Academic posters

Your business card

CORSO DELLA LAUREA MAGISTRALE IN BIOLOGIA
“MALATTIE GENETICHE: DALLA DIAGNOSI ALLA TERAPIA”
Università degli Studi di Milano-Bicocca
04 /06/2024

Elisabetta Citterio, PhD
Department of Biotechnology and Biosciences

"THE MORE STRIKINGLY VISUAL YOUR PRESENTATION IS, THE MORE PEOPLE WILL REMEMBER IT. AND MORE IMPORTANTLY, THEY WILL REMEMBER YOU."

— Paul Arden

Direttore creativo britannico

Summary

01 What is a scientific poster?

02 Why present a research poster?

03 Who is your audience?

04 Structure and contents

05 Poster Design

06 Presenting your research

01. What is a scientific poster?

A concise and visual summary of your research.

Its purpose is to be accessible and to drive attention to your research.

A scientific poster is a visual abstract of your research

Insekten als Indikatoren für Renaturierungserfolge

Vasco Elbrecht (Vasco.Elbrecht@rub.de), **Ralph Tollrian** & **Florian Leese**
Ruhr-Universität Bochum, Lehrstuhl für Evolutionsökologie und Biodiversität der Tiere
GeneStream

Hintergrund

Gewässer werden derzeit europaweit renaturiert, um unsere Trinkwasserversorgung auch in Zukunft zu sichern. Oft kehren Stress-sensitive Arten nach den meist kostspieligen Maßnahmen nicht unmittelbar in die Gewässer zurück, wobei nicht klar ist ob:

a) die Renaturierung unzureichend war oder
b) die Organismen das renaturierte Gewässer nicht selbständig wiederbesiedeln können.

Informationen über die Mobilität von empfindlichen Indikatorarten, wie z. B. der Steinfliege *Dinocras cephalotes*, sind folglich essentiell um den Erfolg von Renaturierungen zu bewerten (Abb. 1).

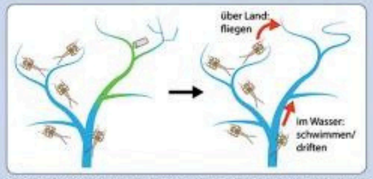


Abbildung 1: Ob und wie Indikatorarten (schematisch in braun dargestellt) renaturierte Gewässer wiederbesiedeln können ist oft nicht bekannt. Diese Informationen sind jedoch essentiell, um Abschnitte für konkrete Maßnahmen zu identifizieren sowie den Erfolg von Renaturierungen zu bewerten.

Ziel dieser Masterarbeit war es, das Ausbreitungspotenzial der Steinfliegenart *D. cephalotes* und somit ihre Eignung als Indikatorart mit molekularen Methoden zu untersuchen.

Material und Methoden

Die Ausbreitungsfähigkeit und -wege von Insekten können durch reines Beobachten nur schwer bzw. gar nicht ermittelt werden. Mit modernen molekularen, DNA-basierten Methoden, ist dies jedoch präzise und kostengünstig möglich. Zusätzlich können über den sogenannten „genetischen Fingerabdruck" Aussagen zum Zustand und Bedrohung von Populationen gemacht werden.

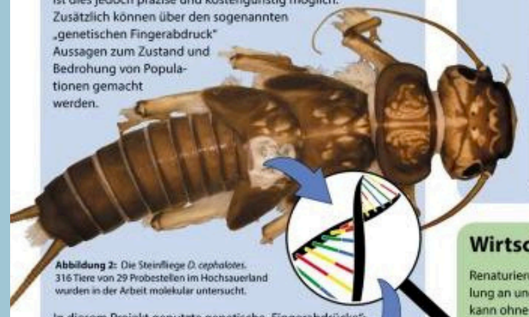


Abbildung 2: Die Steinfliege *D. cephalotes*. 316 Tiere von 29 Probestellen im Hochsauerland wurden in der Arbeit molekular untersucht.

In diesem Projekt genutzte genetische „Fingerabdrücke":

„CO1" Gen
- Mitochondriales Gen
- Historische Prozesse (Tausende von Jahren)

Mikrosatelliten
- Variable Kern DNA
- Vergleichbar mit Vaterschaftstest
- Rezente Prozesse (wenige Jahre)

Ergebnisse und Diskussion

Analysen des CO1 Gens zeigen, dass *D. cephalotes* aus zwei genetisch diversen Gruppen besteht (A und B in Abb. 3). Vertreter beider Gruppen sind über das Untersuchungsgebiet relativ gleichmäßig verteilt, was eine gute Ausbreitungsfähigkeit der Individuen belegt. Die Analyse der hochauflösenden Mikrosatelliten bestätigt dies, da alle Populationen genetisch sehr ähnlich sind (Abb. 4).

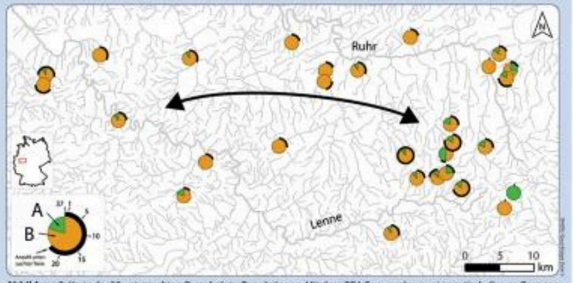


Abbildung 3: Karte der 29 untersuchten *D. cephalotes* Populationen. Mit dem CO1 Gen wurden zwei genetisch diverse Gruppen gefunden: A (grün) und B (orange) deren relative Häufigkeit im Kreisdiagram dargestellt ist. Beide genetischen Gruppen sind im Untersuchungsgebiet gleichmäßig verteilt und in den Populationen sind oft beide Gruppen vertreten. Dies deutet auf einen genetischen Austausch zwischen den Populationen hin, also dass ausgewachsene Steinfliegen zu anderen benachbarten Populationen fliegen können (mit einem Pfeil schematisch dargestellt). Ohne Austausch zwischen Populationen wären deutlichere Muster zu erwarten, d.h. die Kreise hätten entweder nur die Farbe Orange oder Grün.

- CO1 und Mikrosatelliten-Untersuchungen zeigen, dass alle Populationen genetisch sehr ähnlich sind. Dies belegt, dass *D. cephalotes* eine **gute Ausbreitungsfähigkeit** hat.
- Über adulte fliegende Tiere kann *D. cephalotes* innerhalb weniger Jahre renaturierte Gewässerabschnitte von benachbarten Bächen wiederbesiedeln.
- Somit eignet sich die Art als **guter Indikator**, um den Erfolg von Renaturierungen zu überprüfen.
- Diese Arbeit zeigt das - bislang weitgehend ungenutzte - Potenzial genetischer Methoden.

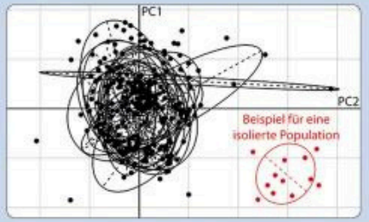


Abbildung 4: Hauptkomponentenanalyse (PCA) für die Mikrosatelliten-Daten, gruppiert nach Sammelorten. Alle Punkte (=untersuchte Individuen) überlagern sich, was auf eine große genetische Ähnlichkeit und somit eine gute Ausbreitungsfähigkeit der Organismen hindeutet.

Hätte die Steinfliege eine schlechte Ausbreitungsfähigkeit, so wären genetisch distinkte Populationen (rote Gruppe) zu erwarten.

Wirtschaftliche Bedeutung

Renaturierungen sind fast immer **teuer** jedoch oft **nicht direkt erfolgreich**. Ob eine Wiederbesiedlung an ungenügenden Maßnahmen oder der fehlenden Nähe von Quellpopulationen scheitert, kann ohne Kenntnis über die Ausbreitungsfähigkeit der Arten nicht eindeutig bestimmt werden. Die Ergebnisse dieser Masterarbeit zeigen, dass:

- sich über die genetische Diversität die **Gefährdung von Populationen** (und somit auch die Gefährdung ihrer Funktion im Ökosystem) durch anthropogene Einflüsse feststellen lässt,
- genetische Daten entscheidende **Hinweise über die Isolation und Wiederbesiedelbarkeit** von Gewässerabschnitten geben können,
- anhand von genetischen Landkarten geeignete **Stellen mit hohem Wiederbesiedlungspotenzial identifiziert** werden können,
- diese wichtigen Informationen durch molekulare Methoden mit vergleichsweise **geringem Aufwand und kostengünstig** gewonnen werden können.

Video-Poster

Dreiminütiges Video zu der Masterarbeit (auf Englisch).
www.goo.gl/4K7Pbt

Veröffentlichungen aus der Masterarbeit

Elbrecht, Feld, Gies, Hering, Sondermann, Tollrian & Leese (2016) Genetic diversity and dispersal potential of the stonefly *Dinocras cephalotes* in a central European low mountain range. Freshwater Sciences.
Elbrecht, Postler, Jahn & Leese (2015) The complete mitochondrial genome of the stonefly *Dinocras cephalotes* (Plecoptera, Perlidae). Mitochondrial DNA.

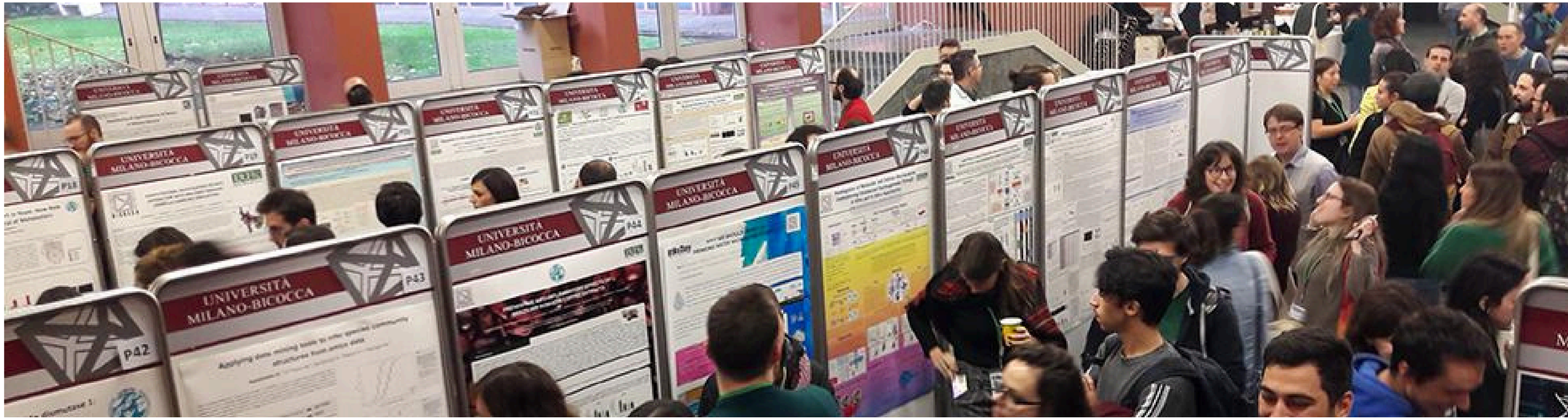
Über den Autor

Vasco Elbrecht ist Doktorand im „GeneStream" Projekt. Im Rahmen der Arbeit entwickelt und testet er modernste genetische Methoden zur Fließgewässerbewertung. www.GeneStream.de

Projektpartner

Wir danken Christian K. Feld, Maria Gies, Daniel Hering und Martin Sondermann (Universität Duisburg-Essen) für die Zusammenarbeit in diesem Projekt.

02. Why present a scientific poster?



Communication and connection

Share key results

It’s not to summarize every detail of your research

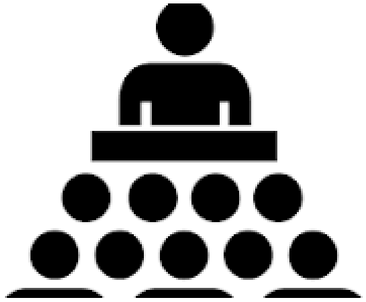
Networking tool

It serves as a conversation starter

Communication tool

It should use visuals to draw people from a distance.

It enhances your research and CV



Practice public speaking

Present research in a formal setting

Direct interactions

Get feedback from peers; share ideas and create collaborations

Enhance your resume

Conference poster abstract are searchable; poster award with cash price

Why are academic posters important? Responsibility!

01.
Represents you and your lab

at conferences, symposia, hallways, informational days

02.
Demonstrate attention to detail

03.
Demonstrate expertise

04.
Enhance your resume

IMPORTANT:
Work with your research supervisor

Vital for your research group/University: it represents their laboratory.
Should be involved in editing and final approval.

Other experts in your field or a related field?

They will read it, even if it’s bad

People who don’t know your research?

A good poster can attract previously uninterested passersby and may open new contacts, at the intersections with other disciplines

The broader public?
Increases your potential audience and impact

03. Who is your audience?

04. Structure and content

CLEAR ORGANIZATION AND A CONCISE CENTRAL ARGUMENT.

Focus on findings that support your argument and validate your conclusion.

Main elements of a poster
-Hierarchy and the ‘must haves’ -

1.Title

2.Names of people involved and affiliations

3.Abstract (max 200 words)

What is known; what is not known; what you set out to do; how you have done it and the key findings and results; conclusions and significance.

Main elements of a poster

4.Introduction

background knowledge
in the field; open questions;
project aim(s);
a couple of literature references

5.Methods/Approach

basis of techniques used,
keep it short;

6.Results
(3-4 Figures)

Show examples
of key experiments
with Figure legends
specifying experimental
details (type of exp.,
samples, etc..)

Main elements of a poster

7.Conclusions/future perspectives
(bullet points)

summarize the research findings and impact/significance

8.Acknowledgements

grant funding, research programs, laboratory, mentors, collaborators

(smaller at the bottom)

9.References

A few literature references

(smaller at the bottom)

10.Contact

your contact email;lab website; QR code; foto

(smaller at the bottom)

05. Poster design

Concept - first draft

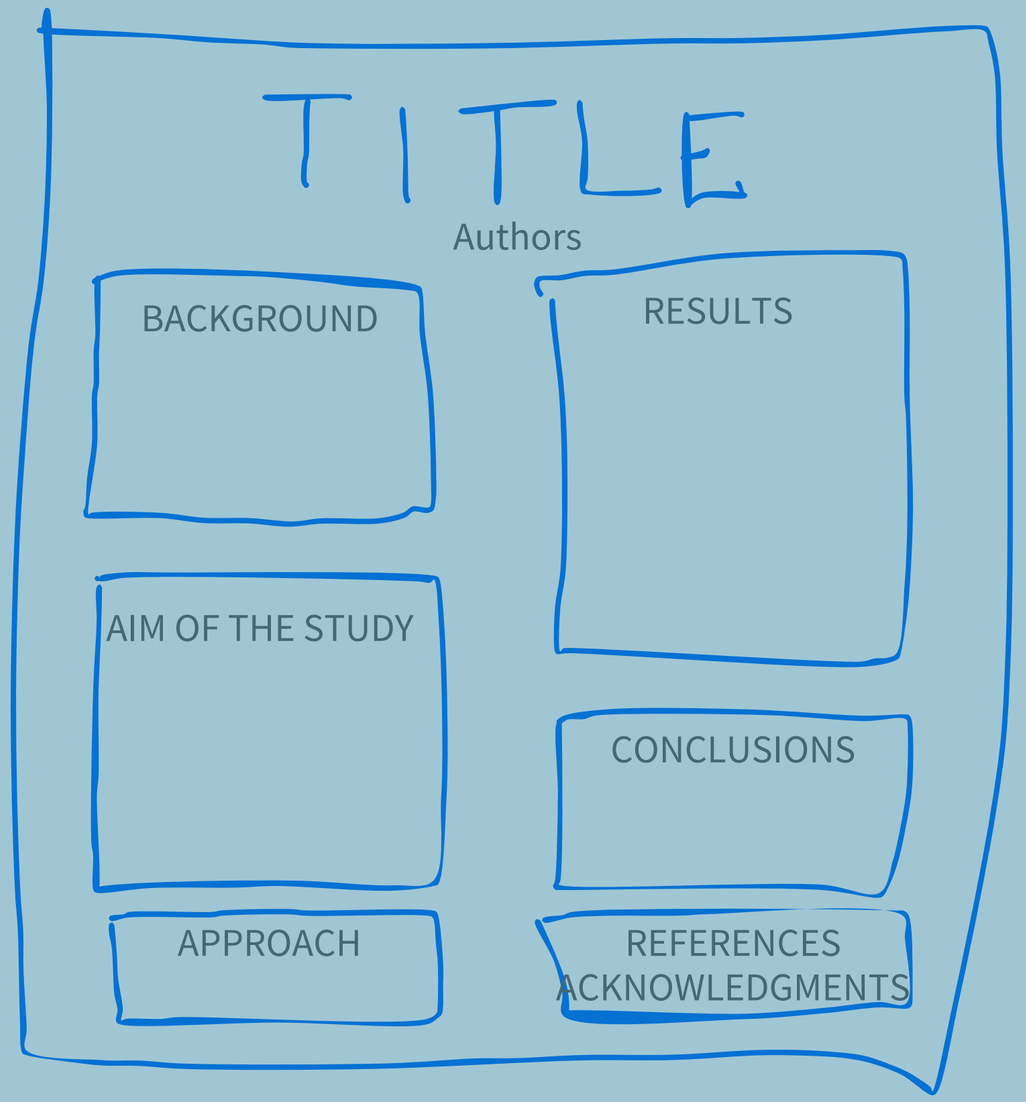
Ask yourself questions:

- What findings I want people to focus on?
- What are two or three background knowledge items to know?
- What knowledge is missing?
- What is the main goal of my research?
- How did I approach the research?
- What are the key results I obtained?
- What is the impact/relevance of my results in the field?

Concept - first draft

Create storyboard:

- Headings, sections
- Experiments to present (3-4)
- Prepare visuals (photos, graphs...)
- Prepare text; concise; figure legends



Layout and size

Check with organizers
(A1 vertical: W594 x L841 cm
W23.4 x L33.1 Inch)

Panels

Use numbered headings

Negative space

Leave space at the edges;
leave clear space between
sections

Poster must be “stand alone”

Eye-catching visuals

One visual related to your research visible from far

Colors

Use a limited number (3-4). Background color; accent color to draw attention

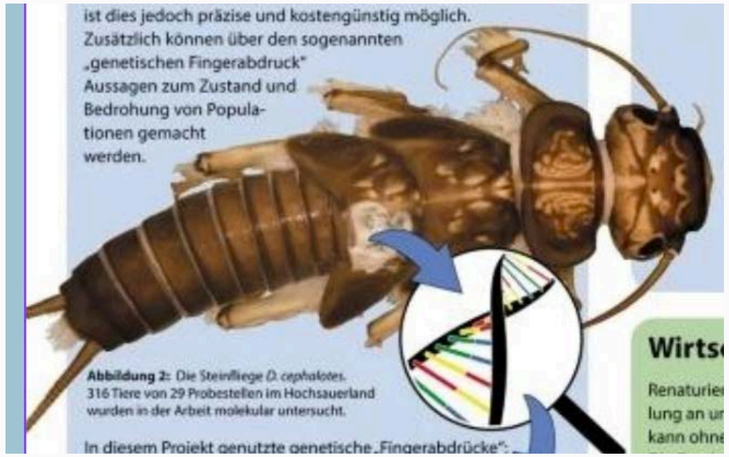
Alignment

Align sections

Font

-legible fonts like Times New Roman, Arial..
-use same fonts throughout the poster
-visible from 2-3 meters:

- 72 points font for title
- 48 points font for headings
- 24 points font for body text



Which would you choose?

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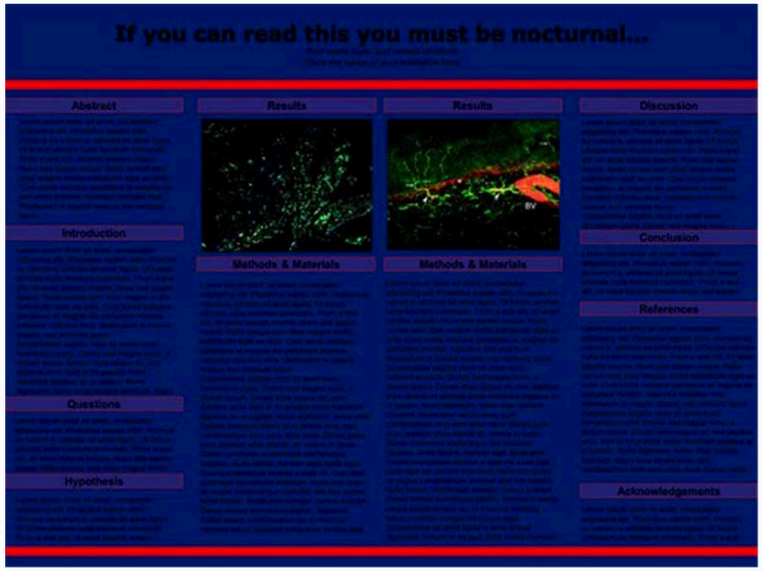

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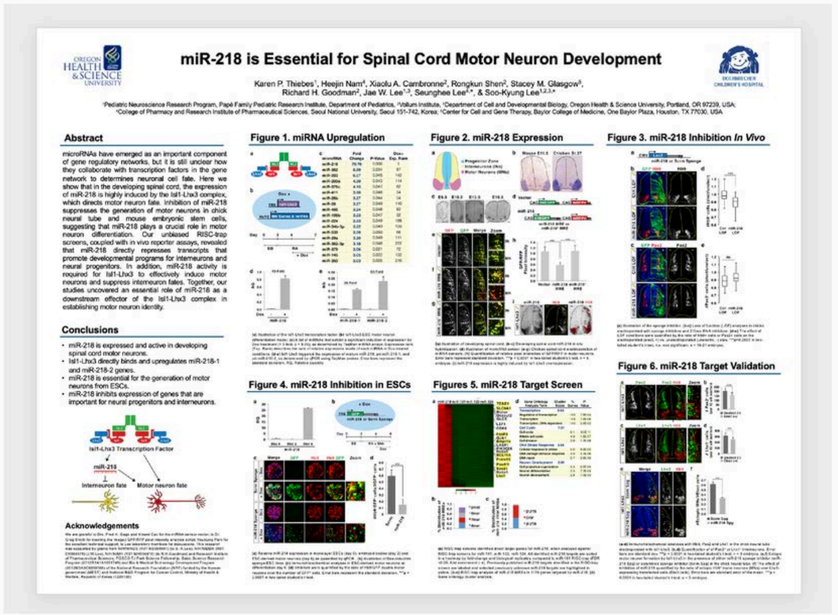

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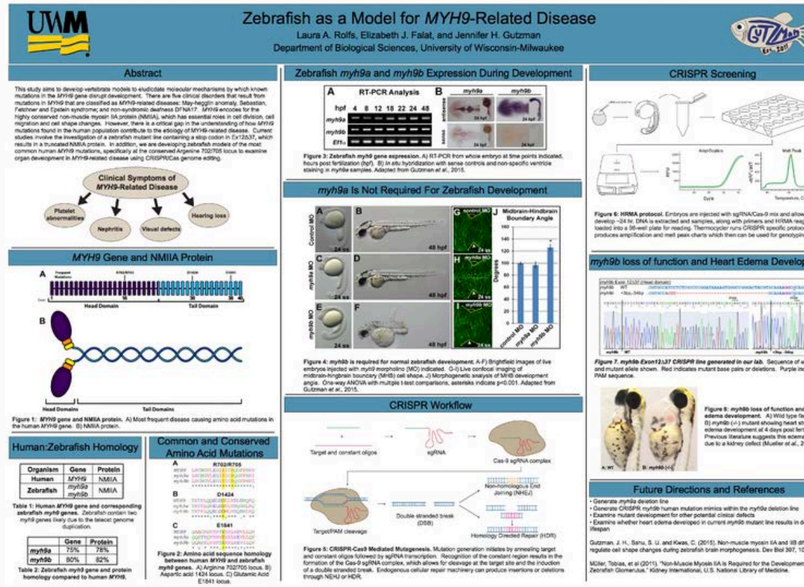

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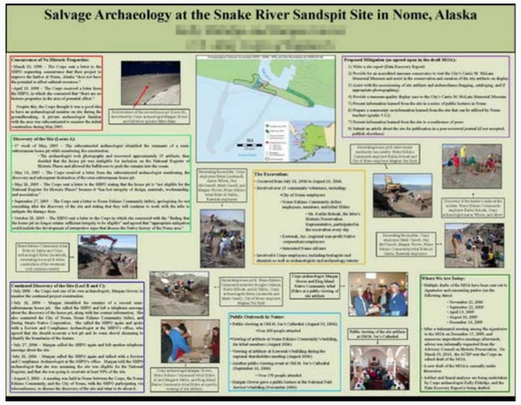

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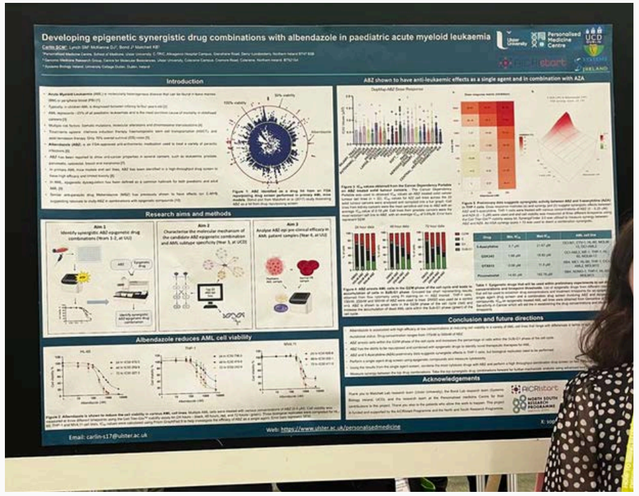

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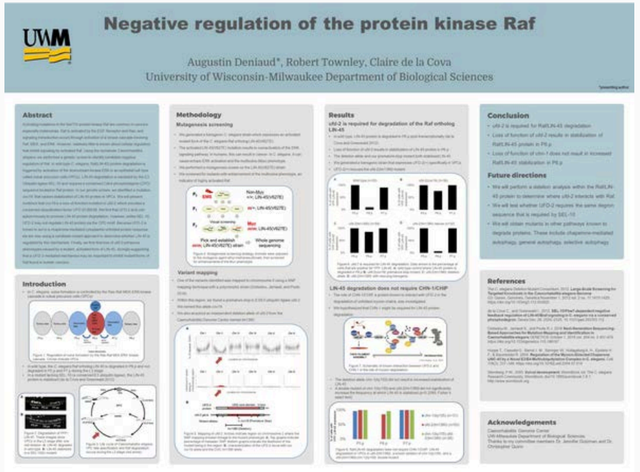

7.

Examples

What do you think should be fixed about this poster?

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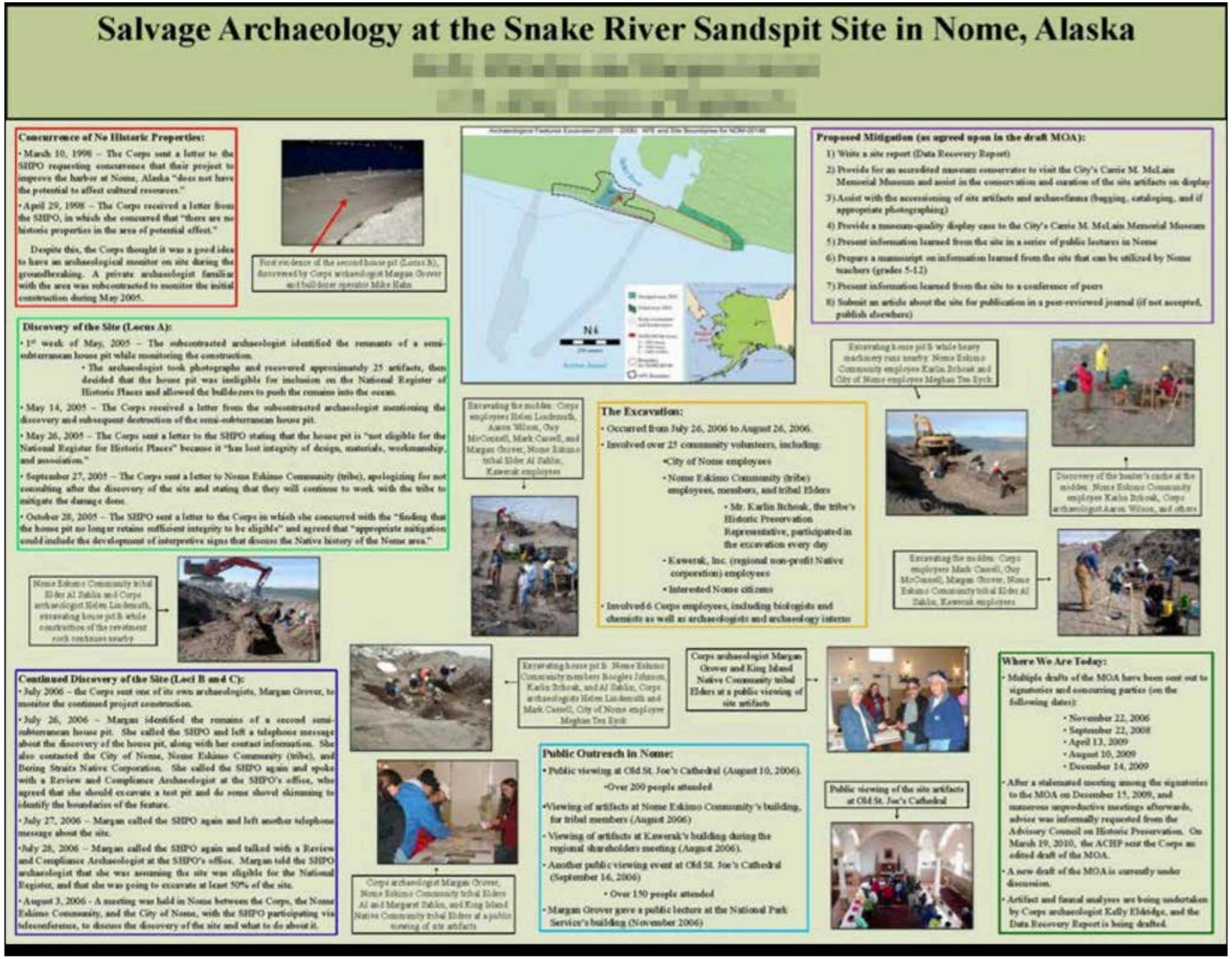
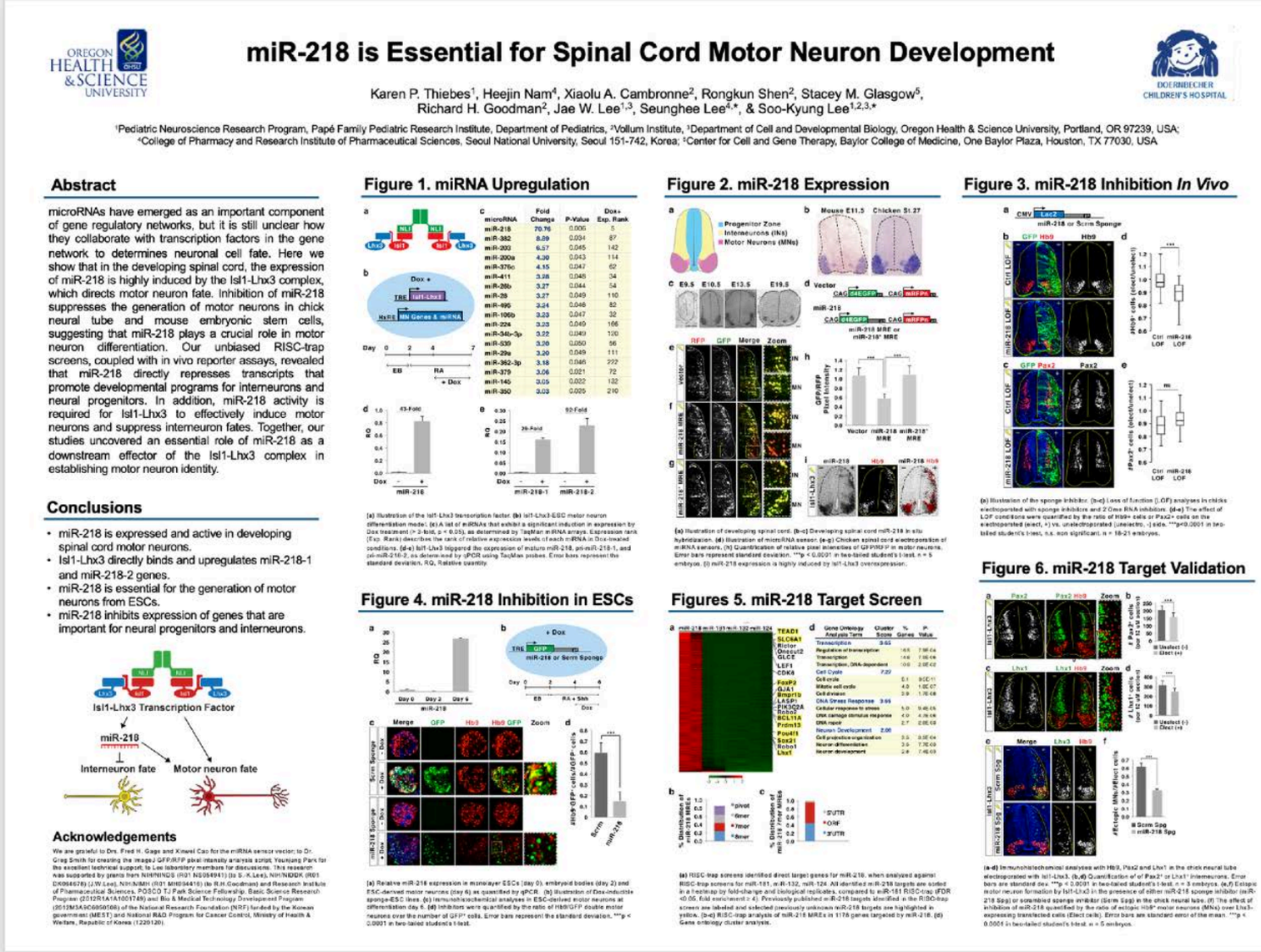


Examples of better posters

What do you think about this poster?



miR-218 is Essential for Spinal Cord Motor Neuron Development

Karen P. Thiebes[1], Heejin Nam[4], Xiaolu A. Cambronne[2], Rongkun Shen[2], Stacey M. Glasgow[5], Richard H. Goodman[2], Jae W. Lee[1,3], Seunghee Lee[4,*], & Soo-Kyung Lee[1,2,3,*]

[1]Pediatric Neuroscience Research Program, Papé Family Pediatric Research Institute, Department of Pediatrics, [2]Vollum Institute, [3]Department of Cell and Developmental Biology, Oregon Health & Science University, Portland, OR 97239, USA; [4]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Korea; [5]Center for Cell and Gene Therapy, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Abstract

microRNAs have emerged as an important component of gene regulatory networks, but it is still unclear how they collaborate with transcription factors in the gene network to determines neuronal cell fate. Here we show that in the developing spinal cord, the expression of miR-218 is highly induced by the Isl1-Lhx3 complex, which directs motor neuron fate. Inhibition of miR-218 suppresses the generation of motor neurons in chick neural tube and mouse embryonic stem cells, suggesting that miR-218 plays a crucial role in motor neuron differentiation. Our unbiased RISC-trap screens, coupled with in vivo reporter assays, revealed that miR-218 directly represses transcripts that promote developmental programs for interneurons and neural progenitors. In addition, miR-218 activity is required for Isl1-Lhx3 to effectively induce motor neurons and suppress interneuron fates. Together, our studies uncovered an essential role of miR-218 as a downstream effector of the Isl1-Lhx3 complex in establishing motor neuron identity.

Conclusions

- miR-218 is expressed and active in developing spinal cord motor neurons.
- Isl1-Lhx3 directly binds and upregulates miR-218-1 and miR-218-2 genes.
- miR-218 is essential for the generation of motor neurons from ESCs.
- miR-218 inhibits expression of genes that are important for neural progenitors and interneurons.

Acknowledgements

We are grateful to Drs. Fred H. Gage and Xinwei Cao for the miRNA sensor vector; to Dr. Greg Smith for creating the ImageJ GFP/RFP pixel intensity analysis script, Youngjung Park for the excellent technical support, to Lee laboratory members for discussions. This research was supported by grants from NIH/NINDS (R01 NS064941) (to S.-K.Lee), NIH/NIDDK (R01 DK064678) (J.W.Lee), NIH/NIMH (R01 MH094418) (to R.H.Goodman) and Research Institute of Pharmaceutical Sciences, POSCO TJ Park Science Fellowship, Basic Science Research Program (2012R1A1A1001749) and Bio & Medical Technology Development Program (2013M3A9C6050168) of the National Research Foundation (NRF) funded by the Korean government (MEST) and National R&D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea (1220120).

Figure 1. miRNA Upregulation

microRNA	Fold Change	P-Value	Dox+ Exp. Rank
miR-218	70.76	0.000	5
miR-382	8.09	0.034	87
miR-200	6.57	0.045	142
miR-200a	4.30	0.043	114
miR-376c	4.15	0.047	62
miR-411	3.38	0.048	34
miR-26b	3.27	0.044	54
miR-26	3.27	0.049	110
miR-99b	3.24	0.048	82
miR-106b	3.23	0.047	32
miR-224	3.23	0.049	166
miR-340-3p	3.22	0.049	100
miR-539	3.20	0.050	56
miR-29a	3.20	0.049	111
miR-362-3p	3.18	0.046	222
miR-379	3.06	0.021	72
miR-145	3.05	0.022	132
miR-350	3.03	0.029	210

(a) Illustration of the Isl1-Lhx3 transcription factor. (b) Isl1-Lhx3-ESC motor neuron differentiation mode. (c) A list of miRNAs that exhibit a significant induction in expression by Dox treatment (> 3-fold, p < 0.05), as determined by TaqMan miRNA arrays. Expression rank (Exp. Rank) describes the rank of relative expression levels of each miRNA in Dox-treated conditions. (d-e) Isl1-Lhx3 triggered the expression of mature miR-218, pri-miR-218-1, and pri-miR-218-2, as detected by qPCR using TaqMan probes. Error bars represent the standard deviation; RQ, Relative quantity.

Figure 2. miR-218 Expression

(a) Illustration of developing spinal cord. (b–c) Developing spinal cord miR-218 in situ hybridization. (d) Illustration of microRNA sensor. (e-g) Chicken spinal cord electroporation of miRNA sensors. (h) Quantification of relative pixel intensities of GFP/RFP in motor neurons. Error bars represent standard deviation. ***p < 0.0001 in two-tailed student's t-test. n = 5 embryos. (i) miR-218 expression is highly induced by Isl1-Lhx3 overexpression.

Figure 3. miR-218 Inhibition *In Vivo*

(a) Illustration of the sponge inhibitor. (b-c) Loss of function (LOF) analyses in chicks electroporated with sponge inhibitors and 2'Ome RNA inhibitors. (d-e) The effect of LOF conditions were quantified by the ratio of Hb9+ cells or Pax2+ cells on the electroporated (elect, +) vs. unelectroporated (unelectro, -) side. ***p<0.0001 in two-tailed student's t-test, n.s. non significant. n = 18-21 embryos.

Figure 4. miR-218 Inhibition in ESCs

(a) Relative miR-218 expression in monolayer ESCs (day 0), embryoid bodies (day 2) and ESC-derived motor neurons (day 6) as quantified by qPCR. (b) Illustration of Dox-inducible sponge-ESC lines. (c) Immunohistochemical analyses in ESC-derived motor neurons at differentiation day 6. (d) Inhibitors were quantified by the ratio of Hb9/GFP double motor neurons over the number of GFP+ cells. Error bars represent the standard deviation. ***p < 0.0001 in two-tailed student's t-test.

Figures 5. miR-218 Target Screen

Gene Ontology Analysis Term	Cluster Score	% Genes	P-Value
Transcription	3.65		
Regulation of transcription		16.6	7.9E-04
Transcription		14.6	7.3E-04
Transcription, DNA-dependent		13.6	2.0E-03
Cell Cycle	1.22		
Cell cycle		8.1	9.0E-01
Mitotic cell cycle		4.0	1.0E-01
Cell division		3.9	1.7E-01
DNA Stress Response	3.05		
Cellular response to stress		5.0	9.4E-05
DNA damage stimulus response		4.0	4.7E-04
DNA repair		2.7	2.0E-03
Neuron Development	2.06		
Cell projection organization		3.5	3.3E-04
Neuron differentiation		3.5	7.7E-03
Neuron development		2.9	7.4E-03

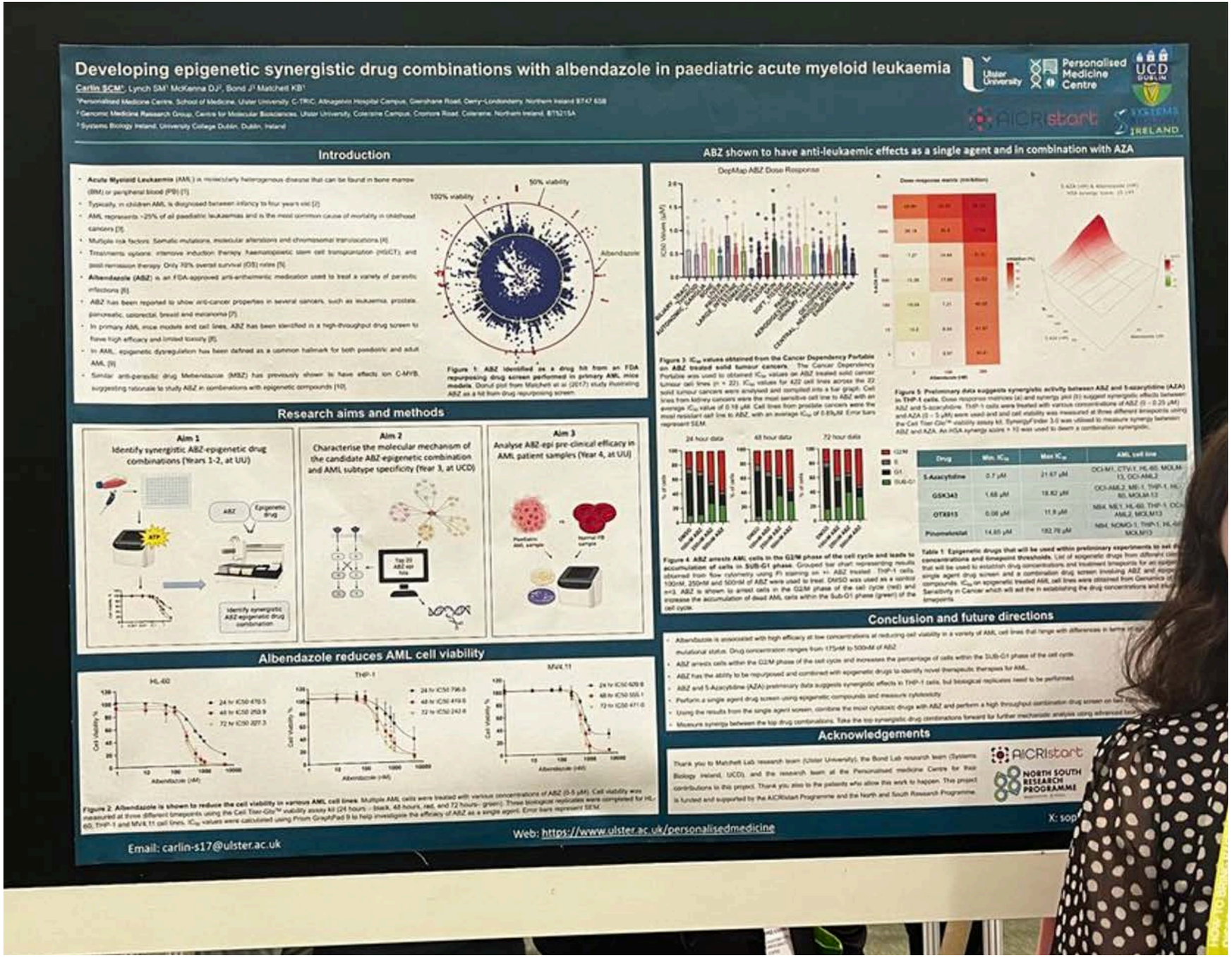
(a) RISC-trap screens identified direct target genes for miR-218, when analyzed against RISC-trap screens for miR-181, miR-132, miR-124. All identified miR-218 targets are sorted in a heatmap by fold-change and biological replicates, compared to miR-181 RISC-trap (FDR <0.05, fold enrichment > 4). Previously published miR-218 targets identified in the RISC-trap screen are labeled and selected previously unknown miR-218 targets are highlighted in yellow. (b-c) RISC-trap analysis of miR-218 MREs in 1178 genes targeted by miR-218. (d) Gene ontology cluster analysis.

Figure 6. miR-218 Target Validation

(a-d) Immunohistochemical analyses with Hb9, Pax2 and Lhx1 in the chick neural tube electroporated with Isl1-Lhx3. (b,d) Quantification of # of Pax2+ or Lhx1+ interneurons. Error bars are standard dev. **p < 0.0001 in two-tailed student's t-test. n = 3 embryos. (e,f) Ectopic motor neuron formation by Isl1-Lhx3 in the presence of either miR-218 sponge inhibitor (miR-218 Spg) or scrambled sponge inhibitor (Scrm Spg) in the chick neural tube. (f) The effect of inhibition of miR-218 quantified by the ratio of ectopic Hb9+ motor neurons (MNs) over Lhx3-expressing transfected cells (Elect cells). Error bars are standard error of the mean. ***p < 0.0001 in two-tailed student's t-test. n = 5 embryos.

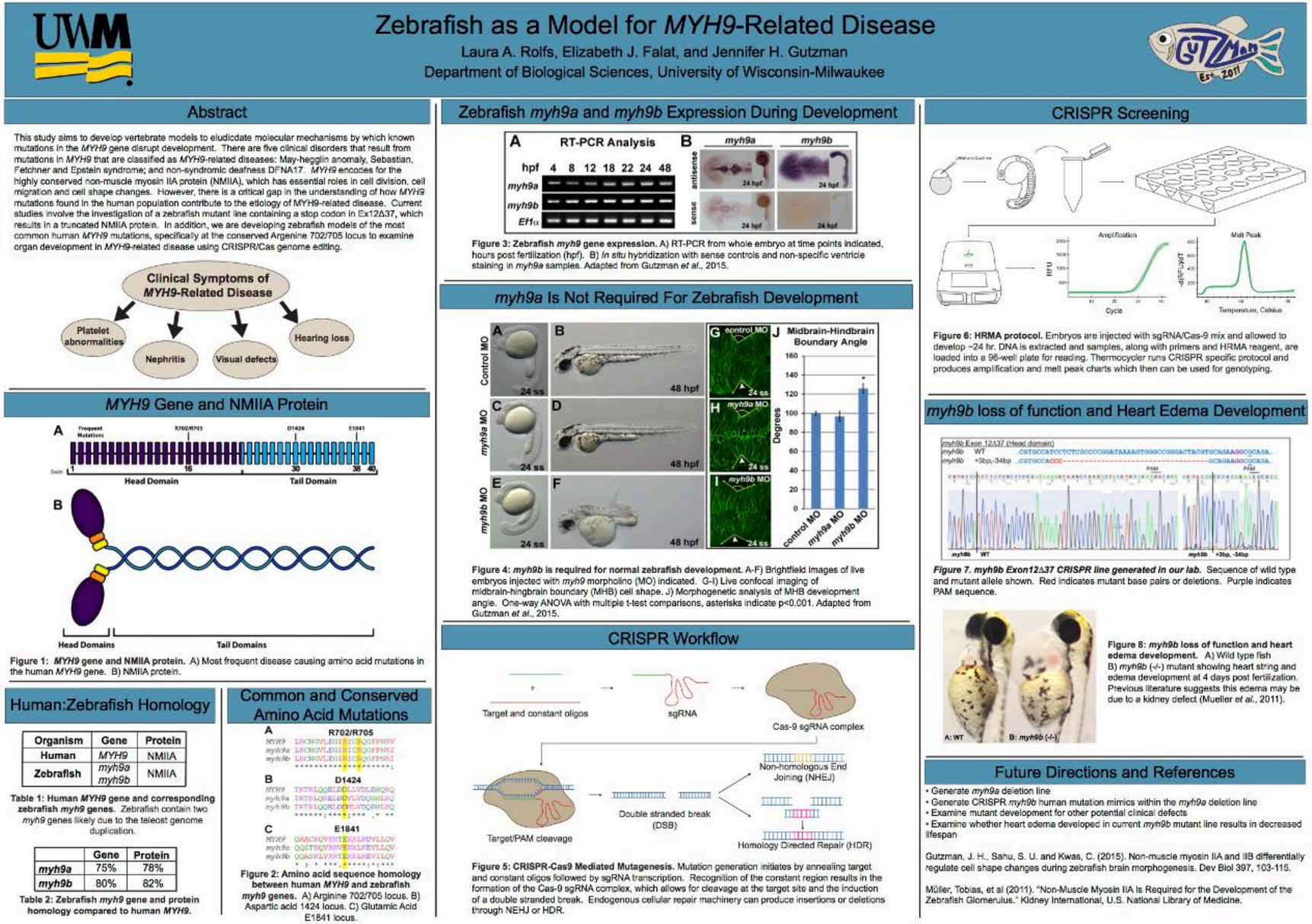
Examples of better posters

What do you think about this poster?



Examples of good posters

What do you think about this poster?



Zebrafish as a Model for *MYH9*-Related Disease

Laura A. Rolfs, Elizabeth J. Falat, and Jennifer H. Gutzman
Department of Biological Sciences, University of Wisconsin-Milwaukee

Abstract

This study aims to develop vertebrate models to eludicdate molecular mechanisms by which known mutations in the *MYH9* gene disrupt development. There are five clinical disorders that result from mutations in *MYH9* that are classified as *MYH9*-related diseases: May-hegglin anomaly, Sebastian, Fetchner and Epstein syndrome; and non-syndromic deafness DFNA17. *MYH9* encodes for the highly conserved non-muscle myosin IIA protein (NMIIA), which has essential roles in cell division, cell migration and cell shape changes. However, there is a critical gap in the understanding of how *MYH9* mutations found in the human population contribute to the etiology of MYH9-related disease. Current studies involve the investigation of a zebrafish mutant line containing a stop codon in Ex12Δ37, which results in a truncated NMIIA protein. In addition, we are developing zebrafish models of the most common human *MYH9* mutations, specifically at the conserved Argenine 702/705 locus to examine organ development in *MYH9*-related disease using CRISPR/Cas genome editing.

MYH9 Gene and NMIIA Protein

Figure 1: *MYH9* gene and NMIIA protein. A) Most frequent disease causing amino acid mutations in the human *MYH9* gene. B) NMIIA protein.

Human:Zebrafish Homology

Organism	Gene	Protein
Human	*MYH9*	NMIIA
Zebrafish	*myh9a* *myh9b*	NMIIA

Table 1: Human *MYH9* gene and corresponding zebrafish *myh9* genes. Zebrafish contain two *myh9* genes likely due to the teleost genome duplication.

	Gene	Protein
myh9a	75%	78%
myh9b	80%	82%

Table 2: Zebrafish *myh9* gene and protein homology compared to human *MYH9*.

Common and Conserved Amino Acid Mutations

Figure 2: Amino acid sequence homology between human *MYH9* and zebrafish *myh9* genes. A) Arginine 702/705 locus. B) Aspartic acid 1424 locus. C) Glutamic Acid E1841 locus.

Zebrafish *myh9a* and *myh9b* Expression During Development

Figure 3: Zebrafish *myh9* gene expression. A) RT-PCR from whole embryo at time points indicated, hours post fertilization (hpf). B) *In situ* hybridization with sense controls and non-specific ventricle staining in *myh9a* samples. Adapted from Gutzman *et al.*, 2015.

myh9a Is Not Required For Zebrafish Development

Figure 4: *myh9b* is required for normal zebrafish development. A-F) Brightfield images of live embryos injected with *myh9* morpholino (MO) indicated. G-I) Live confocal imaging of midbrain-hingbrain boundary (MHB) cell shape. J) Morphogenetic analysis of MHB development angle. One-way ANOVA with multiple t-test comparisons, asterisks indicate p<0.001. Adapted from Gutzman *et al.*, 2015.

CRISPR Workflow

Figure 5: CRISPR-Cas9 Mediated Mutagenesis. Mutation generation initiates by annealing target and constant oligos followed by sgRNA transcription. Recognition of the constant region results in the formation of the Cas-9 sgRNA complex, which allows for cleavage at the target site and the induction of a double stranded break. Endogenous cellular repair machinery can produce insertions or deletions through NEHJ or HDR.

CRISPR Screening

Figure 6: HRMA protocol. Embryos are injected with sgRNA/Cas-9 mix and allowed to develop ~24 hr. DNA is extracted and samples, along with primers and HRMA reagent, are loaded into a 96-well plate for reading. Thermocycler runs CRISPR specific protocol and produces amplification and melt peak charts which then can be used for genotyping.

myh9b loss of function and Heart Edema Development

Figure 7. myh9b Exon12Δ37 CRISPR line generated in our lab. Sequence of wild type and mutant allele shown. Red indicates mutant base pairs or deletions. Purple indicates PAM sequence.

Figure 8: *myh9b* loss of function and heart edema development. A) Wild type fish B) *myh9b* (-/-) mutant showing heart string and edema development at 4 days post fertilization. Previous literature suggests this edema may be due to a kidney defect (Mueller *et al.*, 2011).

Future Directions and References

- Generate *myh9a* deletion line
- Generate CRISPR *myh9b* human mutation mimics within the *myh9a* deletion line
- Examine mutant development for other potential clinical defects
- Examine whether heart edema developed in current *myh9b* mutant line results in decreased lifespan

Gutzman, J. H., Sahu, S. U. and Kwas, C. (2015). Non-muscle myosin IIA and IIB differentially regulate cell shape changes during zebrafish brain morphogenesis. Dev Biol 397, 103-115.

Müller, Tobias, et al (2011). "Non-Muscle Myosin IIA Is Required for the Development of the Zebrafish Glomerulus." Kidney International, U.S. National Library of Medicine.

Examples of good posters

What do you think about this poster?

Negative regulation of the protein kinase Raf

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University of Wisconsin-Milwaukee Department of Biological Sciences
*presenting author

Abstract

Activating mutations in the Ser/Thr protein kinase Raf are common in cancers, especially melanomas. Raf is activated by the EGF Receptor and Ras, and signaling transduction occurs through activation of a kinase cascade involving Raf, MEK, and ERK. However, relatively little is known about cellular regulators that inhibit signaling by activated Raf. Using the nematode *Caenorhabditis elegans*, we performed a genetic screen to identify candidate negative regulators of Raf. In wild-type *C. elegans*, Raf(LIN-45) protein degradation is triggered by activation of the downstream kinase ERK in an epithelial cell type called vulval precursor cells (VPCs). LIN-45 degradation is mediated by the E3 Ubiquitin ligase SEL-10 and requires a conserved Cdc4-phosphodegron (CPD) sequence located in Raf protein. In our genetic screen, we identified a mutation, *cov19*, that causes stabilization of LIN-45 protein in VPCs. We will present evidence that *cov19* is a loss-of-function mutation in *ufd-2*, which encodes a conserved ubiquitination factor UFD-2/UBE4B. We find that UFD-2 acts cell-autonomously to promote LIN-45 protein degradation. However, unlike SEL-10, UFD-2 may not regulate LIN-45 protein via the CPD motif. Because UFD-2 is known to act in a chaperone-mediated cytoplasmic unfolded protein response, we are now using a candidate mutant approach to determine whether LIN-45 is regulated by this mechanism. Finaly, we find that loss of *ufd-2* enhances phenotypes caused by a mutant, activated form of LIN-45, strongly suggesting that a UFD-2-mediated mechanism may be important to inhibit mutant forms of Raf found in human cancers.

Introduction

- In *C. elegans*, vulva formation is controlled by the Ras-Raf-MEK-ERK kinase cascade in vulval precursor cells (VPCs)

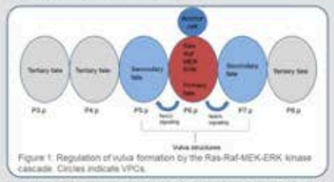

Figure 1. Regulation of vulva formation by the Ras-Raf-MEK-ERK kinase cascade. Circles indicate VPCs.

- In wild type, the *C. elegans* Raf ortholog LIN-45 is degraded in P6.p and not degraded in P5.p and P7.p during the L3 stage
- In a mutant lacking SEL-10 (a conserved E3 ubiquitin ligase), the LIN-45 protein is stabilized (de la Cova and Greenwald 2012)

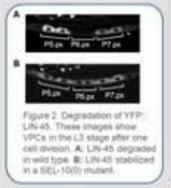

Figure 2. Degradation of YFP::LIN-45. These images show VPCs in the L3 stage after one cell division. **A**: LIN-45 degraded in wild type. **B**: LIN-45 stabilized in a SEL-10(0) mutant.

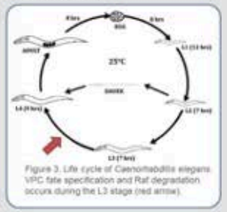

Figure 3. Life cycle of *Caenorhabditis elegans*. VPC fate specification and Raf degradation occurs during the L3 stage (red arrow).

Methodology

Mutagenesis screening

- We generated a transgenic *C. elegans* strain which expresses an activated mutant form of the *C. elegans* Raf ortholog LIN-45(V627E)
- The activated LIN-45(V627E) mutation results in overactivation of the ERK signaling pathway. In humans, this can result in cancer. In *C. elegans*, it can cause ectopic ERK activation and the multivulva (Muv) phenotype
- We performed a mutagenesis screen on the LIN-45(V627E) strain
- We screened for mutants with enhancement of the multivulva phenotype, an indicator of highly activated Raf

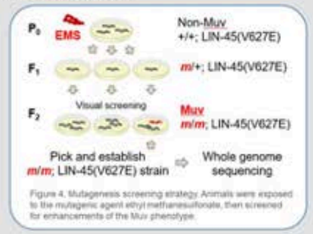

Figure 4. Mutagenesis screening strategy. Animals were exposed to the mutagenic agent ethyl methanesulfonate, then screened for enhancements of the Muv phenotype.

Variant mapping

- One of the variants identified was mapped to chromosome II using a SNP mapping technique with a polymorphic strain (Doitsidou, Jamault, and Poole 2016)
- Within this region, we found a premature stop in E3/E4 ubiquitin ligase *ufd-2*. We named this allele *cov19*.
- We also acquired an independent deletion allele of *ufd-2* from the Caenorhabditis Genome Center named *tm1380*

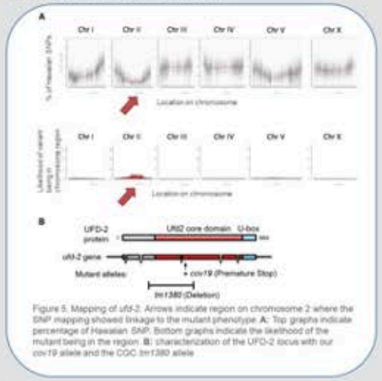

Figure 5. Mapping of *ufd-2*. Arrows indicate region on chromosome 2 where the SNP mapping showed linkage to the mutant phenotype. **A**: Top graphs indicate percentage of Hawaiian SNP. Bottom graphs indicate the likelihood of the mutant being in the region. **B**: characterization of the UFD-2 locus with our *cov19* allele and the CGC *tm1380* allele

Results

ufd-2 is required for degradation of the Raf ortholog LIN-45

- In wild type, LIN-45 protein is degraded in P6.p post-transcriptionally (de la Cova and Greenwald 2012)
- Loss of function of *ufd-2* results in stabilization of LIN-45 protein in P6.p
- The deletion allele and our premature stop mutant both stabilized LIN-45
- We generated a transgenic strain that expresses UFD-2(+) specifically in VPCs
- UFD-2(+) rescues the *ufd-2(tm1380)* mutant

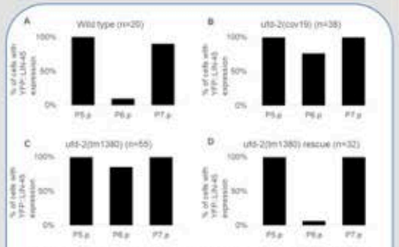

Figure 6. *ufd-2* is required for LIN-45 degradation. Data shown is the percentage of cells that are positive for YFP::LIN-45. **A**: wild-type control where LIN-45 protein is degraded in P6.p **B**: *ufd-2(cov19)* premature stop mutant. **C**: *ufd-2(tm1380)* deletion allele. **D**: *ufd-2(tm1380)* with rescuing transgene.

LIN-45 degradation does not require CHN-1/CHIP

- The role of CHN-1/CHIP, a protein known to interact with UFD-2 in the degradation of unfolded myosin chains, was investigated.
- We hypothesized that CHN-1 might be required for LIN-45 protein degradation

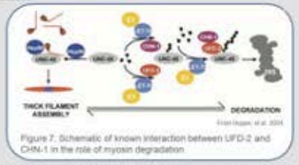

Figure 7. Schematic of known interaction between UFD-2 and CHN-1 in the role of myosin degradation

- The deletion allele *chn-1(by155)* did not result in increased stabilization of LIN-45
- A double mutant of *chn-1(by155)* and *ufd-2(tm1380)* did not significantly increase the frequency at which LIN-45 is stabilized (p=0.2589, Fisher's exact test)

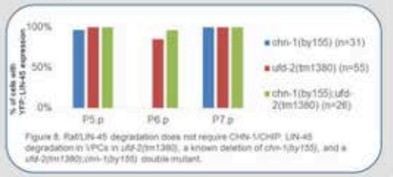

Figure 8. Raf/LIN-45 degradation does not require CHN-1/CHIP. LIN-45 degradation in VPCs in *ufd-2(tm1380)*, a known deletion of *chn-1(by155)*, and a *ufd-2(tm1380);chn-1(by155)* double mutant.

Conclusion

- *ufd-2* is required for Raf/LIN-45 degradation
- Loss of function of *ufd-2* results in stabilization of Raf/LIN-45 protein in P6.p
- Loss of function of *chn-1* does not result in increased Raf/LIN-45 stabilization in P6.p

Future directions

- We will perform a deletion analysis within the Raf/LIN-45 protein to determine where *ufd-2* interacts with Raf.
- We will test whether UFD-2 requires the same degron sequence that is required by SEL-10
- We will obtain mutants in other pathways known to degrade proteins. These include chaperone-mediated autophagy, general autophagy, selective autophagy

References

The C. elegans Deletion Mutant Consortium. 2012. **Large-Scale Screening for Targeted Knockouts in the Caenorhabditis elegans Genome** G3: Genes, Genomes, Genetics November 1, 2012 vol. 2 no. 11 1415-1425; https://doi.org/10.1534/g3.112.003830

de la Cova C., and Greenwald I., 2012. **SEL-10/Fbw7-dependent negative feedback regulation of LIN-45/Braf signaling in C. elegans via a conserved phosphodegron.** Genes Dev. 26: 2524–2535. 10.1101/gad.203703.112

Doitsidou M., Jamault S., and Poole R.J. 2016 **Next-Generation Sequencing-Based Approaches for Mutation Mapping and Identification in Caenorhabditis elegans** GENETICS *October 1, 2016 vol. 204 no. 2 451-474 https://doi.org/10.1534/genetics.115.186197*

Hoppe T., Cassata G., Barral J. M., Springer W., Hutagalung A. H., Epstein H. F., & Baumeister R. 2004. **Regulation of the Myosin-Directed Chaperone UNC-45 by a Novel E3/E4-Multiubiquitylation Complex in C. elegans.** Cell, 118(3), 337–349. https://doi.org/10.1016/j.cell.2004.07.014

Sternberg, P.W., 2005. **Vulval development.** WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.6.1, http://www.wormbook.org.

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Abstract (max 200 words)

Introduction/Research question

Background knowledge in the field

What is the main goal of the research and the research approach.

Can be in bullet points.

Methods/Approach

basis of techniques used, keep it short;.

Results

Show examples of key experiments.

3-4 figures.

Figure legends with specifications of type of experiment, samples etc..

Results

Show examples of key experiments.

Conclusion / Significance

Summarize the research findings/ future perspectives/ impact/significance

Main takeaway messages

Bullet points

In a nutshell (graphical model)

Acknowledgements

Grant funding, research programs, laboratory, mentors

Laboratorio/Istituto ospitante.
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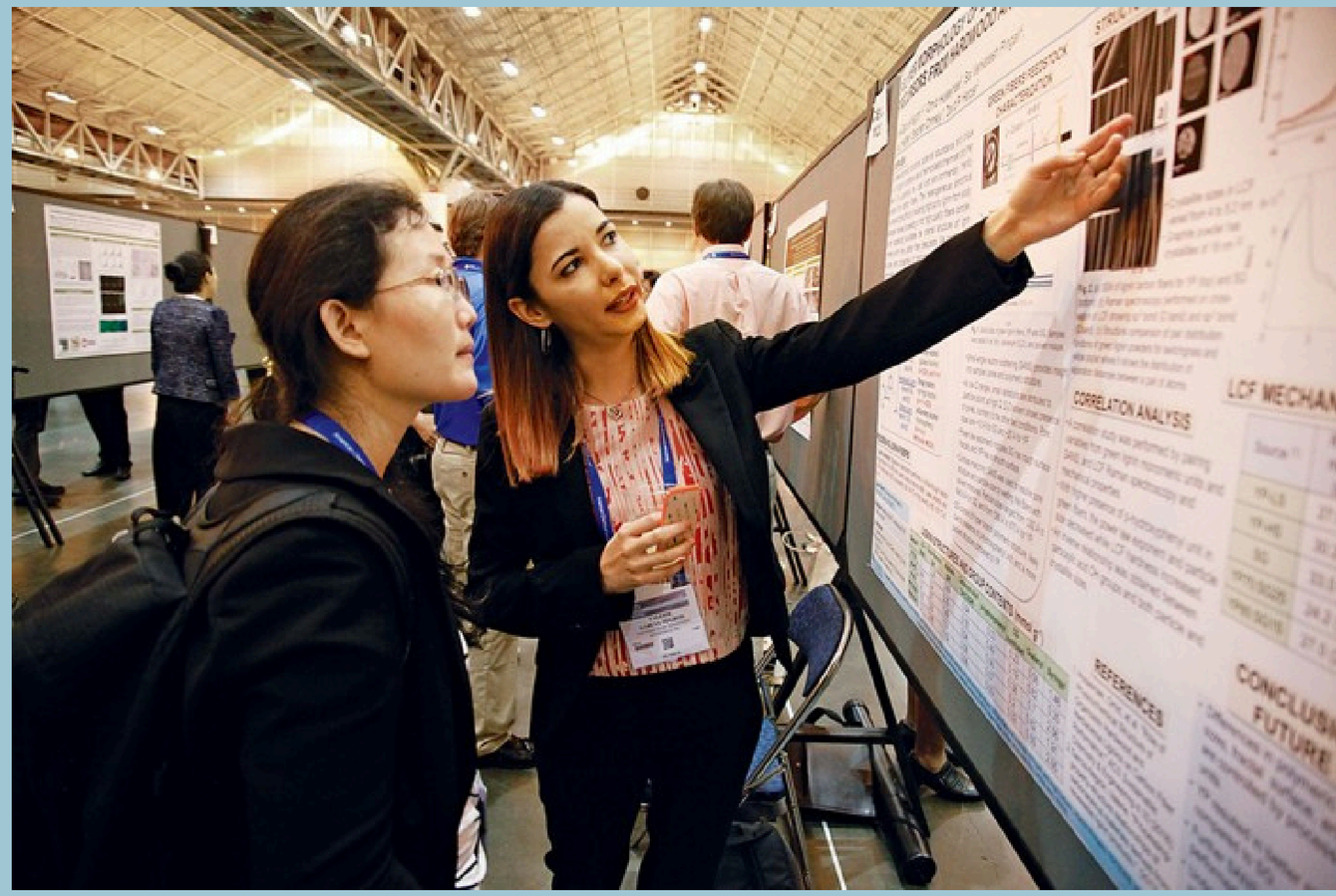
06. Presenting your research



Knowledge

01

Know what to say

for each figure and **transitions** between sections

02

Know all the details of your research

03

Practice for your audience

Walk people through your poster in about 1 min; 5 min; 10 min

04

Master questions (specific and open ended)

Tell me about your research; how does this relate to findings in the field; what are the implications for the future

Practice

Be enthusiastic about your work

Start a conversation asking questions

Resources

- **Posternerd: https://www.posternerd.com/sciposters-templates**

- **Powerpoint**

- **Google slides https://slidesgo.com/search?q=academic+poster#rs=search**
- **https://www.biorender.com/poster-builder**

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- **https://inkscape.org/**
- **Adobe Illustrator**

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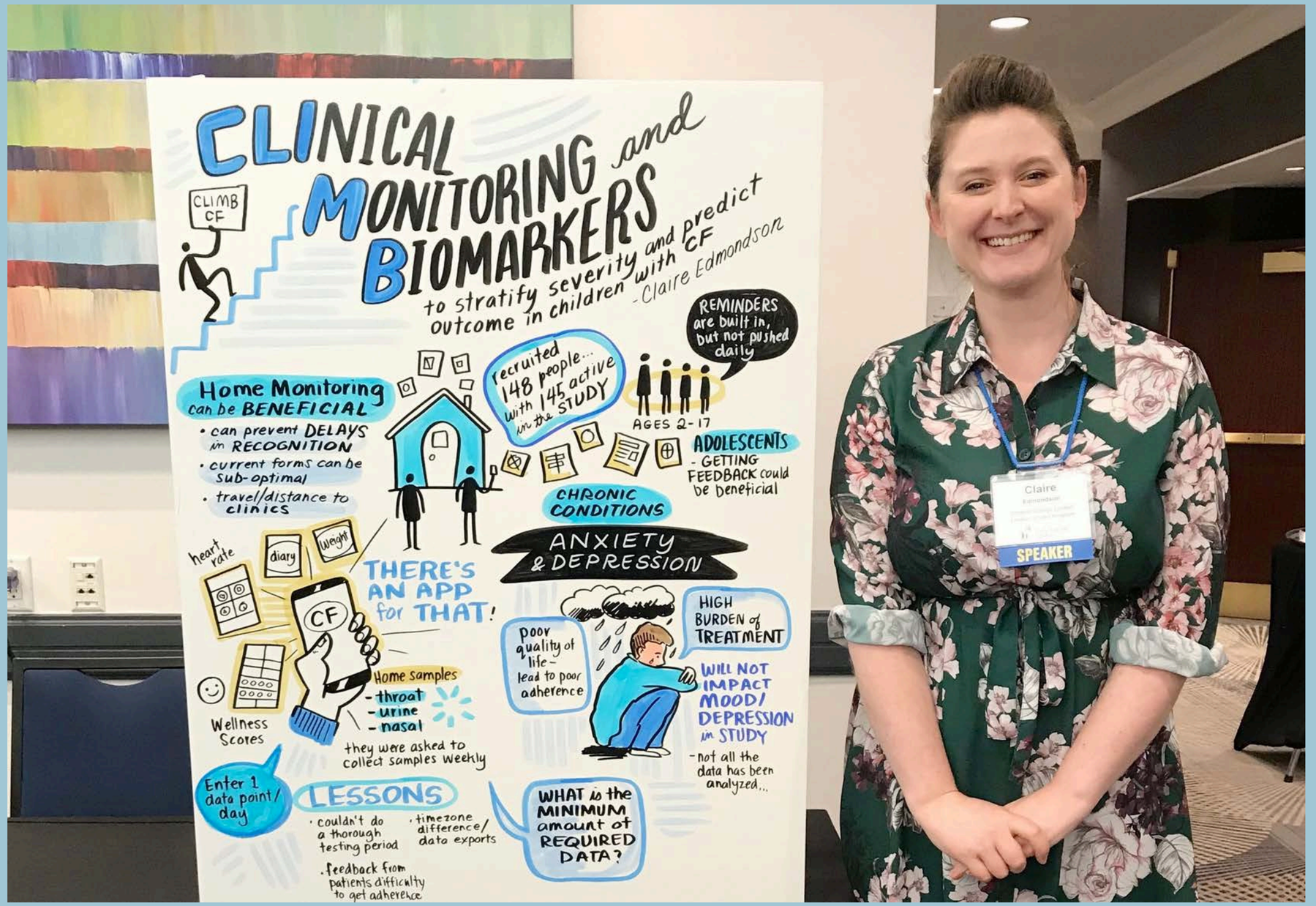
Thank you!

Questions?


CLINICAL MONITORING and BIOMARKERS
to stratify severity and predict outcome in children with CF
-Claire Edmondson
CLIMB CF
Home Monitoring can be BENEFICIAL
• can prevent DELAYS in RECOGNITION
• current forms can be sub-optimal
• travel/distance to clinics
recruited 148 people... with 145 active in the STUDY
REMINDERS are built in, but not pushed daily
AGES 2-17
ADOLESCENTS
- GETTING FEEDBACK could be beneficial
CHRONIC CONDITIONS
ANXIETY & DEPRESSION
heart rate
diary
weight
CF
THERE'S AN APP for THAT!
Wellness Scores
Home samples
- throat
- urine
- nasal
they were asked to collect samples weekly
poor quality of life - lead to poor adherence
HIGH BURDEN of TREATMENT
WILL NOT IMPACT MOOD/ DEPRESSION in STUDY
- not all the data has been analyzed...
Enter 1 data point/ day
LESSONS
• couldn't do a thorough testing period
• timezone difference/ data exports
• feedback from patients difficulty to get adherence
WHAT is the MINIMUM amount of REQUIRED DATA?
Claire
SPEAKER