Lack of transgenerational effects of ionizing radiation exposure in cleanup workers and evacuees of the Chernobyl accident

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Chernobyl Nuclear Power Plant Accident

- April 26, 1986 explosion in reactor 4 during safety test
- Resulting open-air reactor core fire contained 9 days later

Long-standing Controversy on Possible Transgenerational Effect Due to Radiation

Prior Animal Data

- High doses (2-4 Gy) acute exposures tested
- Evidence of double strand breaks
  - NHEJ DNA Repair
- Structural and chromosomal events
  - No single base mutational signature

Prior Human Studies

- Lower/protracted doses
- Small, underpowered studies of a handful of genetic markers
  - Microsatellites
- Nagaski study of three family trios (whole genome sequence)
  - Anecdotal

Study Question:

Is there a transgenerational effect of radiation following the Chernobyl accident?

Yeager et al. *Science* in Revision

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Study Design

Field Study of the Possible Effect of Parental Irradiation on the Germline of Children Born to Cleanup Workers and Evacuees of the Chornobyl Nuclear Accident

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Although transgenerational effects of exposure to ionizing radiation have long been a concern, human research to date has been confined to studies of disease phenotypes in groups exposed to high doses and high dose rates, such as the Japanese atomic bomb survivors. Transgenerational effects of parental irradiation can be addressed using powerful new genomic technologies. In collaboration with the Ukrainian National Research Center for Radiation Medicine, the US National Cancer Institute, in 2014–2018, initiated a genomic alterations study among children born in selected regions of Ukraine to cleanup workers and/or evacuees exposed to low–dose-rate radiation after the 1986 Chornobyl (Chernobyl) nuclear accident. To investigate whether parental radiation exposure is associated with germline mutations and genomic alterations in the offspring, we are collecting biospecimens from father-mother-offspring constellations to study de novo mutations, minisatellite mutations, copy-number changes, structural variants, genomic insertions and deletions, methylation profiles, and telomere length. Genomic alterations are being examined in relation to parental gonadal dose, reconstructed using questionnaire and measurement data. Subjects are being recruited in exposure categories that will allow examination of parental origin, duration, and timing of exposure in relation to conception. Here we describe the study methodology and recruitment results and provide descriptive information on the first 150 families (mother-father-child(ren)) enrolled.

Chornobyl (Chernobyl); genetic risk; germline mutations; low–dose-rate radiation; parent-offspring constellations; preconception exposure; Ukraine

Abbreviations: DOB, date of birth; NCI, National Cancer Institute; NRCRM, National Research Center for Radiation Medicine.

Identifying the full extent of ionizing radiation–related health effects is critical for handling of nuclear accidents, radiation protection standards, and clinical practice. Pioneering experimental studies of transgenerational effects were carried out in 1927 (1) and 1958 (2), but until recently human research on transgenerational radiation effects following parental exposure has been limited. Sievert, in a 1958 paper prepared for the International Commission on Radiological Protection (3), pointed to the scarcity of data on possible genetic effects of radiation in humans. Sixty years later, this remains an important and controversial issue, particularly in the wake of the Chornobyl (Chernobyl) and Fukushima nuclear power plant accidents, which occurred in 1986 and 2011, respectively. Intervening studies have been confined to studies of disease phenotypes and minisatellite mutation rates in Japanese atomic bomb survivors, Chornobyl-exposed groups, and offspring of childhood cancer survivors, mostly exposed to high doses/rates (4). With the

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Partnership with NCRCM in Kiev
Recruit families based on exposure categories
Conduct whole genome sequencing

Goal: Determine if there are genetic ‘footprints’ as a marker of a transgenerational effect

Bazyka et al. *Amer J Epi* 2020

Journal of Radiological Protection

Official journal of the Society for Radiological Protection

ACCEPTED MANUSCRIPT • OPEN ACCESS

Estimation of radiation gonadal doses for the American-Ukrainian trio study of parental irradiation in Chornobyl cleanup workers and evacuees and germline mutations in their offspring

Vadim Chumak[1], Elena Bakhanova[1], Victor Kryuchkov[2], Ivan Golovanov[2], Konstantin Chizhov[3], Dimitry Bazyka[1], Natalia Gudzenko[1], Natalia Trotsuk[1], Kiyohiko Mabuchi[3], Maureen Hatch[3], Elizabeth K. Cahoon[3], Mark P. Little[3], Tatiana Kukhta[4], Amy Berrington de Gonzalez[3], Stephen J. Chanock[3], Vladimir Drozdovitch[3]

Is There a Transgenerational Effect of Protracted Radiation Following Chernobyl Accident?

Approach: Determine de novo mutations in adult children born to
- Liquidators & Evacuees

- Family Structure Design
- Current Analysis
  - 105 families
  - 130 adult children
    - Born 1987-2002
  - High Coverage Whole Genome Sequencing (both generations)
    - Short read (150 bp)
    - 80 X coverage
- Multistep calling algorithm
  - Manual inspection of all putative events

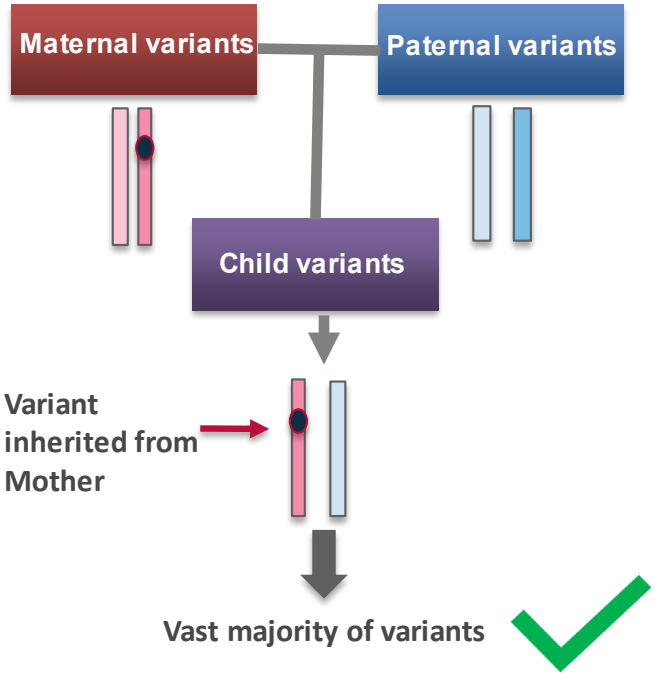
Bazyka et al. *Amer J Epi* 2020

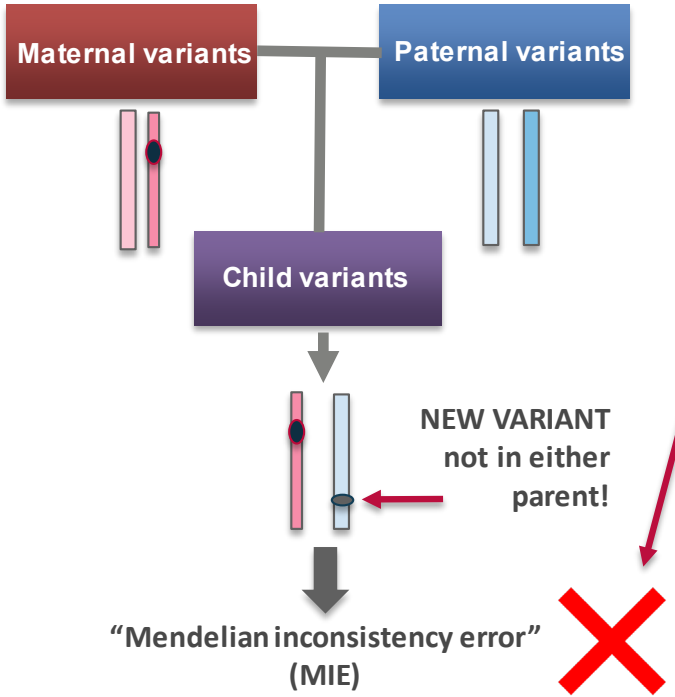
De Novo Mutations (DNMs)

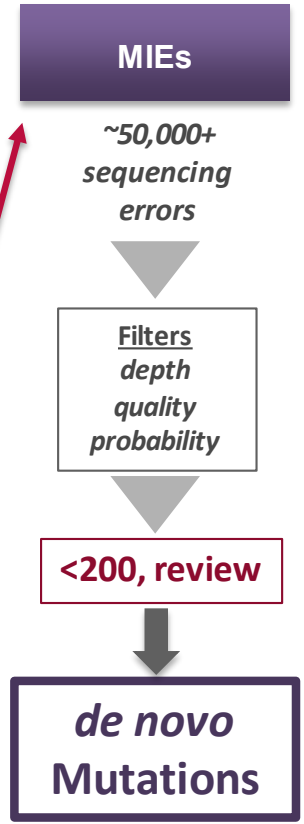
- Due to random mutations in gametes (sperm and oocytes), a small number of variants are generated
- Transmitted variants are known as de novo mutations (DNMs)
- Critical blocks of evolution
- Only class of genomic variation to NOT undergo purifying selection
- DNMs account for a fraction of neurodevelopmental disorders
- DNMs ascertained by analysis of both parents with child(ren)
  - “Seen” only in child but not parents

Principle for Detecting *de novo* mutations (DNMs)

Family Trio Whole Genome Sequencing







Types of De Novo Mutations

• Single Nucleotide Variant (SNVs)	Most common
• Indels (insertions or Deletions)	Next most common
• Microsatellites (type of Indels)	Not common
• Clusters	Unusual
• Complex	Rare

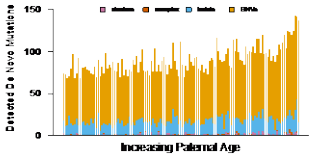
Distribution of Detected DNMs in the Chernobyl Trios

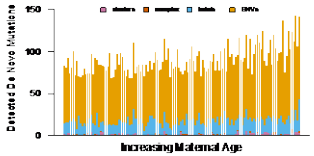
	Mean	Median	Range	Standard Deviation
Number of Clusters	1.39	1	0-6	1.34
Number of Complex	0.38	0	0-5	0.77
Number of Indels	16.18	15	5-38	5.10
Number of Microsatellites	5.62	5.5	0-13	2.49
Number of SNVs	72.22	69.5	47-121	13.36
Total Number of DNMs	90.17	88	69-143	15.94
Phased to Paternal Haplotype	29.33	29	12-53	7.08
Phased to Maternal Haplotype	8.61	8	2-20	4.07
Proportion Phased	42.1%	41.5%	27.6-55.8%	6.3%

Phased= parent of origin can be determined. (Father > Mother)

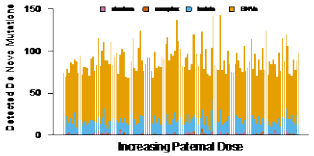
Detected DNMs Per Genome Based on Distributions of Parental Age at Conception

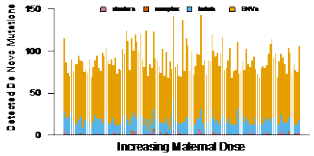
Age at Conception





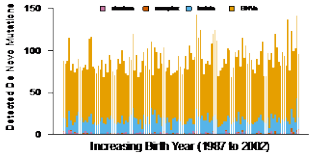
Radiation Dose

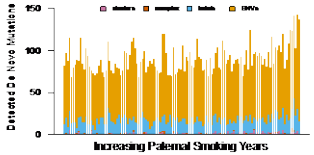




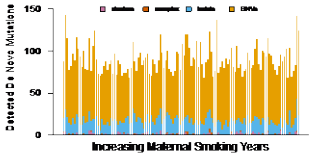
Paternal dose: mean=365 mGy range=0-4080 mGy
Maternal dose: mean=19 mGy range=0-550 mGy

Birth Year of Child





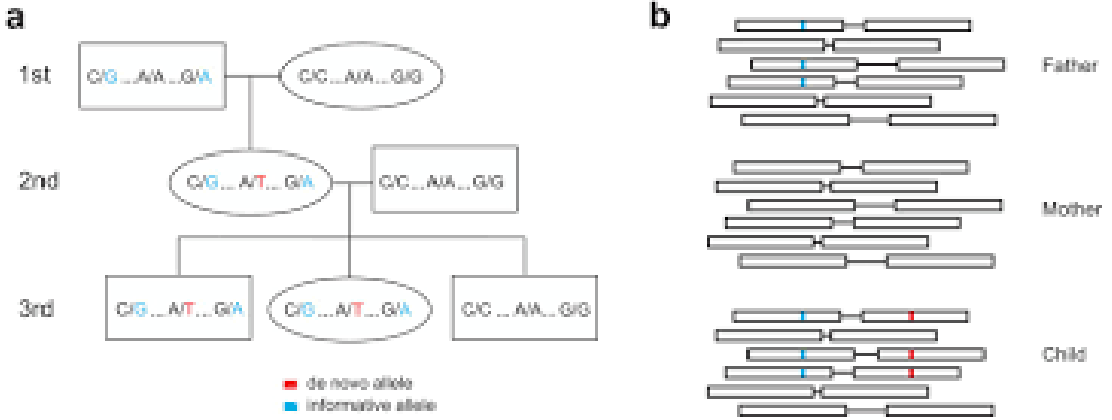
Smoking



Determination of parent-of-origin for DNMs

-Larger fraction derived from paternal origin-

Based on flanking variants that identify parental origin



Parent-of-Origin Estimates for Age at Conception and Cumulative Radiation Dose

42% determined based on an informative nearby germline variant(s) on a parental haplotype

	Estimate	95% Confidence Interval	P-value
Paternal Origin DNMs			
Paternal Age	0.71	0.54, 0.88	3.67E-13
Paternal Dose (mGy)	-0.001	-0.002, 0.0005	0.20
Maternal Origin DNMs			
Maternal Age	0.28	0.15, 0.40	3.24E-05
Maternal Dose (mGy)	0.001	-0.008, 0.01	0.77

Yeager et al Science In Revision

Associations of Age at Conception, Cumulative Ionizing Radiation Dose, and Smoking History with Total DNM Count

	Estimate	95% Confidence Interval	P-value
Age at conception			
Maternal age	0.46	-0.02, 0.93	0.06
Paternal age	1.94	1.51, 2.36	3.65×10^{-15}
Cumulative radiation dose (/mGy)			
Maternal dose	-0.02	-0.04, 0.007	0.17
Paternal dose	-0.0007	-0.003, 0.002	0.56
Smoking history			
Maternal former smoker	-4.13	-10.74, 2.49	0.22
Maternal current smoker	5.31	-0.18, 10.81	0.06
Paternal former smoker	0.91	-5.16, 6.97	0.77
Paternal current smoker	2.91	-0.93, 6.75	0.14

Power Analysis for Radiation Effects

- Power computed based on observed mother-father radiation doses (130 Children and 105 mother-father pairs)
- Power based on a linear dose model with a two-sided test conducted at the 0.05 significance level
- Approximately 80% power to detect a radiation dose of 0.6 DNM per 100mGy of father's dose and 5.5DNM per 100mGy of mother's dose

Sensitivity Analyses of the Impact of Maternal and Paternal Cumulative Radiation Dose Modeling on Association with DNMs

	Estimate	95% CI	P-value
Cumulative radiation dose (/mGy)			
Maternal dose	-0.02	-0.04, 0.007	0.17
Paternal dose	-0.0007	-0.003, 0.002	0.56
Cumulative radiation dose truncated at 1,000 (/mGy)			
Maternal dose	-0.02	-0.04, 0.009	0.21
Paternal dose	-0.003	-0.008, 0.001	0.17
Cumulative log radiation dose (/ln(1+mGy))			
Maternal dose	-0.87	-2.12, 0.39	0.18
Paternal dose	-0.37	-1.07, 0.33	0.30

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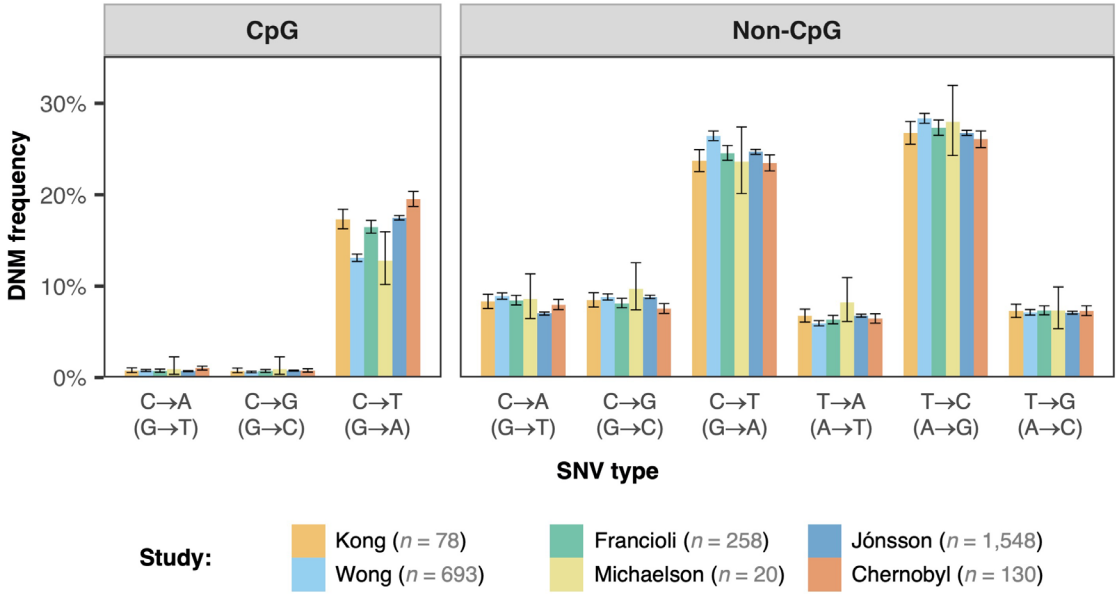
Sensitivity Analysis: Impact of Maternal and Paternal Cumulative Radiation Dose Category on Association with DNMs

Distribution of Estimated Doses

	0-<25mGy		25-<100mGy		100-<500mGy		500-<1000mGy		1000+mGy	
	N	%	N	%	N	%	N	%	N	%
Paternal dose	63	48%	13	10%	24	18%	13	10%	17	13%
Maternal dose	116	89%	11	9%	1	1%	2	2%	0	0%

	Effect	95% CI	P-value
Paternal Dose			
0-<25mGy	(reference)		
25-<100mGy	2.75	-3.37, 8.88	0.37
100-<500 mGy	-1.2	-6.29, 3.90	0.64
500-<1000 mGy	-5.03	-11.35, 1.30	0.12
1000+ mGY	-0.99	-6.48, 4.51	0.72
Maternal Dose			
0-<25mGy	(reference)		
25-<50mGy	-2.56	-11.93, 6.82	0.59
50-<100mGy	-2.58	-10.63, 5.46	0.53
100-<500mGy	-5.11	-25.79, 15.56	0.63
500+ mGY	-6.98	-21.59, 7.62	0.35

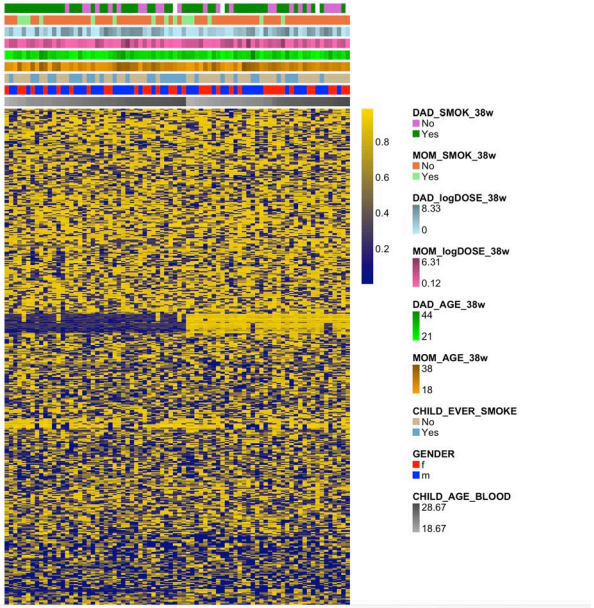
Comparison of DNMs Across Published Studies



(N=2727)

No Material Difference Between Chernobyl Trios and Population-based Studies

Methylation Analyses Illumina 850,000 + probes



Is there a transgenerational effect by:

- Age- chronologic — No
- Age- 'methylation'-biological — No
- Smoking status — No
- Radiation exposure — No (and no evidence for a radiation signature)

Multivariate Associations for qPCR Measured Standardized Relative Telomere Length in Leukocytes of Trio Children:

No substantive effect

Telomeres: Ends of Chromosomes
Altered with age & cancer

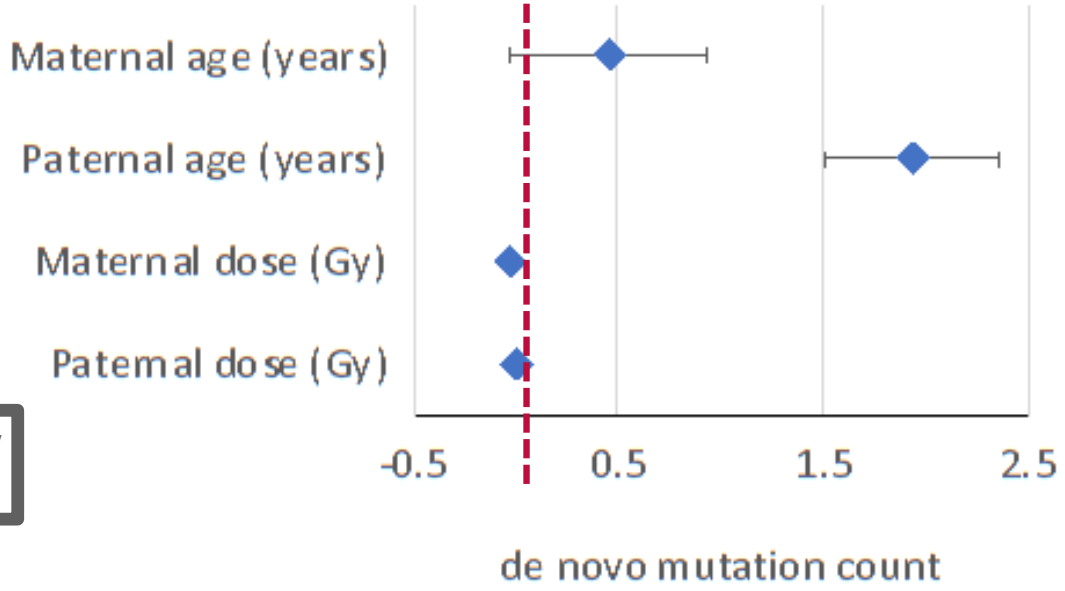
	Estimate	Std. Error	p-value
Child characteristics			
Age at blood draw	1.89E-03	2.95E-03	0.52
Sex (female=0, male=1)	-1.70E-02	1.80E-02	0.35
Former smoker	2.40E-02	2.96E-02	0.42
Current smoker	1.24E-02	2.32E-02	0.60
Age at conception			
Paternal Age	1.32E-04	2.21E-03	0.95
Maternal Age	4.62E-03	2.41E-03	0.06
Cumulative log radiation dose			
Paternal Dose	2.03E-06	1.32E-05	0.88
Maternal Dose	-2.75E-04	1.24E-04	0.03
Smoking history at conception			
Paternal former smoker	2.02E-02	3.15E-02	0.52
Paternal current smoker	2.26-03	1.97E-02	0.91
Maternal former smoker	5.27E-02	3.61E-02	0.15
Maternal current smoker	-3.52E-02	2.86E-02	0.22

Summary of Study in Chernobyl Liquidators/Evacuees

Transgenerational effects of ionizing radiation (Yeager et al., *Science*, In revision)

- N=105 trios (130 children born to parents employed as clean-up workers or exposed from the accident)
- Whole genome sequencing to detect germline de novo mutations

No evidence of increased de novo mutations with increasing radiation dose



Paternal dose: mean=365 mGy range=0-4080 mGy
Maternal dose: mean=19 mGy range=0-550 mGy

Study Considerations

- Evaluation of peripheral blood of adult children conceived months to years after accident
  - No increase in DNMs in children born in 1987
  - Survivor bias among sampled children recruited as adults
- Unable to investigate acute high doses (>2-4Gy)
- No twins analyzed
- Short-read sequencing technology used

Implications of Current Study

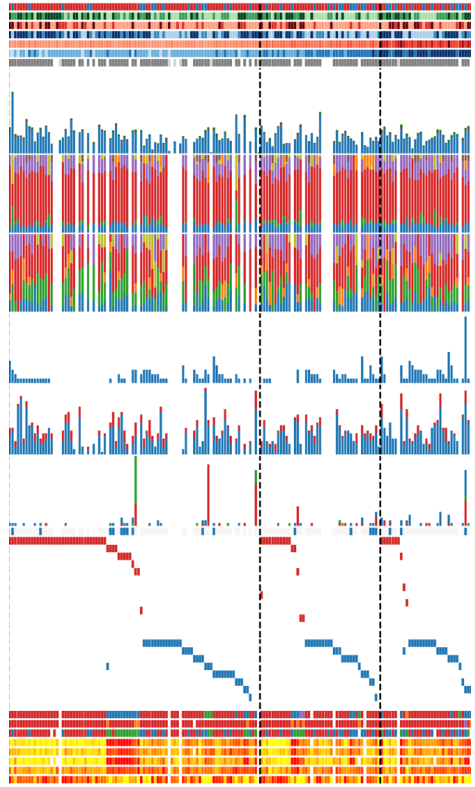
- Adequate power to identify substantive increase in DNMs in adult children born to liquidators/evacuees
- No evidence of radiation-induced single base mutation or epigenetic signature
- Doses were both extended and lower than animal studies
  - Lower rate of gonadal DNM events
- Alter the balance between new gonadal DNMs and DNA repair
- Further studies of higher dose might be informative

Yeager et al. *Science* in Revision

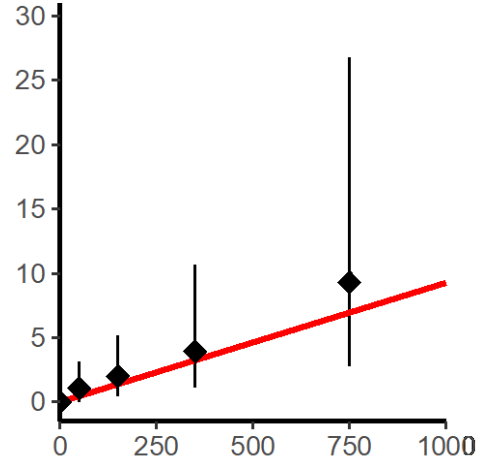
Molecular Studies in Chernobyl

Genomic landscape of papillary thyroid cancer (Morton et al., *Science*, In press)

- N=440 fresh frozen tumor tissues, matched normal tissue (non-tumor thyroid tissue, blood) from the Chernobyl Tissue Bank
- Landscape analysis by radiation dose
  - Whole genome sequencing, mRNA and miRNA sequencing
  - Methylation, SNP array genotyping, relative telomere length



Increasing DNA double-strand breaks with increasing radiation dose



Morton et al. *Science* in press

Key Findings in Thyroid Tissue Analyses

1. >95% of driver mutations in MAPK pathway (ras/raf)
   - Radiation shifts towards fusion drivers
2. Non-homologous end joining DNA repair- predominant in:
   - Fusions and structural variants
3. No field effect (radiation signature or drivers) in companion non-tumor thyroid
4. Epigenetic and gene expression profiles driven by cancer driver
   - No radiation signatures yielded radiation biomarkers

Morton et al. *Science* in press

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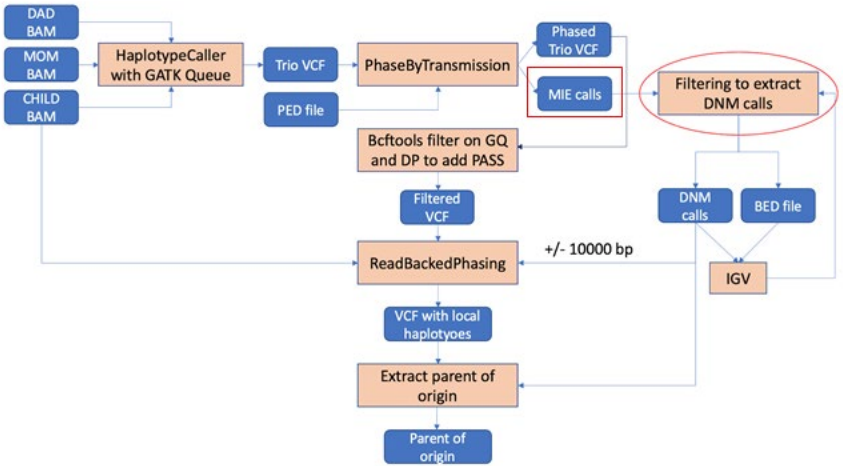
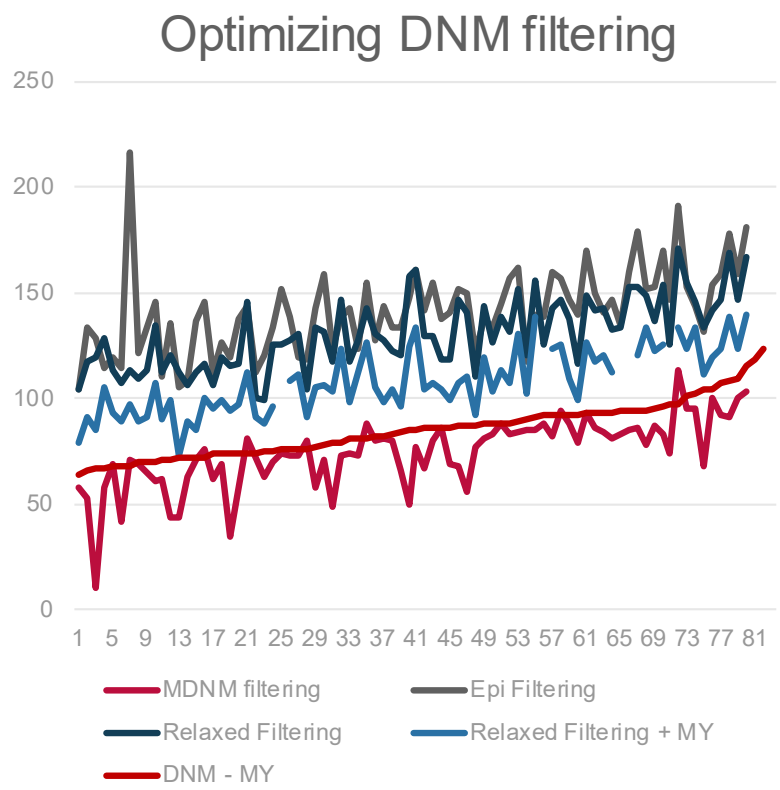
Algorithm for Calling Variants DNMs





ALL Putative DNMs were manually reviewed in IVF