Trainee Omics Group (TOG) • BC Children's Hospital Research Institute • 22 March 2021

Poster Hospital

PRACTICAL DESIGN GUIDELINES FOR CONCEPT AND DATA GRAPHICS ON A LARGE CANVAS

Martin Krzywinski

http://mkweb.bcgsc.ca

Canada's Michael Smith Genome Sciences Centre at BC Cancer

Vancouver, Canada

Poster Hospital http://mkweb.bcgsc.ca/poster.design/

Best titles are short conclusions, not long introductions.

Martin Krzywinski mkweb.bcgsc.ca/poster.design
Canada's Michael Smith Genome Sciences Center bcgsc.ca

POSTER CHILD OF SCIENCE

A poster is your first opportunity to organize and communicate your reasearch to members outside of your lab. It will help you to practise telling and "drawing" your science story and its design should be based on its concepts, themes and transitions.

Most posters are bad not because they are ugly (they are) but because they fail to present concisely what was done and, more importantly, why it was done. Most posters have too much on them. Less is more: get to the point, then stop.

ESTABLISH A STORY PATH and stick to it. Saliently and intuitively code key contrasts (e.g. healthy/disease, wildtype/mutant).

The reader won't know what is important, so tell them.

WHY
WHAT
HOW
ALSO
AND
THIS

USE COLOR FOR THEMES OR DATA ENCODING and not as garnish. The first color to appear should begin the core story. Avoid unintentional emphasis by equalizing for perceptual luminance.

ALL SCIENCE DESERVES EXCELLENT EXPLANATIONS

Explain quickly and clearly. Motivate why the work was done—what is the cost of not doing it?

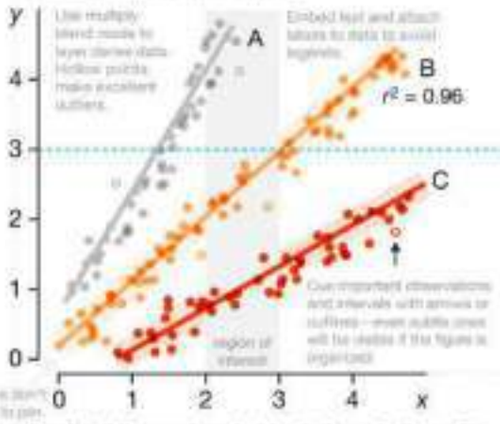
The poster is your prop. In most settings, you will be there to present it. Match its content to the story you will tell.

ONLY YOU CAN STOP POSTER DUMPSTER FIRES

Clip art, pie charts, bullet points, boxes around text, background fills and gradients. Only you can stop it.

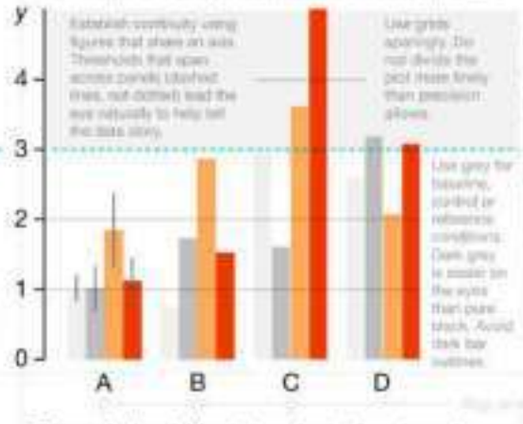
Maintain good Gestalt—similar shapes and colors will form groups. Use them to encode real-world relationships and be on the lookout for unintended accidental groupings.

1 Use figure titles to explain trends, not merely to specify the axes.



▲ Don't tell the reader what is obvious: "a linear fit to a scatter plot" is redundant. Don't tell me what I'm seeing—interpret the figure instead. Italicize variables in fit diagnostics and use shaded bands for confidence intervals. Highlight regions of interests with a solid color (or grey), not outlines.

2 Share axes or align panels to clarify variables or emphasize changing scale.



▲ Categorical variables in bar charts do not need an explicit axis. Specify sample sizes and what error bars represent (e.g. standard error of mean, $n = 5$). Report P-values with effect sizes or confidence intervals. A statistically significant observation isn't necessarily of biological interest.

3 Reveal qualitative trends in small multiples with order, cutoffs and cues.

▲ Look for opportunities to include key observations (●) and explanations in the figure—don't leave it to the main text, where it may be far from the graphic. Emphasize what quantities are important—anticipate the reader's questions and answer them.

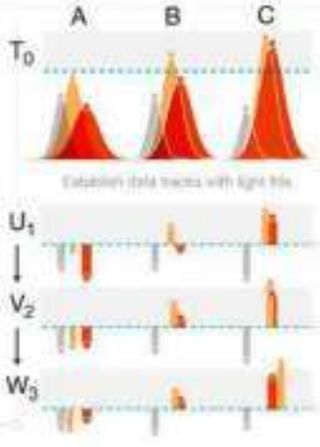
USE SMALL MULTIPLES

Use ink sparingly to make compact figures legible—dense is not necessarily crowded. Explain an encoding once and reuse it. Create a visual key for complex encodings and choose graphical explanations over text.

Avoid visual complications that are not relevant—for example, color blending can create distracting intersections of color. Superimpose white outlines to emphasize shapes with an opaque fill.

TABULATE plots and text seamlessly with a column or row for explanations. Italicize text with care and look for unintended italics in subscripts.

ARROWS imply a relationship or change. Do not use them to guide the eye, which can be achieved with spacing and alignment.

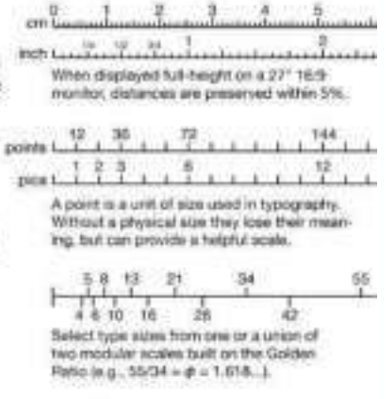


MAINTAIN AND CONTROL PROPORTIONS

This poster is 16" × 12" (1152 × 864 pt), uses Helvetica Neue with a 5, 8, 13, 21, 34, 55 pt scale ladder and is legible on most screens.

Sans-serif is clearer than serif at small sizes and suitable for modest amounts of copy.

Keep line length short and hyphenate instead of fully justifying.



EVERYTHING IS IMPORTANT, BUT SOME THINGS ARE MORE IMPORTANT THAN OTHERS. Establish a visual hierarchy by emphasizing your hypothesis, conclusion and the key points that connect them. Relegate protocols, technical methods, and other minutiae to the bottom of the poster. Always be mindful of what the reader needs to know to understand enough to ask insightful questions and frontload this information.

FORCE LINE BREAKS that split a sentence into noun phrases or offer a natural pause, such as at a comma or a period. Balance layout by shortening sentences—there are many ways to say something and some ways are easier to typeset. Do not let a template bully you into using a specific column width. Change proportions to suit content.

AVOID OBVIOUS HEADINGS such as "references". Citations can be set in a block of text, with bold numbers like this **1**. R. Bringhurst, *Elements of Typographical Style*. 4th ed (2012) and **2**. W. Strunk Jr., *Elements of Style* (1918). Unless a specific citation style is required, use a compact style that also includes the title.

you are the steward of your science

Show your passion for the subject.

Be a good explainer.

Resist visual tropes, fluff and garnish.

design is a process

Thank you for your submissions!

My redesigns are not the “best and only” options. They’re merely better options.

When I first look at a figure, I typically know what needs fixing
but I don’t always know how to fix it.

design is a set of choices

When you speak, you generally know

what / why / why now

you’re saying something.

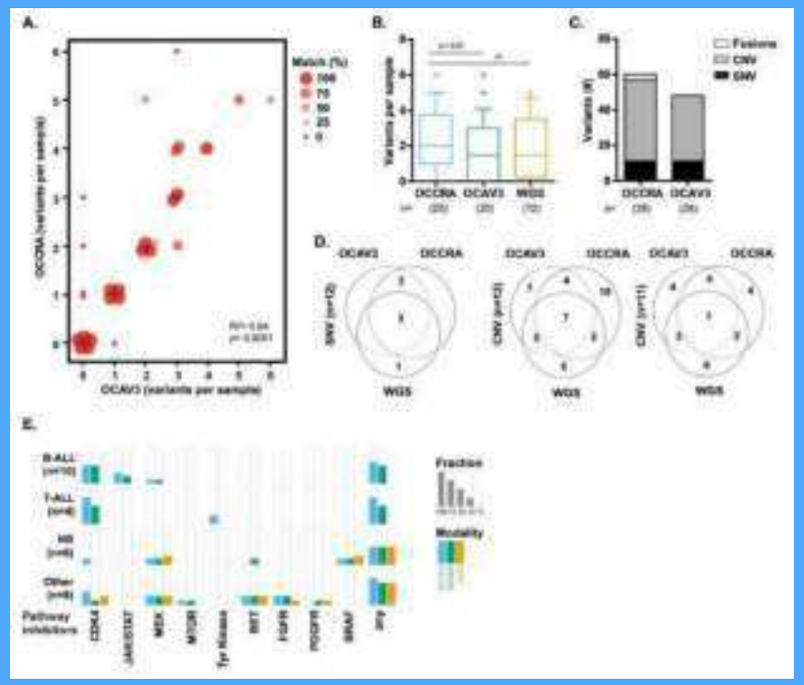
It’s the same with design except time
is replaced by space.

you're 90% of the tool

Good design is never due to software expertise.

Know your software enough to make your ideas possible.

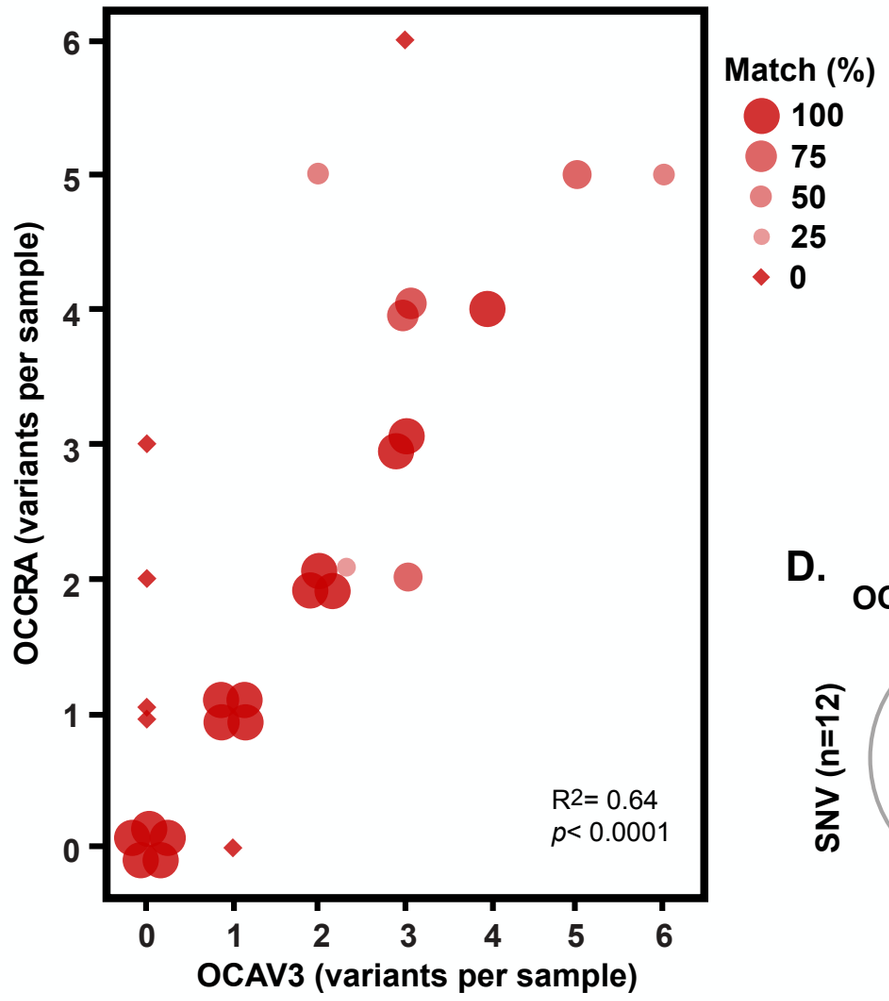
Learning tool XYZ will not make you a better communicator.



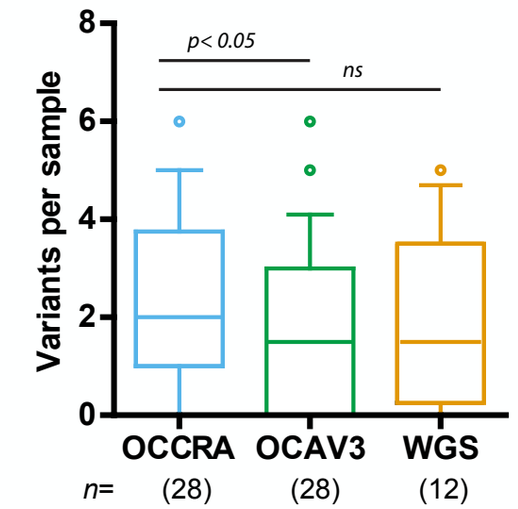
emphasize data

make other elements visually subordinate

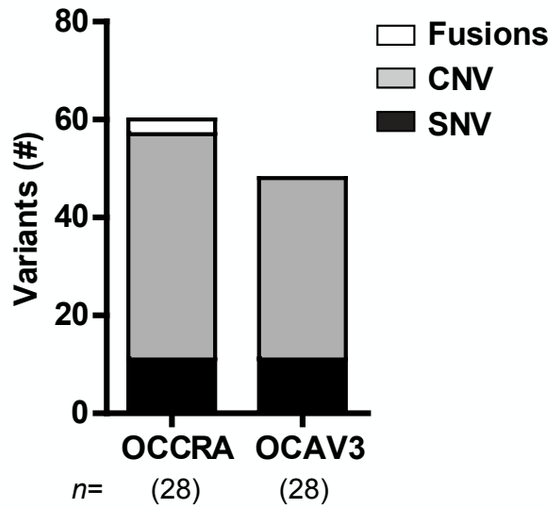
A.


OCCRA (variants per sample)
OCAV3 (variants per sample)
R2= 0.64
p< 0.0001
Match (%)
100
75
50
25
0

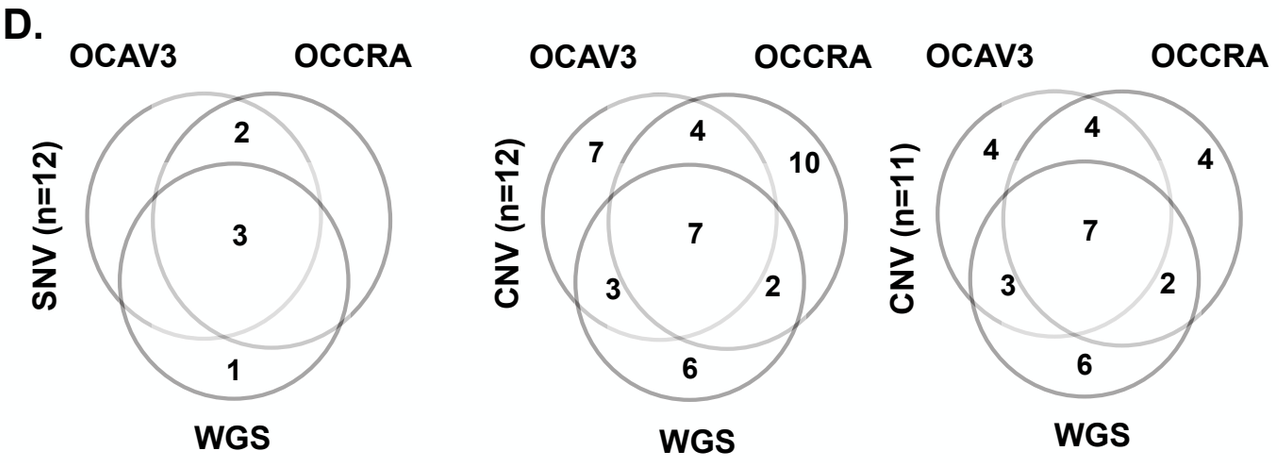
B.


Variants per sample
p< 0.05
ns
OCCRA
OCAV3
WGS
n= (28) (28) (12)

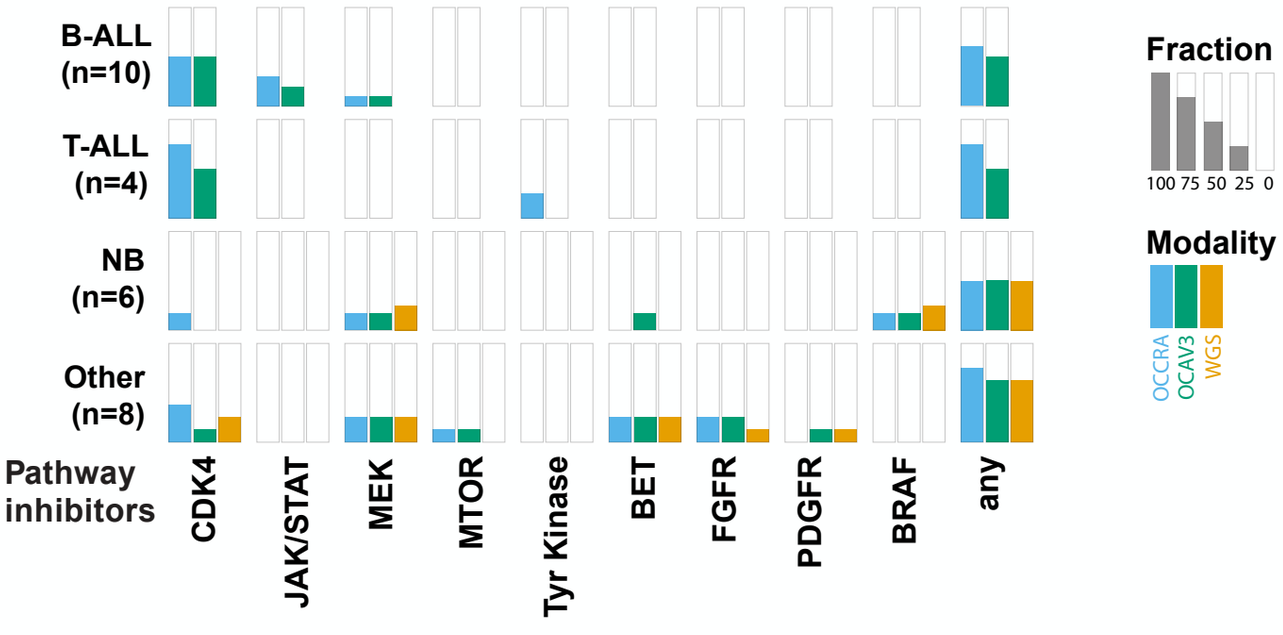
C.


Variants (#)
Fusions
CNV
SNV
OCCRA
OCAV3
n= (28) (28)

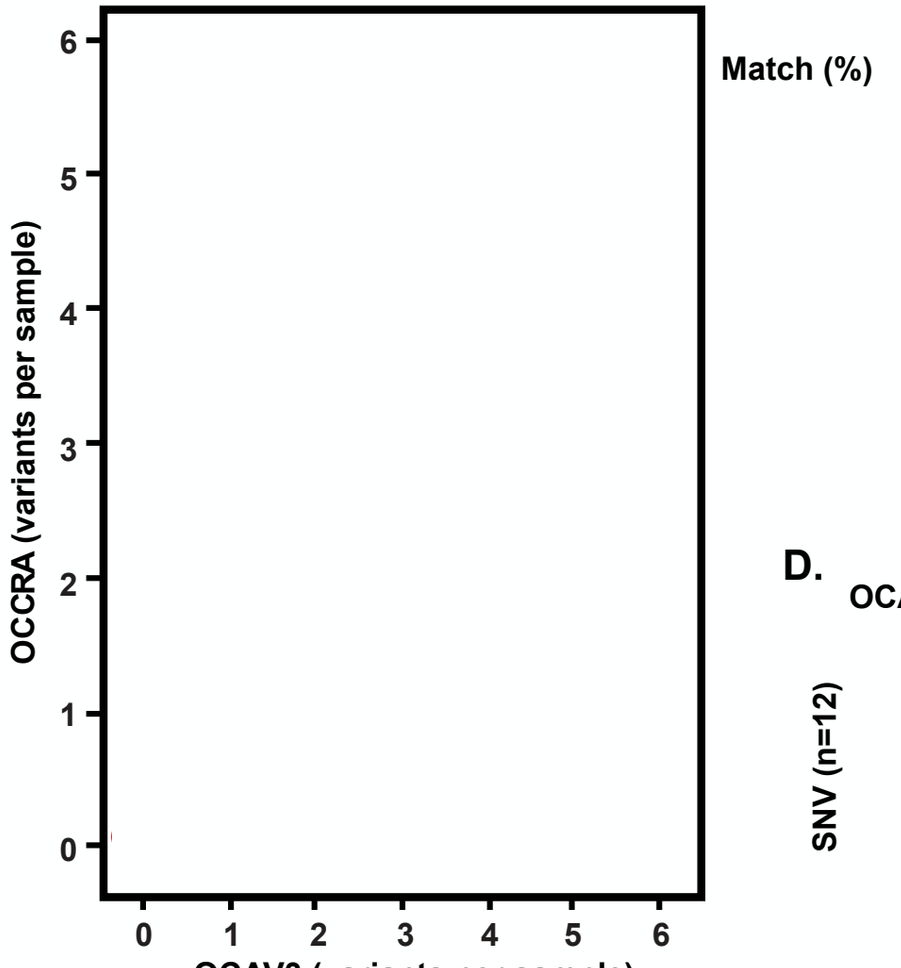
D.


OCAV3
OCCRA
SNV (n=12)
CNV (n=12)
CNV (n=11)
WGS

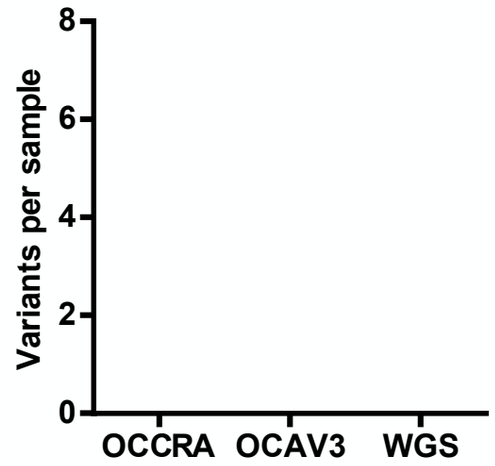
E.


B-ALL (n=10)
T-ALL (n=4)
NB (n=6)
Other (n=8)
Pathway inhibitors
CDK4
JAK/STAT
MEK
MTOR
Tyr Kinase
BET
FGFR
PDGFR
BRAF
any
Fraction
100 75 50 25 0
Modality
OCCRA
OCAV3
WGS

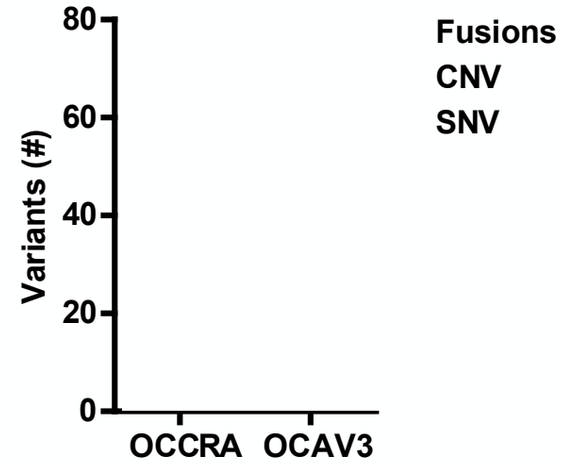
A.


Match (%)
OCCRA (variants per sample)
0
1
2
3
4
5
6
0 1 2 3 4 5 6
OCAV3 (variants per sample)

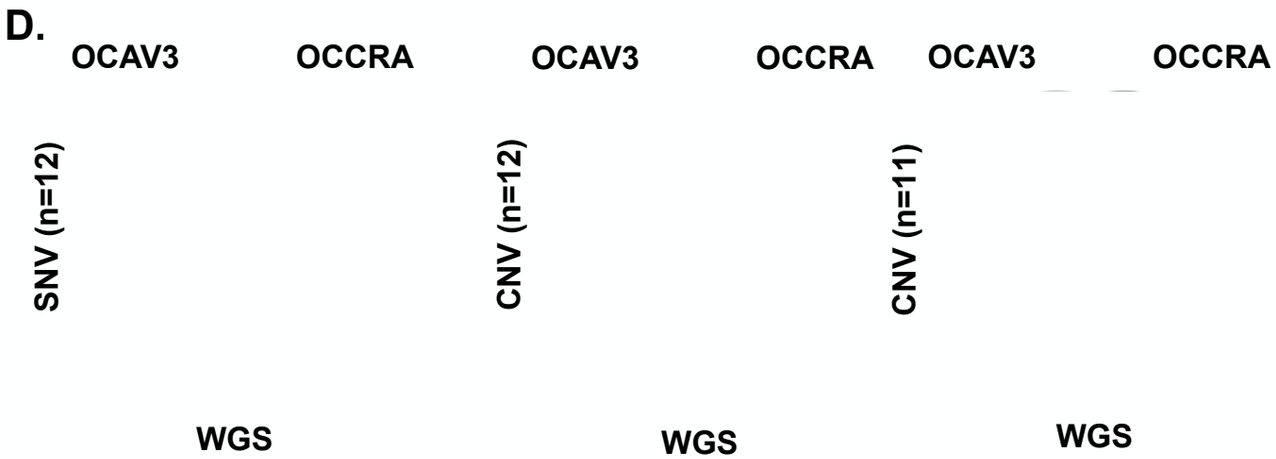
B.


Variants per sample
0
2
4
6
8
OCCRA
OCAV3
WGS

C.

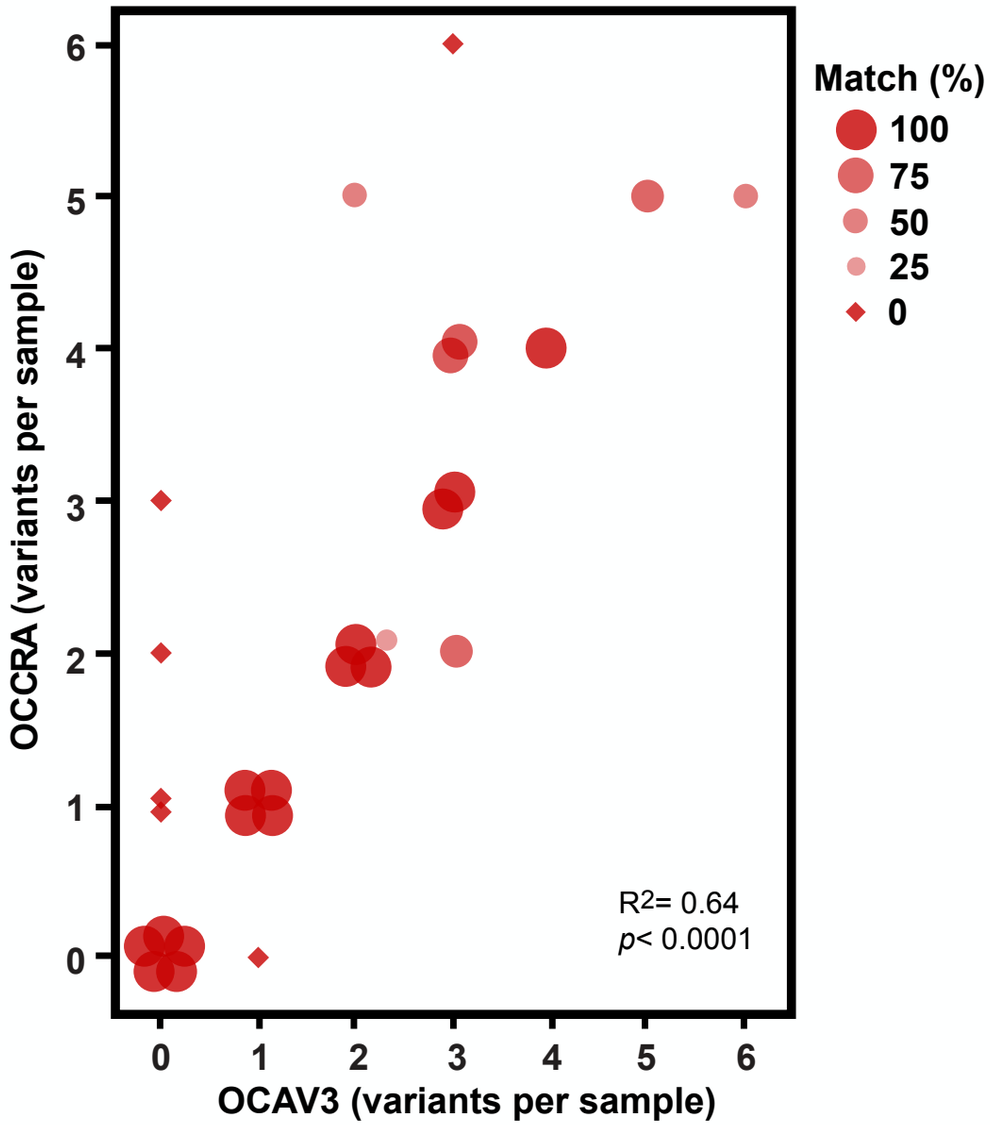

Variants (#)
0
20
40
60
80
OCCRA
OCAV3
Fusions
CNV
SNV

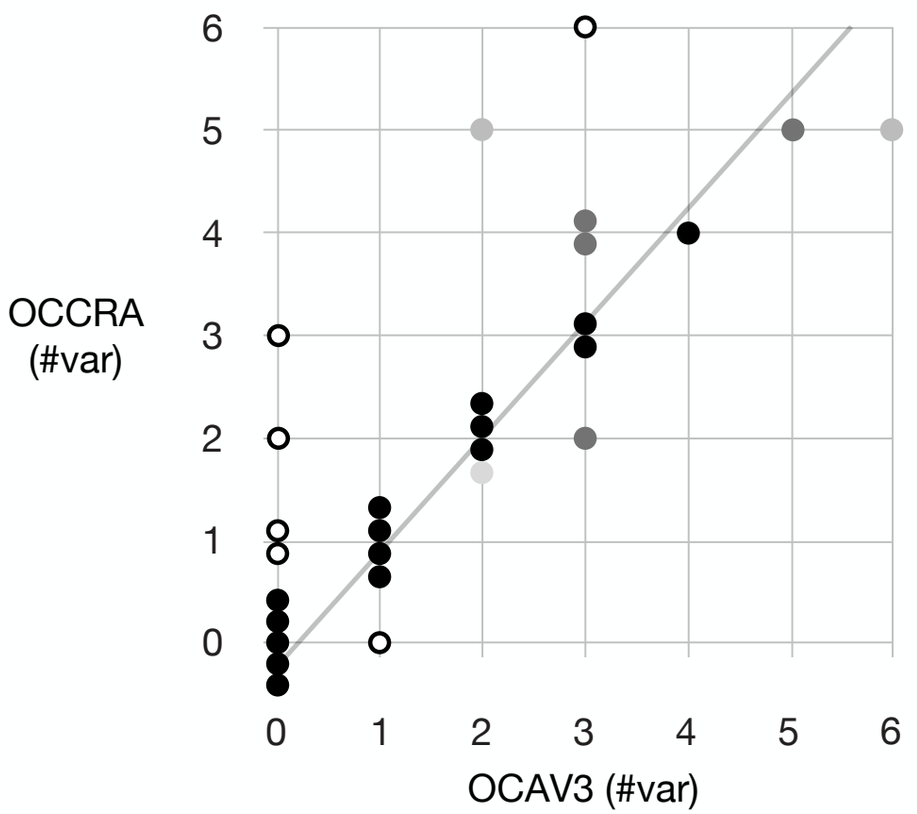
D.

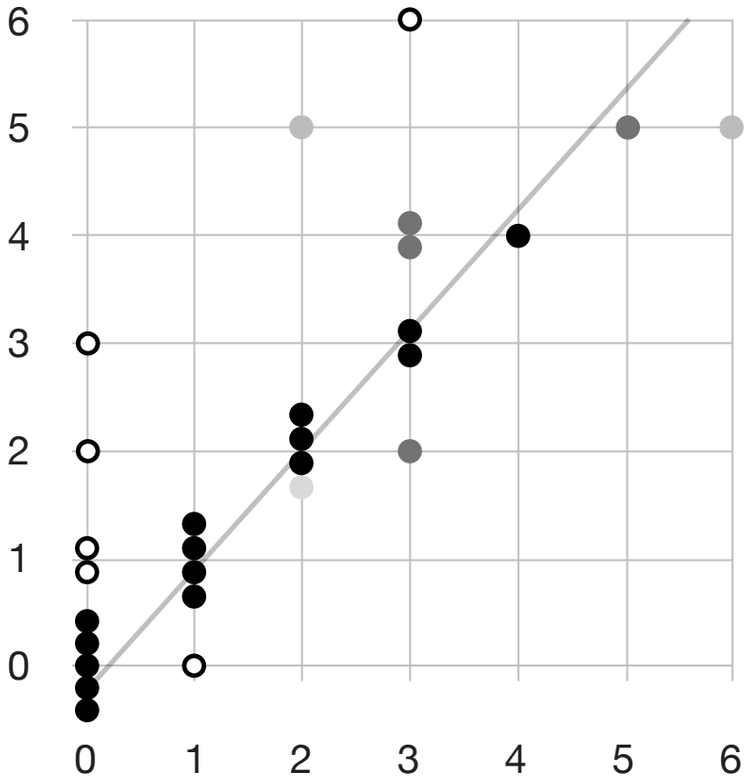

OCAV3
OCCRA
OCAV3
OCCRA
OCAV3
OCCRA
SNV (n=12)
CNV (n=12)
CNV (n=11)
WGS
WGS
WGS

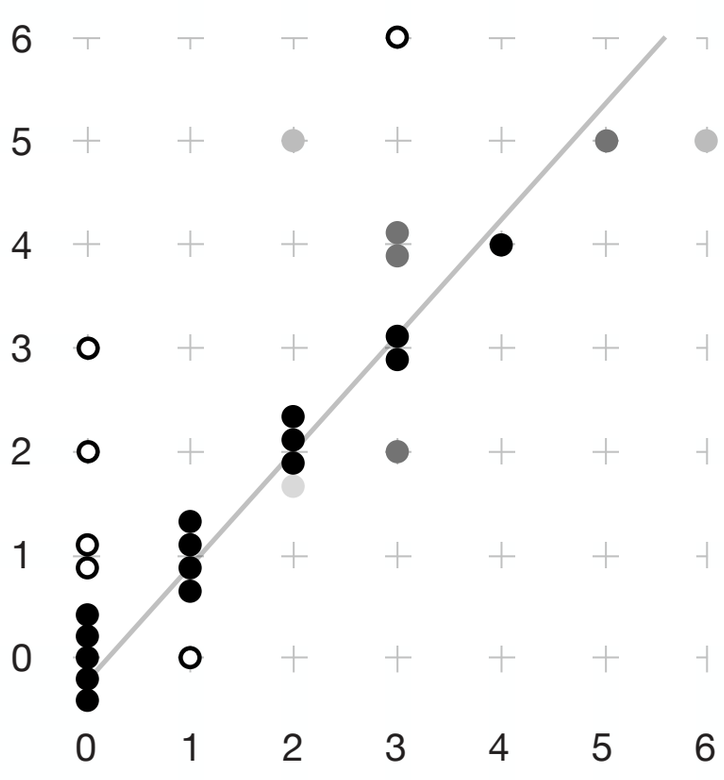
E.

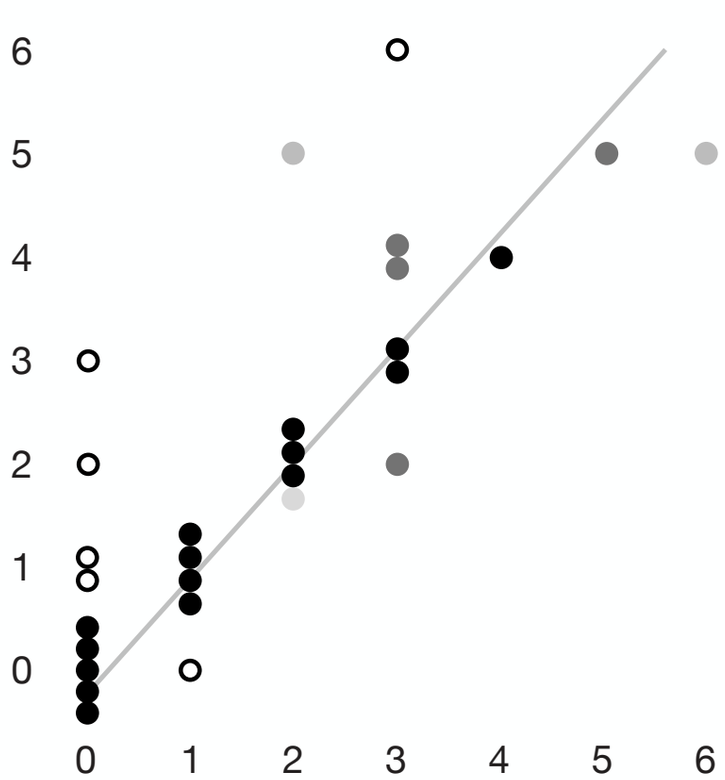

B-ALL (n=10)
T-ALL (n=4)
NB (n=6)
Other (n=8)
Pathway inhibitors
CDK4
JAK/STAT
MEK
MTOR
Tyr Kinase
BET
FGFR
PDGFR
BRAF
any
Fraction
Modality

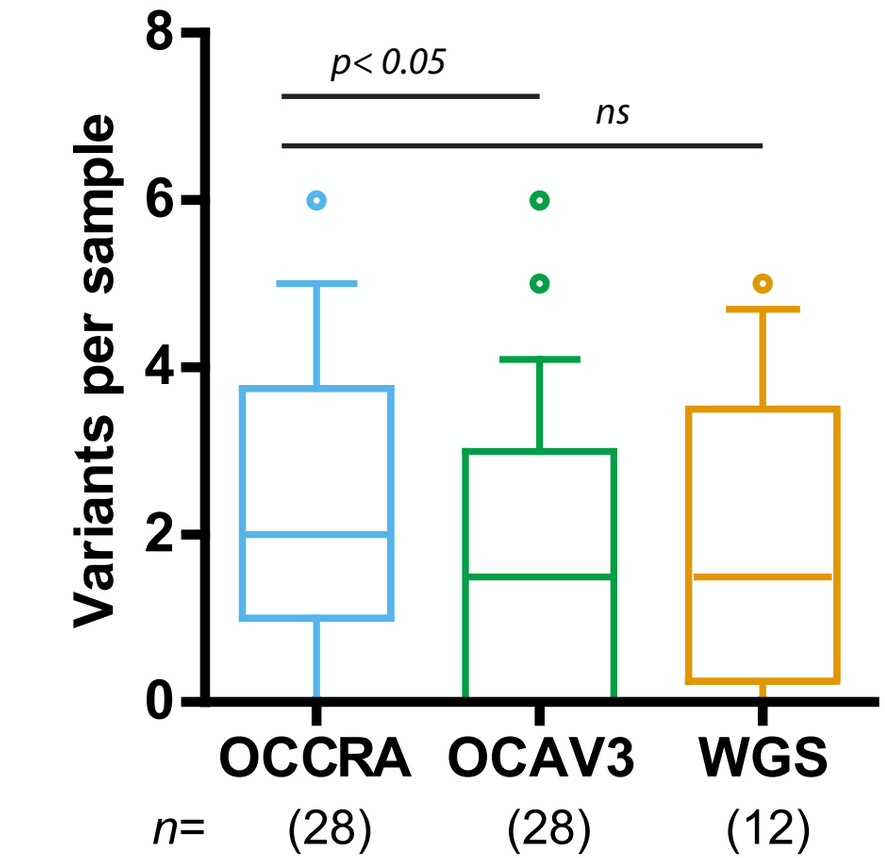

Match (%)
100
75
50
25
0
OCCRA (variants per sample)
OCAV3 (variants per sample)
R2= 0.64
p< 0.0001

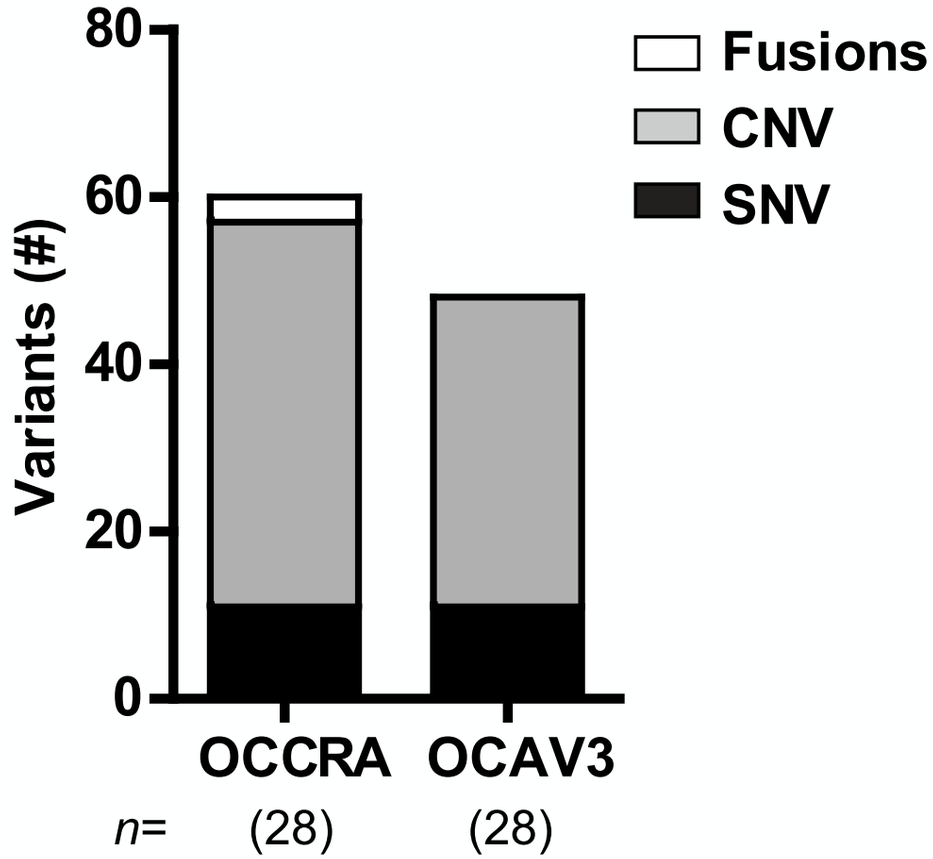

OCCRA
(#var)
match (%)
100
75
50
25
0
R2 = 0.64
p < 0.0001
OCAV3 (#var)

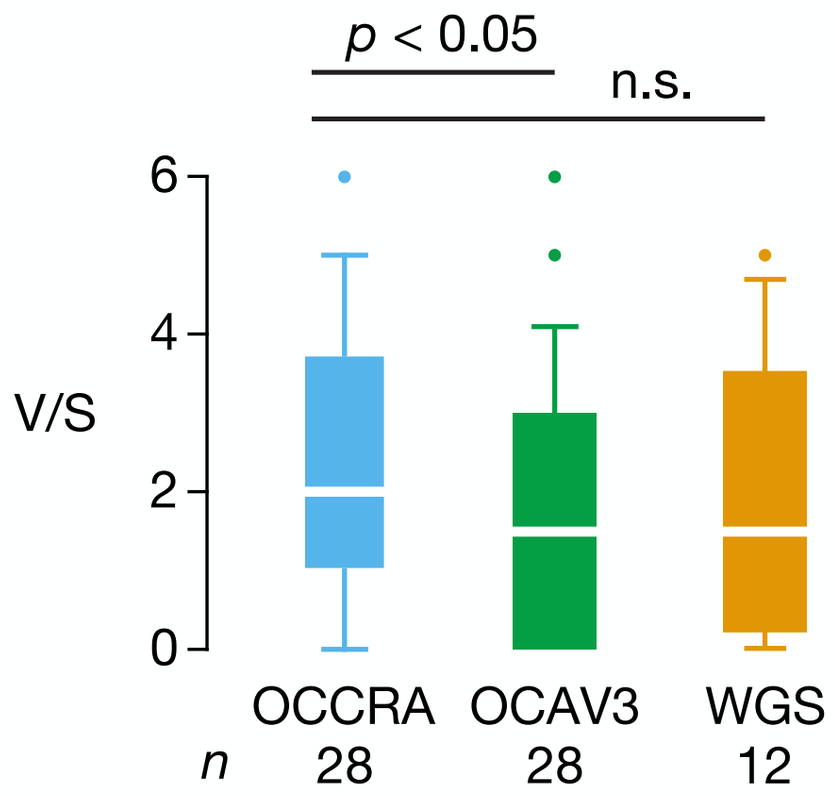

6
5
4
3
2
1
0
0
1
2
3
4
5
6

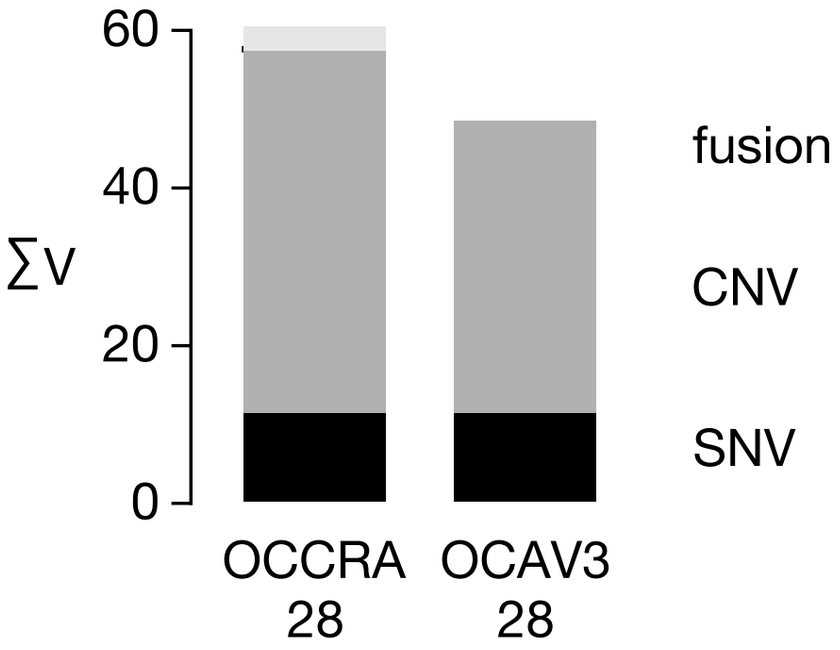

6
5
4
3
2
1
0
0
1
2
3
4
5
6

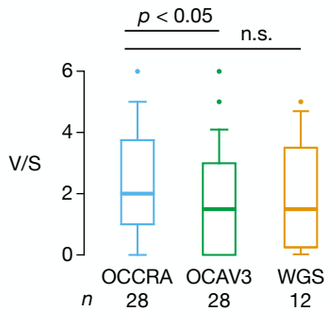

6
5
4
3
2
1
0
0
1
2
3
4
5
6

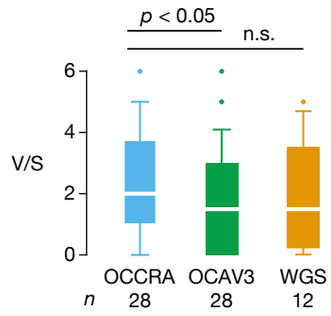

p< 0.05
ns
Variants per sample
8
6
4
2
0
OCCRA
OCAV3
WGS
n=
(28)
(28)
(12)

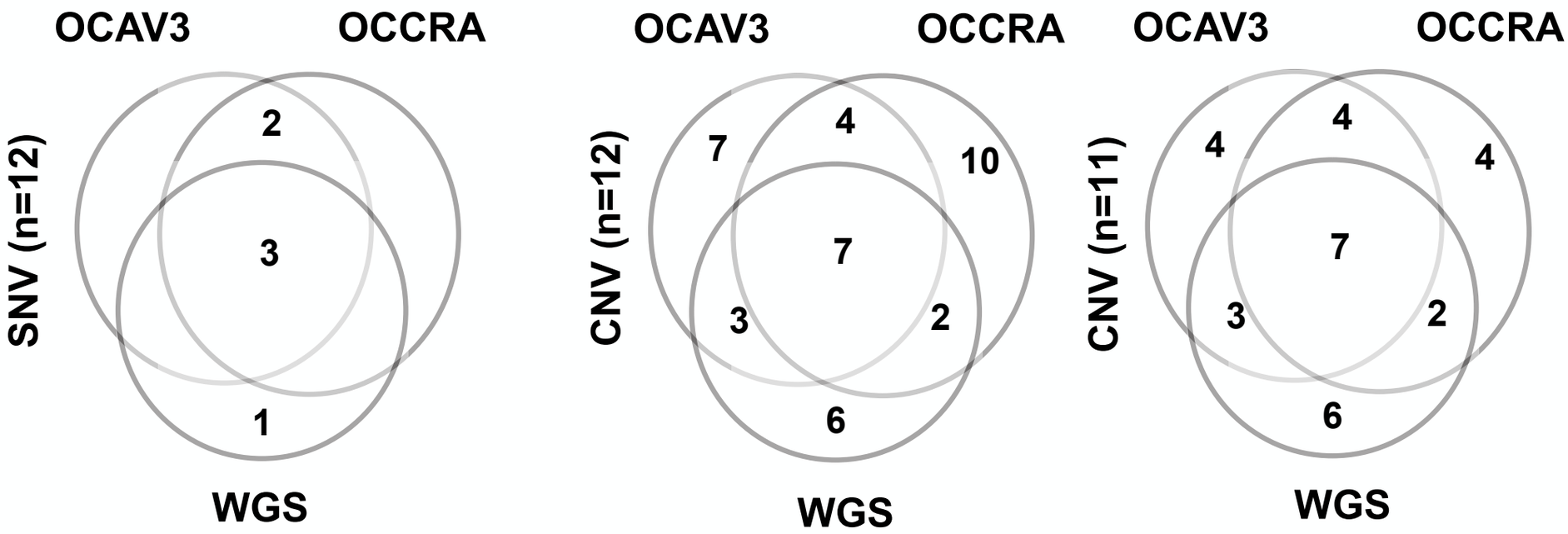

Fusions
CNV
SNV
Variants (#)
80
60
40
20
0
OCCRA
OCAV3
n=
(28)
(28)

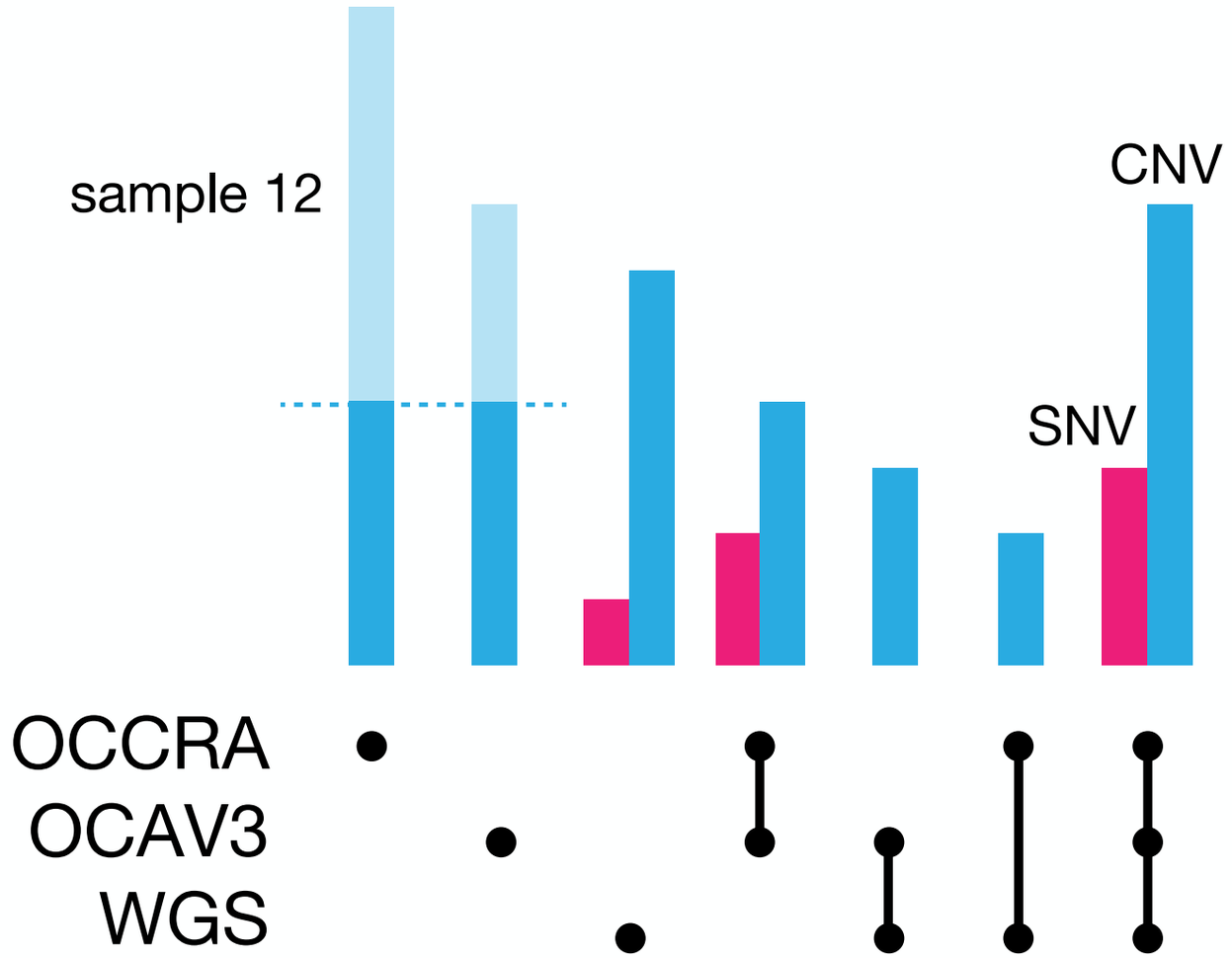

p < 0.05
n.s.
V/S
6
4
2
0
OCCRA
OCAV3
WGS
n
28
28
12

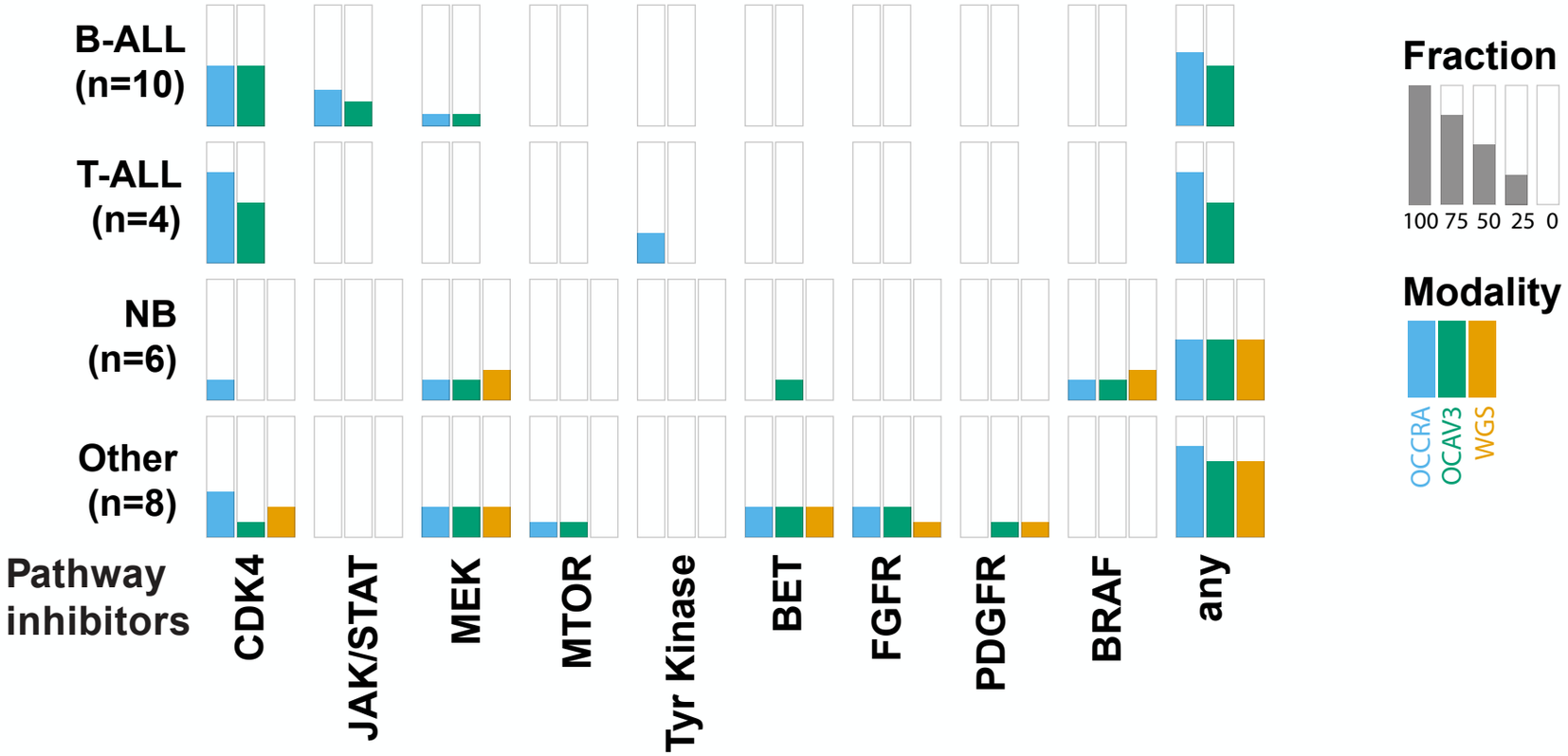

∑V
60
40
20
0
OCCRA
OCAV3
28
28
fusion
CNV
SNV

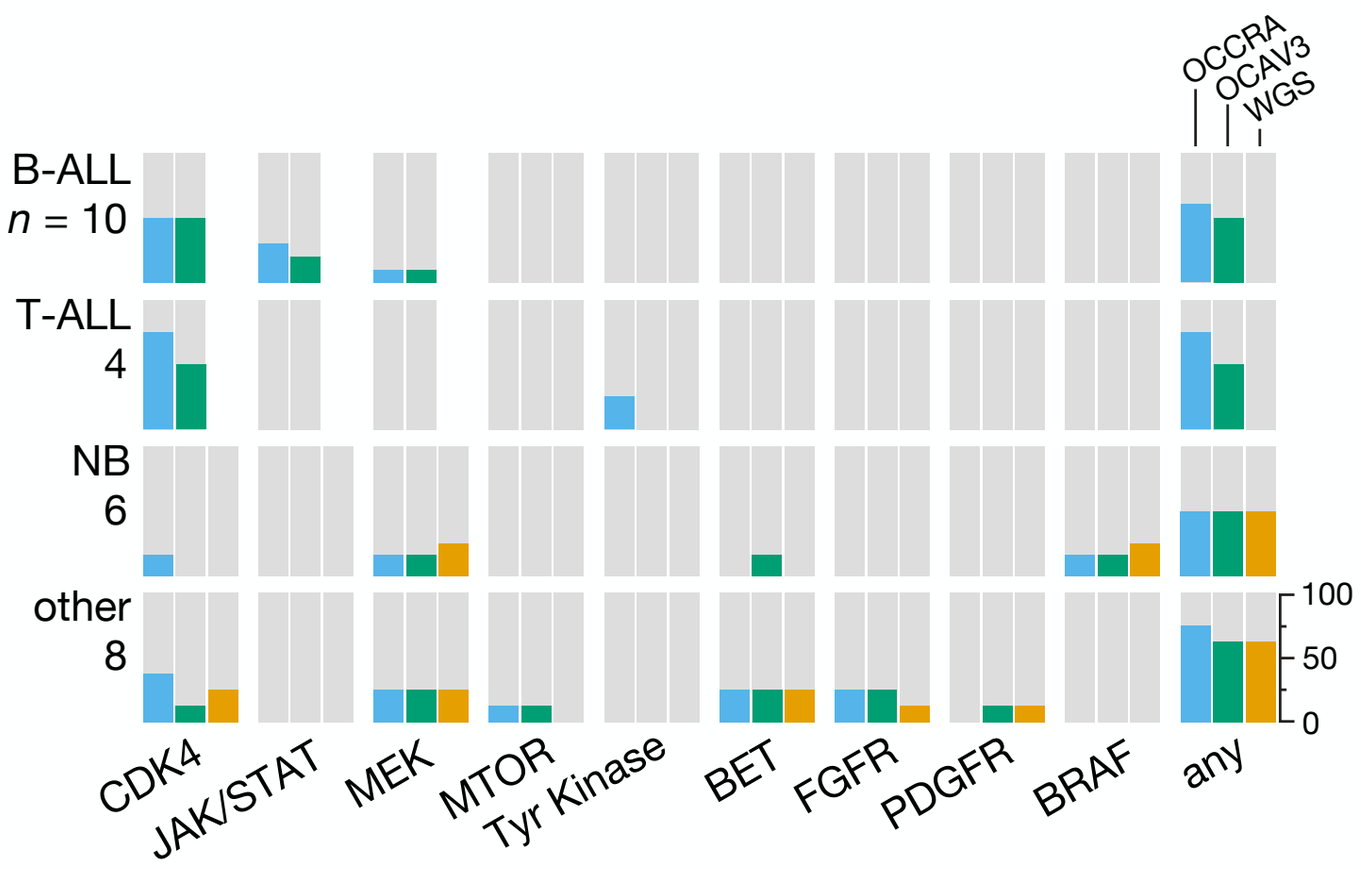

p < 0.05
n.s.
6
4
2
0
V/S
OCCRA
OCAV3
WGS
n
28
28
12

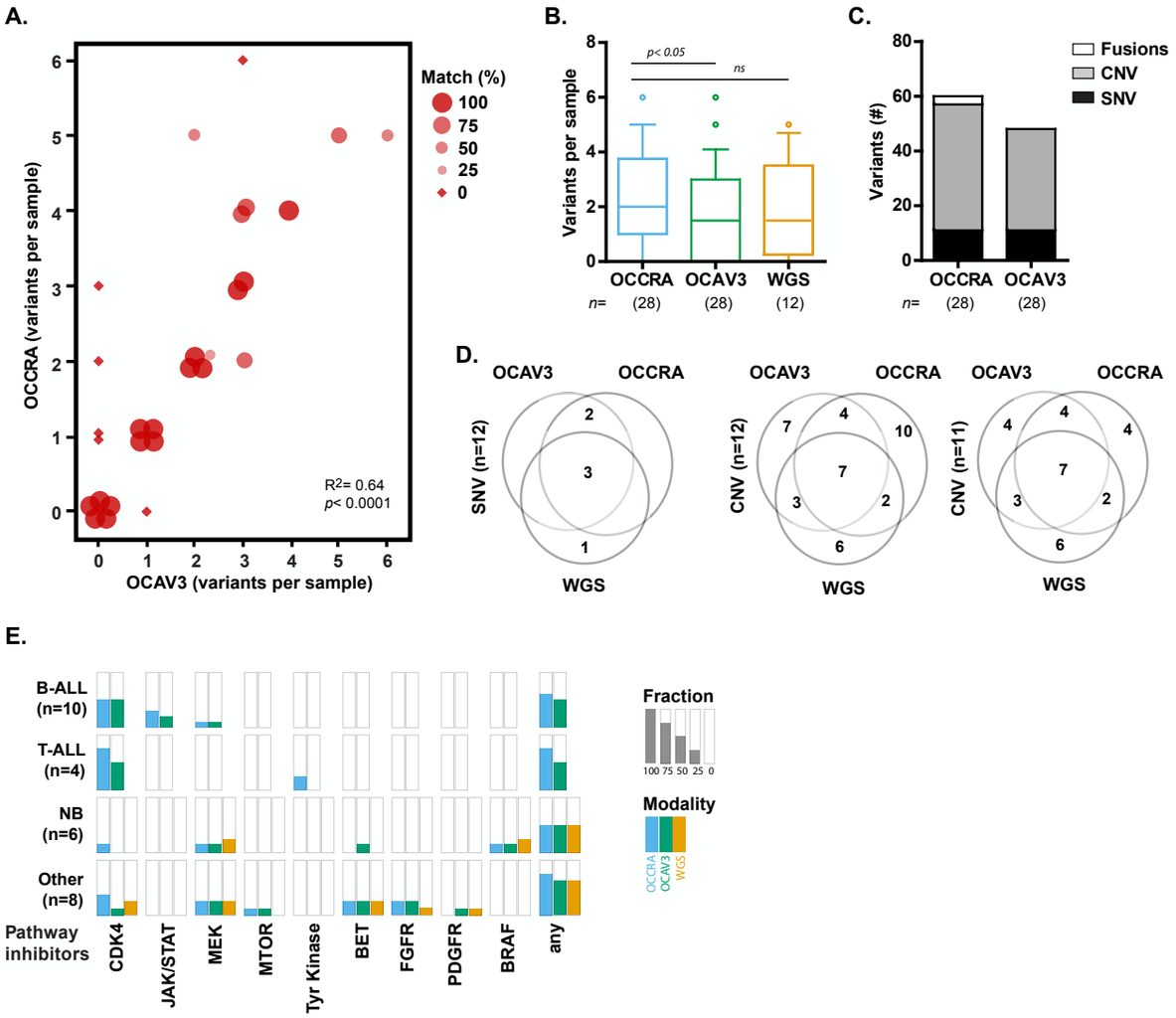

p < 0.05
n.s.
6
4
2
0
V/S
OCCRA
OCAV3
WGS
n
28
28
12

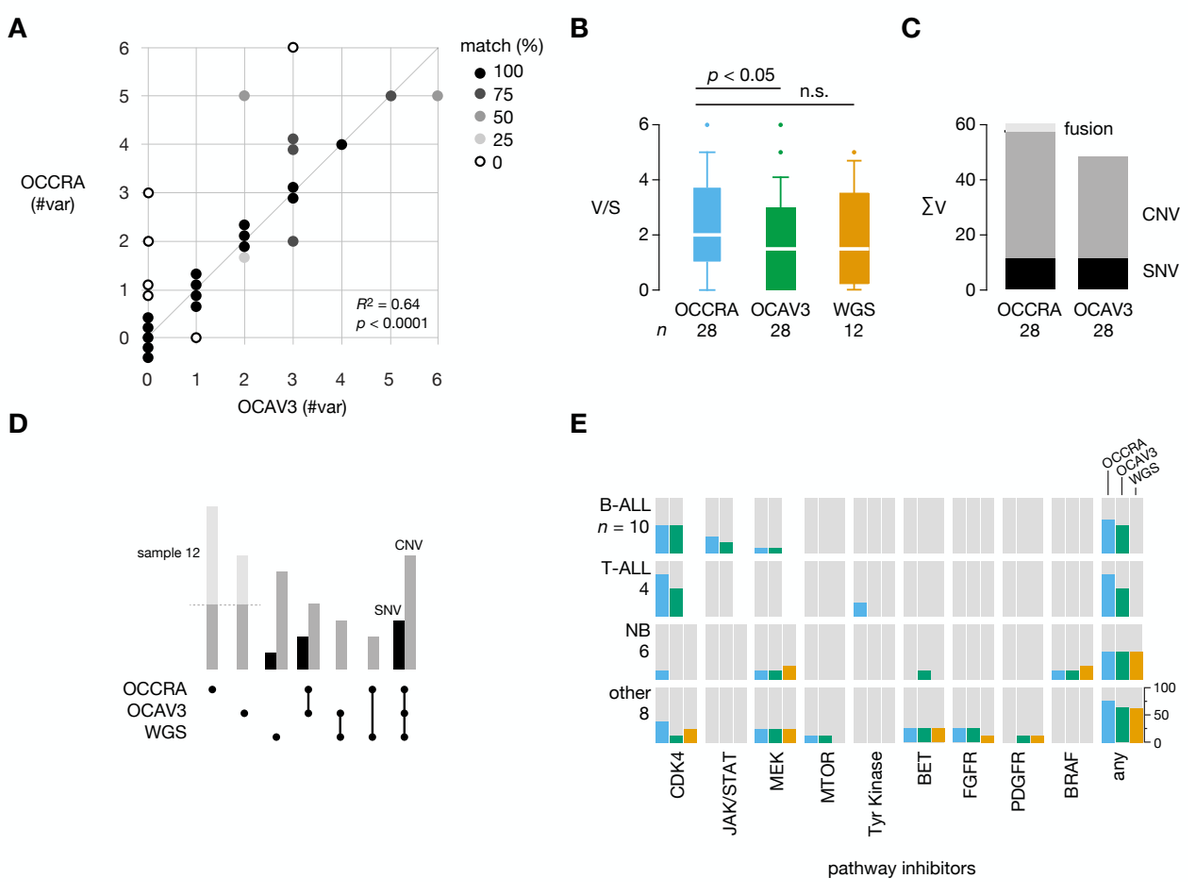

OCAV3
OCCRA
SNV (n=12)
2
3
1
WGS
OCAV3
OCCRA
CNV (n=12)
7
4
10
7
3
2
6
WGS
OCAV3
OCCRA
CNV (n=11)
4
4
4
7
3
2
6
WGS

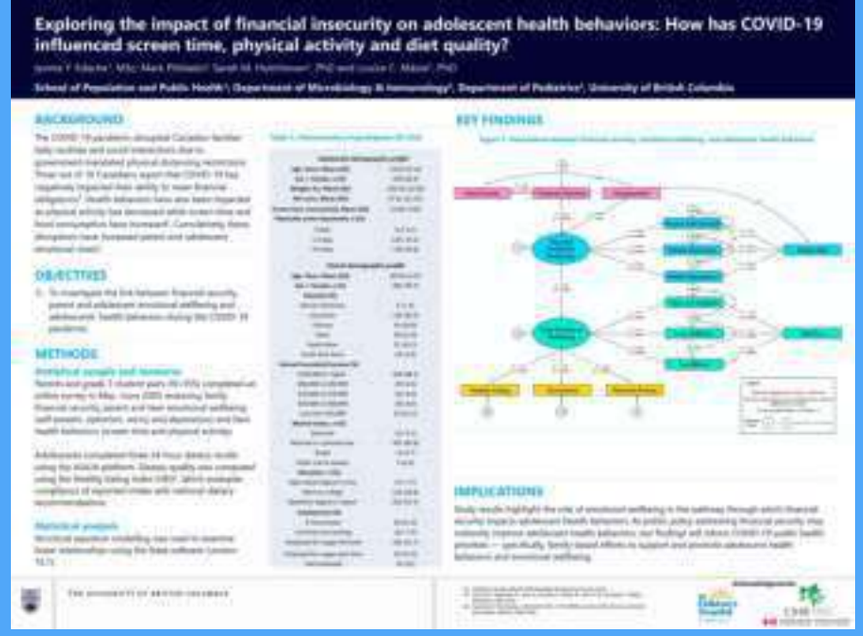

sample 12
CNV
SNV
OCCRA
OCAV3
WGS


B-ALL
(n=10)
T-ALL
(n=4)
NB
(n=6)
Other
(n=8)
Pathway
inhibitors
CDK4
JAK/STAT
MEK
MTOR
Tyr Kinase
BET
FGFR
PDGFR
BRAF
any
Fraction
100 75 50 25 0
Modality
OCCRA
OCAV3
WGS


OCCRA
OCAV3
WGS
B-ALL
n = 10
T-ALL
4
NB
6
other
8
100
50
0
CDK4
JAK/STAT
MEK
MTOR
Tyr Kinase
BET
FGFR
PDGFR
BRAF
any


A.
OCCRA (variants per sample)
OCAV3 (variants per sample)
Match (%)
100
75
50
25
0
R2= 0.64
p< 0.0001
B.
Variants per sample
p< 0.05
ns
OCCRA OCAV3 WGS
n= (28) (28) (12)
C.
Variants (#)
Fusions
CNV
SNV
OCCRA OCAV3
n= (28) (28)
D.
OCAV3 OCCRA WGS
SNV (n=12)
CNV (n=12)
CNV (n=11)
E.
B-ALL (n=10)
T-ALL (n=4)
NB (n=6)
Other (n=8)
Pathway inhibitors
CDK4 JAK/STAT MEK MTOR Tyr Kinase BET FGFR PDGFR BRAF any
Fraction
100 75 50 25 0
Modality
OCCRA
OCAV3
WGS


A
OCCRA (#var)
OCAV3 (#var)
match (%)
100
75
50
25
0
R2 = 0.64
p < 0.0001
B
p < 0.05
n.s.
V/S
OCCRA OCAV3 WGS
n 28 28 12
C
∑V
fusion
CNV
SNV
OCCRA OCAV3
28 28
D
sample 12
CNV
SNV
OCCRA
OCAV3
WGS
E
OCCRA
OCAV3
WGS
B-ALL n = 10
T-ALL 4
NB 6
other 8
100
50
0
CDK4 JAK/STAT MEK MTOR Tyr Kinase BET FGFR PDGFR BRAF any
pathway inhibitors



conclude first

and explain early

don’t squirrel important information
into the least accessible part of the poster

Exploring the impact of financial insecurity on adolescent health behaviors: How has COVID-19 influenced screen time, physical activity and diet quality?

Iyoma Y. Edache[1], MSc; Mark Pitblado[2]; Sarah M. Hutchinson[3], PhD and Louise C. Mâsse[1], PhD

School of Population and Public Health[1]; Department of Microbiology & Immunology[2], Department of Pediatrics[3], University of British Columbia

BACKGROUND

The COVID-19 pandemic disrupted Canadian families' daily routines and social interactions due to government-mandated physical distancing restrictions. Three out of 10 Canadians report that COVID-19 has negatively impacted their ability to meet financial obligations[1]. Health behaviors have also been impacted as physical activity has decreased while screen time and food consumption have increased[2]. Cumulatively, these disruptions have increased parent and adolescent emotional strain[2].

OBJECTIVES

1) To investigate the link between financial security, parent and adolescent emotional wellbeing and adolescents' health behaviors during the COVID-19 pandemic.

METHODS

Analytical sample and measures

Parents and grade 7 student pairs (N=355) completed an online survey in May –June 2020, assessing family financial security, parent and teen emotional wellbeing (self-esteem, optimism, worry and depression) and teen health behaviors (screen time and physical activity).

Adolescents completed three 24-hour dietary recalls using the ASA24 platform. Dietary quality was computed using the Healthy Eating Index (HEI)[3], which evaluates compliance of reported intake with national dietary recommendations.

Statistical analysis

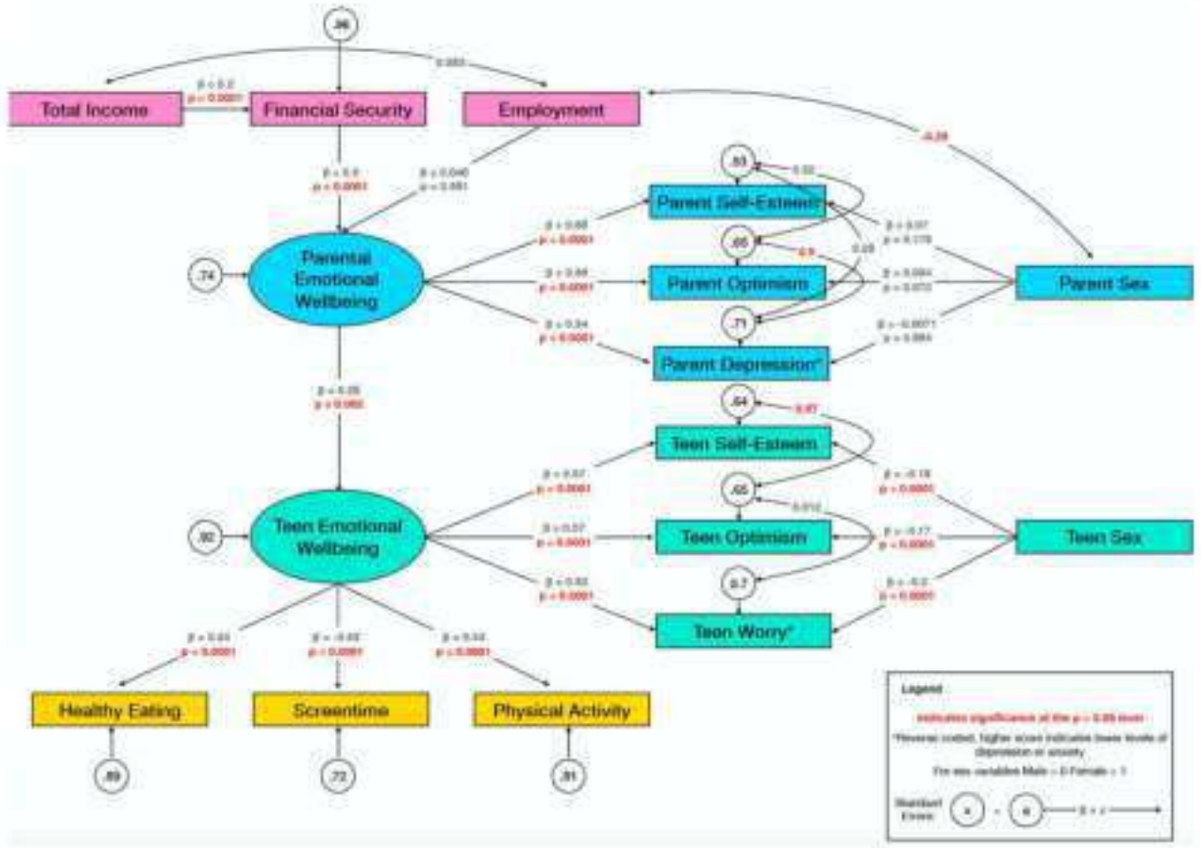
Structural equation modelling was used to examine linear relationships using the Stata software (version 15.1).

Table 1. Characteristics of participants (N=355)

Adolescent demographic profile	
Age, Years, Mean (SD)	13.01 (0.12)
Sex = Female, n (%)	199 (54.0)
Weight, lbs, Mean (SD)	106.56 (22.90)
HEI score, Mean (SD)	57.61 (11.20)
Screen time, hours/week, Mean (SD)	11.84 (7.60)
Physically active days/week, n (%)	
0 days	31 (9.1)
1-3 days	120 (35.3)
4-6 days	118 (34.8)
Parent demographic profile	
Age, Years, Mean (SD)	45.95 (5.42)
Sex = Female, n (%)	285 (79.7)
Ethnicity (%)	
African American	5 (1.4)
Caucasian	126 (36.3)
Chinese	93 (26.8)
Other	48 (13.9)
South Asian	51 (14.7)
South East Asian	24 (6.9)
Annual household income (%)	
$100,000 or higher	169 (48.2)
$80,000 to $99,999	29 (8.3)
$70,000 to $79,999	16 (4.6)
$50,000 to $69,999	28 (8.0)
Less than $50,000	53 (15.1)
Marital status, n (%)	
Divorced	32 (9.1)
Married or common-law	301 (85.8)
Single	13 (3.7)
Prefer not to answer	5 (1.4)
Education, n (%)	
High school degree or less	27 (7.7)
Went to college	122 (34.8)
Bachelors degree or above	202 (57.5)
Employment (%)	
A homemaker	40 (11.4)
Currently not working	26 (7.4)
Employed for wages-full time	181 (51.7)
Employed for wages-part time	43 (12.3)
Self-employed	42 (12)

KEY FINDINGS

Figure 1. Associations between financial security, emotional wellbeing and adolescent health behaviors.



IMPLICATIONS

Study results highlight the role of emotional wellbeing in the pathway through which financial security impacts adolescent health behaviors. As public policy addressing financial security may indirectly improve adolescent health behaviors, our findings will inform COVID-19 public health priorities — specifically, family-based efforts to support and promote adolescent health behaviors and emotional wellbeing.

(1) Statistics Canada's March 2020 Canadian Perspectives Survey Series
(2) Carroll, N., Sadowski, A., Laila, A., Hruska, V., Nixon, M., Ma, D. W., & Haines, J. (2020). Nutrients, 12(8), 2352.
(3) Guenther, P. M., Reedy, J., & Krebs-Smith, S. M. (2008). Journal of the American Dietetic Association, 108(11), 1896-1901.

Acknowledgements

BC Children's Hospital

CIHR IRSC

THE UNIVERSITY OF BRITISH COLUMBIA

Exploring the impact of financial insecurity on adolescent health behaviors: How has COVID-19 influenced screen time, physical activity and diet quality?

Iyoma Y. Edache[1], MSc Mark Pitblado[2] Sarah M. Hutchinson[3], PhD Louise C. Mâsse[1], PhD

[1]School of Population and Public Health [2]Department of Microbiology & Immunology [3]Department of Pediatrics, University of British Columbia

OBJECTIVES

To investigate the link between financial security, parent and adolescent emotional wellbeing and adolescents' health behaviors during the COVID-19 pandemic.

IMPLICATIONS

Study results highlight the role of emotional wellbeing in the pathway through which financial security impacts adolescent health behaviors. As public policy addressing financial security may indirectly improve adolescent health behaviors, our findings will inform COVID-19 public health priorities — specifically, family-based efforts to support and promote adolescent health behaviors and emotional wellbeing.

BACKGROUND

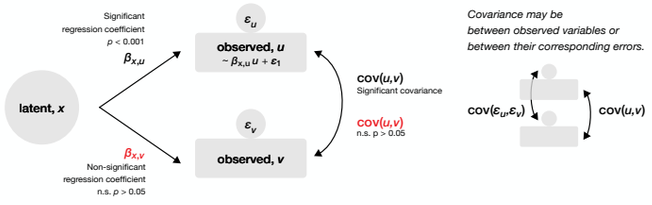
The COVID-19 pandemic disrupted Canadian families' daily routines and social interactions due to government-mandated physical distancing restrictions. Three out of 10 Canadians report that COVID-19 has negatively impacted their ability to meet financial obligations[1]. Health behaviors have also been impacted as physical activity has decreased while screen time and food consumption have increased2. Cumulatively, these disruptions have increased parent and adolescent emotional strain[2].

ANALYTICAL SAMPLE AND MEASURES

Parents and grade 7 student pairs (n = 355) completed an online survey in May –June 2020, assessing family financial security, parent and teen emotional wellbeing (self-esteem, optimism, worry and depression) and teen health behaviors (screen time and physical activity). Adolescents completed three 24-hour dietary recalls using the ASA24 platform. Dietary quality was computed using the Healthy Eating Index (HEI)[3], which evaluates compliance of reported intake with national dietary recommendations.

STATISTICAL ANALYSIS

Structural equation modelling was used to examine linear relationships using the Stata software (version 15.1).



1 Statistics Canada's March 2020 Canadian Perspectives Survey Series

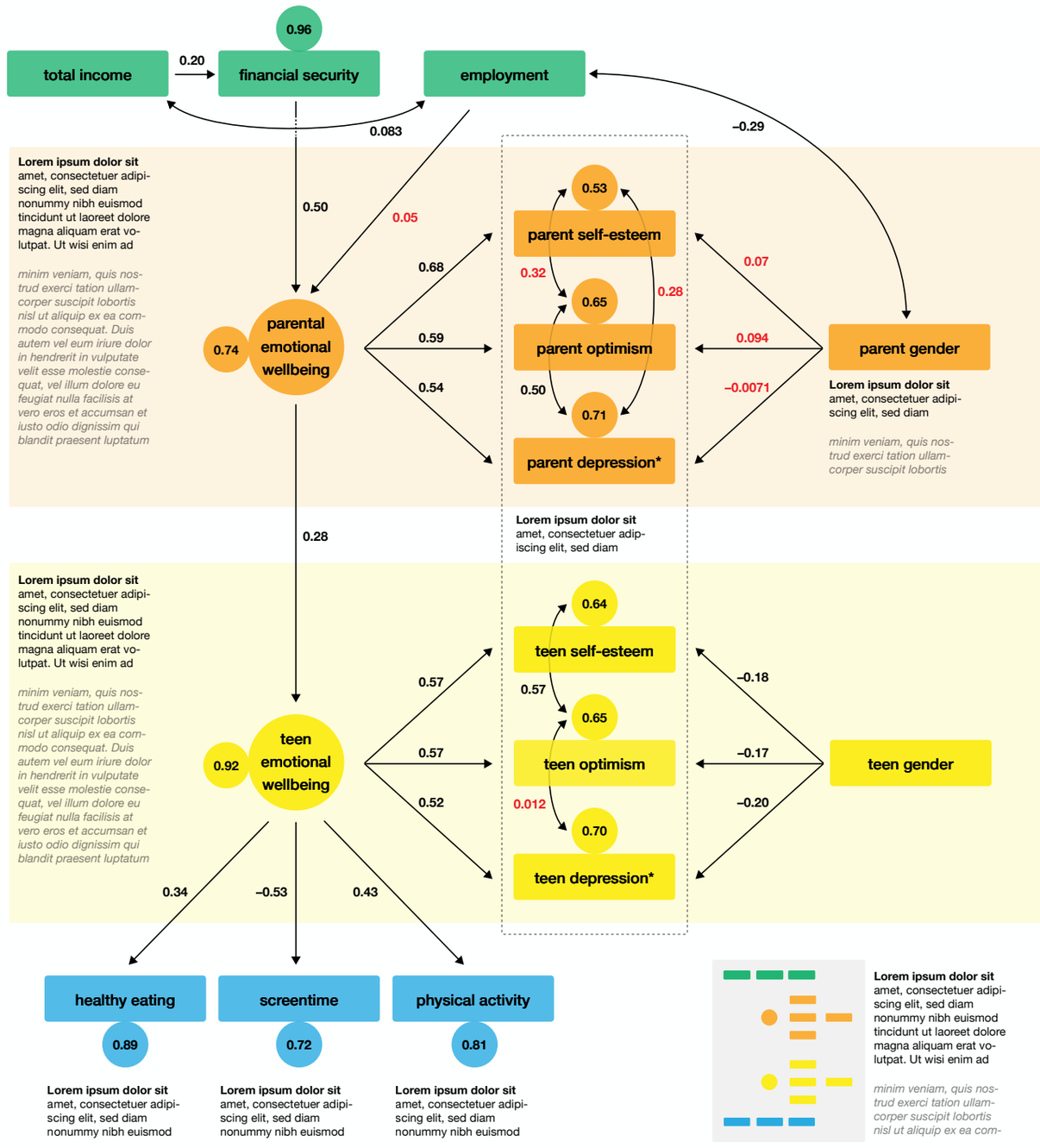
2 Carroll, N., Sadowski, A., Laila, A., Hruska, V., Nixon, M., Ma, D. W., & Haines, J. (2020). Nutrients, 12(8), 2352.

3 Guenther, P. M., Reedy, J., & Krebs-Smith, S. M. (2008). Journal of the American Dietetic Association, 108(11), 1896-1901

Characteristics of participants (n = 355).

ADOLESCENT PROFILE			
age (years)	13.01	0.12	μ, σ
sex (female)	199	54	*n*, %
weight (lbs)	106.6	22.9	μ, σ
HEI score	57.6	11.2	μ, σ
screen time, hours/week	11.8	7.6	μ, σ
physically active days/week			*n*, %
0 days	31	*9.1*	
1-3 days	120	*35*	
4-6 days	118	*35*	
PARENT PROFILE			
age (years)	45.9	5.4	μ, σ
sex (female)	285	80	*n*, %
ethnicity			*n*, %
African American	5	*1.4*	
Caucasian	126	*36.3*	
Chinese	93	*26.8*	
South Asian	51	*14.7*	
South East Asian	24	*6.9*	
other	48	*13.9*	
annual household income			*n*, %
≥ $100,000	169	*48.2*	
$80,000 to $99,999	29	*8.3*	
$70,000 to $79,999	16	*4.6*	
$50,000 to $69,999	28	*8.0*	
< $50,000	53	*15.1*	
marital status			*n*, %
married or common-law	301	*85.8*	
divorced	32	*9.1*	
single	13	*3.7*	
prefer not to answer	5	*1.4*	
education			*n*, %
bachelors degree or above	202	*57.5*	
went to college	122	*34.8*	
high school degree or less	27	*7.7*	
employment			*n*, %
full-time wage	181	*52*	
part-time wage	43	*12*	
self-employed	42	*12*	
homemaker	40	*11*	
currently not working	26	*7*	

Associations between financial security, emotional wellbeing and adolescent health behaviors.



THE UNIVERSITY OF BRITISH COLUMBIA

FINANCIAL SUPPORT amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim.Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam

KIND THANKS TO amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim.Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam

Exploring the impact of fin
influenced screen time, phy

Iyoma Y. Edache[1], MSc; Mark Pitblado[2]; Sarah M. H

School of Population and Public Health[1]; Depa

BACKGROUND

The COVID-19 pandemic disrupted Canadian families' daily routines and social interactions due to government-mandated physical distancing restrictions. Three out of 10 Canadians report that COVID-19 has negatively impacted their ability to meet financial obligations[1]. Health behaviors have also been impacted as physical activity has decreased while screen time and food consumption have increased[2]. Cumulatively, these disruptions have increased parent and adolescent emotional strain[2].

OBJECTIVES

1) To investigate the link between financial security, parent and adolescent emotional wellbeing and adolescents' health behaviors during the COVID-19 pandemic.

METHODS

Analytical sample and measures

Parents and grade 7 student pairs (N=355) completed an online survey in May –June 2020, assessing family financial security, parent and teen emotional wellbeing (self-esteem, optimism, worry and depression) and teen health behaviors (screen time and physical activity).

Adolescents completed three 24-hour dietary recalls using the ASA24 platform. Dietary quality was computed using the Healthy Eating Index (HEI)[3], which evaluates compliance of reported intake with national dietary recommendations.

Statistical analysis

Structural equation modelling was used to examine linear relationships using the Stata software (version 15.1).

Exploring the impact of fi
How has COVID-19 influe

Iyoma Y. Edache[1], MSc Mark Pitblado[2] S

[1]School of Population and Public Health [2]Department of Microbiology

OBJECTIVES

To investigate the link between financial security, parent and adolescent emotional wellbeing and adolescents' health behaviors during the COVID-19 pandemic.

IMPLICATIONS

Study results highlight the role of emotional wellbeing in the pathway through which financial security impacts adolescent health behaviors. As public policy addressing financial security may indirectly improve adolescent health behaviors, our findings will inform COVID-19 public health priorities — specifically, family-based efforts to support and promote adolescent health behaviors and emotional wellbeing.

BACKGROUND

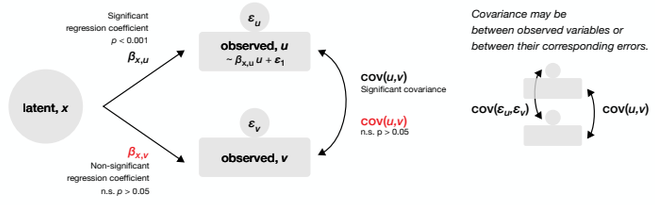
The COVID-19 pandemic disrupted Canadian families' daily routines and social interactions due to government-mandated physical distancing restrictions. Three out of 10 Canadians report that COVID-19 has negatively impacted their ability to meet financial obligations[1]. Health behaviors have also been impacted as physical activity has decreased while screen time and food consumption have increased2. Cumulatively, these disruptions have increased parent and adolescent emotional strain[2].

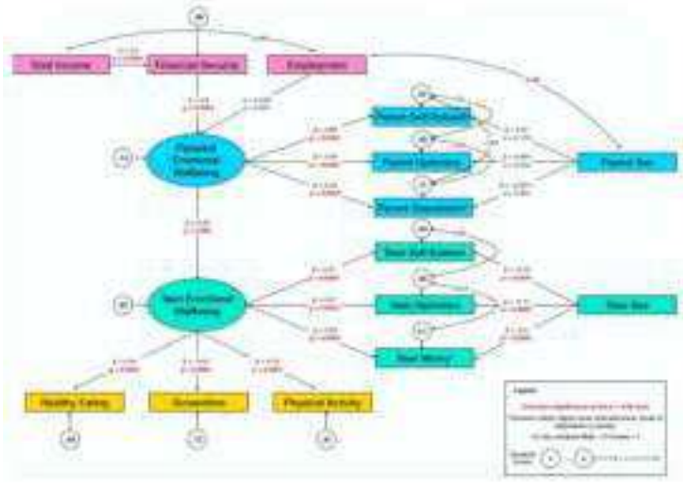
ANALYTICAL SAMPLE AND MEASURES

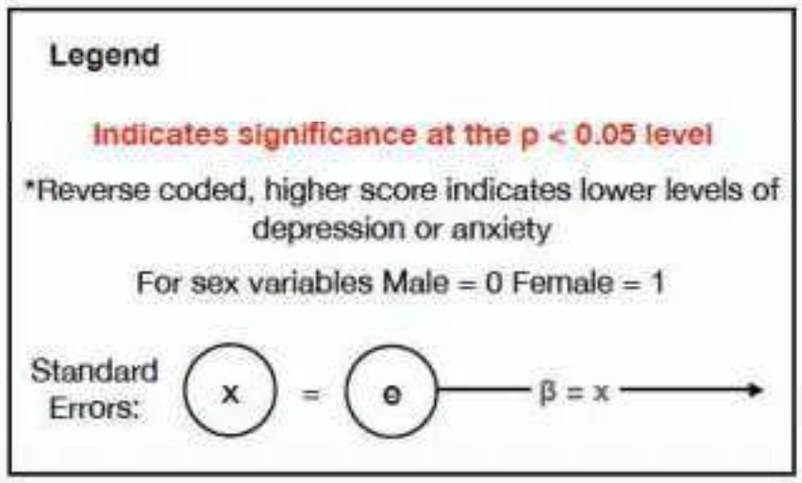
Parents and grade 7 student pairs (n = 355) completed an online survey in May –June 2020, assessing family financial security, parent and teen emotional wellbeing (self-esteem, optimism, worry and depression) and teen health behaviors (screen time and physical activity). Adolescents completed three 24-hour dietary recalls using the ASA24 platform. Dietary quality was computed using the Healthy Eating Index (HEI)[3], which evaluates compliance of reported intake with national dietary recommendations.

STATISTICAL ANALYSIS

Structural equation modelling was used to examine linear relationships using the Stata software (version 15.1).







STATISTICAL ANALYSIS

Structural equation modelling was used to examine linear relationships using the Stata software (version 15.1).

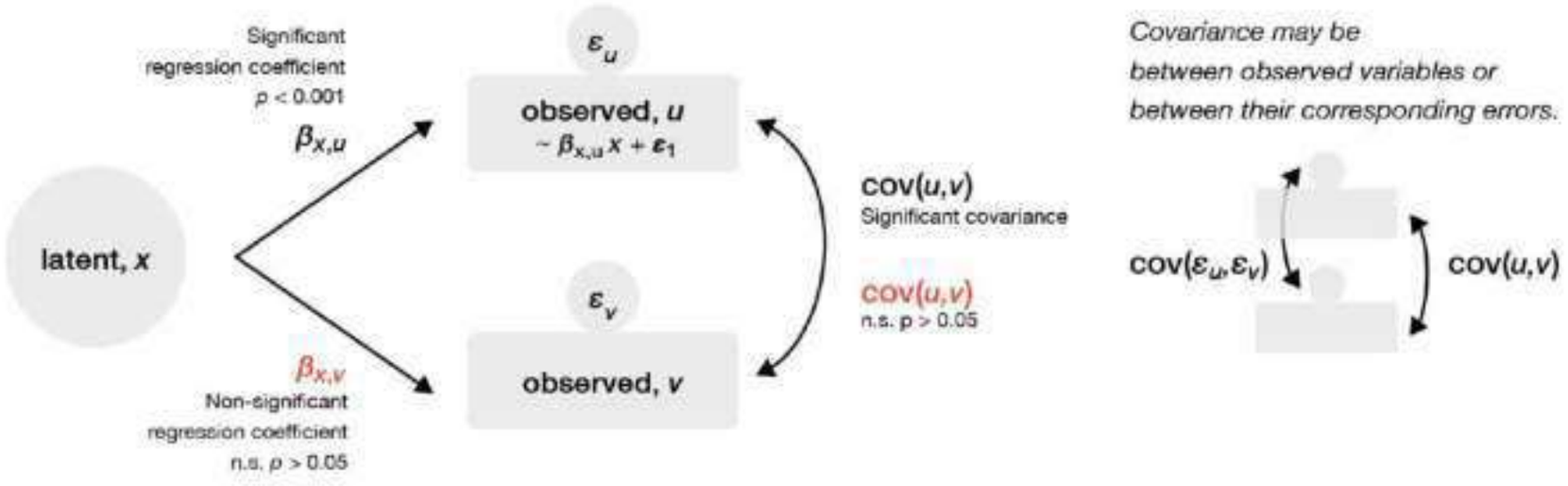


Table 1. Characteristics of participants (N=355)

Adolescent demographic profile	
Age, Years, Mean (SD)	13.01 (0.12)
Sex = Female, n (%)	199 (54.0)
Weight, lbs, Mean (SD)	106.56 (22.90)
HEI score, Mean (SD)	57.61 (11.20)
Screen time, hours/week, Mean (SD)	11.84 (7.60)
Physically active days/week, n (%)	
0 days	31 (9.1)
1-3 days	120 (35.3)
4-6 days	118 (34.8)
Parent demographic profile	
Age, Years, Mean (SD)	45.95 (5.42)
Sex = Female, n (%)	285 (79.7)
Ethnicity (%)	
African American	5 (1.4)
Caucasian	126 (36.3)
Chinese	93 (26.8)
Other	48 (13.9)
South Asian	51 (14.7)
South East Asian	24 (6.9)
Annual household income (%)	
$100,000 or higher	169 (48.2)
$80,000 to $99,999	29 (8.3)
$70,000 to $79,999	16 (4.6)
$50,000 to $69,999	28 (8.0)
Less than $50,000	53 (15.1)
Marital status, n (%)	
Divorced	32 (9.1)
Married or common-law	301 (85.8)
Single	13 (3.7)
Prefer not to answer	5 (1.4)
Education, n (%)	
High school degree or less	27 (7.7)
Went to college	122 (34.8)
Bachelors degree or above	202 (57.5)
Employment (%)	
A homemaker	40 (11.4)
Currently not working	26 (7.4)
Employed for wages-full time	181 (51.7)
Employed for wages-part time	43 (12.3)
Self-employed	42 (12)

Characteristics of participants (n = 355).

ADOLESCENT PROFILE				
age (years)		13.01	0.12	μ, σ
sex (female)		199	54	*n*, %
weight (lbs)		106.6	22.9	μ, σ
HEI score		57.6	11.2	μ, σ
screen time, hours/week		11.8	7.6	μ, σ
physically active days/week				*n*, %
0 days		31	*9.1*	
1-3 days		120	*35*	
4-6 days		118	*35*	
PARENT PROFILE				
age (years)		45.9	5.4	μ, σ
sex (female)		285	80	*n*, %
ethnicity				*n*, %
African American		5	*1.4*	
Caucasian		126	*36.3*	
Chinese		93	*26.8*	
South Asian		51	*14.7*	
South East Asian		24	*6.9*	
other		48	*13.9*	
annual household income				*n*, %
≥ $100,000		169	*48.2*	
$80,000 to $99,999		29	*8.3*	
$70,000 to $79,999		16	*4.6*	
$50,000 to $69,999		28	*8.0*	
< $50,000		53	*15.1*	
marital status				*n*, %
married or common-law		301	*85.8*	
divorced		32	*9.1*	
single		13	*3.7*	
prefer not to answer		5	*1.4*	
education				*n*, %
bachelors degree or above		202	*57.5*	
went to college		122	*34.8*	
high school degree or less		27	*7.7*	
employment				*n*, %
full-time wage		181	*52*	
part-time wage		43	*12*	
self-employed		42	*12*	
homemaker		40	*11*	
currently not working		26	*7*	

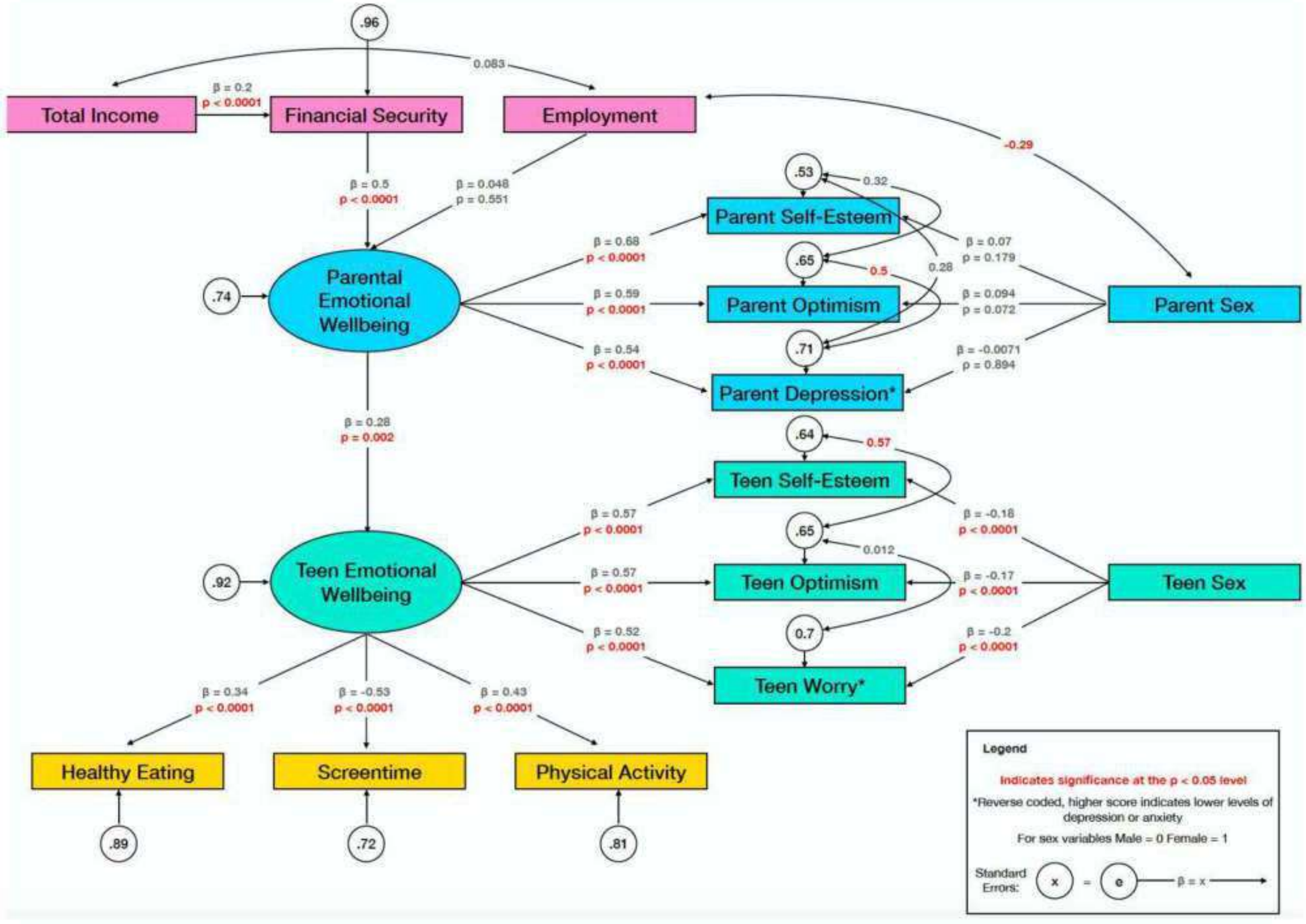
	Yes (%)	*B*	SE	Odds ratio	(95% CI)	*P*
Are you concerned about Newcastle disease? (n = 398)[*]						
Age						0.007
<24 years	5 (17.2)	0	–	1.00	–	
25–34 years	10 (28.6)	1.33	0.86	3.80	(0.8–27.6)	
35–44 years	22 (36.1)	1.37	0.82	3.92	(0.9 –26.9)	
45–54 years	42 (43.8)	1.85	0.79	6.35	(1.7 –42.0)	
55–64 years	36 (50.0)	203	0.80	7.62	(1.9–51.5)	
+65 years	63 (60.0)	2.24	0.79	9.39	(2.4–62.4)	
State						0.042
SA/WA	27 (31.8)	0	–	1.00	–	
NSW	58 (47.5)	0.59	0.35	1.80	(0.9– 3.6)	
QLD	45 (47.4)	0.49	0.36	1.63	(0.8– 3.3)	
TAS	15 (33.3)	−0.23	0.45	0.79	(0.3–1.9)	
VIC	29 (64.4)	1.04	0.45	2.82	(1.2–6.9)	
Do you keep a written record of treatments given to your birds? (n = 398)[*]						
Years owning poultry						0.006
1–5 years	19 (51.4)	0	–	1.00	–	
6–15 years	21 (28.4)	−1.54	0.51	0.21	(0.1–0.6)	
16–29 years	33 (45.2)	−0.75	0.49	0.47	(0.2–1.2)	
+30 years	70 (33.2)	−1.39	0.45	0.25	(0.1–0.6)	
Sex						0.066
Female	38 (44.2)	0	–	1.00	–	
Male	102 (33.9)	−0.56	0.30	0.57	(0.3–1.0)	
Have you contacted a veterinarian in the past 12 months for the health of your birds?(n = 398)[*]						
Years owning poultry						0.006
1–5 years	17 (45.9)	0	–	1.00	–	
6–15 years	22 (29.7)	−0.71	0.51	0.49	(0.2–1.3)	0.017
16–29 years	15 (20.5)	−1.44	0.54	0.24	(0.1–0.7)	
+30 years	38 (18.0)	−1.49	0.48	0.23	(0.1–0.6)	
Sex						
Female	34 (39.5)	0	–	1.00	–	
Male	54 (17.9)	−0.79	0.33	0.46	(0.2–0.9)	
State						0.040
SA/WA	13 (15.3)	0	–	1.00	–	
NSW	37 (30.3)	1.01	0.46	2.75	(1.1–6.6)	
QLD	17 (17.9)	0.18	0.47	1.19	(0.5–3.0)	
TAS	11 (24.4)	0.91	0.52	2.49	(0.9–7.1)	
VIC	14 (31.1)	1.03	0.51	2.79	(1.0–7.8)	

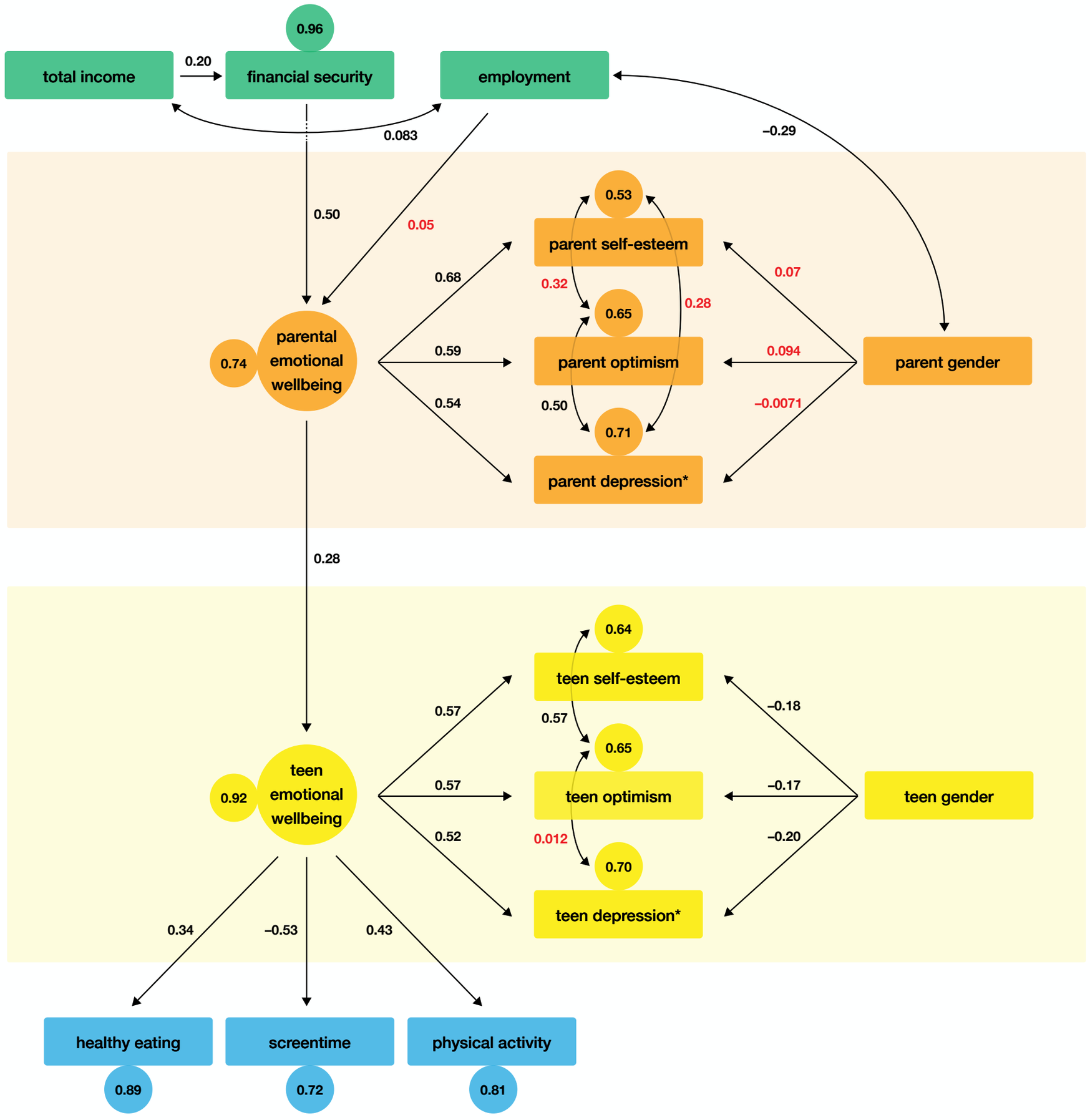
[*] Number of exhibitors contributing to the regression model analysis.

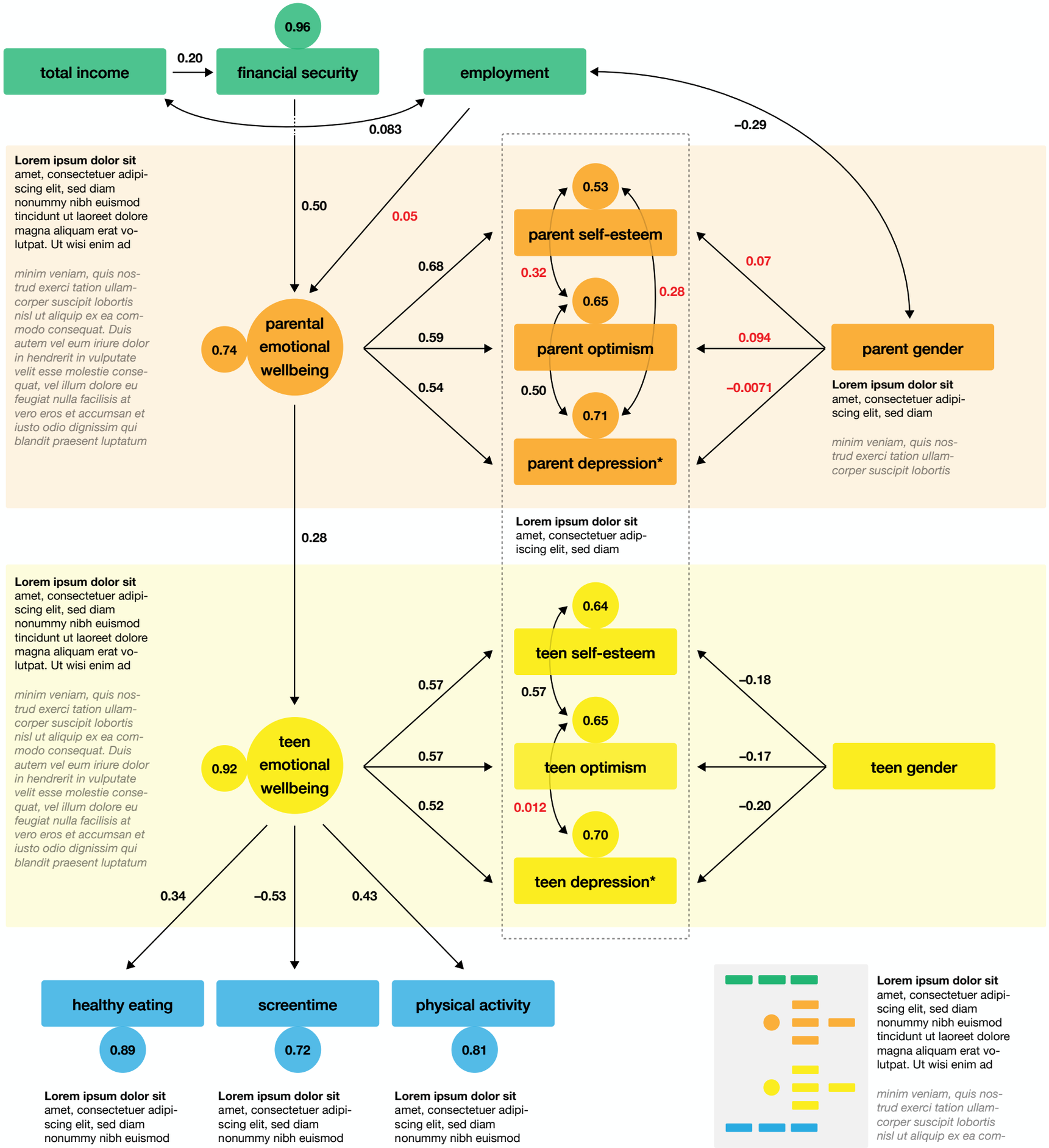
	P	Yes (%) †	β	SE$_\beta$	OR	95% CI
Are you concerned about Newcastle disease?		46.0				
Age (years)	0.007					
≤24 ‡		17.2				
25–34		28.6	1.33	0.86	3.8	0.8 – 27.6
35–44		36.1	1.37	0.82	3.9	0.9 – 26.9
45–54		43.8	1.85	0.79	6.3	1.7 – 42.0
55–64		50.0	203	0.80	7.6	1.9 – 51.5
≥65		60.0	2.24	0.79	9.4	2.4 – 62.4
State	0.007					
SA/WA		31.8				
NSW		47.5	0.59	0.35	1.8	0.9 – 3.6
QLD		47.4	0.49	0.36	1.6	0.8 – 3.3
TAS		33.3	–0.23	0.45	0.8	0.3 – 1.9
VIC		64.4	1.04	0.45	2.8	1.2 – 6.9
Do you keep a written record of treatments given to your birds?		35.2				
Years owning poultry	0.006					
1–5		51.4				
6–15		28.4	–1.54	0.51	0.2	0.1 – 0.6
16–29		45.2	–0.75	0.49	0.5	0.2 – 1.2
≥30		33.2	–1.39	0.45	0.2	0.1 – 0.6
Sex	0.066					
Female		44.2				
Male		33.9	–0.56	0.30	0.6	0.3 – 1.0
Have you contacted a veterinarian in the past 12 months for the health of your birds?		35.2				
Years owning poultry	0.006					
1–5		45.9				
6–15		29.7	–0.71	0.51	0.5	0.2 – 1.3
16–29		20.5	–1.44	0.54	0.2	0.1 – 0.7
≥30		18.0	–1.49	0.48	0.2	0.1 – 0.6
Sex	0.017					
Female		39.5				
Male		17.9	–0.79	0.33	0.5	0.2 – 0.9
State	0.040					
SA/WA		15.3				
NSW		30.3	1.01	0.46	2.8	1.1 – 6.6
QLD		17.9	0.18	0.47	1.2	0.5 – 3.0
TAS		24.4	0.91	0.52	2.5	0.9 – 7.1
VIC		31.1	1.03	0.51	2.8	1.0 – 7.8

† Out of n = 398 survey participants.

‡ For first factor level, $\beta = 0$, OR = 1, SE$_\beta$ and 95% CI are not defined.


.96
0.083
β = 0.2
p < 0.0001
Total Income
Financial Security
Employment
-0.29
β = 0.5
p < 0.0001
β = 0.048
p = 0.551
.53
0.32
Parent Self-Esteem
β = 0.68
p < 0.0001
β = 0.07
p = 0.179
.65
0.5
0.28
.74
Parental Emotional Wellbeing
β = 0.59
p < 0.0001
Parent Optimism
β = 0.094
p = 0.072
Parent Sex
β = 0.54
p < 0.0001
.71
β = -0.0071
p = 0.894
Parent Depression*
β = 0.28
p = 0.002
.64
0.57
Teen Self-Esteem
β = 0.57
p < 0.0001
β = -0.18
p < 0.0001
.65
0.012
.92
Teen Emotional Wellbeing
β = 0.57
p < 0.0001
Teen Optimism
β = -0.17
p < 0.0001
Teen Sex
β = 0.52
p < 0.0001
0.7
β = -0.2
p < 0.0001
Teen Worry*
β = 0.34
p < 0.0001
β = -0.53
p < 0.0001
β = 0.43
p < 0.0001
Healthy Eating
Screentime
Physical Activity
.89
.72
.81
Legend
Indicates significance at the p < 0.05 level
*Reverse coded, higher score indicates lower levels of depression or anxiety
For sex variables Male = 0 Female = 1
Standard Errors:
x
=
e
β = x


0.96
total income
0.20
financial security
employment
0.083
−0.29
0.50
0.05
0.53
parent self-esteem
0.68
0.32
0.28
0.07
0.65
0.59
parent optimism
0.094
parent gender
0.74
parental emotional wellbeing
0.54
0.50
−0.0071
0.71
parent depression*
0.28
0.64
teen self-esteem
0.57
0.57
−0.18
0.65
0.57
teen optimism
−0.17
teen gender
0.92
teen emotional wellbeing
0.52
0.012
−0.20
0.70
teen depression*
0.34
−0.53
0.43
healthy eating
screentime
physical activity
0.89
0.72
0.81



Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad

minim veniam, quis nostrud exerci tation ullamcorper suscipit lobortis nisl ut aliquip ex ea commodo consequat. Duis autem vel eum iriure dolor in hendrerit in vulputate velit esse molestie consequat, vel illum dolore eu feugiat nulla facilisis at vero eros et accumsan et iusto odio dignissim qui blandit praesent luptatum

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam

minim veniam, quis nostrud exerci tation ullamcorper suscipit lobortis

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad

minim veniam, quis nostrud exerci tation ullamcorper suscipit lobortis nisl ut aliquip ex ea commodo consequat. Duis autem vel eum iriure dolor in hendrerit in vulputate velit esse molestie consequat, vel illum dolore eu feugiat nulla facilisis at vero eros et accumsan et iusto odio dignissim qui blandit praesent luptatum

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad

minim veniam, quis nostrud exerci tation ullamcorper suscipit lobortis nisl ut aliquip ex ea com-

Exploring the impact of financial insecurity on adolescent health behaviors: How has COVID-19 influenced screen time, physical activity and diet quality?

Iyoma Y. Edache[1], MSc Mark Pitblado[2] Sarah M. Hutchinson[3], PhD Louise C. Mâsse[1], PhD

[1]School of Population and Public Health [2]Department of Microbiology & Immunology [3]Department of Pediatrics, University of British Columbia

OBJECTIVES

To investigate the link between financial security, parent and adolescent emotional wellbeing and adolescents' health behaviors during the COVID-19 pandemic.

IMPLICATIONS

Study results highlight the role of emotional wellbeing in the pathway through which financial security impacts adolescent health behaviors. As public policy addressing financial security may indirectly improve adolescent health behaviors, our findings will inform COVID-19 public health priorities — specifically, family-based efforts to support and promote adolescent health behaviors and emotional wellbeing.

BACKGROUND

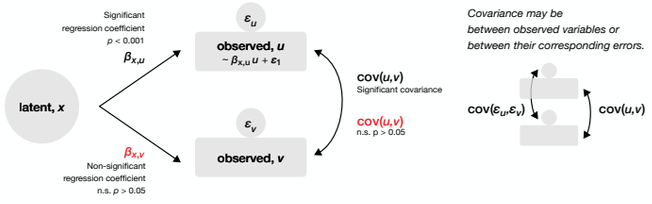
The COVID-19 pandemic disrupted Canadian families' daily routines and social interactions due to government-mandated physical distancing restrictions. Three out of 10 Canadians report that COVID-19 has negatively impacted their ability to meet financial obligations[1]. Health behaviors have also been impacted as physical activity has decreased while screen time and food consumption have increased2. Cumulatively, these disruptions have increased parent and adolescent emotional strain[2].

ANALYTICAL SAMPLE AND MEASURES

Parents and grade 7 student pairs (n = 355) completed an online survey in May –June 2020, assessing family financial security, parent and teen emotional wellbeing (self-esteem, optimism, worry and depression) and teen health behaviors (screen time and physical activity). Adolescents completed three 24-hour dietary recalls using the ASA24 platform. Dietary quality was computed using the Healthy Eating Index (HEI)[3], which evaluates compliance of reported intake with national dietary recommendations.

STATISTICAL ANALYSIS

Structural equation modelling was used to examine linear relationships using the Stata software (version 15.1).



1 Statistics Canada's March 2020 Canadian Perspectives Survey Series

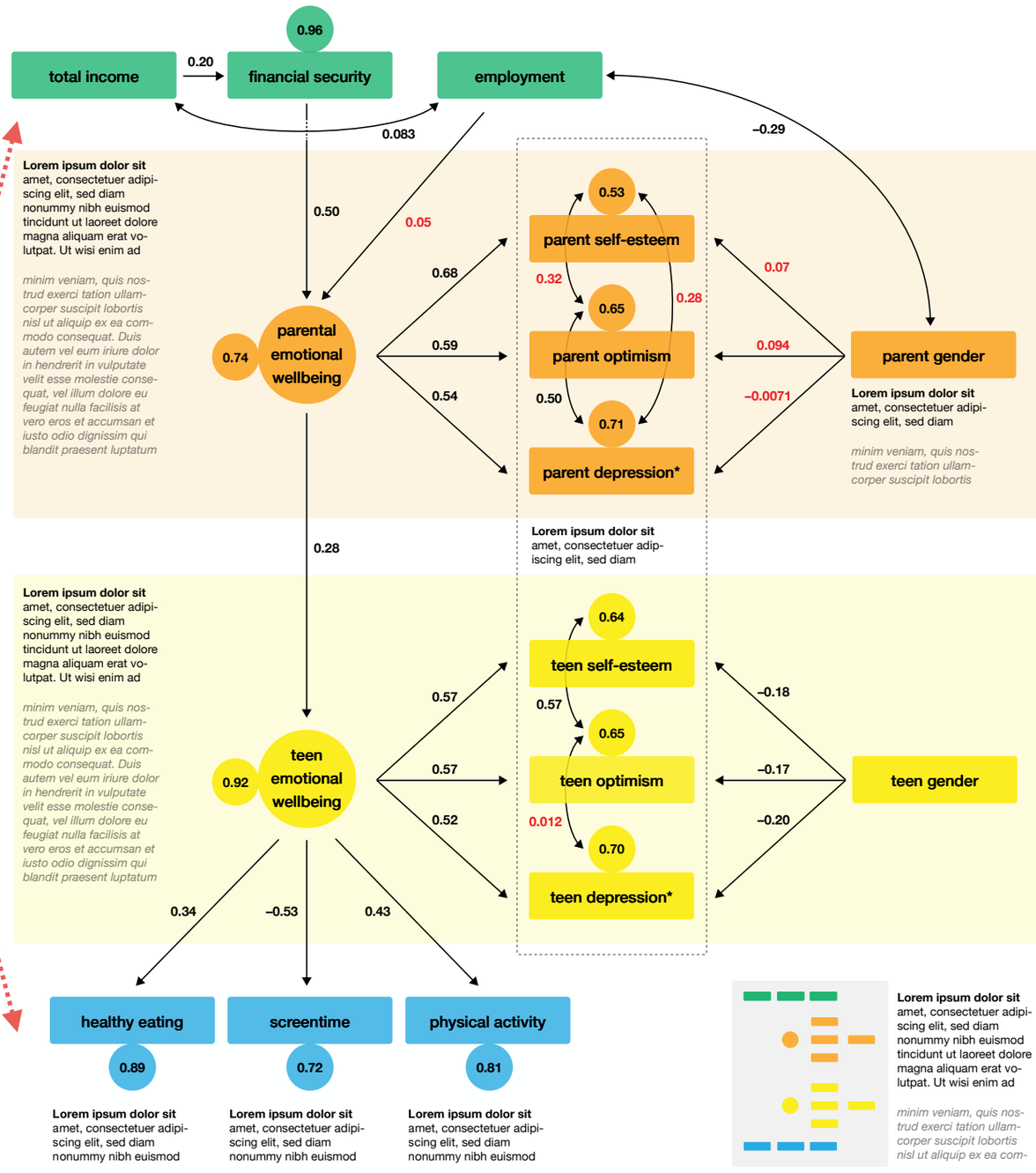
2 Carroll, N., Sadowski, A., Laila, A., Hruska, V., Nixon, M., Ma, D. W., & Haines, J. (2020). Nutrients, 12(8), 2352.

3 Guenther, P. M., Reedy, J., & Krebs-Smith, S. M. (2008). Journal of the American Dietetic Association, 108(11), 1896-1901

Characteristics of participants (n = 355).

ADOLESCENT PROFILE			
age (years)	13.01	0.12	μ, σ
sex (female)	199	54	n, %
weight (lbs)	106.6	22.9	μ, σ
HEI score	57.6	11.2	μ, σ
screen time, hours/week	11.8	7.6	μ, σ
physically active days/week			n, %
0 days	31	*9.1*	
1-3 days	120	*35*	
4-6 days	118	*35*	
PARENT PROFILE			
age (years)	45.9	5.4	μ, σ
sex (female)	285	80	n, %
ethnicity			n, %
African American	5	*1.4*	
Caucasian	126	*36.3*	
Chinese	93	*26.8*	
South Asian	51	*14.7*	
South East Asian	24	*6.9*	
other	48	*13.9*	
annual household income			n, %
≥ $100,000	169	*48.2*	
$80,000 to $99,999	29	*8.3*	
$70,000 to $79,999	16	*4.6*	
$50,000 to $69,999	28	*8.0*	
< $50,000	53	*15.1*	
marital status			n, %
married or common-law	301	*85.8*	
divorced	32	*9.1*	
single	13	*3.7*	
prefer not to answer	5	*1.4*	
education			n, %
bachelors degree or above	202	*57.5*	
went to college	122	*34.8*	
high school degree or less	27	*7.7*	
employment			n, %
full-time wage	181	*52*	
part-time wage	43	*12*	
self-employed	42	*12*	
homemaker	40	*11*	
currently not working	26	*7*	

Associations between financial security, emotional wellbeing and adolescent health behaviors.



THE UNIVERSITY OF BRITISH COLUMBIA

FINANCIAL SUPPORT amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim.Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam

KIND THANKS TO amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim.Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam

BC Children's Hospital

CIHR IRSC

METHODS

Analytical sample and measures

Parents and grade 7 student pairs (N=355) completed an online survey in May –June 2020, assessing family financial security, parent and teen emotional wellbeing (self-esteem, optimism, worry and depression) and teen health behaviors (screen time and physical activity).

Adolescents completed three 24-hour dietary recalls using the ASA24 platform. Dietary quality was computed using the Healthy Eating Index (HEI)[3], which evaluates compliance of reported intake with national dietary recommendations.

Statistical analysis

Structural equation modelling was used to examine linear relationships using the Stata software (version 15.1).

South Asian	51 (14.7)
South East Asian	24 (6.9)
Annual household income (%)	
$100,000 or higher	169 (48.2)
$80,000 to $99,999	29 (8.3)
$70,000 to $79,999	16 (4.6)
$50,000 to $69,999	28 (8.0)
Less than $50,000	53 (15.1)
Marital status, n (%)	
Divorced	32 (9.1)
Married or common-law	301 (85.8)
Single	13 (3.7)
Prefer not to answer	5 (1.4)
Education, n (%)	
High school degree or less	27 (7.7)
Went to college	122 (34.8)
Bachelors degree or above	202 (57.5)
Employment (%)	
A homemaker	40 (11.4)
Currently not working	26 (7.4)
Employed for wages-full time	181 (51.7)
Employed for wages-part time	43 (12.3)
Self-employed	42 (12)

IMPLICATIONS

Study results highlight the role of emotional wellbeing in the pathway through which financial security impacts adolescent health behaviors. As public policy addressing financial security may indirectly improve adolescent health behaviors, our findings will inform COVID-19 public health priorities — specifically, family-based efforts to support and promote adolescent health behaviors and emotional wellbeing.

THE UNIVERSITY OF BRITISH COLUMBIA

(1) Statistics Canada's March 2020 Canadian Perspectives Survey Series
(2) Carroll, N., Sadowski, A., Laila, A., Hruska, V., Nixon, M., Ma, D. W., & Haines, J. (2020). *Nutrients, 12*(8), 2352.
(3) Guenther, P. M., Reedy, J., & Krebs-Smith, S. M. (2008). *Journal of the American Dietetic Association, 108*(11), 1896-1901.

Acknowledgements

FINANCIAL SUPPORT amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim.Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam

KIND THANKS TO amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim.Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam

FINANCIAL SUPPORT amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim.Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam

KIND THANKS TO amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim.Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam

Exploring the impact of fina
How has COVID-19 influen

Iyoma Y. Edache[1], MSc Mark Pitblado[2] Sara

[1]School of Population and Public Health [2]Department of Microbiology & Im

OBJECTIVES

To investigate the link between financial security, parent and adolescent emotional wellbeing and adolescents' health behaviors during the COVID-19 pandemic.

IMPLICATIONS

Study results highlight the role of emotional wellbeing in the pathway through which financial security impacts adolescent health behaviors. As public policy addressing financial security may indirectly improve adolescent health behaviors, our findings will inform COVID-19 public health priorities — specifically, family-based efforts to support and promote adolescent health behaviors and emotional wellbeing.

BACKGROUND

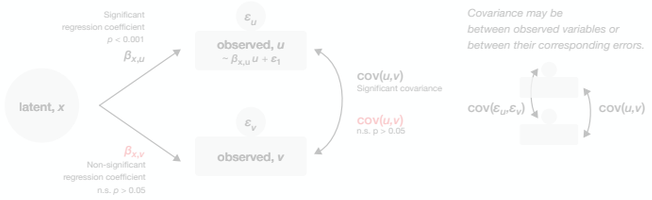
The COVID-19 pandemic disrupted Canadian families' daily routines and social interactions due to government-mandated physical distancing restrictions. Three out of 10 Canadians report that COVID-19 has negatively impacted their ability to meet financial obligations[1]. Health behaviors have also been impacted as physical activity has decreased while screen time and food consumption have increased2. Cumulatively, these disruptions have increased parent and adolescent emotional strain[2].

ANALYTICAL SAMPLE AND MEASURES

Parents and grade 7 student pairs (n = 355) completed an online survey in May –June 2020, assessing family financial security, parent and teen emotional wellbeing (self-esteem, optimism, worry and depression) and teen health behaviors (screen time and physical activity). Adolescents completed three 24-hour dietary recalls using the ASA24 platform. Dietary quality was computed using the Healthy Eating Index (HEI)[3], which evaluates compliance of reported intake with national dietary recommendations.

STATISTICAL ANALYSIS

Structural equation modelling was used to examine linear relationships using the Stata software (version 15.1).



1 Statistics Canada's March 2020 Canadian Perspectives Survey Series

2 Carroll, N., Sadowski, A., Laila, A., Hruska, V., Nixon, M., Ma, D. W., & Haines, J. (2020). Nutrients, 12(8), 2352.

3 Guenther, P. M., Reedy, J., & Krebs-Smith, S. M. (2008). Journal of the American Dietetic Association, 108(11), 1896-1901

THE UNIVERSITY OF BRITISH COLUMBIA

olescent health behaviors: How has COVID-19 quality?

D

², Department of Pediatrics³, University of British Columbia

KEY FINDINGS

Figure 1. Associations between financial security, emotional wellbeing and adolescent health behaviors.

IMPLICATIONS

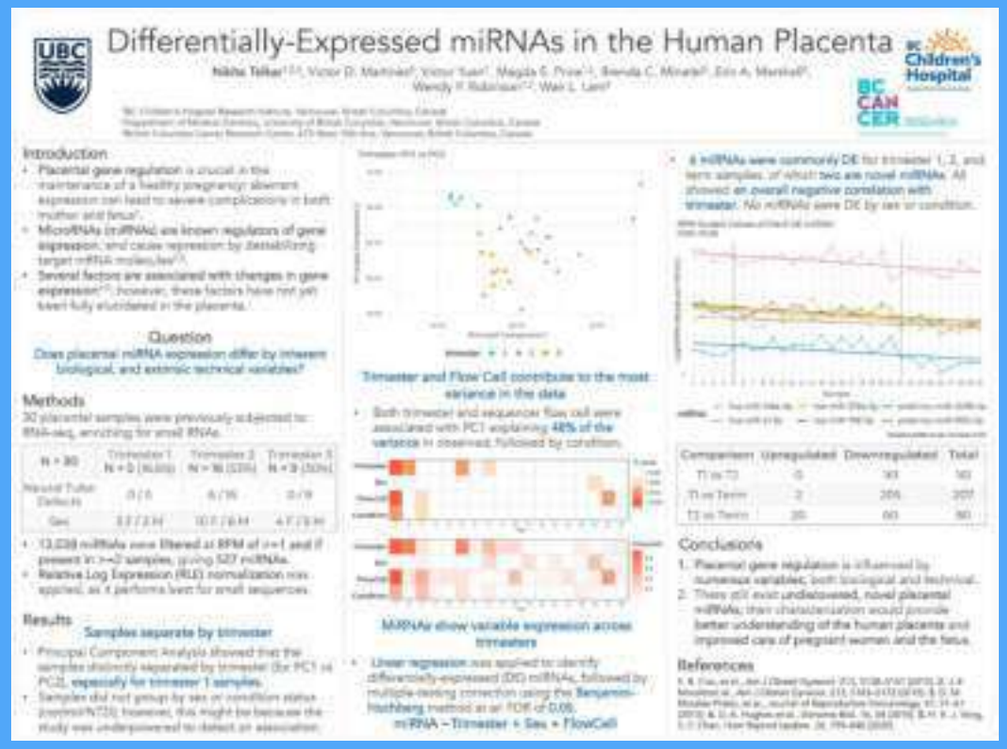
Study results highlight the role of emotional wellbeing in the pathway through which financial security impacts adolescent health behaviors. As public policy addressing financial security may indirectly improve adolescent health behaviors, our findings will inform COVID-19 public health priorities — specifically, family-based efforts to support and promote adolescent health behaviors and emotional wellbeing.

(1) Statistics Canada's March 2020 Canadian Perspectives Survey Series
(2) Carroll, N., Sadowski, A., Laila, A., Hruska, V., Nixon, M., Ma, D. W., & Haines, J. (2020). Nutrients, 12(8), 2352.
(3) Guenther, P. M., Reedy, J., & Krebs-Smith, S. M. (2008). Journal of the American Dietetic Association, 108(11), 1896-1901.

Acknowledgements

BC Children's Hospital

CIHR IRSC
Canadian Institutes of Health Research / Instituts de recherche en santé du Canada



no bullets

establish hierarchy using good text layout

Differentially-Expressed miRNAs in the Human Placenta

Nikita Telkar[1,2,3], Victor D. Martinez[3], Victor Yuan[1], Magda E. Price[1,2], Brenda C. Minatel[3], Erin A. Marshall[3], Wendy P. Robinson[1,2], Wan L. Lam[3]

[1]BC Children's Hospital Research Institute, Vancouver, British Columbia, Canada
[2]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada
[3]British Columbia Cancer Research Centre, 675 West 10th Ave, Vancouver, British Columbia, Canada

Introduction

- Placental gene regulation is crucial in the maintenance of a healthy pregnancy; aberrant expression can lead to severe complications in both mother and fetus[1].
- MicroRNAs (miRNAs) are known regulators of gene expression, and cause repression by destabilizing target mRNA molecules[2,3].
- Several factors are associated with changes in gene expression[4,5]; however, these factors have not yet been fully elucidated in the placenta.

Question

Does placental miRNA expression differ by inherent biological, and extrinsic technical variables?

Methods

30 placental samples were previously subjected to RNA-seq, enriching for small RNAs.

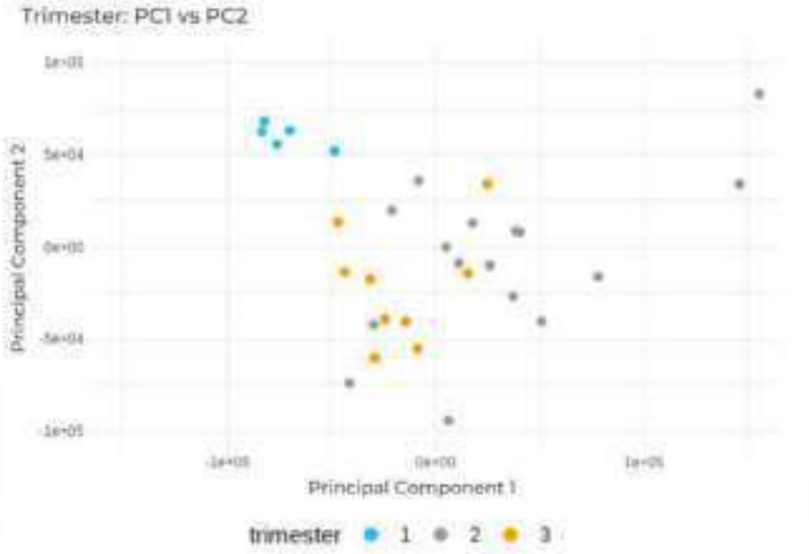
N = 30	Trimester 1 N = 5 (16.6%)	Trimester 2 N = 16 (53%)	Trimester 3 N = 9 (30%)
Neural Tube Defects	0 / 5	6 / 16	0 / 9
Sex	3 F / 2 M	10 F / 6 M	4 F / 5 M

- 13,038 miRNAs were filtered at RPM of >=1 and if present in >=2 samples, giving 527 miRNAs.
- Relative Log Expression (RLE) normalization was applied, as it performs best for small sequences.

Results

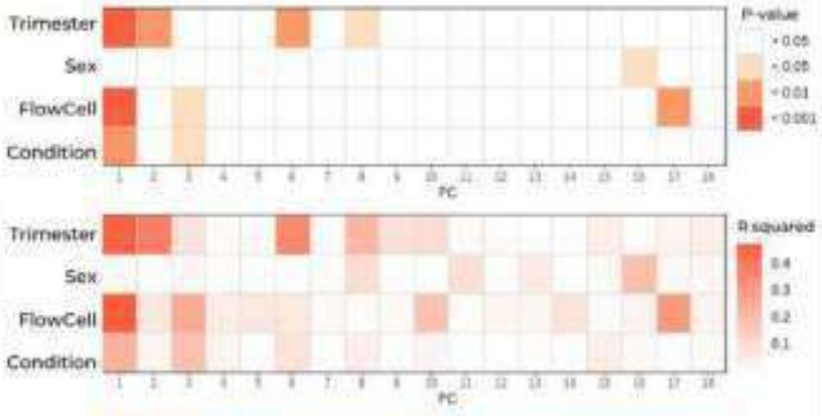
Samples separate by trimester

- Principal Component Analysis showed that the samples distinctly separated by trimester (for PC1 vs PC2), **especially for trimester 1 samples.**
- *Samples did not group by sex or condition status (control/NTD)*; however, this might be because the study was underpowered to detect an association.



Trimester and Flow Cell contribute to the most variance in the data

- Both trimester and sequencer flow cell were associated with PC1 explaining **48% of the variance** in observed, followed by condition.

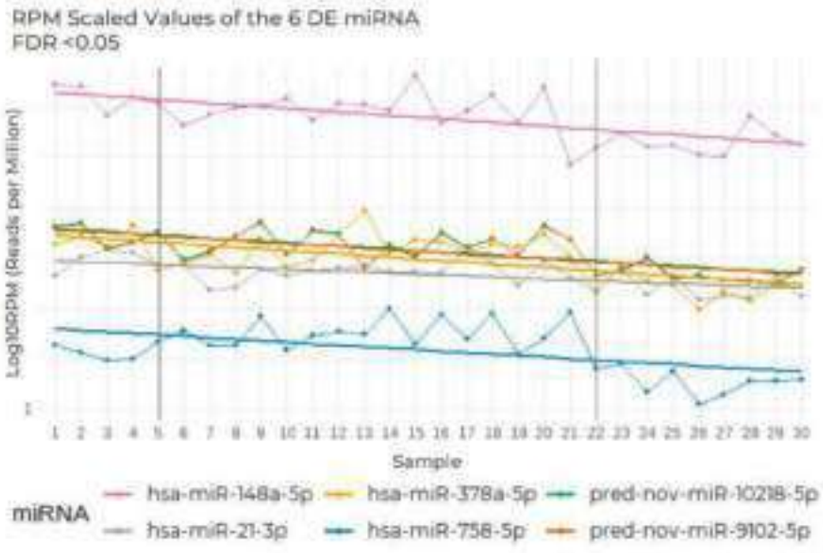


MiRNAs show variable expression across trimesters

- **Linear regression** was applied to identify differentially-expressed (DE) miRNAs, followed by multiple-testing correction using the **Benjamini-Hochberg** method at an FDR of **0.05**.

miRNA ~Trimester + Sex + FlowCell

- **6 miRNAs were commonly DE** for trimester 1, 2, and term samples, of which **two are novel miRNAs.** All showed **an overall negative correlation with trimester.** *No miRNAs were DE by sex or condition.*



Comparison	Upregulated	Downregulated	Total
T1 vs T2	0	161	161
T1 vs Term	2	205	207
T2 vs Term	20	60	80

Conclusions

1. Placental gene regulation is influenced by numerous variables, both biological and technical.
2. There still exist undiscovered, novel placental miRNAs; their characterization would provide better understanding of the human placenta and improved care of pregnant women and the fetus.

References

1. B. Cox, et al., Am J Obstet Gynecol. 213, S138–S151 (2015). **2.** J.-F. Moulletet al., Am J Obstet Gynecol. 213, S163–S172 (2015). **3.** D. M. Morales-Prieto, et al., Journal of Reproductive Immunology. 97, 51–61 (2013). **4.** D. A. Hughes et al., Genome Biol. 16, 54 (2015). **5.** H. E. J. Yong, S.-Y. Chan, Hum Reprod Update. 26, 799–840 (2020).

Does placental miRNA expression differ by inherent biological and extrinsic technical variables?

Yes, but it's more complicated than that.

Nikita Telkar[1,2,3], Victor D. Martinez[3], Victor Yuan[1], Magda E. Price[1,2], Brenda C. Minatel[3], Erin A. Marshall[3], Wendy P. Robinson[1,2], Wan L. Lam[3]

[1]BC Children's Hospital Research Institute, Vancouver, British Columbia, Canada; [2]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada; [3]British Columbia Cancer Research Centre, 675 West 10th Ave, Vancouver, British Columbia, Canada

INTRODUCTION

Placental gene regulation is crucial in the maintenance of a healthy pregnancy; aberrant expression can lead to severe complications in both mother and fetus [1].

MicroRNAs (miRNAs) are known regulators of gene expression, and cause repression by destabilizing target mRNA molecules [2,3].

Several factors are associated with changes in gene expression [4,5]. However, these factors have not yet been fully elucidated in the placenta.

KEY OBSERVATIONS

Placental gene regulation is influenced by numerous variables, both biological and technical.

	up	down	total
T1 vs T2	0	161	161
T1 vs Term	2	205	207
T2 vs Term	20	60	80

There still exist undiscovered, novel placental miRNAs; their characterization would provide better understanding of the human placenta and improved care of pregnant women and the fetus.

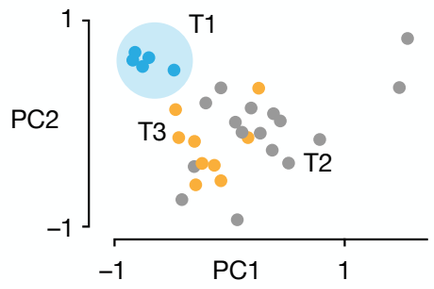
METHODS

30 placental samples were previously subjected to RNA-seq, enriching for small RNAs.

trimester	*n*		sex F	sex M	neural tube defects
1	5	17%	3	2	0
2	16	53%	10	6	6
3	9	30%	4	5	9

527/13,038 miRNAs were present in more than one sample with RPM ≥1. Relative Log Expression (RLE) normalization was applied, which performs best for small sequences.

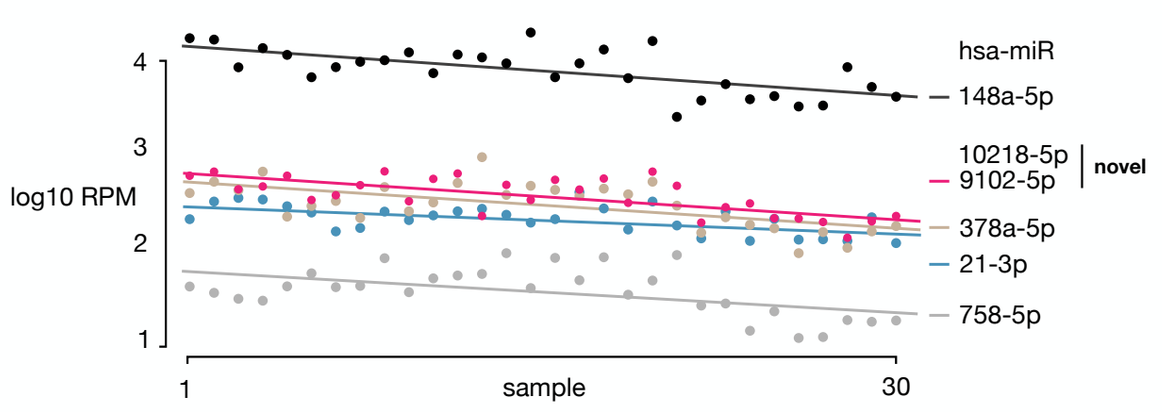
SAMPLES SEPARATE BY TRIMESTER



Samples distinctly separate by trimester, especially for T1.

Samples did not group by sex or condition status (control/NTD). This may be due to lack of power to detect this association.

MIRNAS SHOW VARIABLE EXPRESSION ACROSS TRIMESTERS



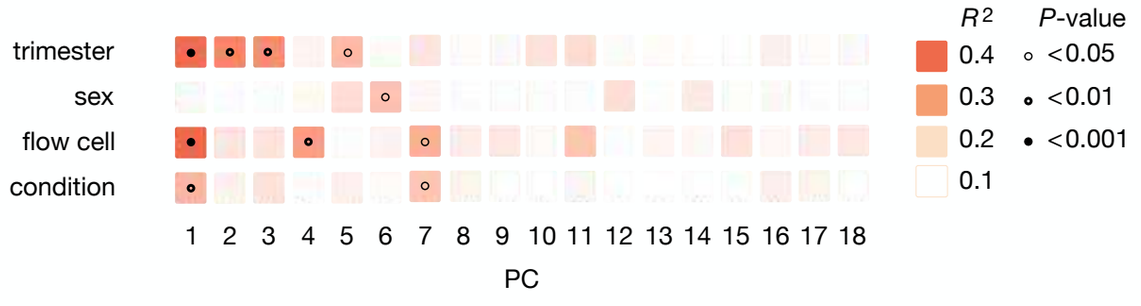
`miRNA ~ Trimester + Sex + Flow Cell`

Linear regression was applied to identify Differentially-expressed (DE) miRNAs were identified using linear regression with Benjamini-Hochberg multiple test correction at FDR = 0.05.

6 miRNAs were commonly DE for T1, T2 and Term samples. Two were novel.

All showed an overall negative correlation with trimester. No miRNAs were DE by sex or condition.

TRIMESTER AND FLOW CELL CONTRIBUTE TO THE MOST VARIANCE IN THE DATA



Both trimester and sequencer flow cell were associated with PC1, explaining 48% of the variance in observed, followed by condition.

[1] B Cox et al. *Am J Obstet Gynecol* (2015) **213**:S138–S151 (2015) **[2]** J-F Mouilletet et al. *Am J Obstet Gynecol* (2015) **213**:S163–S172 **[3]** DM Morales-Prieto et al. *J Rep Immun* (2013) **97**:51–61 **[4]** DA Hughes et al. *Genome Biol* (2015) **16**:54 **[5]** HEJ Yong & S-Y Chan *Hum Reprod Update* (2020) **26:** 799–840

Introduction

- Placental gene regulation is crucial in the maintenance of a healthy pregnancy; aberrant expression can lead to severe complications in both mother and fetus[1].
- MicroRNAs (miRNAs) are known regulators of gene expression, and cause repression by destabilizing target mRNA molecules[2,3].
- Several factors are associated with changes in gene expression[4,5]; however, these factors have not yet been fully elucidated in the placenta.

Question

Does placental miRNA expression differ by inherent biological, and extrinsic technical variables?

Methods

30 placental samples were previously subjected to RNA-seq, enriching for small RNAs.

N = 30	Trimester 1 N = 5 (16.6%)	Trimester 2 N = 16 (53%)	Trimester 3 N = 9 (30%)
Neural Tube Defects	0 / 5	6 / 16	0 / 9
Sex	3 F / 2 M	10 F / 6 M	4 F / 5 M

- 13,038 miRNAs were filtered at RPM of >=1 and if present in >=2 samples, giving 527 miRNAs.
- Relative Log Expression (RLE) normalization was applied, as it performs best for small sequences.

Results

Samples separate by trimester

- Principal Component Analysis showed that the samples distinctly separated by trimester (for PC1 vs PC2), especially for trimester 1 samples.
- *Samples did not group by sex or condition status (control/NTD)*; however, this might be because the study was underpowered to detect an association.

Does placental miRNA expression differ by inherent biological and extrinsic technical variables?

Yes, but it's more complicated than that.

INTRODUCTION

Placental gene regulation is crucial in the maintenance of a healthy pregnancy; aberrant expression can lead to severe complications in both mother and fetus [1].

MicroRNAs (miRNAs) are known regulators of gene expression, and cause repression by destabilizing target mRNA molecules [2,3].

Several factors are associated with changes in gene expression [4,5]. However, these factors have not yet been fully elucidated in the placenta.

KEY OBSERVATIONS

Placental gene regulation is influenced by numerous variables, both biological and technical.

	up	down	total
T1 vs T2	0	161	161
T1 vs Term	2	205	207
T2 vs Term	20	60	80

There still exist undiscovered, novel placental miRNAs; their characterization would provide better understanding of the human placenta and improved care of pregnant women and the fetus.

METHODS

30 placental samples were previously subjected to RNA-seq, enriching for small RNAs.

trimester	*n*		sex F	sex M	neural tube defects
1	5	17%	3	2	0
2	16	53%	10	6	6
3	9	30%	4	5	9

527/13,038 miRNAs were present in more than one sample with RPM ≥1. Relative Log Expression (RLE) normalization was applied, which performs best for small sequences.

[1] B Cox et al. *Am J Obstet Gynecol* (2015) **213**:S138–S151 (2015) **[2]** J-F Mouilletet et al. *Am J Obstet Gynecol* (2015) **213**:S163–S172 **[3]** DM Morales-Prieto et al. *J Rep Immun* (2013) **97**:51–61 **[4]** DA Hughes et al. *Genome Biol* (2015) **16**:54 **[5]** HEJ Yong & S-Y Chan *Hum Reprod Update* (2020) **26:** 799–840

N = 30	Trimester 1 N = 5 (16.6%)	Trimester 2 N = 16 (53%)	Trimester 3 N = 9 (30%)
Neural Tube Defects	0 / 5	6 / 16	0 / 9
Sex	3 F / 2 M	10 F / 6 M	4 F / 5 M

Comparison	Upregulated	Downregulated	Total
T1 vs T2	0	161	161
T1 vs Term	2	205	207
T2 vs Term	20	60	80

	up	down	total
T1 vs T2	0	161	161
T1 vs Term	2	205	207
T2 vs Term	20	60	80

trimester	*n*		sex F	sex M	neural tube defects
1	5	17%	3	2	0
2	16	53%	10	6	6
3	9	30%	4	5	9

KEY OBSERVATIONS

Placental gene regulation is influenced by numerous variables, both biological and technical.

	up	down	total
T1 vs T2	0	161	161
T1 vs Term	2	205	207
T2 vs Term	20	60	80

There still exist undiscovered, novel placental miRNAs; their characterization would provide better understanding of the human placenta and improved care of pregnant women and the fetus.

METHODS

30 placental samples were previously subjected to RNA-seq, enriching for small RNAs.

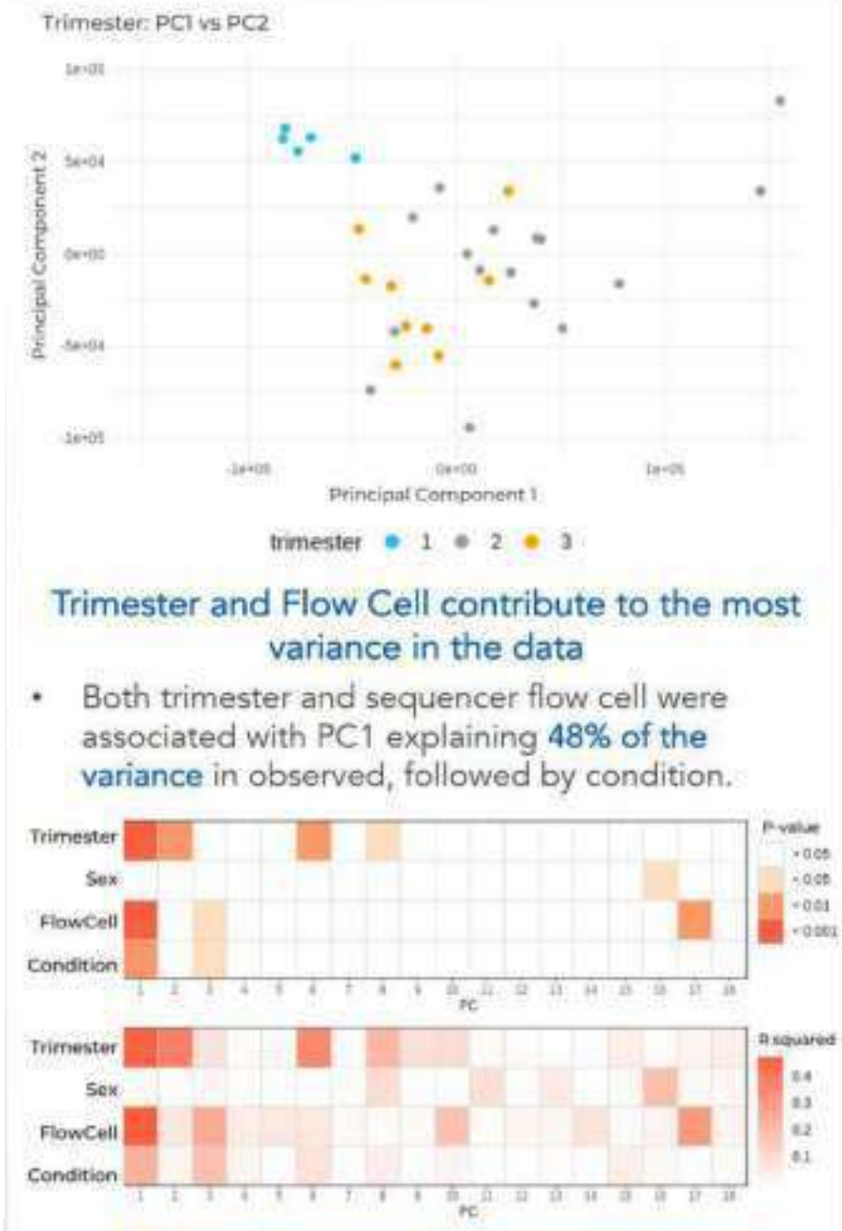
trimester	*n*		sex F	sex M	neural tube defects
1	5	17%	3	2	0
2	16	53%	10	6	6
3	9	30%	4	5	9

527/13,038 miRNAs were present in more than one sample with RPM ≥1. Relative Log Expression (RLE) normalization was applied, which performs best for small sequences.

Results

Samples separate by trimester

- Principal Component Analysis showed that the samples distinctly separated by trimester (for PC1 vs PC2), **especially for trimester 1 samples.**
- *Samples did not group by sex or condition status (control/NTD)*; however, this might be because the study was underpowered to detect an association.



Trimester and Flow Cell contribute to the most variance in the data

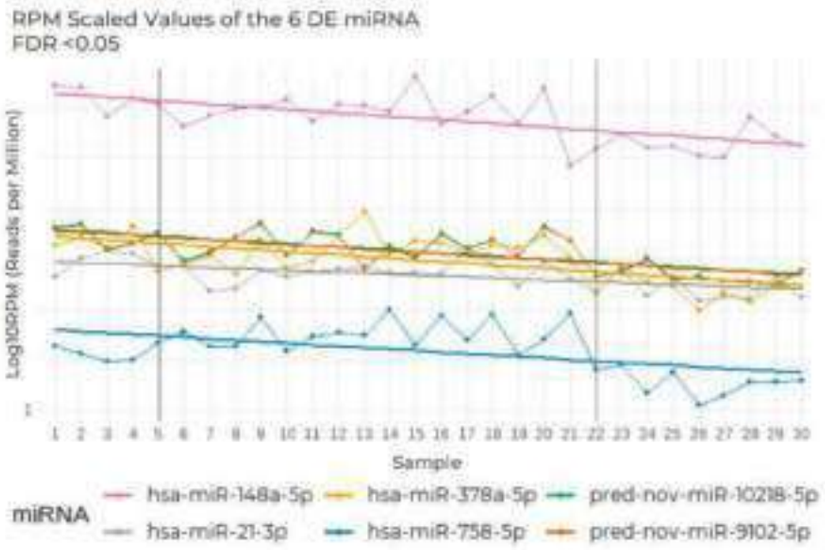
- Both trimester and sequencer flow cell were associated with PC1 explaining **48% of the variance** in observed, followed by condition.

MiRNAs show variable expression across trimesters

- **Linear regression** was applied to identify differentially-expressed (DE) miRNAs, followed by multiple-testing correction using the **Benjamini-Hochberg** method at an FDR of **0.05**.

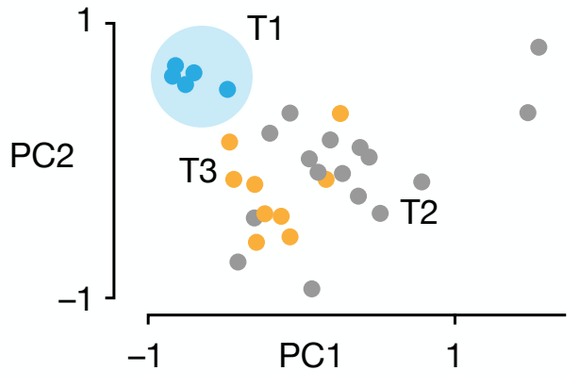
miRNA ~Trimester + Sex + FlowCell

- **6 miRNAs were commonly DE** for trimester 1, 2, and term samples, of which **two are novel miRNAs**. All showed **an overall negative correlation with trimester**. *No miRNAs were DE by sex or condition.*



Comparison	Upregulated	Downregulated	Total
T1 vs T2	0	161	161
T1 vs Term	2	205	207
T2 vs Term	20	60	80

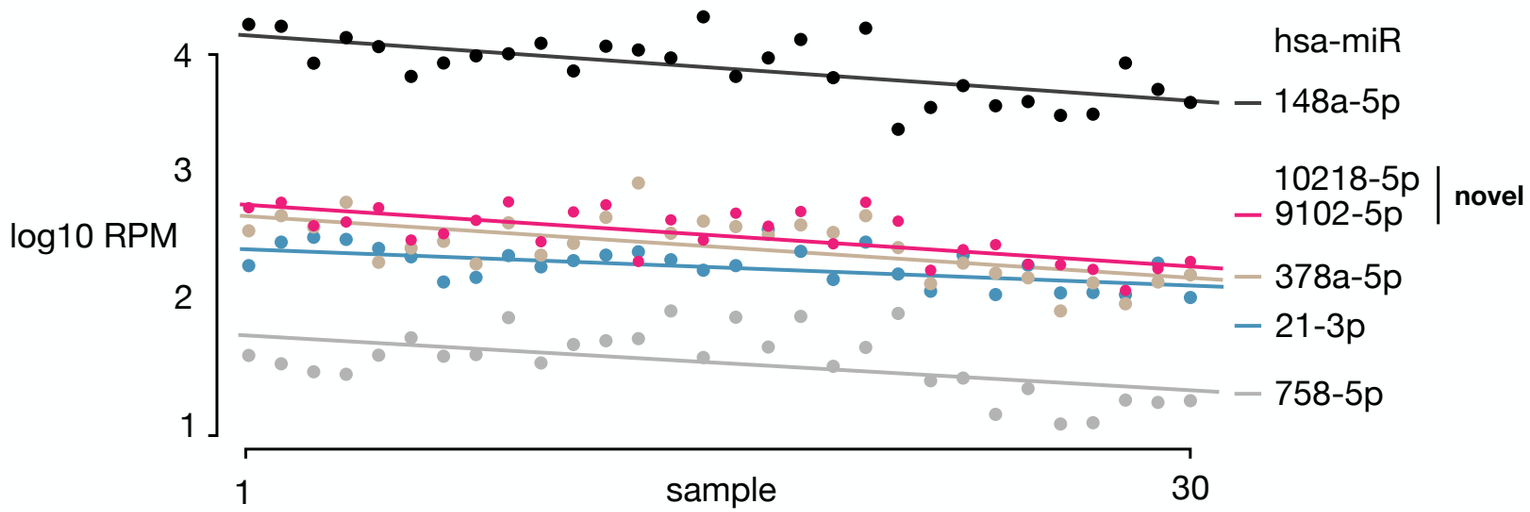
SAMPLES SEPARATE BY TRIMESTER



Samples distinctly separate by trimester, especially for T1.

Samples did not group by sex or condition status (control/NTD). This may be due to lack of power to detect this association.

MIRNAS SHOW VARIABLE EXPRESSION ACROSS TRIMESTERS



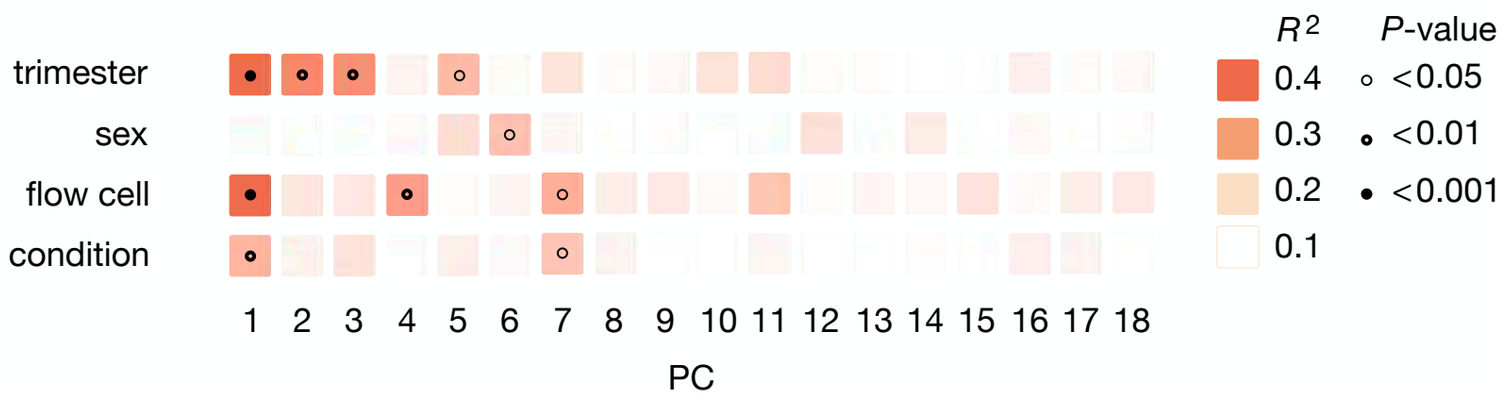
`miRNA ~ Trimester + Sex + Flow Cell`

Linear regression was applied to identify Differentially-expressed (DE) miRNAs were identified using linear regression with Benjamini-Hochberg multiple test correction at FDR = 0.05.

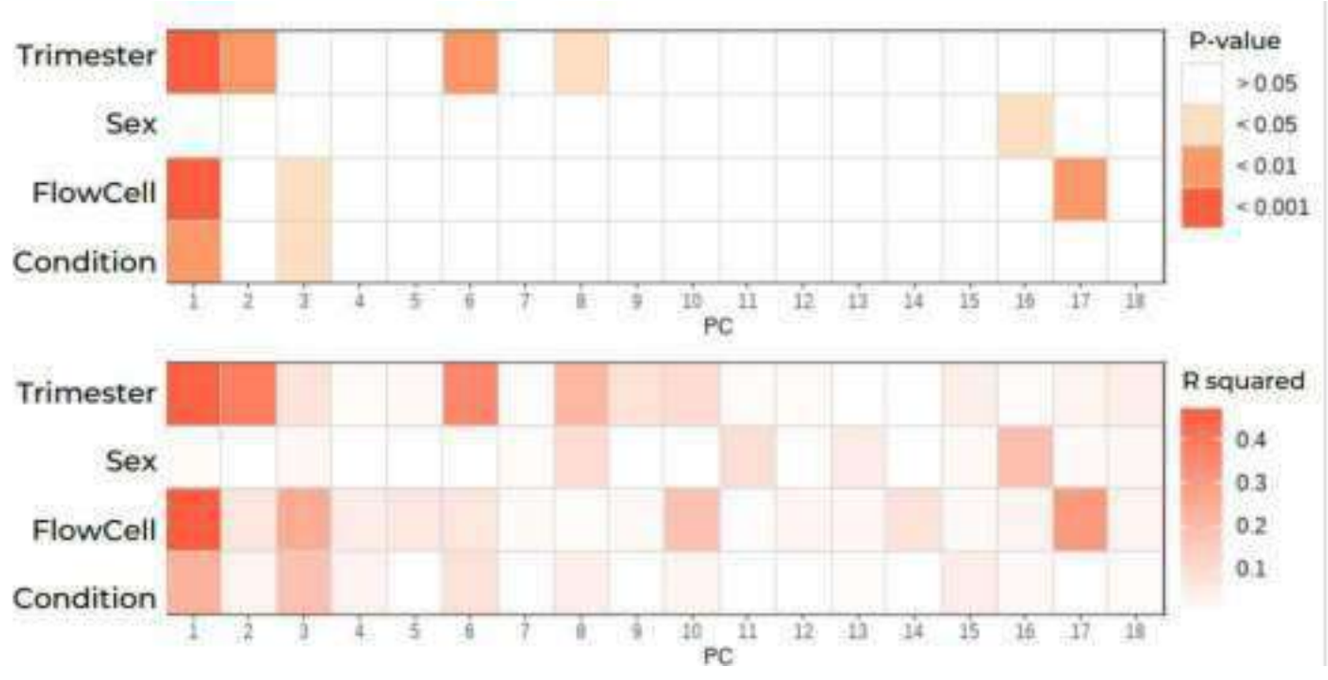
6 miRNAs were commonly DE for T1, T2 and Term samples. Two were novel.

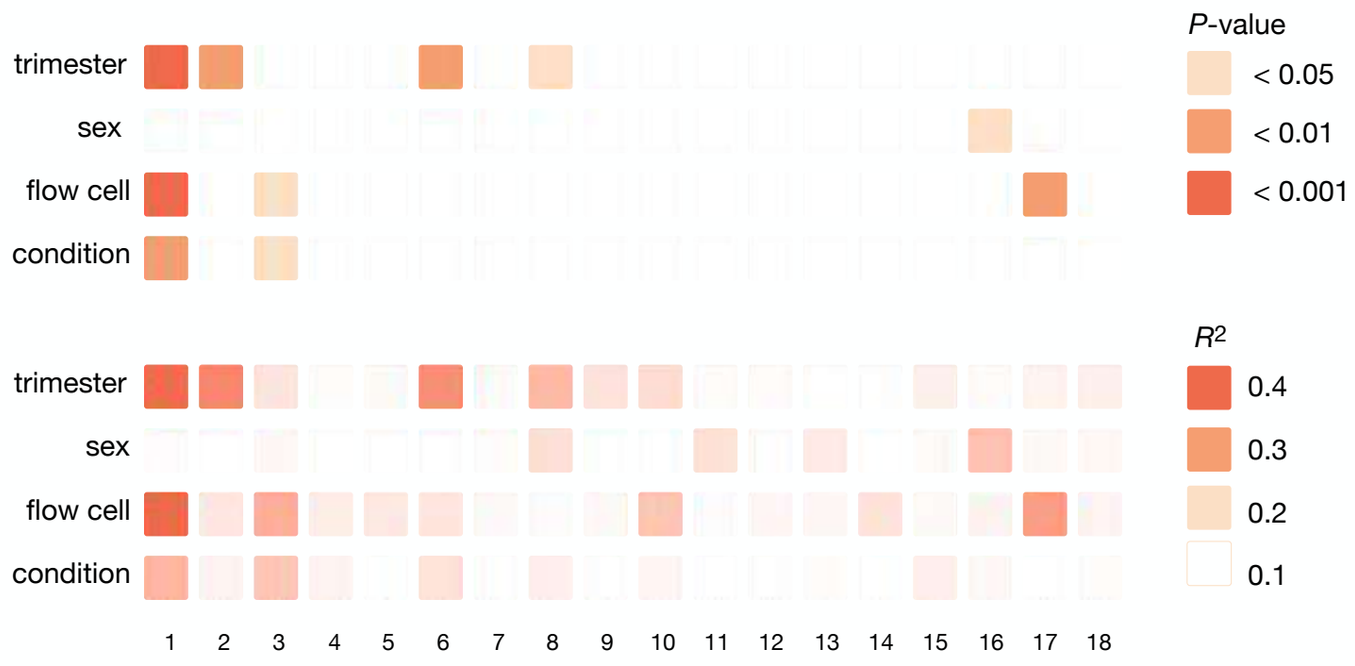
All showed an overall negative correlation with trimester. No miRNAs were DE by sex or condition.

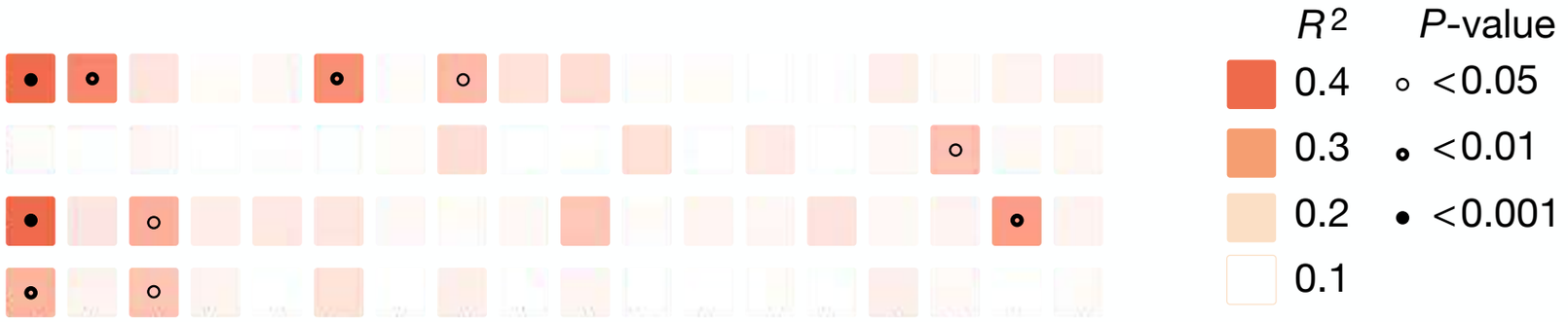
TRIMESTER AND FLOW CELL CONTRIBUTE TO THE MOST VARIANCE IN THE DATA

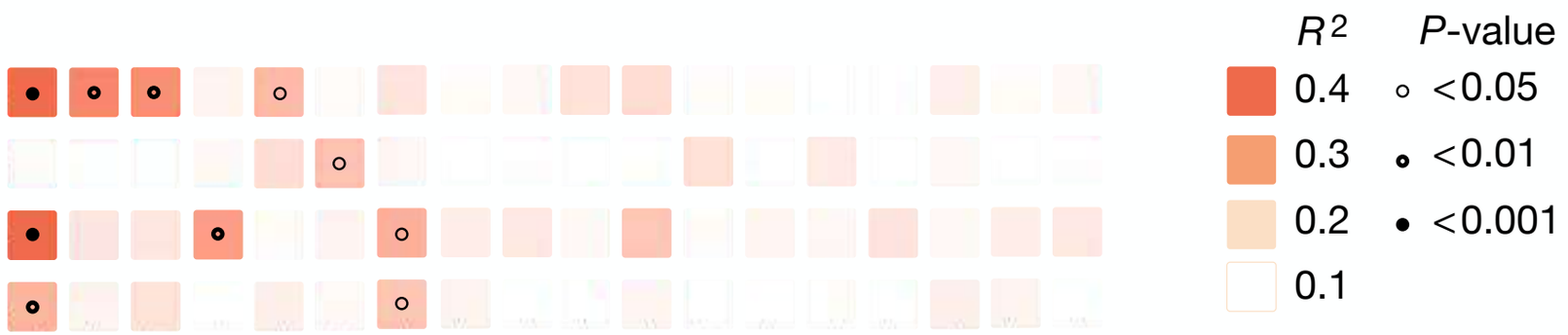


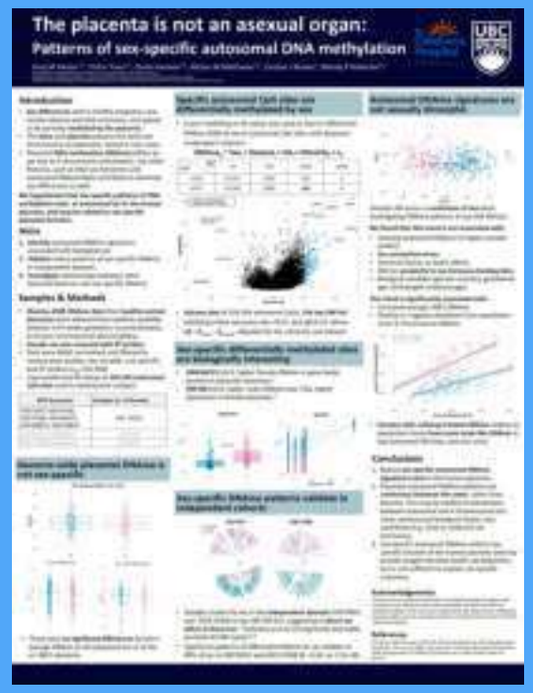
Both trimester and sequencer flow cell were associated with PC1, explaining 48% of the variance in observed, followed by condition.


Trimester
Sex
FlowCell
Condition
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
PC
P-value
> 0.05
< 0.05
< 0.01
< 0.001
Trimester
Sex
FlowCell
Condition
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
PC
R squared
0.4
0.3
0.2
0.1


trimester
sex
flow cell
condition
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
P-value
< 0.05
< 0.01
< 0.001
R2
0.4
0.3
0.2
0.1


R^2
0.4
0.3
0.2
0.1
P-value
<0.05
<0.01
<0.001


R^2
0.4
0.3
0.2
0.1
P-value
<0.05
<0.01
<0.001



ink-heavy templates

shout over data

explain the science, brand later (or never)

The placenta is not an asexual organ:
Patterns of sex-specific autosomal DNA methylation

Amy M Inkster[1,2], Victor Yuan[1,2], Chaini Konwar[1,2], Allison M Matthews[1,3], Carolyn J Brown[2], Wendy P Robinson[1,2]

Introduction

- **Sex differences** exist in healthy pregnancy and certain adverse perinatal outcomes, and appear to be partially **mediated by the placenta**.[1]
- The **fetus** and **placenta** possess the same sex chromosome complement, except in rare cases.
- Placental **DNA methylation (DNAme)** differs by sex due to X chromosome inactivation, but other features, such as fetal sex hormones and autosomal DNAme likely contribute to placental sex differences as well.

We hypothesize that sex-specific patterns of DNA methylation exist at autosomal loci in the human placenta, and may be related to sex-specific placental function.

Aims

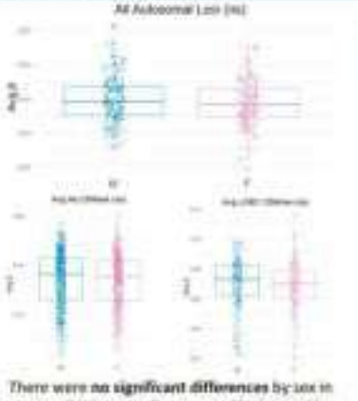
1. **Identify** autosomal DNAme signatures associated with biological sex
2. **Validate** robust patterns of sex-specific DNAme in independent datasets
3. **Investigate** relationships between other placental features and sex-specific DNAme

Samples & Methods

- **Illumina 450K DNAme data** from **healthy normal placentas** were obtained from publicly available datasets (>37 weeks gestation, no preeclampsia, no known chromosomal abnormalities).
- **Sample sex was assessed with XY probes.**
- Data were BMIQ normalized and filtered to remove poor quality, non-variable, non-specific, and XY probes (n_{fil}=161,408).
- Log-transformed M values at **324,104 autosomal CpG sites** used in downstream analysis.

GEO Accession	Samples (n, % female)
GSE73375, GSE74738, GSE75248, GSE100197, GSE100857, GSE128827	841 (53%)
GSE70453 (validation)	72 (47%)
GSE115508 (validation)	44 (45%)

Genome-wide placental DNAme is not sex-specific



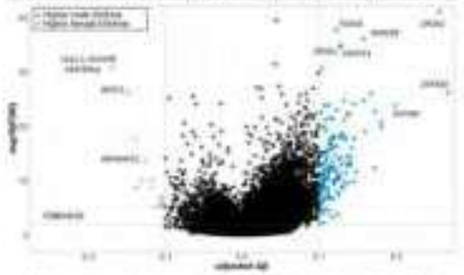
- There were **no significant differences** by sex in average DNAme at all autosomal loci or at Alu or LINE1 elements.

Specific autosomal CpG sites are differentially methylated by sex

- Linear modelling on M-values was used to test for differential DNAme (DM) by sex at autosomal CpG sites, with Bayesian-moderated t-statistics.

$$\mathbf{DNAme}_{ij} \sim \mathbf{Sex}_i + \mathbf{Dataset}_i + \mathbf{GA}_i + \mathbf{Ethnicity}_i + \epsilon_{ij}$$

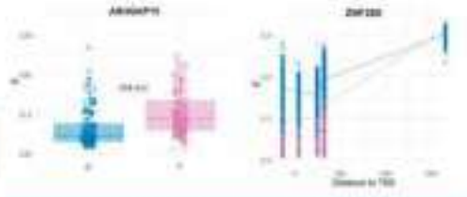
FDR \ Δβ	>0	>5%	>10%	>20%
<0.05	24,715	2,942	166	4
<0.01	14,108	2,682	**166**	4



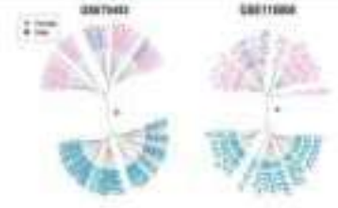
- **Volcano plot** of 324,104 autosomal CpGs, **166 top DM loci** satisfying a false discovery rate <0.01, and Δβ>0.10, where $\Delta\bar{\beta} = \bar{\beta}_{male} - \bar{\beta}_{female}$. Adjusted for GA, ethnicity, and dataset.

Sex-specific differentially methylated sites are biologically interesting

- **ARHGAP15** (chr2, higher female DNAme in gene body) present in placental exosomes.[3]
- **ZNF300** (chr6, higher male DNAme near TSS), higher expression in female placentas.[3]

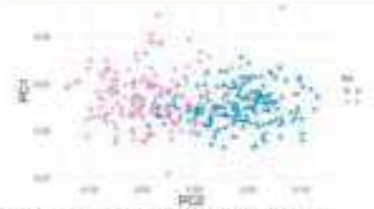


Sex-specific DNAme patterns validate in independent cohorts



- Samples cluster by sex in two **independent datasets** GSE70453 and GSE115508 at top 166 DM loci, suggesting a **robust sex effect at these loci**. *Indicates p<2.2e-16 (sigClust2) and stable (pvclust) AU>80 cluster.[3,4]
- Significant patterns of differential DNAme by sex validate at 90% of loci in GSE70453 and GSE115508 ($R = 0.62$, $p < 2.2e\text{-}16$).

Autosomal DNAme signatures are not sexually dimorphic



Samples fall across a **continuum of sex** when investigating DNAme patterns at top 166 DM loci.

We found that this trend is not associated with:

- Outlying autosomal DNAme (% highly variable probes)
- **Sex annotation errors**
- Technical factors or batch effects.
- DM loci **proximity to sex-hormone binding sites.**
- Biological variables (genetic ancestry, gestational age, birthweight, maternal age).

This trend is significantly associated with:

- Increased average LINE1 DNAme
- Positive or negative deviations from population mean X chromosome DNAme.

- **Females with outlying X-linked DNAme** relative to population trends **have more male-like DNAme** at top autosomal DM sites, and vice-versa.

Conclusions

1. Robust **sex-specific autosomal DNAme signatures exist** in the human placenta.
2. Placental autosomal DNAme patterns are **continuous between the sexes**, rather than discrete. This may be related to interactions between autosomal and X chromosomal loci; other unmeasured biological factors may contribute (e.g. fetal or maternal sex hormones).
3. Sex-specific autosomal DNAme reflects sex-specific function of the human placenta and may provide insight into fetal health sex disparities, but is not sufficient to explain sex-specific outcomes.

Acknowledgements

Thank you to all patients and families who kindly donated samples, and members of the Robinson Lab, especially WPR, VY, MSP, and GDG for valuable feedback. This work was supported by the Department of Medical Genetics (UBC) and the Canadian Institutes of Health Research (ICT-163379, CIHR SVB-158613).

References

[1] Clifton (2010). Placenta 31:S33-S39. [2] Teschendorff et al. 2013. Bioinformatics 29:189-96. [3] Liu et al. 2008. J Am Stat Assoc 103:1281. [4] Suzuki & Shimodaira 2006. Bioinformatics 12:1540-42. [5] Kuleshov et al. 2016. Nucleic Acids Res gkw377.

THE PLACENTA IS NOT AN ASEXUAL ORGAN
Patterns of sex-specific autosomal DNA methylation

Amy M Inkster[1,2], Victor Yuan[1,2], Chaini Konwar[1,2], Allison M Matthews[1,3], Carolyn J Brown[2], Wendy P Robinson[1,2]

[1]BC Children's Hospital Research Institute, Vancouver, British Columbia, Canada
[2]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada
[3]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

SEX-SPECIFIC PATTERNS OF DNA METHYLATION

Sex differences exist in healthy pregnancy and certain adverse perinatal outcomes, and appear to be partially mediated by the placenta[1].

The fetus and placenta possess the same sex chromosome complement, except in rare cases.

Placental DNA methylation (DNAme) differs by sex due to X chromosome inactivation, but other features, such as fetal sex hormones and autosomal DNAme likely contribute to placental sex differences as well.

We hypothesize that sex-specific patterns of DNA methylation exist at autosomal loci in the human placenta, and may be related to sex-specific placental function.

ROBUST SEX-SPECIFIC AUTOSOMAL DNAme SIGNATURES EXIST IN THE HUMAN PLACENTA.

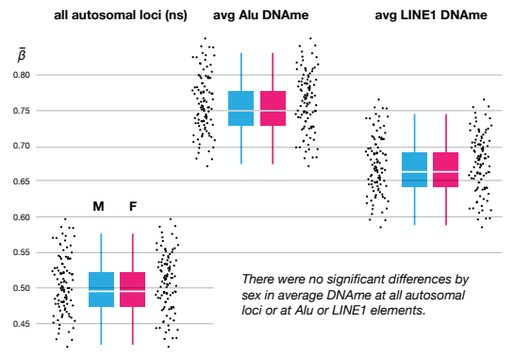
Placental autosomal DNAme patterns are continuous between the sexes, rather than discrete. This may be related to interactions between autosomal and X chromosomal loci; other unmeasured biological factors may contribute (e.g. Fetal or maternal sex hormones).

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad minim veniam, quis.

Sex-specific autosomal DNAme reflects sex-specific function of the human placenta and may provide insight into fetal health sex disparities, but is not sufficient to explain sex-specific outcomes.

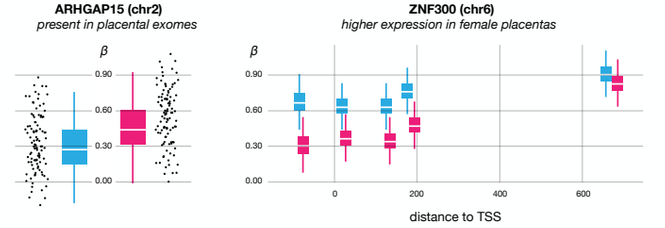
Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad minim veniam, quis.

Genome-wide placental DNAme is not sex-specific



There were no significant differences by sex in average DNAme at all autosomal loci or at Alu or LINE1 elements.

Sex-specific differentially methylated sites are biologically interesting



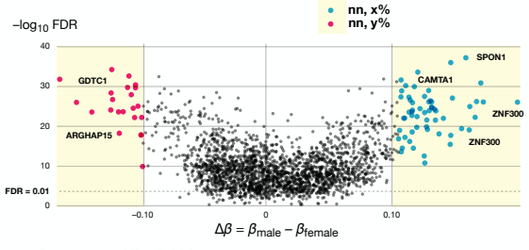
Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad minim veniam, quis nostrud exerci tation ullamcorper suscipit lobortis nisl ut aliquip ex ea commodo consequat. Duis autem vel eum iriure dolor in hendrerit in vulputate velit esse molestie consequat, vel illum dolore eu feugiat nulla facilisis at vero eros et accumsan et iusto odio dignissim

Specific autosomal CpG sites are differentially methylated by sex

$$\mathbf{DNAme}_{ij} \sim \mathbf{sex}_i + \mathbf{dataset}_i + \mathbf{GA}_i + \mathbf{ethnicity}_i + \epsilon_{ij}$$

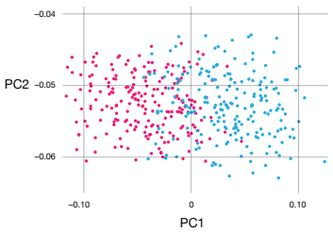
324,104 autosomal CpGs. Adjusted for GA, ethnicity, and dataset.

FDR	Δβ	> 0	> 5%	> 10%	> 20%
< 0.05		24,715	2,942	166	4
< 0.01		14,108	2,682	**166**	4



Autosomal DNAme signatures are not sexually dimorphic

Samples fall across a continuum of sex when investigating DNAme patterns at top 166 DM loci.

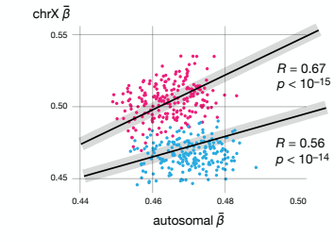


This trend **is significantly associated** with

- Increased average LINE1 DNAme
- Positive or negative deviations from population mean X chromosome DNAme.

This trend **is not associated** with

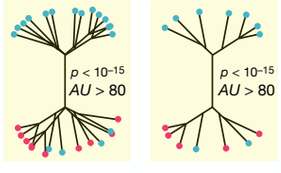
- Outlying autosomal DNAme (% highly variable probes)
- Sex annotation errors
- Technical factors or batch effects.
- DM loci proximity to sex-hormone binding sites.
- Biological variables (genetic ancestry, gestational age, birthweight, maternal age).



Females with outlying X-linked DNAme relative to population trends have more male-like DNAme at top autosomal DM sites, and vice-versa.

Sex-specific DNAme patterns validate in independent cohorts

Samples cluster by sex in two independent datasets at top 166 DM loci, suggesting a robust sex effect at these loci. P-value from sigClust2. Cluster stable (pvclust) at AU > 80. Significant patterns of differential DNAme by sex validate at 90% of loci in GSE70453 and GSE115508 (R = 0.62, p< 2.2e-16).



Samples & Methods

GEO Accession Samples (*n*, % female)
GSE73375, GSE74738, GSE75248, GSE100197, GSE100857, GSE128827 (841, 51%)
GSE70453 (validation) (72, 47%)
GSE115508 (validation) (44, 45%)

Illumina 450K DNAme data from healthy normal placentas were obtained from publicly available datasets (>37 weeks gestation, no preeclampsia, no known chromosomal abnormalities). Sample sex was assessed with XY probes. Data were BMIQ normalized and filtered to remove poor quality, non-variable, non-specific, and XY probes (nfilt=161,408). Log-transformed M values at 324,104 autosomal CpG sites used in downstream analysis.

[1] Clifton 2010. Placenta 21:S33-S39. [2] Teschendorff et al. 2013. Bioinformatics 29:189-96. [3] Liu et al. 2008. J Am Stat Assoc 103:1281. [4] Suzuki & Shimodaira 2006. Bioinformatics 12:1540-42. [5] Kuleshov et al. 2016. Nucleic Acids Res gkw377.

Thank you to all patients and families who kindly donated samples, and members of the Robinson Lab, especially WPR, VY, MSP, and GDG for valuable feedback. This work was supported by the Department of Medical Genetics (UBC) and the Canadian Institutes of Health Research (ICT-163379,CIHR SVB-158613).

The placenta is not an asexual organ:
Patterns of sex-specific autosomal DNA methylation

Amy M Inkster[1,2], Victor Yuan[1,2], Chaini Konwar[1,2], Allison M Matthews[1,3], Carolyn J Brown[2], Wendy P Robinson[1,2]
[illegible]

Introduction

- **Sex differences** exist in healthy pregnancy and certain adverse perinatal outcomes, and appear to be partially **mediated by the placenta**.[1]
- The **fetus** and **placenta** possess the same sex chromosome complement, except in rare cases.
- Placental **DNA methylation (DNAme)** differs by sex due to X chromosome inactivation, but other features, such as fetal sex hormones and autosomal DNAme likely contribute to placental sex differences as well.

We hypothesize that sex-specific patterns of DNA methylation exist at autosomal loci in the human placenta, and may be related to sex-specific placental function.

Aims

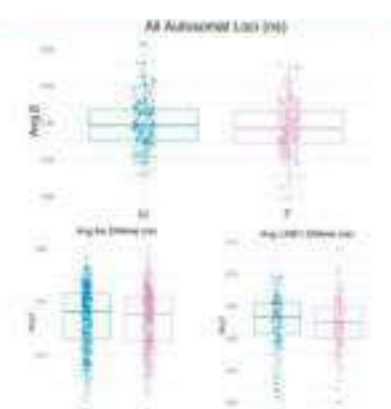
1. **Identify** autosomal DNAme signatures associated with biological sex
2. **Validate** robust patterns of sex-specific DNAme in independent datasets
3. **Investigate** relationships between other placental features and sex-specific DNAme

Samples & Methods

- **Illumina 450K DNAme data** from **healthy normal placentas** were obtained from publicly available datasets (>37 weeks gestation, no preeclampsia, no known chromosomal abnormalities).
- **Sample sex was assessed with XY probes.**
- Data were BMIQ normalized and filtered to remove poor quality, non-variable, non-specific, and XY probes (n_{rm}=161,408).
- Log-transformed M values at **324,104 autosomal CpG sites** used in downstream analysis.

GEO Accession	Samples (n, % female)
GSE73375, GSE74738, GSE75248, GSE100197, GSE108567, GSE128827	341 (51%)
[illegible] (validation)	[illegible]
[illegible] (validation)	[illegible]

Genome-wide placental DNAme is not sex-specific



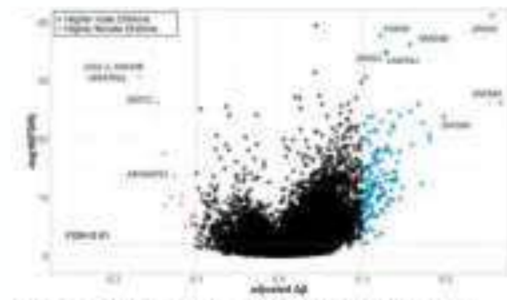
- There were **no significant differences** by sex in average DNAme at all autosomal loci or at Alu or LINE1 elements.

Specific autosomal CpG sites are differentially methylated by sex

- Linear modelling on M-values was used to test for differential DNAme (DM) by sex at autosomal CpG sites, with Bayesian-moderated t-statistics.

$$DNAme_{ij} \sim Sex_i + Dataset_i + GA_i + Ethnicity_i + \varepsilon_{ij}$$

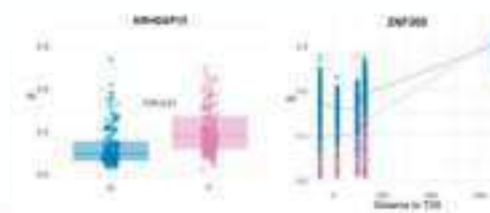
FDR \ Δβ	>0	>5%	>10%	>20%
<0.05	24,715	2,942	[illegible]	4
<0.01	14,108	2,682	**166**	4

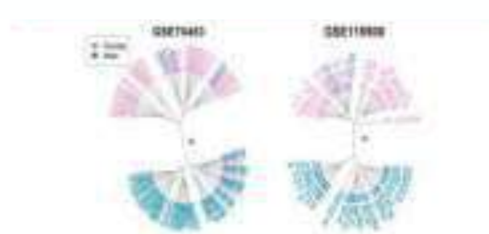


- **Volcano plot** of 324,104 autosomal CpGs, **166 top DM loci** satisfying a false discovery rate <0.01, and $\Delta\beta$>0.10, where $\Delta\beta = \beta_{male} - \beta_{female}$. Adjusted for GA, ethnicity, and dataset.

Sex-specific differentially methylated sites are biologically interesting

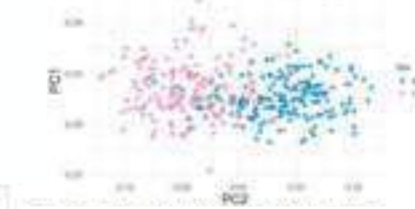
- ***ARHGAP15*** (chr2, higher female DNAme in gene body) present in placental exosomes.[3]
- ***ZNF300*** (chr6, higher male DNAme near TSS), higher expression in female placentas.[5]





- Samples cluster by sex in two **independent datasets** GSE70453 and GSE115508 at top 166 DM loci, suggesting a **robust sex effect at these loci**. *Indicates p<2.2e-16 (sigClust2) and stable (pvclust) AU>80 cluster.[3,4]
- Significant patterns of differential DNAme by sex validate at 90% of loci in GSE70453 and GSE115508 (R = 0.62, p< 2.2e-16).

Autosomal DNAme signatures are not sexually dimorphic



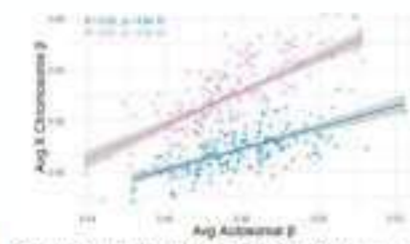
Samples fall across a **continuum of sex** when investigating DNAme patterns at top 166 DM loci.

We found that this trend *is not* associated with:

- Outlying autosomal DNAme (% highly variable probes)
- **Sex annotation errors**
- Technical factors or batch effects.
- DM loci **proximity to sex-hormone binding sites.**
- Biological variables (genetic ancestry, gestational age, birthweight, maternal age).

This trend *is* significantly associated with:

- Increased average LINE1 DNAme
- Positive or negative deviations from population mean X chromosome DNAme.



- **Females with outlying X-linked DNAme** relative to population trends **have more male-like DNAme** at top autosomal DM sites, and vice-versa.

Conclusions

1. Robust **sex-specific autosomal DNAme signatures exist** in the human placenta.
2. Placental autosomal DNAme patterns are **continuous between the sexes**, rather than discrete. This may be related to interactions between autosomal and X chromosomal loci; other unmeasured biological factors may contribute (e.g. Fetal or maternal sex hormones).
3. Sex-specific autosomal DNAme reflects sex-specific function of the human placenta and may provide insight into fetal health sex disparities, but is not sufficient to explain sex-specific outcomes.

Acknowledgements

Thank you to all patients and families who kindly donated samples, and members of the Robinson Lab [illegible] for valuable feedback. This work was supported by the Department of Medical Genetics [illegible] and the Canadian Institutes of Health Research [illegible].

References

[illegible]

THE PLACENTA IS NOT AN ASEXUAL ORGAN
Patterns of sex-specific autosomal DNA methylation

Amy M Inkster[1,2], Victor Yuan[1,2], Chaini Konwar[1,2], Allison M Matthews[1,3], Carolyn J Brown[2], Wendy P Robinson[1,2]

[1]BC Children's Hospital Research Institute, Vancouver, British Columbia, Canada
[2]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada
[3]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

SEX-SPECIFIC PATTERNS OF DNA METHYLATION

Sex differences exist in healthy pregnancy and certain adverse perinatal outcomes, and appear to be partially mediated by the placenta[1].

The fetus and placenta possess the same sex chromosome complement, except in rare cases.

Placental DNA methylation (DNAme) differs by sex due to X chromosome inactivation, but other features, such as fetal sex hormones and autosomal DNAme likely contribute to placental sex differences as well.

We hypothesize that sex-specific patterns of DNA methylation exist at autosomal loci in the human placenta, and may be related to sex-specific placental function.

ROBUST SEX-SPECIFIC AUTOSOMAL DNAme SIGNATURES EXIST IN THE HUMAN PLACENTA.

Placental autosomal DNAme patterns are continuous between the sexes, rather than discrete. This may be related to interactions between autosomal and X chromosomal loci; other unmeasured biological factors may contribute (e.g. Fetal or maternal sex hormones).

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad minim veniam, quis.

Sex-specific autosomal DNAme reflects sex-specific function of the human placenta and may provide insight into fetal health sex disparities, but is not sufficient to explain sex-specific outcomes.

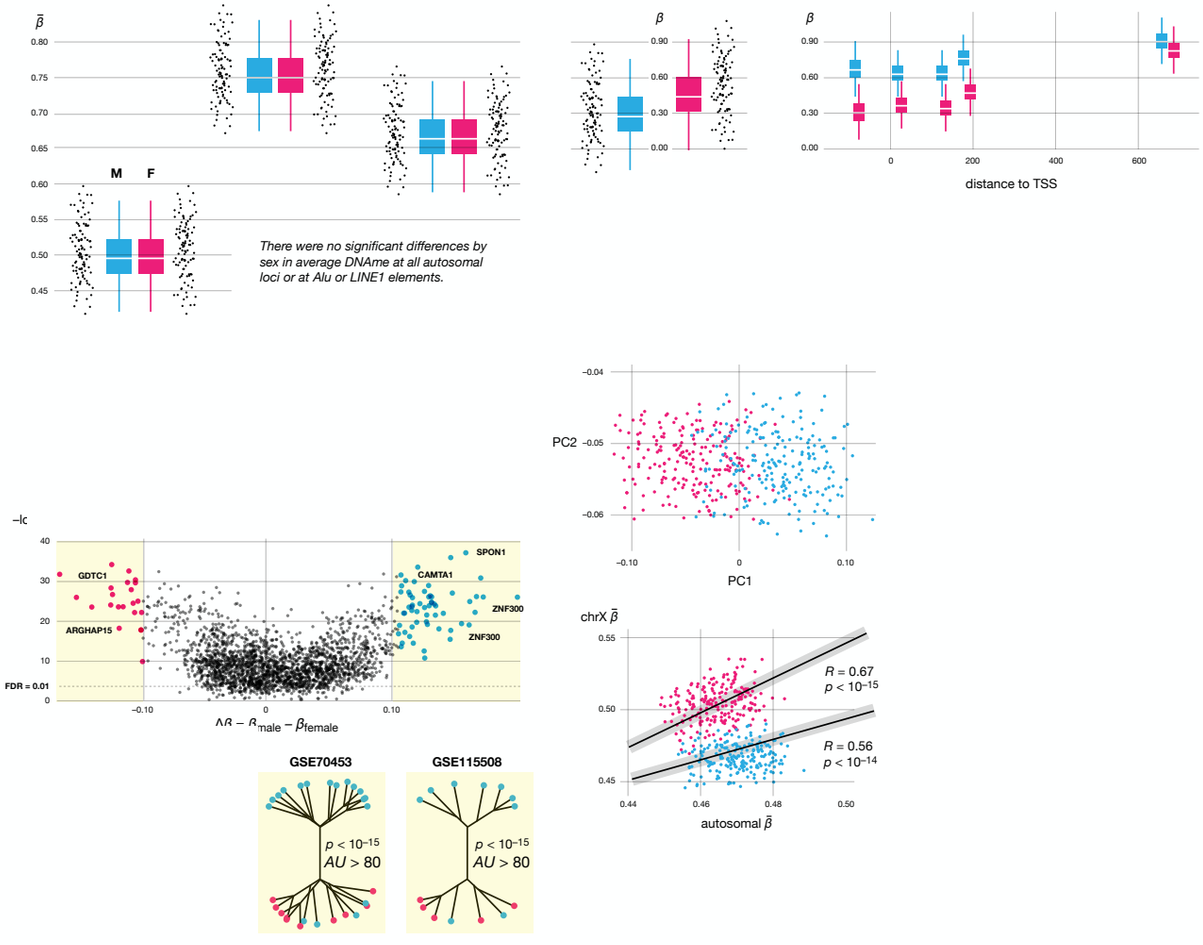
Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad minim veniam, quis.

Genome-wide placental DNAme is not sex-specific

Sex-specific differentially methylated sites are biologically interesting

ARHGAP15 (chr2)
present in placental exomes

ZNF300 (chr6)
higher expression in female placentas



There were no significant differences by sex in average DNAme at all autosomal loci or at Alu or LINE1 elements.

Specific autosomal CpG sites are differentially methylated by sex

$DNAme_{ij} \sim sex_i + dataset_i + GA_i + ethnicity_i + \varepsilon_{ij}$

324,104 autosomal CpGs. Adjusted for GA, ethnicity, and dataset.

FDR	Δβ	> 0	> 5%	> 10%	> 20%
< 0.05		24,715	2,942	166	4
< 0.01		14,108	2,682	**166**	4

Autosomal DNAme signatures are not sexually dimorphic

Samples fall across a continuum of sex when investigating DNAme patterns at top 166 DM loci.

This trend **is significantly associated** with
- Increased average LINE1 DNAme
- Positive or negative deviations from population mean X chromosome DNAme.

This trend **is not associated** with
- Outlying autosomal DNAme (% highly variable probes)
- Sex annotation errors
- Technical factors or batch effects.
- DM loci proximity to sex-hormone binding sites.
- Biological variables (genetic ancestry, gestational age, birthweight, maternal age).

Females with outlying X-linked DNAme relative to population trends have more male-like DNAme at top autosomal DM sites, and vice-versa.

Sex-specific DNAme patterns validate in independent cohorts

Samples cluster by sex in two independent datasets at top 166 DM loci, suggesting a robust sex effect at these loci. P-value from sigClust2. Cluster stable (pvclust) at AU > 80. Significant patterns of differential DNAme by sex validate at 90% of loci in GSE70453 and GSE115508 (R = 0.62, p< 2.2e-16).

Samples & Methods

GEO Accession Samples (*n*, % female)
GSE73375, GSE74738, GSE75248, GSE100197, GSE100857, GSE128827 (341, 51%)
GSE70453 (validation) (72, 47%)
GSE115508 (validation) (44, 45%)

Illumina 450K DNAme data from healthy normal placentas were obtained from publicly available datasets (>37 weeks gestation, no preeclampsia, no known chromosomal abnormalities). Sample sex was assessed with XY probes. Data were BMIQ normalized and filtered to remove poor quality, non-variable, non-specific, and XY probes (nfilt=161,408). Log-transformed M values at 324,104 autosomal CpG sites used in downstream analysis.

[1] Clifton 2010. Placenta 21:S33-S39. [2] Teschendorff et al. 2013. Bioinformatics 29:189-96. [3] Liu et al. 2008. J Am Stat Assoc 103:1281. [4] Suzuki & Shimodaira 2006. Bioinformatics 12:1540-42. [5] Kuleshov et al. 2016. Nucleic Acids Res gkw377.

Thank you to all patients and families who kindly donated samples, and members of the Robinson Lab, especially WPR, VY, MSP, and GDG for valuable feedback. This work was supported by the Department of Medical Genetics (UBC) and the Canadian Institutes of Health Research (ICT-163379,CIHR SVB-158613).

THE PLACENTA IS NOT AN ASEXUAL ORGAN
Patterns of sex-specific autosomal DNA methylation

Amy M Inkster[1,2], Victor Yuan[1,2], Chaini Konwar[1,2], Allison M Matthews[1,3], Carolyn J Brown[2], Wendy P Robinson[1,2]

[1]BC Children's Hospital Research Institute, Vancouver, British Columbia, Canada
[2]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada
[3]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

SEX-SPECIFIC PATTERNS OF DNA METHYLATION

Sex differences exist in healthy pregnancy and certain adverse perinatal outcomes, and appear to be partially mediated by the placenta[1].

The fetus and placenta possess the same sex chromosome complement, except in rare cases.

Placental DNA methylation (DNAme) differs by sex due to X chromosome inactivation, but other features, such as fetal sex hormones and autosomal DNAme likely contribute to placental sex differences as well.

We hypothesize that sex-specific patterns of DNA methylation exist at autosomal loci in the human placenta, and may be related to sex-specific placental function.

ROBUST SEX-SPECIFIC AUTOSOMAL DNAme SIGNATURES EXIST IN THE HUMAN PLACENTA.

Placental autosomal DNAme patterns are continuous between the sexes, rather than discrete. This may be related to interactions between autosomal and X chromosomal loci; other unmeasured biological factors may contribute (e.g. Fetal or maternal sex hormones).

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad minim veniam, quis.

Sex-specific autosomal DNAme reflects sex-specific function of the human placenta and may provide insight into fetal health sex disparities, but is not sufficient to explain sex-specific outcomes.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad minim veniam, quis.

Samples & Methods

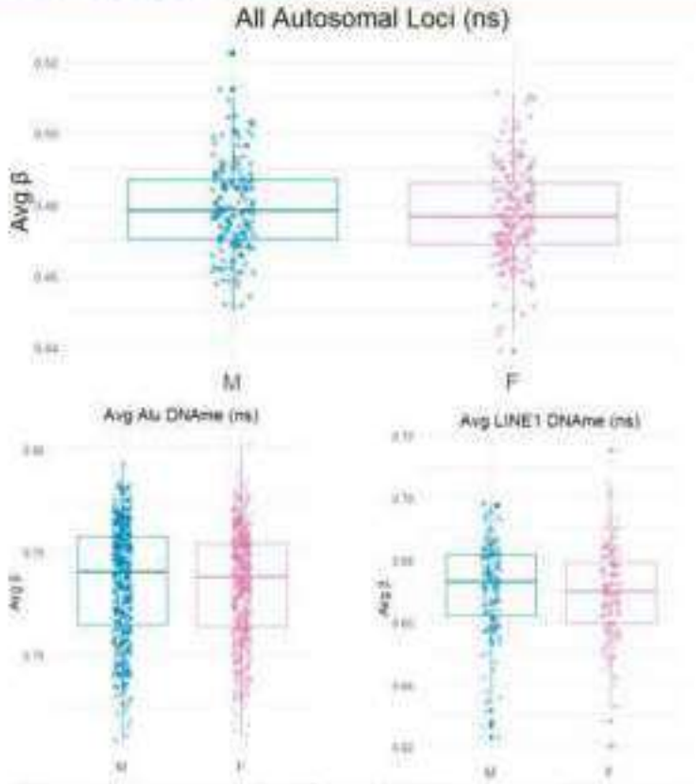
GEO Accession Samples (*n*, % female)
GSE73375, GSE74738, GSE75248, GSE100197, GSE100857, GSE128827 (341, 51%)
GSE70453 (validation) (72, 47%)
GSE115508 (validation) (44, 45%)

Illumina 450K DNAme data from healthy normal placentas were obtained from publicly available datasets (>37 weeks gestation, no preeclampsia, no known chromosomal abnormalities). Sample sex was assessed with XY probes. Data were BMIQ normalized and filtered to remove poor quality, non-variable, non-specific, and XY probes (nfilt=161,408). Log-transformed M values at 324,104 autosomal CpG sites used in downstream analysis.

Thank you to all patients and families who kindly donated samples, and members of the Robinson Lab, especially WPR, VY, MSP, and GDG for valuable feedback. This work was supported by the Department of Medical Genetics (UBC) and the Canadian Institutes of Health Research (ICT-163379,CIHR SVB-158613).

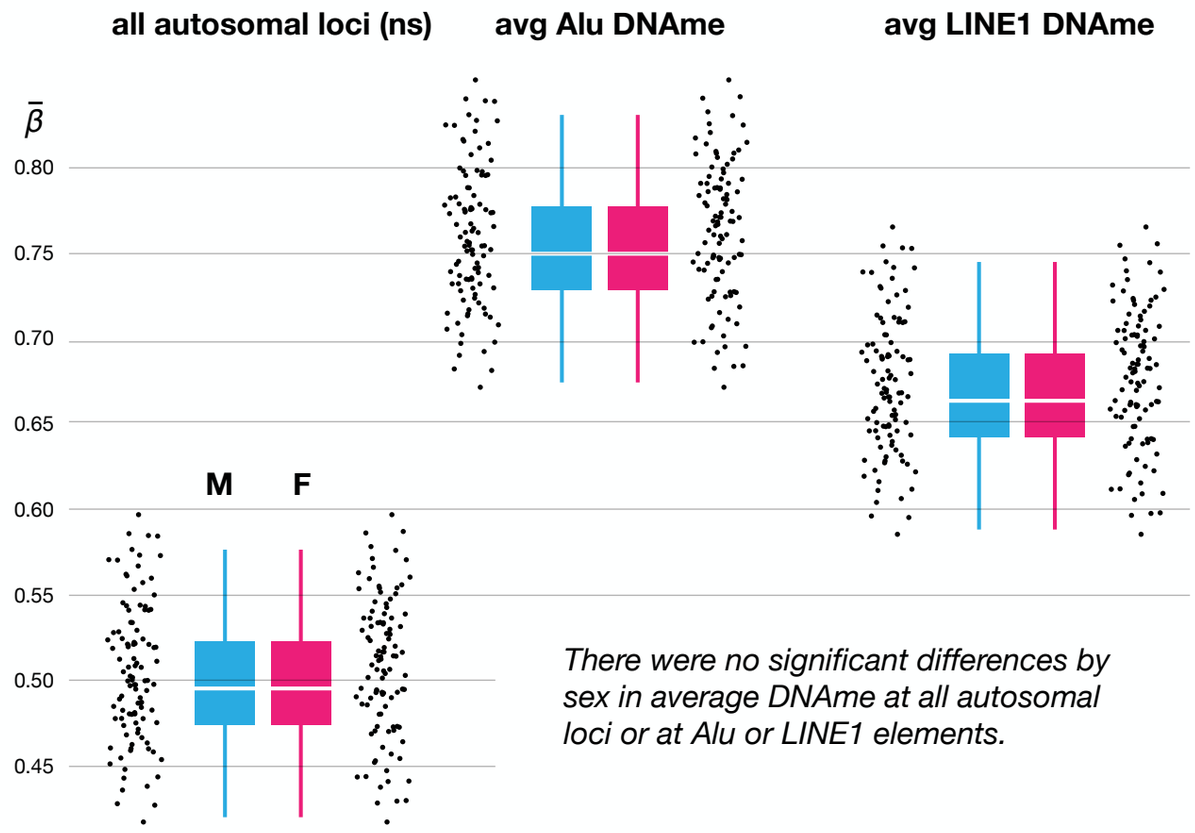
The placenta is
Patterns of sex-spe
Amy M Inkster[1,2], Victor Yuan[1,2], Chaini Kon
Introduction
• Sex differences exist in healthy pregnancy and certain adverse perinatal outcomes, and appear to be partially mediated by the placenta.[1]
• The fetus and placenta possess the same sex chromosome complement, except in rare cases.
• Placental DNA methylation (DNAme) differs by sex due to X chromosome inactivation, but other features, such as fetal sex hormones and autosomal DNAme likely contribute to placental sex differences as well.
We hypothesize that sex-specific patterns of DNA methylation exist at autosomal loci in the human placenta, and may be related to sex-specific placental function.
Aims
1. Identify autosomal DNAme signatures associated with biological sex
2. Validate robust patterns of sex-specific DNAme in independent datasets
3. Investigate relationships between other placental features and sex-specific DNAme
Samples & Methods
• Illumina 450K DNAme data from healthy normal placentas were obtained from publicly available datasets (>37 weeks gestation, no preeclampsia, no known chromosomal abnormalities).
• Sample sex was assessed with XY probes.
• Data were BMIQ normalized and filtered to remove poor quality, non-variable, non-specific, and XY probes (n_{fil}=161,408).
• Log-transformed M values at 324,104 autosomal CpG sites used in downstream analysis.
Conclusions
1. Robust sex-specific autosomal DNAme signatures exist in the human placenta.
2. Placental autosomal DNAme patterns are continuous between the sexes, rather than discrete. This may be related to interactions between autosomal and X chromosomal loci; other unmeasured biological factors may contribute (e.g. Fetal or maternal sex hormones).
3. Sex-specific autosomal DNAme reflects sex-specific function of the human placenta and may provide insight into fetal health sex disparities, but is not sufficient to explain sex-specific outcomes.

Genome-wide placental DNAme is not sex-specific



- There were **no significant differences** by sex in average DNAme at all autosomal loci or at Alu or LINE1 elements.

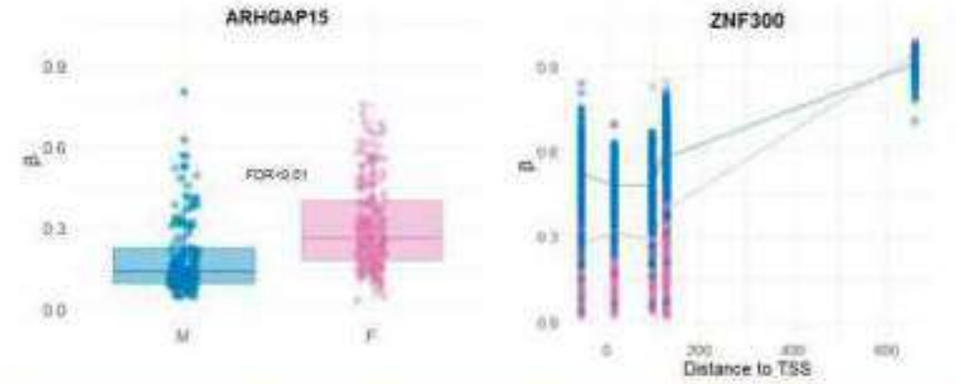
Genome-wide placental DNAme is not sex-specific



There were no significant differences by sex in average DNAme at all autosomal loci or at Alu or LINE1 elements.

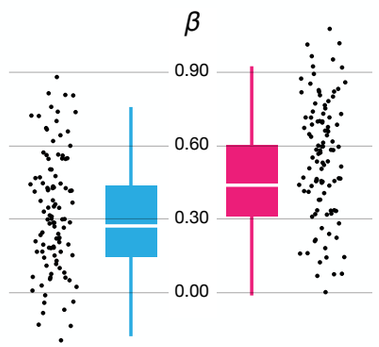
Sex-specific differentially methylated sites are biologically interesting

- ***ARHGAP15*** (chr2, higher female DNAme in gene body) present in placental exosomes.[5]
- ***ZNF300*** (chr6, higher male DNAme near TSS), higher expression in female placentas.[5]

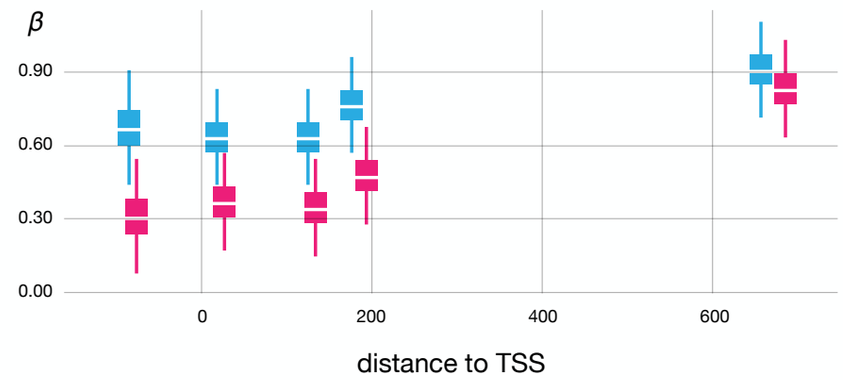


Sex-specific differentially methylated sites are biologically interesting

ARHGAP15 (chr2)
present in placental exomes



ZNF300 (chr6)
higher expression in female placentas



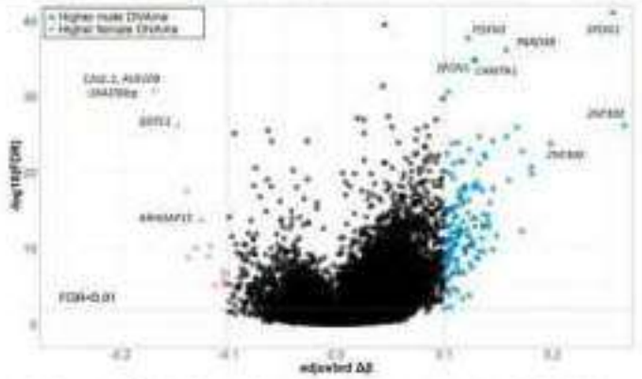
Lorem ipsum dolor sit amet, consectetuer adipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet dolore magna aliquam erat volutpat. Ut wisi enim ad minim veniam, quis nostrud exerci tation ullamcorper suscipit lobortis nisl ut aliquip ex ea commodo consequat. Duis autem vel eum iriure dolor in hendrerit in vulputate velit esse molestie consequat, vel illum dolore eu feugiat nulla facilisis at vero eros et accumsan et iusto odio dignissim

Specific autosomal CpG sites are differentially methylated by sex

- Linear modelling on M-values was used to test for differential DNAme (DM) by sex at autosomal CpG sites, with Bayesian-moderated t-statistics.

$$DNAme_{ij} \sim Sex_i + Dataset_i + GA_i + Ethnicity_i + \varepsilon_{ij}$$

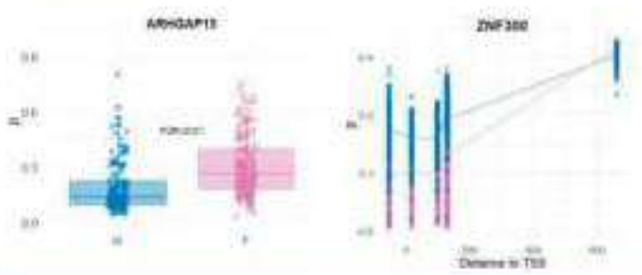
FDR \ Δβ	>0	>5%	>10%	>20%
<0.05	24,715	2,942	166	4
<0.01	14,108	2,682	**166**	4



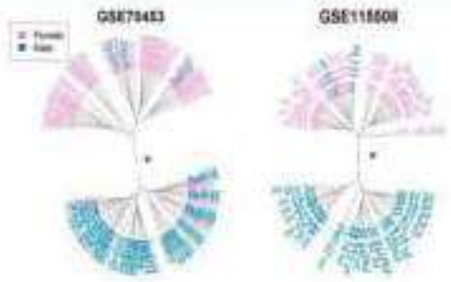
- **Volcano plot** of 324,104 autosomal CpGs, **166 top DM loci** satisfying a false discovery rate <0.01, and Δβ>0.10, where $\Delta\beta = \beta_{male} - \beta_{female}$. Adjusted for GA, ethnicity, and dataset.

Sex-specific differentially methylated sites are biologically interesting

- ***ARHGAP15*** (chr2, higher female DNAme in gene body) present in placental exosomes,[5]
- ***ZNF300*** (chr6, higher male DNAme near TSS), higher expression in female placentas.[5]



Sex-specific DNAme patterns validate in independent cohorts



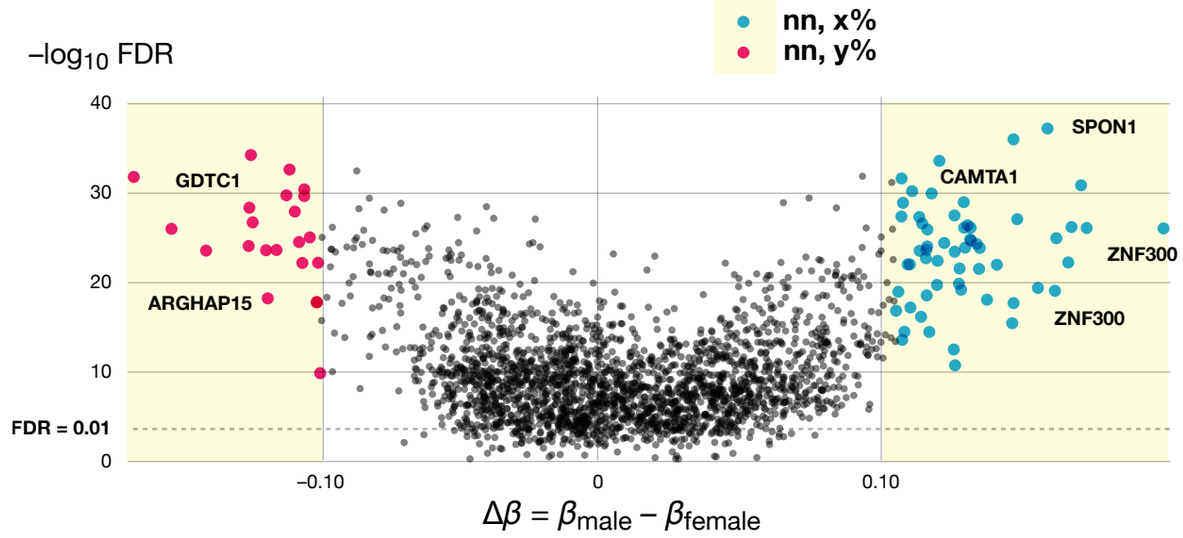
- Samples cluster by sex in two **independent datasets** GSE70453 and GSE115508 at top 166 DM loci, suggesting a **robust sex effect at these loci**. *Indicates p<2.2e-16 (*sigClust2*) and stable (*pvclust*) AU>80 cluster.[3,4]
- Significant patterns of differential DNAme by sex validate at

Specific autosomal CpG sites are differentially methylated by sex

$DNAme_{ij} \sim sex_i + dataset_i + GA_i + ethnicity_i + \varepsilon_{ij}$

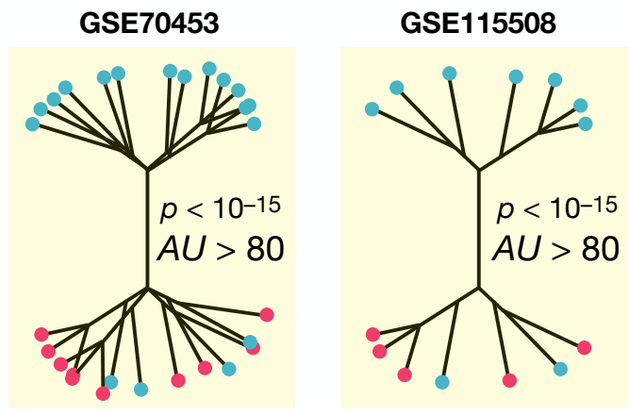
324,104 autosomal CpGs. Adjusted for GA, ethnicity, and dataset.

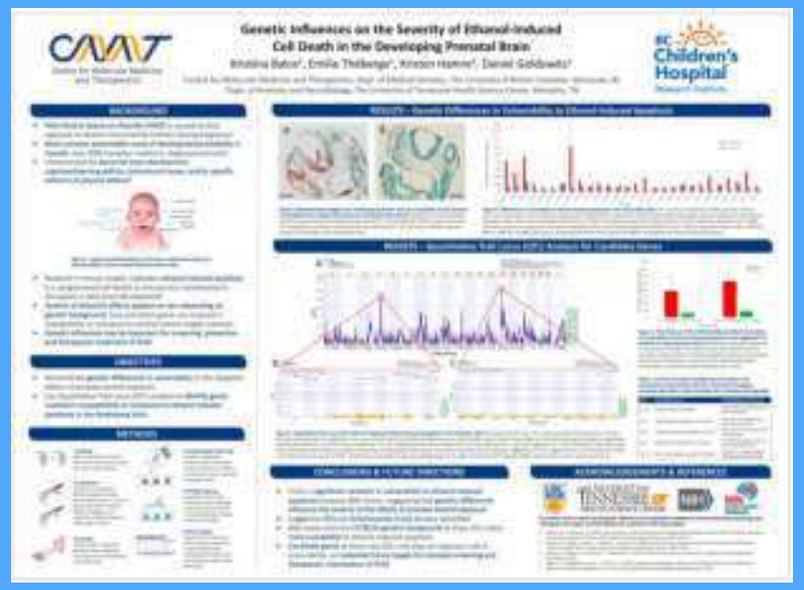
FDR	Δβ	> 0	> 5%	> 10%	> 20%
< 0.05		24,715	2,942	166	4
< 0.01		14,108	2,682	**166**	4



Sex-specific DNAme patterns validate in independent cohorts

Samples cluster by sex in two independent datasets at top 166 DM loci, suggesting a robust sex effect at these loci. P-value from sigClust2. Cluster stable (pvclust) at AU > 80. Significant patterns of differential DNAme by sex validate at 90% of loci in GSE70453 and GSE115508 (R = 0.62, p< 2.2e-16).





arrange and rearrange your puzzle pieces

use color for key themes — and for nothing else

Genetic Influences on the Severity of Ethanol-Induced Cell Death in the Developing Prenatal Brain

Kristina Balce[1], Emilie Théberge[1], Kristen Hamre[2], Daniel Goldowitz[1]

[1]Centre for Molecular Medicine and Therapeutics, Dept. of Medical Genetics, The University of British Columbia, Vancouver, BC
[2]Dept. of Anatomy and Neurobiology, The University of Tennessee Health Science Center, Memphis, TN

Centre for Molecular Medicine and Therapeutics

BC Children's Hospital Research Institute

BACKGROUND

- Fetal Alcohol Spectrum Disorder (FASD) is caused by fetal exposure to alcohol consumed by mothers during pregnancy[1]
- Most common preventable cause of developmental disability in Canada; over 3000 Canadian newborns diagnosed annually[2]
- Characterized by abnormal brain development, cognitive/learning deficits, behavioural issues, and/or specific patterns of physical defects[3]

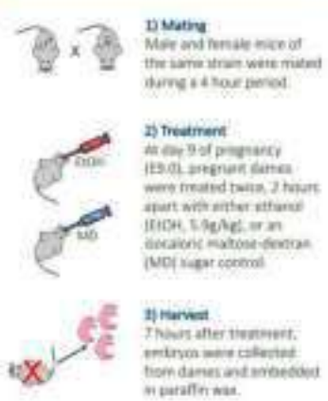
Figure 1. Typical craniofacial defects of full blown Fetal Alcohol Spectrum Disorder (FASD), known as Fetal Alcohol Syndrome (FAS).

- Research in mouse models implicates ethanol-induced apoptosis (i.e. programmed cell death) as one process contributing to disruption in early brain development[4]
- Severity of ethanol's effects appears to vary depending on genetic background; how and which genes are involved in susceptibility or resistance to alcohol remain largely unknown
- Genetic influences may be important for screening, prevention, and therapeutic treatment of FASD

OBJECTIVES

- Demonstrate genetic differences in vulnerability to the apoptotic effects of prenatal alcohol exposure
- Use Quantitative Trait Locus (QTL) analysis to identify genes involved in susceptibility or resistance to ethanol-induced apoptosis in the developing brain

METHODS



RESULTS – Genetic Differences in Vulnerability to Ethanol-Induced Apoptosis

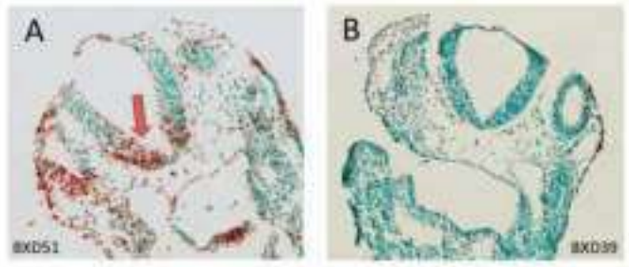


Figure 2. Representative images of the developing brainstem from (A) susceptible and (B) resistant mouse BXD strains, treated with ethanol on embryonic day 9 (E9.0). Apoptotic cells were labelled using the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Cell nuclei of nonapoptotic cells were counterstained with methyl green. The red arrow indicates a greater amount of cell death in the susceptible strain.

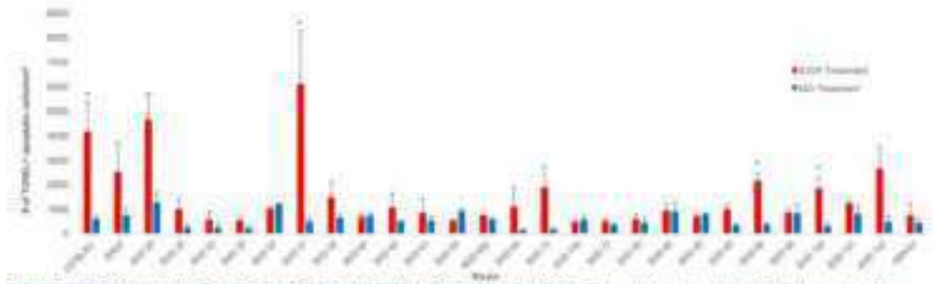


Figure 3. Differences in vulnerability to ethanol-induced apoptosis in the mouse BXD panel. The number of apoptotic (TUNEL positive) cells per mm² was measured in mouse embryos treated with ethanol (EtOH, red) and a maltose-dextran (MD, blue) sugar control at embryonic day 9 (E9.0). Between strains, there was a significant difference ($p < 0.001$) in apoptosis means after EtOH treatment, but no significant difference ($p = 0.084$) after MD treatment. Comparisons between treatments within strain reported significant differences ($p < 0.05$) in apoptosis means in the C57BL/6J, BXD 51, BXD 96, and BXD 100 strains, indicating that these strains show the highest vulnerability to ethanol-induced apoptosis.

RESULTS – Quantitative Trait Locus (QTL) Analysis for Candidate Genes

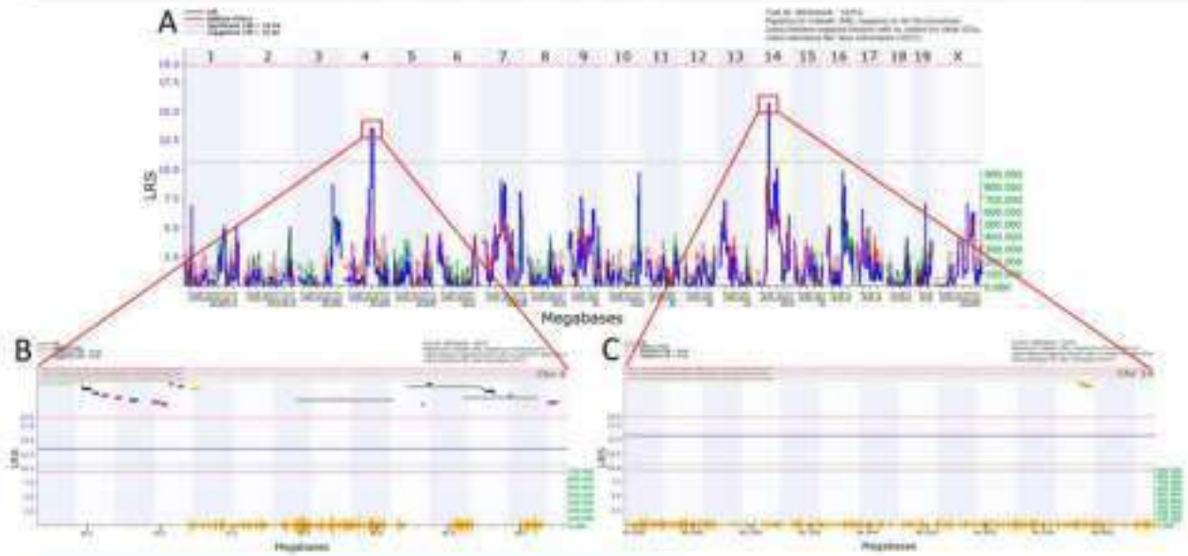


Figure 4. Quantitative Trait Locus (QTL) interval mapping of ethanol-induced apoptosis in the brainstem. **(A)** Whole genome QTL map. The x-axis represents chromosomes 1–19, & X and their physical maps in megabases. The y-axis and the blue line indicate the likelihood ratio statistic (LRS), which reports the strength of association between variations in genotype and the phenotype (i.e. ethanol-induced apoptosis). The red and gray horizontal lines respectively mark the significant ($p = 0.05$) and suggestive ($p = 0.63$) thresholds. Peaks that reach these thresholds indicate genome regions containing candidate genes that may be implicated in ethanol-induced apoptosis. **(B, C)** Maps of the suggestive QTLs on chromosome 4 (LRS = 13.510) and chromosome 14 (LRS = 15.655) were expanded to analyze candidate genes and single nucleotide polymorphisms (SNPs, yellow peaks on the x-axis) at each locus.

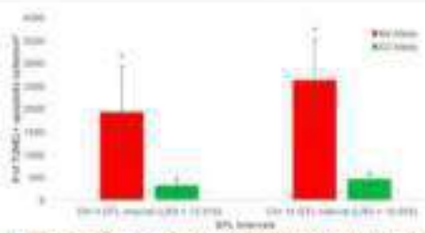


Figure 5. The influence of the C57BL/6J (B6) and DBA/2 (D2) alleles on vulnerability to ethanol-induced apoptosis at the suggestive QTL intervals on chromosomes 4 and 14. BXD mice were grouped based on presence of the B6 or D2 allele at each QTL interval, and the average number of ethanol-induced apoptotic cells were compared. Inheritance of B6 alleles is associated with significantly higher susceptibility to ethanol-induced apoptosis ($p < 0.01$).

Table 1. Summary of select candidate genes located in the chromosome 4 and 14 QTL intervals, and relevant biological processes described in Gene Ontology (GO, www.geneontology.org).

QTL	Candidate Gene	GO Biological Processes
Chr 4	Nuclear factor I/A (Nfia)	Transcription regulation and DNA replication
Chr 4	TNF2 domain containing 1 (Tnfrsf1)	Induction of apoptosis by extracellular signals
Chr 4	Ubiquitin specific peptidase 1 (Usp 1)	DNA repair and response to DNA damage
Chr 4	Dedicator of cytokinesis 7 (Dock7)	Cell differentiation, nervous system development
Chr 14	Bone morphogenic protein 4 (Bmp4)	Involved in a wide range of developmental processes

CONCLUSIONS & FUTURE DIRECTIONS

- There is significant variation in vulnerability to ethanol-induced apoptosis between BXD strains, suggesting that genetic differences influence the severity of the effects of prenatal alcohol exposure
- Suggestive QTLs on chromosomes 4 and 14 were identified
- BXD strains with the C57BL/6J genetic background at these QTLs were more susceptible to ethanol-induced apoptosis
- Candidate genes at these two QTLs may play an important role in vulnerability; are potential future targets for prenatal screening and therapeutic intervention of FASD

ACKNOWLEDGEMENTS & REFERENCES

The authors wish to thank Aria Shokoohi, Mike Wu and Julia Boyle for assistance in tissue processing, sectioning, and staining for this project, and Rob Williams for assistance in QTL data analysis.

1. Roberts, G., & Nanson, J. (2000). Best practices. Fetal alcohol syndrome/fetal alcohol effects and the effects of other substance use during pregnancy. Ottawa, ON: Canada's Drug Strategy Division, Health Canada.
2. Popova, S., Lange, S., Burd, L., & Rehm, J. (2015). The Burden and Economic Impact of Fetal Alcohol Spectrum Disorder in Canada. Toronto, ON, Canada: Centre for Addiction and Mental Health. ISBN: 978-1.
3. Dörrie, N., Föcker, M., Freunscht, I., Hebebrand, J. (2014). Fetal alcohol spectrum disorders. European Child & Adolescent Psychiatry, 23(10).
4. Ogawa, T., Kuwagata, M., Ruiz, J., Zhou, F. C. (2005). Differential teratogenic effects of alcohol on embryonic development between C57BL/6J and DBA/2 mice: A new view. Alcohol Clinical & Experimental Research, 29(1).

Genetic Influences on the Severity of Ethanol-induced Cell Death in the Developing Prenatal Brain

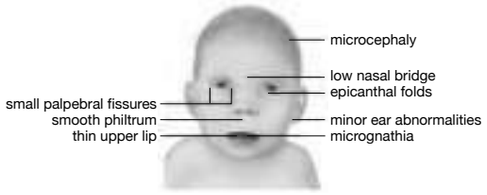
Kristina Balce[1], Emilie Théberge[1], Kristen Hamre[2], Daniel Goldowitz[1]

[1]Centre for Molecular Medicine and Therapeutics, Dept. of Medical Genetics, The University of British Columbia, Vancouver, BC
[2]Dept. of Anatomy and Neurobiology, The University of Tennessee Health Science Center, Memphis, TN

The authors wish to thank Aria Shokoohi, Mike Wu and Julia Boyle for assistance in tissue processing, sectioning, and staining for this project, and Rob Williams for assistance in QTL data analysis.

Fetal Alcohol Spectrum Disorder

Fetal Alcohol Spectrum Disorder (FASD) is caused by fetal exposure to alcohol consumed by mothers during pregnancy [1]. It is the most common preventable cause of developmental disability in Canada — over 3000 Canadian newborns diagnosed annually [2]. FASD is characterized by abnormal brain development, cognitive/learning deficits, behavioural issues, and/or specific patterns of physical defects.



Research in mouse models implicates ethanol-induced apoptosis (i.e. programmed cell death) as one process contributing to disruption in early brain development [4]. Severity of ethanol's effects appears to vary depending on genetic background; how and which genes are involved in susceptibility or resistance to alcohol remain largely unknown. Genetic influences may be important for screening, prevention, and therapeutic treatment of FASD.

1. Roberts, G., & Nanson, J. (2000). Best practices. Fetal alcohol syndrome/fetal alcohol effects and the effects of other substance use during pregnancy. Onawa, ON: Canada's Drug Strategy Division, Health Canada. **2**. Popova, S., Lange, S., Burd, L., & Rehm, J. (2015). The Burden and Economic Impact of Fetal Alcohol Spectrum Disorder in Canada. Toronto, ON, Canada: Centre for Addiction and Mental Health. ISBN, 978-1. **3**. Dörrie, N., Föcker, M., Freunscht, I., Hebebrand, J. (2014). Fetal alcohol spectrum disorders. European Child & Adolescent Psychiatry, 23(10). **4**. Ogawa, T., Kuwagata, M., Ruiz, J., Zhou, F. C. (2005). Differential teratogenic effects of alcohol on embryonic development between C57BL/6J and DBA/2 mice: A new view. Alcohol Clinical & Experimental Research, 29(5)

Objectives

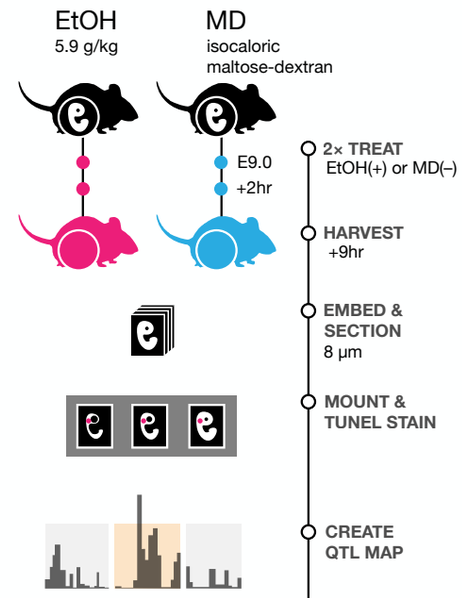
Demonstrate genetic differences in vulnerability to the apoptotic effects of prenatal alcohol exposure. Use Quantitative Trait Locus (QTL) analysis to identify genes involved in susceptibility or resistance to ethanol-induced apoptosis in the developing brain.

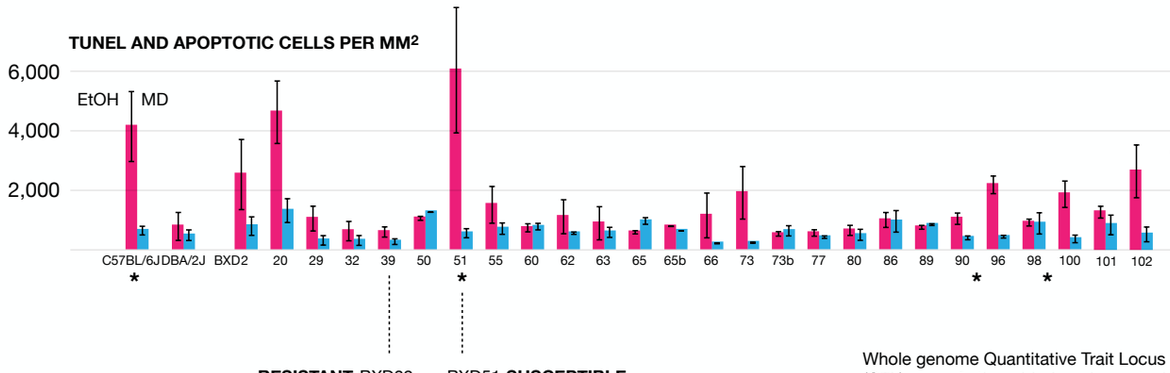
Conclusions

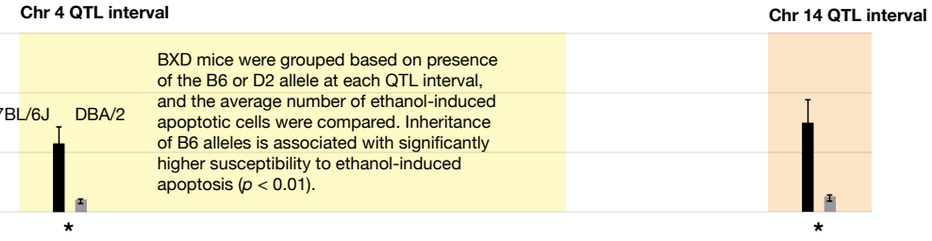
There is significant variation in vulnerability to ethanol-induced apoptosis between BXD strains, suggesting that genetic differences influence the severity of the effects of prenatal alcohol exposure.

Suggestive QTLs on chromosomes 4 and 14 were identified BXD strains with the C57BL/6J genetic background at these QTLs were more susceptible to ethanol-induced apoptosis.

Candidate genes at these two QTLs may play an important role in vulnerability; are potential future targets for prenatal screening and therapeutic intervention of FASD.



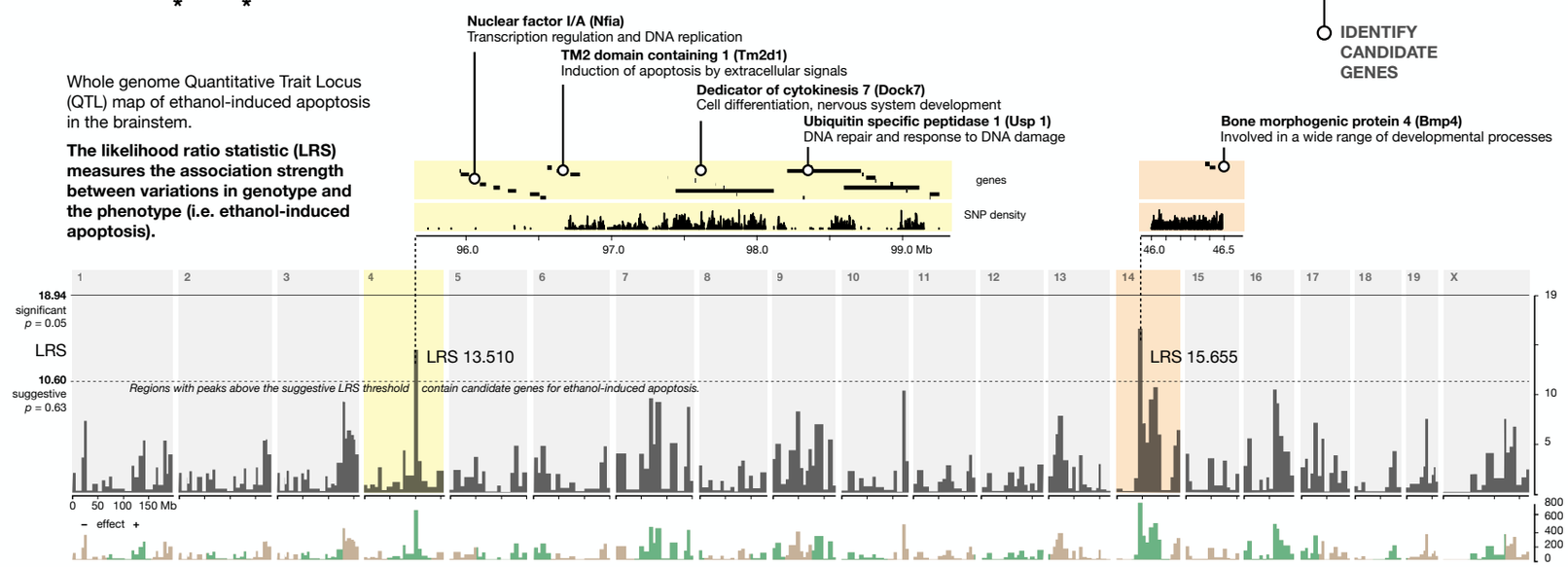




Images of the developing brainstem from a susceptible and resistant mouse BXD strain, treated with ethanol on embryonic day 9 (E9.0). Apoptotic cells were labelled using the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Cell nuclei of nonapoptotic cells were counterstained with methyl green. The arrow indicates a greater amount of cell death in the susceptible strain.

Whole genome Quantitative Trait Locus (QTL) map of ethanol-induced apoptosis in the brainstem.

The likelihood ratio statistic (LRS) measures the association strength between variations in genotype and the phenotype (i.e. ethanol-induced apoptosis).



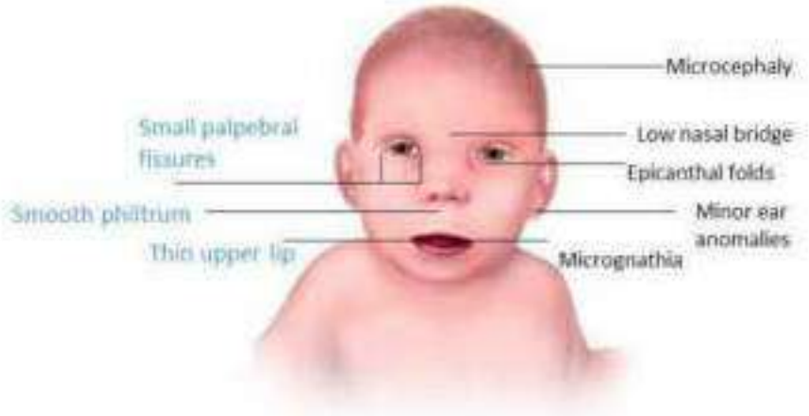
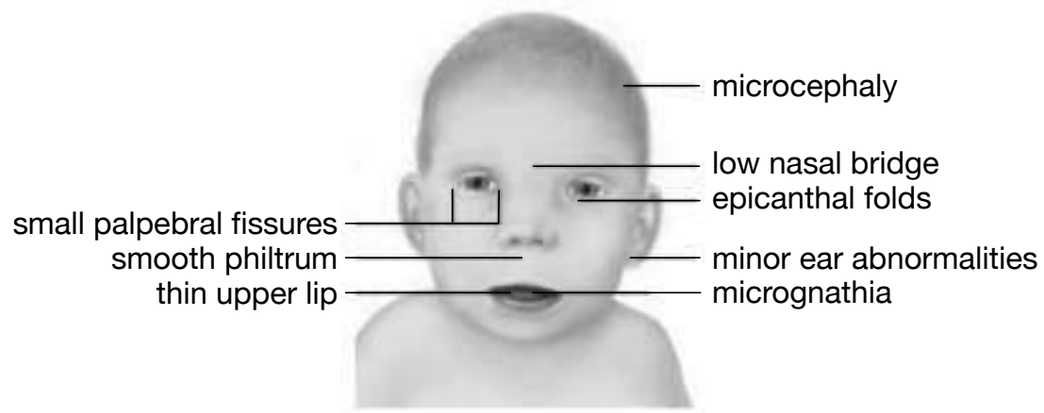
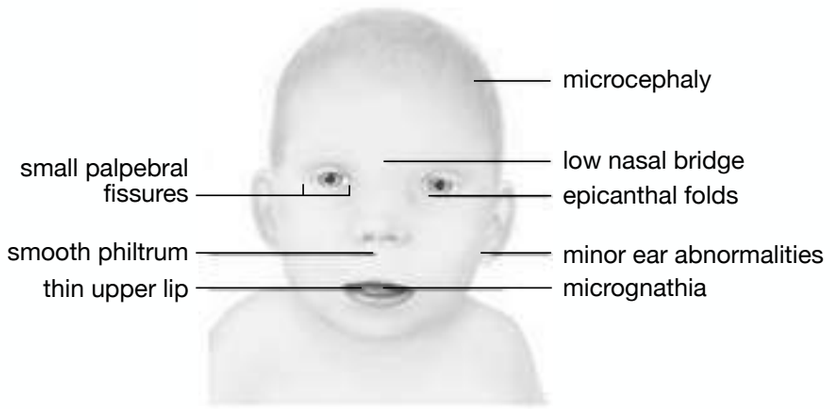
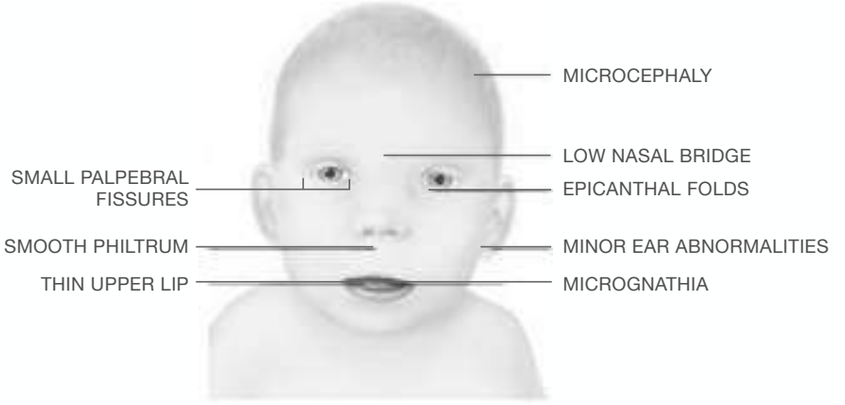


Figure 1. Typical craniofacial defects of full blown Fetal Alcohol Spectrum Disorder (FASD), known as Fetal Alcohol Syndrome (FAS).







MICROCEPHALY
LOW NASAL BRIDGE
SMALL PALPEBRAL FISSURES
EPICANTHAL FOLDS
SMOOTH PHILTRUM
MINOR EAR ABNORMALITIES
THIN UPPER LIP
MICROGNATHIA

LOREM IPSUM DOLOR SIT AMET, CONSECTETUER ADIPISCING ELIT, SED DIAM NONUMMY NIBH EUISMOD TINCIDUNT UT LAOREET DOLORE MAGNA ALIQUAM ERAT VOLUTPAT. UT WISI ENIM AD MINIM VENIAM, QUIS NOSTRUD EXERCI TATION ULLAMCORPER SUSCIPIT LOBORTIS NISL UT ALIQUIP EX EA COMMODO CONSEQUAT. DUIS AUTEM VEL EUM IRIURE DOLOR IN HENDRERIT IN VULPUTATE VELIT ESSE MOLESTIE CONSEQUAT, VEL ILLUM DOLORE EU FEUGIAT NULLA FACILISIS AT VERO EROS ET ACCUMSAN ET IUSTO ODIO DIGNISSIM QUI BLANDIT PRAESENT LUPTATUM ZZRIL DELENIT AUGUE DUIS DOLORE TE FEUGAIT NULLA FACILISI. LOREM IPSUM DOLOR SIT AMET, CONSECTETUER ADIPISCING ELIT, SED DIAM NONUMMY NIBH EUISMOD TINCIDUNT UT LAOREET DOLORE MAGNA ALIQUAM ERAT VOLUTPAT. UT WISI ENIM AD MINIM VENIAM, QUIS NOSTRUD EXERCI TATION ULLAMCORPER SUSCIPIT LOBORTIS NISL UT ALIQUIP EX EA COMMODO CONSEQUAT. DUIS AUTEM VEL EUM IRIURE DOLOR IN HENDRERIT IN VULPUTATE VELIT ESSE MOLESTIE CON-

Genetic Influences on the Severity of Ethanol-Induced Cell Death in the Developing Prenatal Brain

Kristina Balce[1], Emilie Théberge[1], Kristen Hamre[2], Daniel Goldowitz[1]

[1]Centre for Molecular Medicine and Therapeutics, Dept. of Medical Genetics, The University of British Columbia, Vancouver, BC
[2]Dept. of Anatomy and Neurobiology, The University of Tennessee Health Science Center, Memphis, TN

BACKGROUND

- Fetal Alcohol Spectrum Disorder (FASD) is caused by fetal exposure to alcohol consumed by mothers during pregnancy[1]
- Most common preventable cause of developmental disability in Canada; over 3000 Canadian newborns diagnosed annually[2]
- Characterized by abnormal brain development, cognitive/learning deficits, behavioural issues, and/or specific patterns of physical defects[3]

Figure 1. Typical craniofacial defects of full blown Fetal Alcohol Spectrum Disorder (FASD), known as Fetal Alcohol Syndrome (FAS).

- Research in mouse models implicates ethanol-induced apoptosis (i.e. programmed cell death) as one process contributing to disruption in early brain development[4]
- Severity of ethanol's effects appears to vary depending on genetic background; how and which genes are involved in susceptibility or resistance to alcohol remain largely unknown
- Genetic influences may be important for screening, prevention, and therapeutic treatment of FASD

OBJECTIVES

- Demonstrate genetic differences in vulnerability to the apoptotic effects of prenatal alcohol exposure
- Use Quantitative Trait Locus (QTL) analysis to identify genes involved in susceptibility or resistance to ethanol-induced apoptosis in the developing brain

METHODS

RESULTS – Genetic Differences in Vulnerability to Ethanol-Induced Apoptosis

RESULTS – Quantitative Trait Locus (QTL) Analysis for Candidate Genes

CONCLUSIONS & FUTURE DIRECTIONS

- There is significant variation in vulnerability to ethanol-induced apoptosis between BXD strains, suggesting that genetic differences influence the severity of the effects of prenatal alcohol exposure
- Suggestive QTLs on chromosomes 4 and 14 were identified
- BXD strains with the C57BL/6J genetic background at these QTLs were more susceptible to ethanol-induced apoptosis
- Candidate genes at these two QTLs may play an important role in vulnerability; are potential future targets for prenatal screening and therapeutic intervention of FASD

ACKNOWLEDGEMENTS & REFERENCES

Genetic Influences on the Severity of Ethanol-induced Cell Death in the Developing Prenatal Brain

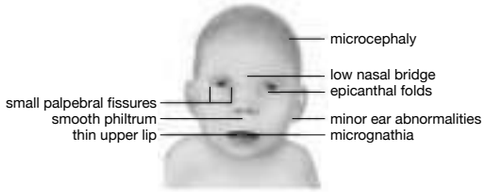
Kristina Balce[1], Emilie Théberge[1], Kristen Hamre[2], Daniel Goldowitz[1]

[1]Centre for Molecular Medicine and Therapeutics, Dept. of Medical Genetics, The University of British Columbia, Vancouver, BC
[2]Dept. of Anatomy and Neurobiology, The University of Tennessee Health Science Center, Memphis, TN

The authors wish to thank Aria Shokoohi, Mike Wu and Julia Boyle for assistance in tissue processing, sectioning, and staining for this project, and Rob Williams for assistance in QTL data analysis.

Fetal Alcohol Spectrum Disorder

Fetal Alcohol Spectrum Disorder (FASD) is caused by fetal exposure to alcohol consumed by mothers during pregnancy [1]. It is the most common preventable cause of developmental disability in Canada — over 3000 Canadian newborns diagnosed annually [2]. FASD is characterized by abnormal brain development, cognitive/learning deficits, behavioural issues, and/or specific patterns of physical defects.



Research in mouse models implicates ethanol-induced apoptosis (i.e. programmed cell death) as one process contributing to disruption in early brain development [4]. Severity of ethanol's effects appears to vary depending on genetic background; how and which genes are involved in susceptibility or resistance to alcohol remain largely unknown. Genetic influences may be important for screening, prevention, and therapeutic treatment of FASD.

1. Roberts, G., & Nanson, J. (2000). Best practices. Fetal alcohol syndrome/fetal alcohol effects and the effects of other substance use during pregnancy. Onawa, ON: Canada's Drug Strategy Division, Health Canada. **2**. Popova, S., Lange, S., Burd, L., & Rehm, J. (2015). The Burden and Economic Impact of Fetal Alcohol Spectrum Disorder in Canada. Toronto, ON, Canada: Centre for Addiction and Mental Health. ISBN, 978-1. **3**. Dörrie, N., Föcker, M., Freunscht, I., Hebebrand, J. (2014). Fetal alcohol spectrum disorders. European Child & Adolescent Psychiatry, 23(10). **4**. Ogawa, T., Kuwagata, M., Ruiz, J., Zhou, F. C. (2005). Differential teratogenic effects of alcohol on embryonic development between C57BL/6J and DBA/2 mice: A new view. Alcohol Clinical & Experimental Research, 29(5)

Objectives

Demonstrate genetic differences in vulnerability to the apoptotic effects of prenatal alcohol exposure. Use Quantitative Trait Locus (QTL) analysis to identify genes involved in susceptibility or resistance to ethanol-induced apoptosis in the developing brain.

Conclusions

There is significant variation in vulnerability to ethanol-induced apoptosis between BXD strains, suggesting that genetic differences influence the severity of the effects of prenatal alcohol exposure.

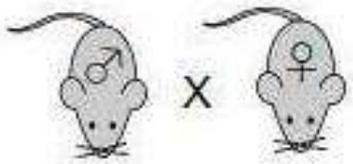
Suggestive QTLs on chromosomes 4 and 14 were identified BXD strains with the C57BL/6J genetic background at these QTLs were more susceptible to ethanol-induced apoptosis.

Candidate genes at these two QTLs may play an important role in vulnerability; are potential future targets for prenatal screening and therapeutic intervention of FASD.

Images of the developing brainstem from a susceptible and resistant mouse BXD strain, treated with ethanol on embryonic day 9 (E9.0). Apoptotic cells were labelled using the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Cell nuclei of nonapoptotic cells were counterstained with methyl green. The arrow indicates a greater amount of cell death in the susceptible strain.

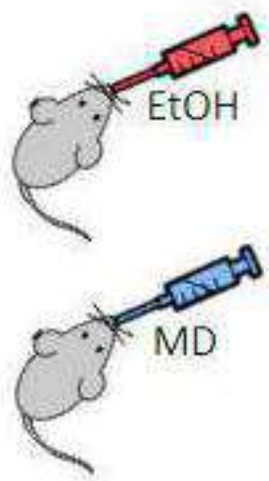
Whole genome Quantitative Trait Locus (QTL) map of ethanol-induced apoptosis in the brainstem.

The likelihood ratio statistic (LRS) measures the association strength between variations in genotype and the phenotype (i.e. ethanol-induced apoptosis).



1) Mating

Male and female mice of the same strain were mated during a 4 hour period.

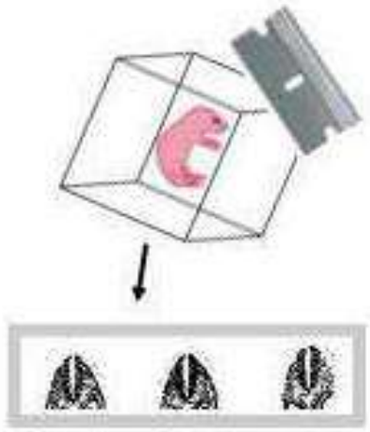


2) Treatment

At day 9 of pregnancy (E9.0), pregnant dames were treated twice, 2 hours apart with either ethanol (EtOH, 5.9g/kg), or an isocaloric maltose-dextran (MD) sugar control.

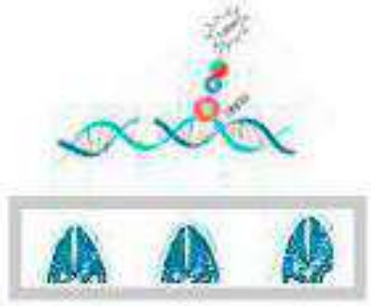
3) Harvest

7 hours after treatment, embryos were collected from dames and embedded in paraffin wax.



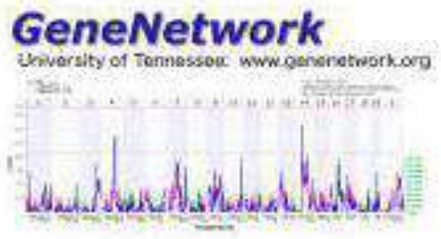
4) Sectioning & Mounting

Paraffin embedded embryos were sectioned using a microtome at 8µm, and sections were mounted onto glass slides.



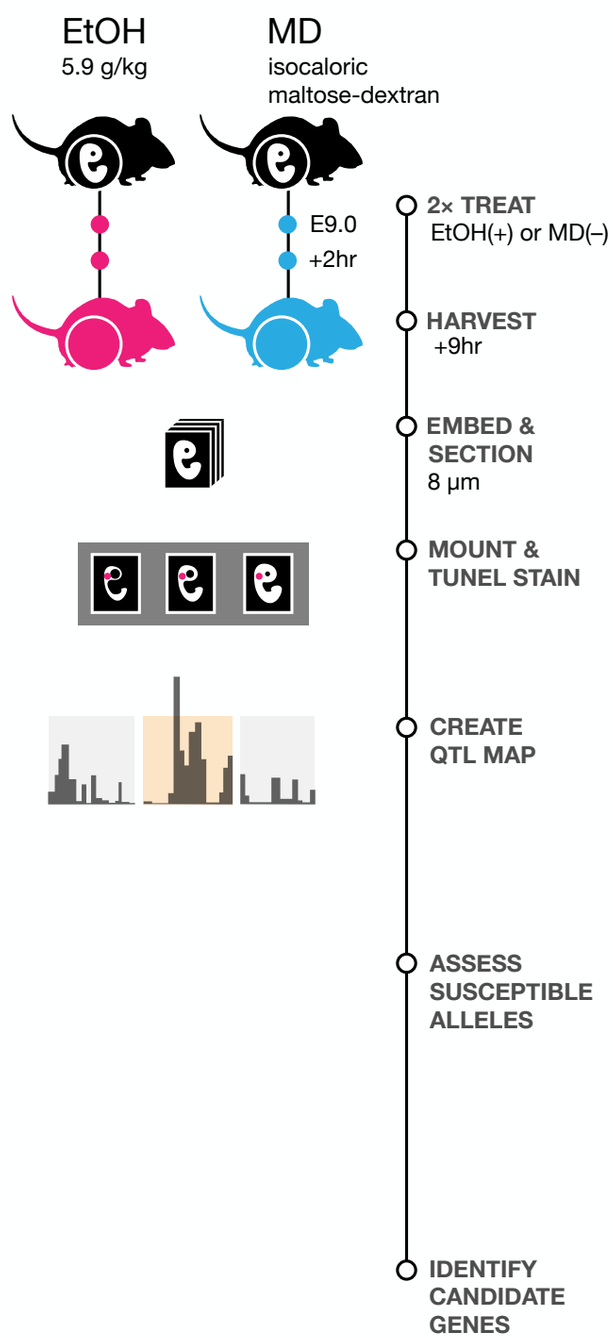
5) TUNEL Staining

Apoptotic cells in the brainstem were labelled using the terminal dUTP nick-end labeling (TUNEL) assay and counted.



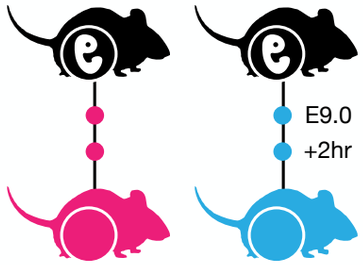
6) QTL Analysis

Quantitative Trait Locus (QTL) analysis was done using GeneNetwork (www.genenetwork.org), and candidate genes were identified.


EtOH
5.9 g/kg
MD
isocaloric
maltose-dextran
E9.0
+2hr
2× TREAT
EtOH(+) or MD(–)
HARVEST
+9hr
EMBED &
SECTION
8 µm
MOUNT &
TUNEL STAIN
CREATE
QTL MAP
ASSESS
SUSCEPTIBLE
ALLELES
IDENTIFY
CANDIDATE
GENES

EtOH
5.9 g/kg

MD
isocaloric
maltose-dextran



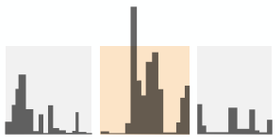
E9.0
+2hr

2× TREAT
EtOH(+) or MD(–)

HARVEST
+9hr

EMBED & SECTION
8 µm

MOUNT & TUNEL STAIN



CREATE QTL MAP

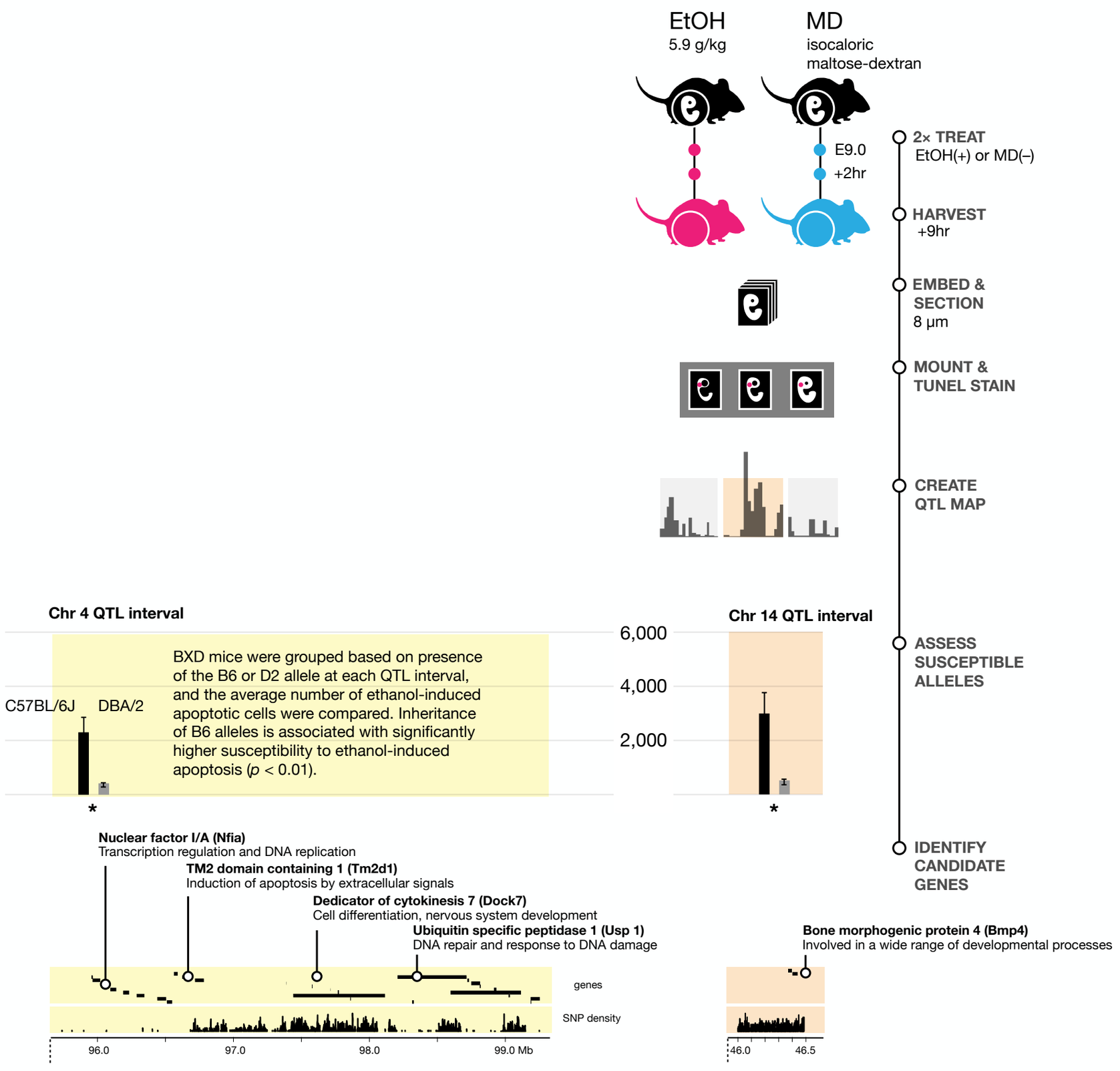
Chr 4 QTL interval

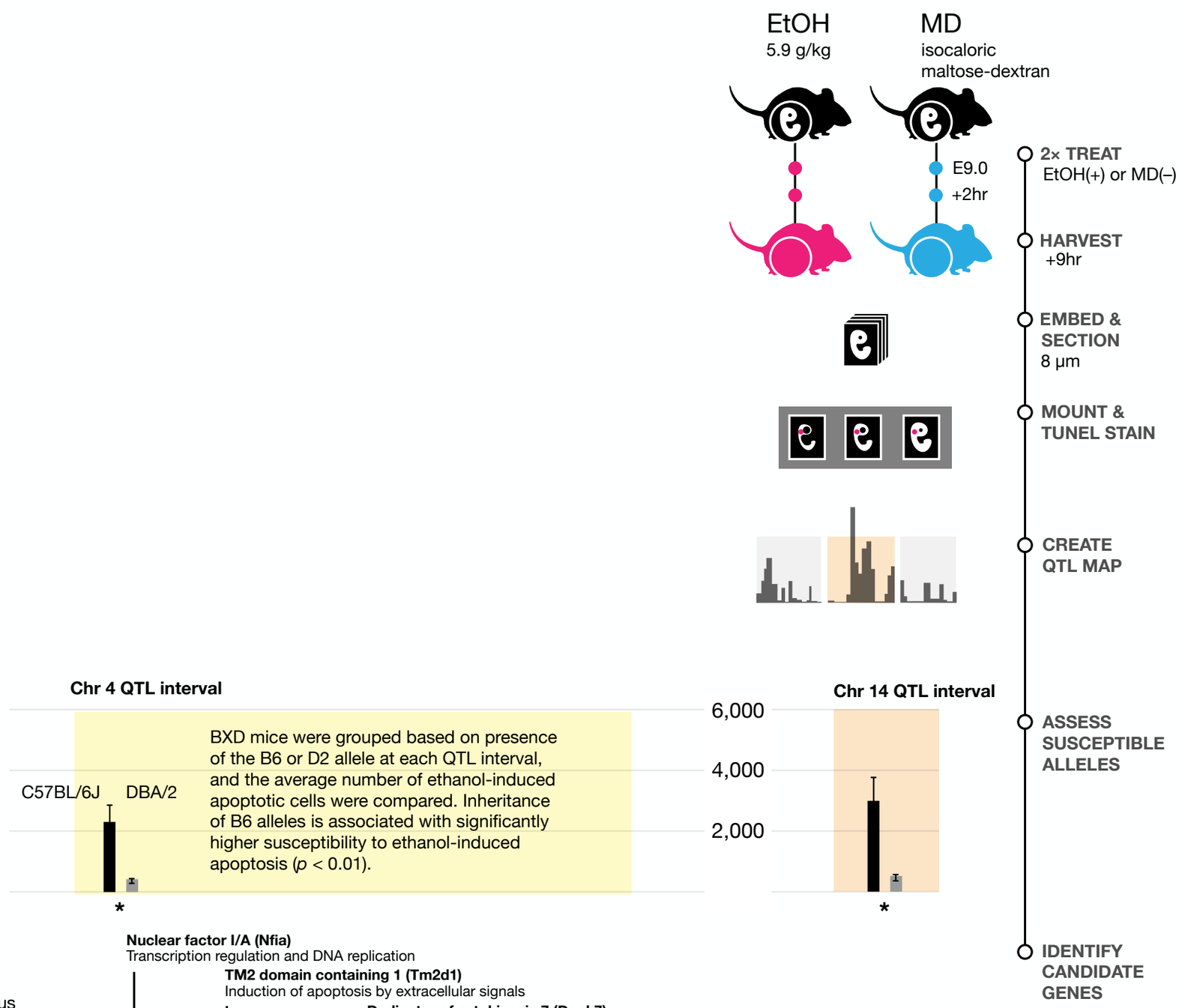
Chr 14 QTL interval

BXD mice were grouped based on presence of the B6 or D2 allele at each QTL interval, and the average number of ethanol-induced apoptotic cells were compared. Inheritance of B6 alleles is associated with significantly higher susceptibility to ethanol-induced apoptosis ($p < 0.01$).

ASSESS SUSCEPTIBLE ALLELES

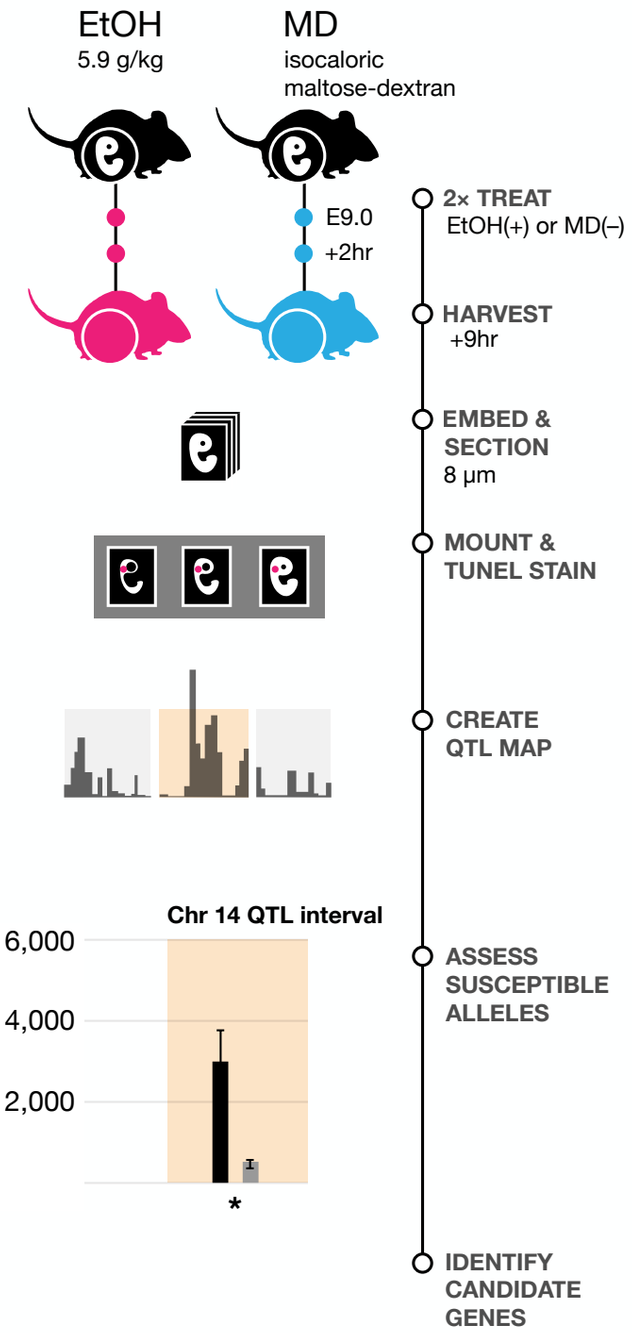
IFY DATE

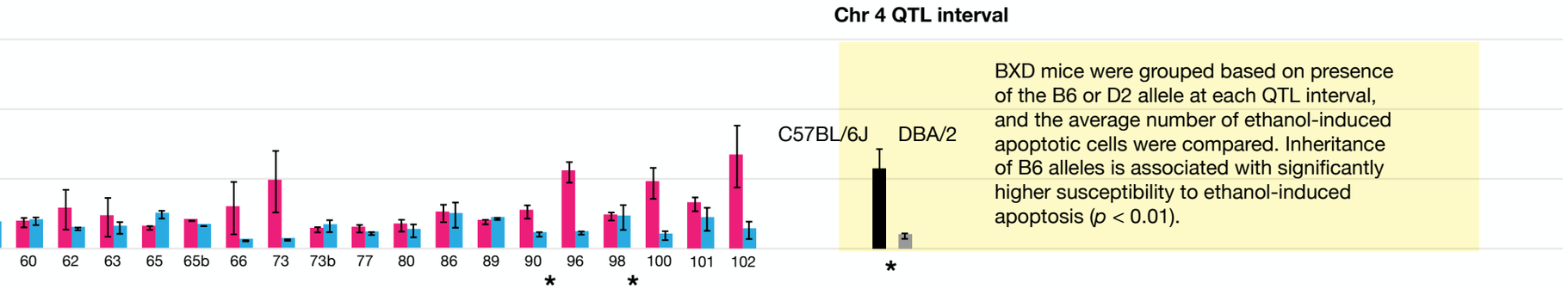

EtOH
5.9 g/kg
MD
isocaloric
maltose-dextran
E9.0
+2hr
2× TREAT
EtOH(+) or MD(–)
HARVEST
+9hr
EMBED &
SECTION
8 µm
MOUNT &
TUNEL STAIN
CREATE
QTL MAP
ASSESS
SUSCEPTIBLE
ALLELES
IDENTIFY
CANDIDATE
GENES
Chr 4 QTL interval
Chr 14 QTL interval
6,000
4,000
2,000
C57BL/6J
DBA/2
BXD mice were grouped based on presence of the B6 or D2 allele at each QTL interval, and the average number of ethanol-induced apoptotic cells were compared. Inheritance of B6 alleles is associated with significantly higher susceptibility to ethanol-induced apoptosis (p < 0.01).
*
*
Nuclear factor I/A (Nfia)
Transcription regulation and DNA replication
TM2 domain containing 1 (Tm2d1)
Induction of apoptosis by extracellular signals
Dedicator of cytokinesis 7 (Dock7)
Cell differentiation, nervous system development
Ubiquitin specific peptidase 1 (Usp 1)
DNA repair and response to DNA damage
Bone morphogenic protein 4 (Bmp4)
Involved in a wide range of developmental processes
genes
SNP density
96.0
97.0
98.0
99.0 Mb
46.0
46.5



Whole genome Quantitative Trait Locus (QTL) map of ethanol-induced apoptosis in the brainstem.

The likelihood ratio statistic (LRS) measures the association strength between variations in genotype and the phenotype (i.e. ethanol-induced apoptosis).





Whole genome Quantitative Trait Locus (QTL) map of ethanol-induced apoptosis in the brainstem.

The likelihood ratio statistic (LRS) measures the association strength between variations in genotype and the phenotype (i.e. ethanol-induced apoptosis).

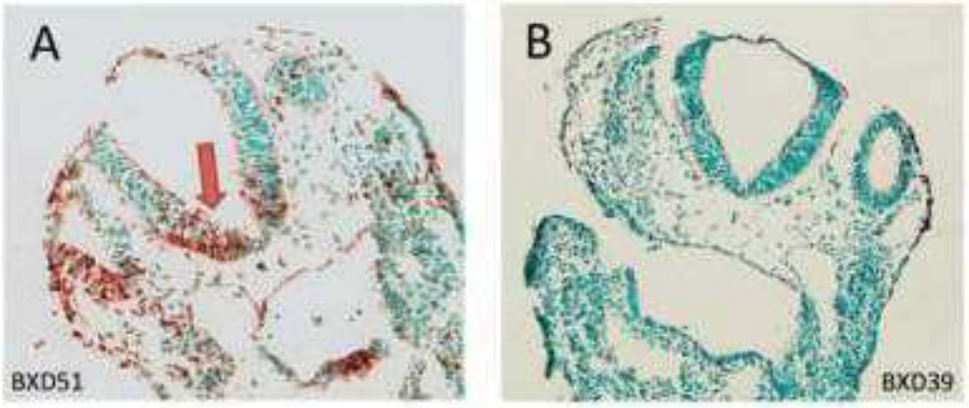


Figure 2. Representative images of the developing brainstem from (A) susceptible and (B) resistant mouse BXD strains, treated with ethanol on embryonic day 9 (E9.0). Apoptotic cells were labelled using the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Cell nuclei of nonapoptotic cells were counterstained with methyl green. The red arrow indicates a greater amount of cell death in the susceptible strain.

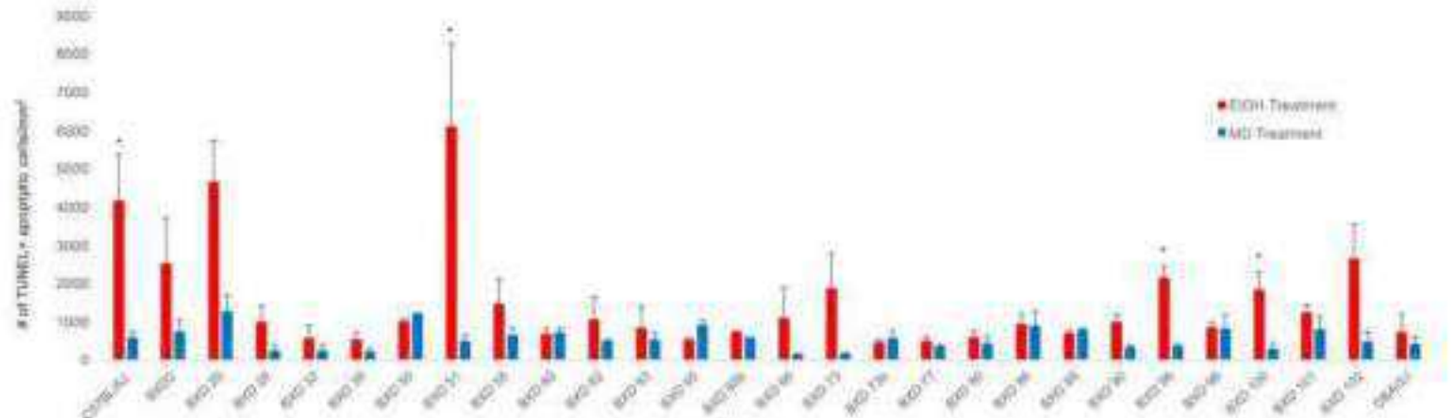
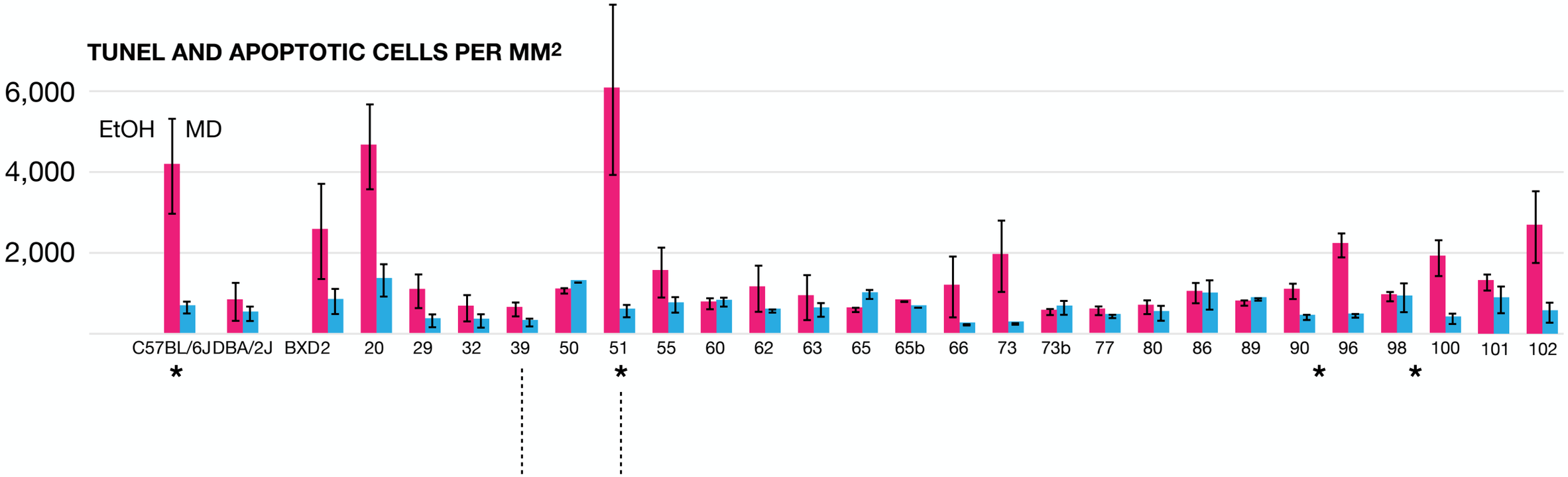
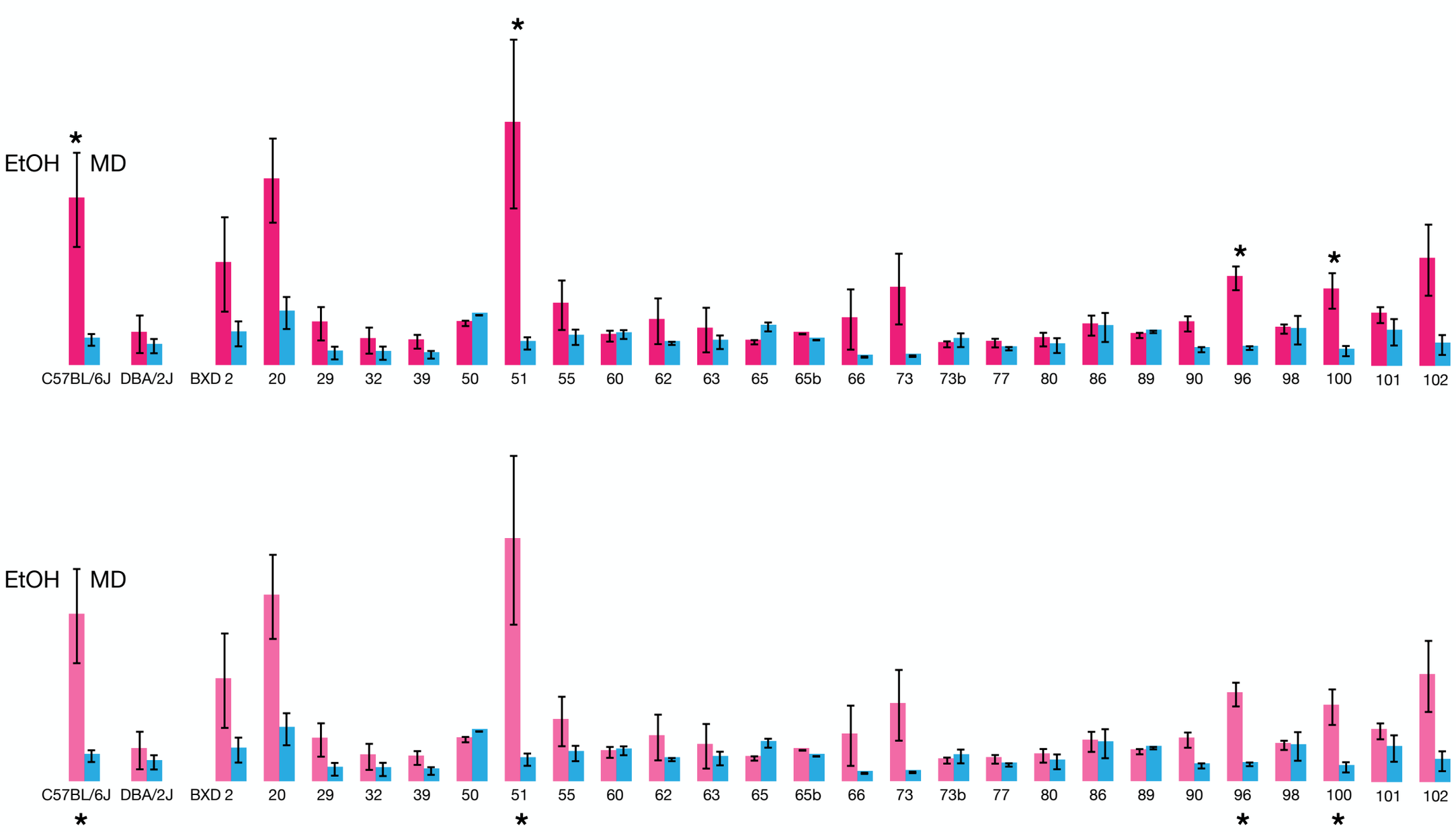
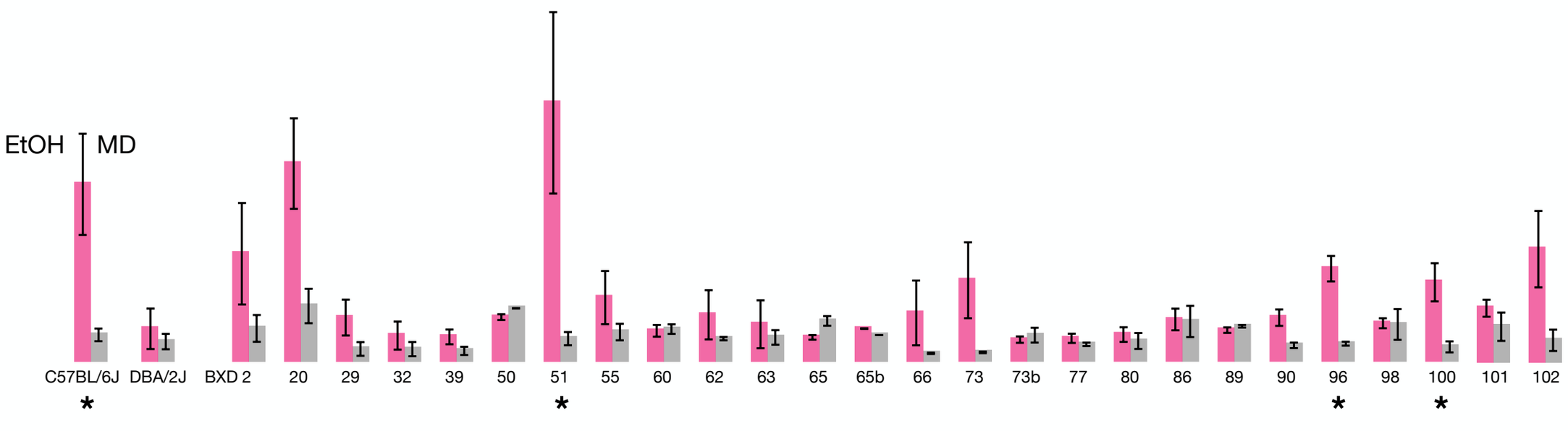


Figure 3. Differences in vulnerability to ethanol-induced apoptosis in the mouse BXD panel. The number of apoptotic (TUNEL positive) cells per mm^3 was measured in mouse embryos treated with ethanol (EtOH, red) and a maltose-dextran (MD, blue) sugar control at embryonic day 9 (E9.0). Between strains, there was a significant difference ($p < 0.001$) in apoptosis means after EtOH treatment, but no significant difference ($p = 0.084$) after MD treatment. Comparisons between treatments within strain reported significant differences ($p < 0.05$) in apoptosis means in the C57BL/6J, BXD 51, BXD 96, and BXD 100 strains, indicating that these strains show the highest vulnerability to ethanol-induced apoptosis..



Images of the developing brainstem from a susceptible and resistant mouse BXD strain, treated with ethanol on embryonic day 9 (E9.0). Apoptotic cells were labelled using the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Cell nuclei of nonapoptotic cells were counterstained with methyl green. The arrow indicates a greater amount of cell death in the susceptible strain.


EtOH
MD
*
*
*
*
C57BL/6J
DBA/2J
BXD 2
20
29
32
39
50
51
55
60
62
63
65
65b
66
73
73b
77
80
86
89
90
96
98
100
101
102


EtOH
MD
*
*
*
*
C57BL/6J
DBA/2J
BXD 2
20
29
32
39
50
51
55
60
62
63
65
65b
66
73
73b
77
80
86
89
90
96
98
100
101
102

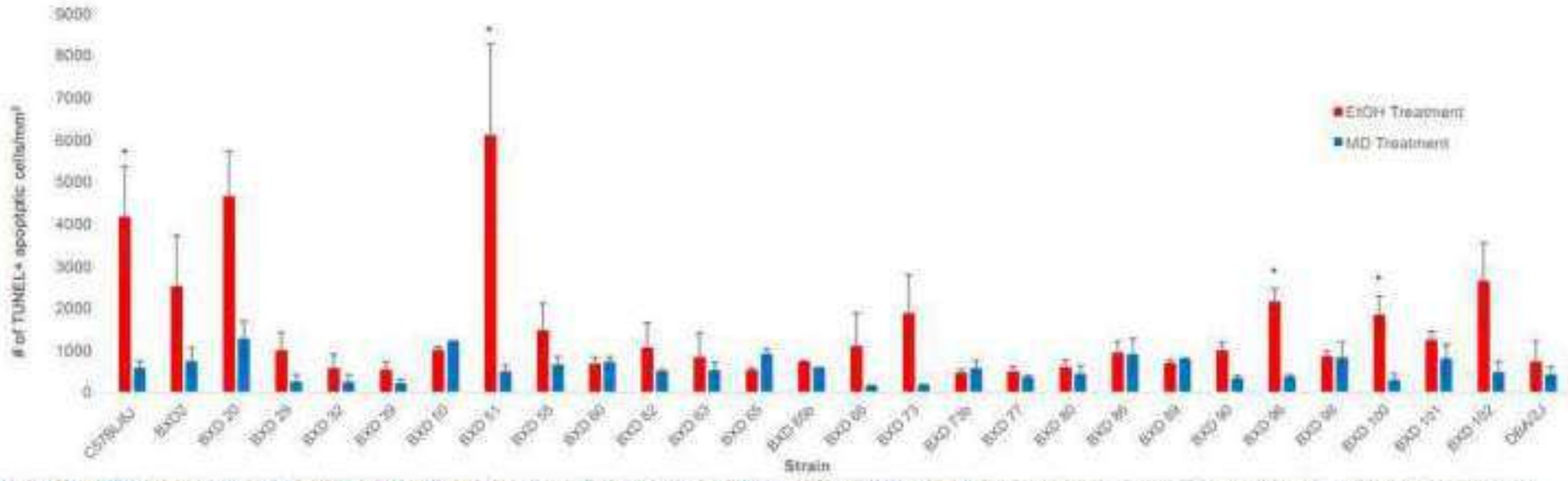


Figure 3. Differences in vulnerability to ethanol-induced apoptosis in the mouse BXD panel. The number of apoptotic (TUNEL positive) cells per mm^2 was measured in mouse embryos treated with ethanol (EtOH, red) and a maltose-dextran (MD, blue) sugar control at embryonic day 9 (E9.0). Between strains, there was a significant difference ($p < 0.001$) in apoptosis means after EtOH treatment, but no significant difference ($p = 0.084$) after MD treatment. Comparisons between treatments within strain reported significant differences ($p < 0.05$) in apoptosis means in the C57BL/6J, BXD 51, BXD 96, and BXD 100 strains, indicating that these strains show the highest vulnerability to ethanol-induced apoptosis..

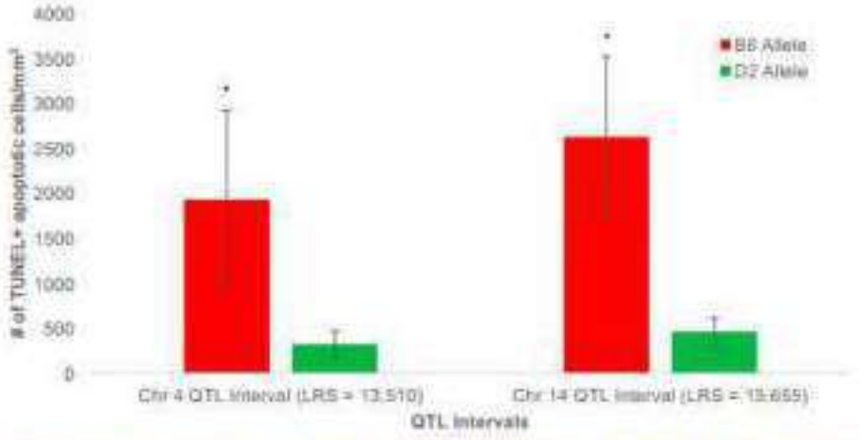


Figure 5. The influence of the C57BL/6J (B6) and DBA/2 (D2) alleles on vulnerability to ethanol-induced apoptosis at the suggestive QTL intervals on chromosomes 4 and 14. BXD mice were grouped based on presence of the B6 or D2 allele at each QTL interval, and the average number of ethanol-induced apoptotic cells were compared. Inheritance of B6 alleles is associated with significantly higher susceptibility to ethanol-induced apoptosis ($p < 0.01$).

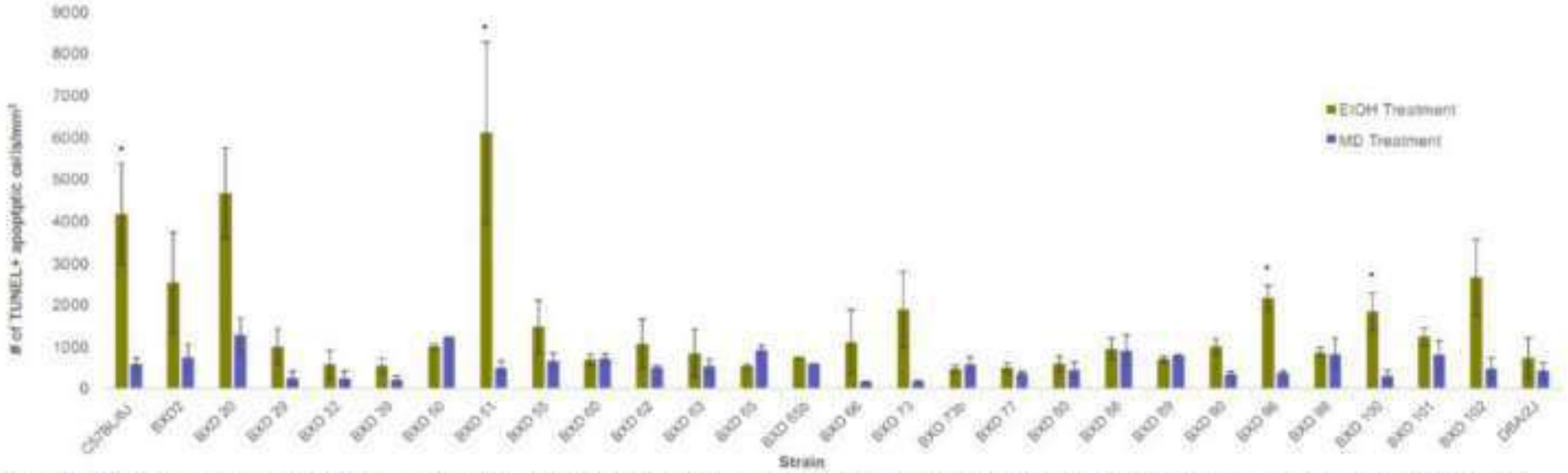


Figure 3. Differences in vulnerability to ethanol-induced apoptosis in the mouse BXD panel. The number of apoptotic (TUNEL positive) cells per mm^2 was measured in mouse embryos treated with ethanol (EtOH, red) and a maltose-dextran (MD, blue) sugar control at embryonic day 9 (E9.0). Between strains, there was a significant difference ($p < 0.001$) in apoptosis means after EtOH treatment, but no significant difference ($p = 0.084$) after MD treatment. Comparisons between treatments within strain reported significant differences ($p < 0.05$) in apoptosis means in the C57BL/6J, BXD 51, BXD 96, and BXD 100 strains, indicating that these strains show the highest vulnerability to ethanol-induced apoptosis..

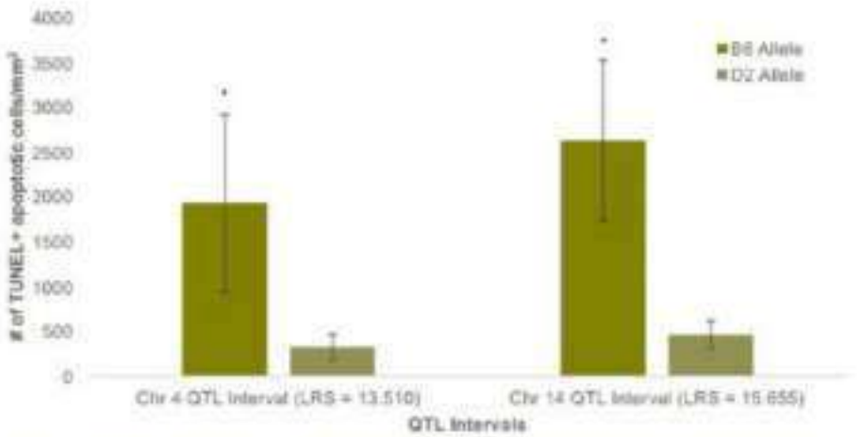
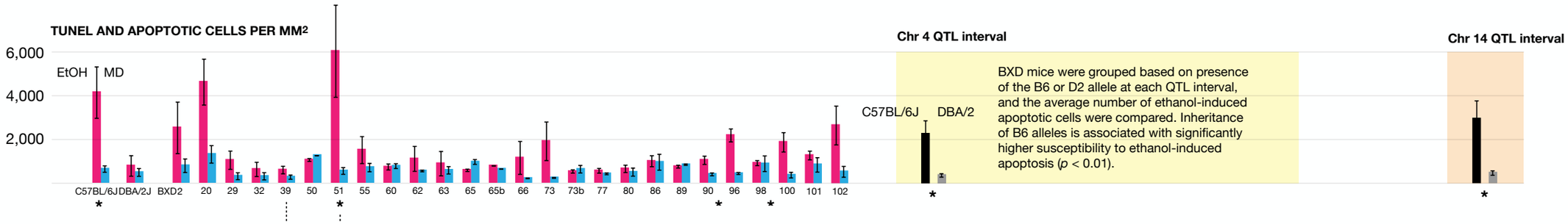
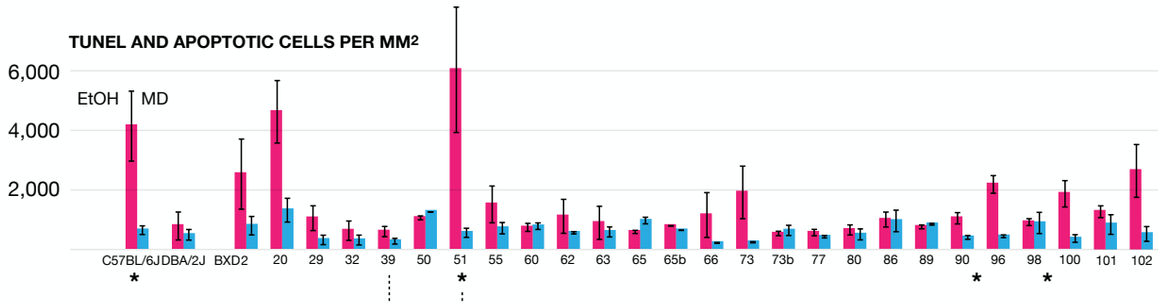
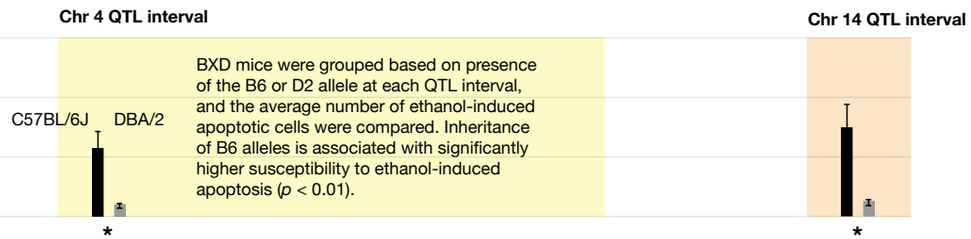
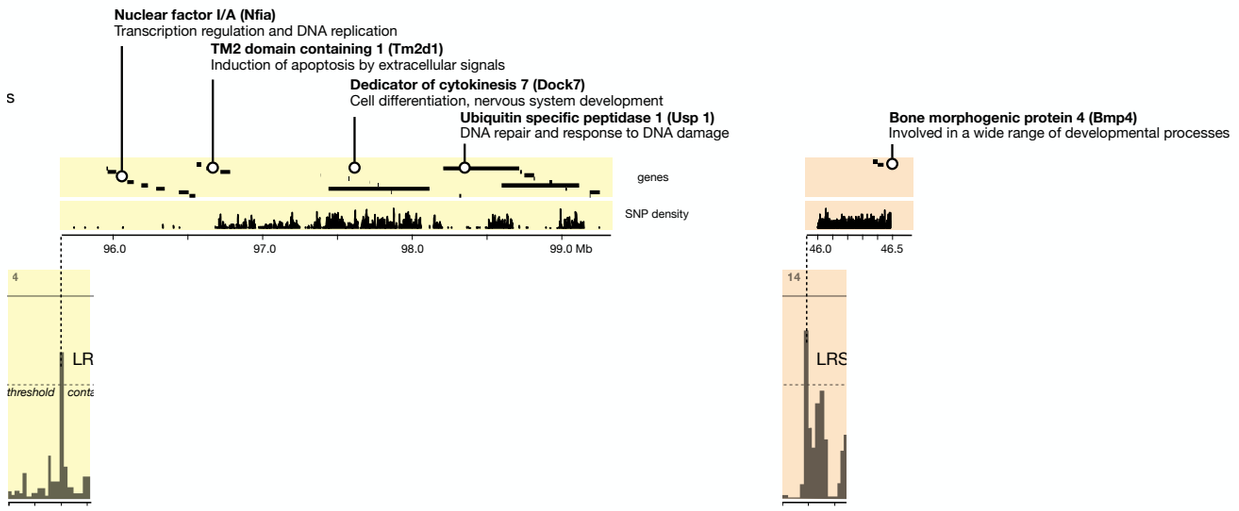


Figure 5. The influence of the C57BL/6J (B6) and DBA/2 (D2) alleles on vulnerability to ethanol-induced apoptosis at the suggestive QTL intervals on chromosomes 4 and 14. BXD mice were grouped based on presence of the B6 or D2 allele at each QTL interval, and the average number of ethanol-induced apoptotic cells were compared. Inheritance of B6 alleles is associated with significantly higher susceptibility to ethanol-induced apoptosis ($p < 0.01$).


TUNEL AND APOPTOTIC CELLS PER MM2
6,000
4,000
2,000
EtOH
MD
C57BL/6J
DBA/2J
BXD2
20
29
32
39
50
51
55
60
62
63
65
65b
66
73
73b
77
80
86
89
90
96
98
100
101
102
Chr 4 QTL interval
C57BL/6J
DBA/2
BXD mice were grouped based on presence of the B6 or D2 allele at each QTL interval, and the average number of ethanol-induced apoptotic cells were compared. Inheritance of B6 alleles is associated with significantly higher susceptibility to ethanol-induced apoptosis ($p < 0.01$).
Chr 14 QTL interval


TUNEL AND APOPTOTIC CELLS PER MM2
6,000
4,000
2,000
EtOH
MD
C57BL/6J
DBA/2J
BXD2
20
29
32
39
50
51
55
60
62
63
65
65b
66
73
73b
77
80
86
89
90
96
98
100
101
102


Chr 4 QTL interval
C57BL/6J
DBA/2
BXD mice were grouped based on presence of the B6 or D2 allele at each QTL interval, and the average number of ethanol-induced apoptotic cells were compared. Inheritance of B6 alleles is associated with significantly higher susceptibility to ethanol-induced apoptosis (p < 0.01).
Chr 14 QTL interval


Nuclear factor I/A (Nfia)
Transcription regulation and DNA replication
TM2 domain containing 1 (Tm2d1)
Induction of apoptosis by extracellular signals
Dedicator of cytokinesis 7 (Dock7)
Cell differentiation, nervous system development
Ubiquitin specific peptidase 1 (Usp 1)
DNA repair and response to DNA damage
Bone morphogenic protein 4 (Bmp4)
Involved in a wide range of developmental processes
genes
SNP density
96.0
97.0
98.0
99.0 Mb
46.0
46.5
4
14
LR
threshold
LRS

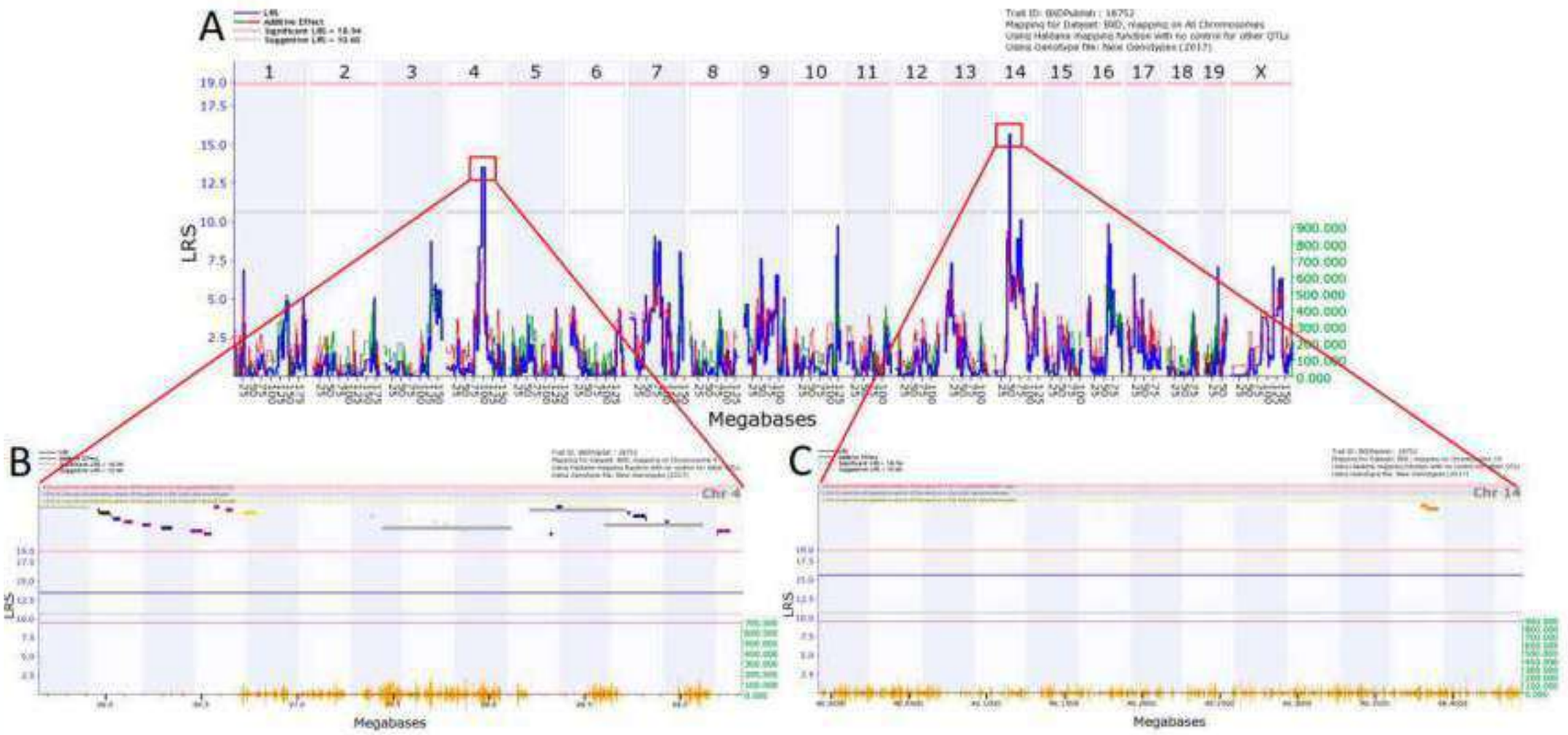


Figure 4. Quantitative Trait Locus (QTL) interval mapping of ethanol-induced apoptosis in the brainstem. **(A)** Whole genome QTL map. The x-axis represents chromosomes 1–19, & X and their physical maps in megabases. The y-axis and the blue line indicate the likelihood ratio statistic (LRS), which reports the strength of association between variations in genotype and the phenotype (i.e. ethanol-induced apoptosis). The red and gray horizontal lines respectively mark the significant (p = 0.05) and suggestive (p = 0.63) thresholds. Peaks that reach these thresholds indicate genome regions containing candidate genes that may be implicated in ethanol-induced apoptosis. **(B, C)** Maps of the suggestive QTLs on chromosome 4 (LRS = 13.510) and chromosome 14 (LRS = 15.655) were expanded to analyze candidate genes and single nucleotide polymorphisms (SNPs, yellow peaks on the x-axis) at each locus.

Whole genome Quantitative Trait Locus (QTL) map of ethanol-induced apoptosis in the brainstem.

The likelihood ratio statistic (LRS) measures the association strength between variations in genotype and the phenotype (i.e. ethanol-induced apoptosis).

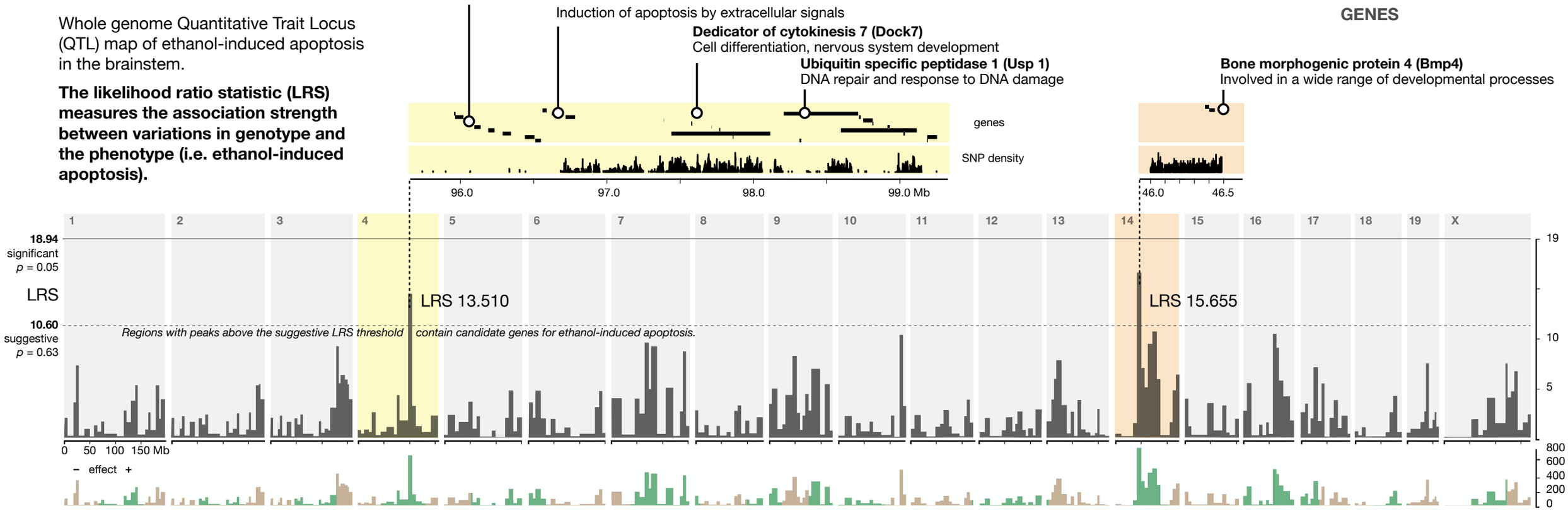


Figure 4. Quantitative Trait Locus (QTL) interval mapping of ethanol-induced apoptosis in the brainstem. (A) Whole genome QTL map. The x-axis represents chromosomes 1–19, & X and their physical maps in megabases. The y-axis and the blue line indicate the likelihood ratio statistic (LRS), which reports the strength of association between variations in genotype and the phenotype (i.e. ethanol-induced apoptosis). The red and gray horizontal lines respectively mark the significant (p = 0.05) and suggestive (p = 0.63) thresholds. Peaks that reach these thresholds indicate genome regions containing candidate genes that may be implicated in ethanol-induced apoptosis. **(B, C)** Maps of the suggestive QTLs on chromosome 4 (LRS = 13.510) and chromosome 14 (LRS = 15.655) were expanded to analyze candidate genes and single nucleotide polymorphisms (SNPs, yellow peaks on the x-axis) at each locus.

Genetic Influences on the Severity of Ethanol-induced Cell Death in the Developing Prenatal Brain

Kristina Balce[1], Emilie Théberge[1], Kristen Hamre[2], Daniel Goldowitz[1]

[1]Centre for Molecular Medicine and Therapeutics, Dept. of Medical Genetics, The University of British Columbia, Vancouver, BC
[2]Dept. of Anatomy and Neurobiology, The University of Tennessee Health Science Center, Memphis, TN

The authors wish to thank Aria Shokoohi, Mike Wu and Julia Boyle for assistance in tissue processing, sectioning, and staining for this project, and Rob Williams for assistance in QTL data analysis.

Fetal Alcohol Spectrum Disorder

Fetal Alcohol Spectrum Disorder (FASD) is caused by fetal exposure to alcohol consumed by mothers during pregnancy [1]. It is the most common preventable cause of developmental disability in Canada — over 3000 Canadian newborns diagnosed annually [2].

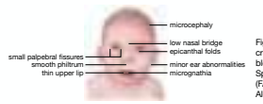


Figure 1. Typical craniofacial defects of full blown Fetal Alcohol Spectrum Disorder (FASD), known as Fetal Alcohol Syndrome (FAS).

FASD is characterized by abnormal brain development, cognitive/learning deficits, behavioural issues, and/or specific patterns of physical defects.

Research in mouse models implicates ethanol-induced apoptosis (i.e. programmed cell death) as one process contributing to disruption in early brain development [4]. Severity of ethanol's effects appears to vary depending on genetic background; how and which genes are involved in susceptibility or resistance to alcohol remain largely unknown. Genetic influences may be important for screening, prevention, and therapeutic treatment of FASD.

Objectives

Demonstrate genetic differences in vulnerability to the apoptotic effects of prenatal alcohol exposure.

Use Quantitative Trait Locus (QTL) analysis to identify genes involved in susceptibility or resistance to ethanol-induced apoptosis in the developing brain.

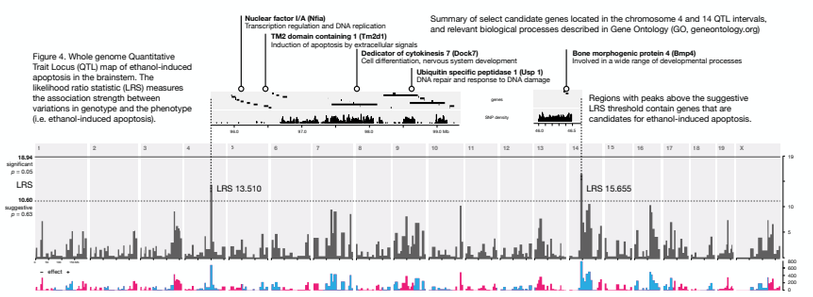
Conclusions

There is significant variation in vulnerability to ethanol-induced apoptosis between BXD strains, suggesting that genetic differences influence the severity of the effects of prenatal alcohol exposure.

Suggestive QTLs on chromosomes 4 and 14 were identified BXD strains with the C57BL/6J genetic background at these QTLs were more susceptible to ethanol-induced apoptosis.

Candidate genes at these two QTLs may play an important role in vulnerability; are potential future targets for prenatal screening and therapeutic intervention of FASD.

1. Roberts, G., & Hanson, J. (2003). Best practices. Fetal alcohol syndrome/fetal alcohol effects and the effects of other substance use during pregnancy. Orsawa, ON: Canada's Drug Strategy Division, Health Canada. 2. Popova, S., Lange, S., Burd, L., & Rehm, J. (2015). The Burden and Economic Impact of Fetal Alcohol Spectrum Disorder in Canada. Toronto, ON, Canada: Centre for Addiction and Mental Health. ISBN, 978-1. 3. Dörrie, N., Föcker, M., Freunscht, I., Hebebrand, J. (2014). Fetal alcohol spectrum disorders. European Child & Adolescent Psychiatry, 23(10). 4. Ogawa, T., Kuwagata, M., Ruiz, J., Zhou, F. C. (2005). Differential teratogenic effects of alcohol on embryonic development between C57BL/6J and DBA/2 mice: A new view. Alcohol Clinical & Experimental Research, 29(5)



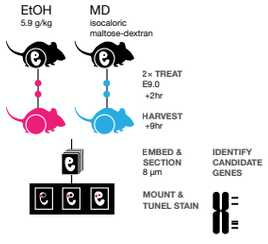


Figure 2. Representative images of the developing brainstem from a susceptible and resistant mouse BXD strain, treated with ethanol on embryonic day 9 (E9.0). Apoptotic cells were labelled using the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Cell nuclei of nonapoptotic cells were counterstained with methyl green. The arrow indicates a greater amount of cell death in the susceptible strain.

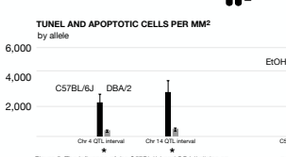


Figure 5. The influence of the C57BL/6J and DBA/2alleles on vulnerability to ethanol-induced apoptosis at the suggestive QTL intervals on chromosomes 4 and 14. BXD mice were grouped based on presence of the B6 or D2 allele at each QTL interval, and the average number of ethanol-induced apoptotic cells were compared. Inheritance of B6 alleles is associated with significantly higher susceptibility to ethanol-induced apoptosis ($p < 0.01$).

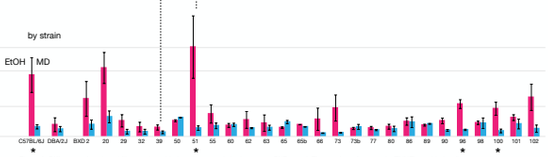


Figure 3. Differences in vulnerability to ethanol-induced apoptosis in the mouse BXD panel. The number of apoptotic (TUNEL positive) cells per mm² was measured in mouse embryos treated with ethanol (EtOH, red) and a maltose-dextran (MD, blue) sugar control at embryonic day 9 (E9.0). Between strains, there was a significant difference ($p < 0.001$) in apoptosis means after EtOH treatment, but no significant difference ($p = 0.084$) after MD treatment. Comparisons between treatments within strain reported significant differences ($p < 0.05$) in apoptosis means in the C57BL/6J, BXD 51, BXD 96, and BXD 100 strains, indicating that these strains show the highest vulnerability to ethanol-induced apoptosis.

Genetic Influences on the Severity of Ethanol-induced Cell Death in the Developing Prenatal Brain

Kristina Balce[1], Emilie Théberge[1], Kristen Hamre[2], Daniel Goldowitz[1]

[1]Centre for Molecular Medicine and Therapeutics, Dept. of Medical Genetics, The University of British Columbia, Vancouver, BC
[2]Dept. of Anatomy and Neurobiology, The University of Tennessee Health Science Center, Memphis, TN

The authors wish to thank Aria Shokoohi, Mike Wu and Julia Boyle for assistance in tissue processing, sectioning, and staining for this project, and Rob Williams for assistance in QTL data analysis.

Fetal Alcohol Spectrum Disorder

Fetal Alcohol Spectrum Disorder (FASD) is caused by fetal exposure to alcohol consumed by mothers during pregnancy [1]. It is the most common preventable cause of developmental disability in Canada — over 3000 Canadian newborns diagnosed annually [2]. FASD is characterized by abnormal brain development, cognitive/learning deficits, behavioural issues, and/or specific patterns of physical defects.

Research in mouse models implicates ethanol-induced apoptosis (i.e. programmed cell death) as one process contributing to disruption in early brain development [4]. Severity of ethanol's effects appears to vary depending on genetic background; how and which genes are involved in susceptibility or resistance to alcohol remain largely unknown. Genetic influences may be important for screening, prevention, and therapeutic treatment of FASD.

Objectives

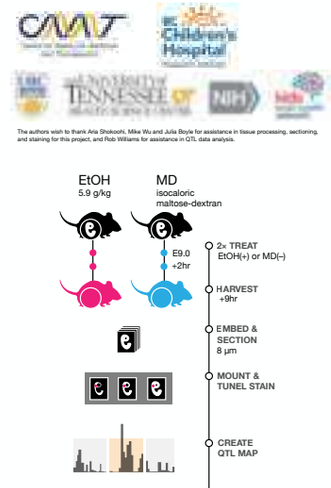
Demonstrate genetic differences in vulnerability to the apoptotic effects of prenatal alcohol exposure. Use Quantitative Trait Locus (QTL) analysis to identify genes involved in susceptibility or resistance to ethanol-induced apoptosis in the developing brain.

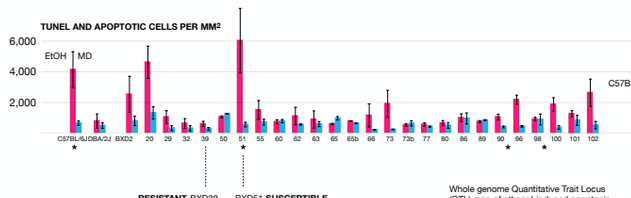
Conclusions

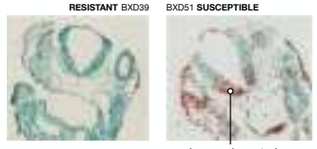
There is significant variation in vulnerability to ethanol-induced apoptosis between BXD strains, suggesting that genetic differences influence the severity of the effects of prenatal alcohol exposure.

Suggestive QTLs on chromosomes 4 and 14 were identified BXD strains with the C57BL/6J genetic background at these QTLs were more susceptible to ethanol-induced apoptosis.

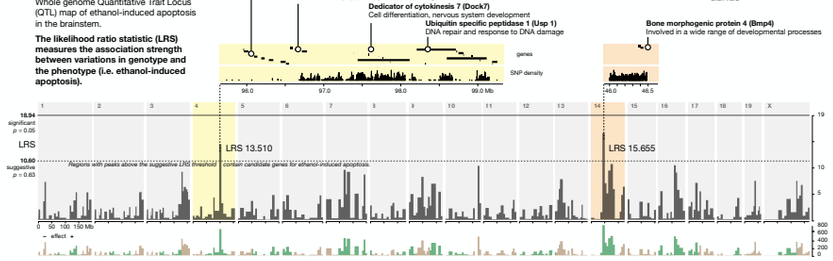
Candidate genes at these two QTLs may play an important role in vulnerability; are potential future targets for prenatal screening and therapeutic intervention of FASD.







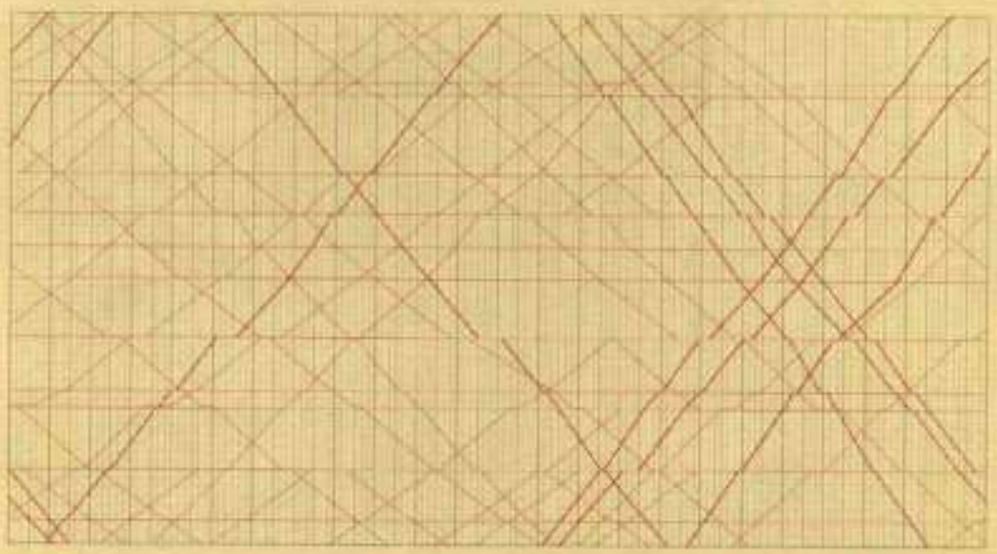
Images of the developing brainstem from a susceptible and resistant mouse BXD strain, treated with ethanol on embryonic day 9 (E9.0). Apoptotic cells were labelled using the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Cell nuclei of nonapoptotic cells were counterstained with methyl green. The arrow indicates a greater amount of cell death in the susceptible strain.



The Elements of Typographic Style

version 3.0

Robert Bringhurst



The Visual Display of Quantitative Information

EDWARD R. TUFTE

Gordon Andrews (designer)
Gazelle chair (c. 1950) designed, 1957 manufactured
plywood, aluminium, wool
74.0 x 48.0 x 55.0 cm
Museum of Applied Arts and Sciences, Sydney
Purchased, 1989 (89/499)