RESEARCH ARTICLE

# Higher *ATM* expression in lymphoblastoid cell lines from centenarian compared with younger women

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**Abstract**

With increased life expectancies in developed countries, cancer rates are becoming more common among the elderly. Cancer is typically driven by a combination of germline and somatic mutations accumulating during an individual's lifetime. Yet, many centenarians reach exceptionally old age without experiencing cancer. It was suggested that centenarians have more robust DNA repair and mitochondrial function, allowing improved maintenance of DNA stability. In this study, we applied real-time quantitative PCR to examine the expression of *ATM* in lymphoblastoid cell lines (LCLs) from 15 healthy female centenarians and 24 younger female donors aged 21–88 years. We observed higher *ATM* mRNA expression of in LCLs from female centenarians compared with both women aged 21–48 years (FD = 2.0, $p$ = .0016) and women aged 56–88 years (FD = 1.8, $p$ = .0094). Positive correlation was found between *ATM* mRNA expression and donors age ($p$ = .0028). Levels of hsa-miR-181a-5p, which targets *ATM*, were lower in LCLs from centenarians compared with younger women. Our findings suggest a role for ATM in protection from age-related diseases, possibly reflecting more effective DNA repair, thereby reducing somatic mutation accumulation during aging. Further studies are required for analyzing additional DNA repair pathways in biosamples from centenarians and younger age men and women.

**KEYWORDS**

*ATM*, centenarians, DNA repair, healthy aging, lymphoblastoid cell lines, miR-181a-5p

## 1 | INTRODUCTION

Life expectancies in developed countries have increased in recent decades, owing to improved healthcare, the availability of better therapeutics, and healthier lifestyles (Abrams et al., 2021; Garmany

et al., 2021). However, improved longevity contributes to higher cancer incidence (Mariotto et al., 2018). Cancer is typically driven by a combination of germline (heritable) and somatic (acquired) mutations; somatic mutations accumulate during an individual's life and increase cancer risk above the risk posed by germline mutations

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alone (Qing et al., 2020; Tesch et al., 2020). Yet, some individuals reach exceptional ages, above 100 years, in relatively good health and without experiencing cancer (or other life-threatening diseases). Centenarians have become the focus of studies aimed at understanding both genetic and nongenetic factors underlying exceptional longevity (Barak et al., 2021; Marron et al., 2019; Z. D. Zhang et al., 2020). Human longevity is partially heritable, and several genes were suggested as implicated in exceptional longevity (Marron et al., 2019; Z. D. Zhang et al., 2020). Effective DNA repair was shown to enable better maintenance of DNA stability, reduce cancer risk and contribute to longevity (D. E. Kim et al., 2020; Ma et al., 2018; Stead & Bjedov, 2021).

Most published studies on the genetic and environmental factors implicated in longevity were done in animal models, such as *Caenorhabditis elegans* (Frakes et al., 2020), Drosophila (Garschall et al., 2017), and mice (Fernández et al., 2018). Relatively little is known about the genomic landscape of longevity in humans. *ATM* (ataxia-telangiectasia mutated) is among key genes associated with longevity in humans and animals. It encodes a member of the PI3/PI4-kinase family, which plays key role in DNA damage repair, thereby contributing to healthy aging (Gavish-Izakson et al., 2018; Y. C. Kim et al., 2009; Shiloh & Ziv, 2013; Shiloh, 2003). Here we report on *ATM* mRNA expression levels in lymphoblastoid cell lines (LCLs; 19) derived from B lymphocytes of women from various ages, including centenarian women.

## 2 | MATERIALS AND METHODS

### 2.1 | Cell culture and reagents

LCLs were generated from peripheral blood B lymphocytes donated by consenting individuals, maintained in optimal growth conditions as described in our earlier study (Hadar et al., 2018). LCLs from 24 healthy female donors aged 21 to 88 years were obtained from the National Laboratory for the Genetics of Israeli Populations (http://yoran.tau.ac.il/nlgip/) at Tel-Aviv University, Israel (Hadar et al., 2018). LCLs from 15 centenarian women were obtained from the International Institute of Molecular and Cell Biology in Warsaw, Poland (Hadar et al., 2018). Tissue-culture reagents were purchased from Biological Industries (Beit-Haemek).

### 2.2 | RNA extraction and real-time quantitative PCR

RNAs were extracted using the phenol-chloroform method as described (Hadar et al., 2018). Briefly, cells were collected by centrifugation and lysed using Tri-reagent (T9424, Sigma-Aldrich), followed by RNA separation and washing with 80% of cold ethanol. RNA samples were quantified by NanoDrop spectrophotometer (NanoDrop), with both 260/280 nm and 260/230 nm parameters >2.0, and stored at −80°C for analysis.

Real-time quantitative PCR (qPCR) reactions for *ATM* were performed with cDNA samples prepared from 1 μg RNA samples using qScript cDNA Synthesis Kit (Quanta Bio). Reverse transcription was performed using a thermal cycler over three steps (22°C for 5 min, followed by 42°C for 30 min and 85°C for 5 min). Real-time PCR reactions were done with 10 μl mixtures containing 10 ng of cDNA, PerfeCTa SYBR® Green FastMix Kit (Quanta Bio) and Integrated DNA Technologies, Inc. GUSB (Glucuronidase beta) was used as a reference gene. Real-time PCR product sizes of both human *ATM* and human *GUSB* were 82 base pairs when using the following SYBR® Green primer sequences:

*ATM* forward: ATCTGCTGCCGTCAACTAGAA
*ATM* reverse: GATCTCGAATCAGGCGCTTAAA
*GUSB* forward: CTGCTGGCTACTACTTGAAGATG
*GUSB* reverse: GAGTTGCTCACAAAGGTCAC

For measuring hsa-miR-181a-5p expression levels, cDNA samples were prepared from 350 ng RNA samples using High Capacity cDNA Reverse Transcription kit (Applied Biosystems) in accordance with the protocol for Creating Custom RT Pools using MicroRNA Assays from Applied Biosystems. Real-time PCR reactions were done with 10μl mixtures containing 10 ng of cDNA, TaqMan™ Fast Advanced Master Mix (Applied Biosystems) and TaqMan™ Primers (TaqMan™ MicroRNA Assay ID 000480; Applied Biosystems). TaqMan™ U6 snRNA (Assay ID 001973; Applied Biosystems) was used as the reference gene.

### 2.3 | Statistical analyses

Real-time quantitative PCR data analysis was conducted using the GraphPad Prism v.8. Normality of data distribution was evaluated using the Shapiro–Wilk test; continuous variables between more than two groups were analyzed by ANOVA, followed by a multiple comparison correction Tukey's test; outliers were removed following Grubbs test. When data were not normally distributed, the Kruskal–Wallis test was used, followed by a multiple comparison correction Dunn's test. $p ≤ .05$ were considered as significant.

## 3 | RESULTS

Our qPCR experiments indicated 2-fold ($p = .0016$) and 1.8-fold ($p = .0094$) higher *ATM* mRNA levels in LCLs from centenarian women compared with LCLs from women aged 21–48 years or 56–88 years, respectively (Figure 1a). Combining all samples, *ATM* mRNA levels were correlated with women's age ($R = 0.465$; $p = .0028$; Figure 1b). Additionally, we compared *ATM* mRNA from the current study with *SIRT*1 mRNA levels reported in our earlier study using the same LCLs (Hadar et al., 2018). We observed positive correlations between *ATM* and *SIRT1* mRNA levels in both centenarian LCLs (Figure 2a) or LCLs from younger women (Figure 2b). This positive correlation suggests that a common microRNA may regulate the expression of *ATM* and *SIRT1*.
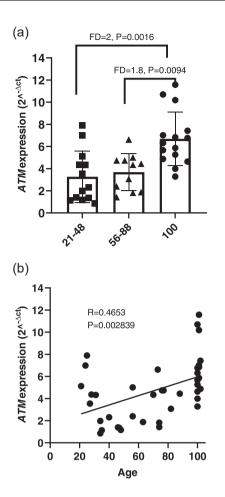
**FIGURE 1** Higher expression of ATM mRNA in lymphoblastoid cell lines (LCLs) from female centenarians versus younger females. (A). LCLs from centenarian women express higher *ATM* mRNA compared with LCLs from two age groups of younger women (FD = 1.8, *p* = .0016) and FD = 1.8, *p* = .0094) compared with women aged 21–48 years or 56–88 years, respectively). (B). *ATM* mRNA levels in female LCLs positively correlate with their age.



**FIGURE 2** *ATM* and *SIRT1* mRNA expression levels positively correlate in lymphoblastoid cell lines (LCLs) from female centenarians and in younger women. Positive correlations for *ATM* and *SIRT1* mRNA expression levels are shown for LCLs from female centenarians (A; *p* = .003) and younger females (B; *p* = .001). *SIRT1* mRNA expression levels in the same LCLs were taken from Hadar et al. (2018) (20).

We used three miRNA target prediction tools for searching a common human miRNA which may bind both *SIRT1* and *ATM*: TargetScan (targetscan.org), miRDB (mirdb.org), and miRTarBase (mirtarbase.cuhk.e-du.cn). These three tools indicated that hsa-miR-181a-5p binds to a common 3'UTR nucleotide sequence (tgaatgt) of both human *SIRT1* and *ATM*. Indeed, hsa-miR-181a-5p was shown to down-regulate the expression of *ATM* mRNA in human gastric cancer SGC-7901 cells (Liu et al., 2016; X. Zhang et al., 2014). We, therefore, measured hsa-miR-181a-5p expression in the same LCLs. Figure 3 shows that LCLs from centenarian women expressed lower hsa-miR-181a-5p (FD = 1.45; *p* = .04) compared with LCLs from younger women.

## 4 | DISCUSSION

Increasing life expectancies in developed countries have led to increased cancer morbidity and mortality. Cancer is typically driven by a co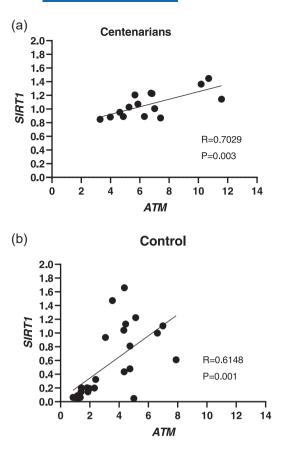mbination of inherited (germline) and somatic mutations accumulating during an individual's lifetime. Somatic mutations reflect the effects of environmental DNA-damaging agents, along with aging-related imperfect DNA repair during cell replication, thereby taking part in driving cancer. Yet, most centenarians reach exceptionally old ages without experiencing cancer (Barak et al., 2021; Marron et al., 2019; Z. D. Zhang et al., 2020). In this study, we compared the expression levels of *ATM* between LCLs derived from healthy female donors of different age groups, including centenarian women, and observed about two-fold higher expression of *ATM* in LCLs of centenarian compared with younger women (Figure 1a). Moreover, *ATM* expression levels positively correlated with donors age (Figure 1b).

ATM takes crucial parts in the cell cycle and in DNA repair pathways. It regulates the response to double-strand DNA breaks, and *ATM* mutations were linked to cancer, sterility, radiosensitivity, and premature aging-related disorders (Gavish-Izakson et al., 2018; Shiloh & Ziv, 2013; Shiloh, 2003). ATM is associated with chromatin in somatic cells, where it monitors DNA damage and activates the DNA damage response pathway (Y. C. Kim et al., 2009). Distinct polymorphic *ATM*
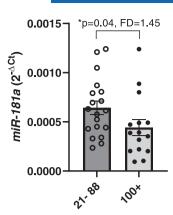
**FIGURE 3** Lower expression levels of hsa-miR-181a-5p in LCLs from female centenarians compared with younger women. See Methods for further details.

alleles were implicated with longevity in humans (Chen et al., 2010; Piaceri et al., 2013), *C. elegans*, and mice (Qian et al., 2018). However, to our knowledge, our current study is the first demonstration of higher *ATM* mRNA expression levels in blood-derived human cells from centenarians compared with younger individuals.

As the *ATM* gene codes for a protein essential for DNA damage repair, its role in cancer is well-established (Gavish-Izakson et al., 2018; Y. C. Kim et al., 2009; Shiloh, 2003; Shiloh & Ziv, 2013), with loss-of-function mutations contributing to many types of solid cancers (Helgason et al., 2015; Russell et al., 2015). Cancer risk increases with age due to the accumulation of somatic mutations during an individual's life; hence, it is likely that the higher *ATM* levels observed here in centenarians contribute to their exceptional longevity, among other reasons, by reducing cancer risk.

The robust correlations observed for the mRNA expression of *ATM* and *SIRT1* in LCLs from both female centenarians and younger women (Figure 2a,b) suggest that these genes may be targeted by a shared miRNA. Our bioinformatics and literature search identified hsa-miR-181a-5p as targeting both *ATM* and *SIRT1*. Indeed, hsa-miR-181a-5p was shown to decrease the expression of both *ATM* (Liu et al., 2016; X. Zhang et al., 2014) and *SIRT1* (M. Zhang et al., 2017; Zhou et al., 2012) in human cells. Our real-time qPCR experiments indicated that LCLs from female centenarians expressed lower hsa-miR-181a-5p compared with LCLs from younger women ($p = .04$; Figure 3).

The current study has certain limitations that should be considered by future studies on the involvement of higher cellular *ATM* expression in human longevity. First, the LCLs applied for this study were derived from the donors` peripheral blood B lymphocytes by immortalization with EBV; while LCLs are among the most popular research tools for studying human genomics and their interindividual variations (Gurwitz, 2016), ATM studies with blood samples, ideally including its protein level measurements, are required for addressing the role of ATM in human longevity. Second, our study included LCLs derived only from female donors, as we could not obtain sufficient numbers of LCLs from

male centenarians. The relevance of ATM expression to human longevity should thus be independently assessed in men, for whom different factors, such as hormones and the presence of a single X chromosome, could be implicated in their shorter average lifespan (Austad, 2006; Perls, 2017). Lastly, future studies should analyze DNA repair pathways and somatic mutation accumulation in cultured cells from female and male centenarians compared with younger individuals.

In conclusion, the higher *ATM* mRNA levels observed in LCLs from female centenarians compared with younger women may reflect more robust DNA repair capacity, which may, in turn, allow lower somatic mutation accumulation, and thereby lower cancer risk, heathier aging, and exceptional longevity. Elucidating the mechanisms underlying the higher ATM expression levels in centenarians requires further studies, and may lead to the discovery of longevity-promoting therapeutics.

## CONFLICT OF INTEREST
The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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