Norwegian Institute of Public Health

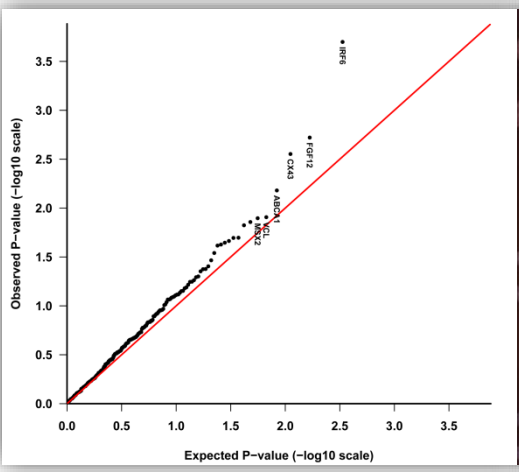
UNIVERSITY OF BERGEN

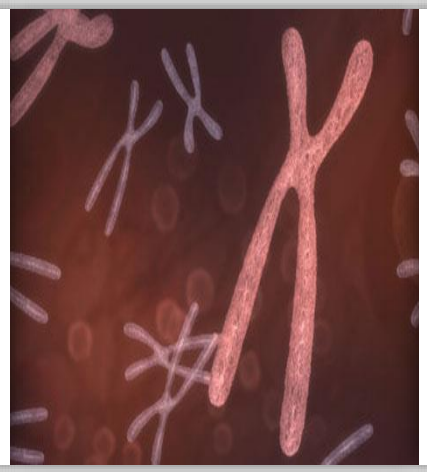
GENTLE INTRODUCTION TO GENETIC EPIDEMIOLOGY

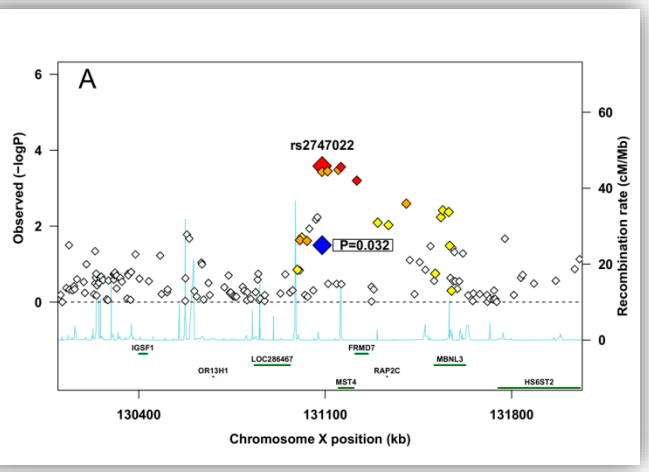
— LECTURE 3 —

Anil Jugessur

Senior scientist, Norwegian Institute of Public Health, Oslo







Lecture Outline

- PART I: Modeling effects
  - Genetic and gene-environmental effects
  - Additive and multiplicative models

- PART II: Modeling effects in family triads
  - Child effects
  - Maternal effects
  - Parent of origin (PoO) effects
  - Interaction of PoO effects by environmental factor (PoOxE)
  - Gene by methylation interaction (GxM) effects

How Do We Define «Exposed» In GEPI Studies?

In essence:

A genetic risk factor is treated as any other epidemiological risk factor.

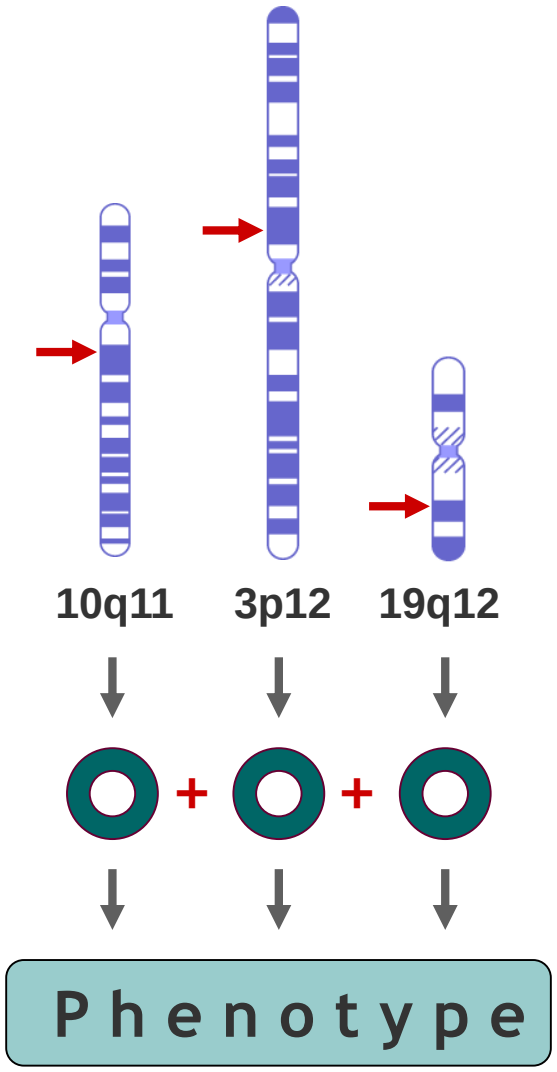
	Disease	No Disease	Total
Exposed	a	b	a + b
Not Exposed	c	d	c + d
	a + c	b + d	N

Odds ratio = ad/bc

- It can represent exposure to an environmental risk factor
  - E.g. smoking, alcohol, drugs, pollutants, low level of essential vitamins, etc.
- It can represent exposure to a genetic risk factor
  - E.g. presence of a particular allele, genotype or haplotype that increase disease risk.

Models Of Genetic Interaction

Additive



Reference: Modified from Passarge (2002). Nature Genetics; 31; 11-12

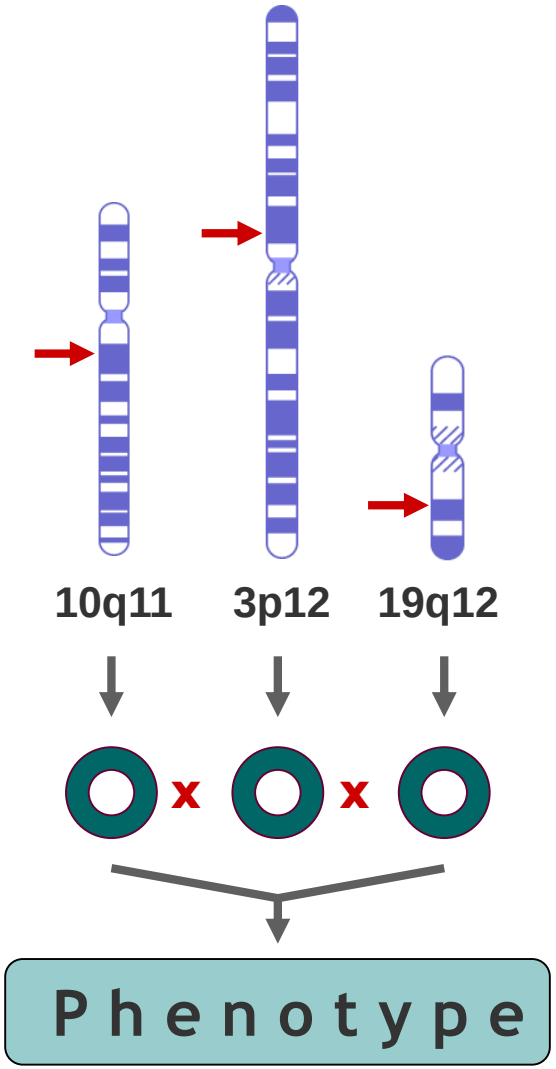
3 susceptibility loci on 3 different chromosomes contribute to the risk of having a disease.

The additive model assumes an individual effect of each locus

The effects of the 3 loci are simply added.

Models Of Genetic Interaction – *Contd..*

Multiplicative



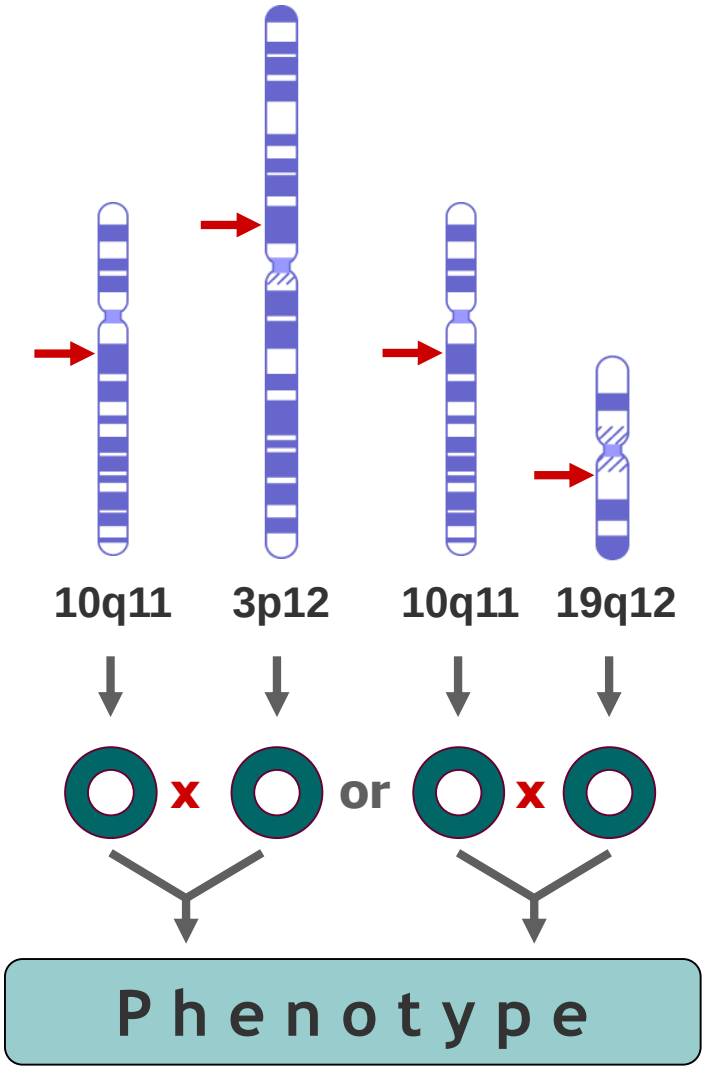
Reference: Modified from Passarge (2002). Nature Genetics; 31; 11-12

All three loci are jointly involved (effects in combination only).

Multiply the individual effects from each locus.

Models Of Genetic Interaction – *Contd..*

Mixed multiplicative



Reference: Modified from Passarge (2002). Nature Genetics; 31; 11-12

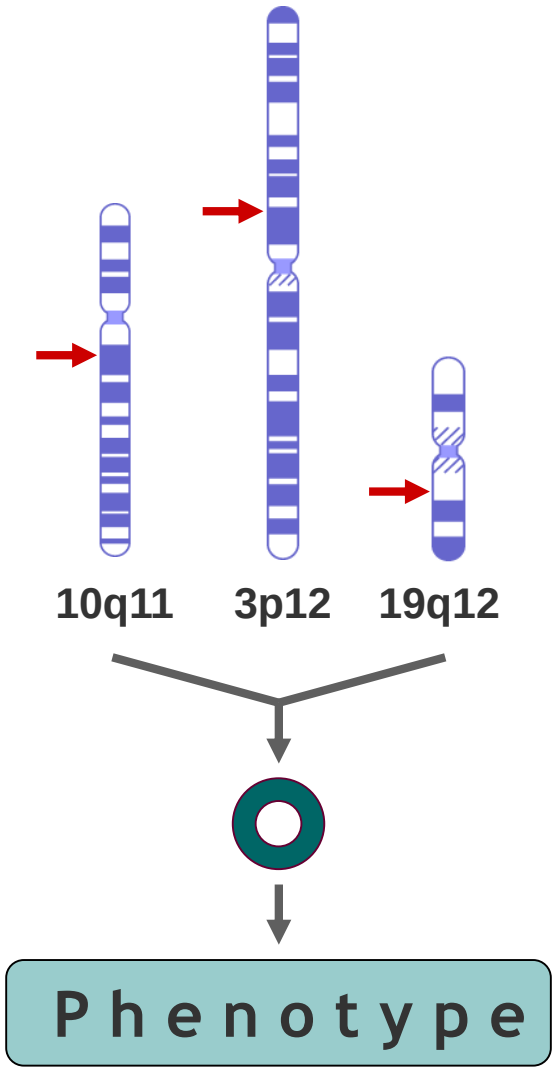
A. In some families: 10q11 + 3p12
B. In other families: 10q11 + 19q12

Effects in either combination A or B only

Product of individual effects between 2 loci

Models Of Genetic Interaction – *Contd..*

Epistasis



Reference: Modified from Passarge (2002). Nature Genetics; 31; 11-12

No individual effects of loci

For an effect, interaction is necessary between all 3 loci

Modeling Interaction

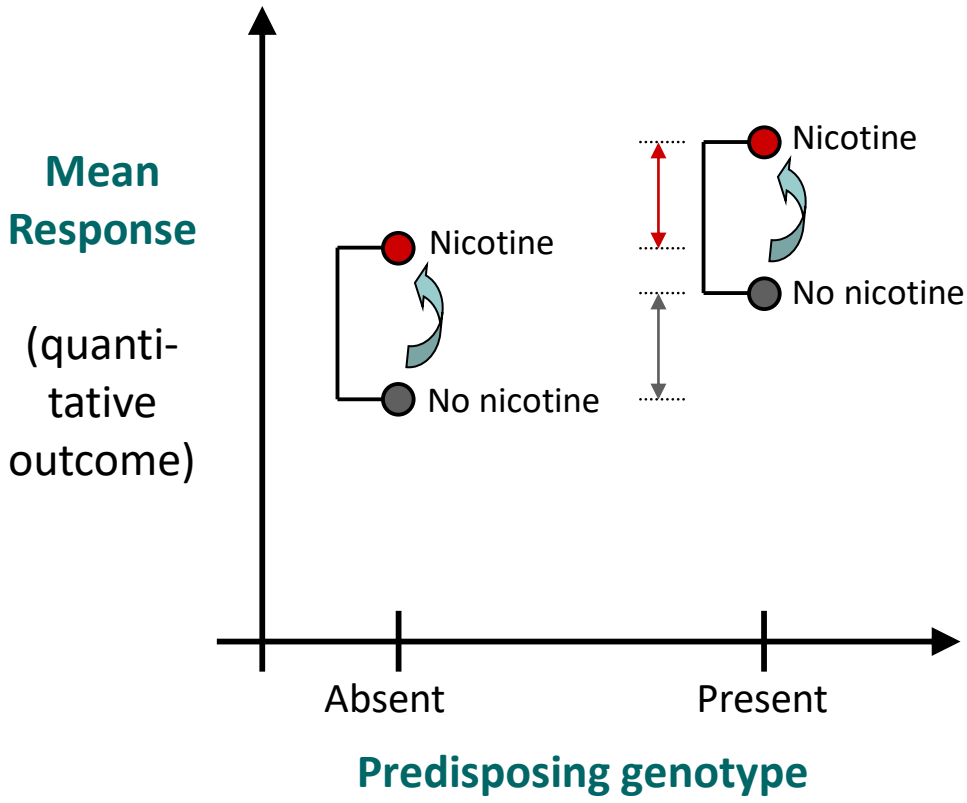
- Statistical interaction: One cannot accurately describe the effect of one factor on an outcome of interest without specifying the level (or value) of the other factor.

- Lack of interaction between two factors, in terms of their impact on an outcome of interest, is referred to as «additivity» of the two factors.
  - Departure from additivity $\Rightarrow$ interaction

MODELING GXE INTERACTION – CH. 11

– AN EXAMPLE OF «ADDITIVITY» –

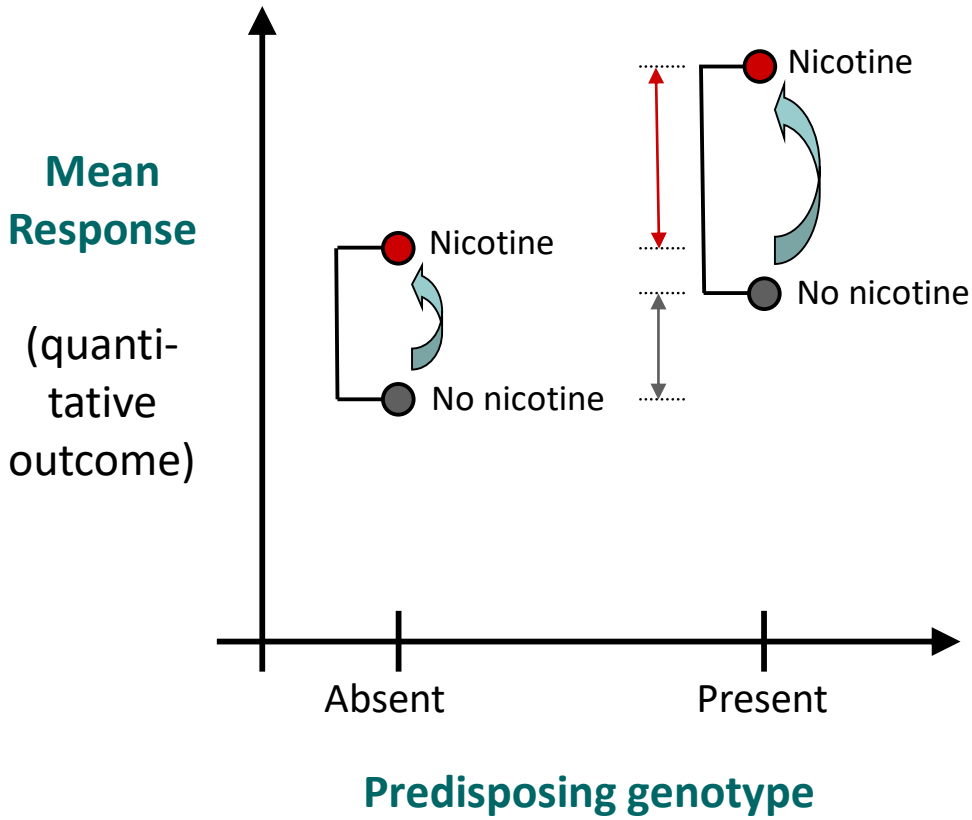
No interaction between the effects of smoking and genotype.



- There is an effect of genotype on risk, but it is independent of nicotine (and vice versa).
- Effect of nicotine is the same regardless of whether the predisposing genotype is present or not (and *vice versa*).
- Here, we can talk about the effect of the genotype <u>without reference</u> to the effect of nicotine.
- This is an example of «***additivity***».

MODELING GXE INTERACTION
– 1ST EXAMPLE OF INTERACTION –

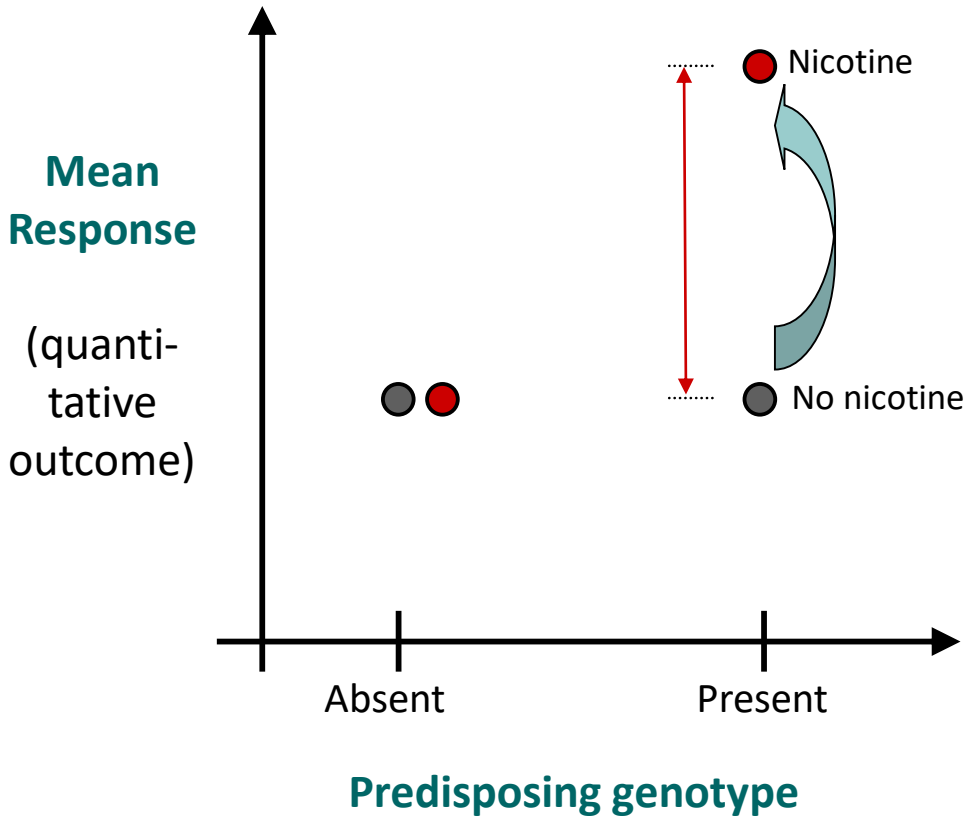
Interaction between the effects of smoking and genotype.



- There is a greater effect of nicotine in the presence of the predisposing genotype
- Here, the effect of nicotine is different depending on the presence/absence of the predisposing genotype (and *vice-versa*)
- One cannot accurately describe the effect of one factor without specifying the level of another factor.
- This is an example of ***non-additivity*** or ***interaction***.

Modeling GxE Interaction
– 2nd Example Of Interaction –

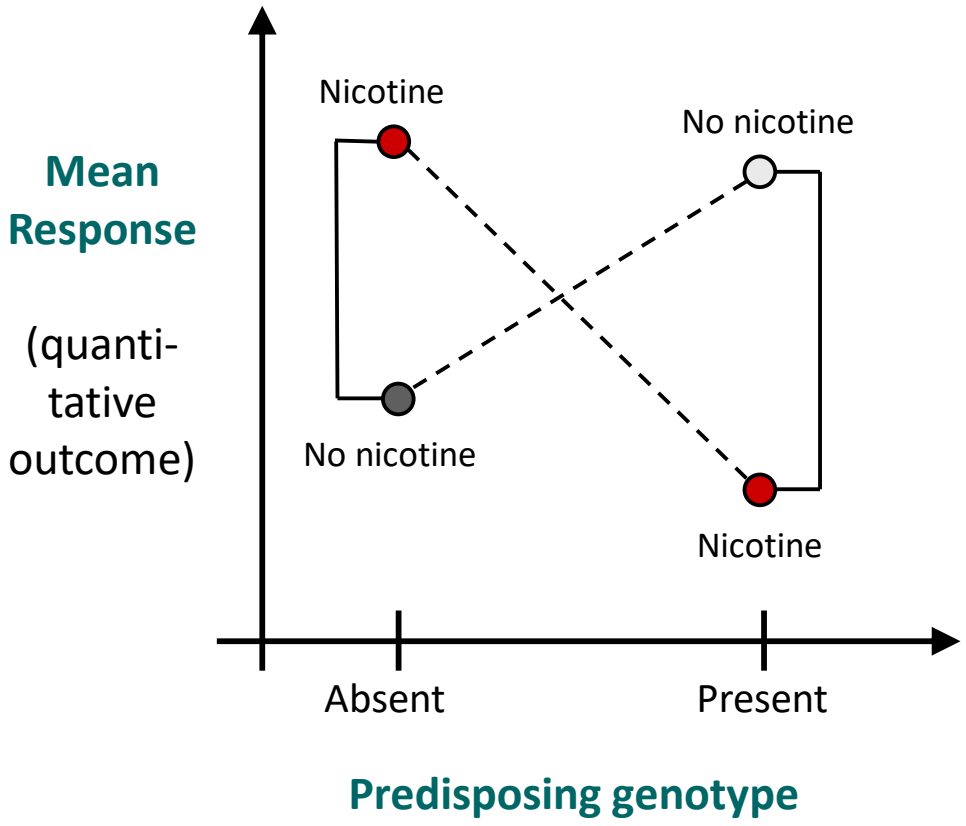
Both nicotine and the predisposing genotype must be present.



- Here ***both*** the environmental factor ***and*** the genotype must be present to increase risk.
- One cannot accurately describe the effect of one factor without specifying the level of another factor.
- This is another example of ***non-additivity*** or ***interaction***.

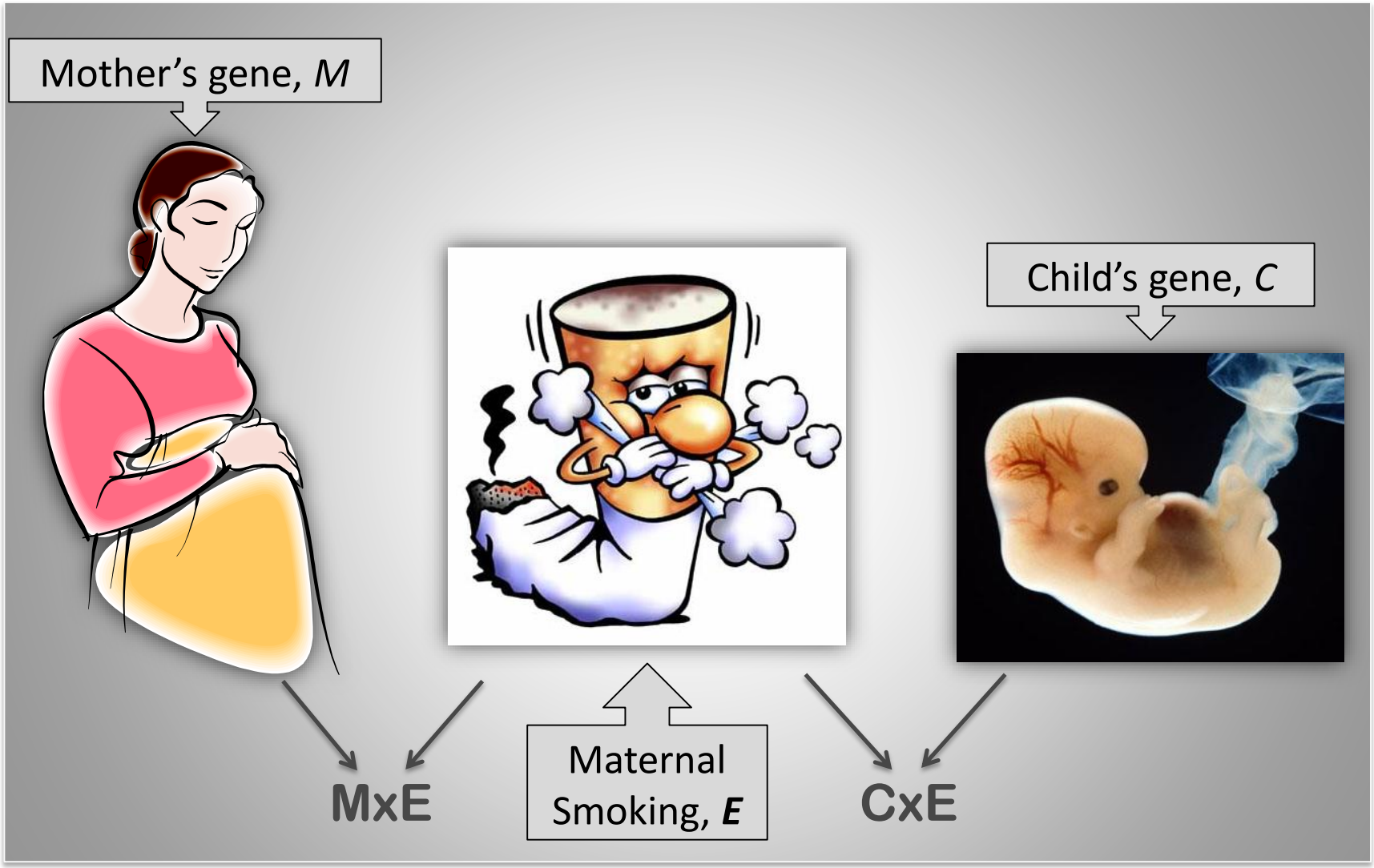
Modeling GxE Interaction
– 3rd Example Of Interaction –

Reversal of effects



- Here the effect of nicotine on outcome is ***reversed***, depending on whether or not the genotype of interest is present!
- This is yet another example of ***non-additivity*** or ***interaction***.

Several Effects to Consider
– e.g. For perinatal disorders –



Two Genomes Moderate Fetal Exposure

Important to examine both fetal and maternal gene-effects:

- Both mother and fetus can metabolize mother's exposures, thus both can affect fetal environment.

- Letterio et al. [1] showed that maternal Tgfb1 could cross the placenta and rescue *Tgfb1*$^{-/-}$ mice.

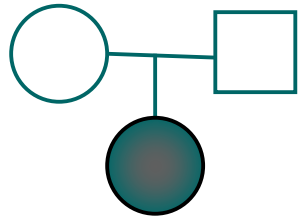
- Popliker et al. [2] showed that maternal epidermal growth factor (Egf) could be transported to the fetus via the placenta.

1. Letterio et al. (1994). Maternal rescue of transforming growth factor-beta 1 null mice. *Science* 264: 1936–1938.

2. Popliker et al. (1987). Onset of endogenous synthesis of epidermal growth factor in neonatal mice. *Dev Biol* 119

ESTIMATING FETAL AND MATERNAL GENE-EFFECTS

The offspring-parent triad design allows modeling both maternal and fetal gene-effects without confounding from one another.



- Several ways in which a variant allele can **increase** risk for disease:

  1) *Only* when carried by the fetus ⇒ "fetal gene-effect".
     - The variant allele will be over-represented in *cases* vs. *biological parents*.

  2) *Only* when carried by the mother ⇒ "maternal gene-effect".
     - The variant allele will be over-represented in *case-mothers* vs. *case-fathers*.

  3) *Both* when carried by the fetus and by the mother.
     - The relative risks for the fetal and maternal contributions can be multiplied together to obtain the joint risk of disease.

ESTIMATING GxE INTERACTION EFFECTS

- **A risk-conferring allele *A* can interact with an environmental agent *E* as follows:**

  1) ***A*** increases risk *only* when carried by the fetus and, at the same time, the fetus is exposed to ***E***.

  - Positive interactive effect between the child's genotype ***C*** and environmental exposure ***E*** $\Rightarrow$ ***CxE* effect**

  2) ***A*** increases risk *only* when carried by the mother and, at the same time, the fetus exposed to the environmental agent ***E*** via the mother.

  - An interactive effect between the mother's genotype ***M*** and the environmental exposure ***E*** $\Rightarrow$ ***MxE* effect**

  3) Mixed scenarios of the above.

- In case-parent triads, GxE interaction is assessed by comparing transmission of risk-allele or risk-haplotype to affected offspring in triads of exposed *vs.* unexposed mothers.
  - Statistically significant difference between the two transmissions would suggest a multiplicative interaction.

g Haplin

OPEN ACCESS Freely available online

PLoS one

Maternal Genes and Facial Clefts in Offspring: A Comprehensive Search for Genetic Associations in Two Population-Based Cleft Studies from Scandinavia

Astanand Jugessur[1,2☯], Min Shi[3☯], Håkon Kristian Gjes[1,4], [4,5], [6]
Clarice Ring Weinberg[3], Kaare Christensen[7], Abee Low
Nguyen[5], Lene Christiansen[7], Andrew Carl Lidral[9], Jeff

OPEN ACCESS Freely available online

PLoS one

Genetic Determinants of Facial Clefting: Analysis of 357 Candidate Genes Using Two National Cleft Studies from Scandinavia

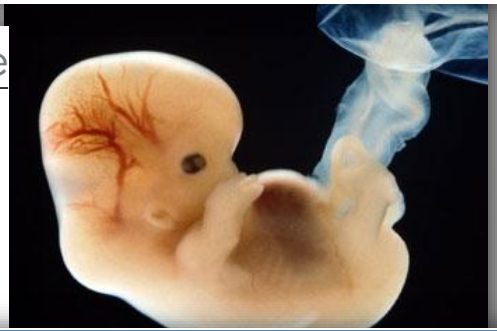
Astanand Jugessur[1☯], Min Shi[2☯], Håkon Kristian Gjessing[3,4], Rolv Terje Lie[4,5], Allen James Wilcox[6], Clarice Ring Weinberg[2], Kaare Christensen[7], Abee Lowman Boyles[6], Sandra Daack-Hirsch[8], Truc Nguyen Trung[5], Camilla Bille[7], Andrew Carl Lidral[9], Jeffrey Clark Murray[7,9*]

OPEN ACCESS Freely available online

PLoS one

X-Linked Genes and Risk of Orofacial Clefts: Evidence from Two Population-Based Studies in Scandinavia

Astanand Jugessur[1,2*☯], Øivind Skare[1,3☯], Rolv T. Lie[3,4], Allen J. Wilcox[5], Kaare Christensen[6,7,8], Lene Christiansen[6], Truc Trung Nguyen[4], Jeffrey C. Murray[9], Håkon K. Gjessing[1,3]



Annals of human genetics

doi: 10.1111/j.1469-1809.2012.00707.x

Application of a Novel Hybrid Study Design to Explore Gene-Environment Interactions in Orofacial Clefts

Øivind Skare[1,2,*,†], Astanand Jugessur[1,3,†], Rolv Terje Lie[2,4], Allen James Wilcox[5], Jeffrey Clark Murray[6], Astrid Lunde[2], Truc Trung Nguyen[4] and Håkon Kristian Gjessing[1,2*]

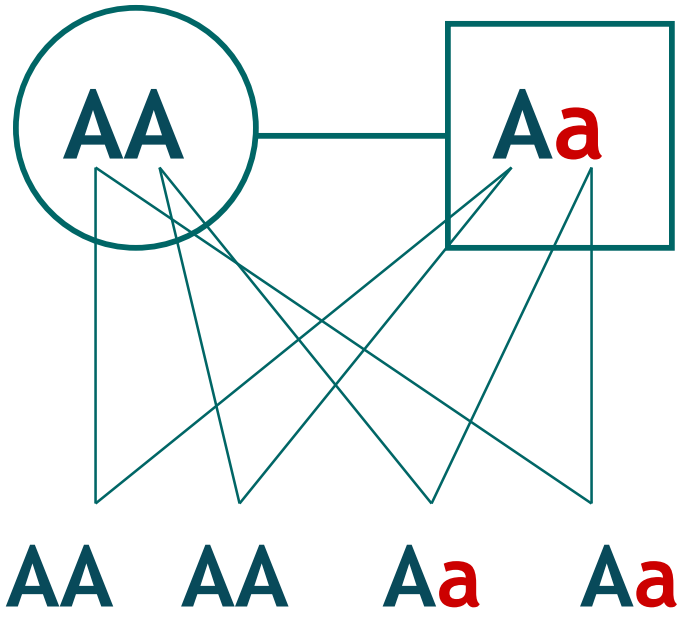
Lecture Outline

PART I: Modeling effects
- Genetic and gene-environmental effects
- Additive and multiplicative models

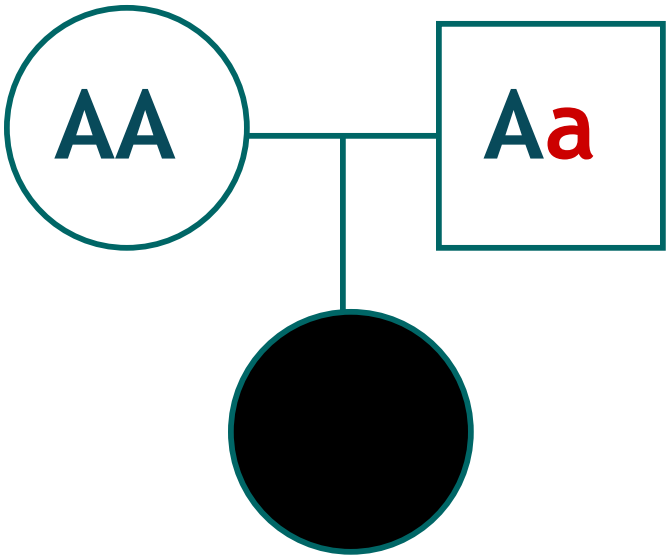
- PART II: Modeling effects in family triads
  - Child effects
  - Maternal effects
  - Parent of origin (PoO) effects
  - Interaction of PoO effects by environmental factor (PoOxE)
  - Gene by methylation interaction (GxM) effects

FAMILY-BASED STUDY-DESIGNS

– A «TRIAD» AS UNIT OF ANALYSIS –



A = normal allele
a = disease-causing allele



Equal #s of **AA** og **Aa** among cases?
Test for this asymmetry!

- Mendelian inheritance tells us that allele **a** and **A** have an equal chance of being transmitted to the next generation.
- If the variant allele **a** is associated with disease risk, it will be overrepresented among affected offspring.

Key assumptions for offspring-parent triad design

1) Mendelian transmission of alleles
   - Alleles **a** and **A** assumed transmitted with equal probability to next generation
   - No differential survival with a given genotype (no survival bias)

2) Mating is symmetric with regard to genotype and choice of partner is independent of genotype (i.e., non-assortative mating)
   - Especially relevant when studying parent-of-origin effects!

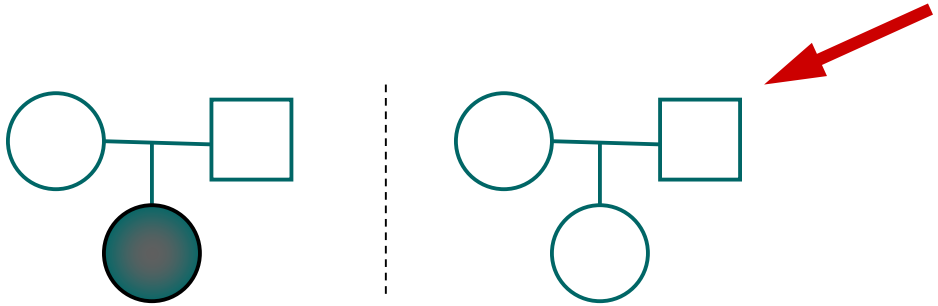
3) In GxE interaction studies, the genotype and environmental exposure are assumed to be independent
   - Distortions may occur if a genetic variant influences the tendency for an individual to be exposed!

Statistical analyses

What can the «case-parent triad» design offer us?

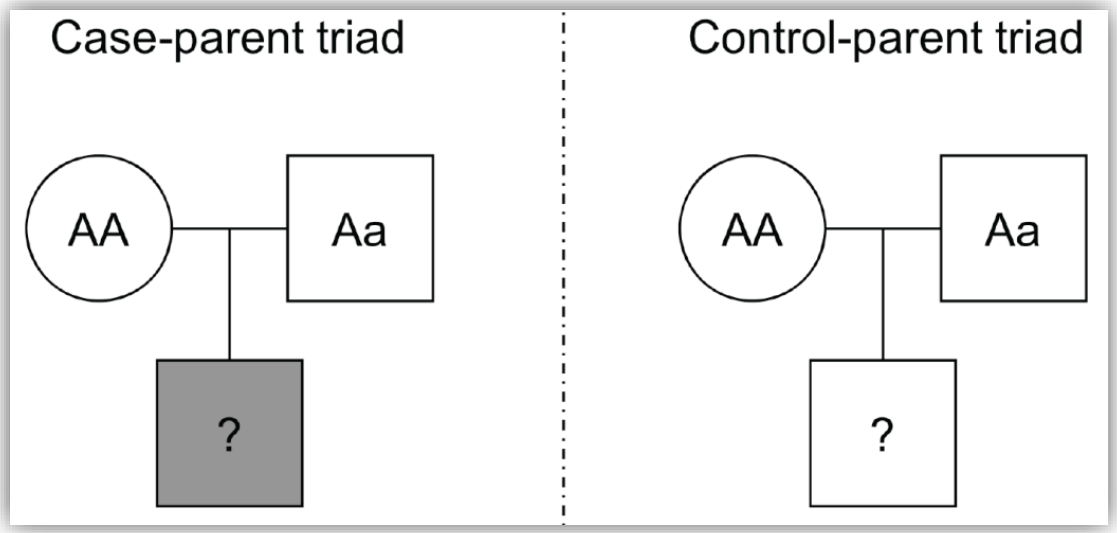
- ✓ Estimate the effects of an allele in the fetus, in the mother, and the effects of imprinted genes (parent-of-origin effects).
- ✓ Interaction of an allele with another allele, or with an environmental exposure (*GxG* and *GxE* interactions).
- ✓ Cannot estimate the *main effect* of an exposure with case-parent triads alone

  $\Rightarrow$ for this we need independent control-parent triads.



Offspring-parent triad design – «Hybrid design»

- Genetic and environmental data collected on 2 groups:
  - Affected offspring and their biological parents (case group)
  - Unaffected offspring and their parents (control group).



- Prerequisites for offspring-parent triad design:
  - It must be possible to obtain DNA from the child's parents
    - Not always possible if disease is typically late-onset – e.g., Alzheimers disease
      - More suitable for early-onset diseases

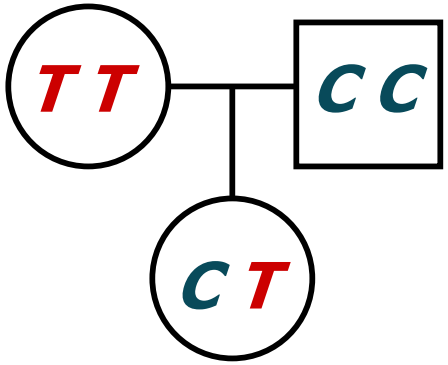
Overview of the 15 possible triad types

M-F C (a-alleles)	Mating-type *	Probability (H-W) **	Probability (Not H-W)
2-2 2	1	p^4	μ_1
2-1 2	2	$p^3(1-p)$	μ_2
2-1 1	2	$p^3(1-p)$	μ_2
1-2 2	2	$p^3(1-p)$	μ_2
1-2 1	2	$p^3(1-p)$	μ_2
2-0 1	3	$p^2(1-p)^2$	μ_3
0-2 1	3	$p^2(1-p)^2$	μ_3
1-1 2	4	$p^2(1-p)^2$	μ_4
1-1 1	4	$2p^2(1-p)^2$	$2\mu_4$
1-1 0	4	$p^2(1-p)^2$	μ_4
1-0 1	5	$p(1-p)^3$	μ_5
1-0 0	5	$p(1-p)^3$	μ_5
0-1 1	5	$p(1-p)^3$	μ_5
0-1 0	5	$p(1-p)^3$	μ_5
0-0 0	6	$(1-p)^4$	μ_6

* Mating symmetry
** Choice of partner is independent of genotype and the allele is in HWE

For e.g., *C*677*T*

M-F C: 2-0 1



Source: Wilcox, Weinberg, and Lie (1998). *Am J Epidemiol* 148;893-901

Case-parent triads – effects of child's alleles

M-F C (a-alleles)	Mating type	Probability (Not H-W)
2-2 2	1	$R_2\mu_1$
2-1 2	2	$R_2\mu_2$
2-1 1	2	$R_1\mu_2$
1-2 2	2	$R_2\mu_2$
1-2 1	2	$R_1\mu_2$
2-0 1	3	$R_1\mu_3$
0-2 1	3	$R_1\mu_3$
1-1 2	4	$R_2\mu_4$
1-1 1	4	$2R_1\mu_4$
1-1 0	4	μ_4
1-0 1	5	$R_1\mu_5$
1-0 0	5	μ_5
0-1 1	5	$R_1\mu_5$
0-1 0	5	μ_5
0-0 0	6	μ_6

Assumption: Within each mating type (1-6), the different triad types (M-F C) are equally probable.

R_2 = effect of **TWO** "a" alleles in the child

R_1 = effect of **ONE** "a" allele in the child

μ_1-μ_6= unknown frequencies

MAIN IDEA: If the allele is associated with disease, the # of triads of a particular mating type will be increased over the expected.

Two genomes moderate fetal exposure

It is thus important to examine both fetal and maternal gene-effects:

- Both mother and fetus can metabolize mother's exposures, thus both can affect fetal environment.
- Letterio et al. [1] showed that maternal Tgfb1 was able to cross the placenta and rescue *Tgfb1*$^{-/-}$ mice.
- Popliker et al. [2] showed that maternal epidermal growth factor (Egf) could be transported to the fetus via the placenta [18].

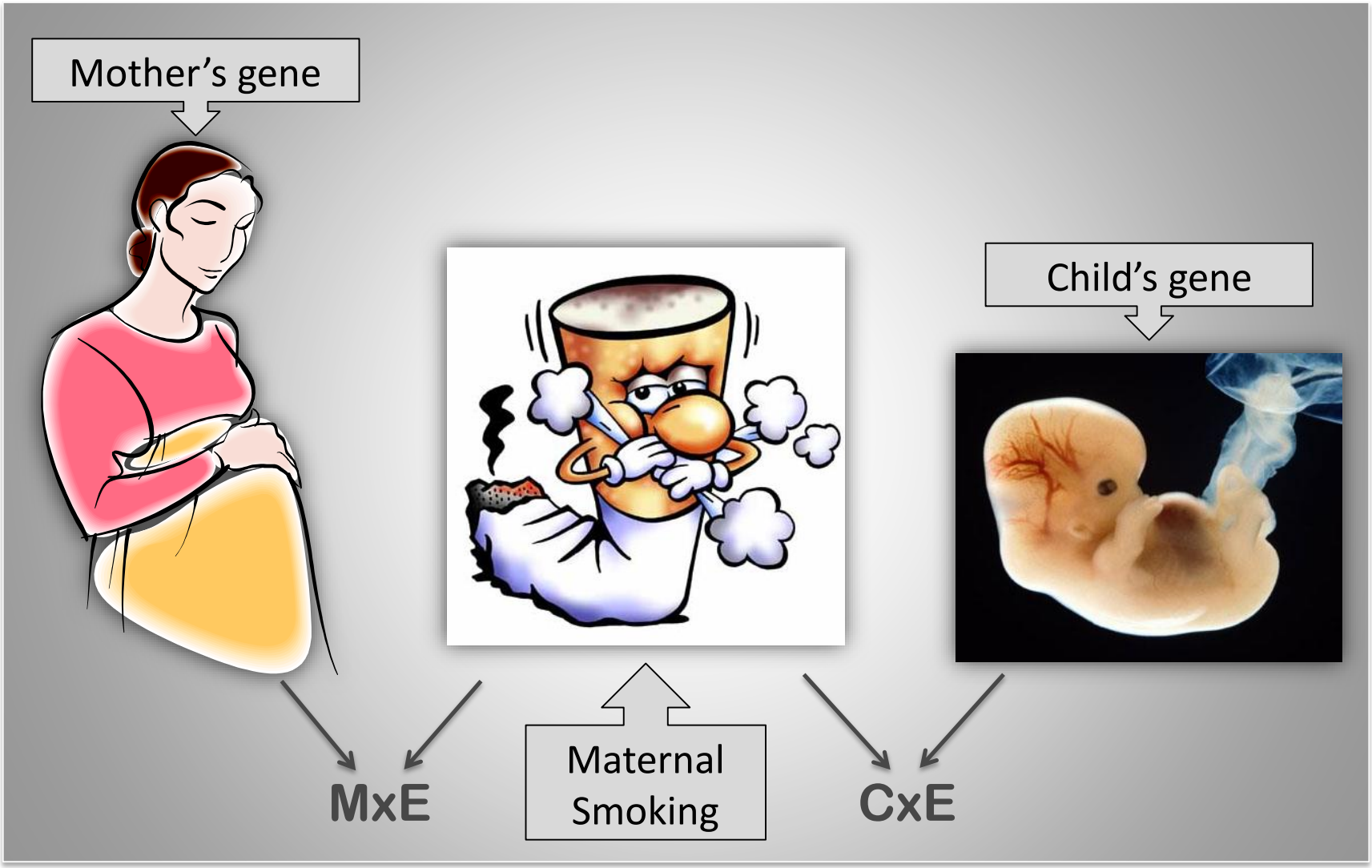
1. Letterio et al. (1994) Maternal rescue of transforming growth factor-beta 1 null mice. Science 264: 1936–1938.

2. Popliker et al. (1987) Onset of endogenous synthesis of epidermal growth factor in neonatal mice. Dev Biol 119

Child and maternal gene effects

- The offspring-parent triad design allows a straightforward modeling of both maternal and fetal gene-effects without confounding from one another.
- There are several ways in which a variant allele can increase risk
  1) The variant allele increases risk *only* if carried by the fetus.
     - "**Fetal gene-effect**": The variant allele will be over-represented in the cases compared to the biological parents.
  2) The variant allele increases risk *only* if carried by the mother.
     - "**Maternal gene-effect**": The variant allele will be over-represented in the case mothers compared to the case fathers.
  3) The variant allele increases risk *both* when carried by the fetus and by the mother.
     - The relative risks for the fetal and maternal contributions can be multiplied together to obtain the joint risk of disease.

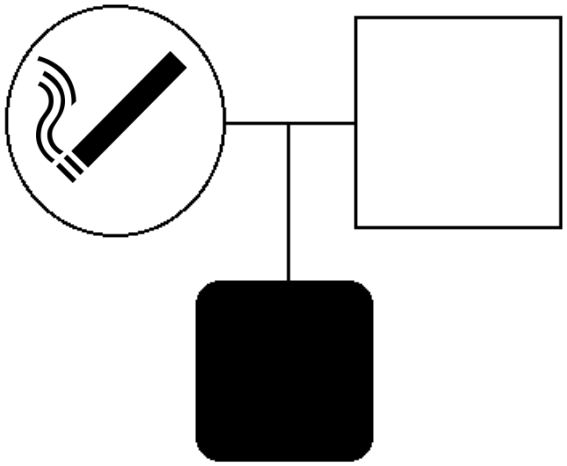
Gene-environmental (GxE) effects
- Maternal 1st-trimester smoking as an example -



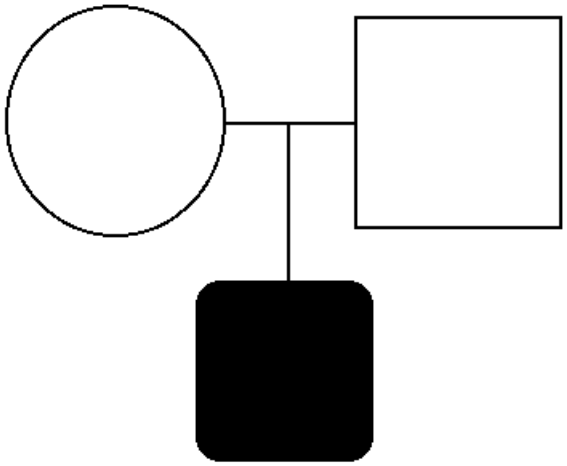
Interaction with environmental exposure – Case-parent triad design –

- Separate triads into ”**exposed**” and ”**unexposed**” groups, and analyze the change in gene-effects across the two strata.
- Compare the transmission of the risk-allele or risk-haplotype to affected offspring in triads of **exposed** *vs.* **unexposed** mothers.
- A statistically significant difference between the two transmissions would suggest a GxE interaction.

Exposed



Unexposed



Interpretation of a GxE interaction analysis

- **A risk-conferring allele can interact with an environmental exposure as follows:**

  1) The allele increases risk *only* when carried by the fetus and, at the same time, the fetus is exposed to the environmental agent E.

  - Here we expect to observe a positive interactive effect between the child's genotype and the environmental exposure – **CxE effect**

  2) The allele increases risk *only* when carried by the mother and, at the same time, she is exposed to the environmental agent E.

  - Here we expect to observe a positive interactive effect between the mother's environmental exposure and her genotype – **MxE effect**

  3) Mixed scenarios of the above.

Summary of effects to model/estimate

- Major gene effects
  - Fetal gene effects, ***C***
  - Maternal gene effects, ***M***
- Gene-gene (GxG) interactive effects
  - Interactions between genes in a specific biological pathway
- Gene-environment (GxE) interactive effects
  - Fetal gene ⇔ Maternal exposure – ***CxE***
  - Maternal gene ⇔ Maternal exposure – ***MxE***
- Parent-of-origin (PoO) effects
  - Effect of imprinted genes
  - PoOxE effects

Lecture Outline

- PART I: Modeling effects
  - Genetic and gene-environmental effects
  - Additive and multiplicative models

- PART II: Modeling effects in family triads
  - Child effects
  - Maternal effects
  - Parent of origin (PoO) effects
  - Interaction of PoO effects by environmental factor (PoOxE)
  - Gene by methylation interaction (GxM) effects