Supplementary Materials and Methods

DNA isolation, PCR and Direct Sequencing of PCR products

Peripheral blood mononuclear cells (PBMCs) were isolated from 8 ml of venous blood and DNA extracted with FlexiGene DNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Exons 1α, 1β, 2 and 3 of CDKN2A to give PCR fragments of 340 bp, 678 bp, 576 bp and 319 bp for exons 1α, 1β ,2 and 3, respectively. PCR conditions were: initial denaturation and DNA polymerase activation at 95^{o} C for 6 min followed by 40 cycles of 95^{o} C for 10 sec, 61^{o} C, 59^{o} C, 60^{o} C or 62^{o} C (for exons 1α, 2 and 3, respectively) for 20 sec and 72 o C for 30 sec. The cycling was followed by 5 min. incubation at 72^{o} C then soak at 4^{o} C. The PCRs consisted of 1X PCR buffer, 0.2 mM dNTPs, 1.5 mM MgCl2, 1 U of Platinum Taq polymerase (all reagents from Invitrogen, Carlsbad, CA), 1 M of betaine (exons 1 and 2) or 5% of DMSO (exon 3) (both Sigma-Aldrich Chemie GmbH, Steinheim, Germany), or no additives with 20 pmoles of each primer (Eurofins-MWG GmbH, Ebersberg, Germany) and 50 ng of genomic DNA in a total volume of 20 µl. Exon 1 β PCR was run using the PCRx Enhancer System™ (Invitrogen, Carlsbad, CA) at final PCRx Enhancer solution concentration of 1X, in 1X PCRx Amplification buffer, 0.2 mM dNTPs, 1.5 mM $MgSO_4$, 30 pmoles of each primer and 3 U of Platinum Taq polymerase (all reagents Invitrogen, Carlsbad, CA) with PCR conditions as above except with an annealing temperature at 56^{o} C. 3 µl of each PCR product was run on a 1.6 % agarose gel to confirm PCR specificity. Ten µl of the PCR product was purified using 2 U of exonuclease I and 1 U of FastAP alkaline phosphatase (both Thermo Fisher Scientific, Gothenburg, Sweden). The purification conditions were 50 min at 37^{o} C followed by 20 min at 80^{o} C then soak at 4^{o} C. 0.5 to1.0 µl of the purified PCR corresponding to approximately 25 to 50 ng of PCR product was used in a sequencing reaction utilizing Applied Biosystems BigDye Terminator Cycle Sequencing Kit version 1.1 according to a 1:4 protocol with 1 µl of BigDye Terminator™ in a final 0.75X BigDye Terminator sequencing buffer (reagents Applied Biosystems, Foster City, CA) and 4 pmole of each primer (Eurofins-MWG GmbH, Ebersberg, Germany) in total volume of 10 µl. The sequencing

reactions were analyzed in ABI Prism® 3700 genetic analyzer (Applied Biosystems, Foster City, CA) All PCR products were sequenced bi-directionally, with analyses of electropherograms using Mutation Surveyor v.3.97 software (Softgenetics LLC, State College, PA).

Supplementary Table 1. Sex and age distribution in the study cohorts

	Sex, males/females (%)	Median year of birth
p.Arg112dup families	51/49	1957
Carriers	47/53	1959
FDRs	52/48	1955
SDRs	53/47	1957
Control population	51/49	1959
Controls	47/53	1959
cFDRs*	51/49	1958
cSDRs†	50/50	1960

*cFDRs=first degree relatives of controls and

†cSDRs=second degree relatives of controls.

Supplementary Table 2. Specification of all cancer occurrences in table 4 and RRs in *CDKN2A* p.Arg112dup kindreds (carriers, FDRs and SDRs) compared to controls

Types of cancer	Carriers n=120	FDR n=275	SDR n=321	Controls n=3976	p.Arg112dup fam vs. Ctrl RR (95% CI)
Breast	4	8	7	62	1.7 (1.0-2.8)
Central nervous system	1	3		21	1.0 (0.4-3.0)
Astrocytoma	1			6	0.9 (0.1-7.5)
Craniopharyngioma				4	0
Meningioma		2		5	2.2 (0.4-11.2)
Neurinoma				3	0
Ocular tumor (non melanoma)				3	0
Unspecified		1			0
Connective tissue		1	1	5	2.2 (0.4-11.2)
Mesothelioma			1	1	5.4 (0.3-8.7)
Sarcoma		1		4	1.4 (0.2-12.2)
Digestive -lower	2	5	2	64	0.8 (0.4-1.5)
Large Intesitine	1	2	1	41	0.5 (0.2-1.5)
Rectum	1	3	1	19	1.4 (0.5-3.8)
Small intestine and appendix				4	0
Digestive -upper	15	20	9	42	5.7 (3.7-8.7)
Tongue and oral cavity	5	1		7	4.7 (1.6-13.9)
Pharynx			1	1	5.4 (0.3-8.7)
Esophagus	2	1		3	5.4 (1.1-27.0)
Stomach	1	4	2	15	2.5 (1.0-6.2)
Pancreas	7	13	3	9	13.9 (6.4-30.1)
Liver			2	4	2.7 (0.5-14.9)
Gall bladder		1	1	1	10.9 (0.9-12.0)
Endocrine	2	4		20	1.6 (0.7-4.1)
Adrenal gland	1				0
Carcinoid		1		4	1.4 (0.2-12.2)
Hypophysis				3	0
Malignant thymoma				1	0
Neuroendocrine tumor				1	0
Parathyroid		3		6	2.7 (0.7-10.9)
Thyroid	1			5	1.1 (0.1-9.3)
Gynecological	11	7	8	98	1.5 (1.0-2.3)
Cervix	9	5	7	74	1.5 (1.0-2.6)
Ovaries and salpinges	2		1	13	1.3 (0.4-11.2)
Endometrium		2		5	2.2 (0.4-11.2)
Vagina and vulva				6	0
Hematopoietic or Lymphatic	4	2	2	27	1.6 (0.7-3.5)
Leukemia	2		2	10	2.2 (0.7-6.9)
Lymphoma	2			13	0.8 (0.2-3.8)
Myeloma				4	0
Unknown primary tumor		3		17	1.0 (0.3-3.3)
Respiratory	6	9	5	23	4.7 (2.4-8.7)
Larynx	2	2		1	21.8 (2.4-194.7)
Lung and bronchi	4	7	5	22	4.0 (2.1-7.5)
Skin	64	32	8	61	9.3 (6.8-12.8)
Melanoma	60	28	8	21	24.6 (15.3-40.0)
Basal cell carcinoma				2	0
Squamous cell skin cancer	4	4		37	1.2 (0.5-2.5)
Skin adnexal tumors				3	0
Urinary	3	8	12	107	1.2 (0.7-1.8)
Kidney	1		4	16	1.7 (0.6-4.6)
Urinary bladder and ureters		1	1	23	0.5 (0.1-2.0)
Prostate	2	7	7	64	1.4 (0.8-2.4)
Testis				4	0

Supplementary Table 3. Cumulative incidence for each age group in *CDKN2A* p.Arg112dup carriers.

	10y	20y	30y	40y	50y	60y	70y	80y
Non melanoma cancers	0.00	0.00	0.03	0.12	0.20	0.31	0.44	0.76
Respiratory and upper digestive	0.00	0.00	0.00	0.02	0.07	0.10	0.16	0.53