Genetic Landscape of Somatic Myeloid Mutations in the Presence of Rare *TERT* Variants and their Relation with Myeloid Neoplasia

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Abstract

Context: Germline variants in the *TERT* gene may lead to impaired telomere maintenance implicated in the pathogenesis of myelodysplastic syndromes (MDS) by unknown mechanisms. **Objective:** To explore the clinical and genetic associations of germline *TERT* variants in patients with suspicion of myeloid neoplasm. **Design:** We selected patients tested through our institutional amplicon-based sequencing panel (42 myeloid genes, including exons 2 to 16 of *TERT*, read depth >250X) that carried a *TERT* variant with allele frequency between 35% and 65%. Variants were classified into those with CADD score >20 and <20 to identify the top 1% according to predicted deleteriousness. Results were correlated with MDS diagnosis, clinical and survival information. **Setting:** Division of Hematology (Mayo Clinic). Research protocol approved by Institutional Review Board. **Patients:** Unselected cohort of patients screened with a myeloid mutation gene panel due to clinical suspicion of myeloid neoplasia. **Results:** 55 different *TERT* variants (46 missense, 3 splice sites, 4 synonymous and 2 inframe deletions) were identified in 148 individuals from April, 2016 to November, 2021. None of these patients were diagnosed with a telomere biology disorder and no telomere length measurement was available. All variants were clinically classified as uncertain significance. 17 (12%) patients carried CADD>20 *TERT* variants of which, 59% (10 patients) were diagnosed with MDS. [5 with <5% bone marrow blasts (4 with normal karyotype) and 5 with 5-19% blasts (all with complex karyotype)] This percentage was higher compared to CADD<20 *TERT* carriers (34%, 49 patients, p = 0.048 by Chi-square test). No major differences were found in age at diagnosis (median 70 vs 73 year-old, p = 0.2305), AML-free survival (median 10 vs 15.5 months since diagnosis, p = 0.4674), or percentage of carriers of additional variants in myeloid-related genes (60% vs 64%). The most frequenly mutated genes in CADD>20 carriers were *TP53* (22%), *TET2* (18%) and *RUNX1* (9%) while in CADD<20 carriers were *TET2* (14%), *ASXL1* (11%) and *SRSF2* (9%). *TP53* variants were significantly enriched in CADD>20 *TERT* carriers. (22.7% vs 7.4%, p = 0.0169) **Conclusion:** CADD>20 *TERT* variant carriers were more likely to be associated with a diagnosis of MDS and with somatic *TP53* mutations than CADD<20 carriers.

Background

- *TERT* gene encodes telomerase reverse transcriptase, the main component involved in telomere length maintenance. (1)
- Germline genetic variants in *TERT* may lead to abnormal shortening of telomeres resulting in telomere biology diseases. (TBD) (2)
- TBD patients present higher risk of developing myelodysplastic syndromes (MDS) by a mechanism not yet fully understood. (3)

Objective

To describe clinical outcomes and co-occurring somatic mutations in the presence of *TERT* germline variants in an unselected cohort of patients screened with a myeloid mutation next generation sequencing (NGS) panel due to clinical suspicion of a myeloid neoplasia.

Methods

- This research was approved by Mayo Clinic Institutional Review Board.
- A retrospective chart review was performed to assemble a cohort of unselected individuals within all historical data at Mayo Clinic that had undergone somatic mutational analysis for suspected myeloid neoplasia using our institutional amplicon based NGS panel (42 genes, including exons 2 to 16 of *TERT*, with a read depth of at least 250X).
- *TERT* variants were classified into those with CADD score >20 and <20 in line with previous literature (4) to identify variants within the top 1% according to their predicted deleteriousness.
- We identified patients that received a diagnosis of MDS, clinical and survival information.

Figure 1

- Lollipop graph of all variants identified (CADD>20 in red and <20 in blue) in our cohort (top) and all pathogenic variants described in ClinVar (bottom). Predicted pathogenic variants (CADD>20) were not preferentially found in any functional domain or gene region. Similarly, presence of additional somatic variants was not linked to any specific gene region.

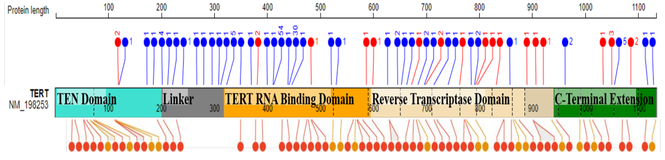


Figure 2

- Patients carrying *TERT* genetic variants with CADD>20 presented higher percentage of MDS. (22.7% vs 7.4%, p = 0.0169)

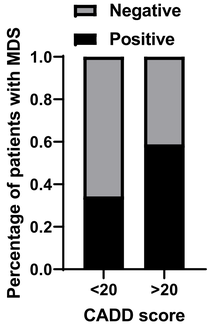


Figure 3

- Percentage of somatic variants in myeloid genes identified in the study.
- Percentages are divided between those found in the presence of a *TERT* variant with CADD <20 (Blue) and >20 (red).
- *TP53* variants co-occurred with higher frequency in patients carrying *TERT* germline variants with CADD >20 compared to carriers of CADD<20 variants.

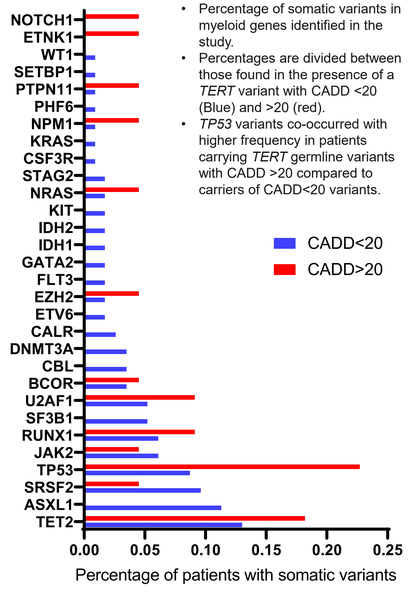


Table 1

cDNA Change (NM_198253)	Predicted Protein Impact	CADD	Number of patients	MAF <0.001 in any gnomAD population	Co-occurrence with somatic pathogenic variants	TERT domain
c.2495C>T	p.Pro832Leu	25.3	1	Yes	Yes	Reverse Transcriptase Domain
c.2720T>A	p.Phe907Tyr	24.6	1	Yes	Yes	Reverse Transcriptase Domain
c.3150G>C	p.Lys1050Asn	24.5	3	No	Yes	C-Terminal ExTEN Domain
c.1795C>T	p.Arg599Trp	24.1	1	Yes	Yes	Reverse Transcriptase Domain
c.2092C>T	p.Arg698Trp	24	1	Yes	Yes	Reverse Transcriptase Domain
c.1142G>C	p.Arg381Pro	23.7	2	Yes	Yes	TERT RNA Binding Domain
c.2668G>A	p.Gly890Ser	23.7	1	No	No	Reverse Transcriptase Domain
c.2762A>C	p.Gln921Pro	23.3	1	Yes	Yes	Reverse Transcriptase Domain
c.2014C>T	p.Arg672Cys	22.8	1	Yes	Yes	Reverse Transcriptase Domain
c.1760T>C	p.Ile587Thr	22.5	1	Yes	Yes	TERT RNA Binding Domain
c.2386T>C	p.Ser796Pro	22.4	1	Yes	Yes	Reverse Transcriptase Domain
c.3100C>T	p.Arg1034Cys	21.2	1	Yes	Yes	C-Terminal ExTEN Domain
c.3257G>A	p.Arg1086His	21	2	Yes	No	C-Terminal ExTEN Domain
c.3352G>A	p.Ala1118Thr	18.52	1	Yes	No	C-Terminal ExTEN Domain
c.2051A>G	p.Asp684Gly	17.99	2	No	No	Reverse Transcriptase Domain
c.2884C>T	p.Arg962Cys	17.88	2	Yes	No	C-Terminal ExTEN Domainsion
c.358C>T	p.Arg120Cys	17.66	1	Yes	Yes	TEN Domain
c.1234C>T	p.His412Tyr	17.59	48	No	No	TERT RNA Binding Domain
c.2221G>A	p.Val741Met	17.36	1	Yes	Yes	Reverse Transcriptase Domain
c.355G>C	p.Val119Leu	16.08	1	No	No	TEN Domain
c.1562G>A	p.Arg521His	15.35	1	No	No	TERT RNA Binding Domain
c.3164C>T	p.Ser1055Leu	15.23	5	Yes	Yes	C-Terminal ExTEN Domainsion
c.1957C>T	p.Arg653Cys	14.87	1	Yes	Yes	Reverse Transcriptase Domain
c.2371G>A	p.Val791Ile	14.81	2	Yes	No	Reverse Transcriptase Domain
c.1880C>G	p.Pro627Arg	14.14	1	Yes	Yes	Reverse Transcriptase Domain
c.1232C>T	p.Thr411Met	13.36	1	No	No	TERT RNA Binding Domain
c.320_328del	p.Ala107_Gly109del	12.8	1	Yes	Yes	TEN Domain
c.1936C>T	p.Arg646Cys	12.35	1	Yes	No	Reverse Transcriptase Domain
c.2573G>A	p.Arg858Gln	12	1	Yes	Yes	Reverse Transcriptase Domain
c.1046C>T	p.Ser349Phe	11.73	1	Yes	Yes	TERT RNA Binding Domain
c.2354C>G	p.Pro785Arg	11.33	1	Yes	Yes	Reverse Transcriptase Domain
c.2087G>C	p.Arg696Pro	11.31	1	No	No	Reverse Transcriptase Domain
c.1108C>T	p.Pro370Ser	10.45	1	Yes	Yes	TERT RNA Binding Domain
c.1937G>A	p.Arg646His	9.374	2	Yes	No	Reverse Transcriptase Domain
c.554G>T	p.Arg185Leu	7.843	1	Yes	Yes	TEN Domain
c.2287-5G>A	N/A	7.199	1	Yes	Yes	N/A
c.604G>A	p.Ala202Thr	7.162	1	No	No	Linker
c.718C>T	p.Arg240Cys	7.12	1	No	No	Linker
c.634G>A	p.Val212Ile	5.809	1	No	No	Linker
c.519G>T	p.Pro173=	5.355	1	No	No	TEN Domain
c.2141C>T	p.Thr714Met	5.293	1	Yes	Yes	Reverse Transcriptase Domain
c.1315G>C	p.Glu439Gln	3.862	1	Yes	Yes	TERT RNA Binding Domain
c.935G>A	p.Arg312Gln	3.312	1	Yes	Yes	Linker
c.2582+7C>T	N/A	2.938	1	Yes	Yes	Reverse Transcriptase Domain
c.887A>C	p.His296Pro	2.038	1	Yes	No	Linker
c.969G>A	p.Pro323=	1.176	4	No	Yes	TERT RNA Binding Domain
c.3296-8C>A	N/A	0.959	1	Yes	No	N/A
c.1200C>T	p.His400=	0.848	1	Yes	Yes	TERT RNA Binding Domain
c.663G>T	p.Ala221=	0.761	1	Yes	Yes	Linker
c.838G>A	p.Glu280Lys	0.126	1	No	No	Linker
c.800A>G	p.Asp267Gly	0.098	1	No	Yes	Linker
c.1336C>A	p.Arg446Ser	0.026	1	No	No	TERT RNA Binding Domain
c.922C>G	p.Pro308Ala	0.025	1	Yes	No	Linker

Results

- We identified 55 different *TERT* variants in 148 individuals tested from April of 2016 to November of 2021 (Fig. 1, Table 1).
- Of the 148 total patients, 17 (12%) carried *TERT* variants with CADD>20 that were distributed thorough all the gene domains with no clustering or presence of hotspots (Fig.1).
- All variants were considered germline since their allele frequency was near 50%, although no germline confirmation was performed. All variants were clinically classified as uncertain significance according to the American College of Medical Genetics guidelines.
- None of the patients identified were diagnosed with a bona fide TBD and no clinical information about telomere length status was available.
- The percentage of patients presenting additional somatic variants in other myeloid-related genes was similar between CADD<20 and CADD>20 *TERT* variant carriers (60% vs 64%).
- 59% (10 patients) of CADD>20 variants carriers were diagnosed with MDS: 5 patients with <5% bone marrow blasts (4 with normal karyotype) and 5 patients with 5-19% blasts, all with complex karyotype. This percentage was significantly higher compared to CADD<20 variant carriers (34%, 49 patients, p = 0.048 by Chi-square test, Fig. 2).
- No major differences were found between the two groups in terms of age at diagnosis (median 70 vs 73 years old, p = 0.2305) or AML-free survival (median 10 vs 15.5 months since diagnosis, p = 0.4674).
- Our data showed that TP53 pathogenic variants were significantly enriched in CADD>20 *TERT* variant carriers compared to CADD<20 carriers. (22.7% vs 7.4%, p = 0.0169, Fig. 3).
- The two most frequently mutated genes in CADD>20 carriers were *TP53* (22%), *TET2* (18%), *RUNX1* (9%), *U2AF1* (9%) and *BCOR* (9%) while in the CADD<20 carriers were *TET2* (14%), *ASXL1* (11%), *SRSF2* (9%), *TP53* (7%) and *JAK2* (6%).

Conclusion

- Carriers of *TERT* variants with CADD score of >20 were more likely to be associated with a diagnosis of MDS
- Somatic *TP53* mutations were enriched in carriers with *TERT* variants with CADD score >20.
- Functional validation and prospective studies are currently underway to validate these results.

Acknowledge

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