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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 germ line 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/TCF transcription factors near the transcription start and in reporter gene assays, caused up to 2-fold increase in transcription. We then screened the *TERT* promoter in sporadic melanoma and observed recurrent UV signature somatic mutations in 125/168 (74%) of human cell lines derived from metastatic melanomas, corresponding metastatic tumor tissues (45/53, 85%) and in 25/77 (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 germ line mutations that co-segregate 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 germ line mutations in *CDKN2A* or *CDK4*, two known melanoma genes (Fig. 1). Multipoint linkage analysis showed a possible 2.2Mb linkage region on chromosome 5p with maximal LOD-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 non-gene 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 -57bp from ATG translation start site of the *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 (TCF) Elk1 and Elk4 (*2*, *3*). To exclude the possibility that the detected promoter mutation in *TERT* is a common germ line variant, we screened germ line 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 database (data available for 18 individuals, ENSEMBL).

The familial mutation in the *TERT* promoter was in complete allelic linkage with a common polymorphism rs2853669 (G>A) at -246bp 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 -57bp of the *TERT* promoter was increased 1.5 fold and 1.2 fold over the wild type construct in Ma-Mel-86a and HEK293T 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 germ line 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 cancer susceptibility among carriers of the *TERT* mutation (*5*) (see 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 UV-signature mutations in the *TERT* core promoter in 74% (125 of 168) of the cell lines. The mutations were located within a 49bp region starting from -100bp upstream of the ATG start site (Table 1, Fig. 2, fig. S2 and table S4). There were two frequent mutations at -124bp (G>A; C>T on opposite strand) and -146bp (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/-125bp and -138/-139bp were observed at a frequency of 9%. The tandem mutation at positions -138/-139bp could also be generated by a single base mutation at -138bp, as the base change at -139bp 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 (PBMC) 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%CI, 1.3-8.2). Concomitant mutations in *TERT*, *BRAF* and *CDKN2A* were observed in 30% of cell lines compared to the expected frequency of such occurrence of 9% (OR 5.6, 95% 2.4-13.8). 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

The *TERT* promoter mutations were also detected in 25 out of 77 (33%) paraffin embedded primary melanoma tumors (Table 1 and table S5) at -124bp (7/77; 9%) and -146bp (5/77; 7%). Four primary tumors carried the GG>AA tandem mutations at -124/-125bp and eight primary tumors carried the GG>AA tandem mutations at -138/-139bp. Reduced sensitivity to detect mutations in paraffin embedded primary tumors due to 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 -101bp, 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*). Finally, 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.

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**Supplementary Materials**
www.sciencemag.org/cgi/content/full/science.1230062/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S7
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References (*14*–*33*)

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**Table 1.** Most frequent *TERT* core promoter mutations in screened metastatic melanoma cell lines and paraffin embedded primary tumors.

| 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 |

*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.

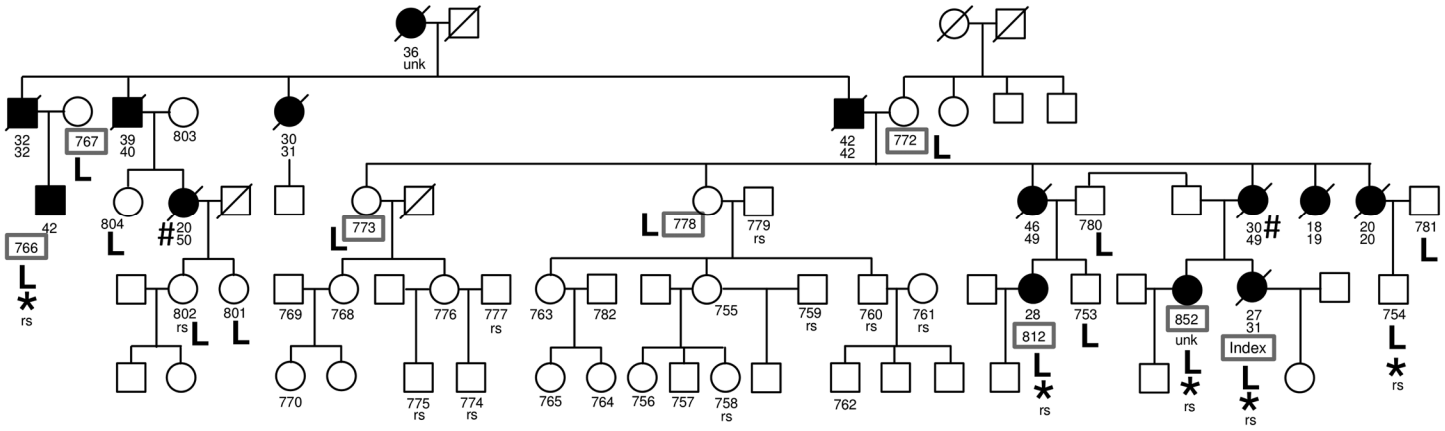


**Fig. 1.** Pedigree of melanoma prone family. Four generations were affected by melanoma (filled symbols; circles represent females and squares represent males). After linkage analysis carried out on 15 family members (L), high throughput sequencing was performed on four affected and four unaffected individuals (boxed samples). A mutation in the *TERT* promoter was identified in all affected members and one unaffected individual (stars). Strikethrough symbols: deceased. Two digit numbers: 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. Index: index patient.

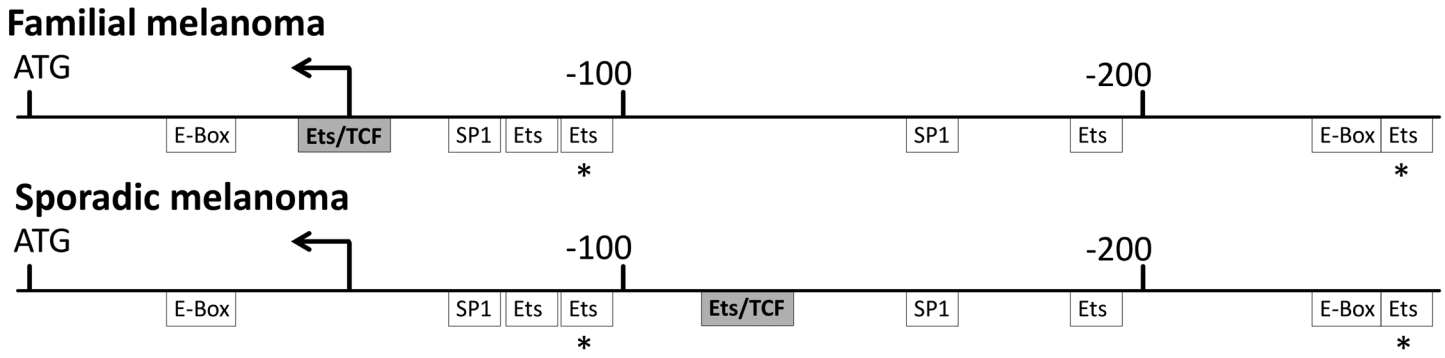


**Fig. 2.** The *TERT* core promoter in melanoma. Mutations creating Ets/TCF binding motifs were found in affected family members (-57bp) immediately next to the transcription start site and in sporadic metastatic melanoma (-124bp to -149bp, sequence details in fig. S2). Binding sites for c-Myc (E-Box),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

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