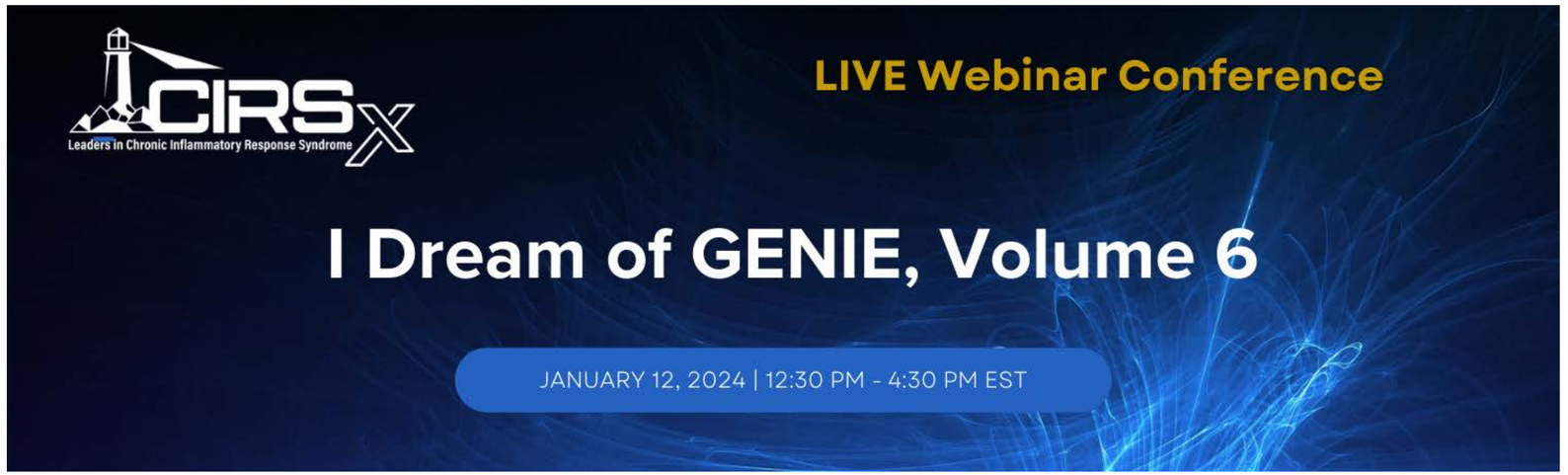

Unlocking the power of GENIE

Integrating an mRNA Expression test into your CIRS practice

IDOG Vol 6
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Louise Carder, BSc Nutr. Med., Pg Dip., mNNA, CNHC Reg., IFMCP, mBSEM, mNAP

Disclosure

- Director of Colab Services Ltd, based in the UK but works globally
- Owner of LCNH Ltd Clinical Practice also in the UK

About

Louise Carder, Registered Nutritional Therapist, UK

- BA (Hons), BSc Nutritional Medicine, PgDip. (Masters Course starts late 2024)
- CNHC Reg. mNNA (previous national Director of BANT & inaugural Regulatory Committee Chair & Head of Communications)
- mBSEM, mNAP, IFMCP and fellow of the Royal Society of Medicine
- Bredesen Trained Practitioner 2017
- First Shoemaker Certified practitioner in Europe and the first Nutrition Professional to be Certified (2018)
- Co-author of one published book
- Leads a European CIRS Working Group and provides European training on CIRS in association with Dr Shoemaker & Surviving Mold Team and Dr McMahon/Dr Dorninger

Overview

Part 1:

- The need and value of testing
- Clinical integration and survey responses

Part 2- mRNA testing in more detail

- What are we considering when we do mRNA testing
- How I approach mRNA Gene expression testing:

1. CIRS Pattern Assessment
2. Possible Triggers/mapping this relating to the CIRS pattern/triggers
3. Effects of the CIRS picture
4. Underlying Consideration: Specialty
5. Next Steps e.g. treatment

- Returning to consider the differential diagnosis
- Closing statements

“*If you don’t know the transcriptomics, you don’t know the illness*”
Dr R Shoemaker

GENIE: GENE EXPRESSION INFLAMMATION EXPLAINED

1) Metabolism
Ratio for metabolic gene families compared to normal controls. 1 equals control value.

Absolute IF	ATP synth	COX	Mito Large	Mito Small	mTranslocase
0.73	0.67	0.74	0.77	0.78	0.75

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The system that controls circulating blood sugar as well as sugar entry into the cell including binding proteins, receptors and growth factors.

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1.24	-0.27	-0.17	-0.52	1.78

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Can be triggered by mild cellular injury and by various factors internal or external to the cell; the damaged cells are then disposed of in an orderly fashion.

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FOXO3	MAP3K5	MAPK9	RIPK1
-0.34	-0.28	-1.37	-1.73

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Also known as clotting is the process by which blood changes from a liquid to a gel forming a blood clot. It potentially results in hemostasis.

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PF4	SELP	THBS1	TREML1
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Signaling molecules that direct immune function.

CCL5	IL1B	JAK1	NFKB1	RELA	SOCS1
-0.43	-0.6	0.87	1.84	2.31	0.18

TGFB1	TGFBR1	TGFBR2	TGFBR3	TNF
1.97	-0.47	-0.27	-0.91	-0.22

6) Lyme
These genes were found to be changed in patients with acute and post antibiotics Lyme disease.

CD40LG	EIF4g2	IL1B	NFKB1	RELA	TRIM31
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TICAM1	TNF
-0.75	-1.03

7) GZMS/DEF
Granzymes are proteases used by NK and Cytotoxic T cells to destroy unhealthy cells. Defensins are antimicrobial peptides.

DEFA1B	DEFA4	GZMA	GZMB	GZMK	GZMM
-0.92	-1.06	-0.81	0.63	-0.44	1.2

8) Ikaros
A family of transcription factors important for lymphocyte proliferation and senescence.

IKZF1	IKZF2	IKZF3	VIPR1
0.31	-0.1	0.3	0.35

9) MAP Kinase
MAPKs are involved in directing cellular responses to a diverse array of stimuli.

MAP2K3	MAP3K5	MAPK1	MAPK14	MAPK3	MAPK9
0.19	-1.54	-0.72	-1.54	-1.25	-0.21

10) Toll Receptors
Different TLRs recognize different surface and intracellular components of microorganisms and are important in innate defense.

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Most often associated with antibody production.

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Most often associated with cell to cell combat and immunosynapse with APCs.

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-0.76	-0.98

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Genes important to CIRS typically found upregulated

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NEAT1	RPS10-NUDT3	THBS1	TREML1	TUBB1	YLPM1
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SETPIN	UQCRQ
1	-1.3

15) PTSD
Post Traumatic Stress Disorder

FKBP5
-0.5

16) Histamine
Inlflammatory vasodilator

CCL5	HDC
2.2	-0.2

17) Cytoskeleton
Interlinking protein filaments that support cellular structure.

TUBA4A	TUBB1
1.5	1.4

18) Treg
Regulatory T cell membrane receptors

CD127	CD25	CD4
0.7	-1.4	1

19) Dispersion
Variance of normalization. Scores below 1 are best.

Dispersion
0.95

Red= up-regulated
Blue= down-regulated
White= normal range

Part 1

The Value of Testing:

“ 70% of clinical decisions rely on laboratory testing”

Hallmark, Mike J. The 70% claim: what’s the evidence base? Ann Clin. Biochem 2011; 48:487-8

Rohr UP, Binder C, Dieterle T, Giusti F, Messina CG, Toerien E, et al. The Value of In Vitro Diagnostic Testing in Medical Practice: A Status Report. PLoS One 2016;11:e0149856

Sikaris KA Enhancing the Clinical Value of Medical Laboratory Testing. David Curnow Plenary Lecture, Australian Association of Clinical Biochemists Annual Scientific Meeting 2016 accessed https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5759162/pdf/cbr-38-107.pdf

Part 1

The value of testing

“ The original data from the Mayo Clinic stated that the relative amount of data on the Mayo Electronic Result Enquiry System was: pathology: 94%, radiology: 3%, **patient data: 1%**, electrocardiogram: 1% and surgery: 1%”

Hallmark, Mike J. The 70% claim: what’s the evidence base? Ann Clin. Biochem 2011; 48:487-8

Part 1

The Value of Testing:

A recent study on Chat GPT by Mass General Brigham has shown that overall Chat GPT was 72% accurate and in making a final diagnosis it was 77% accurate. However, it was lowest, at 60% in accurately making differential diagnoses and 68% accurate in clinical management decisions e.g. treatment path.

Rao A, Pang M, Kim J, Kamineni M, Lie W, Prasad AK, Landman A, Dreyer K, Succi MD
Assessing the Utility of ChatGPT Throughout the Entire Clinical Workflow: Development and Usability Study
J Med Internet Res 2023;25:e48659

How do we explain value of testing to our clients?

‘*Disease prevention, early detection? Accurate diagnosis, treatment selection, minimising delays in treatment, supporting recovery, reducing disability, prevent relapse, slowing disease progression, reducing long term care needs*?’

Relating to mRNA testing, we can learn: staging information, clarity on MHM, support CIRS diagnosis (or not), clues about possible trigger(s)/differential diagnosis, how to map the fall-out across multi-systems that includes mapping an association with other tests e.g. NQ

The Value of Laboratory Medicine to Health Care. Chapter 1. In: *The Lewin Group: Laboratory Medicine – A National Status Report*. May 2008:19–65.

How do we explain value of testing to our CIRS clients?

- US GAO “Point 3 “There must be laboratory testing results similar to those seen in peer- reviewed, published studies”

  We have a Diagnostic Criteria for CIRS- if we don’t do some testing, are we just guessing?

  Opinion 1: Sx cluster+VCS deficits- 98.5% indicative of CIRS- good enough for being clear on likelihood of exposure and step 1 being required

  Opinion 2: Testing allows for a more personalised approach that can facilitate the necessary detail for a legal case and more formal diagnosis, as well as greater clarity to individualise a therapeutic protocol/treatment direction

“GAO Report to the Chairman, Committee on Health, Education, Labor and Pensions, U.S. Senate, Indoor Mold” (September 2008). http://www.gao.gov/ new.items/d08980.pdf.

Integrating mRNA testing into your practice

Practitioner	Patient
Feeling ready to start using it? Tech Support call before start using it? Education options?	What is the value of this? How will it specifically help me? Research conversation
Which patients?	Practicalities of having it done e.g. draw etc
How to integrate with existing test roster? and managing re-testing?	Results process/Managed expectations
Results process: time to review/get support/ creating own crib sheet	Results Appointment/Dr S call listen in?
Actions from the result: further testing/tx programme creation	Further tests/Start of Protocol

Survey Responses

16 Healthcare Practitioner Responses

Used mRNA testing? Yes/No	16 replied yes	1 replied only tested self	8 replied tested only patients	7 replied tested both self/patients	
Key reasons for use	To guide next steps generally, especially for those who are complex/ stuck	Precision knowledge/adding to evidence base	To add to exposure /etiology assessment	Tracking of progress/staging	Ease of access
	Assess key areas of gene expression eg. VIP/ histamine considerations	e.g. supporting personalised care decisions	e.g. determine if patient is still in exposure/re-	e.g. CIRS Staging	e.g. cheaper than other CIRS labs
	e.g. MARCoNS tx	e.g. supporting priorities	e.g. specifics of exposure e.g. actinos/ mycotoxins.endos	e.g. for tracking with other key tests e.g. NQ	e.g. easier to get an mRNA draw done
	e.g. PTSD onward referrals	e.g. if not done previously by another practitioner	e.g. Lyme		e.g. something a client can do themselves

Additional Survey Comments

1. Tests are expensive, so understanding them and being able to explain their value is important for both practitioner and patient. But sometimes they are still out of budget so simply cannot be accessed. Concern also that GENIE may lead to further testing e.g. actinos so cascade effect of cost comes into play for some.

2. General feeling of not knowing enough and a desire for more training/support. Much information on mRNA gene expression testing is not taught in current centralised practitioner education pathways so Shoemaker group is the only place to get this information, unless self-taught, which brings its own insecurity around ‘getting it right’.

Confidence

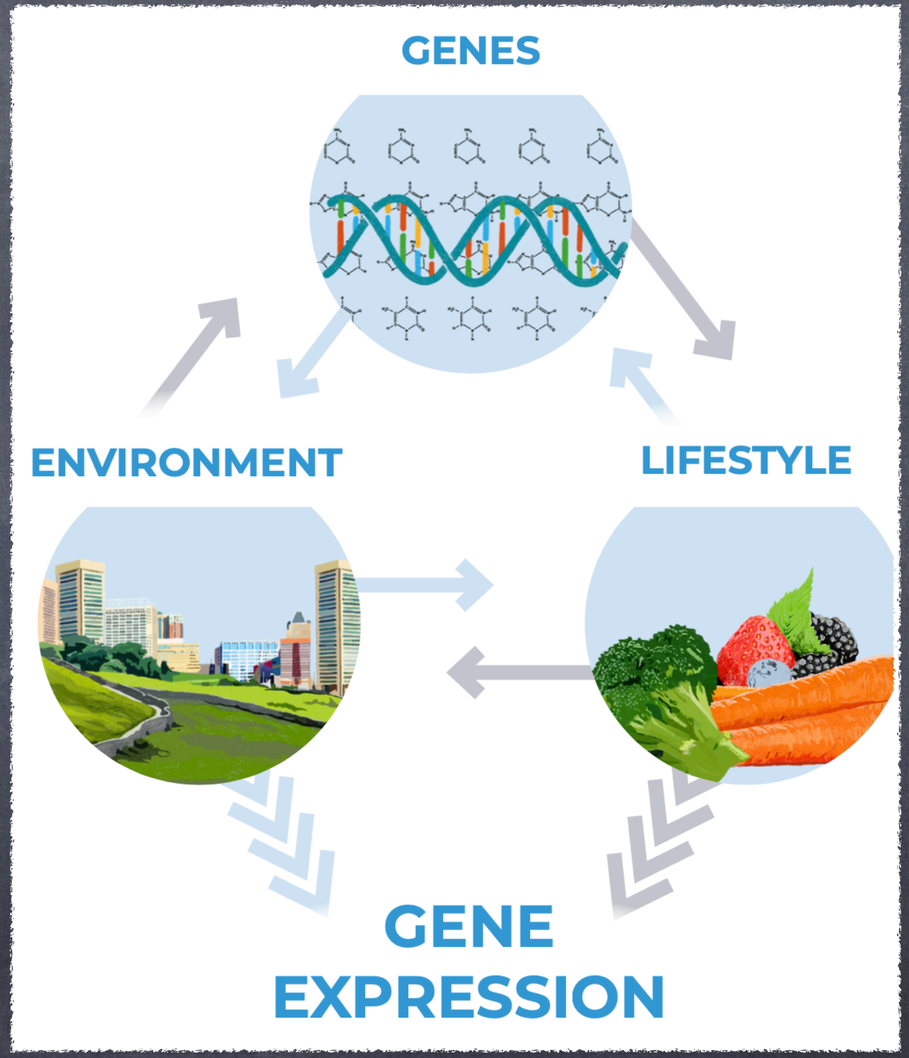
Ultimately it all comes down to this once value has been established

Practitioner:
Do you have confidence in the test and in your ability to work with it?

Patient:
Does your patient have confidence the test could deliver information to help guide their care?

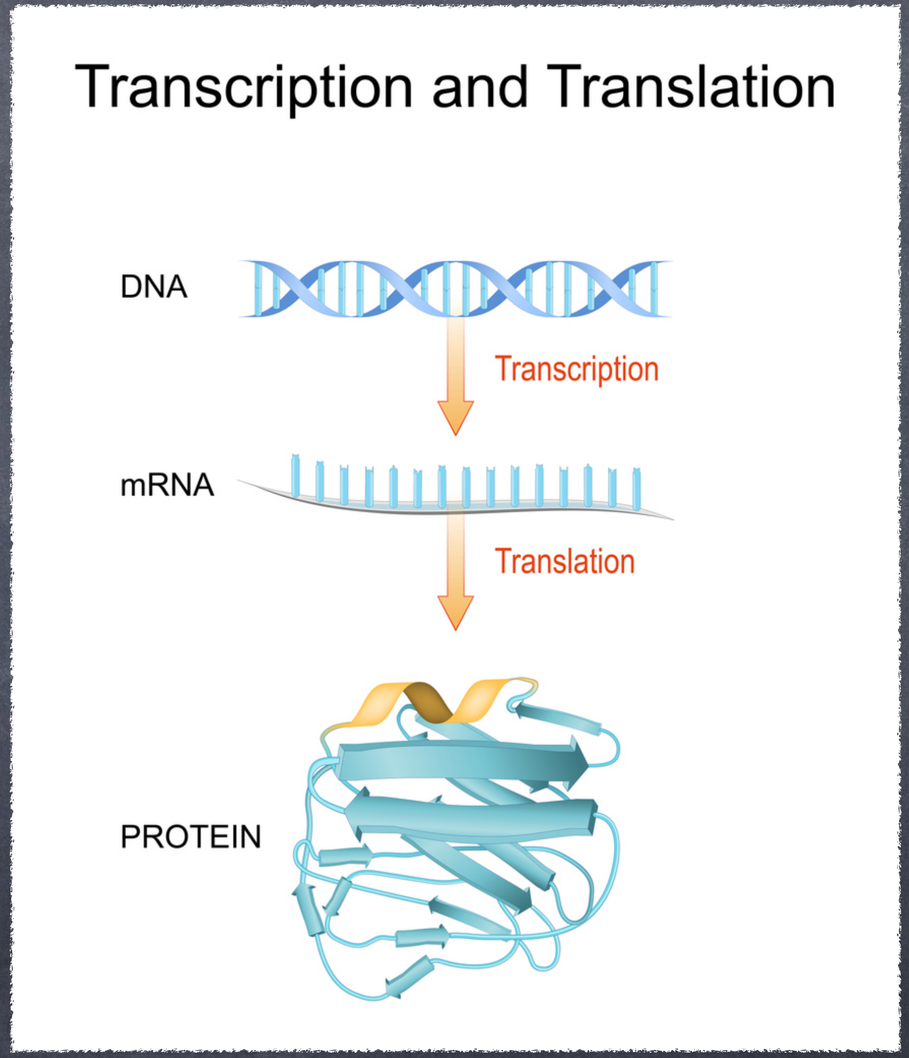
Genes, Environment & Lifestyle

(Genes are not entirely fixed)



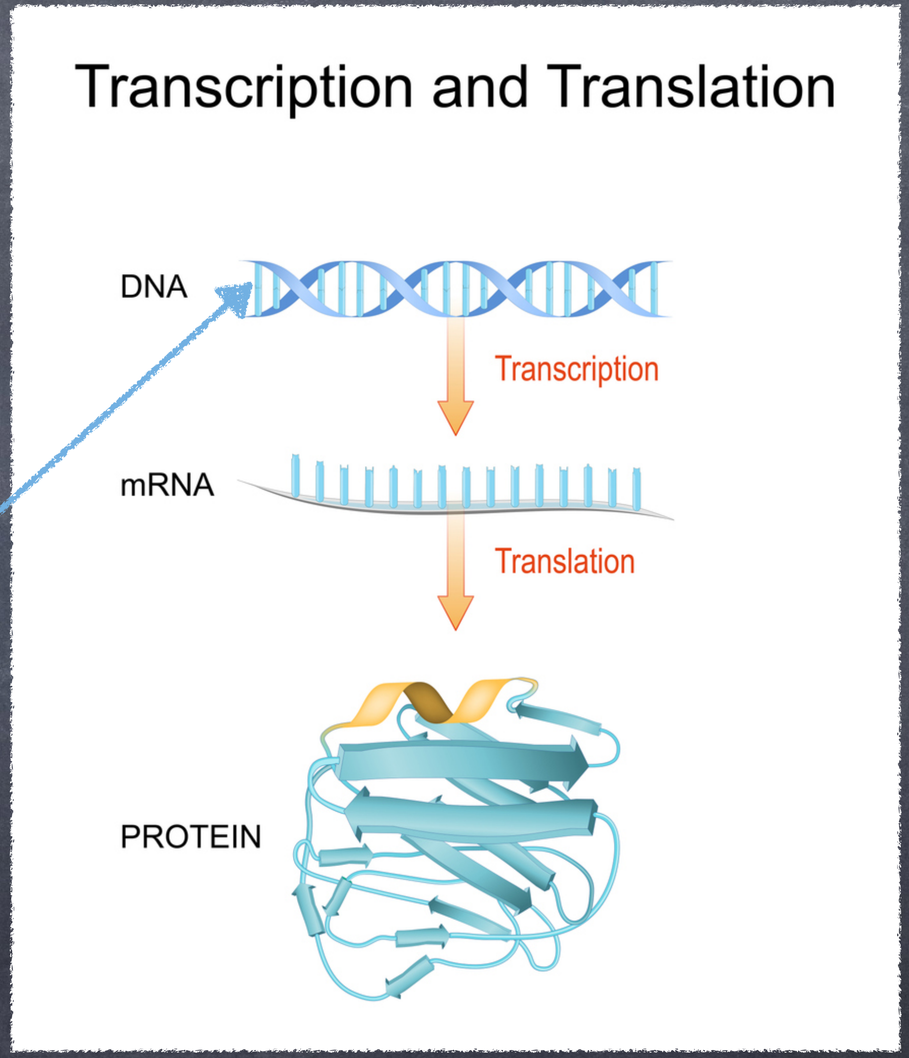
Genes/SNPs

Genes are part of the coding of our DNA; we have variants in our DNA that make us who we are



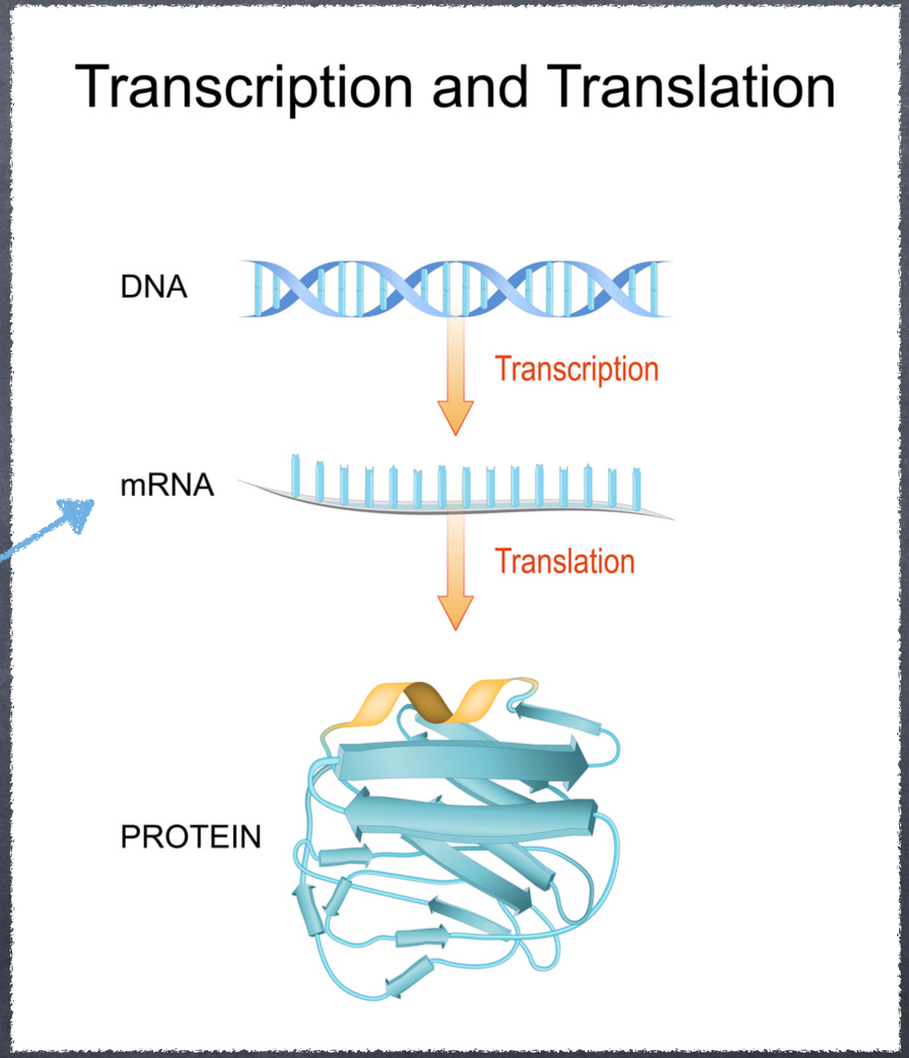
Genes

Our DNA bathes in the nucleus of our cells, so whatever our genes bathe in changes how they express... ie how active or not they are



Transcription to mRNA

DNA information converts through transcription to messenger RNA.... this is then converted into proteins that help repair and replicate in our bodies to keep us functioning



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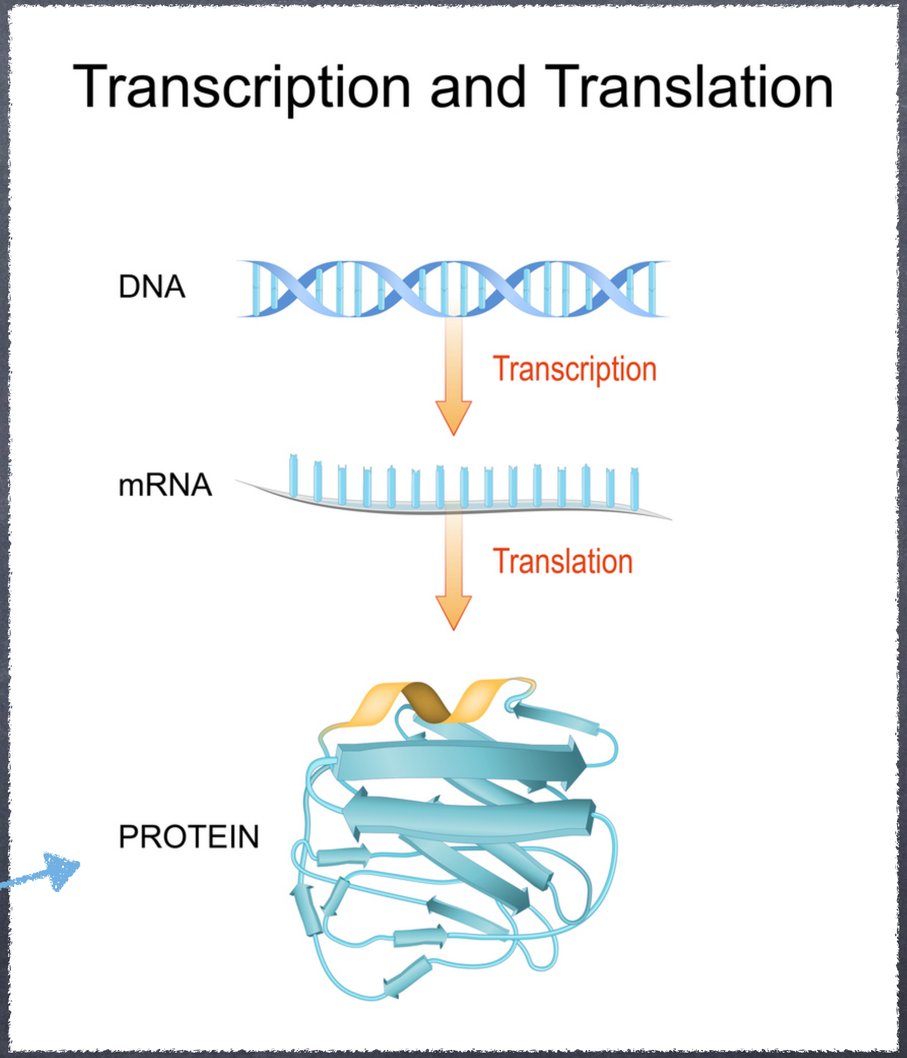
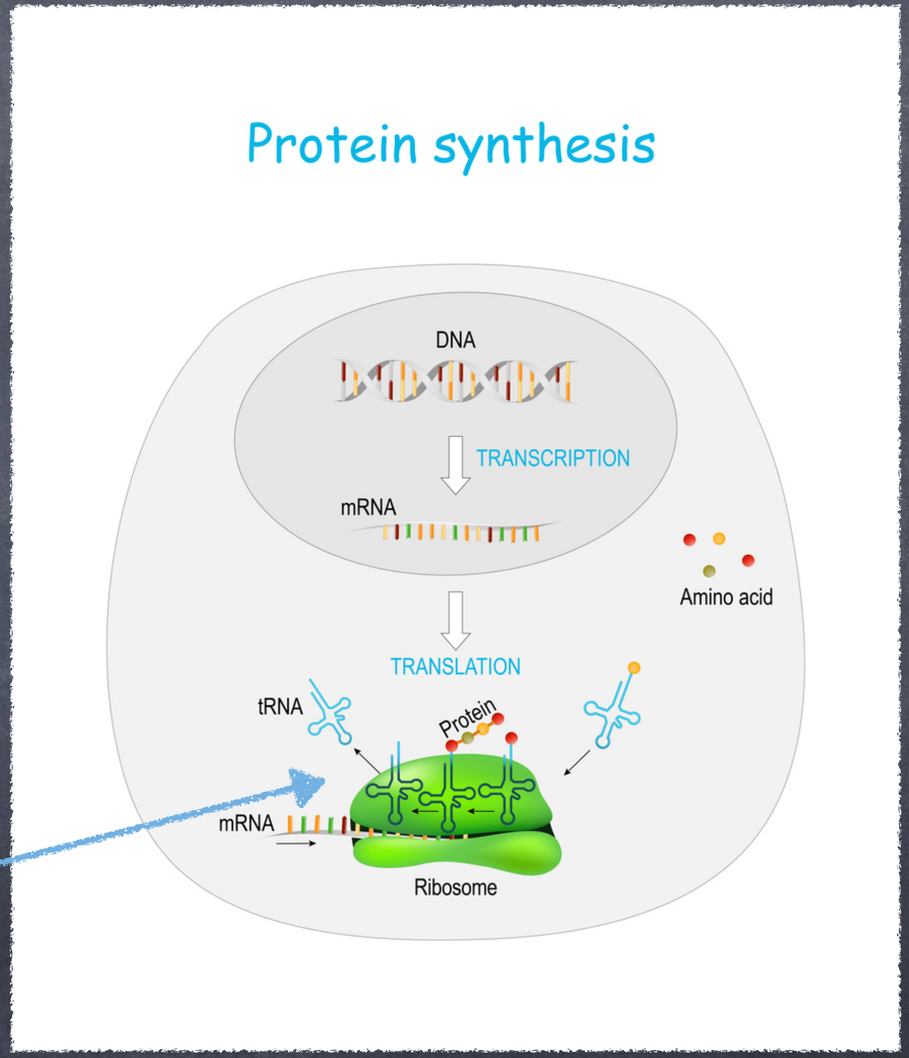


Gene SNPs

How mRNA genes are expressing as they are going through the transcription process could tell us so much about how our bodies are functioning....., if only we could learn more about what messages are being sent out by mRNA..... and how well Ribosomes are functioning (or not)....and understand how that might in turn affect proteins....



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Variance of normalization. Scores below 1 are best.

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Red= up-regulated
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White= normal range

How I approach mRNA testing

1. CIRS Assessment: Sections 1,12,13/14, 19

2. Possible Triggers: Sections 1, 3, 5, 6, 9, 10, 11, 16

3. Effects of the CIRS picture: Section 2, 3, 4/17, 5, 6, 15, 16

4. Underlying Consideration Specialty: Section 1, 6, 8, 16

Pulling the picture together (with other test results) to decide on priorities (can mean a call with Dr Shoemaker) and creating a summary for the client based around these 5 factors, at that point there is clarity on:

5. Next Steps: Further testing, Protocol initial steps, Environmental considerations

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Underlying Considerations

1. Consideration: CIRS ‘pattern’ assessment

(CIRS Assessment based on the published work of Dr Shoemaker and Dr James Ryan)

Section 1-
1. Ribose Large/Small is the darkest blue, therefore down-regulated and likely affected by a Biotoxin
2. NDUF, ATP synth, COX and mTranslocase are also down-regulated as expected. A **clear hypometabolic picture is indicated** with this result with Citric Acid cycle/ATP creation all down-regulated too (we also see CoQ10B and UQCRQ down-regulated in section 14 relating to energy production/ATP)
3. Mito Large (& Small) down-regulation indicates possible risk of MARCoNS

Section 12-
1. CD3D and CD48 are both negative values and down-regulated. CD3D relates to your immune system capacity to create antibodies

Section 13/14-
1. CIRS Biomarkers- we expect to see up-regulation of 4 genes for a stage I CIRS case. When we do not see this it raises the possibility that someone is out of primary exposure or that medication may be impacting on the result- or that this is not a CIRS case
2. CD52 as a negative/down-regulated value

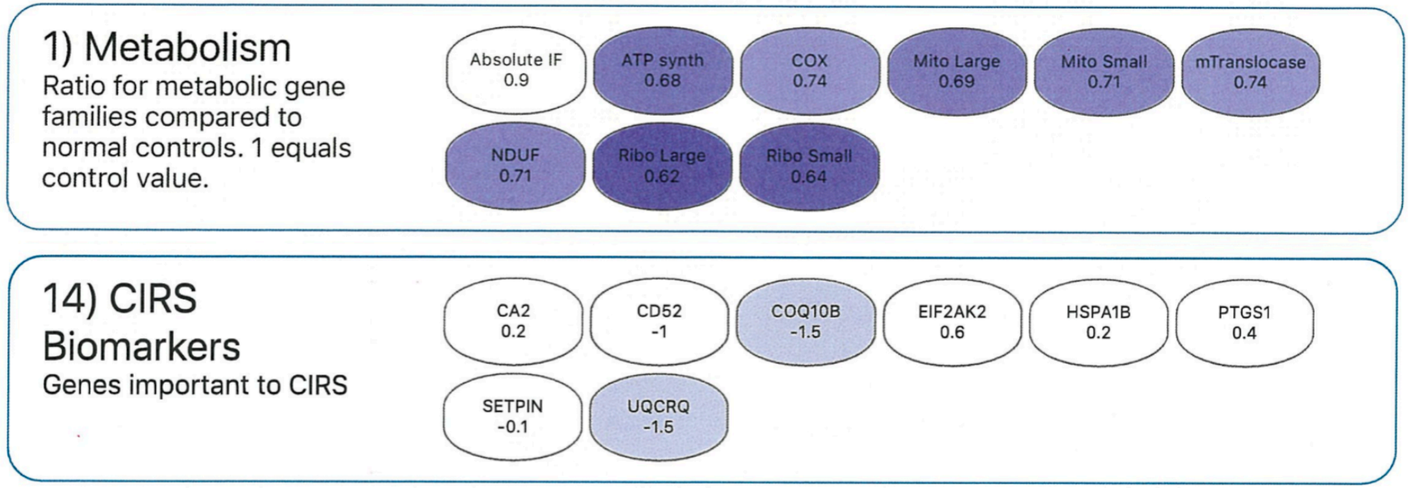
Section 19-
1. Dispersion- has to be under 1 for a valid result, which it is.

CIRS Assessment

1. Hypometabolism

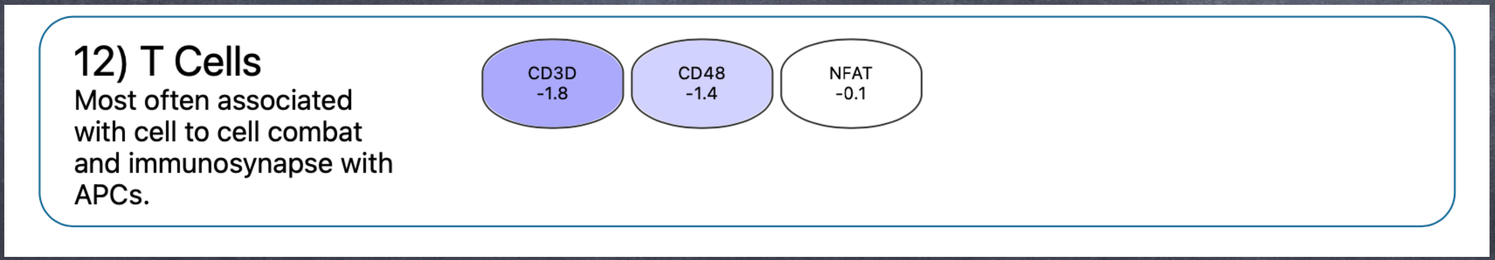
- Hypometabolism- Ribosome (large and small subunit), mito-ribosome (large and small), COX, mTranslocase, NDUF, ATP and Absolute IF

- Section 14- CoQ10B and UQCRQ



CIRS ASSESSMENT

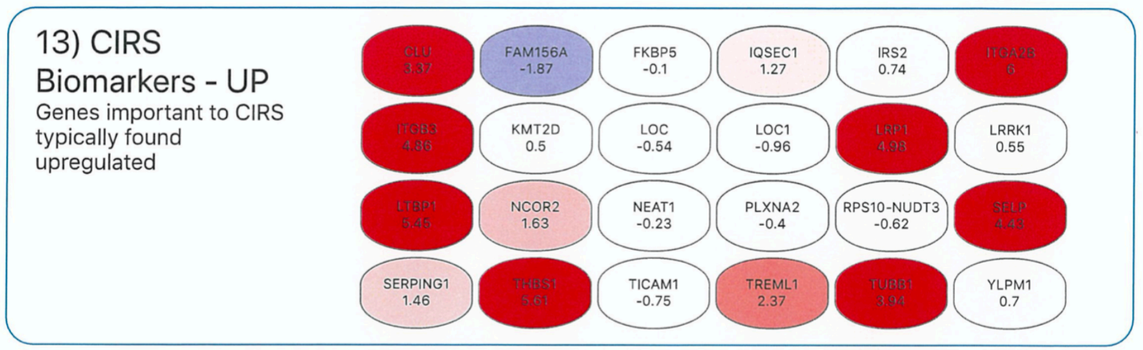
2. T cell down-regulation (blue or at least negative value)



CIRS ASSESSMENT

3. More than 4 sections up-regulated (ie red)

- More than 4 CIRS Biomarkers up-regulated



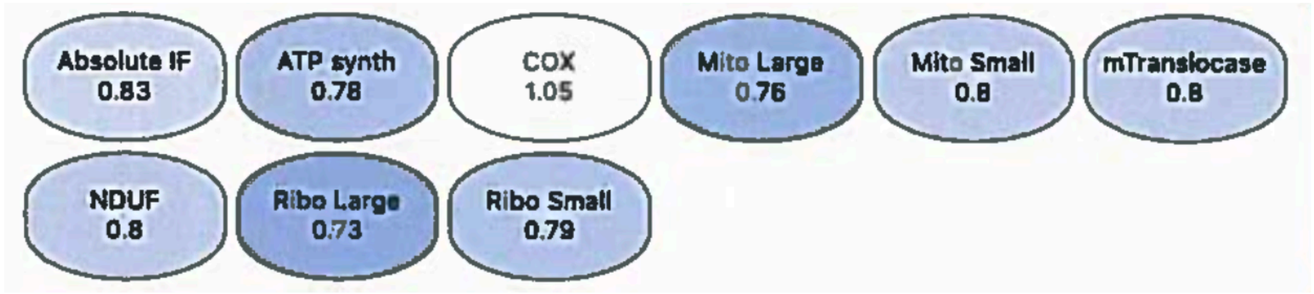
3. Negative value for CD52 in section 14

CIRS ASSESSMENT

4. Normal Dispersion level

1. Mapping with Proteomics/Swab Testing

1. Clear hypometabolism is seen:



2. Maps with CIRS proteomics- Fits CIRS DX
C4a 12,666 ng/mL
TGFb1 5,780 pg/mL
aMSH 16 pg/mL
MMP9 655 ng/mL
Osmolality 321 mOsm/kg

3. MARCoNS seen on swab testing

NARES CULTURE

SOURCE	NARES
ORGANISM #1	STAPH COAG NEGATIVE-SMALL AMOUNT
	MARCONS POSITIVE

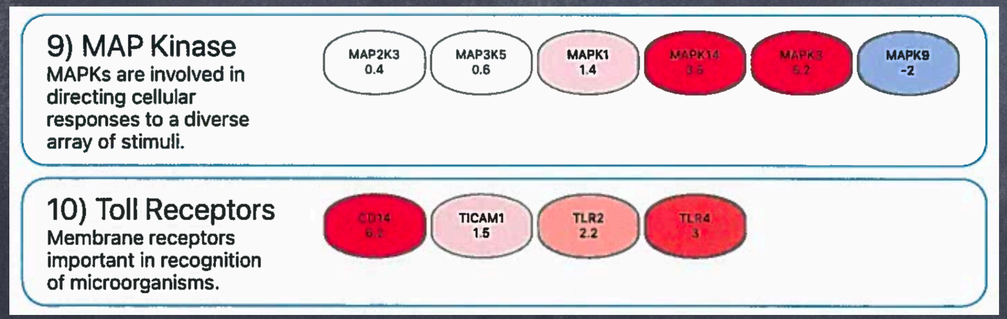
2. Consideration: Triggers

(Sections 1, 3, 5, 6, 7, 9, 10, 11, 16)

Possible Triggers-

1. MARCoNS- there is still an indication for MARCoNS in section 1 with the Mito Large down-regulation
2. Section 5/16 have CCL5- infection/underlying histamine trigger may be present
3. Environmental triggers: TGFBR1 and TGFBR3 up-regulation in section 5 can be associated with exposures to endotoxins and actinomycetes, and is read in conjunction with MAPkinase up-regulation in section 9 & TLR up-regulation in section 10
4. Section 6 is the Lyme section
5. Section 3 has RIPK1, when up-regulated is associated with auto-inflammatory process
6. Granzymes- infection
7. CD81 up-regulation in section 11. Could possibly associate with viral factors- but also something that someone was exposed to prior to CIRS changes on antibody respons

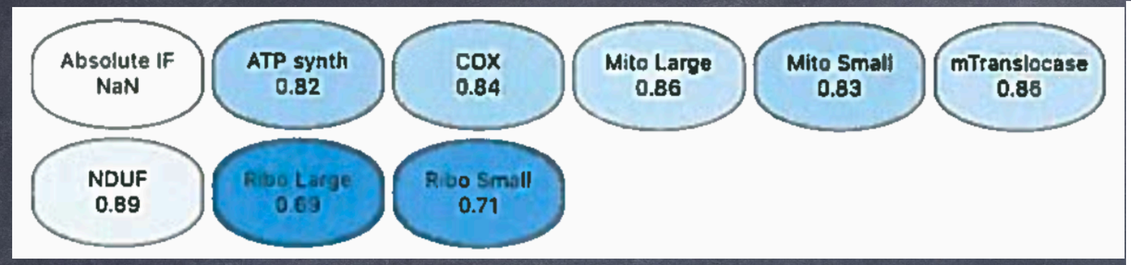
Mapping mRNA + Skin Swab Testing (actinos/endos)



Actino Skin Results

	Genus	Species	B.E/ml	
1	Corynebacterium	Amycolatum	13,298	*
2	Corynebacterium	Simulans	108	
3	Corynebacterium	Tuberculostearicum	5,812	
4	Corynebacterium	Xerosis	N. D.	
5	Propionibacterium	Acnes	876,284	

Mapping mRNA + Proteomics + Environmental Results



C4a 24,884 ng/mL
TGFb1 4,220 pg/mL
MARCoNS positive
HLA 13-6-52A & 14-5-52B (mould/multi)

Actino Score

Actino Score	21

Color-coded interpretation	
10 or below	Indicative of a Healthy Building
Between 11 to 15	Further investigation needed
Greater than 15	Suggestive of Building Related Illness.

Species	Spore E./mg		Weighting
Aspergillus penicillioides	223		6
Aspergillus versicolor	66	*	4
Chaetomium globosum	2		0
Stachybotrys chartarum	7		4
Wallemia sebi	85		0
HERTSMI-2 Score =			**14**

IEP Survey

2 responses only

“Demonstrates team effort. In my experience (and feedback from clients), whenever the patient witnesses docs and IEPs on the same page regarding their case, it adds confidence to the entire process and tends to naturally increase the level of hope and determination/drive that the client experiences”

5. Consideration: Next Steps

Underlying considerations

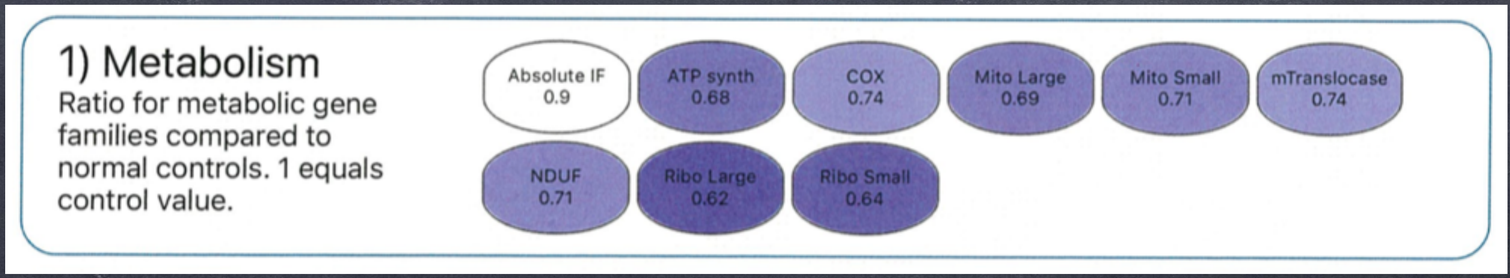
1. Mis-match of positive to negative values in the IKAROS section indicates increased risk of a foundational/genetic-environmental sensitivity risk. You may therefore be more at risk of reacting to your environment/MCS sensitivity.

2. Absolute IF- down or up-regulated

UNDERLYING CONSIDERATIONS

1. IKAROS MIS-MATCH

2. ABSOLUTE IF



Differential Diagnosis

(when systematic processes are used to identify the correct diagnosis from a set of possible diagnoses)

- Always return to the differential…. This is why the proteomics are still important.
- We still need the proteomics for the diagnosis…the dot to dot
- mRNA testing adds the colour
- Critically important to consider what else ‘it might be’ and consider a link with any previous diagnoses

A Patient History Survey

Patient History Survey

Tube ID #s
On the tube labels — A/B

Ordering Health Care Provider:	
Email:	Phone:
Address:	

Patient Information

Progene DX will use the Tube ID #s (above) to communicate with you about this assay. Be sure to record the Tube ID #s and Order # (above) in your patient's file. When filling the tubes be sure to use only the tubes that came with this order – you can tell by the Tube ID #s on the tubes. **Do not provide the patient's name, address, SSN or any personally identifiable information on any paperwork including additional studies you send us.**

Sex (circle): Male / Female Birth year (yyyy only)_____ Height:_____ Weight:_____

Racial Heritage:_____ Years of education (high school=12; college=16)

Top 10 symptoms

Differential Diagnosis Considered

Biotoxin exposure

If mold, what was HERTSMI-2? _____ What was ERMI? _____Attach copy please

I. was there visible mold? ..... Yes No
II. were there musty smells? ..... Yes No
III. was actinomycetes testing performed? ..... Yes No Please attach
IV. was endotoxin testing performed? ..... Yes No Results_____

If patient is CIRS-WDB, when was last exposure to WDB prior to GENIE draw:_____

If Lyme, was there any ECM rash? ..... Yes No

Positive Western Blot? (from Quest, LabCorp or Stony Brook) Yes No

Patient History Survey

Circle the number indicating the stage of CIRS therapy:

1. Naïve (prior to CSM protocol) 2. After removal from exposure and started CSM protocol
3. Currently on VIP 4. Finished VIP 5. Relapse

Diagnostic studies

IMPORTANT For best results, we request the following tests be conducted at the same time as the GENIE assay; within 1 week is acceptable. In the case that your patient is unable to provide concurrent tests please indicate the date of the test results you are providing.

If you are conducting concurrent tests, please copy this page and retain in your patient's file while waiting for test results. When you have received all the test results, please complete your copy of this page (along with supporting pages i.e. VCS, NeuroQuant, etc.) and mail to CRBAI, 500 Market St., Suite 103, Pocomoke City, MD 21851. Be sure to only identify additional pages using the Tube ID #s above.

TEST		Test Date (mmm/dd/yy)
HLA DR by PCR	_____	
MARCoNS: Positive / Negative (circle one and attach report)		Date_____
VCS: Positive / Negative (circle one and attach report)		Date_____
MSH	_____	Date_____
TGF beta-1	_____	Date_____
MMP-9	_____	Date_____
VEGF	_____	Date_____
C3a (Quest only)	_____	Date_____
C4a (Quest only)	_____	Date_____
ADH/osmolality	_____	Date_____
ACTH/cortisol	_____	Date_____
AGA	_____	Date_____
von Willebrand's profile (Quest only)_____		Date_____
Pulmonary stress test (please attach) V02 max _____		Date_____
Stress ECG (please attach) PASP Before_____PASP After _____		Date_____
NeuroQuant (attach copy of General Morphometry Report)		Date_____

Prior use of anti-fungals Y / N (circle one). If yes, type and route _____

Pertinent additional studies (please attach).

Re-testing

CIRS Staging

Test can also be utilised to help place results on a timeline, allowing for tracking of progress through a therapeutic programme:

Stage 1: Still in exposure with evidence of hypo-metabolism and before any intervention has been undertaken

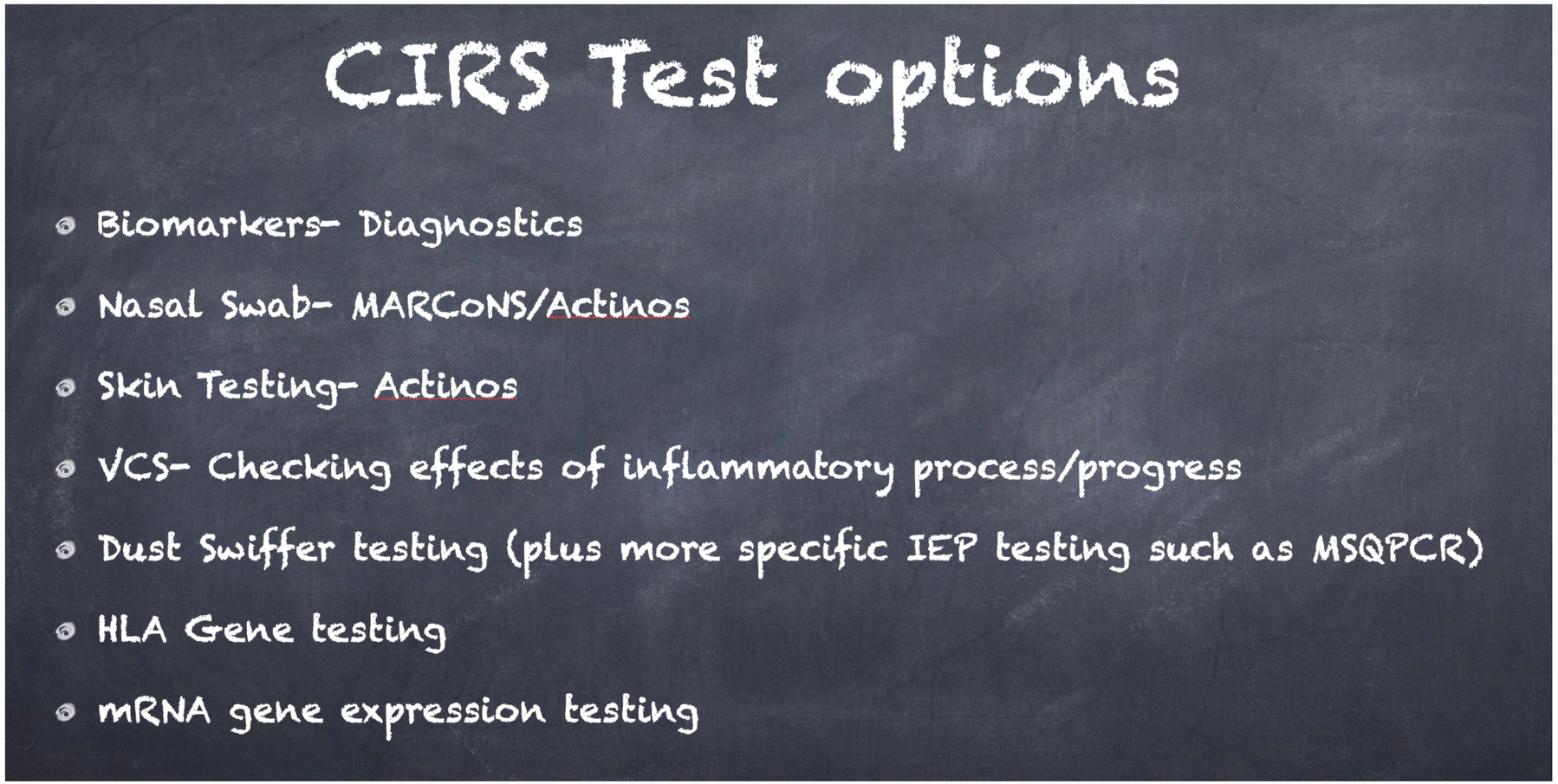
Stage 2: After the initial steps of the programme some hypo-metabolism may still be seen

Stage 3: If VIP has been started (if required/available)

Stage 4: After completion of VIP (if required/available)

Ultimate in Personalised Medicine

Dr Shoemaker's work gives us so much



Survey

For healthcare providers:

1. Have you used mRNA testing?
2. If yes, was this just for yourself, clients or both?
3. If yes, what is the main reason why you use mRNA testing
4. If you have not used mRNA testing yet, what is the main reason why not? (please share up to 3 reasons)
5. If you do not use mRNA testing which other tests do you use the most?
6. If you do not utilise regular testing what is the main reason for this? (please share up to 3 reasons)

For IEPs:

1. Do you work with healthcare provider(s) who communicate mRNA findings to support work with a client?
2. If no, do you feel you know enough about mRNA testing to be able to work with a healthcare provider on a case?
3. If yes, what has been the best thing about this level of joined-up data?

Thank you

For questions/comments/contact/survey responses please email me via

louise.carder@colabeu.com

OR for clinical enquiries

Clinical practice: info@louisecarder.com