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Clinical Significance of Germline Telomere Length and Associated Genetic Factors in Patients with Neuroblastoma

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Research Article

Keywords: telomere length, neuroblastoma, genetic marker, survival duration, genome-wide association study

Posted Date: March 3rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1360590/v1

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Abstract

Background: Studies investigating the relationship between germline telomere length and the clinical characteristics of tumors are very limited. This study evaluated the relationship between germline telomere length and the clinical characteristics of neuroblastoma. In addition, a genome-wide association study (GWAS) was performed to investigate the genetic factors associated with telomere length.

Methods: The telomere length of peripheral blood mononuclear cells from 186 patients with neuroblastoma was measured by quantitative PCR. The association between germline telomere length and clinical characteristics, including long-term survival, was investigated. For the GWAS, genotyping was performed with a high-density Illumina bead chip. After strict quality control checks of the samples, an association analysis was conducted.

Results: An extra-abdominal primary site, lower stage (stage 1/2), MYCN non-amplified tumor, and being in the low-risk group were associated with longer telomere lengths (P = 0.015, 0.007, 0.046 and 0.042, respectively). Patients with longer third telomeres had longer event-free survival than other patients (P = 0.032). In the GWAS, rs2492082, located in the 3'UTR of the *HIVEP*3 gene, showed the highest association with telomere length (P = 1.7E-06). Patients with long telomeres had a recessive allele A. A gene-based analysis using the VEGAS2 algorithm showed that the *CNTN4* gene had the most significant association with telomere length (P = 1.0E-06). Patients with the CC homozygote in the rs71311728 marker within the *CNTN4* gene had a longer telomere length than those with the TC heterozygote. A gene ontology analysis showed that genetically susceptible genes associated with telomere length were mainly distributed in neurite morphogenesis and neuron development.

Conclusions: Longer germline telomere length is associated with favorable prognostic factors at diagnosis and eventually better event-free survival in patients with neuroblastoma. In addition, the GWAS demonstrated that genetic markers and genes related to telomere length are associated with neurite morphogenesis and neuron development. A further study with a larger cohort of patients and functional studies are needed.

Introduction

Telomeres are DNA-protein complexes at the ends of chromosomes that determine the lifespans of cells. As a repeat sequence (TTAGGC) present at the end of the genome, telomeres are known to play a role in preventing damage to the genome [1]. They protect chromosomes from end-to-end fusion [2] and are involved in a variety of other functions, including cell death, cell senescence, abnormal cell proliferation, and separation during meiosis [3,4]. Telomere length has been reported to be inherited and has been found to vary between individuals of the same age [5]. Telomere length decreases when cells divide, and as telomere length decreases, cells age and die. Thus, telomere length has a profound relationship with age-related diseases and cancer [6–8]. Aging caused by telomere shortening differs from individual to individual and is known to be affected by environmental [9] and genetic differences [10].

Many genetic epidemiologic studies have been reported the results of analysis of the association between telomere length and cancer risk [11–14]. A significant association of short telomere length with cancer risk in peripheral blood leucocytes, lung cancer, bladder, head and neck, lung, and renal cell cancers, and breast cancer has been reported [11,12,15–17]. Functional studies using mouse model have shown that shorter telomere lengths are associated with an increased risk of cancers such as epithelial caner, prostate cancer [12,18–20]. A shorter telomere length may promote cell aging and inhibit cancer progression. However, when the critical telomere length is reached, it would cause genomic instability expanding and malignant transformation potential through the fusion bridge breakage cycle [21]. Conversely, conflicting results have also been reported, suggesting that longer telomere lengths increase cancer risk [22–25]. A genetic studies that measures the telomere length in various type of cancer for various populations and analyzes the association with telomere length with clinical characteristics of cancer patients is necessary to obtain more accurate conclusion. In particular, in the case of neuroblastoma, attempts to analyze the association between clinical features by measuring the telomere length of a cancer patient are very insufficient.

Telomere length, which is associated with chromosomal instability and thus the degree of cell protection from external stimuli, might also be associated with the clinical characteristics of tumors, including treatment outcomes. However, studies investigating the relationship between germline telomere length and the clinical characteristics of tumors are very limited. This study evaluated the relationship between germline telomere length and the clinical characteristics of neuroblastoma. In addition, a genome-wide association study (GWAS) was performed to investigate the genetic factors associated with telomere length.

Methods

Patients

A total of 186 patients who were diagnosed with neuroblastoma between May 2007 and July 2016 and had peripheral blood samples already cryopreserved at the Samsung Medical Center Biobank were enrolled in this study. Medical records were reviewed to obtain detailed clinical and biological data, such as the clinical presentation at diagnosis, tumor biology (including MYCN amplification status), tumor histology using the International Neuroblastoma Pathology Classification, and survival.

This study was approved by the Institutional Review Board of Samsung Medical Center (IRB No. SMC 2015-11-053-035) and conducted according to the Declaration of Helsinki. All patients signed an informed consent before enrolled in this research and all methods were performed in accordance with the relevant guidelines and regulations.

Genome-wide genotyping

Genomic DNA was extracted from the collected blood using a Wizard Genomic DNA Purification Kit (Promega, WI, USA). DNA quantification was measured by fluorescence using Qubit equipment, and DNA integrity was checked using TapeStation equipment (Agilent, Santa Clara, USA). 200 ng of DNA that passed quality control (QC) was used for the Illumina Infinium assay. The chip used in this study is an Illumina Infinium Exome-24 BeadChip array (Illumina, San Diego, USA) containing 547,644 markers. The samples were processed according to the Illumina Infinium assay manual. Each sample was whole-genome amplified, fragmented, precipitated, and resuspended in an appropriate hybridization buffer. The denatured samples were then hybridized on a prepared GSA BeadChip for a minimum of 16 h at 48°C. Following hybridization, the bead chips were processed for the single-base extension reaction, staining, and imaging on an Illumina iScan system. The normalized bead intensity data obtained for each sample were uploaded to GenomeStudio software (Illumina, San Diego, USA), which converted the fluorescent intensities into single nucleotide polymorphism (SNP) genotypes. Sample quality was checked using a sample call rate of > 95%. In GenomeStudio (Illumina, San Diego, USA), the cluster quality was measured using GenTrain scores, and then high-quality markers (> 0.7) were used.

Telomere length measurement

Quantitative real-time polymerase chain reaction (qPCR) was used to measure telomere length [26]. The relative telomere lengths were measured as the ratio of the number of telomere (T) repeat copies to the number of single copy gene (S) copies (T:S ratio) in a given sample. Telomere length was calculated as the ratio between the length of the test DNA and the pooled reference DNA. Three replicates were performed on patient samples to calculate the average T:S ratio.

Statistics

The clinical variables are summarized using mean ± standard deviation or median (range), as appropriate (Table 1). In HelixTree (Golden Helix Inc., Bozeman, MT, USA), marker filtering was performed based on the following criteria: (1) call rate > 0.85, (2) minor allele frequency > 0.05, and Hardy Weinberg equilibrium *P* value > 0.001. High-quality markers with values above those QC criteria were used in the following analyses. For the GWAS, genotype distributions were compared using multivariable regression analyses in HelixTree software (Golden Helix Inc., Bozeman, MT, USA). The gene-based analysis was performed using the VEGAS2 algorithm (https://vegas2.qimrberghofer.edu.au/). In the VEGAS2 options, we choose 1000G

Asians for SNPs and all Asians for sub-population. The analysis was carried out for three groups: all and 10% and 20% extreme groups. A gene pathway analysis was done using VEGAS2. The event-free survival (EFS) and overall survival (OS) rates were estimated using the Kaplan–Meier method, and differences in survival curves were compared using the log-rank test. A multivariate analysis for EFS was performed using a Cox regression analysis. Clinical characteristics were compared between two groups using the Pearson chi-square test or Fisher's exact test for categorical variables and the t test or Kruskal-Wallis rank-sum test for continuous variables. *P* values < 0.05 were considered significant.

Table 1
Telomere length according to clinical characteristics

Clinical Characteristics	No. (%)	Median (range)	*P*-value
Sex			0.820
Male	99 (53.2)	17.51 (8.28–29.36)	
Female	87 (46.8)	17.18 (8.41–26.73)	
Age			0.113
< 1.5 years	73 (39.2)	18.05 (8.41–29.36)	
> 1.5 years	113 (60.8)	17.22 (8.28–25.17)	
Primary site			**0.015**
Abdomen	141 (75.8)	17.18 (8.41–27.46)	
Others	45 (24.2)	18.78 (8.28–29.36)	
Stage			**0.007**
1, 2	50 (26.9)	18.44 (8.28–29.36)	
3, 4, 4S	136 (73.1)	17.11 (8.41–25.70)	
Differentiation (N = 166)			0.891
GNB	39 (23.5)	18.00 (8.28–25.17)	
Differentiating	37 (22.3)	17.85 (9.93–25.10)	
PD/UD	90 (54.2)	17.65 (8.48–29.36)	
MYCN amplification (N = 183)			**0.046**
Absent	155 (84.7)	17.85 (8.28–29.36)	
Present	28 (15.3)	16.20 (8.48–24.73)	
1p deletion (N = 99)			0.856
Absent	86 (86.9)	18.41 (11.07–27.46)	
Present	13 (13.1)	17.80 (15.20–25.17)	
11q deletion (N = 98)			0.893
Absent	73 (74.5)	18.57 (11.07–27.46)	
Present	25 (25.5)	18.10 (12.72–25.70)	
17q gain (N = 97)			0.300
Absent	66 (68.0)	18.41 (11.07–27.46)	
Present	31 (32.0)	18.46 (15.41–25.70)	
Risk group			**0.042**

Abbreviations: Ganglioneuroblastoma (GNB), poorly differentiated (PD), undifferentiated (UD)

Stage 1, 2, and 4S tumors were stratified into the low-risk group if MYCN was not amplified, whereas stage 4 tumors in patients older than 12 months (until 2008) or 18 months (since 2009) or any tumors with amplified MYCN were classified as the high-risk group. The intermediate-risk group includes all other tumors not mentioned above.

Clinical Characteristics	No. (%)	Median (range)	*P*-value
Low	50 (26.9)	18.36 (8.28–29.36)	
Intermediate	49 (26.3)	17.18 (8.41–25.70)	
High	87 (46.8)	17.44 (8.28–29.36)	
Abbreviations: Ganglioneuroblastoma (GNB), poorly differentiated (PD), undifferentiated (UD)			
Stage 1, 2, and 4S tumors were stratified into the low-risk group if MYCN was not amplified, whereas stage 4 tumors in patients older than 12 months (until 2008) or 18 months (since 2009) or any tumors with amplified MYCN were classified as the high-risk group. The intermediate-risk group includes all other tumors not mentioned above.			

Results

Clinical characteristics and their association with telomere length

The clinical characteristics of the 186 patients are given in Table 1. The median age was 2.1 years (range, 0.0–19.3), and 87 (46.8%) patients were categorized into the high-risk group. In addition, 28 (15.3%) patients had *MYCN* amplification. We tested the association between telomere length and ten important clinical characteristics in neuroblastoma: sex, age, primary site, tumor stage, tumor differentiation, *MYCN* amplification, 1p deletion, 11q deletion, 17q gain, and risk group. We found that an extra-abdominal primary site, lower stage (stage 1/2), MYCN non-amplified tumor, and low-risk group categorization were associated with longer telomere length ($P = 0.015$, 0.007, 0.046, and 0.042, respectively). Patients with longer third telomeres had longer EFS than other patients ($P = 0.032$), but there was no difference in OS (Fig. 1). Table 2 lists the results of the multivariate analysis for EFS. Age older than 1.5 years (hazard ratio 3.08, $P = 0.017$), stage 4 (hazard ratio 4.75, $P = 0.003$), and longer third telomere length (hazard ratio 0.037, $P = 0.046$) were independent prognostic factors for EFS.

Table 2
Multivariate analysis for event-free survival (EFS)

Risk Factors	Hazard ratio (range)	*P*-value
Age > 1.5 years	3.08 (1.22–7.78)	**0.017**
Stage 4	4.75 (1.70–13.26)	**0.003**
MYCN amplification	1.40 (0.65–3.03)	0.392
Differentiation		0.456
GND	1.00	
Differentiating	0.88 (0.28–2.85)	0.840
PD/UD	1.46 (0.53–4.00)	0.466
Telomere length		0.131
Shorter third	1.00	
Middle third	0.71 (0.36–1.42)	0.338
Longer third	0.37 (0.14–0.98)	**0.046**

Genetic association analysis of telomere length

We tested associations with telomere length in neuroblastoma patients in a multivariable regression analysis using age and sex as covariates. After marker filtering, 248,399 markers were used in the association analysis. We found that telomere length correlated with several significant genes: *HIVEP3*, *LRRTM4*, *ADGRV1*, *RAB30*, and *CHRNA4* (Fig. 2A). The significant

markers for telomere length are summarized in Table 3. In the GWAS, the most highly associated marker was rs10842679 (P = 4.7E-07). The gene nearest rs10842679 was *BHLHE41*, which is known as a putative regulator of neuronal differentiation [27]. Interestingly, markers located in the 3'UTR of the *HIVEP3* gene showed a strong association with telomere length (Table 3). The telomere length in the markers tends to increase from the dominant allele to the recessive allele. We analyzed the regional associations of 400 kb around *HIVEP3* on chromosome 1p34.2 (Fig. 2B) and found that the rs2492082 marker had a relatively robust association signal (*P* = 1.7.E-06, Table 3, Fig. 2B).

Table 3
Single nucleotide polymorphisms associated with telomere length in neuroblastoma patients

SNP	CHR	BP	Alleles	Gene	Region	MAF	DD(TL)	Dd(TL)	dd(TL)	*P*-value
rs10842679	12	26281858	G > C			0.084	155(16.6)	29(20.1)	1(25.2)	4.7E-07
rs10890075	1	41970768	T > C			0.395	67(15.6)	90(17.7)	28(19.5)	1.2E-06
rs11210339	1	41971212	A > G			0.392	68(15.6)	89(17.7)	28(19.5)	1.7E-06
rs2492082	1	41972198	T > A	HIVEP3	UTR3	0.392	68(15.6)	89(17.7)	28(19.5)	1.7E-06
rs1383116	12	26278079	A > C			0.081	156(16.7)	28(20.0)	1(25.2)	1.8E-06
rs71420970	2	77012296	C > G	LRRTM4	intron	0.070	159(16.7)	26(20.5)		3.5E-06
rs6679278	1	70851205	G > A			0.254	106(16.1)	64(18.5)	15(19.6)	3.6E-06
rs11243436	9	134449467	C > T			0.100	152(16.6)	29(19.6)	4(22.3)	3.8E-06
rs11243437	9	134450073	C > G			0.100	152(16.6)	29(19.6)	4(22.3)	3.8E-06
rs12806316	11	113495279	C > A			0.414	63(15.4)	91(17.9)	31(18.9)	3.9E-06
rs2492080	1	41971867	A > C			0.395	67(15.7)	90(17.7)	28(19.3)	5.3E-06
rs35359723	2	77015092	G > A	LRRTM4	intron	0.065	161(16.7)	24(20.6)		5.5E-06
rs2731765	16	58276591	C > A			0.300	87(18.2)	85(16.8)	13(12.9)	6.5E-06
rs2257931	16	58279244	A > G			0.300	87(18.2)	85(16.8)	13(12.9)	6.5E-06
rs1969749	1	41973908	T > C	HIVEP3	UTR3	0.405	63(15.7)	94(17.5)	28(19.6)	6.6E-06
rs2810587	1	41973095	G > A	HIVEP3	UTR3	0.400	64(15.7)	94(17.6)	27(19.4)	7.3E-06
rs1383112	12	26287230	T > C			0.092	152(16.7)	32(19.6)	1(25.2)	8.4E-06
rs1383113	12	26287240	A > G			0.092	152(16.7)	32(19.6)	1(25.2)	8.4E-06
rs2729628	12	26290639	T > C			0.092	152(16.7)	32(19.6)	1(25.2)	8.4E-06
rs1780432	10	34184022	C > T			0.208	112(18.2)	69(15.9)	4(13.3)	8.5E-06
rs1740718	10	34184861	C > A			0.208	112(18.2)	69(15.9)	4(13.3)	8.5E-06

Abbreviation: CHR (chromosome), BP (base pair), TL (telomere length)

SNP	CHR	BP	Alleles	Gene	Region	MAF	DD(TL)	Dd(TL)	dd(TL)	*P*-value
rs956613	10	34185869	G > C			0.208	112(18.2)	69(15.9)	4(13.3)	8.5E-06
rs74386538	22	27516762	A > G			0.257	101(18.2)	73(16.4)	11(13.7)	8.6E-06
rs417693	4	111211272	G > A			0.124	140(16.5)	44(19.2)	1(24.4)	9.2E-06
rs10915048	1	30682811	A > G			0.254	99(16.1)	78(18.4)	8(20.0)	9.2E-06
rs3809140	12	26278444	G > A			0.089	153(16.7)	31(19.6)	1(25.2)	1.1E-05
rs11243434	9	134448487	G > A			0.097	153(16.7)	28(19.5)	4(22.3)	1.1E-05
rs80083893	22	27516984	G > A			0.265	99(18.2)	74(16.4)	12(13.9)	1.3E-05
rs12788951	11	113471957	T > C			0.416	63(15.4)	90(18.0)	32(18.7)	1.5E-05
rs7931613	11	82694032	T > C	RAB30	intron	0.403	63(16.0)	95(17.2)	27(20.0)	1.5E-05
Abbreviation: CHR (chromosome), BP (base pair), TL (telomere length)										

To investigate the effects of multiple SNPs on telomere length in our neuroblastoma patients, we performed a gene-based assay using the VEGAS2 algorithm [28]. We found that the *CNTN4* gene had the most significant association (*P* = 1.0E-06). The results of the gene-based analysis are summarized in Supplementary Table 1.

Discussion

In this study, we investigated the association between telomere length in peripheral blood mononuclear cells, not tumor cells, and the clinical characteristics of tumors at diagnosis. Patients with an extra-abdominal primary tumor, lower stage tumor, MYCN non-amplified tumor, or low-risk tumor had longer germline telomere length than other patients. These clinical features are usually associated with better prognosis, and thus, it was unsurprising that longer telomere length was associated with better EFS and was an independent prognostic factor for EFS in our multivariate analysis. This is the first study to elucidate the clinical significance of germline telomere length in patients with neuroblastoma. We cannot explain the reason for the association between germline telomere length and the clinical characteristics of tumors. However, our results suggest that germline genomic characteristics, including telomere length, might affect the clinical characteristics of tumors at diagnosis and the treatment response.

For this reason, we performed genome-wide genotyping with a high-density bead chip to identify the genetic factors related to telomere length. In the GWAS analysis, novel risk SNPs, including *HIVEP3* and *LRRTM4*, were significantly associated with telomere length (Table 3). The most significant association was with rs10842679 (*P* = 4.7E-07). That marker was adjacent to the *BHLHE41* (basic helix-loop-helix family member e41) gene. *BHLHE41* is known to be a transcription factor implicated in cellular functions such as proliferation, differentiation, and tumorigenesis [27]. It is possible that this marker is linked to genetic factors that directly or indirectly affect the expression of the *BHLHE41* gene. Follow-up functional studies would be required to clarify the exact mechanism. Table 3 summarizes information about the genes with significant markers. Among them, many genes significant to the *HIVEP3* gene were found. No direct functional association between neuroblastoma and the *HIVEP3* gene has yet been reported. However, studies of other carcinomas have reported that they have higher levels of *HIVEP3* and *SOX9* messenger RNA expression than non-carcinoma cells [29]. In particular, patients with *HIVEP3* and *SOX9* overexpression

showed a lower survival rate. In this study, patients with the recessive allele of the *HIVEP3* gene had long telomere lengths. Follow-up studies comparing therapeutic effects and survival rates according to the expression of the *HIVEP3* gene and telomere length changes are needed to clarify how telomere length is related to the function of the *HIVEP3* gene.

A gene-based analysis was performed using the VEGAS2 algorithm, and the *CNTN4* gene showed the most significant association (P = 1.0E-06; Supplementary Table 1). The rs71311728 marker within the *CNTN4* gene also showed a significant association in the GWAS analysis (P = 0.0001). The rs71311728 marker within *CNTN4* gene is a new marker with no reported association with other cancers. The *CNTN4* gene encodes contactin 4, a member of the immunoglobulin superfamily [30]. The functions of these proteins are suggested to involve synaptic plasticity. In addition, they are known to be involved in axon growth, guidance, and fasciculation [31]. Although the details remain unclear, the effect of *CNTN4* on the development of nerve endings and the development of neuroblastoma should be examined in future functional studies. To investigate the biological functions of the significant genes we identified, we used VEGAS2 to perform a gene ontology analysis. The results are summarized in Supplementary Table 2. The statistically significant GO categories (empirical P < 0.00005) were cadherin binding, cell to cell pathway, cell leading edge, neurite morphogenesis, cell part morphogenesis, and neuron development.

This is the first GWAS study to investigate an association with telomere length in neuroblastoma patients, and we found that the *CNTN4* gene is associated with changes in telomere length in Korean neuroblastoma patients. Our number of samples was insufficient due to the rarity of neuroblastoma. However, the new genes and markers discovered through this study will contribute to other GWAS studies that can be conducted in various ethnicities in the future.

In conclusion, we found that longer germline telomere length is associated with favorable prognostic factors at diagnosis and eventually a better EFS in patients with neuroblastoma. In addition, the GWAS demonstrated that genetic markers and genes related to telomere length were associated with neurite morphogenesis and neuron development in neuroblastoma. A further study with a larger cohort of patients and functional studies are needed.

Declarations

Acknowledgement

This work was supported by a grant from the National Research Foundation of Korea (NRF), which is funded by the Korean government (NRF-2019R1F1A1041365). The samples used in this study were obtained from the Samsung Medical Center Biobank.

Competing Interest

The authors declare that they have no competing interests.

Data availability

All data analyzed or generated during this study are included in the article.

References

1. Blackburn, E. H. Switching and signaling at the telomere. Cell **106**, 661–673. http://doi.org/10.1016/s0092-8674(01)00492-5 (2001).
2. Palm, W. & de Lange, T. How shelterin protects mammalian telomeres. Annu Rev Genet **42**, 301–334. http://doi.org/10.1146/annurev.genet.41.110306.130350 (2008).

3. Siderakis, M. & Tarsounas, M. Telomere regulation and function during meiosis. Chromosome Res **15**, 667–679. http://doi.org/10.1007/s10577-007-1149-7 (2007).
4. Autexier, C. & Lue, N. F. The structure and function of telomerase reverse transcriptase. Annu Rev Biochem **75**, 493–517. http://doi.org/10.1146/annurev.biochem.75.103004.142412 (2006).
5. Njajou, O. T. *et al.* Telomere length is paternally inherited and is associated with parental lifespan. Proc Natl Acad Sci U S A **104**, 12135–12139. http://doi.org/10.1073/pnas.0702703104 (2007).
6. Bojesen, S. E. Telomeres and human health. J Intern Med **274**, 399–413. http://doi.org/10.1111/joim.12083 (2013).
7. Herrmann, M., Pusceddu, I., Marz, W. & Herrmann, W. Telomere biology and age-related diseases. Clin Chem Lab Med **56**, 1210–1222. http://doi.org/10.1515/cclm-2017-0870 (2018).
8. Maciejowski, J. & de Lange, T. Telomeres in cancer: tumour suppression and genome instability. Nat Rev Mol Cell Biol **18**, 175–186. http://doi.org/10.1038/nrm.2016.171 (2017).
9. Patel, C. J., Manrai, A. K., Corona, E. & Kohane, I. S. Systematic correlation of environmental exposure and physiological and self-reported behaviour factors with leukocyte telomere length. Int J Epidemiol **46**, 44–56. http://doi.org/10.1093/ije/dyw043 (2017).
10. Codd, V. *et al.* Identification of seven loci affecting mean telomere length and their association with disease. Nat Genet **45**, 422–427, 427e421-422. http://doi.org/10.1038/ng.2528 (2013).
11. Frias, C. *et al.* Telomere shortening is associated with poor prognosis and telomerase activity correlates with DNA repair impairment in non-small cell lung cancer. Lung Cancer **60**, 416–425. http://doi.org/10.1016/j.lungcan.2007.11.001 (2008).
12. D'Mello, M. J. *et al.* Association between shortened leukocyte telomere length and cardiometabolic outcomes: systematic review and meta-analysis. Circ Cardiovasc Genet **8**, 82–90. http://doi.org/10.1161/CIRCGENETICS.113.000485 (2015).
13. Jang, J. S. *et al.* Telomere length and the risk of lung cancer. Cancer Sci **99**, 1385–1389. http://doi.org/10.1111/j.1349-7006.2008.00831.x (2008).
14. Shen, M. *et al.* A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of lung cancer. Lung Cancer **73**, 133–137. http://doi.org/10.1016/j.lungcan.2010.11.009 (2011).
15. Ma, H. *et al.* Shortened telomere length is associated with increased risk of cancer: a meta-analysis. PLoS One **6**, e20466. http://doi.org/10.1371/journal.pone.0020466 (2011).
16. Karimi, B., Yunesian, M., Nabizadeh, R., Mehdipour, P. & Aghaie, A. Is Leukocyte Telomere Length Related with Lung Cancer Risk?: A Meta-Analysis. Iran Biomed J **21**, 142–153. http://doi.org/10.18869/acadpub.ibj.21.3.142 (2017).
17. Shen, J. *et al.* Telomere length, oxidative damage, antioxidants and breast cancer risk. Int J Cancer **124**, 1637–1643. http://doi.org/10.1002/ijc.24105 (2009).
18. Sommerfeld, H. J. *et al.* Telomerase activity: a prevalent marker of malignant human prostate tissue. Cancer Res **56**, 218–222 (1996).
19. Artandi, S. E. *et al.* Constitutive telomerase expression promotes mammary carcinomas in aging mice. Proc Natl Acad Sci U S A **99**, 8191–8196. http://doi.org/10.1073/pnas.112515399 (2002).
20. Blasco, M. A. *et al.* Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell **91**, 25–34. http://doi.org/10.1016/s0092-8674(01)80006-4 (1997).
21. Babizhayev, M. A., Savel'yeva, E. L., Moskvina, S. N. & Yegorov, Y. E. Telomere length is a biomarker of cumulative oxidative stress, biologic age, and an independent predictor of survival and therapeutic treatment requirement associated with smoking behavior. Am J Ther **18**, e209-226. http://doi.org/10.1097/MJT.0b013e3181cf8ebb (2011).
22. Seow, W. J. *et al.* Telomere length in white blood cell DNA and lung cancer: a pooled analysis of three prospective cohorts. Cancer Res **74**, 4090–4098. http://doi.org/10.1158/0008-5472.CAN-14-0459 (2014).
23. Sanchez-Espiridion, B. *et al.* Telomere length in peripheral blood leukocytes and lung cancer risk: a large case-control study in Caucasians. Cancer Res **74**, 2476–2486. http://doi.org/10.1158/0008-5472.CAN-13-2968 (2014).

24. Gramatges, M. M., Telli, M. L., Balise, R. & Ford, J. M. Longer relative telomere length in blood from women with sporadic and familial breast cancer compared with healthy controls. Cancer Epidemiol Biomarkers Prev **19**, 605–613. http://doi.org/10.1158/1055-9965.EPI-09-0896 (2010).
25. Zheng, Y. L. *et al.* Telomere length in blood cells and breast cancer risk: investigations in two case-control studies. Breast Cancer Res Treat **120**, 769–775. http://doi.org/10.1007/s10549-009-0440-z (2010).
26. Cawthon, R. M. Telomere measurement by quantitative PCR. Nucleic Acids Res **30**, e47. http://doi.org/10.1093/nar/30.10.e47 (2002).
27. Sun, Y. *et al.* Loss of the basic helix-loop-helix transcription factor Bhlhe41 induces cell death and impairs neurite outgrowth in Neuro2a cells. Mol Cell Biochem **450**, 167–174. http://doi.org/10.1007/s11010-018-3383-z (2019).
28. Mishra, A. & Macgregor, S. VEGAS2: Software for More Flexible Gene-Based Testing. Twin Res Hum Genet **18**, 86–91. http://doi.org/10.1017/thg.2014.79 (2015).
29. Qin, G. Q. *et al.* Combined overexpression of HIVEP3 and SOX9 predicts unfavorable biochemical recurrence-free survival in patients with prostate cancer. Onco Targets Ther **7**, 137–146. http://doi.org/10.2147/OTT.S55432 (2014).
30. Hansford, L. M. *et al.* Cloning and characterization of the human neural cell adhesion molecule, CNTN4 (alias BIG-2). Cytogenet Genome Res **101**, 17–23. http://doi.org/10.1159/000073412 (2003).
31. Yoshihara, Y. *et al.* Overlapping and differential expression of BIG-2, BIG-1, TAG-1, and F3: four members of an axon-associated cell adhesion molecule subgroup of the immunoglobulin superfamily. J Neurobiol **28**, 51–69. http://doi.org/10.1002/neu.480280106 (1995).

Figures

Figure 1.

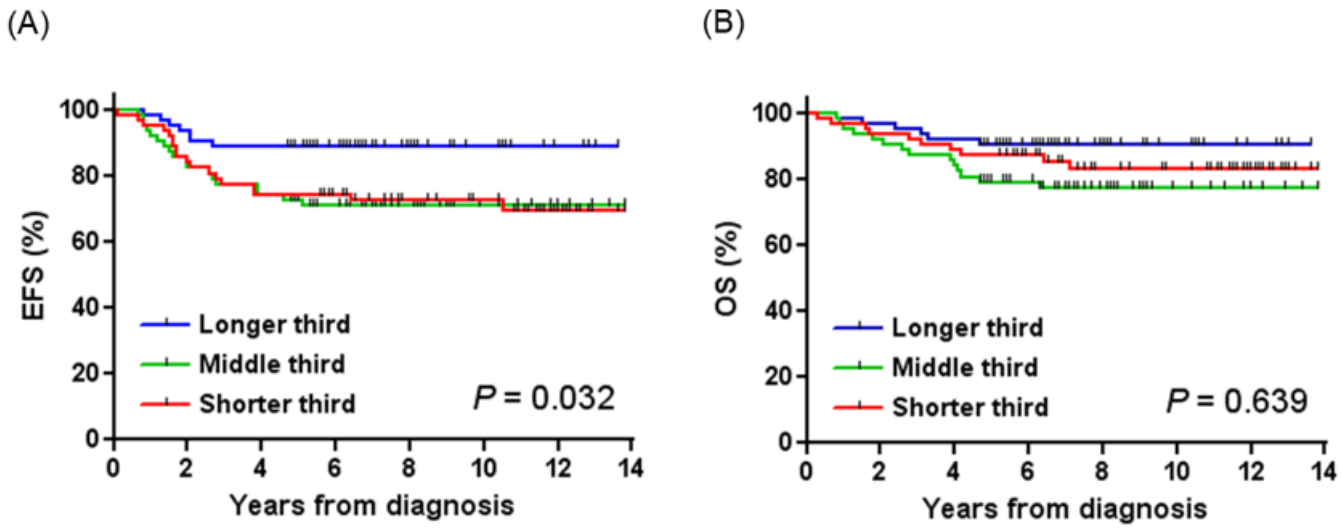

Figure 1

Survival data according to telomere length. (A) Patients with longer third telomeres showed longer event-free survival than other patients, (B) but there was no statistical difference in overall survival.

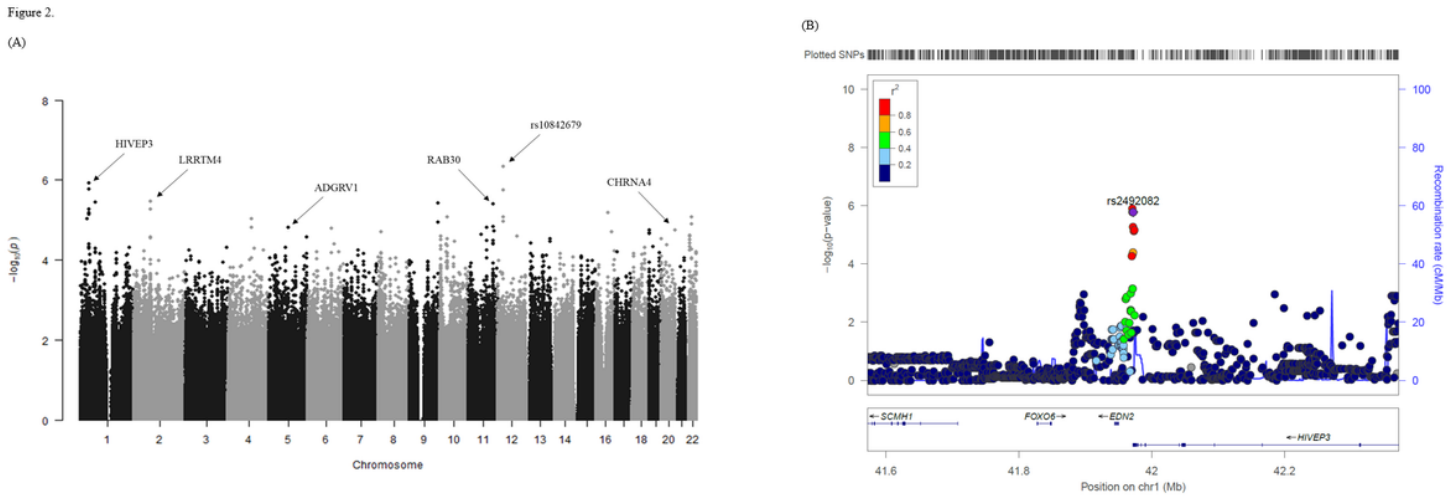

Figure 2

Manhattan plot and regional association plot. (A) *P* values from the genome-wide association study. The Manhattan plot shows the *P* values for the risk of neuroblastoma calculated using a logistic regression analysis. The X-axis represents the single nucleotide polymorphism (SNP) markers on each chromosome. The highest *P* value (*P* = 4.7E-07) was observed for rs10842679 on 12p.12.1. (B) Regional association plots at *CNTN4*. Regional association plots containing both genotypes and SNPs within 400 kb of *CNTN4* were generated by LocusZoom. The significance of the association (−log10-transformed p-values) and recombination rate are plotted. SNPs are colored to reflect pairwise linkage disequilibrium (r^2) with the most significantly associated genotyped SNPs in the 1000 Genomes Project Phase 1 interim release Asian (ASN) population genotypes. The most significant genotyped SNPs are labeled and shown in purple.

Supplementary Files

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