Disproportional transient C3 elevations on Marshallese newborn screenings: what do these mean?

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Republic of the Marshall Islands

1. Dietary habits largely sea fish, sweet potatoes, fruits, such as breadfruit, papaya, coconut, and watermelon (diets rich in vitamins A, C, and E
2. Shared environmental histories, less genetic complexity
3. Maintained island habitation for up to 5000 years

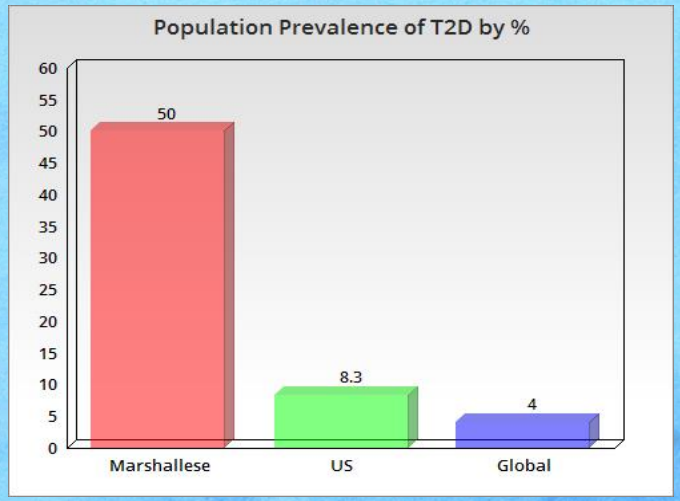
US Nuclear Weapons Testing Background

1. Strategic military use during post-World War II era
2. 1946-1958: CROSSROADS, SANDSTONE, GREENHOUSE, IVY, CASTLE, WIGWAM, REDWING, HARDTACK I
3. March 1, 1954: BRAVO (CASTLE)
4. Project 4.1 – Research prior to informed consent laws

US Migration

1. Compact of Free Association (COFA) 1985
2. Removed travel restrictions to and from US
3. Some states with large Marshallese populations: Arkansas, Oregon, Iowa, Missouri, and Oklahoma
4. 1996-2020 – Health care coverage differed by state

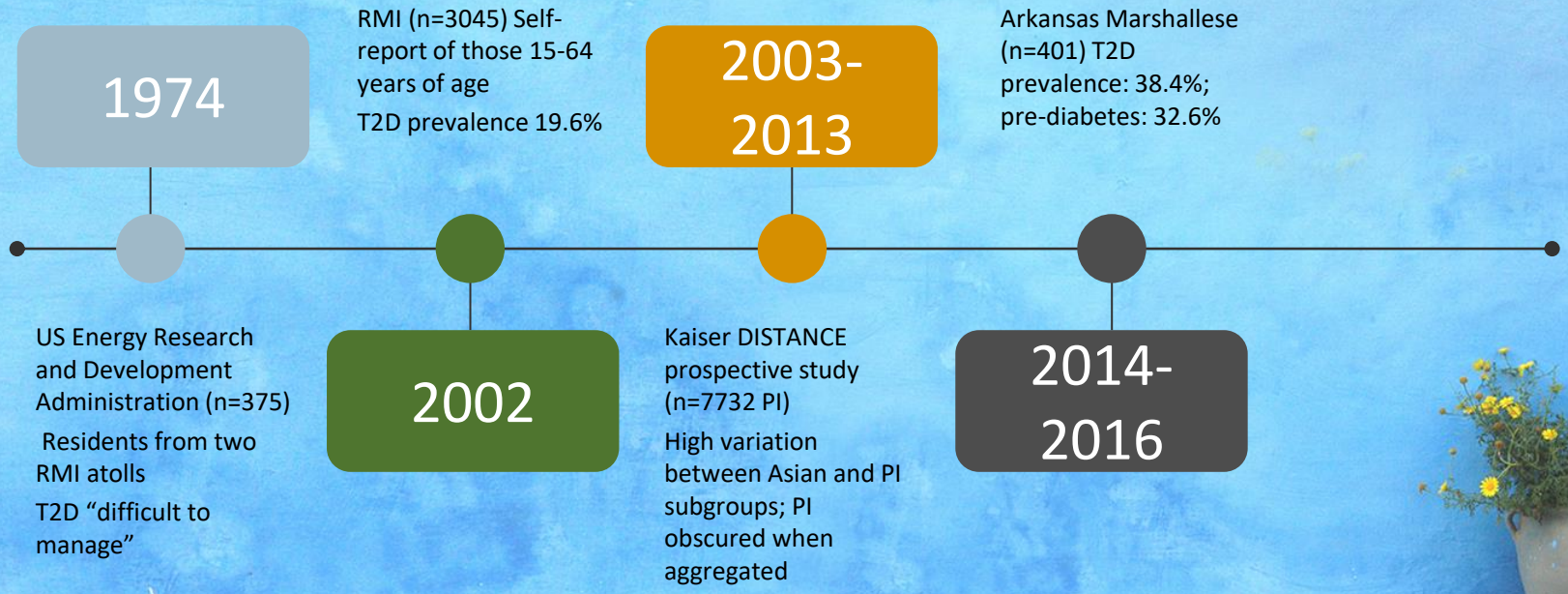
Marshallese prevalence of T2D is greater than 12 times global rate



Unretractable with self-management interventions that are successful in other populations

ADA, 2010; Ichiho, 2013; Karter et al., 2013; McElfish et al; Reddy et al; Yamada & Palafox, 2001

Marshallese T2D disparities



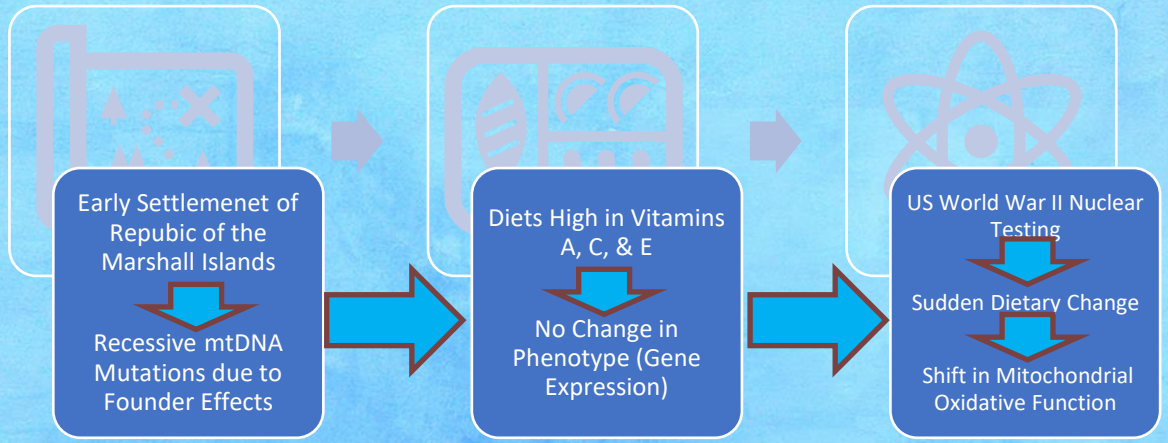
Hypothesis links

- Isolate populations may have amplified founder effects – the effect which occurs when a small subset of a population is removed or isolated from the larger population causing a change in the subset population's gene pool.
- Can elevate a variant from rare to common in the population.
- Founder effects which elevate variants in the mitochondrial DNA in areas of the mitochondrial respiratory chain that affect energy production and regulation of reactive oxygen species can cause imbalance in the tightly regulated process.
- An imbalance can result in oxidative stress, which is associated with multiple diseases, including T2D.

Thrifty genotype concept

- In a population expending high amounts of energy sea fishing or engaged in heavy physical labor required to survive in an environment such as an isolated island, a population-shared genotype conveying efficiency in the intake or processing of food sources would be an asset.
- The same genotype in a sudden scenario of readily available food sources and more sedentary lifestyles may result in greater incidence of metabolic imbalance compared to non-affected individuals given the same scenario.

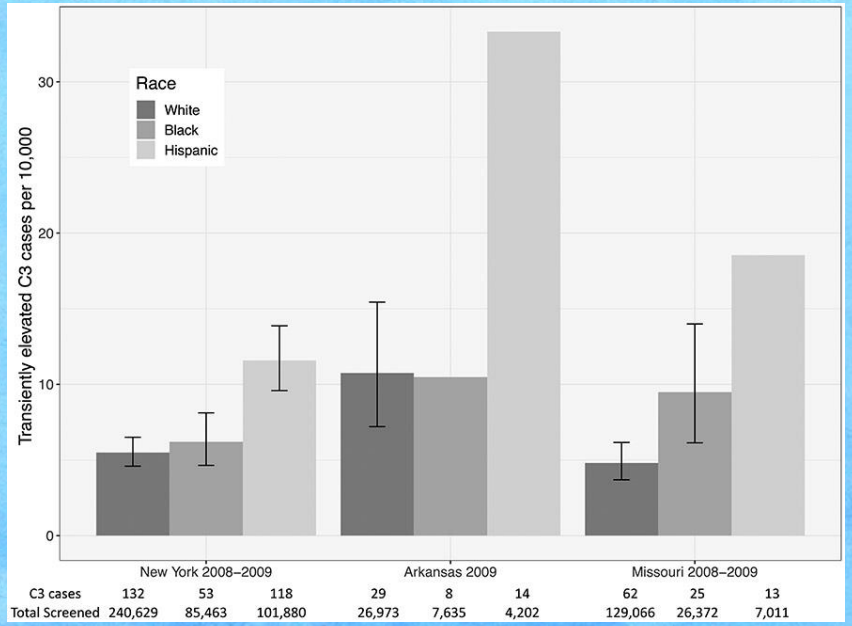
Hypothesis model



Marshallese newborn screening

- *RMI has no NBS public health program.*
- *Marshallese children born in the US are screened according to state mandates.*
- *Arkansas newborns are screened between 24 and 48 hours for 34 rare disorders; 32 of which are achieved through a dried blood spot sample collected and placed on filter paper and sent to the Arkansas Public Health Laboratory in Little Rock for testing.*

Pangilinan F, Watkins D, Bernard D, Chen Y, Dong N, Wu Q, Ozel-Abaan H, Kaur M, Caggana M, Morrissey M, Browne ML, Mills JL, Van Ryzin C, Shchelochkov O, Sloan J, Venditti CP, Sarafoglou K, Rosenblatt DS, Kay DM, Brody LC. **Probing the functional consequence and clinical relevance of CD320 p.E88del, a variant in the transcobalamin receptor gene**. Am J Med Genet A. 2022 Apr;188(4):1124-1141. doi: 10.1002/ajmg.a.62627. Epub 2022 Feb 2. PMID: 35107211; PMCID: PMC8923979.



Newborns with transiently elevated C3 per 10,000 screened newborns by race/ethnicity in three states. Incidence was calculated based on counts of screened newborns with transiently elevated C3 divided by the total number of infants screened by race/ethnicity in each state for the indicated birth years. Vertical bars indicate the 95% confidence interval; this is omitted (NA) when <20 cases were observed.

AR C3 2017-2018

Transient elevation is defined as having a mildly elevated C3 level (5.5-11.9 µmol/L) on initial screening with a subsequently normal follow up screening. Marshallese newborns have an ~10 times higher incidence of transient elevation of this protein identified on newborn screening compared to all other populations in Arkansas.

# of births in AR # of C3 elevations (% affected)	Total	White	African American	Hispanic or Latino	Asian	Marshall Islands	Other
2017	37 520 133 (0.35)	24 089 62 (0.26)	7082 8 (0.11)	4048 26 (0.64)	783 5 (0.64)	**470** **24 (5.11)**	1048 8 (0.76)
2018	36 996 114 (0.31)	23 587 67 (0.28)	6960 8 (0.11)	4097 9 (0.22)	775 0	**501** **23 (4.60)**	1076 7 (0.65)

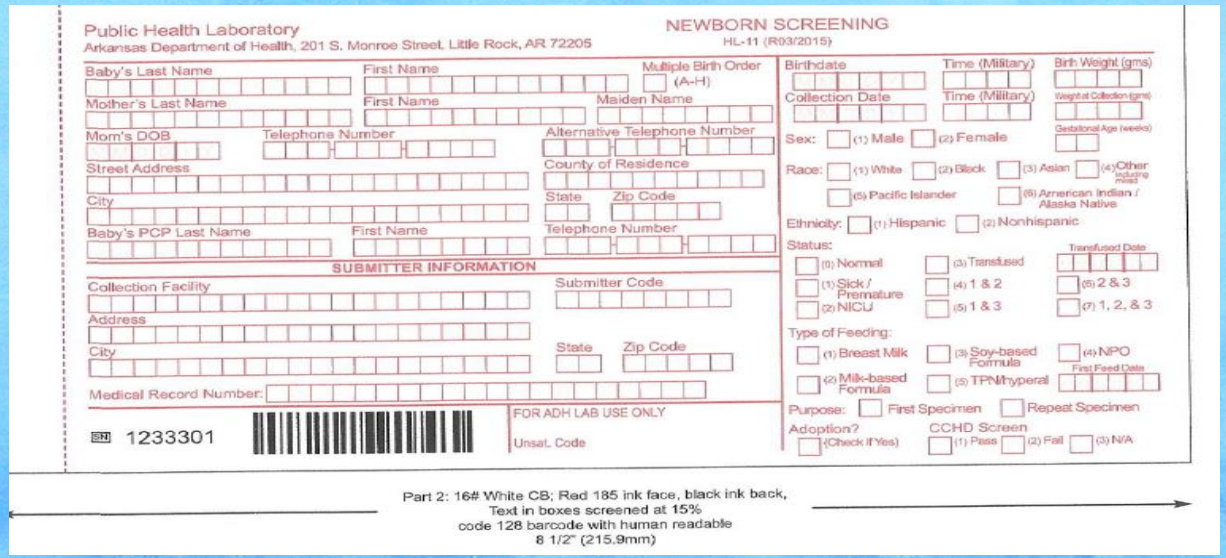
AR NBS Filter paper collection card 2019

Changes to the card included:

Addition of Pacific Islander and American Indian/Alaska Native to Race choices

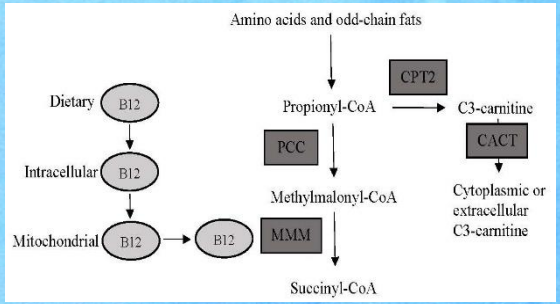
Addition of NICU to Status choices

Addition of CCHD to card as checklist to indicate the screening has been done



01

C3 is a newborn screening marker for propionic acidemia (PA) and methylmalonic acidemia (MMA).



Involvement of vitamin B12 as a co-factor for mitochondria-mediated TCA pathway

02

Transient elevation of this marker may indicate a defect of vitamin B12 synthesis and transport in the neonate.

04

Maternal vitamin B12 deficiency can also result in neonatal vitamin B12 deficiency and an abnormal NBS result in the neonate.

03

The citric acid cycle (TCA) occurs in the matrix of the mitochondria, and disruption in vitamin B12 as a co-factor can cause shifts in this tightly regulated pathway.

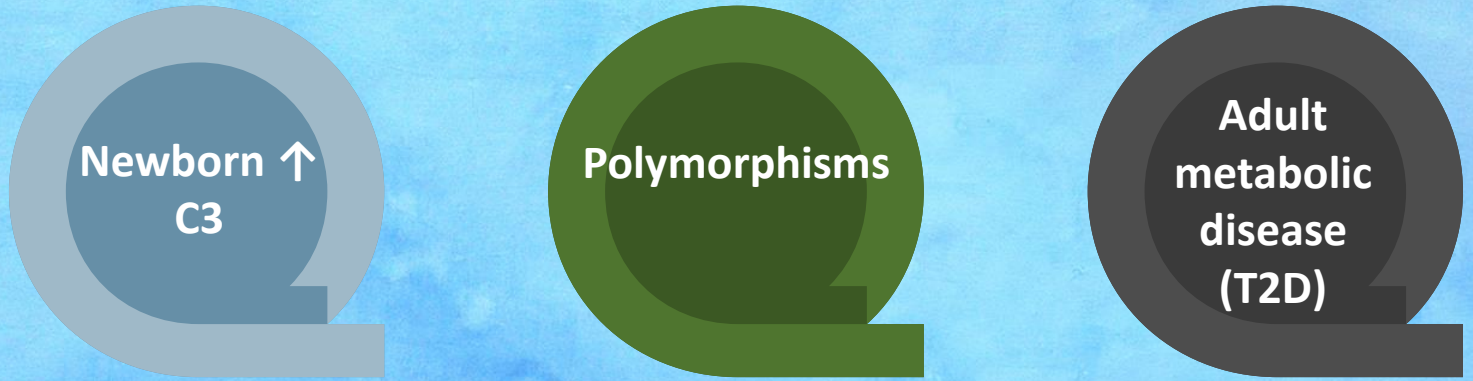
Neonatal vitamin B12 deficiency

Severe vitamin B_{12} deficiency produces a cluster of neurological symptoms in infants, including **irritability, failure to thrive, apathy, anorexia, and developmental regression**, which respond remarkably rapidly to supplementation.

Even though they are born of normal size with apparently normal neurological development, symptoms in vitamin B12-deficient infants typically appear by 4–10 months of age, but may occur as early as 2 months of age. Symptoms include irritability, failure-to-thrive including a falling-off in growth rate, apathy, anorexia, refusal of solid foods, megaloblastic anemia, and developmental regression.

Dror & Allen, 2008 Nutrition Reviews® Vol. 66(5):250–255

Associations that would further investigation for health outcomes of adult disease



Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study

C. S. Yajnik, S. S. Deshpande, A. A. Jackson, H. Refsum, S. Rao, D. J. Fisher, et al.

Diabetologia 2008 Vol. 51 Issue 1 Pages 29-38

- Low maternal vitamin B12 predicted higher insulin resistance in the children. The offspring of mothers with a combination of high folate and low vitamin B12 concentrations were the most insulin resistant at six years of age.

2 Studies

ONE

- Assessed Willingness to Participate
- Established trust
- Built relationships
- Learned attitudes toward genetic testing
- Feasibility of further testing
- Purposive sample of parents of 10 babies born in 2019
- Reached 7/10; all were willing to consent

TWO

- Conducted testing on NBS samples
- Approached same participants
- Asked permission to perform further genetic testing on child's NBS DBS
- Reached 5/7 from first study; obtained 3 samples

Study 2

- DNA extracted at UAMS by Research technician in COM Department of Pharmacology and Toxicology
- Sent to CD Genomics (New York) for mtDNA sequencing
- Each identified mutation compared with MITOMAP and MitImpact 3
- Aligned identified mitochondrial mutations of individuals to the revised Cambridge reference sequence through Haplogrep 2 to obtain each one's haplogroup
- Predictive scoring models applied

Four coding variants in each individual mt sequence

8860A>G ***14766C>T*** ***15326A>G*** ***15746A>G***

B4a1a Haplogroup [NC_012920 rCRS]

Literature

Benton et al. (2012)

Sequenced 20 mtDNA genomes of Maori individuals, members of a population of Polynesians residing in New Zealand

Reduced mt genome diversity and 6 novel mtDNA variants

***Variant A15746G** in MT-CYB in all 20 samples, absent or rare in all ancestral subgroups*

Tabbada et al. (2010); Trejaut et al. (2005)

***Subclade B4a1a** linked to founder effects in Taiwanese groups*

Loo et al. (2014)

Sig associations between haplogroup B4a1a and T2D and between the individual nucleotide variant 15476 and T2D when variant is found within haplogroups B4a1a and G3

Mt genetic effect on health status (T2D) for ATP production, lipid peroxidation, DNA oxidation, mt inner membrane potential, lipid metabolism, and BP

Concluded that mtDNA variation may influence generation of ROS in cells and tissues and therefore the mtDNA profile of an individual may influence these physiological and biochemical measurements

Predictive scoring models

Each of our sample sequences contained the variants 8860A>G; 14766C>T; 15326A>G; and 15746A>G; all missense variants classified deleterious by **Condel**. Condel is a unified classification tool based on a weighted average of normalized scores of five individual tools: Log R Pfam E-value (Logre), MAPP, Mutation Assessor, Polyphen2 (PPH2), and SIFT. Additionally, the 8860A>G variant was classified disease by **Meta-SNP**, a prediction model integrating four methods: PANTHER, PhD-SNP, SIFT, and SNAP, and was classified deleterious by **PROVEAN**, a web-based tool which predicts the functionality of protein variants. The variant 14766C>T was classified deleterious by **CADD**, an annotative program that considers deleteriousness as a metric of the totality of the human genome, with pre-computed C-scores for all 8.6 billion possible human single nucleotide variants, and was classified as disease by the single non-synonymous disease-annotated prediction programs PhD-SNP and SNAP. This variant – 14766C>T – was also found damaging by **Mitoclass.1**, a predictive tool developed specifically for the prediction of pathogenic human mitochondrial DNA missense variants.

Three of the missense variants, 8860A>G, 15326A>G, and 15746A>G, were identified as likely functional mutations by three scoring methods developed to predict the functional impact of cancer-causing somatic variants by first considering baseline tolerance of genes to functional variants. These were PPH2 transformed, which assigned scores of high impact (8860A>G and 15746A>G) and medium impact (15326A>G); SIFT transformed, which assigned scores of medium impact to all three variants; and Mutation Assessor transformed, which assigned scores of medium impact (8860A>G and 15746A>G) and low impact (15326A>G).

Precision approaches

- Can address both individual and population disparities
- Population precision health includes consideration of history and social practices of a population, shared biological factors, exposures and those influences on the population's health
- Population screening supported when variants or single nucleotide polymorphisms occur at a high carrier frequency in a population

Examples of population DNA-based screening

- Ashkenazi Jewish descent
- Screening panels specifically developed for this population
- ~7-9 rare diseases including Tay Sachs disease, Gaucher disease, Canavan disease, Bloom syndrome, and others

Webb et al., 2014

- Sub-Saharan African descent with risk variants in *APOL1* genes
- ↑ risk for chronic kidney disease and poorer clinical outcomes with disease
- Up to 40% of AA are carriers of at least one risk allele – having one confers protection against African sleeping sickness; more than one = sig more risk for chronic kidney disease
- Increased sepsis incidence and severity

Friedman & Pollak, 2016; Kruzel-Dairla et al., 2016; Wu et al., 2021

- Disaggregate data

- Include and recognize small sub-groups of larger populations

- Reducing health care disparities is an ethical obligation, including disparities of health care accessibility and increased disease risk due to genetic susceptibility

Future

- Need to include Marshallese in genomic databases to investigate disease risks
- Disaggregate data
- Research: Do founder effects explain of influence high rates of metabolic disease in the Marshallese?
- Do genetic mutations impact response to clinical management in Marshallese adults with type II diabetes?

Implications

- Interventional trials testing mitochondria-targeted antioxidant therapies could inform treatment for T2D

- Prenatal vitamin B12 supplementation – especially during first trimester – could mitigate VB12 deficiency (even long-term effects for infant) This would be like the impact of folic acid supplementation during pregnancy.

Current work

The Marshallese "FISH for Health" Study
Recruitment Feasibility Pilot

- Aim 1: Develop and test collection tool for a future study.
- Aim 2: Determine feasibility of recruiting Marshallese adults for a future study.
- Aim 3: Collect data and samples for use in future research to achieve case-control candidate gene association study and/or studies investigating genetic links to type II diabetes.

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Thank You

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