Y chromosomes during DNA testing. Sperm residuals, which usually carry the Y chromosome, are the main piece of evidence in cases of sexual assault. Although LOY is a byproduct of mitosis, it is not anticipated to be present in sperm cells because they are the product of meiosis.Given the significance of the Y chromosome in forensic case solving, it is necessary to ascertain whether LOY may have altered the outcomes of other kinds of forensic sample examination.

Several techniques, such as karyotype analysis, in situ hybridization, and SNP-array data analysis, have been used to identify and quantify LOY [Jacobs *et al*., 1963; Holmes *et al*., 1985; Kujawski*et al*., 2004; Al-Saleem*et al*., 2005; Silva Veiga*et al*., 2012]. The most popular method is in situ hybridization, although SNP-array data analysis continuously estimates LOY in DNA samples using probes unique to MSY [Forsberg*et al*., 2014, 2017; Dumanski*et al*., 2015, 2016; Zhou *et al*., 2016; Forsberg, 2017; Haitjema*et al*., 2017]. SNP-array quality at the sample level is necessary because low-quality SNP-array data can cause bias in LOY estimation [Dumanski*et al*., 2016]. By comparing the read depth on the Y chromosome to the entire human genome, whole-genome sequencing (WGS) provides another technique for identifying LOY [Danielsson*et al*., 2020]. A median ploidy is obtained by utilizing read counts to determine the copy number of the male sex chromosome [Danielsson*et al*., 2020].

Compared to other approaches, PCR-based techniques involve less data interpretation and can be a more cost-effective standardized alternative for identifying LOY. A number of investigations have employed a Y/X ratio, which entails amplifying the two AMEL fragments on both the X and Y chromosomes, to

identify LOY. To calculate LOY, capillaryelectrophoresis is used to measure the products and compare them with mixed samples [Noveski*et al*., 2016; Hirata *et al*., 2018; Kimura *et al*., 2018]. Conversely, droplet digital PCR (ddPCR) was described as a method for identifying LOY by Danielsson*et al*. [2020]. Y and X chromosome counts in a particular sample are measured using droplet digital PCR (ddPCR), a TaqMan-based technique[Danielsson*et al*., 2020].HindIII enzyme is used to digest the DNA, which is then diluted with ionized water and combined with all the other components required for PCR amplification.In order to get readings for the Y/X ratio, this combination is placed into a droplet generator and examined by a droplet reader [Danielsson*et al*., 2020]. Male AMELY deletion is rare, though, this could cause problems with AMELY/AMELX ratio measurements and lead to LOY false positives. It will take more research to fully comprehend how the loss of AMELY could interfere.

Real-time PCR (qPCR) is a quick, affordable, and successful way to identify LOY [Schmittgen and Livak, 2008].This kind of PCR is based on a quantitative endpoint called the cycle threshold (Ct), which is the point at which the targeted protein starts to be amplified when the apparatus detects fluorescence [Schmittgen and Livak, 2008].The quantity of amplification product has an inverse relationship with the Ct value. By employing a constitutive Y chromosomal protein like SRY and an additional protein as an endogenous control, this technique might be utilized to evaluate the presence of LOY and enable prompt LOY detection.

Three techniques have been suggested to measure the proportion of cells devoid of the Y chromosome. The median Log R ratio of SNP-probes in the MSY (mLRR-Y) was initially used by Forsberg *et al*. [2014] to convert SNP-array data into LOY percentage.It was calculated that samples exhibiting mLLR-Y values less than –0.139 and –0.40 had over 18 and 35% of their cells impacted by LOY, respectively [Forsberg *et al*., 2014]. The LOY percentage was estimated by Grassmann*et al*. [2020] using a method based on karyotyping and chip genotyping data. Using data from SNP-array, WGS, and ddPCR, Danielssonn*et al*. [2020] created a formula.Multiple techniques, demographic traits, and bodily fluid used for DNA research mean that a standard approach for identifying and measuring LOY has not yet been devised.

Although the theory that LOY results from a complete chromosome loss event is unconfirmed, Heller *et al*.'s 1996 work indicates that fragments of the Y chromosome may still be replicated and transmitted to offspring cells. This may help to explain the differences in LOY between various illnesses and measurement techniques.

Biomarker	**Disorders/ Conditions**
Exposure to smoking and outdoor pollution	Raises the risk of mortality
Environmental factors and genetic susceptibility factors	Familial testicular germ cell tumor (TGCT)
LOY-associated gene variants	Affects hematopoietic stem progenitor cells
Biological age	Expression of aging that assesses one's level of health or a life expectancy predictor
Work/professional factor	Shorten the worker's longevity
Post-mortem blood and brain samples	Showed abnormal LOY rates
Y-STR allelic dropout	Change the signal strength of the analysis
Blood and buccal samples	Utilized as information sources in forensic investigations

SNP-array data analysis	Permit an ongoing assessment of LOY inside a DNA specimen.
AMELY deletion	Disrupt the AMELY/AMELX ratio values and encourage LOY false positives

Conclusion and Prospects for the Future

Research is being done on the application of LOY as a biomarker for TGCT early identification. Various diseases have various LOY levels, with cancer patients between the ages of 15 and 40 having a greater degree of detection. This may contribute to TGCT early detection. However, correlations between LOY and illness differ considerably among research, maybe as a result of sample size effects.Sample size and randomized selection should be taken into account in future research in order to enhance prediction algorithms and more accurately reflect the whole population. Determining LOY in every illness could potentially improve healthcare preventive strategies.

The purpose of the research is to ascertain whether LOY influences Y-SNP and Y-STR studies and whether this can lead to false-negative results or the absence of evidence in forensic situations. Postmortem samples and buccal mucosa can also contain LOY.To prevent sample degradation, more studies with living subjects are required.To fully grasp how LOY affects forensic case solving, testing samples with LOY using various Y-STR kits is also essential.

Potential uses for the biological age biomarker LOY could be found in forensics and medicine. It can assist in assessing longevity and health condition as well as creating health indices. It is more likely than previously believed that life insurance options can also be categorized using LOY.Clients would gain from regulations on this topic, and their human rights would be upheld. An employee'slifetime can also be evaluated with the aid of LOY, since elements that raise LOY percentage can signify deteriorating health as a result of exposure to specific work-related conditions.

The absence of comparison studies between various methodologies and the current lack of a uniform approach for LOY identification and quantification represent important gaps in the field. The creation of a single, accepted technique to determine the percentage of LOY would aid in our understanding of LOY in various demographics. The next stage is standardization, which enables cross-study comparison and leads to more precise findings in subsequent investigations.

Comprehending the function of Y chromosome proteins beyond the male reproductive system is essential to comprehending the relationship between LOY and early male mortality as well as a host of other illnesses. The development of Y chromosome aneuploidy models by the CRISPR/Cas9 system may aid in clarifying the mechanism through which LOY is linked to an elevated risk of death. Pathologies linked to LOY have been identified and causative and consequential links have been assessed in a rigorous assessment of the causes and effects of LOY. To confirm if LOY can have a deleterious effect on human biological activities, functional research including immune system genes and LOY-associated polymorphisms in humans is required.

The goal of LOY research is to use it as a biomarker to help the forensic and medical domains.

Author's contributions:

MM performed the selection of literature, drafted the manuscript. MPK and RG prepared the figures and collected the related references. MM, RG and MPK carried out the design of this review. Dr. SKB and

Dr. SRK reviewed the whole work and manuscript. SKB is the corresponding author. All authors contributed to this manuscript. All authors read and approved the final manuscript.

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