1980 cohorts, and age is not a significant determinant within pre- and post-OCP periods. The results for neuroticism are less robust to checks for age effects (table S16).

Previous research has shown that noncognitive attributes such as conscientiousness, neuroticism, and optimism are important determinants of educational attainment, labor market outcomes, health, and marriage and divorce (*38*–*40*). Prosocial behavior is consistently seen to be an important determinant of social capital and plays a role in institutional development (*41*). A willingness to take risks is an important component of entrepreneurship (*17*). Our data show that being an only child as a result of the OCP is associated with taking less risk in the labor market (table S19).

Although our findings were obtained from a comparison of cohorts in Beijing born directly around the time of the policy's introduction, our results are generalizable to other urban areas of China where the OCP was strictly implemented. Previous work suggests that differences between only children and others in Beijing are similar to those in other urban areas (*26*). The effect of the policy on the behavior of people born long after the policy's introduction may, however, differ from what we found here, because later cohorts will have grown up with very limited extended family and in a society dominated by only children. Under such circumstances, we would expect that the policy's effect would, if anything, be magnified.

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Supplementary Materials

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Highly Recurrent *TERT* Promoter Mutations in Human Melanoma

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Systematic sequencing of human cancer genomes has identified many recurrent mutations in the protein-coding regions of genes but rarely in gene regulatory regions. Here, we describe two independent mutations within the core promoter of *telomerase reverse transcriptase* (*TERT*), the gene coding for the catalytic subunit of telomerase, which collectively occur in 50 of 70 (71%) melanomas examined. These mutations generate de novo consensus binding motifs for E-twenty-six (ETS) transcription factors, and in reporter assays, the mutations increased transcriptional activity from the *TERT* promoter by two- to fourfold. Examination of 150 cancer cell lines derived from diverse tumor types revealed the same mutations in 24 cases (16%), with preliminary evidence of elevated frequency in bladder and hepatocellular cancer cells. Thus, somatic mutations in regulatory regions of the genome may represent an important tumorigenic mechanism.

Systematic characterization of human cancer genomes has led to the discovery of a wide range of mutated genes that contribute to tumor development and progression. Most of the somatic mutations in tumors reside within the protein-coding regions of genes or at splice junctions. To determine whether tumor genomes harbor recurrent mutations outside of protein-coding regions, we systematically queried noncoding somatic mutations using published whole-genome sequencing data.

Analysis of whole-genome sequencing data from malignant melanomas (*1*, *2*) revealed two somatic *telomerase reverse transcriptase* (*TERT*) gene promoter mutations in 17 of 19 (89%) cases examined. The average sequence coverage at the *TERT* promoter locus was 30-fold in normal samples and 60-fold in tumor samples (fig. S1A).

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Each of these promoter mutations resulted in a cytidine-to-thymidine transition at a dipyrimidine motif indicative of ultraviolet (UV) light–induced damage (chr5, 1,295,228 C>T and 1,295,250 C>T; hereafter termed C228T and C250T, respectively), and both mutations localized within 100 base pairs (bp) of the *TERT* transcriptional start site (TSS) (mean allelic fraction, 0.32; range, 0.07 to 0.55) (table S1). We validated these mutations by means of polymerase chain reaction and Sanger sequencing tumor/normal sample pairs from both the discovery set (Fig. 1A and fig. S1, B and C) and an extension set of 51 additional melanoma tumor/normal sample pairs. Within this extension set, 33 tumors (65%) harbored one of the mutations. Moreover, the mutations were mutually exclusive in both the discovery and extension sets ($P = 5.4 \times 10^{-7}$, Fisher's one-sided exact test). Two tumors with a C228T transition also contained an adjacent C>T transition (at position chr5, 1,295,229), which is indicative of a dinucleotide CC>TT transition. Together, these *TERT* promoter mutations were observed in 50 of 70 (71%; 95% confidence interval: 59 to 82%, Clopper-Pearson method) melanomas examined (Fig. 1B and table S1).

Both C228T and C250T generated an identical 11-bp nucleotide stretch (5′-CCCCTTCCGGG-3′) containing a consensus binding site for E-twenty-six (ETS) transcription factors (GGAA, reverse complement) within the *TERT* promoter region. Because ETS transcription factors may become activated through dysregulation of mitogen-activated protein kinase (MAP kinase) signaling, we hypothesized that these promoter mutations might augment gene expression. To test this hypothesis, we used a reporter assay system in which the relevant portion of the mutant or wild-type *TERT* core promoter was cloned upstream of the firefly luciferase gene (*2*). Here, we tested both a core promoter fragment (–132 to +5 relative to the TSS) and the full core promoter (–200 to +73). In comparison to the wild-type *TERT* promoter, both mutations conferred approximately two- to fourfold increased transcriptional activity in five distinct cell line contexts (Fig. 1C and fig. S1D). Thus, each mutation was capable of augmenting transcriptional activity from the *TERT* promoter.

To investigate whether similar *TERT* promoter mutations occur in other cancer types, we examined sequencing data from this locus in 150 cell lines from the Cancer Cell Line Encyclopedia (CCLE) (*3*). Overall, 24 CCLE lines (16%) contained either C228T or C250T (mean allelic fraction, 0.61; range, 0.17 to 1.00) (table S1). An increased frequency in melanoma was again noted (five of six lines tested), with additional evidence suggesting possible heightened prevalence (>25%; one-sided 95% confidence interval) in bladder (three of three lines) and hepatocellular cancer cell lines (four of six lines) (Fig. 1D).

Several lines of evidence support the hypothesis that these promoter mutations may function as driver events that contribute to oncogenesis through TERT dysregulation and undergo positive selection, at least in human melanoma. First, the *TERT* promoter mutations showed a combined frequency that exceeded those of *BRAF* and *NRAS* mutations, which activate known melanoma driver oncogenes (*4*, *5*). In an analysis restricted to somatic mutations present at an allelic fraction of 0.2 or greater [to reduce artifacts of mutation calling (*1*)], the four most recurrent melanoma nucleotide substitutions included *BRAF* [chr7, 140,453,136 A>T (V600E)], *NRAS* [chr1, 115,256,529 T>C (Q61R)], and the *TERT* core promoter mutations C228T and C250T. Second, although highly recurrent, C228T and C250T occurred in a wholly mutually exclusive fashion. This suggests the possibility that the mutations might be functionally redundant. Third, the absence of other recurrent somatic mutations in the 3 kb upstream of the *TERT* transcription start site in the queried melanomas (*1*) coupled with the absence of the described *TERT* promoter mutations in 24 lung adenocarcinomas with comparably high somatic mutation rates (*6*) reduces the possibility that these recurrent *TERT* promoter mutations are solely due to an increased background mutation rate at this locus.

Although the role of telomerase in tumorigenesis is well established, details regarding its dysregulation in cancer cells remain incompletely understood, particularly in melanoma (*7*). The *TERT* promoter mutations identified here may link telomerase gene regulation and tumorigenic activation in this malignancy. The high prevalence of C228T and C250T suggests that these *TERT* promoter mutations may comprise early genetic events in the genesis of melanoma and other cancer types. Although TERT expression alone is not sufficient to bypass oncogene-induced senescence, genomic *TERT* activation may potentiate mechanisms by which melanocytes achieve immortalization in the setting of oncogenic mutations (*8*). These results therefore suggest that renewed efforts to develop clinically effective telomerase inhibitors may be warranted.

At the same time, promoter mutations likely represent only one potential mechanism of *TERT* reactivation in a subset of human cancers. Indeed, recurrent chromosomal copy gains spanning the *TERT* locus have been described previously for several cancers, including melanoma (*9*, *10*).

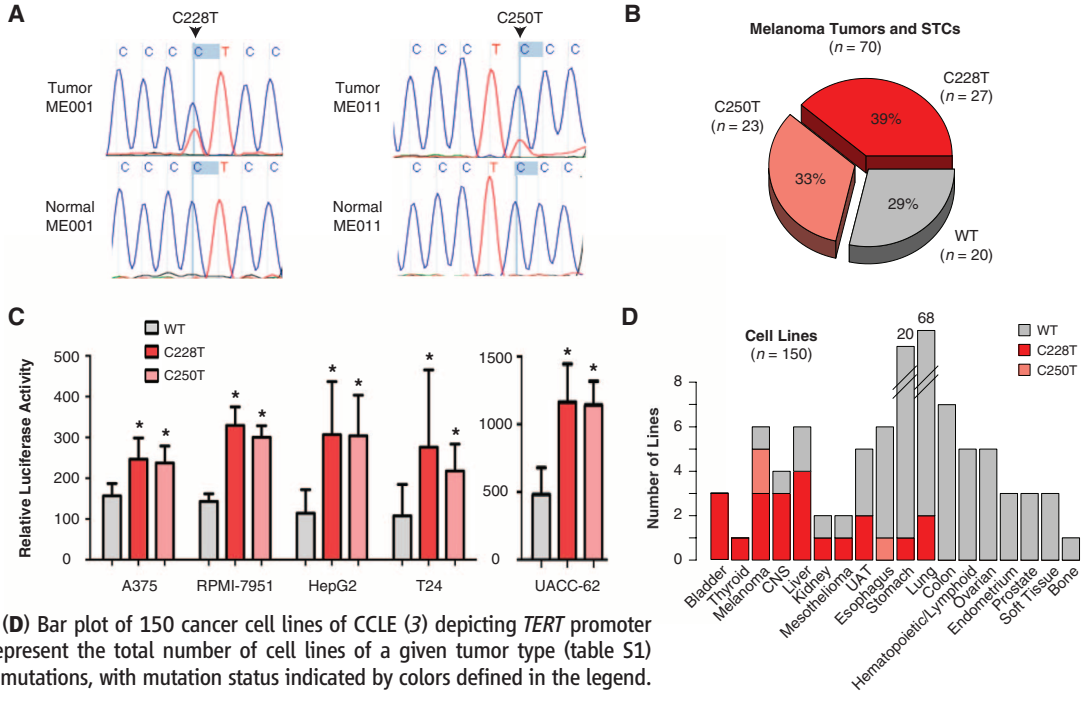

Fig. 1. Identification of *TERT* promoter mutations in melanoma and cancer cell lines. (**A**) Sequence chromatograms of matched tumor and normal DNA representing somatic mutations chr 5 [1,295,228 C>T (C228T)] and chr 5 [1,295,250 C>T (C250T)] in the *TERT* promoter locus. (**B**) Pie chart of C228T and C250T somatic mutation status in 70 surveyed melanoma tumors and short-term cultures. Sum of percentages is greater than 100% because of rounding. (**C**) Luciferase reporter assays for transcriptional activity from the *TERT* core promoter (–200 to +73) with either the C228T or C250T mutation compared with wild-type promoter in A375, RPMI-7951, UACC-62, T24, or HepG2 cell lines. The results depicted are the average of at least three independent experiments. Values are mean ± SD; *$P < 0.05$. (**D**) Bar plot of 150 cancer cell lines of CCLE (*3*) depicting *TERT* promoter mutation status. Individual bars represent the total number of cell lines of a given tumor type (table S1) interrogated for C228T and C250T mutations, with mutation status indicated by colors defined in the legend.

Highly recurrent somatic mutations within a cancer gene promoter region have not previously been described. Similarly, the de novo mutational generation of transcription factor binding motifs in tumor genomes was heretofore unknown, although an ETS transcription factor binding motif was previously associated with a single-nucleotide polymorphism insertion at the MMP-1 locus (*11*). Together, these findings raise the possibility that recurrent somatic mutations involving regulatory regions, in addition to coding sequences, may represent important driver events in cancer.

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Supplementary Materials

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Table S1
References (*12*, *13*)

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TERT Promoter Mutations in Familial and Sporadic Melanoma

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Cutaneous melanoma occurs in both familial and sporadic forms. We investigated a melanoma-prone family through linkage analysis and high-throughput sequencing and identified a disease-segregating germline mutation in the promoter of the *telomerase reverse transcriptase* (*TERT*) gene, which encodes the catalytic subunit of telomerase. The mutation creates a new binding motif for Ets transcription factors and ternary complex factors (TCFs) near the transcription start and, in reporter gene assays, caused up to twofold increase in transcription. We then screened the *TERT* promoter in sporadic melanoma and observed recurrent ultraviolet signature somatic mutations in 125 of 168 (74%) of human cell lines derived from metastatic melanomas, 45 of 53 corresponding metastatic tumor tissues (85%), and 25 of 77 (33%) primary melanomas. The majority of those mutations occurred at two positions in the *TERT* promoter and also generated binding motifs for Ets/TCF transcription factors.

The identification of germline mutations that cosegregate with disease in cancer-prone families often provides genetic and mechanistic insights into the more common, sporadically arising cancers. In a study of cutaneous melanoma, the most malignant skin cancer, we investigated a large pedigree with 14 related melanoma patients who were not carriers of germline mutations in *CDKN2A* or *CDK4*, two known melanoma genes (Fig. 1). Multipoint linkage analysis showed a possible 2.2-Mb linkage region on chromosome 5p with maximal logarithm of the odds ratio for linkage scores of 2.35 at rs1379917 and 2.45 at rs1968011. Target-enriched high-throughput sequencing (HTS) of the region was carried out on constitutional DNA from the four affected and four unaffected members of the family with an average coverage between 55- and 108-fold (table S1) (*1*). The HTS data revealed a single promoter variant, three intronic variants, and three nongene variants previously unknown and unique to the DNA sequences of the affected individuals (table S2). The disease segregating variants, seven in total, were validated by Sanger sequencing of DNA from the individuals sequenced by HTS and of DNA from additional unaffected members of the family. The new variants were also detected in an unaffected member (754, table S3), who was 36 years old and carried multiple nevi. DNA from affected individuals other than those sequenced by HTS was not available for testing.

Of the seven unique variants identified, one variant (T>G), was located in the promoter at –57 base pairs (bp) from ATG translation start site of the *telomerase reverse transcriptase* (*TERT*) gene. The *TERT* gene encodes the catalytic reverse transcriptase subunit of telomerase, the ribonucleoprotein complex that maintains telomere length. The nucleotide change in the sequence CCTGAA>CCGGAA creates a new binding motif for Ets transcription factors, with a general recognition motif GGA(A/T). Beyond the general motif for Ets transcription factors, the familial mutation also generates a binding motif, CCGGAA, for the ternary complex factors (TCFs) Elk1 and Elk4 (*2*, *3*). To exclude the possibility that the detected promoter mutation in *TERT* is a common germline variant, we screened germline DNA from 140 sporadic melanoma cases and 165 healthy controls, and none carried the variant. Screening of DNA from index cases from 34 Spanish melanoma families also did not show any mutations. No carriers were found in dbSNP and the 1000 Genomes databases (data available for 18 individuals were obtained from Ensembl).

The familial mutation in the *TERT* promoter was in complete allelic linkage with a common polymorphism rs2853669 (G>A) at –246 bp upstream from the ATG start site (table S3). In previous work, this polymorphism was reported to disrupt an Ets binding site, and it was associated with low telomerase activity in patients with non–small cell lung cancer (*4*). In luciferase reporter gene assays, we found that the activity of constructs containing the mutation at –57 bp of the *TERT* promoter was increased 1.5-fold and 1.2-fold over the wild-type construct in Ma-Mel-86a and human embryonic kidney (HEK) 293T cells, respectively. A construct with both the *TERT* mutation and the variant allele of the rs2853669 polymorphism showed a 2.2-fold increase in promoter activity in Ma-Mel-86a and and 1.3-fold increase in HEK293 cells (mean from three measurements; details in supplementary text and fig. S1).

The germline occurrence of the promoter mutation, creating an Ets/TCF motif, can result in modification of *TERT* expression in all tissues expressing Ets/TCF. Highest staining for the TCF Elk1 protein has been reported in female-specific tissues, such as ovary and placenta. The increased expression of TCF Elk1 protein in female-specific tissues may cause gender-related differences in

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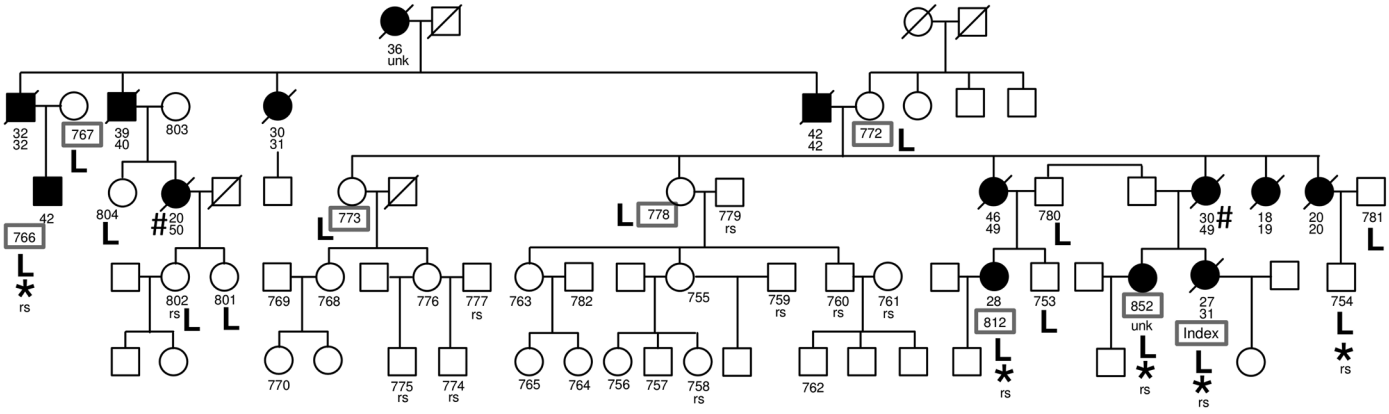

Fig. 1. Pedigree of melanoma-prone family. Four generations were affected by melanoma (solid symbols; circles represent females, and squares represent males). After linkage analysis carried out on 15 family members (L), HTS was performed on four affected and four unaffected individuals (boxed samples). A mutation in the *TERT* promoter was identified in all affected members and one unaffected individual (stars). Strikethrough symbols indicate deceased individuals. Two-digit numbers are age at onset of melanoma and age at death; Unk, unknown; Rs, rs2853669 observed in heterozygous form; three-digit numbers, DNA available; #, affected by other cancers; and index, index patient.

cancer susceptibility among carriers of the *TERT* mutation (*5*) (supplementary text). Two affected members of the family developed several different types of cancer (marked with # in Fig. 1). One affected individual presented with ovarian cancer at age 27 and melanoma at age 30. Another individual was diagnosed with melanoma at age 20; later she developed ovarian cancer, renal cell carcinoma, bladder cancer, mammary carcinoma, and finally bronchial carcinoma, leading to her death at age 50.

The mutation in the melanoma-prone family prompted us to screen melanoma cell lines derived from sporadic cases of metastatic melanoma. None of the cell lines carried the mutation detected in the family. However, we identified recurrent ultraviolet (UV)–signature mutations in the *TERT* core promoter in 74% (125 of 168) of the cell lines. The mutations were located within a 49-bp region starting from –100 bp upstream of the ATG start site (Table 1, Fig. 2, fig. S2, and table S4). There were two frequent mutations at –124 bp (G>A; C>T on opposite strand) and –146 bp (G>A); these mutations were mutually exclusive and occurred in 27 and 38% of cell lines, respectively. Two tandem GG>AA (CC>TT) mutations at positions –124/–125 bp and –138/–139 bp were observed at a frequency of 9%. The tandem mutation at positions –138/–139 bp could also be generated by a single-base mutation at –138 bp, because the base change at –139 bp has been reported as a rare polymorphism (rs35550267). The two most frequent single-base mutations as well as the two tandem mutations also result in the creation of Ets/TCF binding motifs.

Mutations were confirmed in 45 of 53 (85%) available metastasized tumors corresponding to the cell lines. The somatic nature of the mutations was shown by the absence of mutations in corresponding DNA from peripheral blood mononuclear cells available from 23 patients. Somatic mutations in the *TERT* promoter were more frequent than the *BRAF* mutations (53%, 90 of 169), *CDKN2A* alterations (50%, 84 of 169), and *NRAS* mutations (23%, 38 of 169; fig. S3). The occurrence of concomitant mutations in the *TERT* promoter and *BRAF* was more frequent (47%) than by random chance (40%) with an odds ratio (OR) of 3.2 [95% confidence interval (CI) 1.3 to 8.2]. Concomitant mutations in *TERT*, *BRAF*, and *CDKN2A* were observed in 30% of cell lines compared with the expected frequency of such occurrence of 9% (OR 5.6, 95% CI 2.4 to 13.8). The high recurrence and specificity of the *TERT* promoter mutations, together with the preliminary evidence from reporter assays that they have a functional effect on transcription, suggest that these mutations are driver rather than passenger events. Extensive functional studies will be required to validate this hypothesis.

Table 1. Most-frequent *TERT* core promoter mutations in screened metastatic melanoma cell lines and paraffin embedded primary tumors. A total of 169 cell lines were screened. Amplification for the *TERT* promoter failed for one cell line. Of 168 cell lines examined, 125 carried recurrent mutations. Of 77 primary melanomas examined, 24 carried recurrent mutations, and one carried a rare mutation (table S5). Seven rare mutations occurred at other sites in less than 2% of samples. Details of all mutations and polymorphisms are given in tables S4 and S5. Matched normal control DNA corresponding to 23 cell lines did not show mutations. For primary tumors, matched normal control DNA was not available.

Position (hg19) and variant	Distance to start (bp)	Cell lines	%	Primaries	%
1,295,228 G>A	–124	46	27.4	7	9.1
1,295,228 and 1,295,229 GG>AA	–124, –125	7	4.2	4	5.2
1,295,242 and 1,295,243 (rs35550267) GG>AA	–138, –139	8	4.8	8	10.4
1,295,250 G>A	–146	64	38.1	5	6.5

The *TERT* promoter mutations were also detected in 25 out of 77 (33%) paraffin embedded primary melanoma tumors (Table 1 and table S5) at –124 bp (7/77; 9%) and –146 bp (5/77; 7%). Four primary tumors carried the GG>AA tandem mutations at –124/–125 bp, and eight primary tumors carried the GG>AA tandem mutations at –138/–139 bp. Reduced sensitivity to detect mutations in paraffin-embedded primary tumors because of contaminating normal cells cannot be ruled out. Primary tumors harbored five additional mutations in the *TERT* promoter, which were not present in metastases, and those did not generate Ets/TCF binding motifs. We also screened DNA extracted from 25 melanocytic nevi and only one carried a mutation at –101 bp, which did not create an Ets/TCF motif. For both primary tumors and melanocytic nevi, matched normal control DNA was not available for testing.

The *TERT* coding region has been reported to be somatically mutated in 1% of cancers (14 cancer types, 1271 unique samples) (*6*). Mutations creating Ets/TCF binding motifs in the *TERT* promoter in melanoma have not been described in earlier sequencing projects.

TCFs are a subfamily of Ets transcription factors; two members of this subfamily, Elk1 and Elk4, are downstream targets of *BRAF* and regulate the expression of many genes (*7*–*11*). Conceivably, TCF may represent a link between telomerase activity and the frequent *BRAF* activating mutations in melanoma (fig. S4) (*12*, *13*). Lastly, whether *TERT* promoter mutations occur in other cancer types remains to be determined. We did not detect these mutations in a screen of 22 esophageal squamous cell carcinomas, but further analyses are warranted.

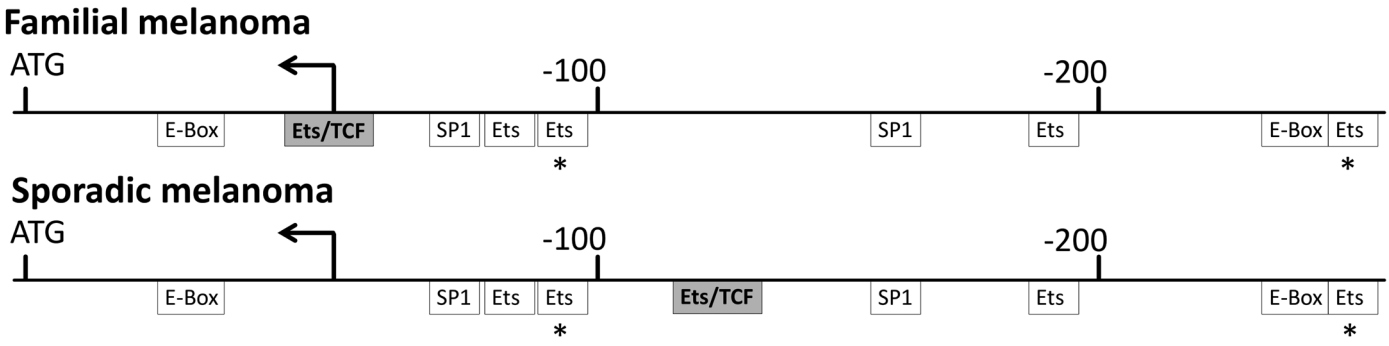

Fig. 2. The *TERT* core promoter in melanoma. Mutations creating Ets/TCF binding motifs were found in affected family members (–57 bp) immediately next to the transcription start site and in sporadic metastatic melanoma (–124 to –149 bp; sequence details in fig. S2). Binding sites for c-Myc (E-Box), SP1, and Ets transcription factors are known to exist in the wild-type *TERT* promoter. Ets2 binding was reported for Ets2 sites at –99 and –243 bp (stars) (*4*). The plus strand of DNA is shown.

References and Notes

1. Materials and methods are available as supplementary materials on *Science* Online.
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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1230062/DC1
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Increases in Adult Life Expectancy in Rural South Africa: Valuing the Scale-Up of HIV Treatment

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The scale-up of antiretroviral therapy (ART) is expected to raise adult life expectancy in populations with high HIV prevalence. Using data from a population cohort of over 101,000 individuals in rural KwaZulu-Natal, South Africa, we measured changes in adult life expectancy for 2000–2011. In 2003, the year before ART became available in the public-sector health system, adult life expectancy was 49.2 years; by 2011, adult life expectancy had increased to 60.5 years—an 11.3-year gain. Based on standard monetary valuation of life, the survival benefits of ART far outweigh the costs of providing treatment in this community. These gains in adult life expectancy signify the social value of ART and have implications for the investment decisions of individuals, governments, and donors.

For most of the 20th century, life expectancy increased in nearly every part of the world (*1*). However, from the late 1980s, the HIV epidemic led to a reversal of this trend in southern Africa, with a large rise in mortality among working-age adults (*1*–*3*). In South Africa, life expectancy at age 15 declined from 67.4 years in 1990 to 58.7 years in 2009; and in Swaziland, from 68.1 to 53.4 years (*2*). In addition to the direct loss of life, these declines in adult life expectancy had profound negative effects on households, communities, and governments, including declines in household wealth; large increases in the number of orphans; the loss of skilled workers, including teachers, doctors, and government officials; and the interruption of intergenerational transmission of knowledge and norms (*4*).

In the early 2000s, southern African nations began to disburse mass antiretroviral therapy (ART) for HIV through public-sector treatment programs, often with support from international donors. Using a combination of three or more drugs, ART interrupts HIV replication, enables immune recovery, and improves survival among people with HIV (*5*). Population-level declines in HIV-related and all-cause mortality have been documented in South Africa (*6*–*9*), Malawi (*10*), and other countries receiving financial assistance for HIV programs from the U.S. government (*11*). However, the impact of ART on population-level adult life expectancy in highly affected communities has not been quantified.

Life expectancy summarizes age patterns of mortality in a single statistic and is commonly used to compare differences in mortality across populations and over time (*1*–*3*). Because HIV predominantly affects working-age adults, adult life expectancy is of particular interest for governments and donors, as well as for individuals and households, whose plans for the future will be influenced by changes in the anticipated length of life. Adult life expectancy is defined as the mean age to which a 15-year-old could expect to live if subjected to the full pattern of age-specific mortality rates observed for a population over a particular period of time. Because future mortality rates are unknown, adult life expectancy cannot be interpreted as the average age to which a cohort will live, except in the limited case in which age-specific mortality rates remain constant into the future. Adult life expectancy is best interpreted as a summary indicator of the mortality experience in a population at a given time.

This paper documents the impact of South Africa's public-sector scale-up of ART on adult life expectancy in a large population cohort in

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