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Medical genetics and epigenetics of telomerase

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Abstract

Telomerase is a specialized reverse transcriptase that extends and maintains the terminal ends of chromosomes, or telomeres. Since its discovery in 1985 by Nobel Laureates Elizabeth Blackburn and Carol Greider, thousands of articles have emerged detailing its significance in telomere function and cell survival. This review provides a current assessment on the importance of telomerase regulation and relates it in terms of medical genetics. In this review, we discuss the recent findings on telomerase regulation, focusing on epigenetics and non-coding RNAs regulation of telomerase, such as microRNAs and the recently discovered telomeric-repeat containing RNA transcripts. Human genetic disorders that develop due to mutations in telomerase subunits, the role of single nucleotide polymorphisms in genes encoding telomerase components and diseases as a result of telomerase regulation going awry are also discussed. Continual investigation of the complex regulation of telomerase will further our insight into the use of controlling telomerase activity in medicine.

Keywords: telomerase • medical genetics • epigenetics • miRNAs • TERRA • SNPs • gene variation

Introduction

The ends of linear chromosomes are protected from genomic instability by the DNA-protein complexes known as telomeres [1]. The DNA component of human telomeres comprised a repetitive TTAGGG sequence (and complimentary sequence on the opposite strand), up to 20 kb in length, and a 3′ G-rich single-stranded overhang [2, 3]. The G-rich overhang invades the double-stranded telomeric repeats to form a t-loop, which protects the telomere from end-to-end fusions and unregulated nuclease digestion of the 3′ overhang [4, 5]. The protein components of human telomeres that recognize and bind to the telomeric repeats, such as Telomeric Repeat Binding Factor 1 and 2, Protection of Telomeres 1, TRF-Interacting Nuclear Factor-2, Tripeptidyl Peptidase 1, and Repressor/Activator Protein 1 (*TRF1*, *TRF2*, *POT1*, *TIN2*, *TPP1* and *Rap1*), are known as the Shelterin complex which is responsible for t-loop formation and telomere protection [6, 7].

Telomerase, the specialized enzyme capable of reverse transcribing DNA, is required for complete replication of telomeres [8]. In human beings, the core enzyme comprises the protein hTERT, which contains a reverse transcriptase domain capable of catalysing the addition of a six nucleotide repeat onto the ends of telomeres [9], and an RNA component, termed hTR or hTERC (TElomerase RNA Component), which contains a template region that is complementary to the human telomere sequence [10]. Telomerase expression is low or absent in normal human somatic cells, but highly expressed in embryonic cells, adult male germline cells, in stem cells of proliferating tissues and over 90% of human malignancies

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[11, 12]. Both *hTR* and *hTERT* are significantly up-regulated in malignant cells compared to normal cell counterparts [13]. Other telomere/telomerase associated proteins [such as dyskerin, NucleOlar Protein-10 (NOP10), Non-Histone Protein-2 (NHP2) and Glycine Arginine Rich-1 (GAR1)] are required for assembly of a functional telomerase holoenzyme complex [14]. How telomerase is regulated in expression and activity beyond transcription control which is covered by several other reviews, and the consequences of its dysfunction, is the focus of the following sections.

Epigenetic regulation of telomerase: recent findings beyond transcriptional control

Regulation of telomerase is very complex as many factors can effect *hTERT* expression and telomerase activity. Telomerase may be regulated through such known methods as transcriptional regulation, post-transcriptional regulation, post-translational regulation, localization within the cell, assembly of the subunits, epigenetic regulation and by telomeric proteins and RNAs [15, 16]. Many new forms of regulation may be yet to be uncovered. In this section we review recent studies of telomerase regulation involving methylation of the *hTERT* promoter and telomeric regions, the possible role of sirtuins and non-coding RNAs including microRNAs (miRNAs) and telomeric-repeat containing RNA (TERRA).

Methylation

Regulation of *hTERT* transcription generally takes place at the promoter region which has been found to contain an abundance of CpG sites, common targets for methylation [17]. DNA methylation and chromatin remodelling are common regulators of gene activity that alter the binding of transcription factors to gene promoters. The field of epigenetics has become very important in the study of telomerase regulation, and several recent studies have begun to shed light on some of the factors involved.

A recent study in HPV-induced carcinogenesis has suggested that a gradual increase in methylation at the *hTERT* promoter may coincide with the progression to a tumorigenic phenotype in cervical cell lines, but the effect was not as significant in clinical samples [18]. This discrepancy may be the result of selection for cells with greater methylation or, as the authors suggest, may be caused by the presence of normal tissue in clinical samples, although it may be related to another complication: researchers have not been able to agree about whether *hTERT* methylation follows the expected relationship. Promoter region methylation is commonly associated with gene silencing, but studying the epigenetics affecting the *hTERT* gene has been difficult. Although some studies have generated the expected results, other researchers have indicated that there is no correlation between methylation and *hTERT* expression [19]. It has been suggested that methylation status and its relation to *hTERT* expression is dependent on the cell type [20, 21], but there is some debate.

In 2007, Zinn *et al.* studied multiple cell types to try to determine if there was some underlying mechanism behind the epigenetic regulation of telomerase in all cancer types [22]. The group tested breast, lung and colon cancer samples using methylation-specific PCR and bisulphite sequencing of the promoter and found that all of the samples maintained at least one allele with less methylation around the transcription start site despite surrounding methylation patterns. They suggest that although much of the *hTERT* promoter may be heavily methylated in some cancer cell types, a region of about 300 bp around the transcription start site remains unmethylated and following the usual pattern of methylation resulting in gene silencing. Although this is an interesting study providing a possible explanation to the complexity of *hTERT* promoter epigenetics, only three tissue types were studied, and some research suggests that we are still not seeing the whole picture [20, 23]. Of course, telomerase regulation is complex, and *hTERT* promoter methylation is just one piece of the puzzle.

Telomerase activity has also been shown to be subjected to regulation by methylation of other regions such as the subtelomeric region [24]. This is the chromosomal region just proximal to the telomeric region which has been found to be highly methylated in mouse and human cells as reviewed previously [24–26]. A recent study has indicated that subtelomeric methylation may influence telomerase activity, and specifically whether a tumour maintains its telomeres with telomerase or through the alternative lengthening of telomeres (ALT) pathway [27]. The specific mechanisms of the ALT pathway are not well known, and it is only utilized by 10–15% of human tumours [28]. Ng *et al.* analysed subtelomeric cytosine methylation, first determining methylation state in normal cells, and then comparing cells using the ALT pathway to cells that were telomerase positive. They found that the average percentage of methylated loci across all normal samples was relatively constant: 81 $\pm$ 3%. When analysing ALT cells, the group found that the amount of methylation varied dramatically, not just across cell lines, but even across loci. However, when analysing telomerase positive cells, it was found that all loci were heavily methylated with little variation: 97 $\pm$ 1% [27]. This indicates that increased methylation of subtelomeric regions may somehow allow for an increase in telomerase activity, although it is not known if this is somehow affecting *hTERT* transcription.

Sirtuins

Recently, work has been done by several groups which suggest that sirtuins may have a role in telomerase regulation, but findings have been unclear. Sirtuins are a family of enzymes that have been shown to play a role in increasing overall health and longevity in organisms [29]. A study from 2003 by Lin and Elledge suggested that Sir2 (a yeast protein and the first sirtuin discovered, shown to increase lifespan by guarding against genome instability [29]) may be an activator of *hTERT* in a small variety of cancer cell lines

tested [30]. *SIRT1* (NAD-dependent deacetylase sirtuin-1, the human analogue to Sir2), however, has been suggested to be an inhibitor of telomerase activity [31]. Likewise, a recent study has shown that an isoform of P63, ΔNP63α, can induce *TERT* promoter activity in mice, and it was suggested that this may be due to the down-regulation of *SIRT1* [32]. Recent studies with resveratrol have also added to the speculation that *SIRT1* may be involved in telomerase regulation. Resveratrol is one of the most commonly used molecules thought to activate sirtuins. It is a small molecule that has been shown to improve health and longevity in many of the same ways as targets of *SIRT1* [33]. It has been suggested that resveratrol promotes health and longevity by acting through sirtuins, although this idea is still debated [34, 35]. It has also been shown that resveratrol activates telomerase in endothelial progenitor cells [35] and decreases telomerase activity in breast cancer cells [36]. Although it has not been shown that these modifications of telomerase activity are directly related to SIRT1 activity, it is an interesting area requiring more study. For more information on current studies looking into the pharmaceutical use of resveratrol as a telomerase inhibitor, refer to the section below.

Non-coding RNAs

Little is known about which non-coding RNAs might regulate telomerase. When studying these factors, the complexity of telomerase regulation is compounded by the complex networks of miRNAs. MicroRNAs, also known as miRNAs or miRs, are non-coding, single stranded RNAs that have recently been shown to play critical roles in many biological processes such as development, differentiation, apoptosis and proliferation. They commonly down-regulate the target messenger RNA of protein-coding genes in a sequence-specific manner [37]. More than 1000 miRNAs are predicted to work in human biology in a complex regulation network, and several studies have shown that many miRNAs are deregulated in cancers [37–40]. However, to this day, miRNA regulation of telomerase remains elusive.

In 2008, Mitomo *et al.* found an association between the down-regulation of miR-138 and overexpression of telomerase in anaplastic thyroid carcinoma cell lines [41]. The researchers selected miRNAs of interest reported to be differentially expressed in thyroid carcinoma when compared to normal thyroid. Each miRNA was studied individually using stem-loop-mediated reverse transcription real-time PCR. miR-138 was found to be significantly down-regulated in anaplastic thyroid carcinoma cell lines compared to papillary thyroid carcinoma cell lines. Using the miRBase online database (http://microrna.sanger.ac.uk), the authors looked for potential targets of miR-138 and focused on *hTERT*. The thyroid carcinoma cell lines were transfected with miR-138 precursor molecules, and effects on hTERT expression were studied using Western blotting and luciferase assay of the promoter activity [41]. It is important to note, however, that it has been shown that many commercially available antibodies used for Western blotting are not specific to telomerase and may result in inaccurate conclusions [42]. Although increasing the amount of miR-138 molecules in the cells did not completely turn off telomerase activity in the cell lines studied, this is most likely just the first of many miRNAs to be associated with telomerase activity. Also, miRNA activities may be cell-type specific, and the disregulation of miR-138 may be important in telomerase activity in thyroid carcinomas, whereas other miRNAs and mechanisms may be more important in regulating telomerase in other cells and tissues.

It has proven to be a difficult task to pinpoint which miRNAs target telomerase. Since Motimo *et al.* 2008 findings, very little headway has been made. Although no other specific miRNAs have been found to date, Miura *et al.* have found a region of interest that they believe alters telomerase activity and is most likely to contain a miRNA precursor gene [43]. Several studies have indicated that human chromosome 10p may contain a gene involved in regulating telomerase activity [44–46]. To determine which genes in this region may be involved, Miura *et al.* studied the region by exon trapping using bacterial artificial chromosome clones and studied the effect these segments had on telomerase activity using human hepatocytes and hepatoma cell lines. One of the genes cloned (*RGM249*-RNA gene for miRNAs, 249 bp in length), located at chromosome 10p15.3 was found to be overexpressed in cancer than in normal liver cells, similar to telomerase expression and was found to inhibit more than 80% of *hTERT* mRNA expression [43].

Both the Mitomo and the Miura studies shed light on a small portion of non-coding RNA molecules that may regulate telomerase activity. Both studies utilize relatively new techniques and technologies which will need to be continually combined and refined as we continue to study miRNA functions. Telomerase activity is such an important step in development and cancer progression that many complex regulatory pathways play a role. miRNAs have become popular topics of study since their discovery, but even more recently, large non-coding RNAs called TERRA have been suggested to play a role in telomerase regulation.

Telomeres have long been thought to be transcriptionally silent regions of the genome. Recently, this notion has been challenged with the discovery of TERRA, or telomeric repeat-containing RNA, also called TelRNA [47, 48]. Interestingly, these molecules have been found to have regulatory effects on telomerase. Mammalian TERRA molecules are large, non-coding RNA containing UUAGGG repeats and range in size from 100 bases to about 9 kb [47, 48]. At least some of the TERRA also contain subtelomeric-derived RNAs well as the telomeric UUAGGG repeats suggesting that transcription of these elements begins at different starting points in the subtelomeric regions and moves towards the chromosome ends [47–49]. Relatively little is known about the functions of TERRA, but they have been shown to be an integral part of telomeric heterochromatin [47, 48], and possibly help maintain telomere architecture [49], provide epigenetic protection of telomeres from DNA repair mechanisms and regulation of telomerase activity [50]. It is thought that TERRA block telomerase activity at the telomeres in at least two ways: by blocking the RNA component with its sequence complementarity [27, 48] and by recruiting heterochromatinizing activities to the telomeric regions [48].

Several recent publications have supported the notion that TERRA molecules may function as telomerase inhibitors.

Researchers have found that TERRA are capable of duplexing with hTERC [48, 50], and it has been shown that there are lower levels of TERRA molecules in tumours than in corresponding normal tissue [48, 51]. It has also been found that shorter telomeres have lower levels of TERRA molecules [48, 50]. Shorter telomeres may transcribe lower amounts of TERRA because of the decrease in template space. This would cause less impairment of telomerase activity and help explain why telomerase has been shown to be selectively active at critically short telomeres [48, 50].

To further elucidate how transcription of telomeres is regulated and how it might be linked to telomere length, Caslini *et al.* have shown that histone 3/lysine 4 (H3/L4) histone methyltransferase and the transcription regulator Mixed-Lineage Leukemia (MLL) may contribute to telomere methylation and their transcription [52]. Local combined activity of H3/L4 and MLL are thought to promote transcription elongation and chromatin maintenance, but MLL had not been previously associated with telomeres. Using ChIP, they found that TTAGGG repeats were capable of pulling down both the N- and C-terminal domains of MLL in human haematopoietic cell lines and human ovarian surface epithelium. The same methods also showed that telomeres were acetylated at H3 and H4 and to be methylated at H3/K4. It was also shown that MLL binding positively correlated to H3K4 methylation and transcription of telomeres, and that knocking down MLL resulted in histone modifications at the telomeres [52].

Interestingly, it has also been shown that there are lower levels of TERRA transcripts in telomerase positive cells than in cancer cells that maintain their telomere lengths through the ALT pathway [27]. Ng *et al.* compared the levels of TERRA in normal cells, ALT cells and telomerase positive cells by hybridizing RNA from each sample set to probes against the UUAGGG TERRA sequence and the antisense CCCUAA sequence and normalizing to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). They found greater amounts of TERRA molecules in the ALT cells than any others. One possibility is that these amounts were higher because ALT cells have longer telomeres and therefore can transcribe more TERRA molecules. Taking this into account, the group corrected for the additional telomeres by measuring telomere lengths in each sample. ALT cells were still found to have more TERRA transcripts. The group had already shown that telomerase positive cells have increased methylation at the subtelomeric region compared to normal and ALT cells. It is possible that these two findings are connected and indicate a mechanism in which TERRA molecule transcription is dampened to allow for more telomerase activity without hindrance from TERRA in telomerase positive cells, whereas normal TERRA transcription is allowed in ALT cells.

Consequences of genetic dysfunction of telomerase: medical genetics of telomerase

Dysfunction due to mutations in the human genes essential for telomerase function (*TERT* and *TERC*) exhausts the proliferative capacity of cells with a high turnover rate, such as the haematopoietic system and other tissues [53]. Extensive work has shown that telomerase dysfunction and/or telomere shortening play an important role in the development of bone marrow disorders, such as dyskeratosis congenita (DC) and aplastic anaemia (AA), and the lung disease idiopathic pulmonary fibrosis (IPF) [14]. Mutations have been found by amplifying the telomerase genes of interest and direct sequencing or denaturing HPLC to find differences between cases and controls (See Table 1 for a summary list of mutations) [66, 68]. More recently, genome-wide association studies have found single nucleotide polymorphisms (SNPs) associated with the diseases [73]. It will be interesting to see how many more mutations can be found with the new, faster, more efficient next-generation sequencing becoming more and more available.

The mutations found thus far are spread throughout *hTERC* and *hTERT* gene sequences, as well as the telomerase-associated protein dyskerin in the case of DC. The mutations in *hTERC* affect the function of telomerase RNA either by disrupting the secondary structure to prevent its folding into a stable conformation or by sequence changes that prevent its interaction with other telomerase complex components [60]. Two mutations (See Table 1) have been found in the template region of *hTERC*, which decrease telomerase activity to levels that are undetectable by the Telomeric Repeat Amplification Protocol (TRAP) assay [55]. The mutations in *hTERT* can affect its reverse transcriptase function, DNA-binding ability and protein–protein interaction strength [74, 75]. *hTERT* mutations show varying degrees of effect on telomerase activity, with both conserved and non-conserved amino acids being able to drastically reduce activity [59]. Dyskerin interacts with *hTR* prior to assembly of the holoenzyme and mutations in Dyskerin 1 (DKC1) are shown to significantly impair this interaction [76]. *hTERT* mutations do not show a strict linkage between mutation and disease, but rather may show an increased risk of disease in individuals with *hTERT* mutations. Oppositely, *hTERC* mutations show a strong linkage between mutation and disease; however, the correlation between genotype and phenotype is unclear [77].

Dyskeratosis congenita

DC is a rare multisystem disorder defined by three clinical characteristics: oral leukoplakia, nail dystrophy and hyerpigmentation of the skin [78]. Patients also display signs of premature aging including hair greying and hair loss, osteoporosis and poor dentition [14]. The vast majority of DC patients (80–90%) develop bone marrow abnormalities by the age of 30 [79] and bone marrow failure is the principal cause of early mortality [56]. Individuals with DC also have a predisposition to cancer with an 11-fold increase in all cancer types, an 1154-fold increase in frequency of tongue cancer, and a 196-fold increase in frequency of acute myeloid leukaemia (AML) compared to the general population [80].

Mutations in patients suffering from DC were initially discovered in the *DKC1* gene (See Table 1) located on the X chromosome, which encodes dyskerin, a protein involved in stabilizing

Table 1 Known mutations in *DKC1*, *TERT* and *TERC* involved in human diseases

Disease	Gene mutated	Domain	Type of mutation	DNA or protein change	Telomerase activity (%)	References
DC	*DKC1*	N-terminal	Missense	A2V	NA	[54]
				P10L	NA	[55]
				Q31K	NA	[55]
				Q31E	NA	[55]
				F36V	NA	[56]
				I38T	NA	[55]
				K39E	NA	[54]
				P40R	NA	[56]
				E41K	NA	[54]
				K43E	NA	[55]
				T49M	NA	[55]
				R65T	NA	[54]
				T66A	NA	[54]
				T67I	NA	[55]
				H68Q	NA	[55]
				L72Y	NA	[56]
			Deletion	L37del	NA	[56]
		TruB domain	Missense	S121G	NA	[56]
				R153W	NA	[56]
		Middle Region	Missense	S280R	NA	[56]
		PUA domain	Missense	K314R	NA	[55]
				L317F	NA	[57]
				L321V	NA	[54]
				R322Q	NA	[57]
				M350T	NA	[54]
				M350I	NA	[54]
				A353V	NA	[54]
				T357A	NA	[55]
				D359N	NA	[55]
		C-terminal region	Missense	P384L	NA	[56]
				P384S	NA	[56]
				A386T	NA	[55]
				L398P	NA	[55]
				G402E	NA	[56]
				G402R	NA	[54]
				T408I	NA	[55]
				P409L	NA	[55]
				S420Y	NA	[55]
			Deletion	Δ from 493	NA	[55]
	hTERT	RT domain	Missense	P702S	13	[58]
				P721R	20–100	[59]
				Y846C	10	[58]
				H876Q	50	[58]
				K902N	0	[59]

Continued

Table 1 Continued

Disease	Gene mutated	Domain	Type of mutation	DNA or protein change	Telomerase activity (%)	References
		C-terminus	Missense	R979W	20–100	[59]
				F1127L	20–100	[59]
	hTERC	Template	Point	48A→G	0	[55]
			Deletion	Δ52–55	0	[55]
		Near template	Point	58G→A	100	[60]
		Core pseudoknot	Point	37A→G	70	[61]
				72 C→G	5	[62]
				116C→T	0	[63]
				143G→A	0	[62]
			Deletion	Δ96–97	0	[62]
			Double Point	107–108GC→AG	5	[64]
		Hypervariable	Deletion	Δ216–229	0	[61]
			Point	228G→A	1	[60]
		CR4-CR5	Deletion	Δ316–451	ND	[62]
		H/ACA box	Deletion	Δ378–451	5	[64]
			Point	408C→G	25	[64]
AA	*hTERT*	N-terminus	Missense	V96L	ND	[65]
				V119L	ND	[65]
				A202T	0	[66]
				A279T	20–100	[59]
				H412Y	36	[58]
				K570N	0	[59]
		RT domain	Missense	G682D	0	[67]
				V694M	0	[66]
				T726M	20–100	[67]
				Y772C	0	[66]
		C-terminus	Missense	V1090M	0	[66]
	hTERC	Near template	Point	58G→A	100	[68]
		Core pseudoknot	Deletion	Δ28–34	20–100	[59]
				Δ79	0	[55]
				Δ110–113	0	[68]
			Point	72C→G	5	[68]
				116C→T	0	[69]
				117A→C	0	[69]
				204C→G	0	[69]
		CR4-CR5 domain	Point	228G→A	100	[69]
				305G→A	1–2	[69]
		H/ACA box	Point	450G→A	20–100	[69]
				467T→C	ND	[70]

Continued

Table 1 Continued

Disease	Gene mutated	Domain	Type of mutation	DNA or protein change	Telomerase activity (%)	References
IPF	*hTERT*	N-terminus	Missense	P33S	80	[71]
				L55Q	40	[72]
				V144M	78	[71]
				R486C	45	[71]
			Deletion	Δ112C	ND	[72]
		RT Domain	Frameshift del	V747fs	2	[71]
			Missense	R865C	20	[71]
				R865H	28	[71]
		C-terminus	Missense	T1110M	50	[72]
			Frameshift del	E1116fs	6	[71]
		Intron	Point	Intron 1 +1 G→A	ND	[72]
			Point	Intron 9–2 A→C	ND	[72]
	hTERC	Core pseudoknot	Point	37A→G	70	[71]
			Point	98G→A	10	[12]

For further review, see http://telomerase.asu.edu/diseases.html.

telomerase RNA and forming the enzyme complex [12]. The most common mutation found in *DKC1* is a C→T nucleotide substitution, which results in the A353V missense mutation in the protein sequence. This mutation can be inherited or arise *de novo* [54]. Approximately half of DC patients have a mutation in the genes of the telomerase holoenzyme complex, *hTERT*, *hTERC* or *DKC1* [81]. It is likely that the remaining 50% of patients have mutations in other telomere maintenance genes, such as the mutations found in *TINF2*, *NHP2* and *NOP10* [82]. The most common form of the disease shows X-linked inheritance (*DKC1* mutations), with 90% of patients being male, although both autosomal dominant (*hTERT*, *hTERC* and *TINF2* mutations) and autosomal recessive (*NHP2* and *NOP10* mutations) inheritance patterns are recognized [83, 84].

Aplastic anaemia

AA is another bone marrow failure disease associated with telomerase mutations. The disease is rare with an incidence of one to five per million and defined by hypocellular bone marrow and low peripheral blood cell counts [63]. A few patients who initially presented with AA have been found to develop DC, suggesting that AA is a marker of more severe phenotypes [68]. The observation that AA patient leucocytes had significantly shorter telomeres than controls led researchers to screen patients for mutations in telomerase components [53]. In acquired AA, approximately 2.5% of patients have mutations in *hTERC* and 3.5% have mutations in *hTERT* (See Table 1 for list) [66]. The majority of *hTERC* mutations cluster in the pseudoknot region, which is essential for *hTERT* binding and telomerase enzymatic activity [53]. The identified *hTERT* mutations are spread throughout the gene and act by haploinsufficiency, not a dominant negative mechanism. *hTERC* mutations decrease telomerase activity through haploinsufficiency as well [66].

Idiopathic pulmonary fibrosis

IPF is a much more common disease, with a prevalence of at least 90,000, than DC or AA, although the aetiology of the disease is unclear, as implied by idiopathic [85]. The clinical course of IPF is progressive and predictable, beginning with massive fibrotic changes and alveolar wall thickening in the lungs and patients ultimately succumbing to respiratory failure [72, 73]. One in five patients with IPF has a family history of the disease and inheritance looks to be autosomal dominant with variable penetrance [53]. In patients with a documented family history of IPF, *hTERC* and *hTERT* mutations account for 8–15% of disease inheritance [86]. Sporadic IPF has detectable telomerase mutations in 1–3% of cases. Although the percentage of patients with telomerase mutations is lower in IPF than dyskeratosis congentia, the higher incidence rate makes pulmonary fibrosis the most common clinical manifestation of telomerase gene mutations [85]. There are fifteen known mutations in *hTERT* and only two in *hTERC* (See Table 1); moreover, the mutations do not appear to cluster in any region or domain of either gene. IPF patients without telomerase mutations also have short telomeres when compared to controls, suggesting that telomere length, rather than telomerase mutations, is a better predictor of disease onset [86].

Acute myeloid leukaemia

Due to AA patients with telomerase mutations often having a family history of AML and patients suffering from AA and DC having a predisposition to AML, researchers looked for and found telomerase mutations in AML patients [87]. AML is a heterogeneous disease due to an acquired somatic mutation in the myeloid lineage of a haematopoietic progenitor cell [88]. Nearly half of AML patients have abnormal karyotypes and genomic instability plays a crucial role in leukemogenesis [87]. Calado *et al.* recently reported that 6.8% of patients with AML had mutations in *hTERT* and no mutations were found in *hTERC*. The A1062T *hTERT* mutation was found to be the most common variant between cases and controls and constituted 60% of all *hTERT* mutations found in AML patients [87]. Kirwan *et al.* have expanded on this work and found another *hTERT* mutation and two *hTERC* mutations in familial forms of AML [89]. The A1062T mutation has been found in multiple haematopoietic cancers and may serve as a surrogate marker for mutations throughout *hTERT* as a screening tool to identify other malignancies with telomerase as a predisposing factor [12].

TERT and TERC variations and disease predisposition

Although mutations in telomerase have been found to have important implications in growth and development, naturally occurring variations in *hTERT* and *hTERC* remain to be fully understood. These SNPs are limited and telomerase genes express less heterozygosity compared to other genes further demonstrating the overall importance of these genes [80, 90]. *hTERT* is located within a locus at chromosome 5p13.33 and *hTERC* is located within a locus at chromosome 3q26. Given the presence of telomerase activity in haematopoietic stem cells, it is likely that variations and/or mutations in the telomerase components would have significant implications for haematopoiesis. In fact, defects in telomerase function contribute to disease pathogenesis particularly in bone-marrow failure syndromes [91]. Studies have also identified an association between telomerase mutations in both *hTERC* and *hTERT* with familial myelodysplastic syndrome and AML as mentioned previously [12, 89]. In addition to the haematopoietic cell compartment, tissue based stem cells might also be affected by variations in telomerase.

Variations in the genes required to form the active telomerase complex may contribute to cancer risk. The correlation between telomerase function and cancer is most likely due to the fact that tumour formation can be driven by telomerase dysfunction. Immortalization of tumour cells can occur through *hTERT* gene rearrangement which may contribute to cancer risk if variations in telomerase promote gene rearrangement and telomerase activation [92]. It is also probable that failure to stabilize telomeres prior to significant shortening could contribute to an increased genomic instability capable of promoting increased tumour development and progression [80]. Variations in *hTERC* and *hTERT* have been found to contribute to overall telomere length implying a potential role for these variations in cancer risk [93, 94]. Recent genome-wide association studies have been performed in order to define whether there is an association between telomerase SNPs and cancer risk.

There is conflicting data regarding the role of *hTERT* variations and increased risk of breast cancer [80]. Although some studies have found an association between breast cancer risk and telomerase SNP [90], the most compelling data involve studies performed on a total of 1656 breast cancer samples and 2019 matched controls [95]. These compelling, high power studies did not find an association between increased breast cancer risk and the SNPs of the telomerase promoter [95, 96]. In line with these findings, Pooley *et al.* did not observe a strong association of the SNP rs401681 in the *TERT-CLPTM1L* locus with cancer risk, related to telomere length [97].

Although breast cancer risk does not appear to be associated with telomerase SNPs, other studies have demonstrated a change in cancer risk associated with *hTERC* and *hTERT* variations. For example, variation in the *TERT-CLPTM1L* genes was found to be associated with a decreased risk of squamous cell carcinoma of the head and neck [98]. Although a decreased risk was observed in this study, other studies have found an association for this region (5p15.33) and increased cancer risk. Additional genome-wide association studies found this region to be associated with increased risk for both pancreatic cancer and lung adenocarcinoma [99–104]. Unlike the studies involving breast cancer, an increased risk of prostate cancer was found to be associated with at least one *hTERT* variation that influences telomerase expression [105, 106]. Other studies have linked cancer risk and telomerase SNP for several cancers including the following: bladder cancer [106, 107], ovarian cancer [108] and cervix cancer [106].

But, what do these reported associations mean? What is the functional consequence of a SNP/variant in *hTERT* or *hTERC*? It is known that telomerase is tightly controlled and complex; mutations or haploinsufficiency of *hTERT*/*hTERC* drive telomere dysfunction and bone marrow failures that are detrimental to the organism's survival. More research is needed to determine whether a variant in the *hTERT* promoter could alter the ability of a repressor to bind and maintain its tight control over its transcription and ultimately activity. Loss of such repression could impact telomere maintenance, immortality, and progression of precancerous lesions. Furthermore, could a SNP in *hTERC* slightly alter structure of the RNA which in turn affects telomerase activity and telomere maintenance? These are questions arising by the observations of the medical geneticists for the telomerase/telomere biology field to address in the laboratory.

Summary and perspectives

Telomerase regulation is complex and has important consequences not just for telomere length, but also for the development of disease.

Obtaining further insight into the role of telomerase gene regulation and therefore telomerase activity in disease development will serve to assist in the generation of enhanced therapies. Understanding how telomerase is regulated will also lead to improved targeting of telomerase as a therapy for genetic diseases and cancer.

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Conflict of interest

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