Using Molecular Epidemiology to Investigate Environmental Exposures and Pediatric Cancer

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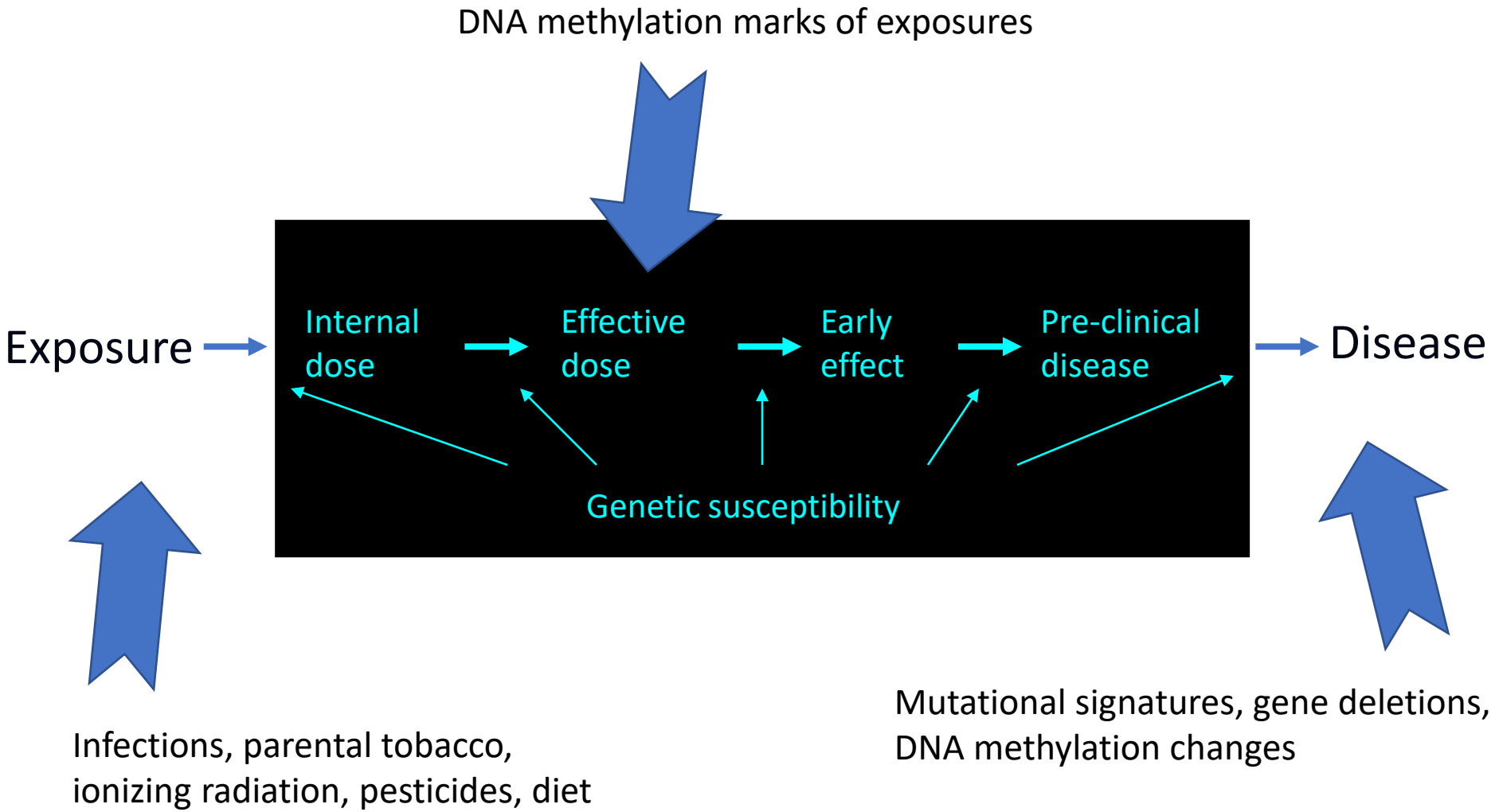
CiRCLE

The Center for Integrative Research on Childhood Leukemia and the Environment

Molecular Epidemiology Paradigm



Leukemia rates are rising: why?

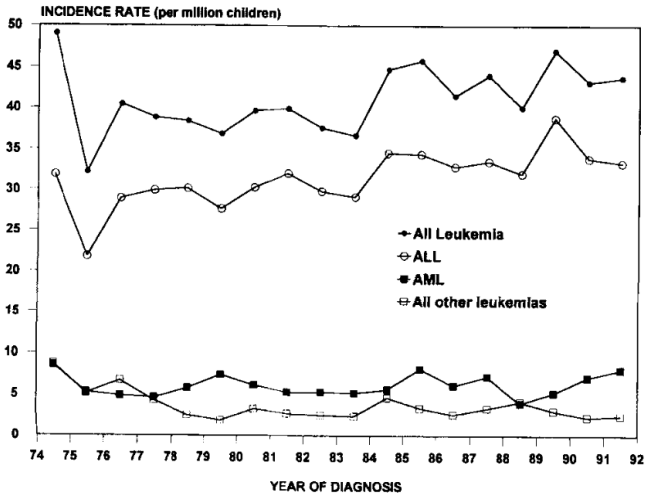
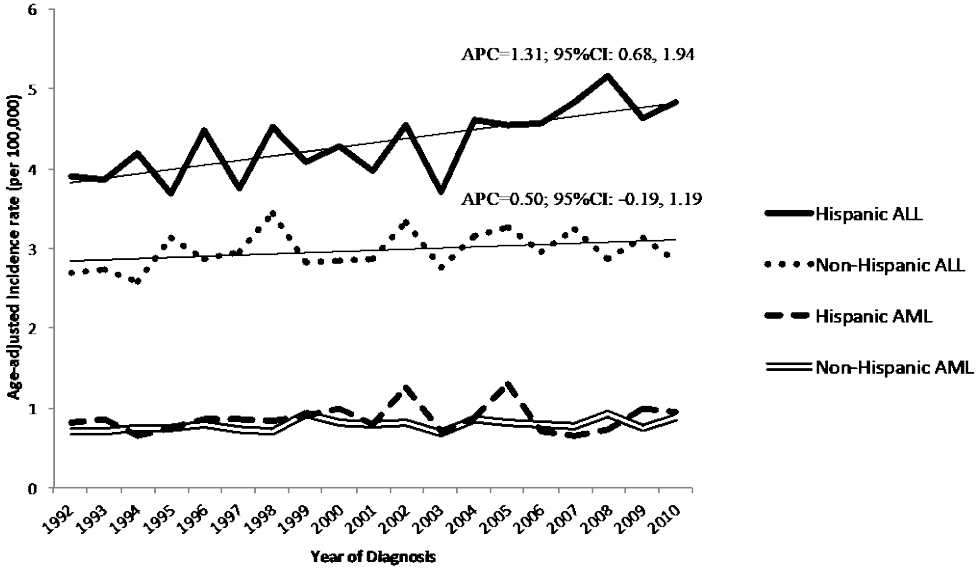


FIGURE 1. Age-standardized leukemia incidence rates per million children age 14 years and younger are shown.

Gurney, *Cancer* 1996

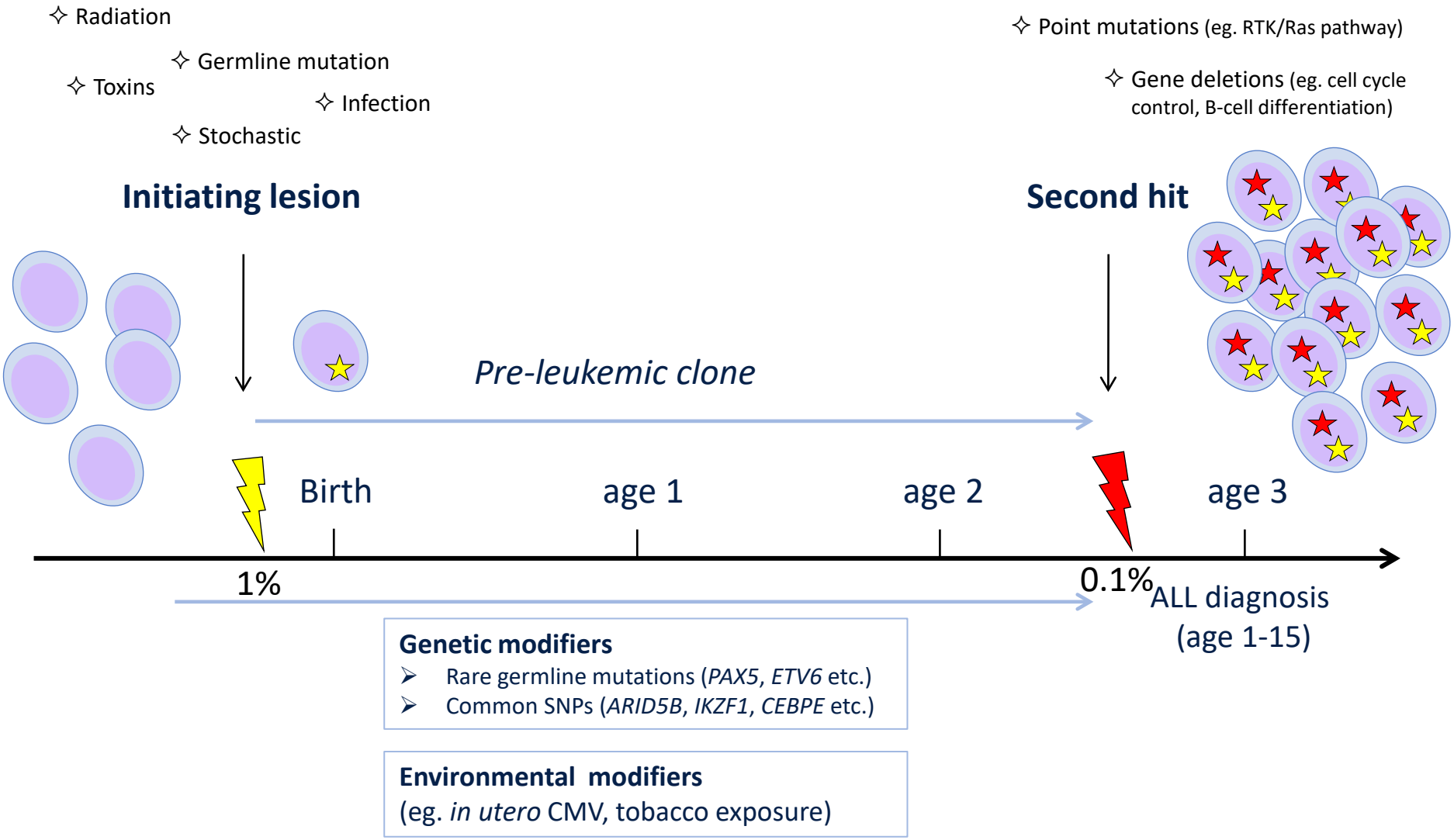


Barrington-Trimis, *Blood* 2015

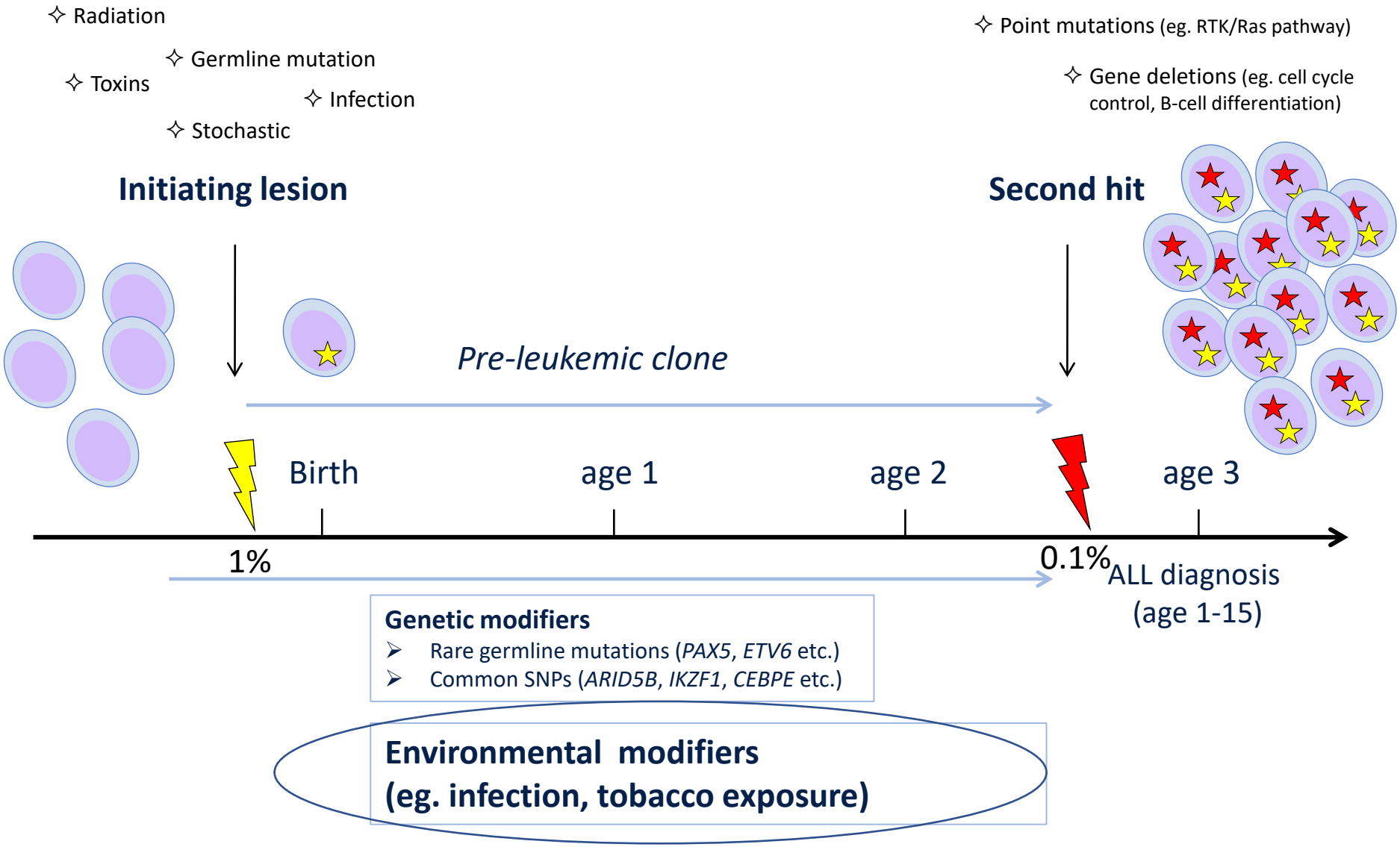
35% rise in rates since 1975, 70% since 1945

Suggests environmental causes

Natural history of childhood ALL



Natural history of childhood ALL

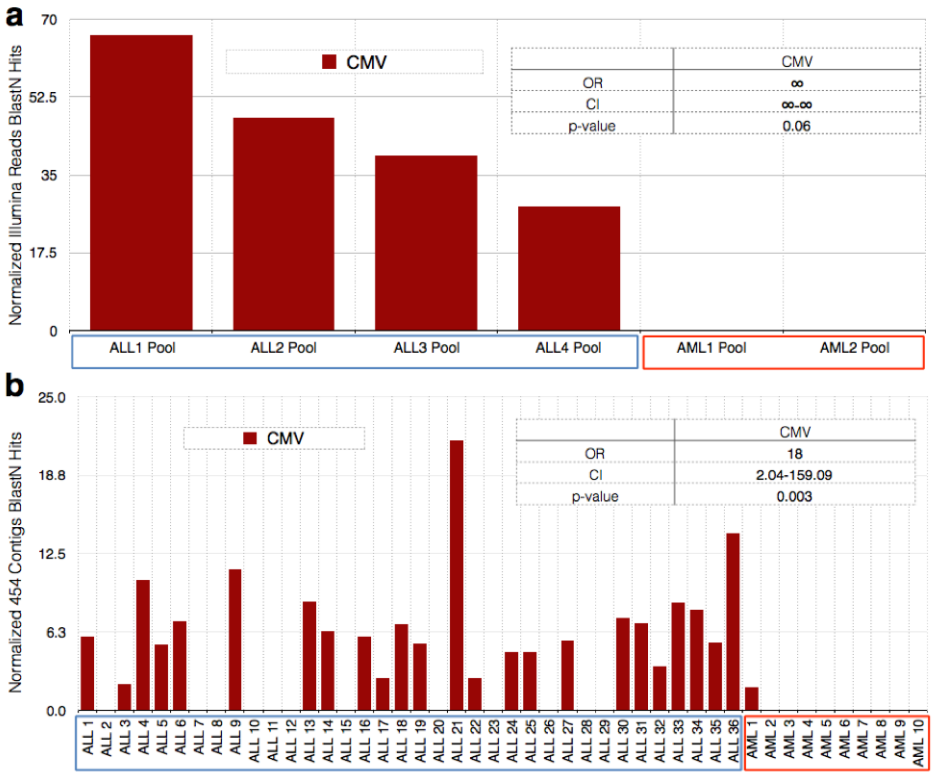


What infections?

RNASeq of leukemia cells,
DNASeq of plasma virions

Leukemia cell RNA

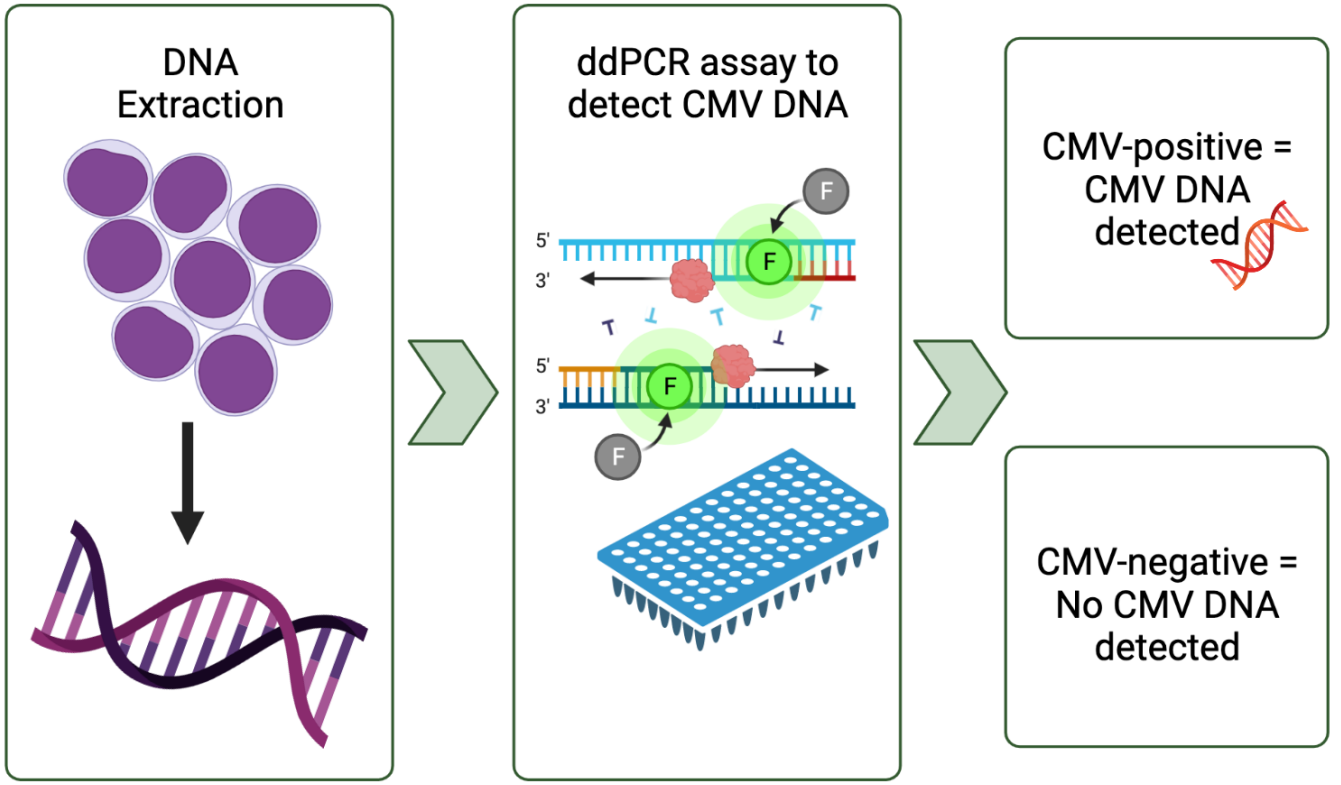
Viral particle DNA



Francis, et al, *Blood* 2017

cytomegalovirus

CMV infection: when does it happen?

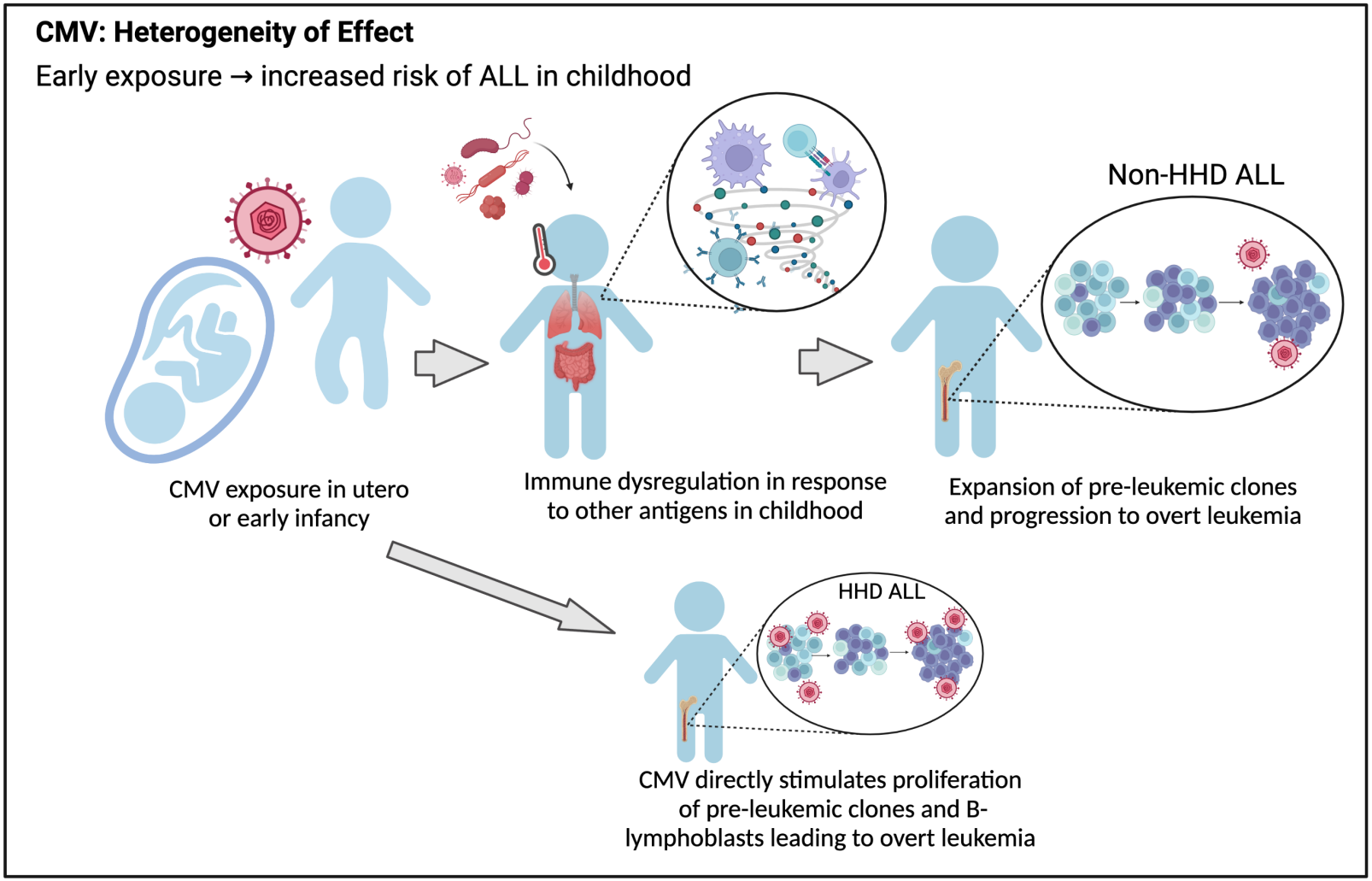


Neonatal CMV is a risk factor for childhood ALL

	CMV -	CMV +	OR (CI)	p-value	EBV -	EBV +	OR (CI)	p-value
Overall*	-	-	-	-	-	-	-	-
ALL	242	26	**3.71 (1.71-8.95)**	0.0016	257	11	1.01 (0.42-2.42)	1
Controls	262	8	-	-	259	11	-	-
By Race	-	-	-	-	-	-	-	-
Hispanic ALL	134	15	**5.90 (1.89-25.96)**	0.006	141	8	1.38 (0.46-4.35)	0.56
Hispanic Controls	152	3	-	-	149	6	-	-
White ALL	71	10	**2.10 (0.69-7.13)**	0.204	79	2	0.53 (0.09-2.94)	0.68
White Controls	82	5	-	-	83	4	-	-

* Logistic regression adjusted for race, age and sex

Francis et al, Blood 2017

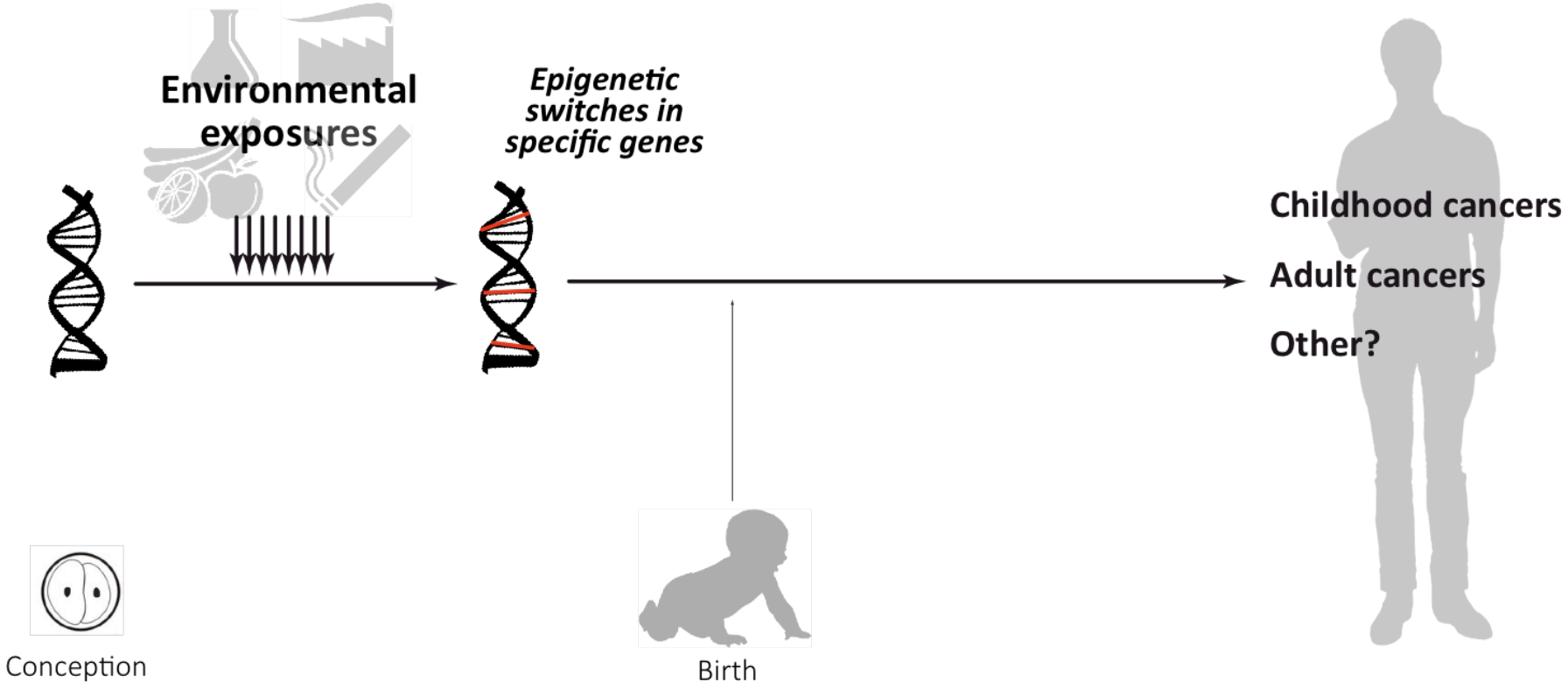


HHD = high hyperdiploidy, the most common subtype of ALL (35%)

Gallant et al, IJC 2023

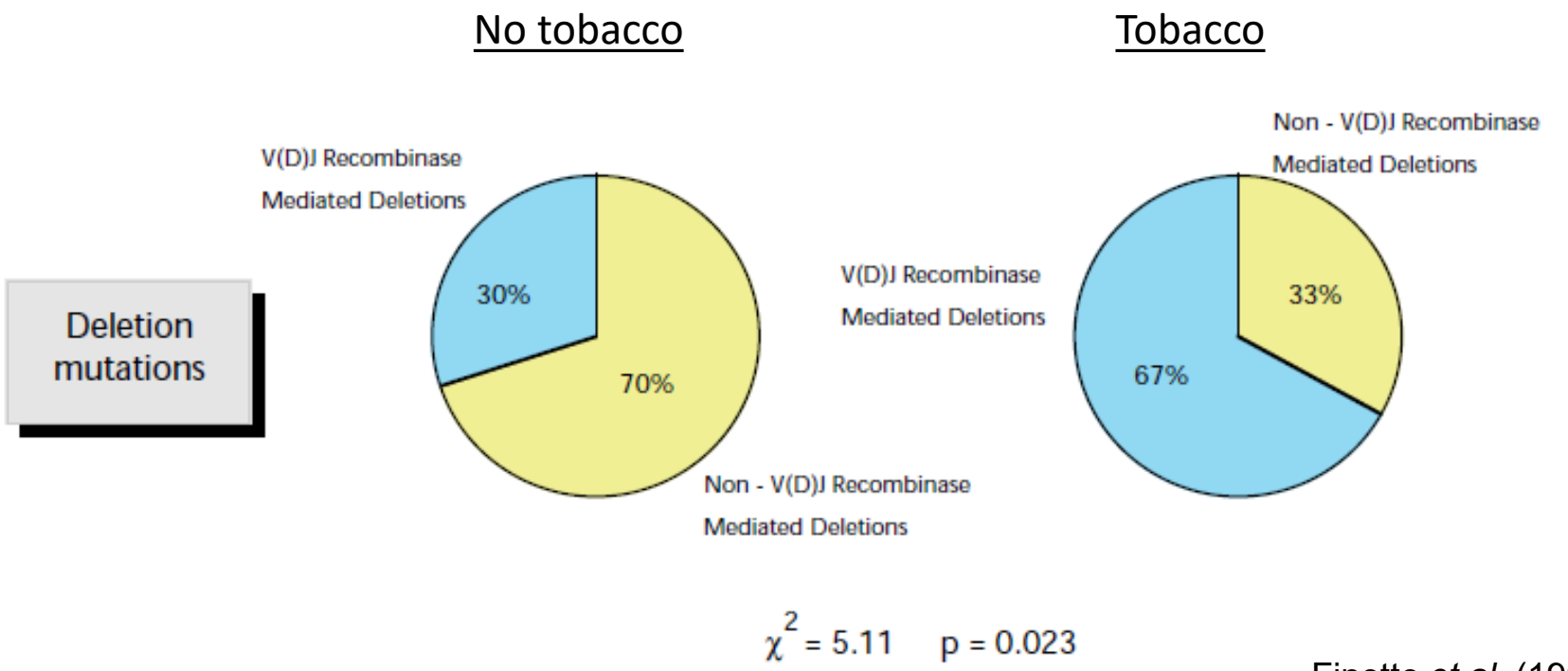
Tobacco and ALL risk

Developmental origins of cancer hypothesis



Tobacco smoke and gene deletions

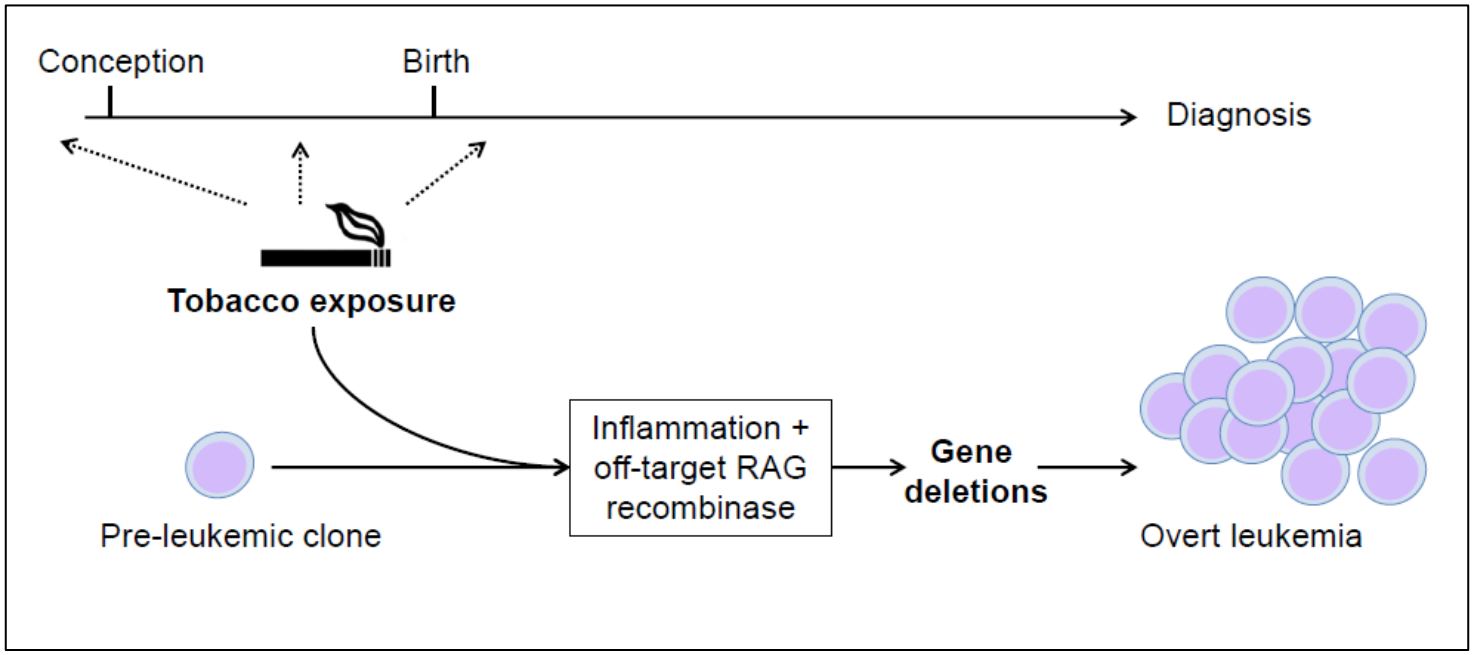
- Finette *et al.* analyzed distribution of somatic mutations in the *HPRT* reporter gene in cord blood lymphocytes from newborns exposed or not exposed to cigarette smoke during pregnancy



Finette *et al.* (1998) *Nat Medicine*. 4:1144-51.

Hypothesis:

Increased parental smoking → increased frequency of gene deletions in ALL cases



ALL tumor copy number analysis

- Multiplex ligation-dependent probe amplification (MLPA) carried out using the Childhood ALL kit (MRC Holland) to assess copy number at 8 genes most commonly lost in ALL tumors:
  - *CDKN2A* (3 probes)
  - *ETV6* (6 probes)
  - *IKZF1* (8 probes)
  - *PAX5* (7 probes)
  - *RB1* (5 probes)
  - *BTG1* (4 probes)
  - PAR1 region (including *CRLF2*) (5 probes)
  - *EBF1* (4 probes)

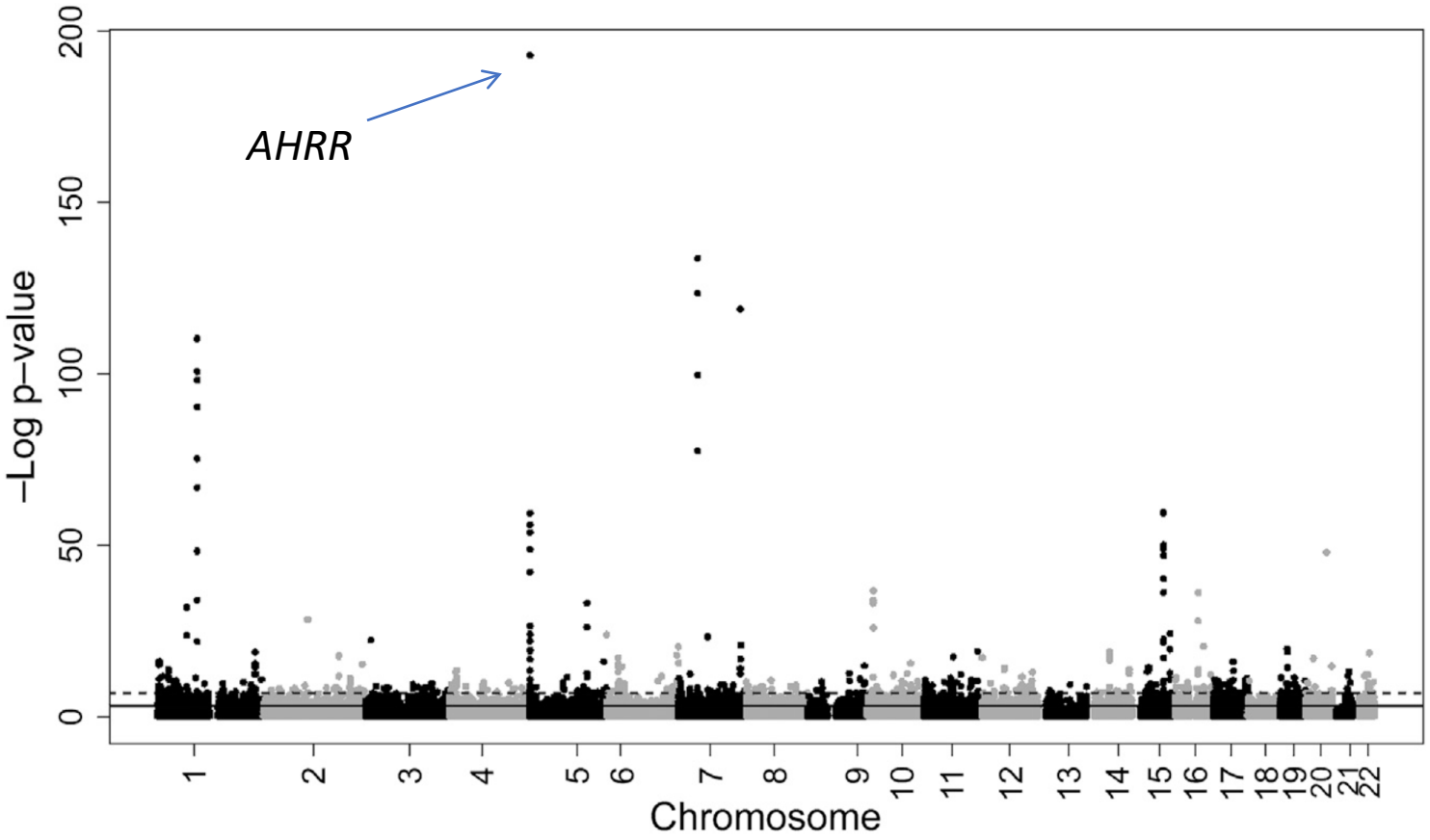
- In 846 diagnostic bone marrow (*i.e.* tumor) samples from CCLS ALL cases

- Multivariable Poisson regression analyses used to analyze tobacco smoke exposures vs. number of deletions, using ratios of means (RM) as the measure of association

Binary tobacco smoking exposures	# Cases	RM	95% CI	P-value[a]
Paternal, ever[bc]				
No	313	1 (ref)		
Yes	213	1.13	(0.95, 1.36)	0.17
Maternal, ever[bc]				
No	425	1 (ref)		
Yes	101	1.24	(1.01, 1.53)	0.04 [d]
Paternal, preconception[c]				
No	394	1 (ref)		
Yes	133	1.21	(0.99, 1.48)	0.06
Maternal, preconception[c]				
No	460	1 (ref)		
Yes	65	1.27	(0.99, 1.65)	0.06
Maternal, pregnancy[c]				
No	482	1 (ref)		
Yes	43	1.4	(1.05, 1.87)	0.02 [d]
Maternal, breastfeeding[c]				
No	509	1 (ref)		
Yes	16	1.94	(1.34, 2.79)	0.0004 [d]
Continuous exposures	**# Cases**	**RM**	**95% CI**	**P-value**
Paternal, preconception (CPD)[c]	511	1.07	(1.00, 1.13)	0.03
Maternal, preconception (CPD)[c]	512	1.05	(0.95, 1.15)	0.34
Maternal, pregnancy (CPD)[c]	511	1.3	(1.00, 1.68)	0.048 [d]
Maternal, breastfeeding (CPD)[c]	493	1.6	(1.18, 2.17)	0.003 [d]

De Smith et al, *Cancer Res* 2017, CEBP 2022

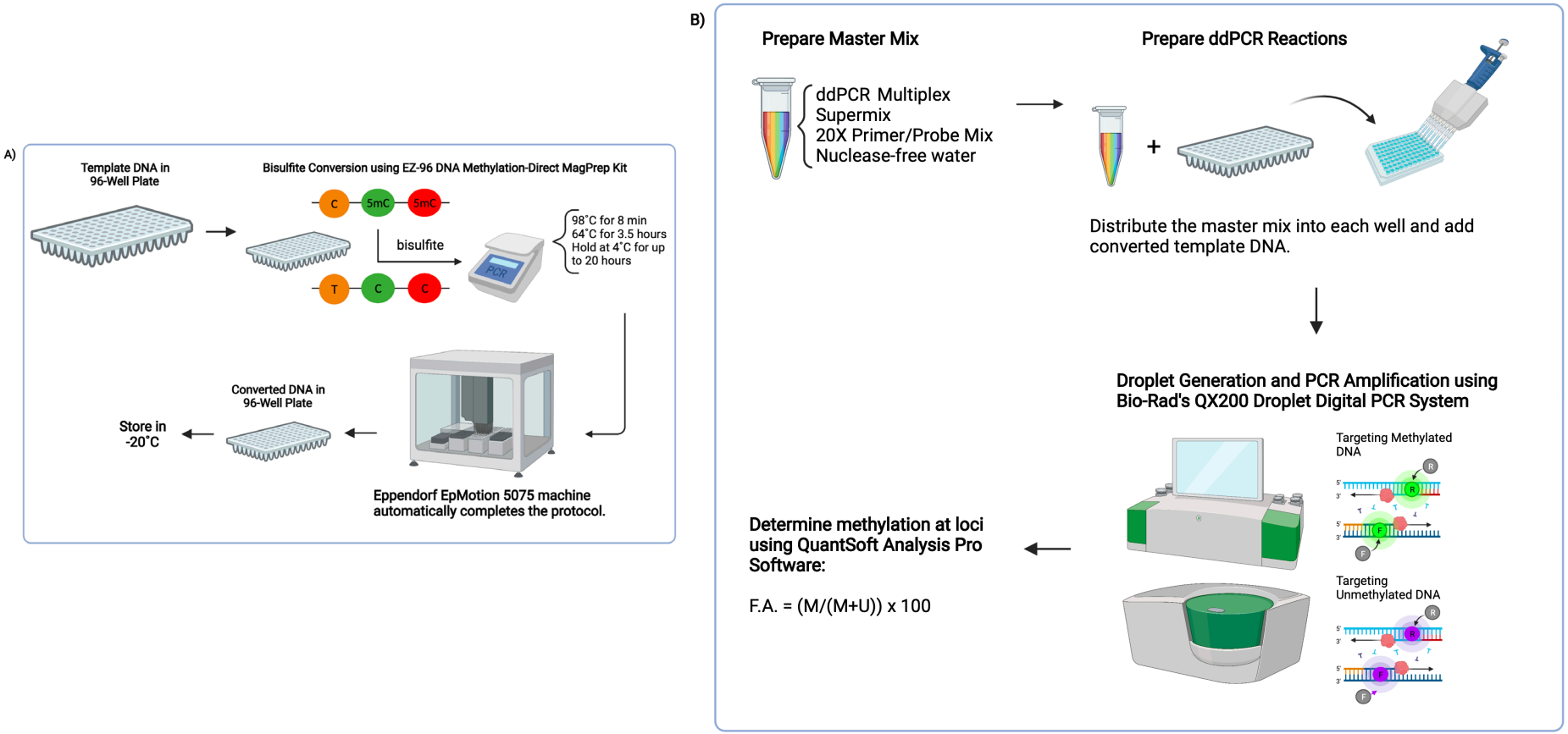
Epigenetic biomarker for maternal smoking



- Meta-analysis of association between sustained maternal smoking during pregnancy and DNA methylation in newborn cord blood
- Aryl hydrocarbon receptor repressor (*AHRR*) CpG cg05575921 = top hit

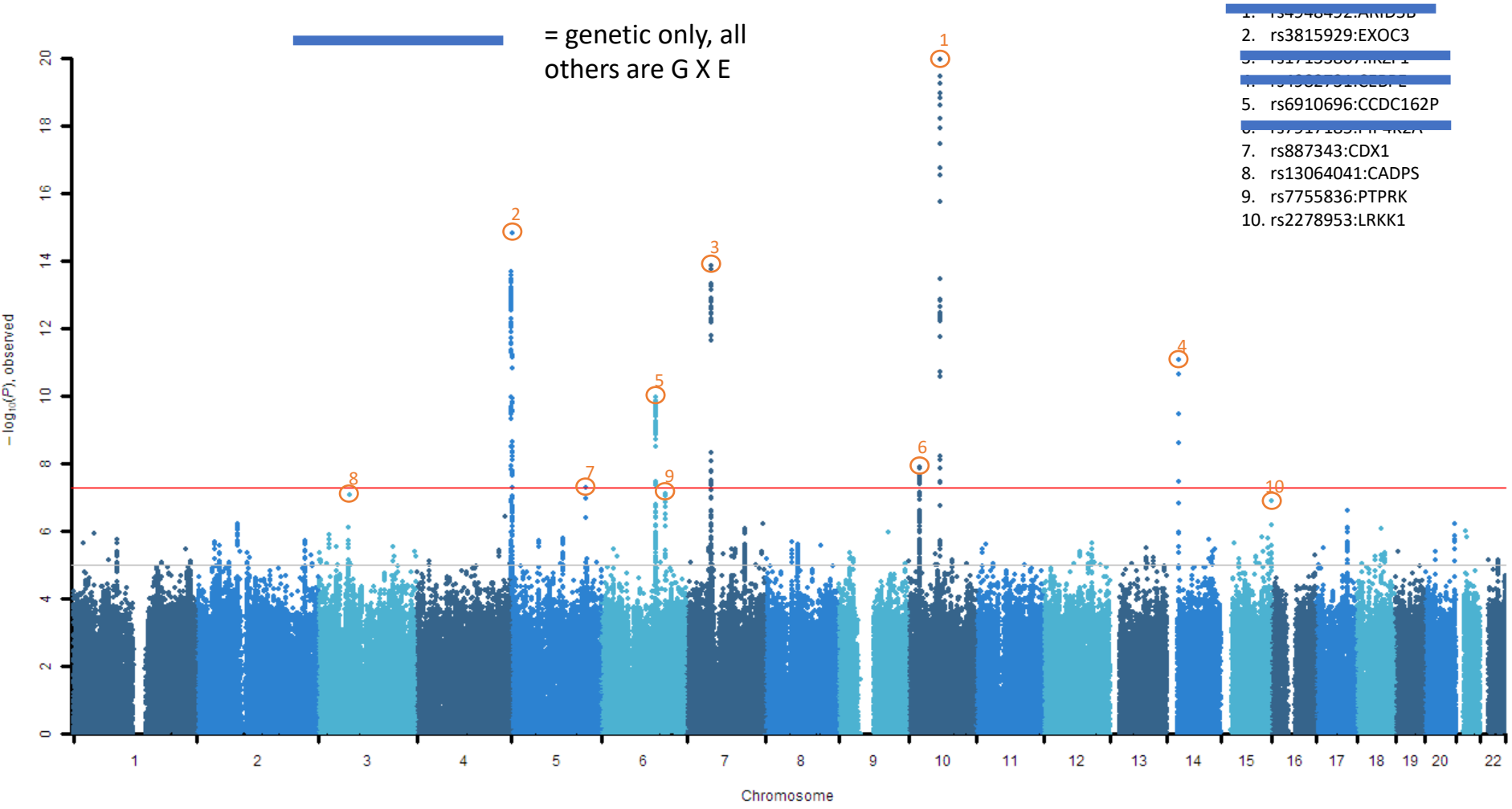
Joubert et al (2016) *Am J Hum Genet*, 98(4):680-96

Development of a smoking biomarker assay based on DNA methylation



Arroyo…… Wiemels, *Biotechniques* 2022

Figure 3. Manhattan plot of 3 degree of freedom test of gene-environment associations and ALL risk

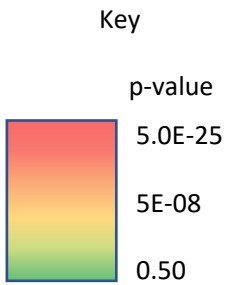


C. Zhong et al. submitted

Germline genetic polymorphisms and tobacco, and leukemia risk

Figure 5. Top 10 Most Significant Regions in 3 Degree of Freedom Gene-Environment Association Test

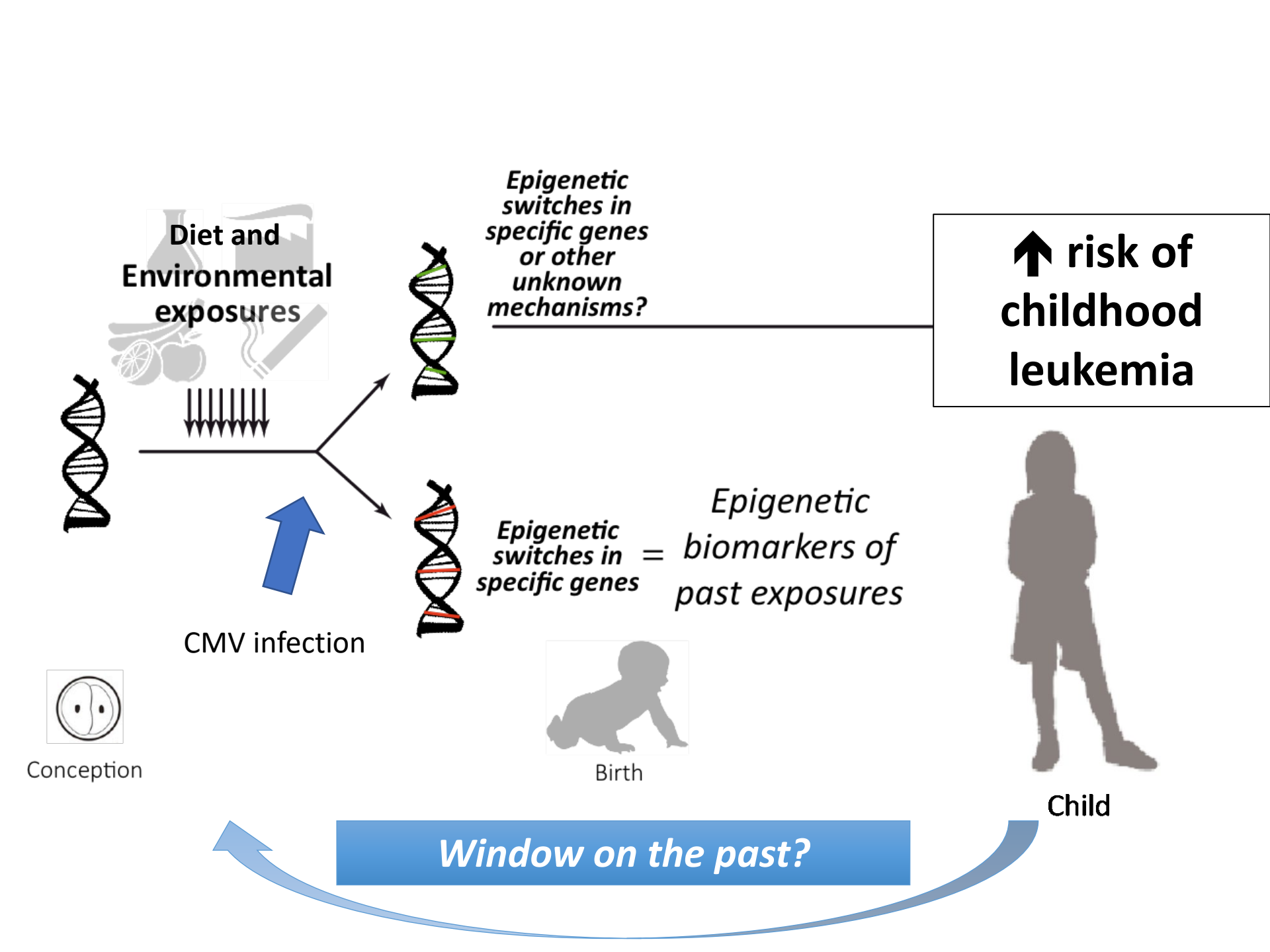
snp	gene		3-DF	D-G	E-G	GxE
rs4948492	ARID5B	Gene only	1.02E-20	3.36E-25	6.68E-01	1.38E-01
rs3815929	EXOC3		1.51E-15	3.81E-01	1.03E-14	6.49E-03
rs17133807	IKZF1	Gene only	1.34E-14	2.70E-18	9.58E-02	5.79E-01
rs4982731	CEBPE		8.10E-12	5.90E-15	1.83E-02	5.18E-01
rs6910696	CCDC162P	Gene only	1.08E-10	6.70E-02	7.57E-14	2.45E-01
rs7917185	PIP4K2A	Gene only	1.22E-08	8.58E-11	9.64E-02	3.58E-01
rs887343	CDX1		5.03E-08	4.14E-04	7.50E-04	7.95E-04
rs13064041	CADPS		8.67E-08	5.27E-06	2.96E-04	9.56E-02
rs7773849	PTPRK	★	9.17E-08	7.67E-01	4.66E-06	1.39E-04
rs2278953	LRRK1		1.24E-07	2.41E-05	1.39E-03	1.28E-03



D – disease (leukemia)
G – gene
E – environment (tobacco)

★ - significant G X E

C. Zhong et al. submitted


Diet and Environmental exposures
Epigenetic switches in specific genes or other unknown mechanisms?
↑ risk of childhood leukemia
Epigenetic switches in specific genes = Epigenetic biomarkers of past exposures
CMV infection
Conception
Birth
Child
Window on the past?

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CIRCLE
The Center for Integrative Research on Childhood Leukemia and the Environment

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