SUPPLEMENTARY APPENDIX:

Supplemental Methods

Cohorts

Families followed for Li Fraumeni Syndrome (LFS) care at one of four academic institutions were reviewed for inclusion in the LFS cohorts (**Table S1, Table S5**). Germline *TP53* variants were identified in four large series of prostate cancer patients from academic or reference laboratories (AL/RL): University of Washington or Seattle Cancer Care Alliance (UW/SCCA)[1], Invitae[2], Memorial Sloan Kettering IMPACT Study (MSKCC-IMPACT)[3], and The Cancer Genome Atlas (TCGA)[4] (**Table S5**). The UW/SCCA cohort was a consecutive series of 831 prostate cancer patients who underwent clinical cancer genetic testing (GT) at University of Washington and Seattle Cancer Care Alliance between 2014 and 2020. Of 831 patients, 497 had advanced prostate cancer and underwent UW-OncoPlex targeted panel Next Generation sequencing (NGS) with germline follow-up, and 334 underwent germline GT via traditional pre-and post-test genetic counseling[1]. The Invitae series was 3,329 men with prostate cancer who underwent germline genetic testing at Invitae between 2013 and 2018[2]. The MSKCC-IMPACT cohort included 2191 prostate cancer patients who underwent combined germline-tumor testing[3]. Finally, The Cancer Genome Atlas prostate cancer patient cohort (n=499) was patients with primary prostate cancer who underwent research combined germline-tumor testing[4].

TP53 Variant Review

All variants in both the LFS and prostate cancer cohorts were referenced with ClinVar, and curated by a molecular pathologist and a cancer genetics trained medical oncologist based on *TP53*-specific American College of Medical Genetics (ACMG) guidelines[5] (Pritchard, Maxwell, **Table S6**). *TP53* variants were classified based on *TP53*-specific American College of Medical Genetics (ACMG) guidelines applying the following criteria: 1) frequency in gnomAD database of patients without cancer (absent=PM2; $\geq$0.1%=BA1; 0.03-0.1%=BS1) and FLOSSIES ($\geq$2=BS2); 2)

Observations in LFS families (PS4 with evidence modifications), de novo status (PS2/PM6), and co-segregation (PP1/BS4); 3) Mutational hotspot (codons 175, 273, 245, 248, 282, 249) or somatic mutation count (n>10) in COSMIC, combined TCGA/ICGC/GENIE somatic counts, or IARC database (PM1); 4) Published functional data (PS3/BS3 flow chart); 5); Missense variant occurring at an amino acid position that has other pathogenic missense changes (PS1;PM5); 6) prediction of splice site disruption or premature protein truncation (PVS1); 7) in silico prediction of splice site disruption or functional impact using BayesDel and AlignGVGD (PP3/BP4/BP7).

As per guidelines, the following ACMG criteria were excluded from classification: PM3 (recessive conditions, cis/trans data), PM4/BP3 (in-frame indels), PP4 (phenotype specificity), PP5/BP6 (other databases), BP1 (missense as not causative of disease), BP5 (alternate molecular basis).

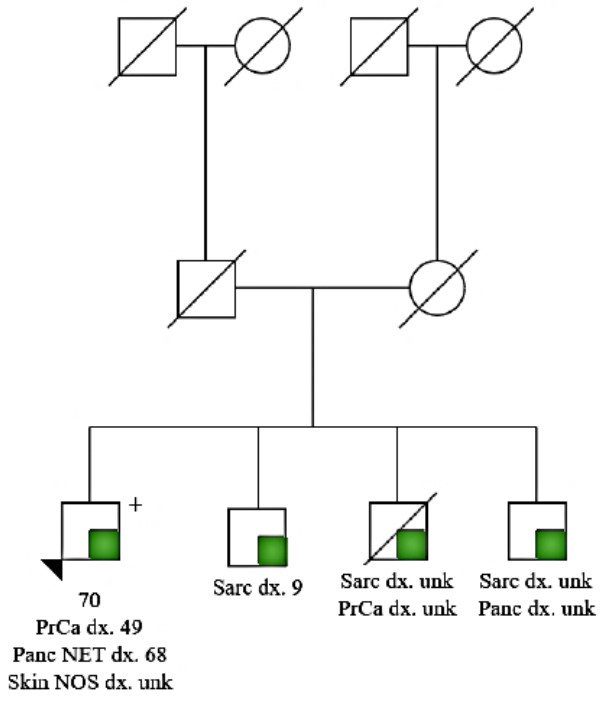
After ACMG criteria were assigned, variants were classified as likely pathogenic or pathogenic if they met one of the following criteria: a) PVS1 + ≥1 Strong OR ≥1 Moderate OR 1 Moderate/1 Supporting OR ≥2 Supporting); b) ≥2 Strong; c) 1 Strong + (≥1 Moderate OR ≥2 Supporting); d) ≥3 Moderate; e) 2 Moderate and ≥2 Supporting; f) 1 Moderate and ≥4 Supporting.

In the LFS cohort, 31 adult males with prostate cancer had 22 *TP53* mutations, all of which were classified as LP/P by the above criteria. In the PrCa cohort, 36 adult males had 18 *TP53* mutations, with 14 classified as LP/P by the above criteria. In addition, four addition mutations - p.D49H, p.I245V, p.R283C and p.Q74Vfs*48 - were reclassified as likely pathogenic alleles and included in this analysis. *TP53* p.D49H is non-functional in a yeast assay. *TP53* p.I254V is a change at a residue where other alterations have been determined to be pathogenic[6]. p.R283C was reclassified as likely pathogenic for attenuated LFS based on review of segregation data in a large family[7], observation of this variant in a second family that included a proband with leiomyosarcoma with associated LOH in tumor, and significant

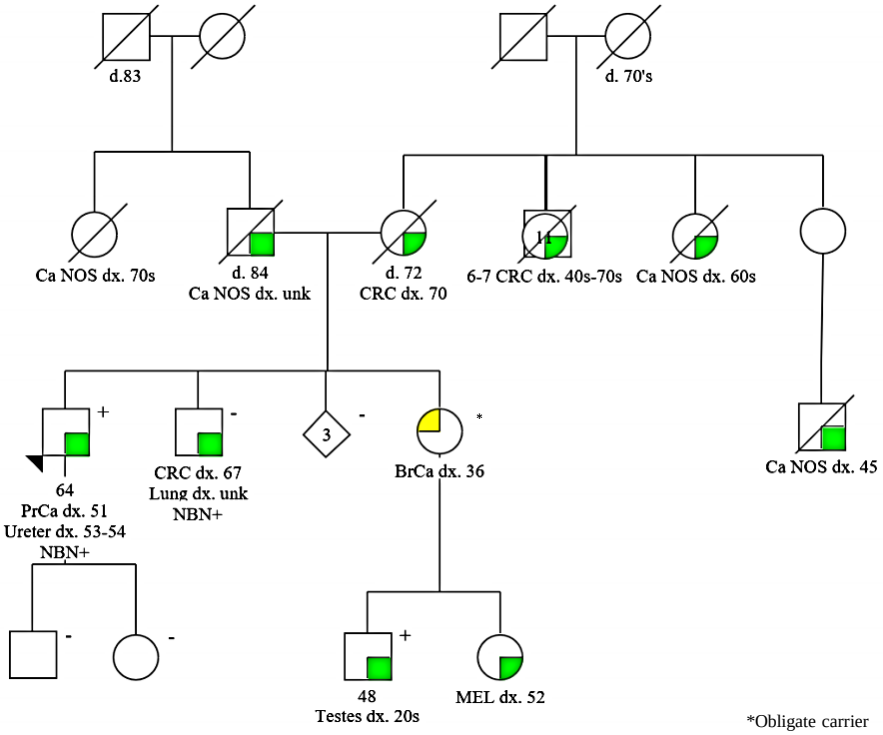
enrichment for this variant in a pan-cancer TCGA analysis[8]. In the pan-cancer analysis, carriage of p.R283C confers a relative risk for cancer of 6.4 compared to gnoMAD non-cancer (95% CI 2.6 to 15.8, *p*=0.0001) and relative risk of 13.0 compared to gnoMAD controls (95% CI 4.0 to 42.3, *p*=<0.0001. Finally, *TP53* p.G374Vfs*48 is a truncating mutation that is predicted to replace the last 20 amino acids of the p53 protein with 47 different amino acids residues, creating a new downstream translational stop signal that extends the length of the protein by 27 amino acids. This is predicted to disrupt the C-terminal regulatory domain (residues 363-393) of p53 that is necessary for full TP53 DNA binding and transactivation activity[9].

Variants in gnomAD were manually curated as P/LP variants as above. Calculated expected frequencies accounted for the number of individuals represented for each allele. Variant classification of *TP53* variants in gnomAD is included in **Table S7.**

a. AL-1.6
g*TP53* exon 7-9 deletion



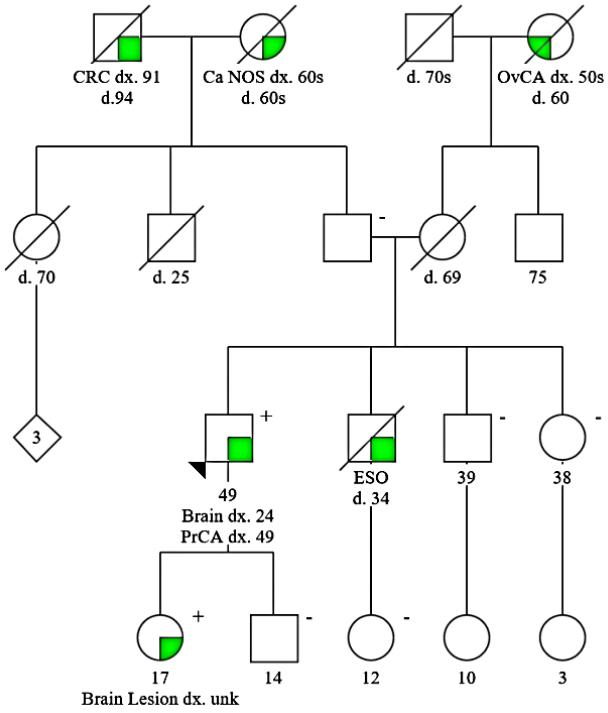
b. LFS-12
g*TP53* c.320_327del; p.Y107Sfs*16



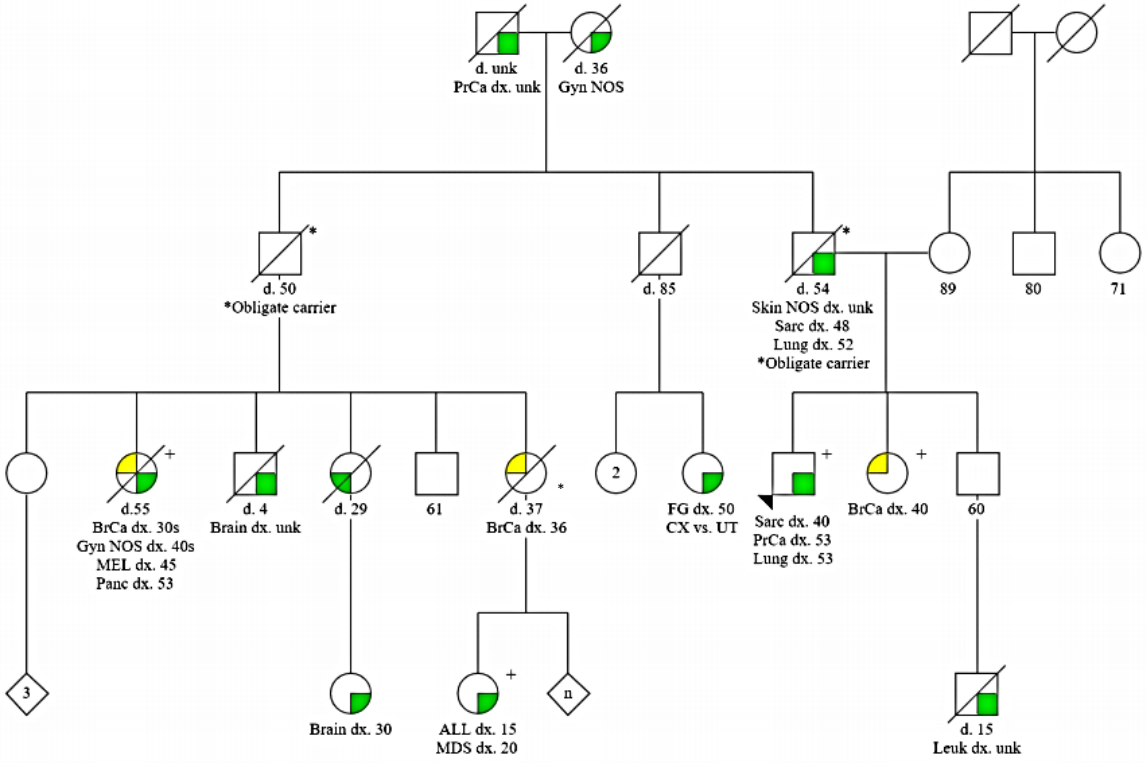
*Obligate carrier

Supplementary Figure 1, cont

c. LFS-28
g*TP53* c.329G>T; p.R110L



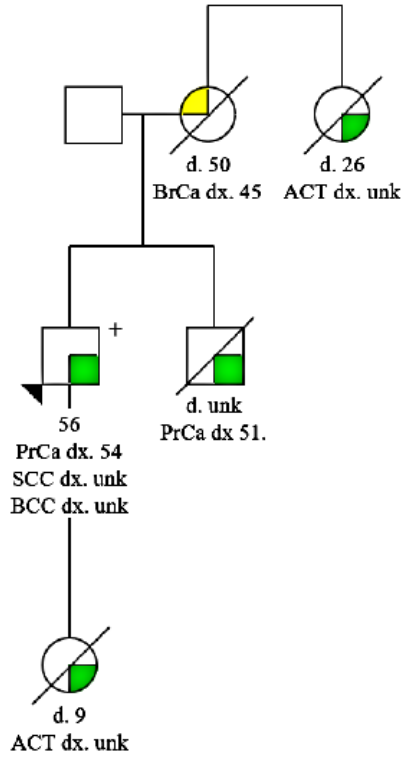
d. LFS-7
g*TP53* c.389T>A; p.L130H



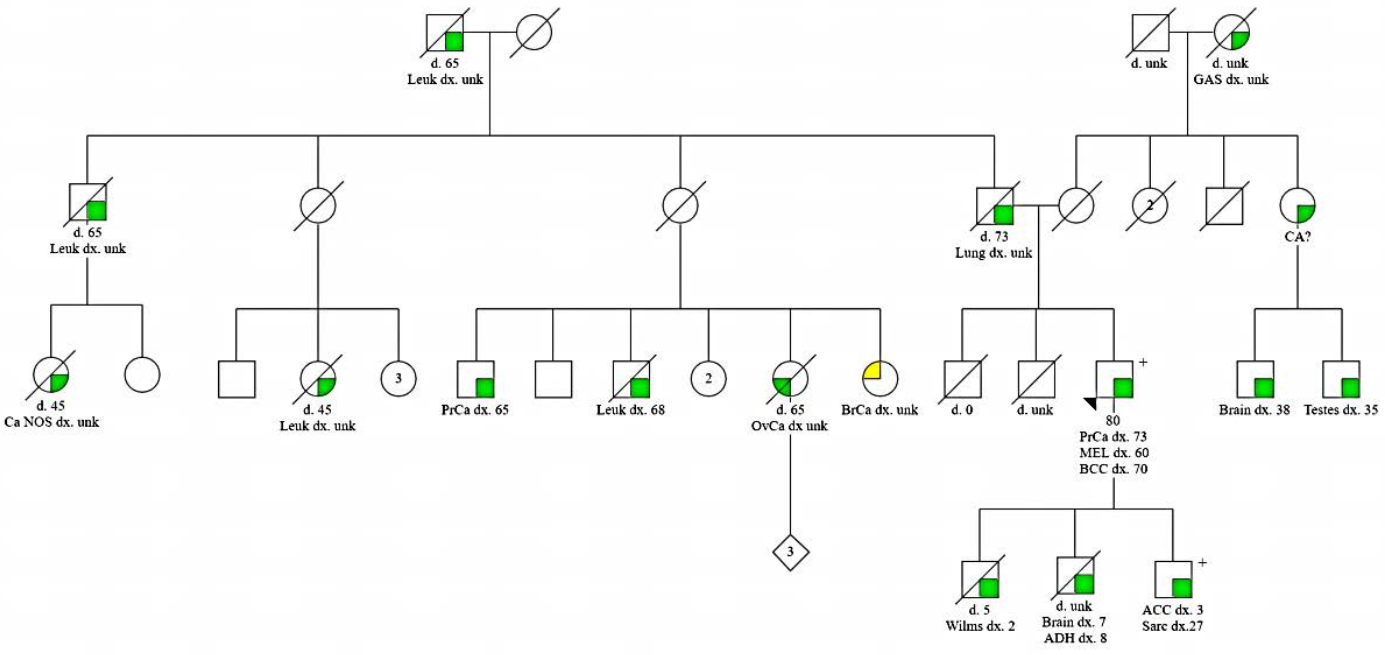
*Obligate carrier

Supplementary Figure 1, cont

e. AL-1.7
g*TP53* c.400T>C; p.F134L

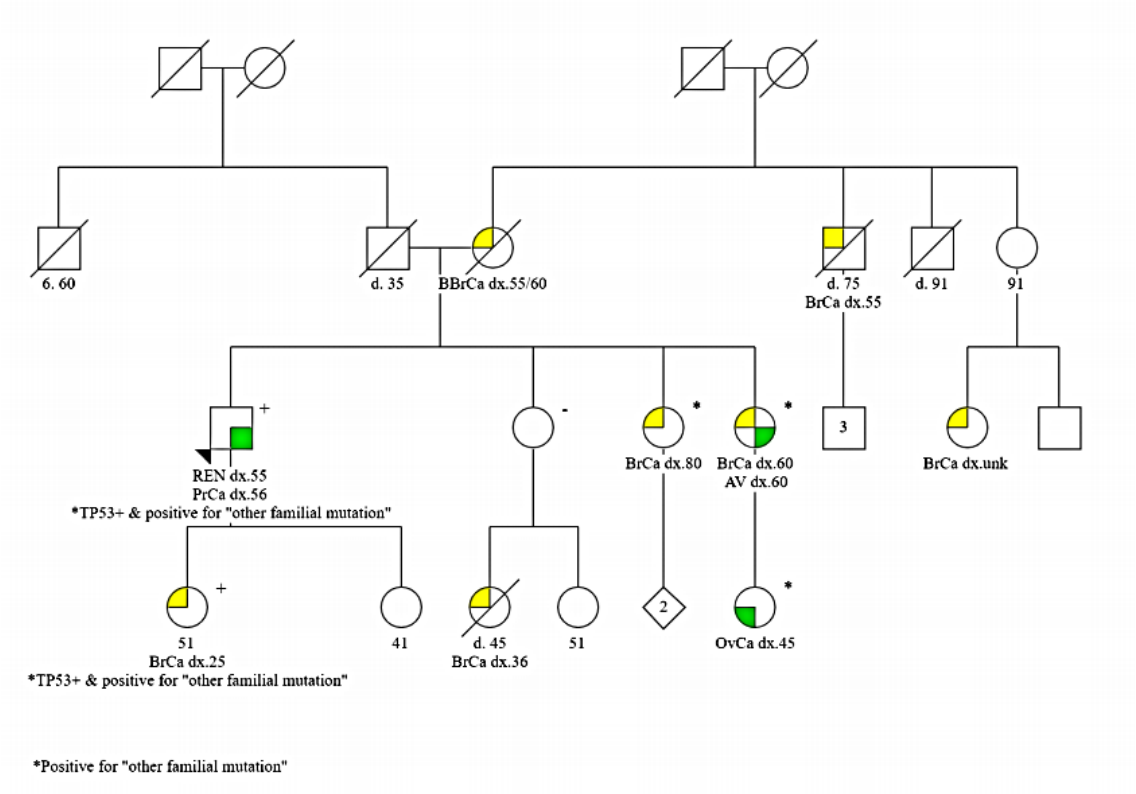


f. LFS-2
g*TP53* c.403_405delTGCinsGG; p.C135Gfs*35

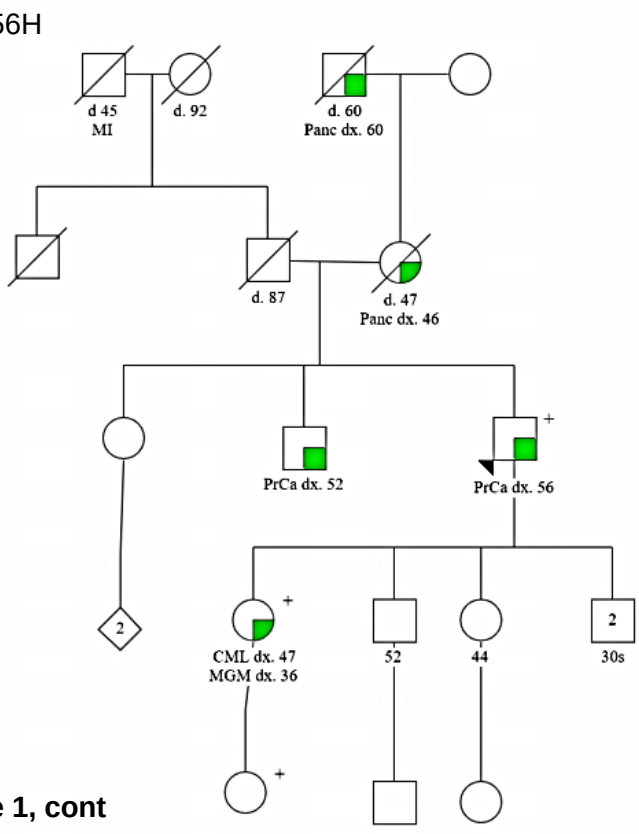


Supplementary Figure 1, cont

g. LFS-6
g*TP53* c.422G>A; p.C141Y

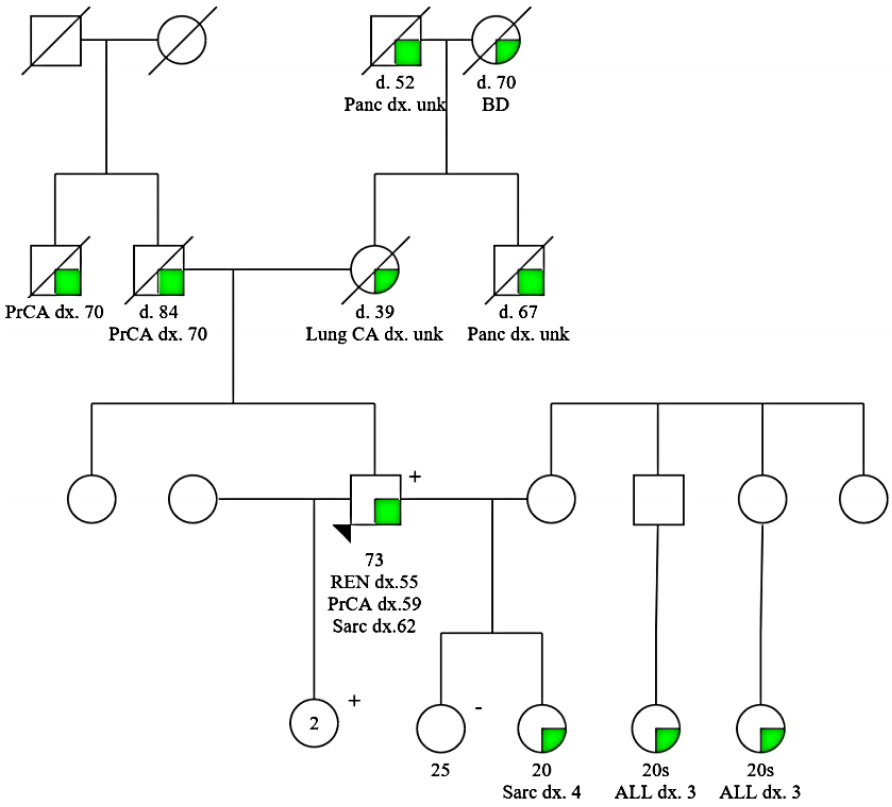


h. LFS-3
g*TP53* c.467G>A; p.R156H

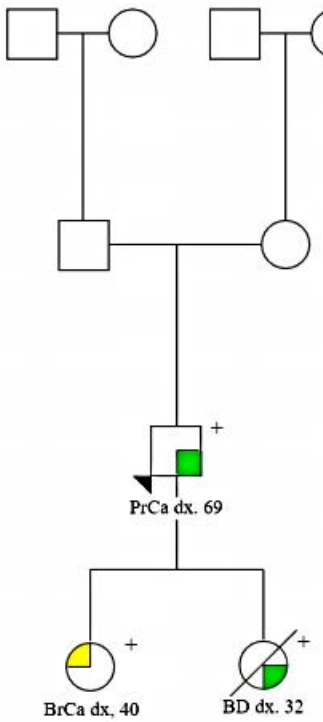


Supplementary Figure 1, cont

i. LFS-21
g*TP53* c.473G>A; p.R158H

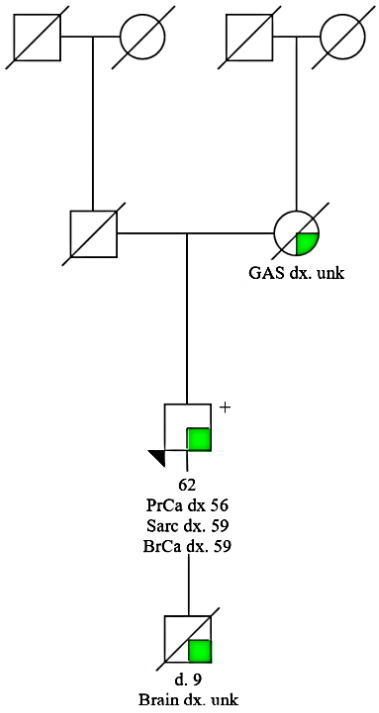


j. LFS-17
g*TP53* c.473G>A; p.R158H

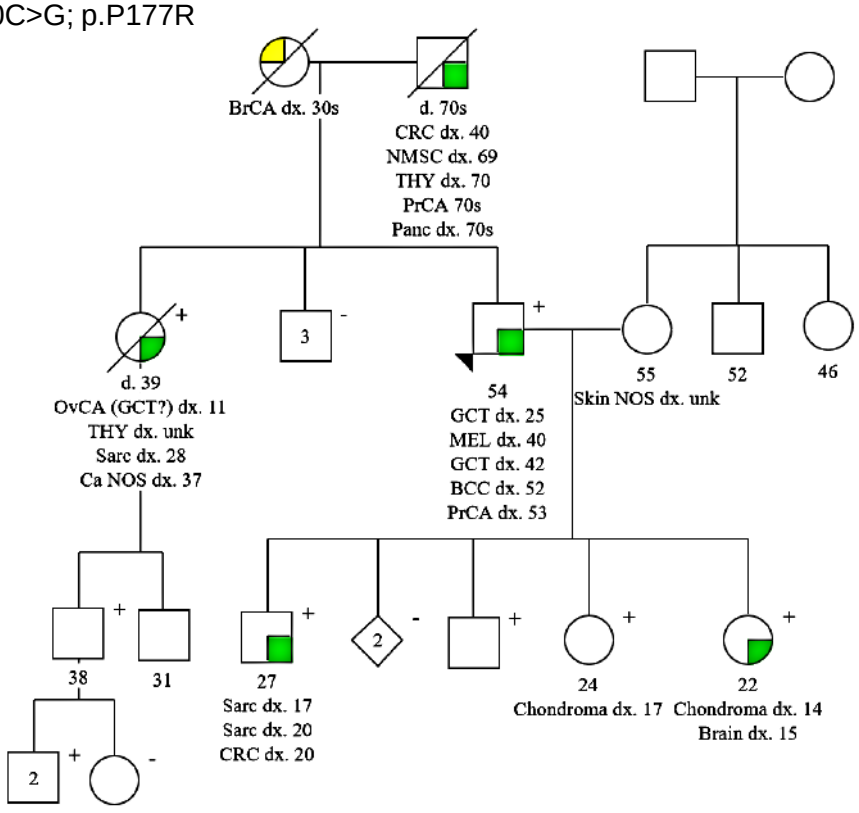


Supplementary Figure 1, cont

k. LFS-22
g*TP53* c.524G>A; p.R175H

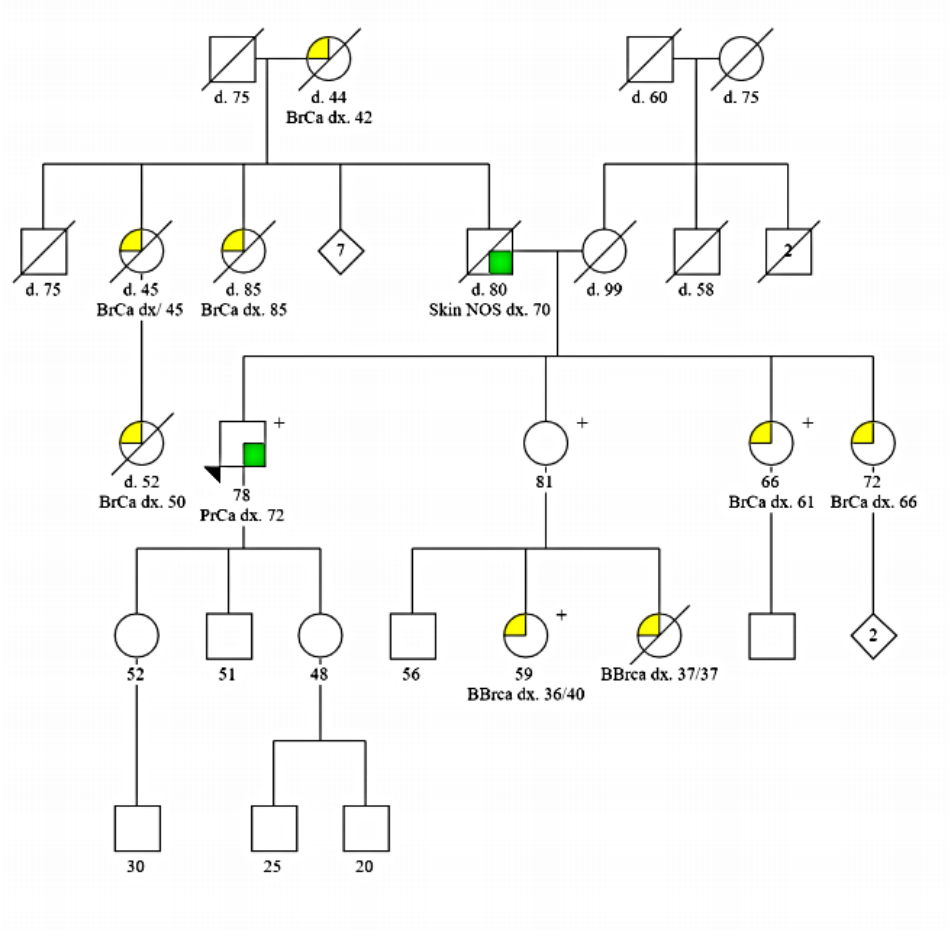


l. LFS-13
g*TP53* c.530C>G; p.P177R

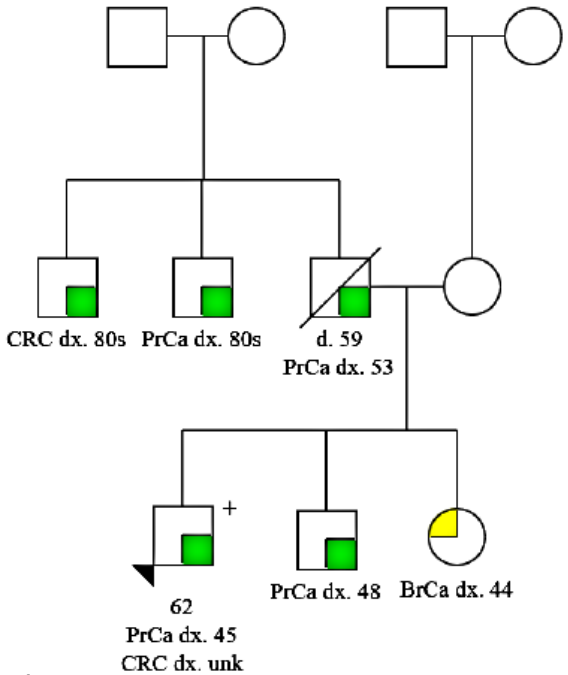


Supplementary Figure 1, cont

m. LFS-1
g*TP53* c.541C>T: p.R181C

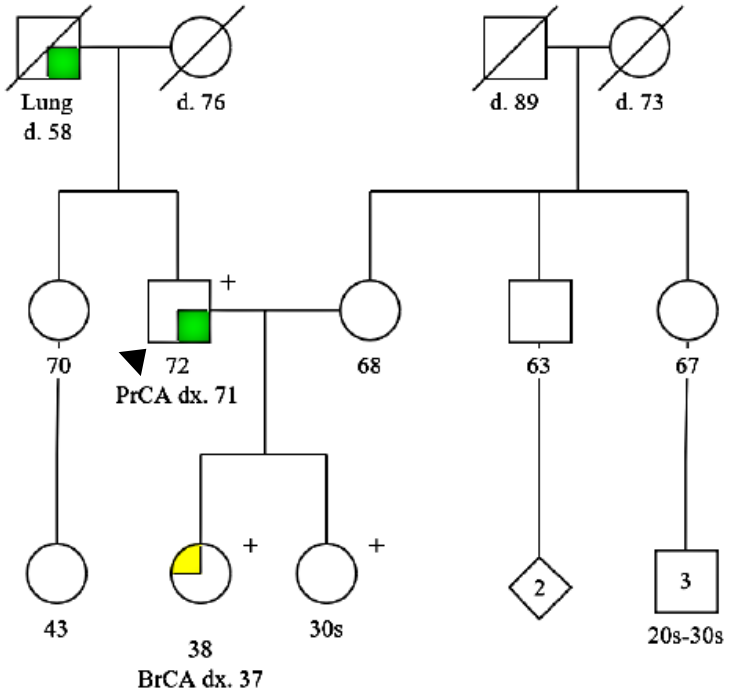


n. AL-1.1
g*TP53* c.541C>T; p.R181C

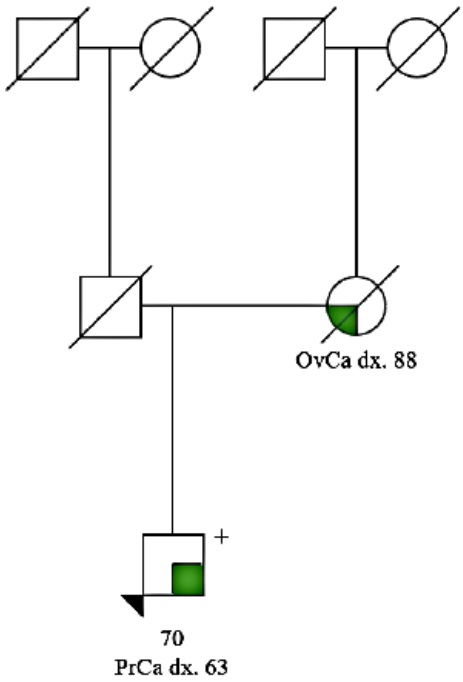


Supplementary Figure 1, cont

o. LFS-25
g*TP53* c.542G>A; p.R181H

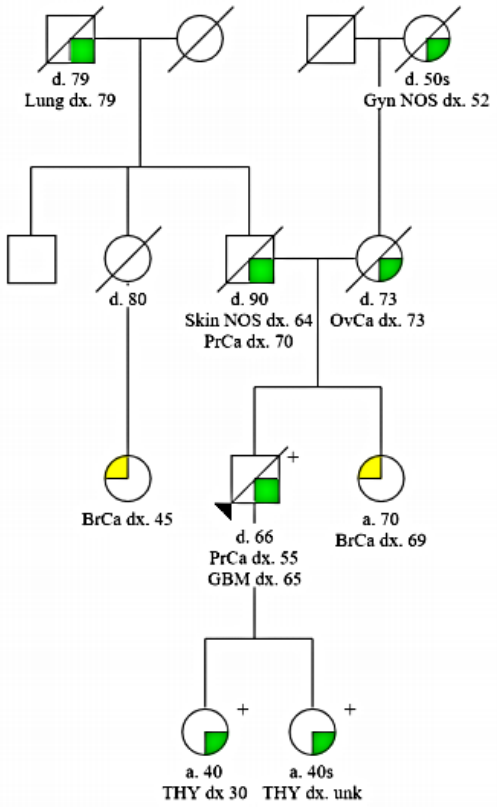


p. AL-1.2
g*TP53* c.542G>A; p.R181H

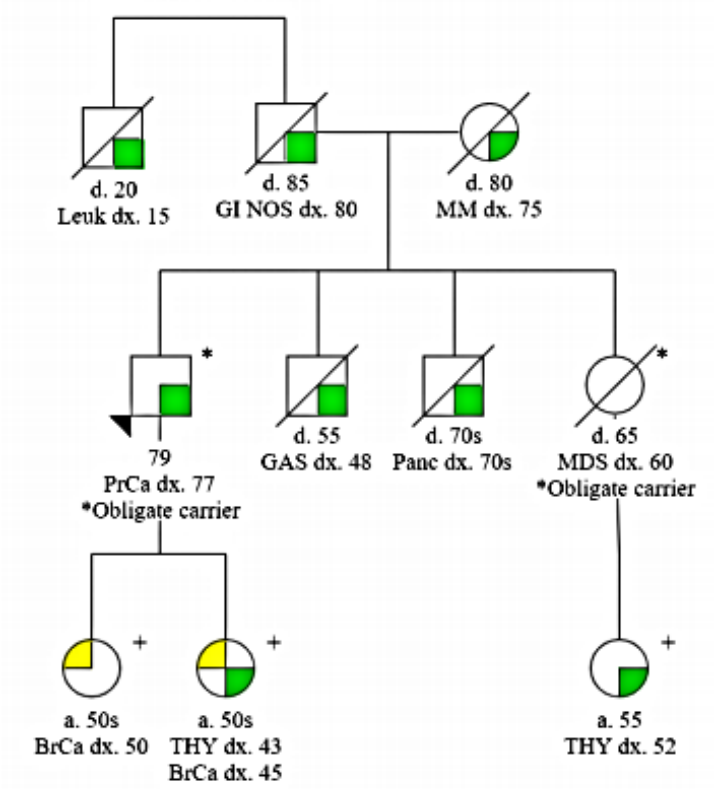


Supplementary Figure 1, cont

q. LFS-9
g*TP53* c.542G>A; p.R181H



r. LFS-10
g*TP53* c.542G>A; p.R181H



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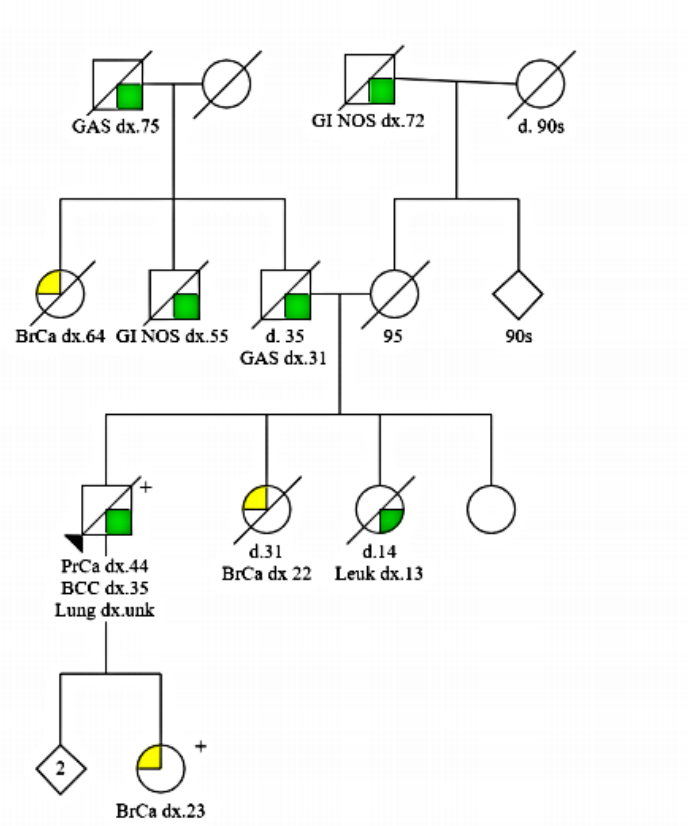
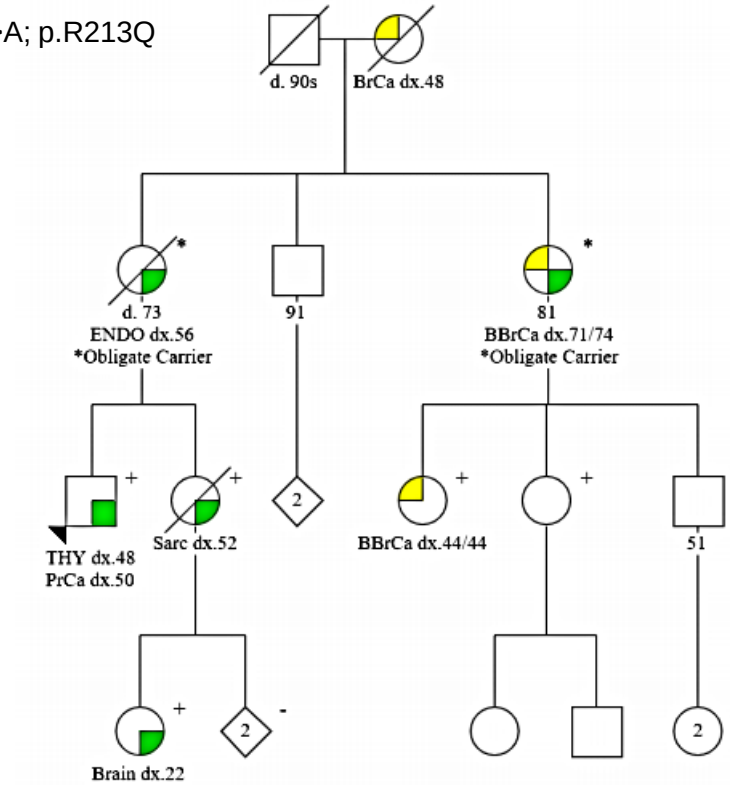
s. LFS-4
g*TP53* c.586C>T; p.R196*

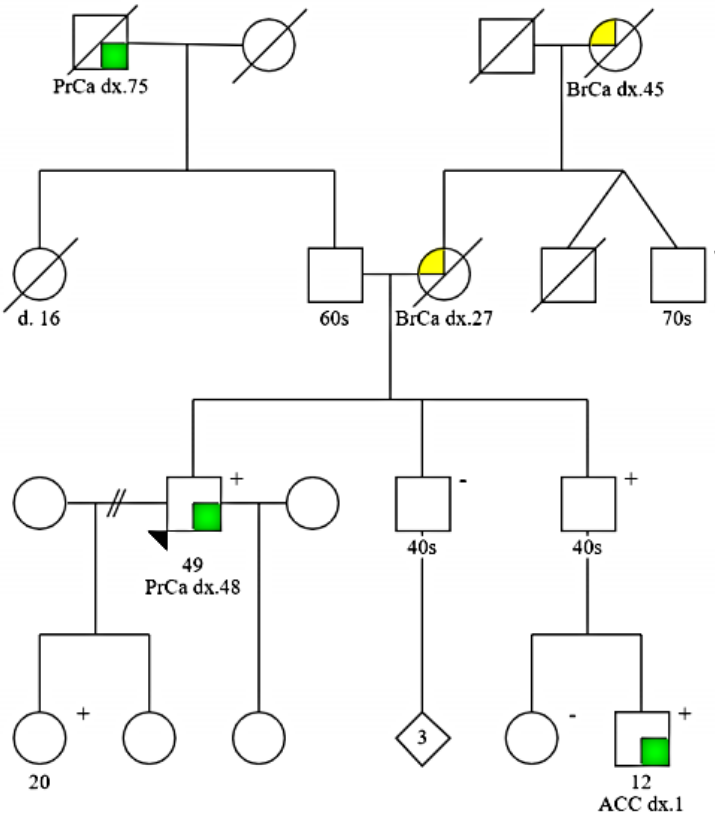


t. LFS-5
g*TP53* c.638G>A; p.R213Q

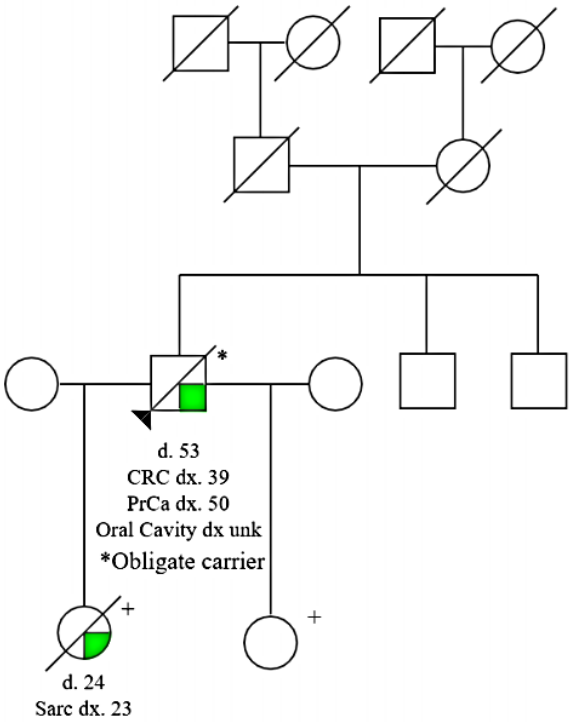


Supplementary Figure 1, cont

u. LFS-8
g*TP53* c.638G>C; p.R213P

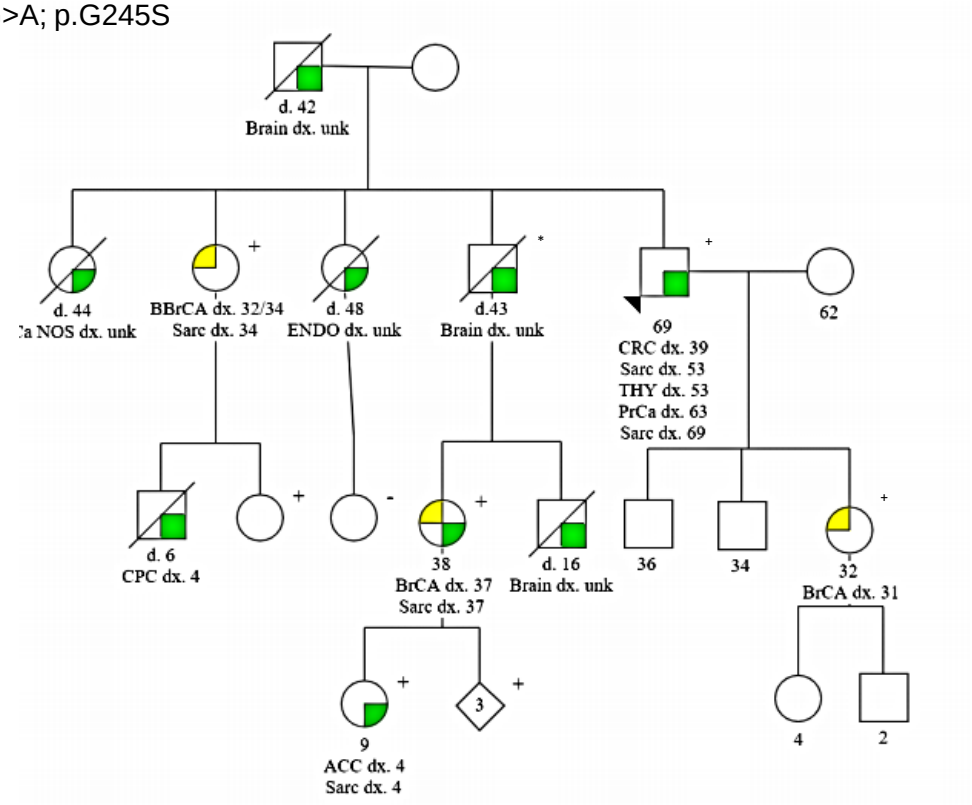


v. LFS-30
c.643G>A; p.S215G

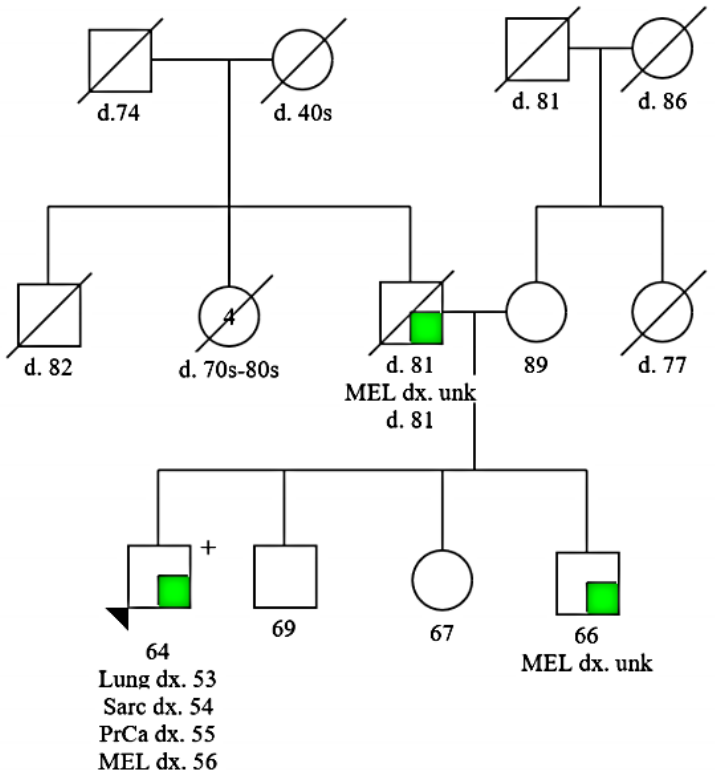


Supplementary Figure 1, cont

w. LFS-23
g*TP53* c.733G>A; p.G245S

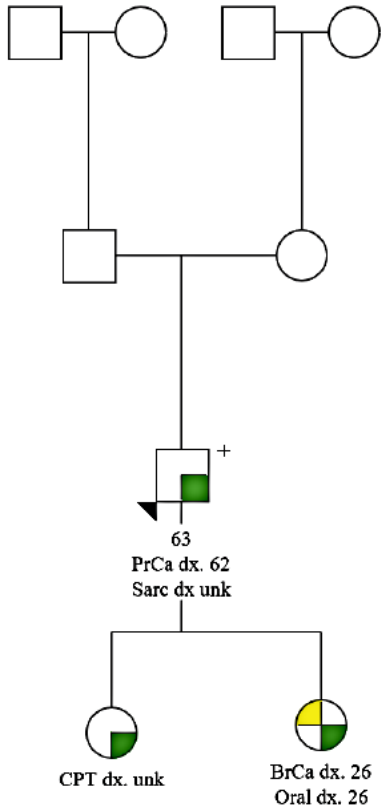


x. LFS-20
g*TP53* c.743G>A; p.R248Q

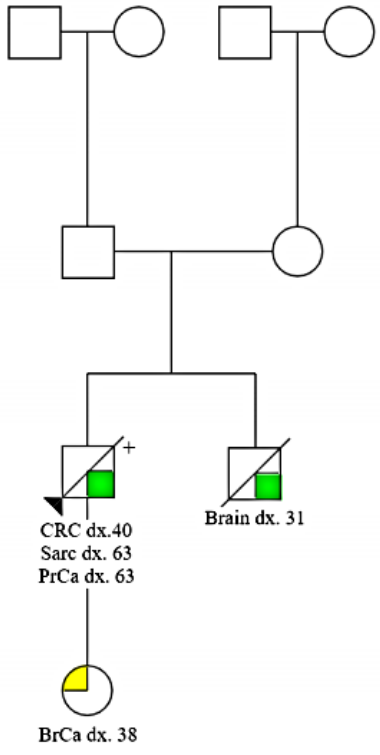


Supplementary Figure 1, cont

y. AL-1.3
g*TP53* c.743G>A; p.R248Q

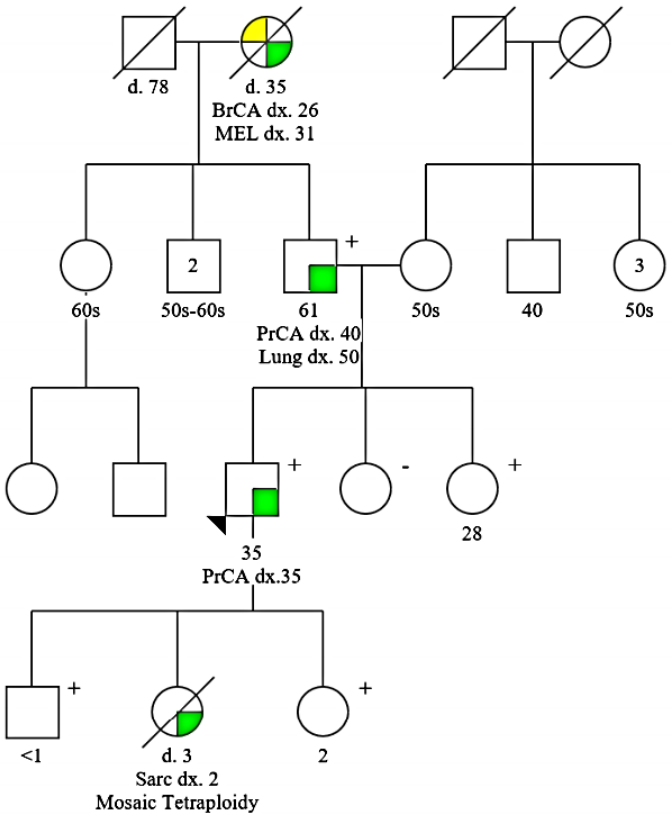


z. LFS-15
g*TP53* c.743G>A; p.R248Q

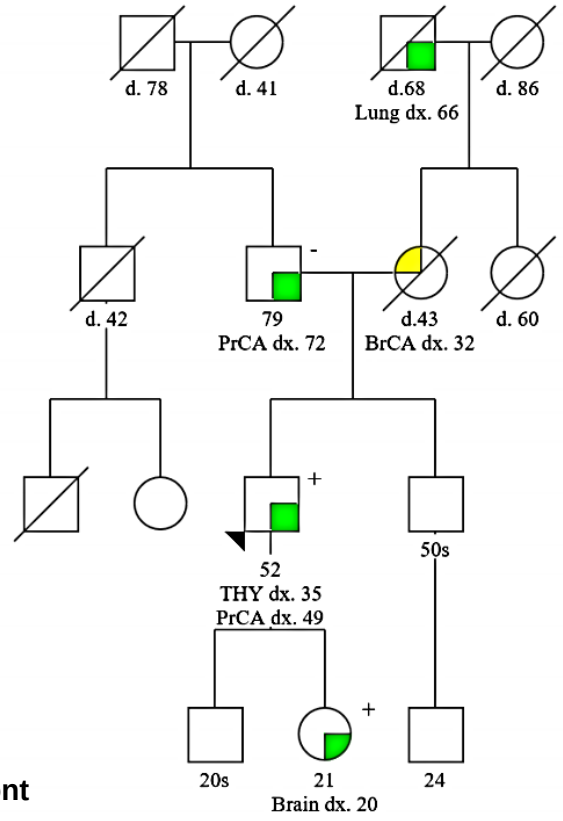


Supplementary Figure 1, cont

aa. LFS-26
g*TP53* c.743G>A; p.R248Q

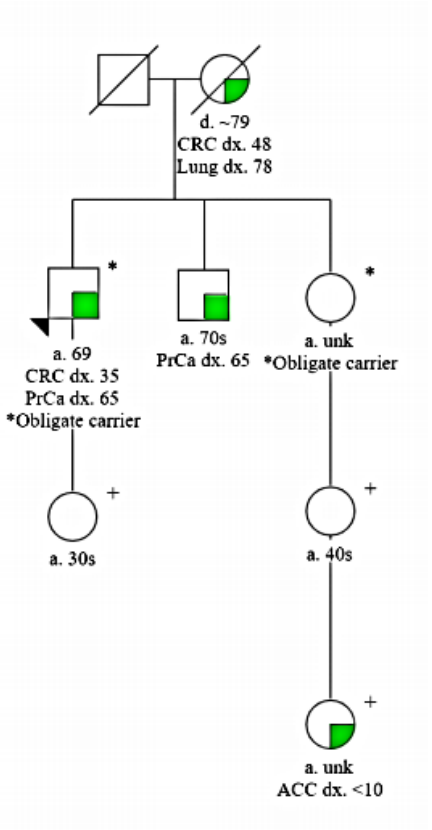


bb. LFS-29
g*TP53* c.794T>A; p.L265Q

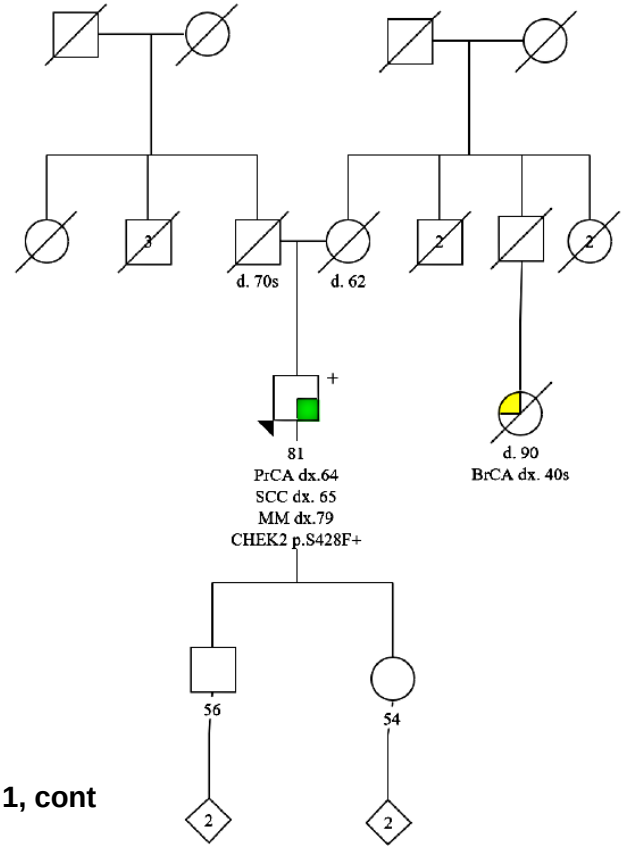


Supplementary Figure 1, cont

cc. LFS-31
c.800G>A; p.R267Q

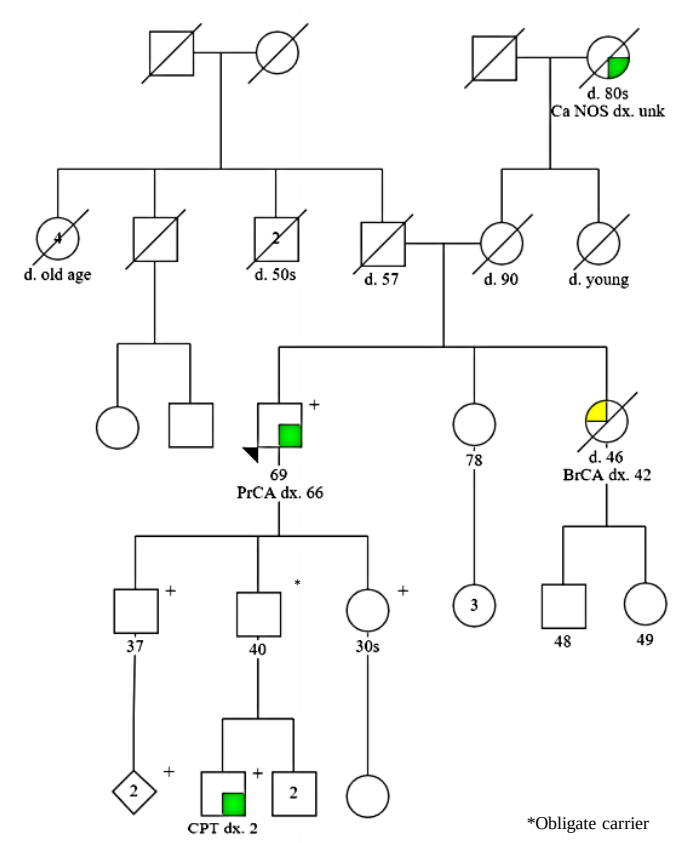


dd. LFS-27
g*TP53* c.1000G>C; p.G334R

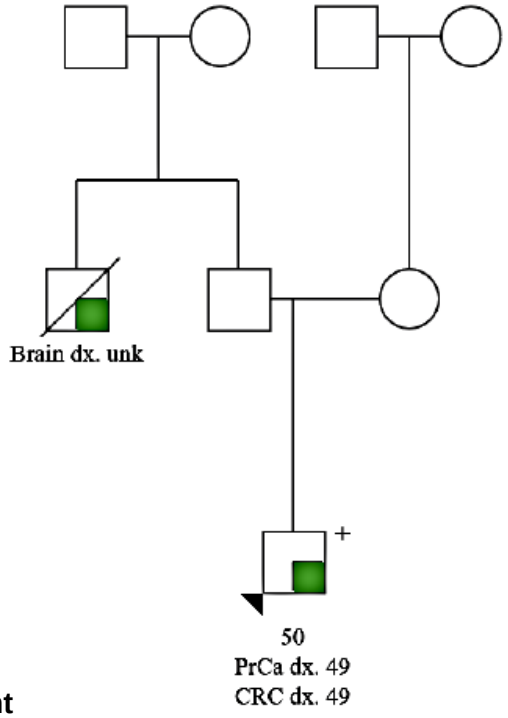


Supplementary Figure 1, cont

ee. LFS-24
g*TP53* c.1010G>A; p.R337H

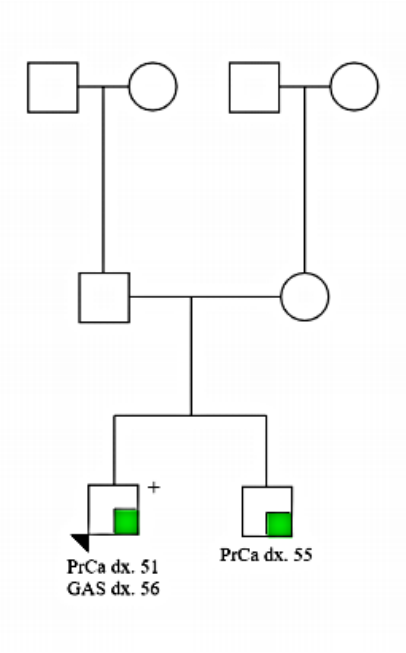


ff. AL-1.4
g*TP53* c.1010G>A; p.R337H

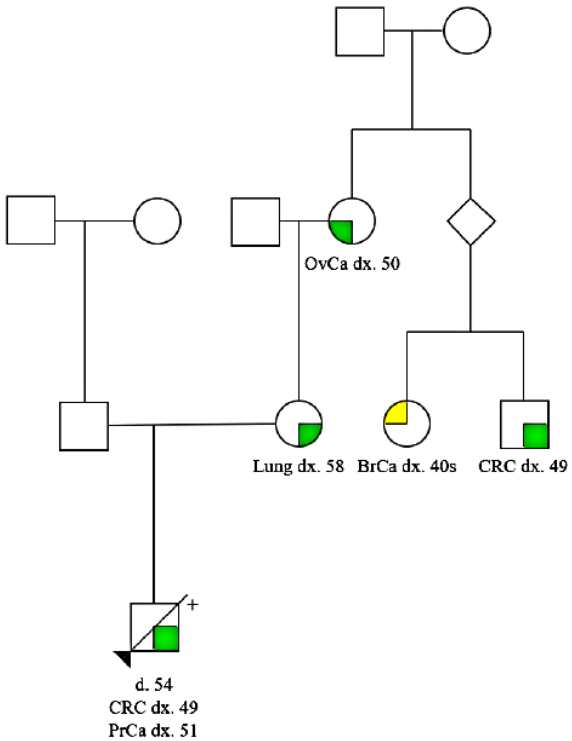


Supplementary Figure 1, cont

gg. LFS-18
g*TP53* c.1010G>A; p.R337H

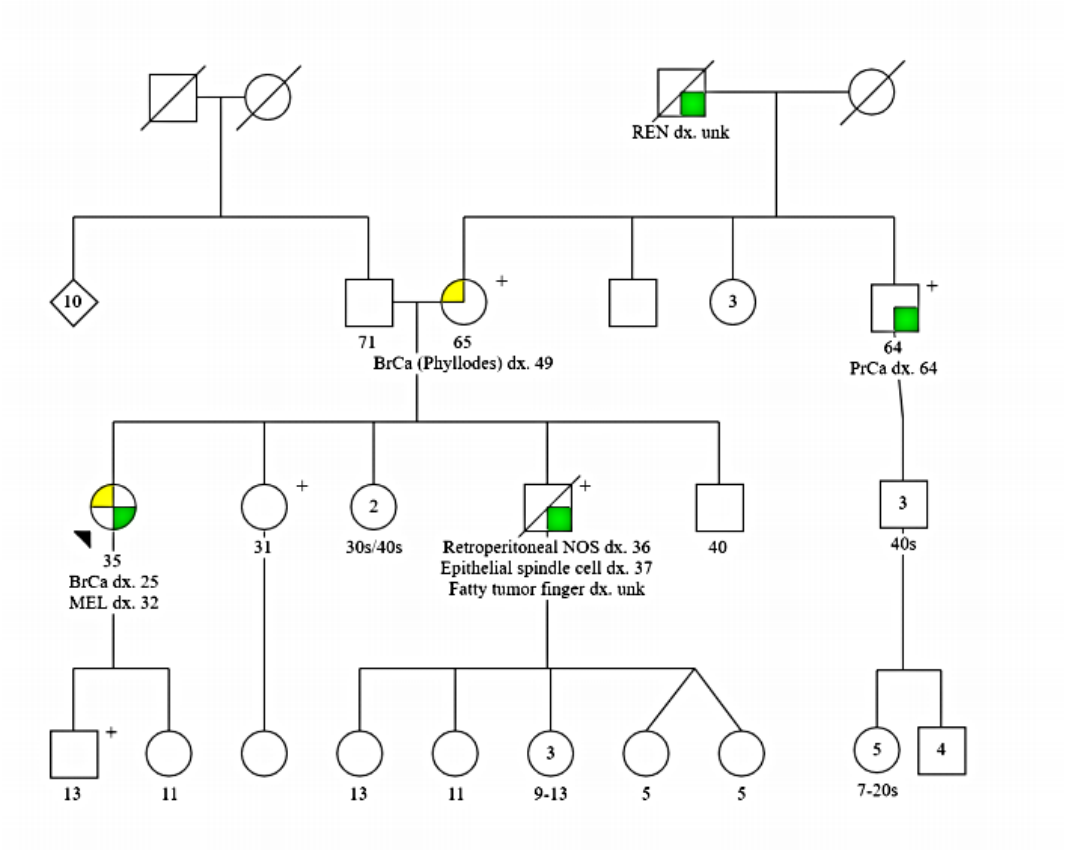


hh. AL-1.5
g*TP53* c.1101-2A>G; splicing

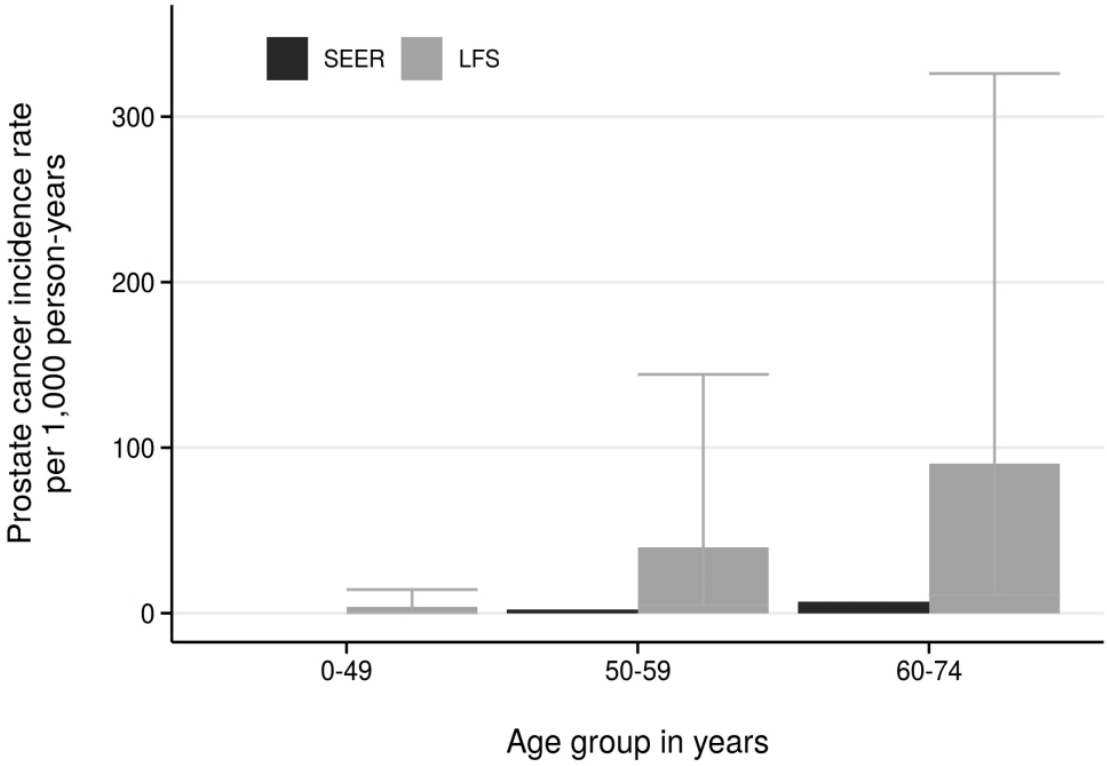


Supplementary Figure 1, cont

ii. LFS-11
g*TP53* c.1177del; p.D393Tfs*29



Supplementary Figure 1: Pedigrees of prostate cancers ascertained via Li-Fraumeni Syndrome (LFS) or at Academic Laboratory #1 (AL-1). Pedigrees are in order of variant location (5' to 3'). Shading orientation: Top left yellow: breast; bottom left green: ovarian; bottom right green: other; Abbreviations: Dx: age at diagnosis; d.: age at death; PrCa: prostate cancer; Panc: pancreatic; NOS: not otherwise specified; Sarc: sarcoma; Ca: cancer; CRC: colorectal; BD: Bile Duct; BrCa: breast cancer; MEL: melanoma; OvCa: ovarian; ESO: esophageal; GI: gastrointestinal; Gyn: gynecological; FG: female genital; CX: cervical; ACC: adrenocortical carcinoma; ALL: acute lymphocytic leukemia; MDS: myelodysplastic syndrome; Leuk: leukemia; ACT: adrenocortical tumor; SCC: squamous cell carcinoma; BCC: basal cell carcinoma; GAS: gastric; REN: renal; CML: chronic myeloid leukemia; MGM: meningioma; MM: multiple myeloma; Unk: unknown; NMSC: nonmelanoma skin cancer; THY: thyroid; GCT: germ cell tumor; BBrca: bilateral breast cancer; ENDO: endometrial; CPT: choroid plexus tumor



Supplementary Figure 2: Prostate cancer incidence in LFS males. Prostate cancer incidence rate per 1,000 males in a cohort of LFS men from four academic institutions restricted to males with no prior PrCa diagnosis before genetic testing and corresponding rates in SEER for selected age groups.

Supplementary References

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