The Addition of Pancreatitis Associated Protein (PAP) in a Two-Tier IRT/DNA Screening Strategy for Cystic Fibrosis is Less Effective in Programs that Screen at 48 hours of Age.

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Government of South Australia
Children, Youth and Women's Health Service

Women's and Children's Hospital Adelaide, South Australia

Summary Statistics	2008/2009
Budget	36.5 million
Emergency attendances	55,502
- Women	20,850
- Children	35,652
Admissions	41,595
- Women	19,480
- Children	22,115
Births	5,895
Beds	316
- Women	123
- Children	220
- ICU/SC	54
Average bed Occupancy	91.5%

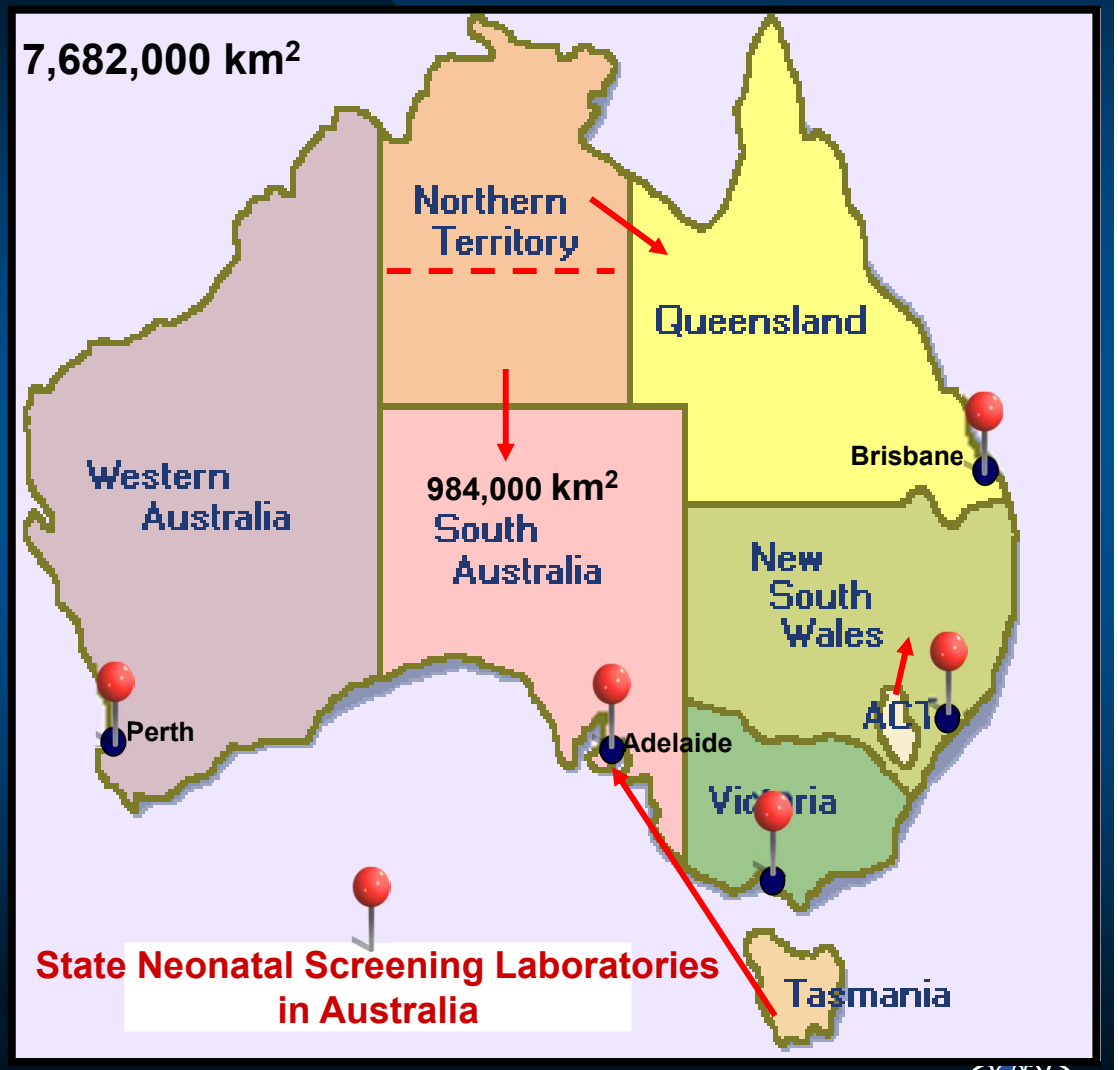
SA PATHOLOGY

Neonatal Screening Laboratories in Australia

Each year 265,000 Australian babies are screened at or near 48hrs in 5 Specialist Paediatric Hospital Centres. Metabolic Clinic with specialist clinical expertise for treatment and monitoring of IEM.

Demographic	Australia	South Australia
Area (,000 km^2)	7,682	984
Population (millions)	21.1	2.2
Residents/km^2	2.33	1.4
Urban Pop'n (millions)		1.2 (86%)
Health Costs ($m)	$41,742	
Health Costs/Person	$2,333	

Newborn screening laboratories in Australia within Paediatric Tertiary Hospitals



(WCH) Women's and Children's Hospital (WCH), Adelaide

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AIMS of the Study

- To determine the value of adding Pancreatitis-Associated protein (PAP) in a newborn screening strategy for CF.
- Does adding PAP to an existing two-tier IRT/DNA strategy improve CF screening:
  - through review of the:-
    - correlation between PAP and CF
    - association between elevated PAP and CFTR carriers
    - correlation of the level of PAP :-
      - » with birth weight & age at collection
      - » Specifically at, or near 48h of age

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Two-Tier IRT/DNA CF Screening Strategy

- A two-tier IRT/DNA screening strategy is in use in all Australian\New Zealand newborn screening laboratories
  - Has been in operation in South Australia since December 1989.

Ranieri et al, Brit Med J. 1991: 302, 1994: 308. Current Topics in CF 1996: Vol. 3, Chapter 9

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Two-Tier IRT/DNA CF Screening Strategy

- Screening Strategy relies upon:
  - **First Tier**: Generous Immunoreactive Trypsin (IRT) cut-off point
    - Top 1%
  - **Second Tier**: High frequency of common CFTR mutations
    - p.F508del ~ 72% of CF Chromosomes in our population

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SA PATHOLOGY

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    - p.F508del ~ 72% of CF Chromosomes in our population
- Detection/miss rate
  - ***Predicted up to 6% of CF neonates missed***
    - IRT<99th centile
    - no CFTR mutations
  - ***Sweat-testing requires expertise***
    - Sufficient number of tests (ideally centralised)
    - Appropriate age-related normal ranges (>4 weeks old to adults)
  - Co-ordinated, timely Genetic Counselling

Ranieri et al, Brit Med J. 1991: 302, 1994: 308. Current Topics in CF 1996: Vol. 3, Chapter 9

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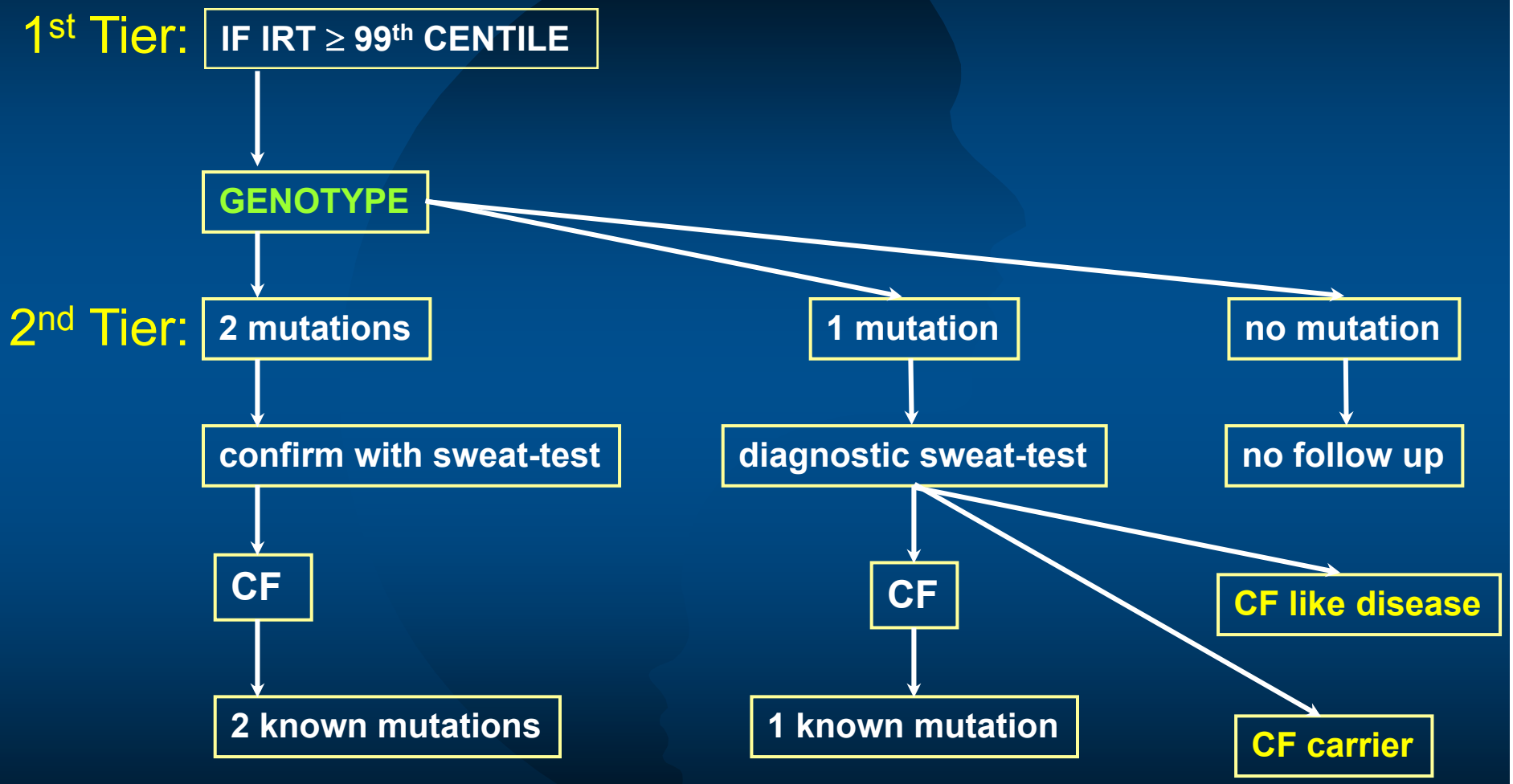
SA NEONATAL CF SCREENING PROGRAMME

Two-Tier IRT/ DNA Screening Strategy



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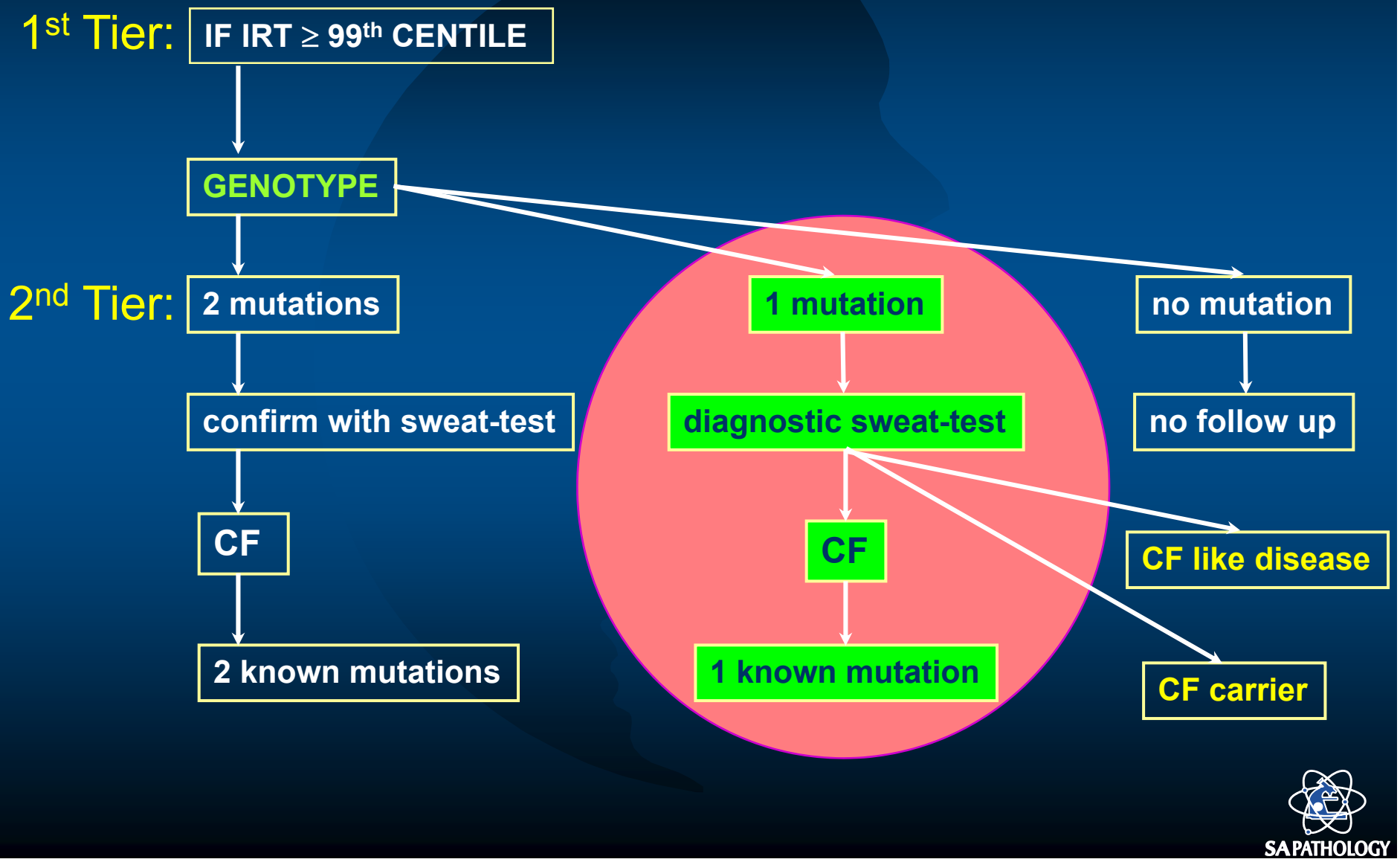

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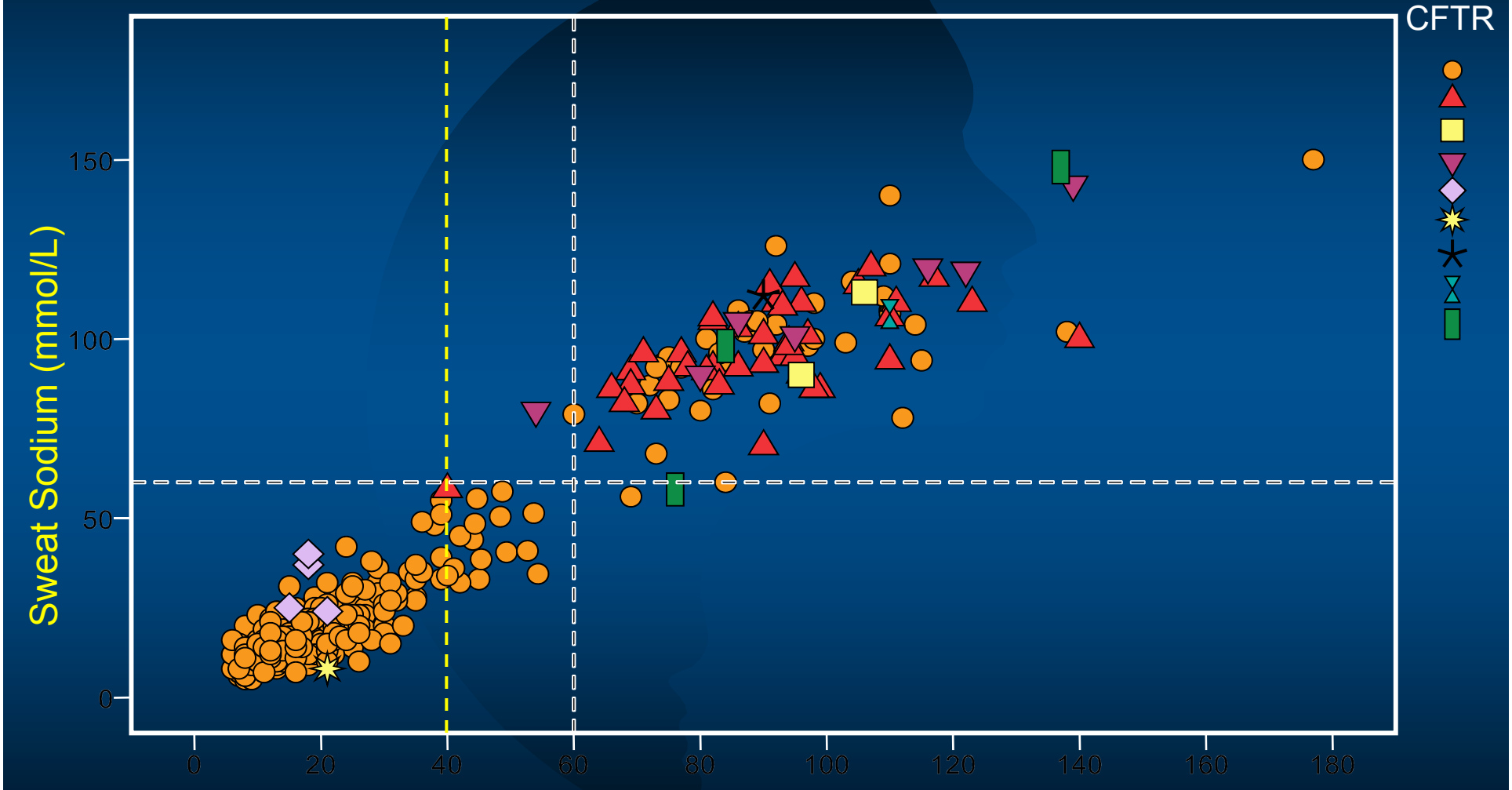
Sweat Test in IRT/DNA Screened Population

Screened cohort with IRT>99th centile and one or two CFTR mutations



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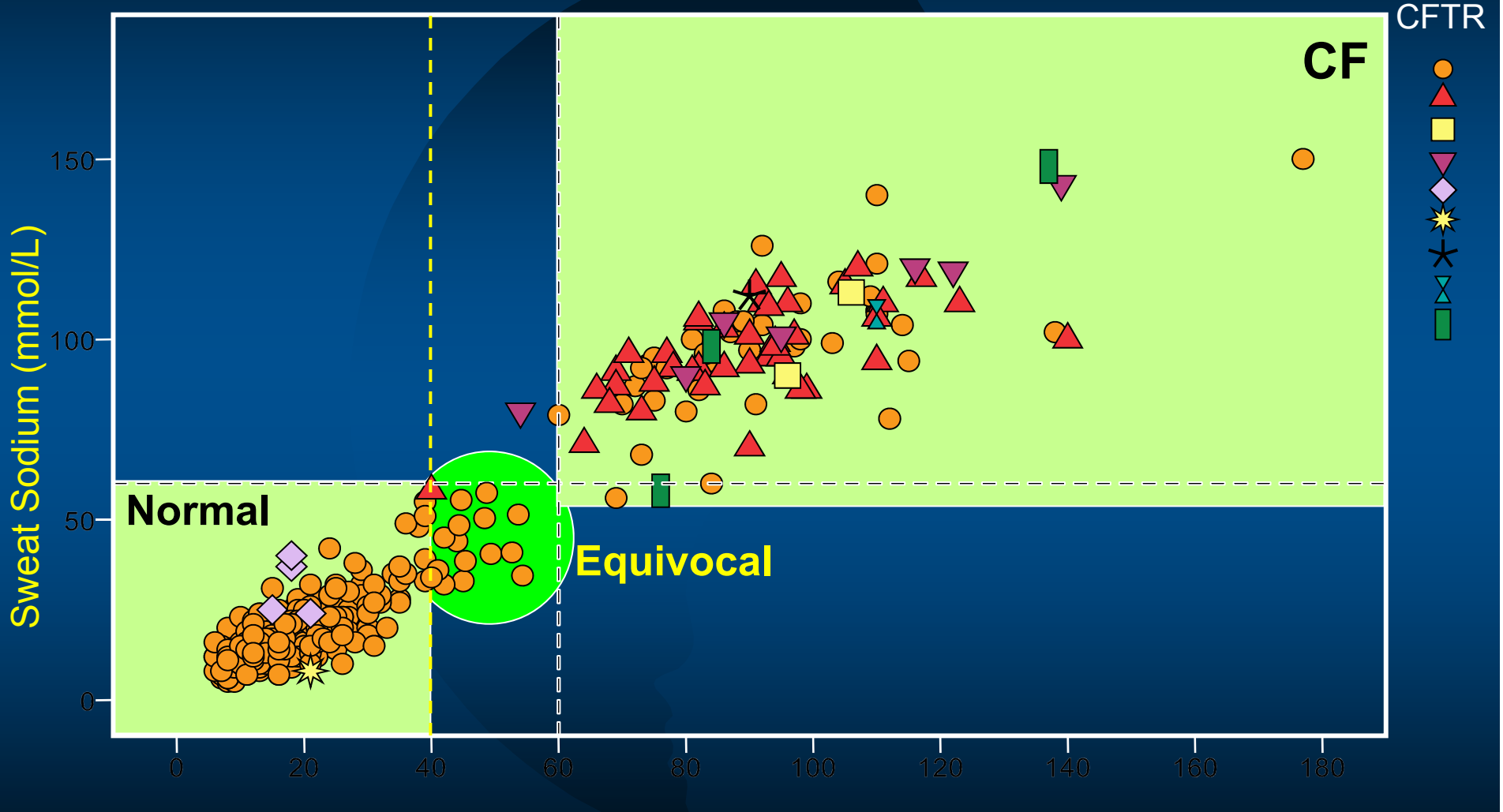

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South Australian CF Screening Programme Performance Data

Description	Number Identified
Number of infants screened	477,904
IRT <99th	472,169
DNA mutation analysis performed	5,735 (1.2%)
No identifiable mutation	5,243
Two identifiable mutations	94
One identifiable mutation	398
Sweat test positive	42
Sweat test negative	356
Carrier frequency	*1 in 13*
Total number of CF infants detected	136
Positive predictive value	34%
Missed (presentation 2 -12 years of age)	7 (4%)
Normal IRT	**3**
Elevated IRT no identified CF mutation	**4**
Sensitivity	95%
Apparent incidence of detected CF infants	1: 3,515
Prenatal diagnosis and termination	26
Overall prevalence of CF	1: 2,770 **(162 cases)**

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NSW CF Screening Programme performance data

Description	Number Identified
Babies screened	925,094
Tested by PCR	10,275
CF	296
p.F508del/p.F508del	168
p.F508del/other	113
Terminations	8 (up to 1999)
Apparent incidence	1:3,000
Missed, False negatives	**18 (5%)**
Normal IRT	**6**
Elevated IRT no p.F508del CFTR mutation	**12***
p.F508del/other, negative sweat test	**595**
Carrier frequency	***1 in 13***
Expected overall number of CF	354

****Data provided by Dr. Veronica Wiley NSW Newborn Screening Programme***

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Neonatal Screening for CF

- Pancreatitis-Associated Protein (PAP) has been reported to be elevated in newborn infants with CF

SA PATHOLOGY

Neonatal Screening for CF

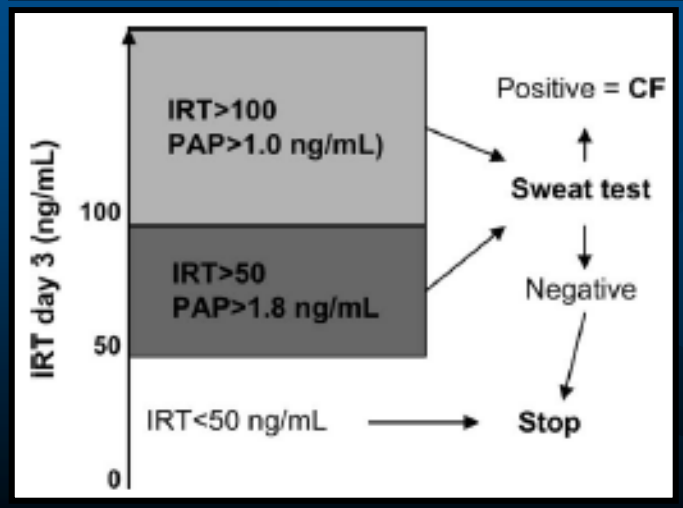
- Pancreatitis-Associated Protein (PAP) has been reported to be elevated in newborn infants with CF

  - *Sarles et al I Pediatr. 147, 302-305 2005*

A:

COMBINING IMMUNOREACTIVE TRYPSINOGEN AND PANCREATITIS-ASSOCIATED PROTEIN ASSAYS, A METHOD OF NEWBORN SCREENING FOR CYSTIC FIBROSIS THAT AVOIDS DNA ANALYSIS

JACQUES SARLES, MD, PATRICE BERTHÉZÈNE, MSC, CHRISTIAN LE LOUARN, BSC, CLAUDE SOMMA, MD, JEAN-MARC PERINI, MD, MICHAEL CATHELINE, MD, SOPHIE MIRALLIÉ, MD, KARINE LUZET, BSC, MICHAEL ROUSSEY, MD, JEAN-PIERRE FARRIAUX, MD, JACQUES BERTHELOT, MD, AND JEAN-CHARLES DAGORN, PHD



Suggested IRT/PAP CF screening strategy
All newborns are tested for IRT:
-Those with levels >50mg/L tested for PAP. or IRT>100µg/L & PAP >1.0ng/mL

-Those with PAP > 1.8ng/mL and with PAP>1.0ng/mL, and IRT >100ng/mL
Recalled for sweat-testing

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Pancreatitis-associated protein (PAP)- a screening marker for CF?

- PAP
  - A lectin-related secretory protein present in small amounts in normal pancreas and over expressed during the acute phase of pancreatitis.
  - In animal models PAP is constitutively expressed in the intestinal tract, but not in other tissues . PAP mRNA could not be evidenced in liver, stomach, salivary glands, brain, kidney or testis.
  - Its pattern of expression during severe pancreatic aggression suggests that it might be a stress protein involved in the control of bacterial proliferation.
  - PAP has been suggested to be a marker of ‘pancreatic sufficiency’ in individuals with CF

SA PATHOLOGY

Two-Phase Study Design

- *Phase I:* to determine South Australian newborn population statistics for PAP.

SA PATHOLOGY

Two-Phase Study Design

- *Phase I:* to determine South Australian newborn population statistics for PAP.

- *Phase II*: to include selected samples from the screening programmes in other Australian states (NSW, QLD & VIC) to form a screen cohort of ~195,000 samples.
  - **Selected for CFTR mutational analysis**
    - **Top 1% and/or >2.5MoM of IRT values**
  - Determination of PAP & repeat IRT in South Australia on coded whole blood-spot samples
  - Stratify by:
    - » CF mutational analysis
    - » Sweat-test negative
    - » CFTR carriers

SA PATHOLOGY

Australian PAP Study Phase I

- Phase I
  - Modification of PAP assay (MucoPAP®, DYNABIO) to use Eu^{3+} labelled strepavidin.
  - South Australian Newborn Population
    - Establish normal PAP population distribution and determine levels for the 90th, 95th & 99th centiles
  - Cohort
    - 2,885 unselected newborn specimens
      - » Normal population statistics
      - » CFTR carriers

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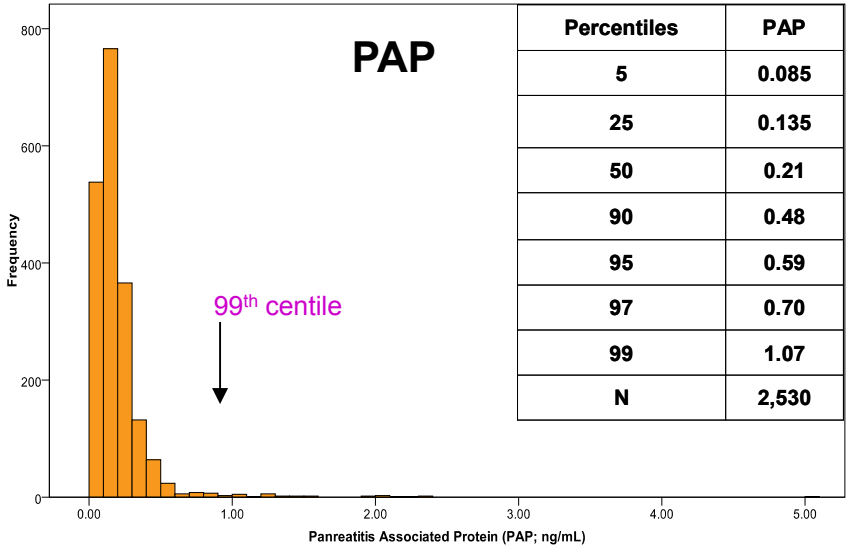
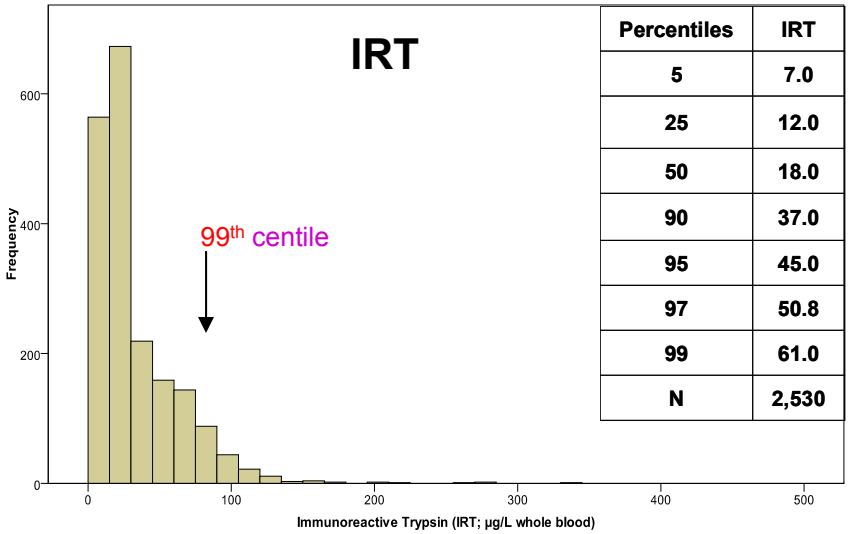
Population distributions for IRT and PAP on the same samples



Percentiles	PAP
5	0.085
25	0.135
50	0.21
90	0.48
95	0.59
97	0.70
99	1.07
N	2,530



Percentiles	IRT
5	7.0
25	12.0
50	18.0
90	37.0
95	45.0
97	50.8
99	61.0
N	2,530

- SA population
  - Establish population reference intervals for IRT and PAP on 2,885 consecutive blood-spot samples
    - 90^{th} ,95^{th} & 99^{th} percentiles
  - Stratify against age at collection birth weight preterm and low gestational age.

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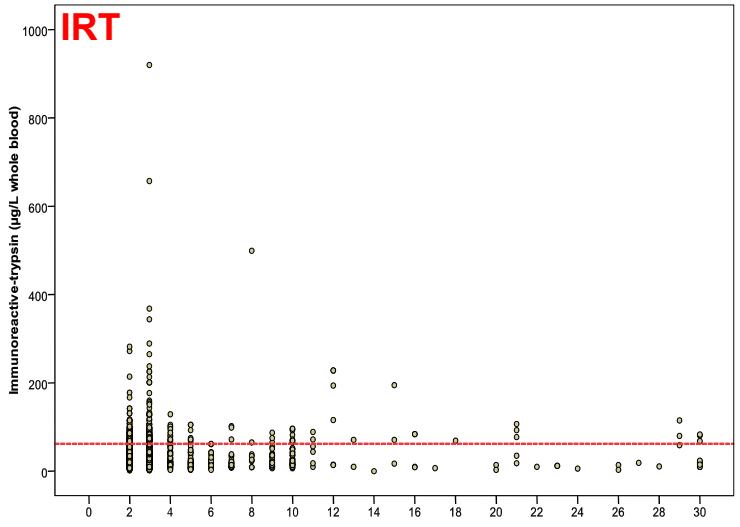
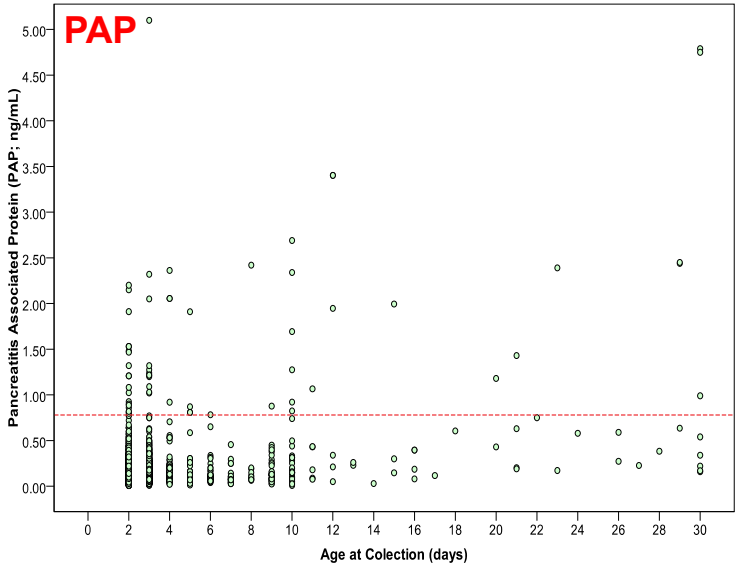
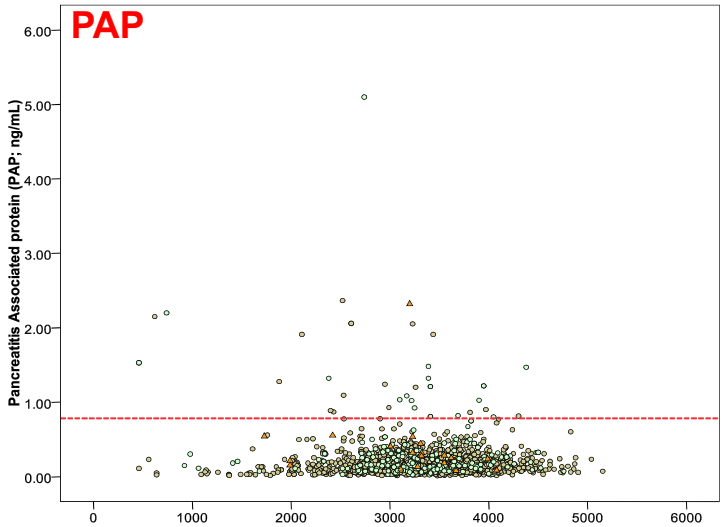
Correlation of IRT and PAP against Age at Collection & Birth Weight

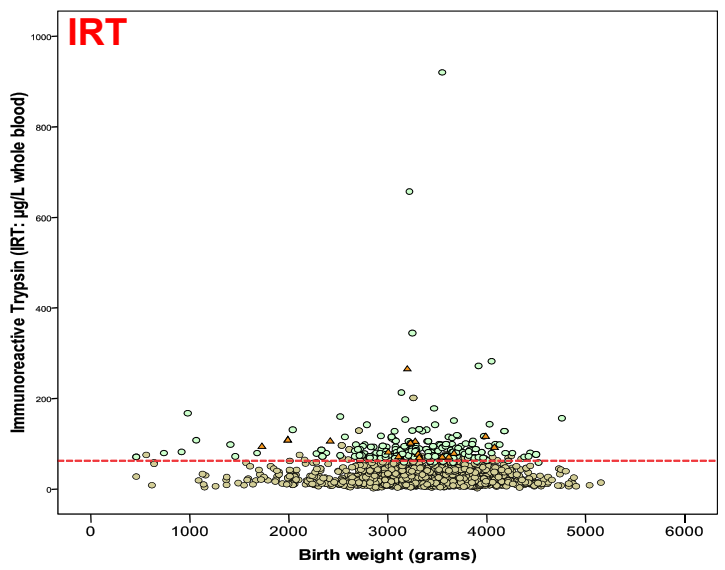
AGE AT COLLECTION





BIRTH WEIGHT





(Plot represents blood-spot samples from the unpartitioned 2,888 in addition to selected cases where N08 represents 8 CFTR mutations.

SA PATHOLOGY

"Clinical Study" Phase II

- Participation by the NSW, QLD & VIC Neonatal Screening Laboratories
  - Provided prospectively collected coded dried blood-spot samples
    - ***Selected IRT population ≥99th centile and or >2.5 MoM***
      - 3 blood spots for each case sent as a weekly batch to the SANSC laboratory for analysis.
        - » Estimated 1x IRT & 2 x PAP
    - Statistical analysis
      - To ascertain sensitivity & specificity

SA PATHOLOGY

Phase II: IRT versus PAP

- Phase II Cohort
  - "Clinical study" cohort (N=1,979 specimens) with **IRT≥ 99th centile and/or >2.5MoM**
    - 1,812 No CFTR mutations
    - 119 with a single CFTR mutation
    - 48 specimens from infants with CF (47)

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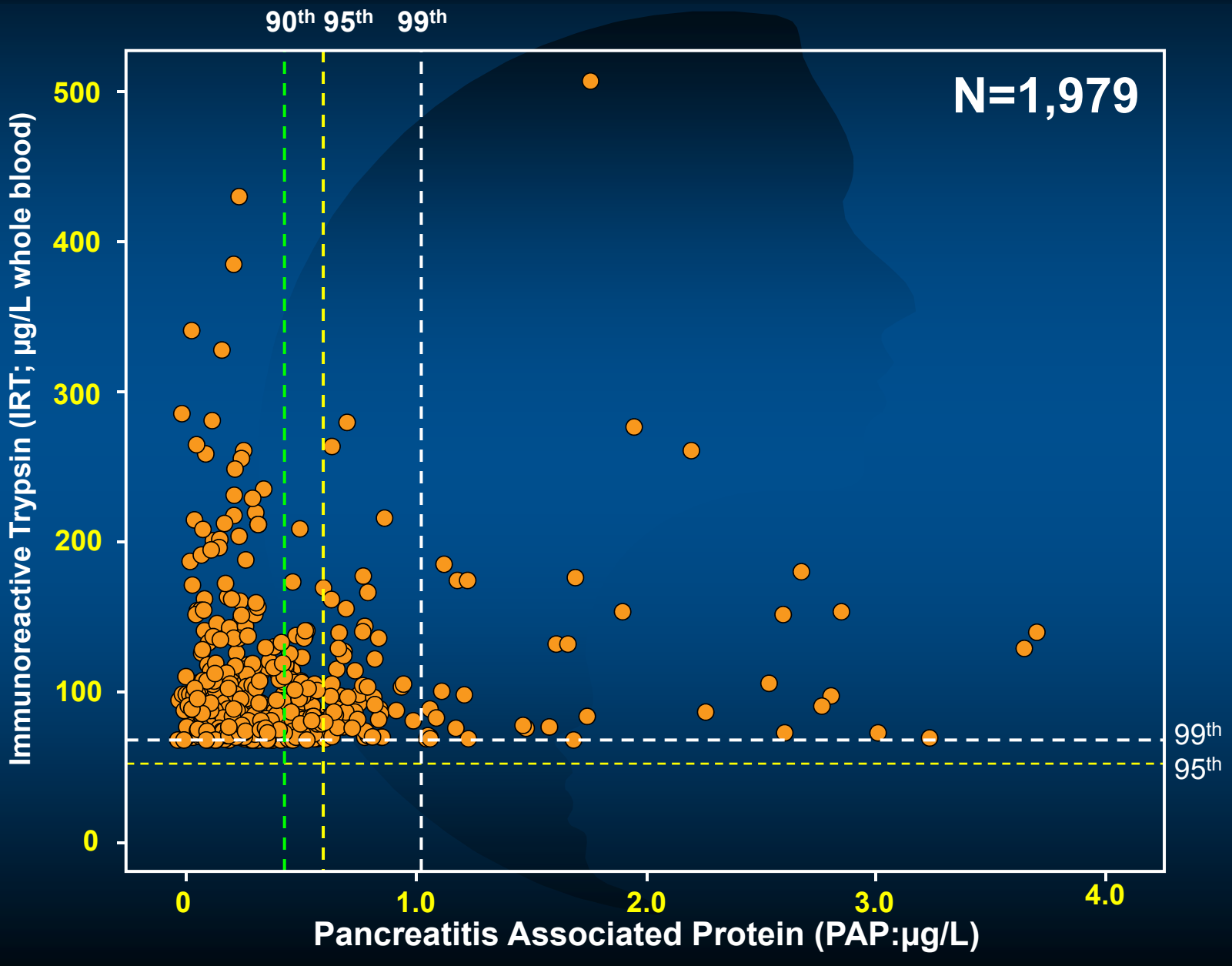
Phase II: IRT versus PAP



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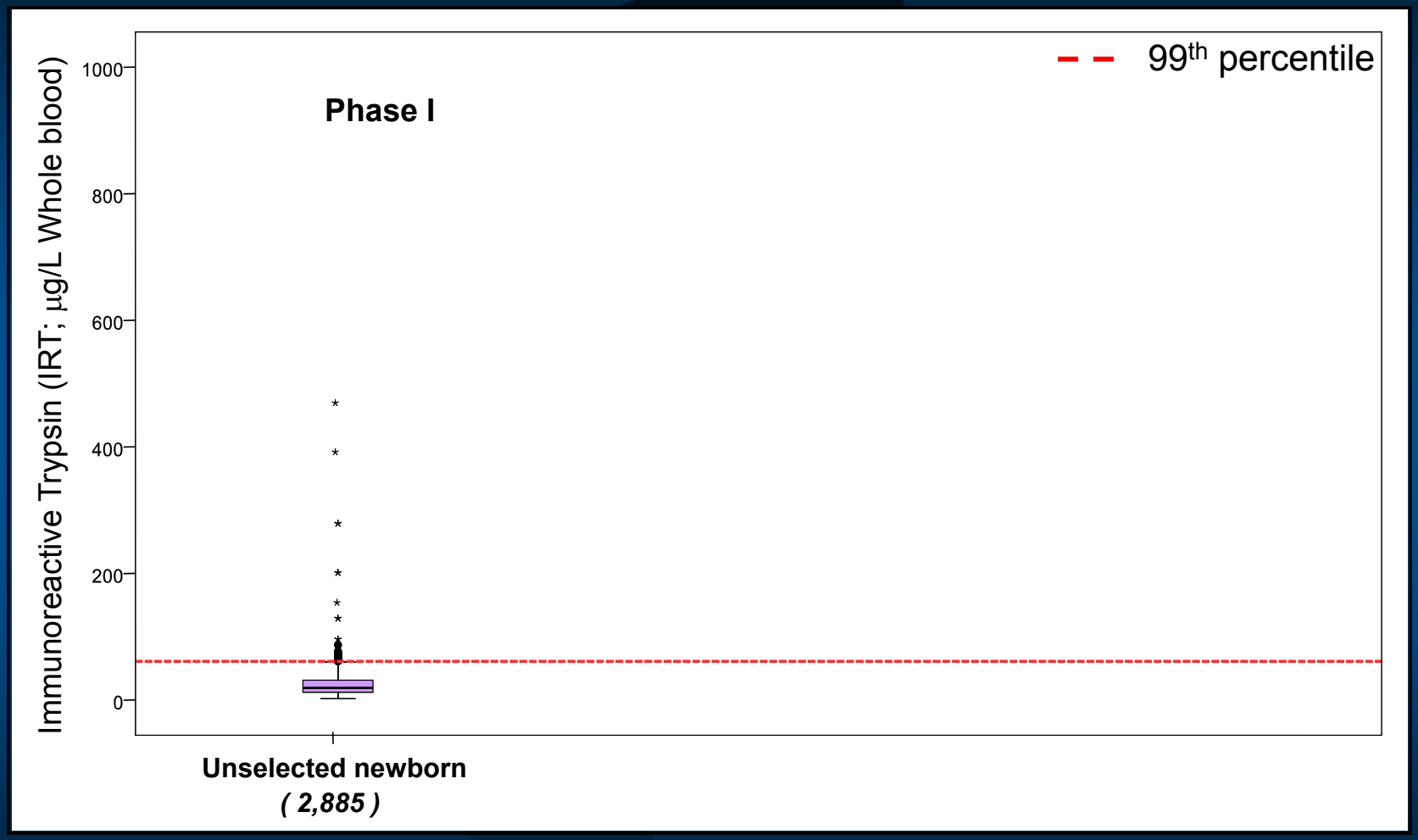
IRT comparison between different groups



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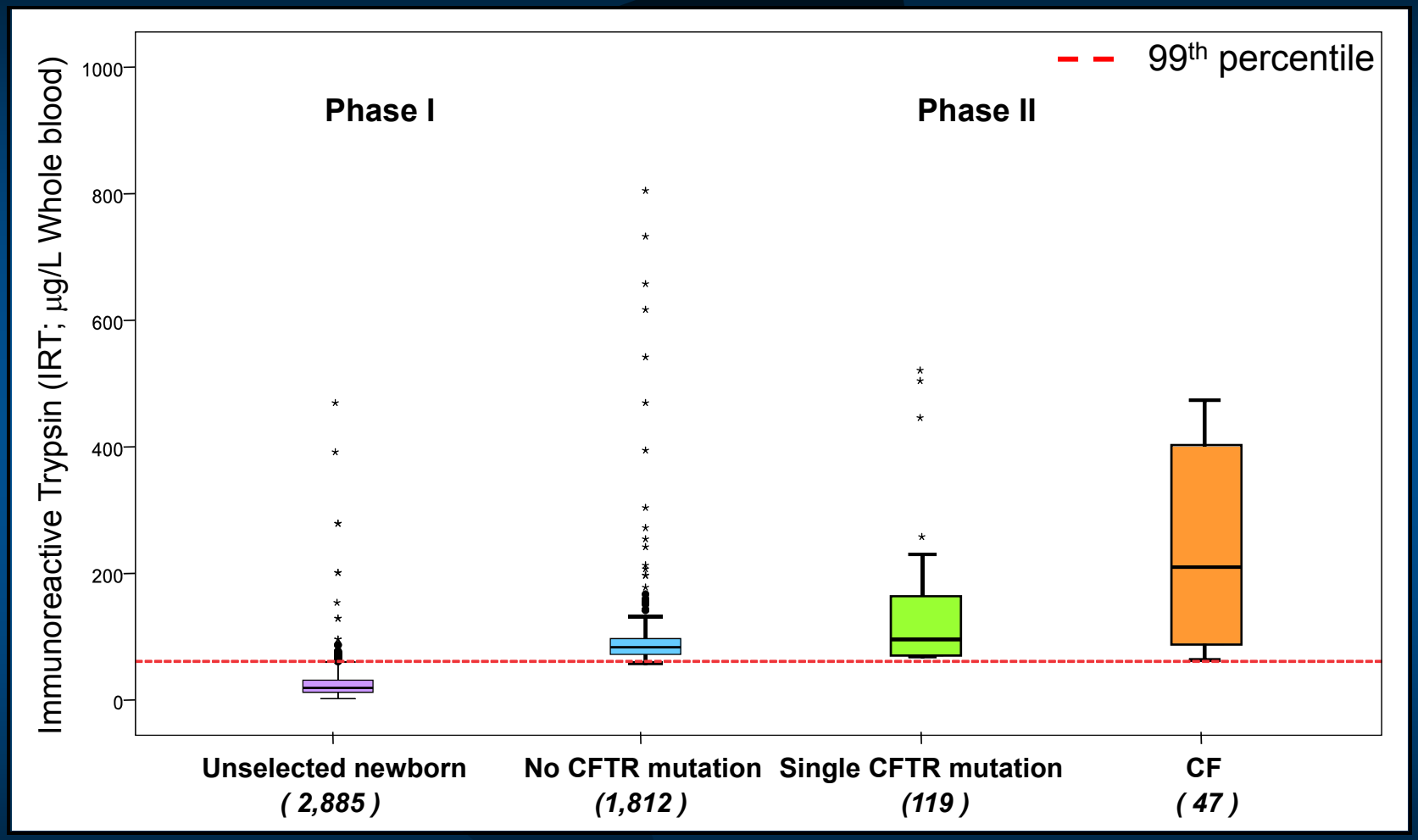

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SA PATHOLOGY

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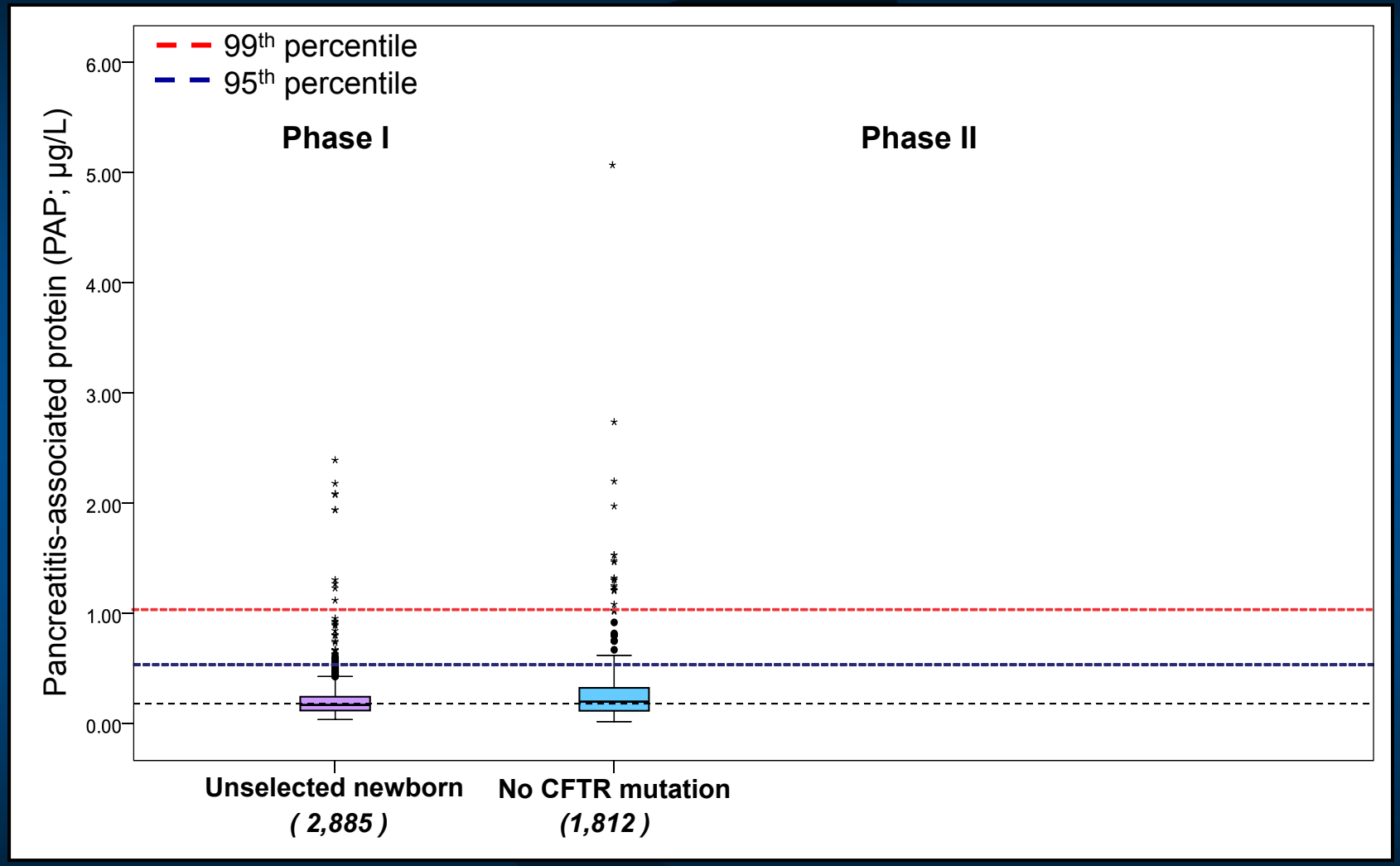

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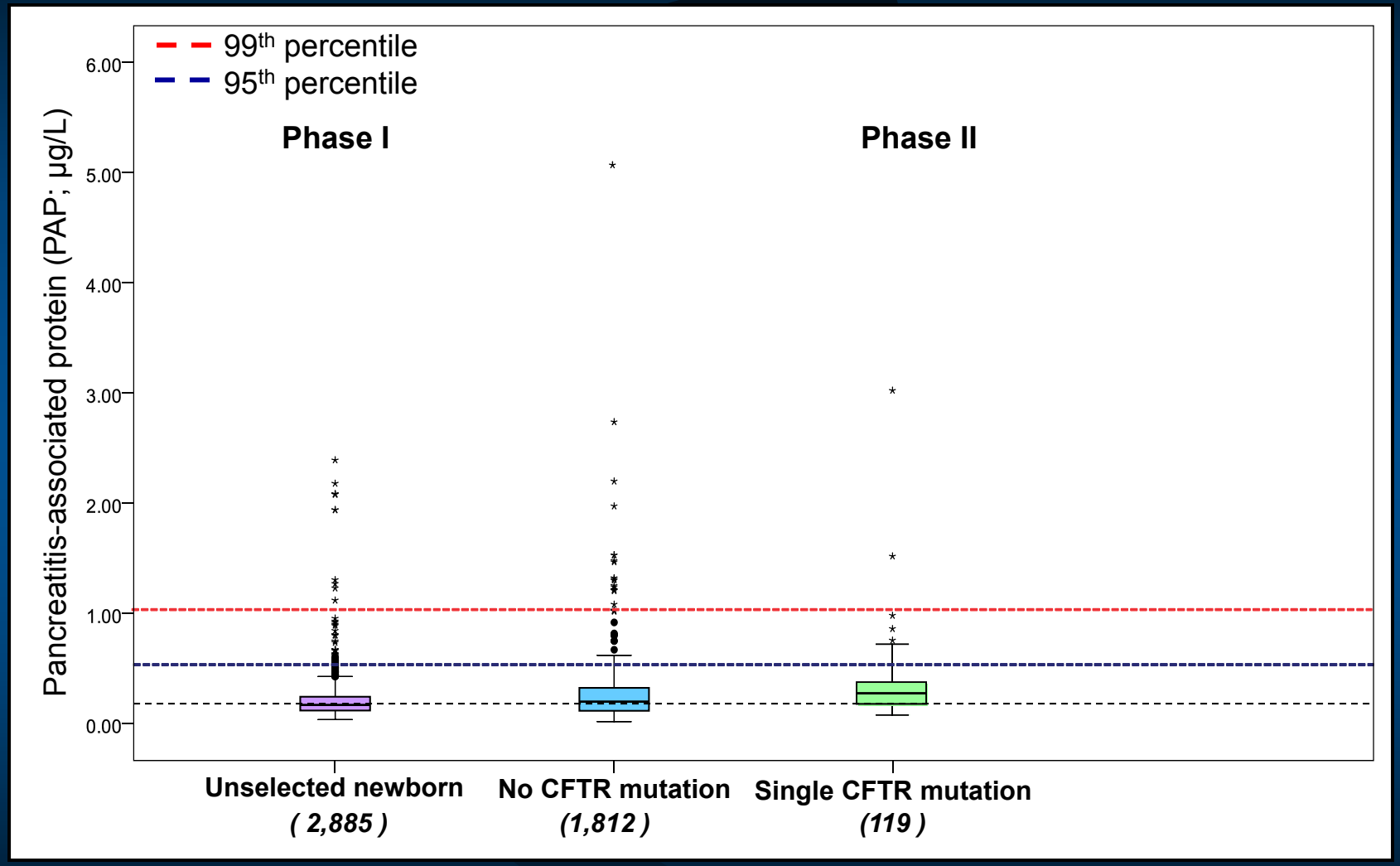

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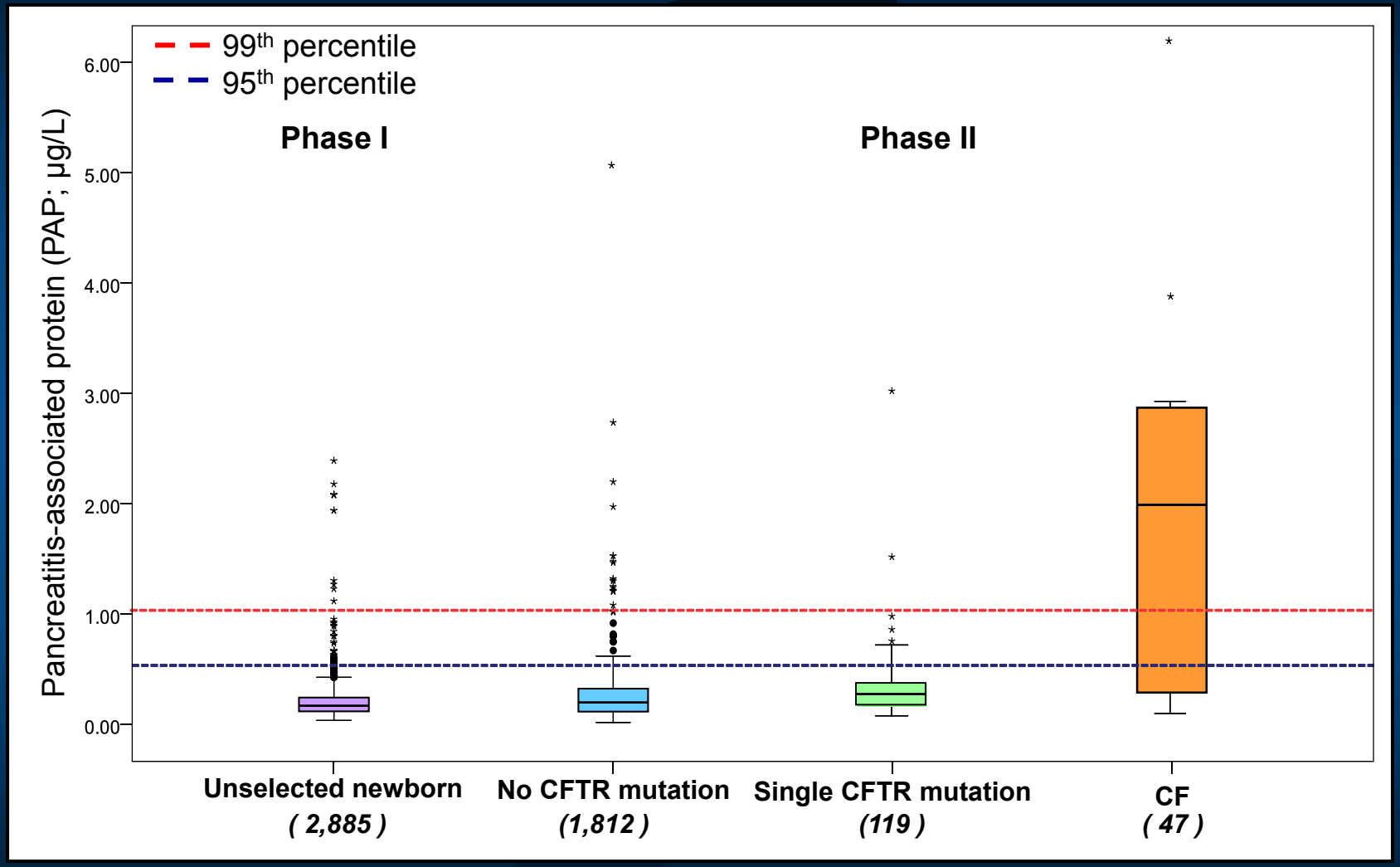

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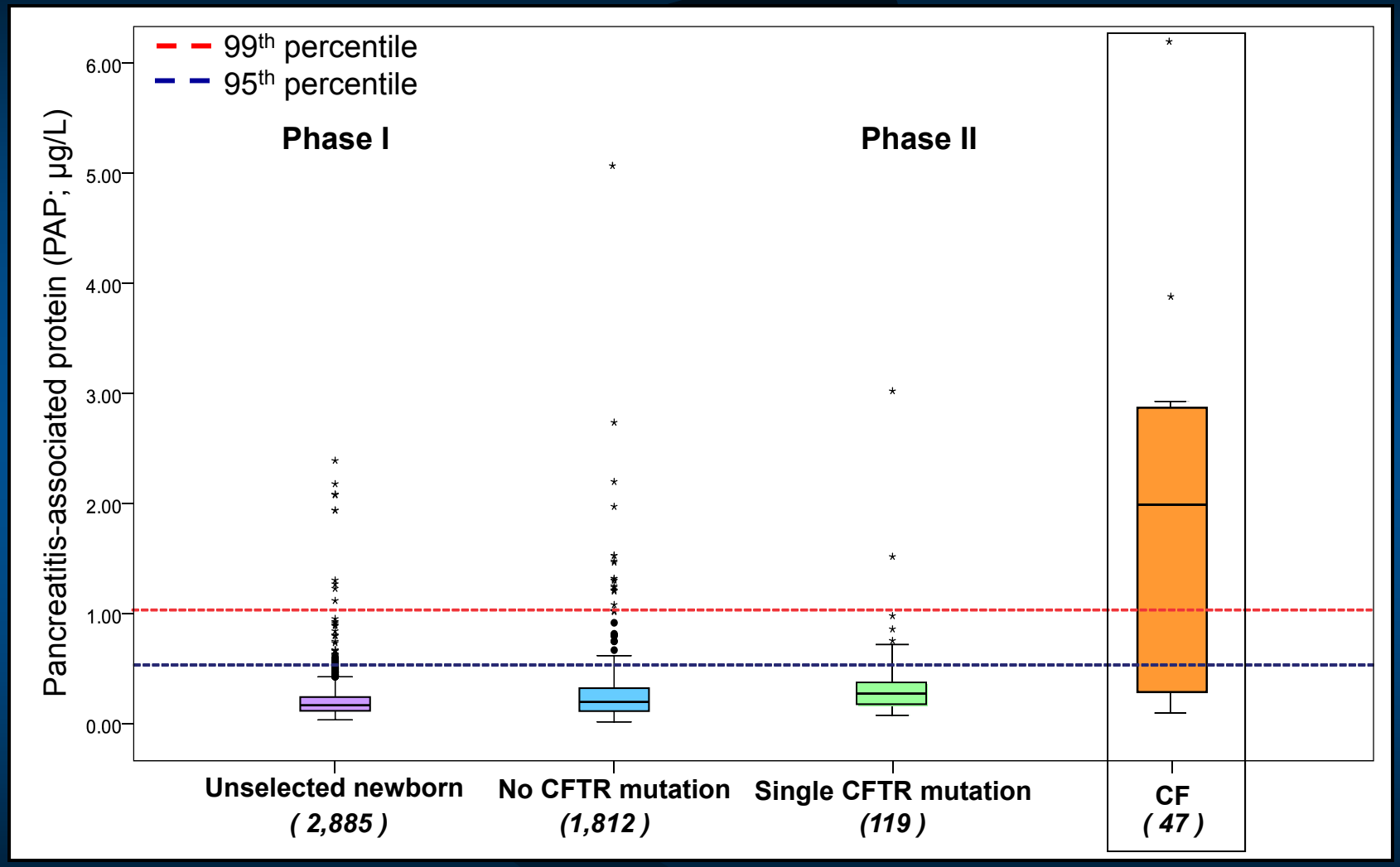

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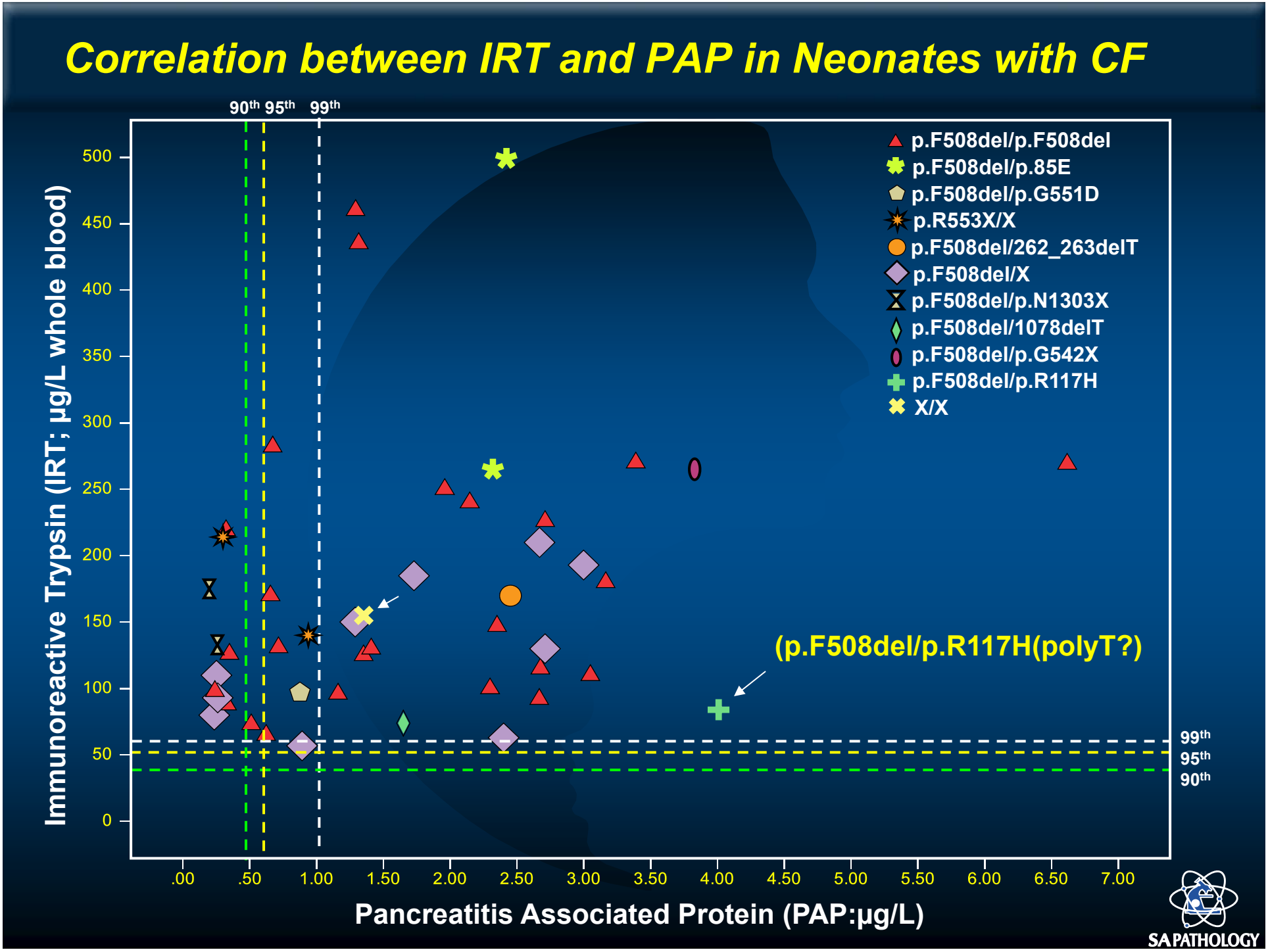
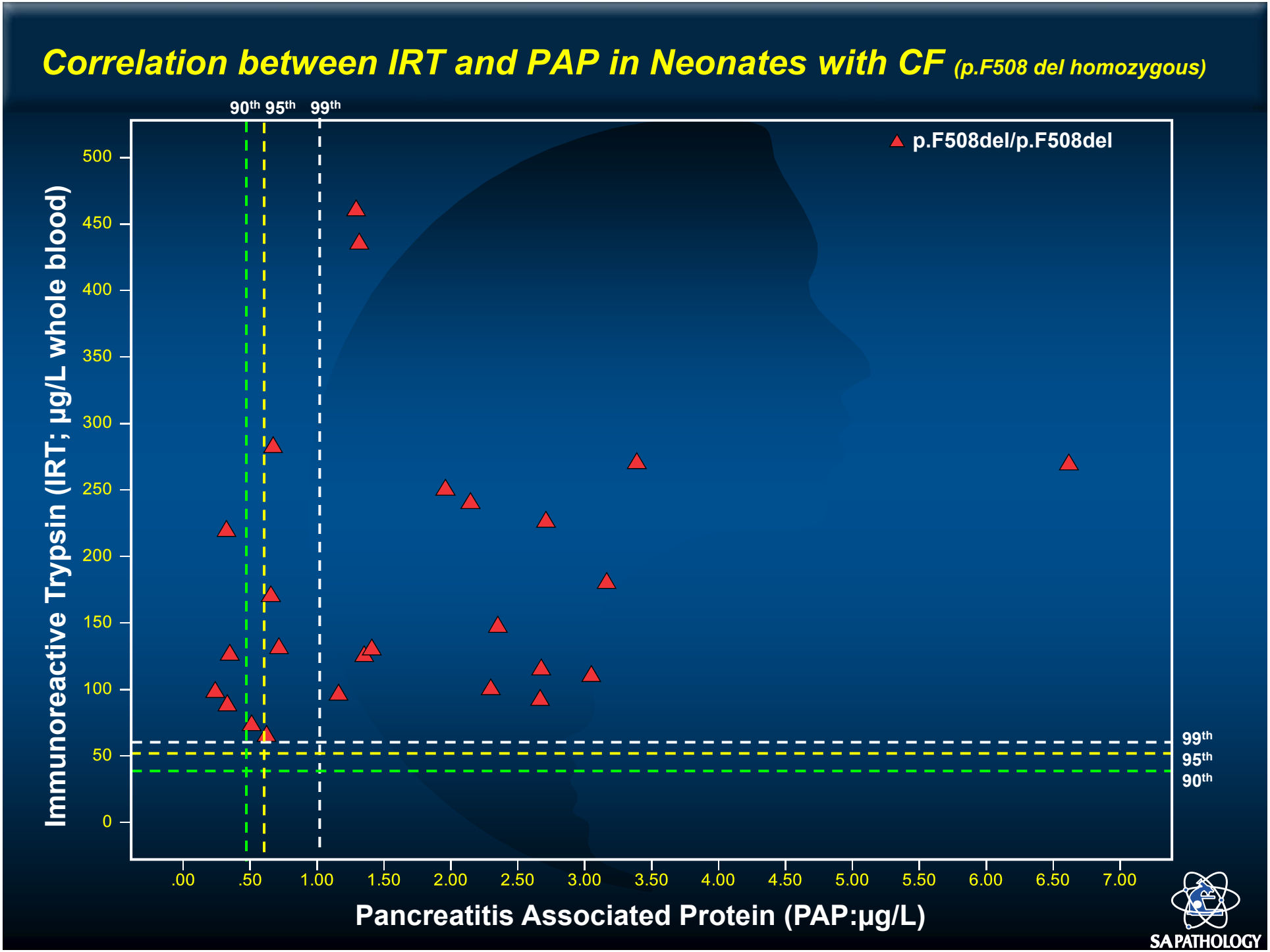
Correlation between IRT and PAP in Neonates with CF



Correlation between IRT and PAP in Neonates with CF (p.F508 del homozygous)



SA PATHOLOGY

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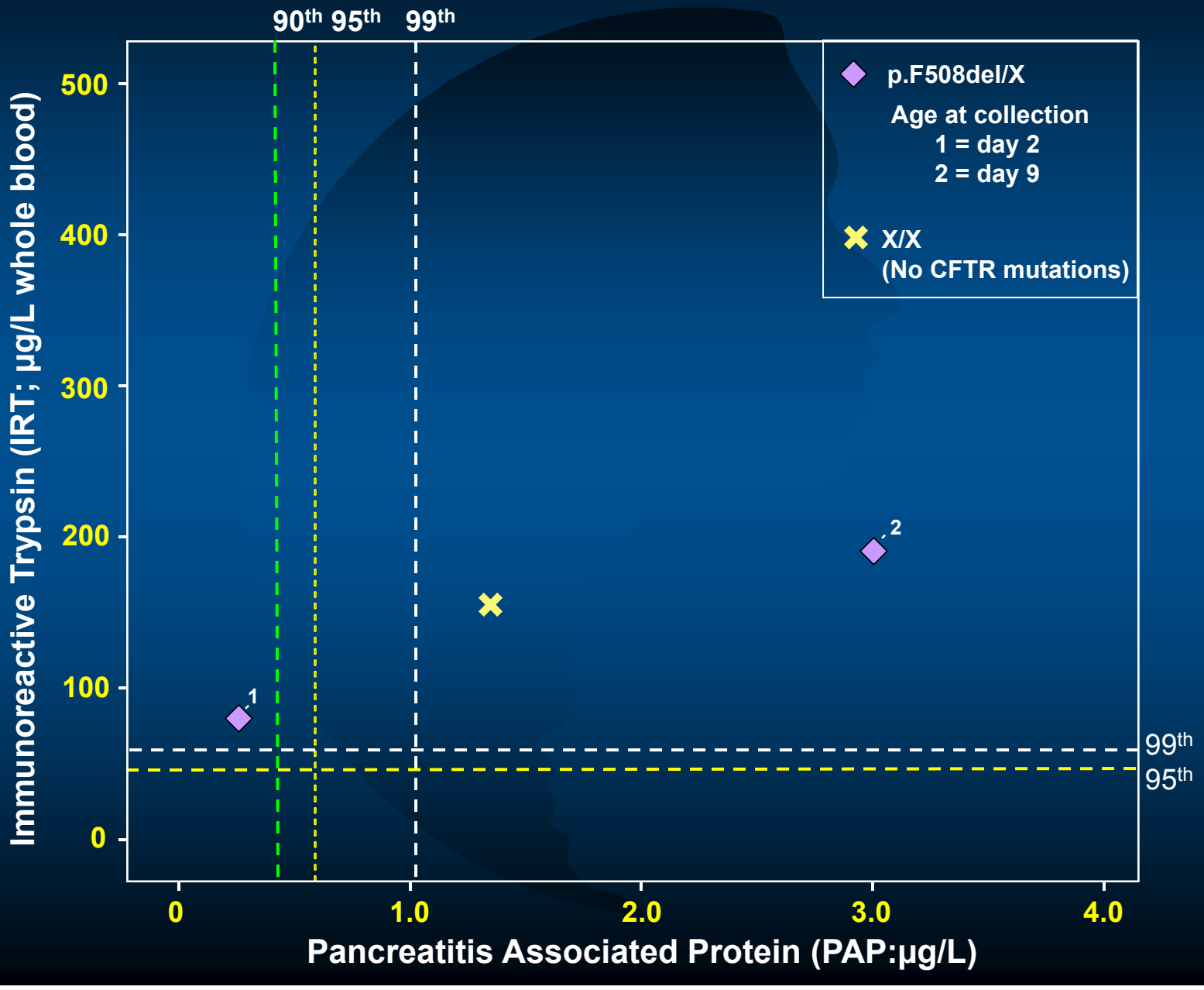

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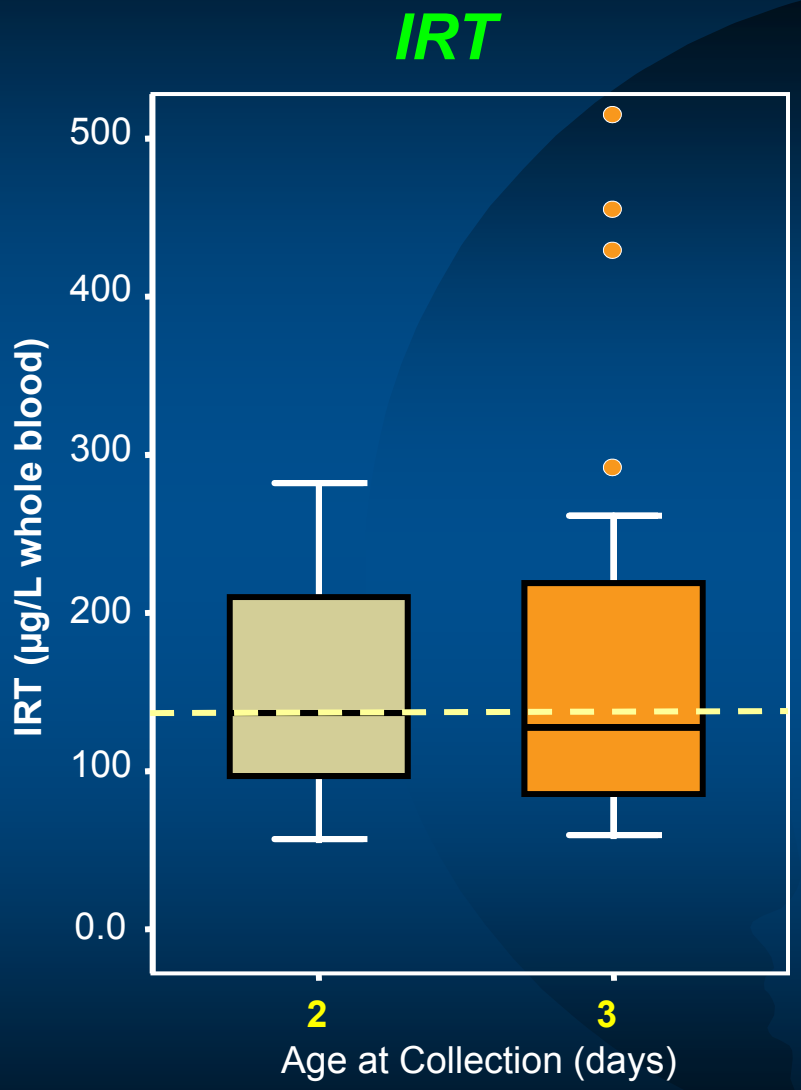
IRT and PAP in Infants with CF at Age of Collection



(not statistically significant at the p<0.05 level)

(Non-parametric K-W median test)

SA PATHOLOGY

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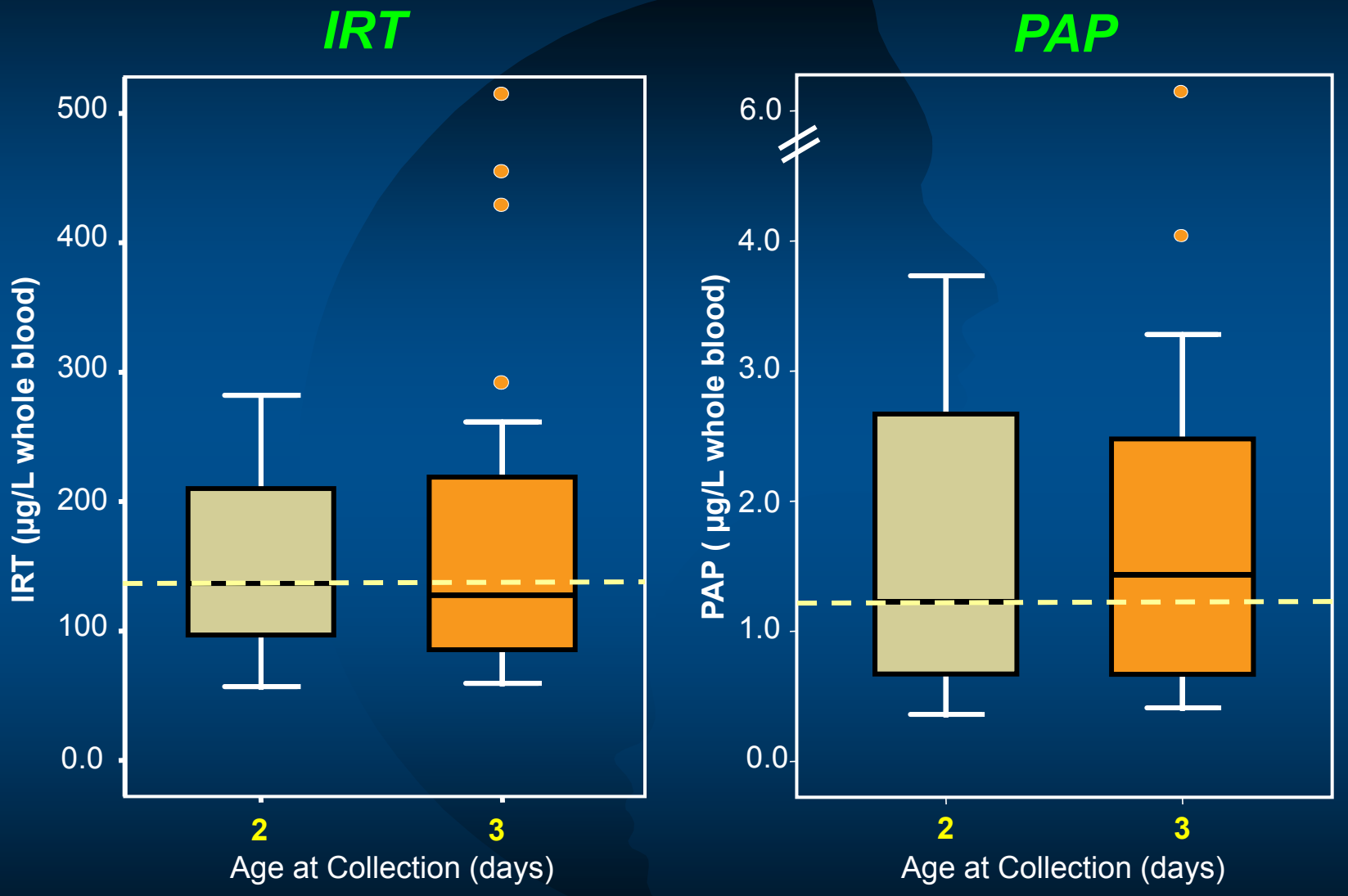

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(Non-parametric K-W median test)

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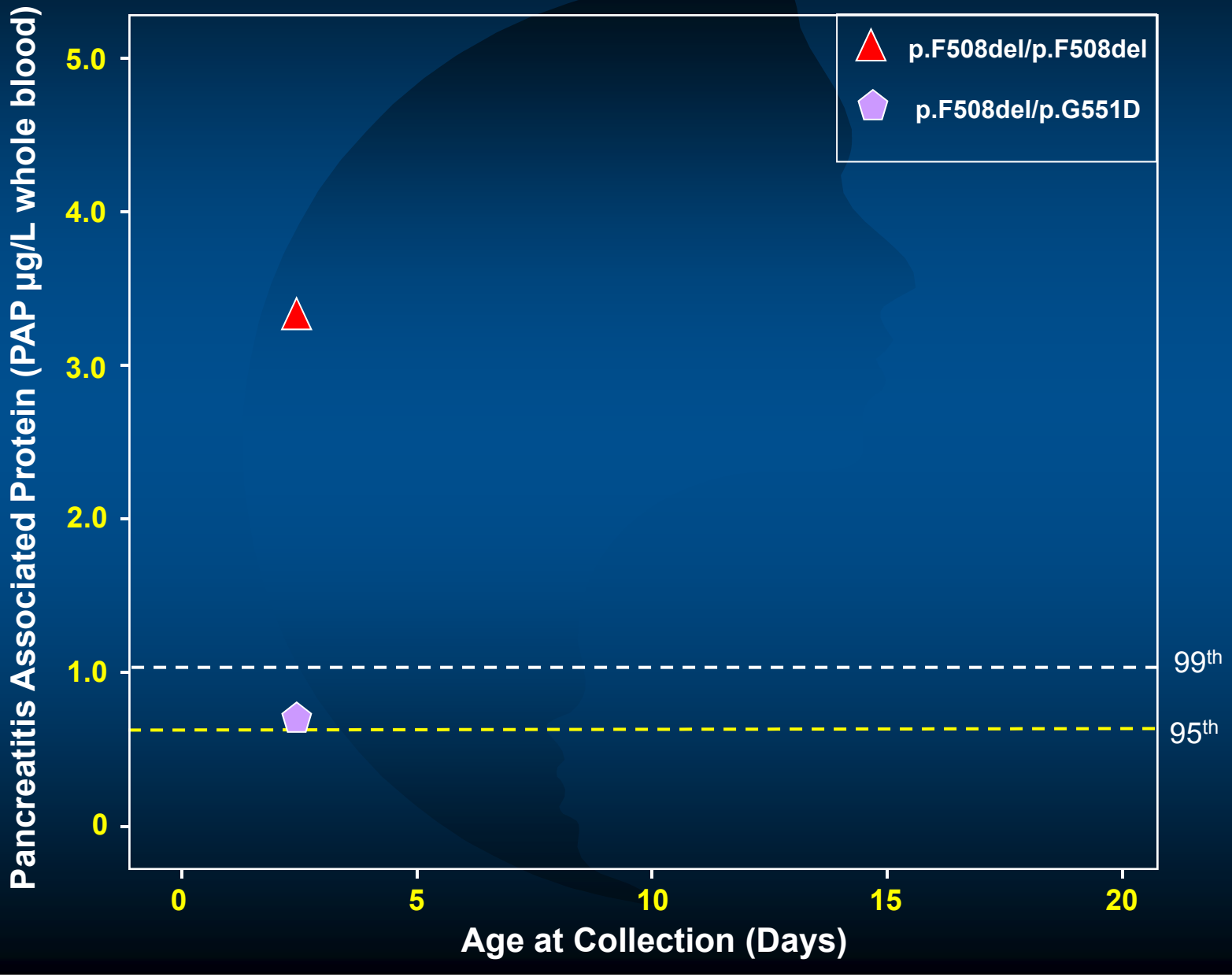
Additional studies of PAP level in Neonates with CF



SA PATHOLOGY

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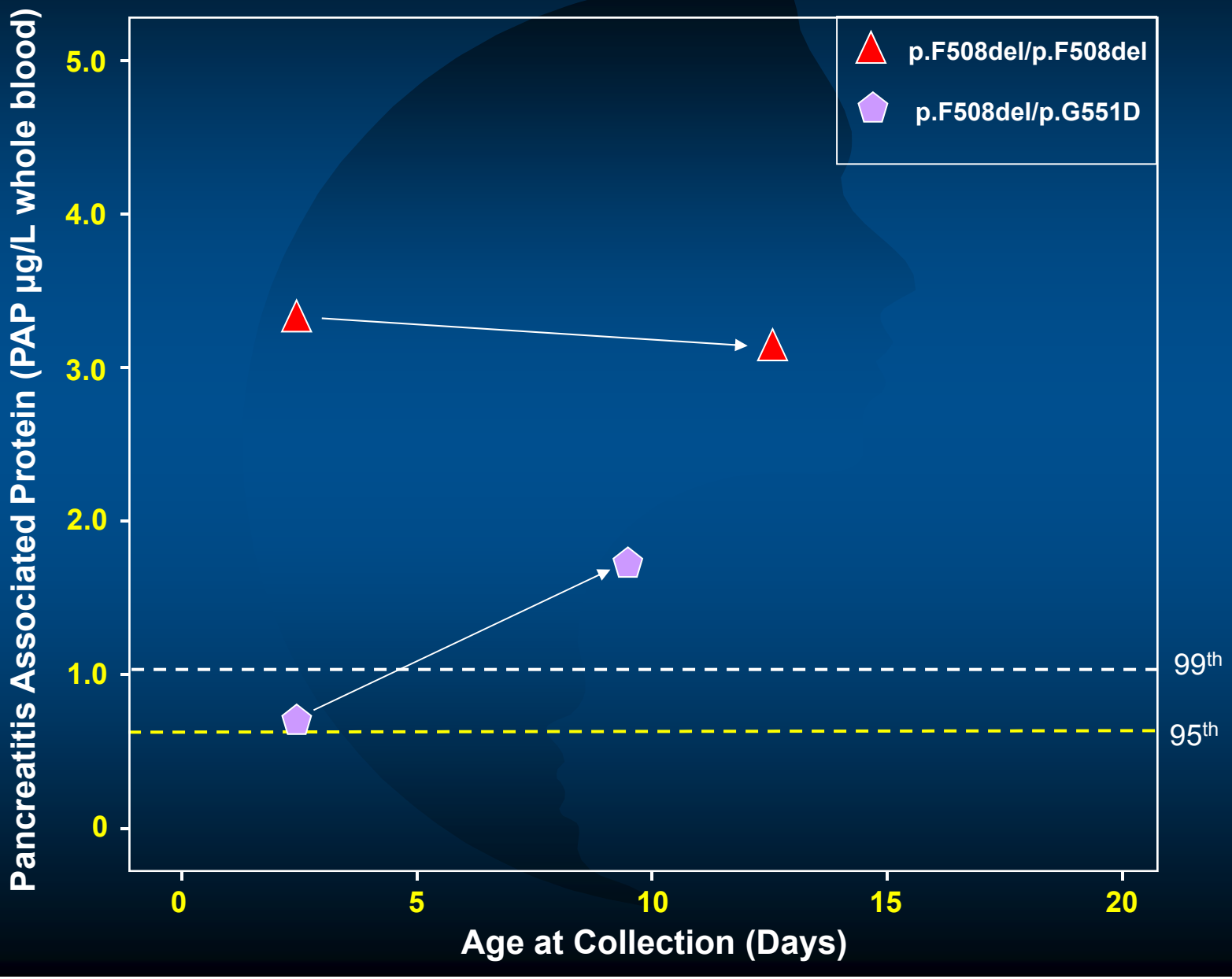

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IRT/DNA versus IRT/PAP/DNA

Total	1,978 *From a projected newborn screened population of ~195,000*	
Analyte	**IRT ≥ 99th percentile**	**PAP≥ 95th percentile**
CFTR Carrier	**119**	**25**
CFTR carrier Frequency	**1 in 16**	**1 in 80**
CF	**47**	**CFTR genotype**
	25	p.F508del/p.F508del
	2	p.F508del p.85E
	1	p.F508del p.G542X
	1	p.F508del p.G511D
	10	p.F508/X
	2	p.R553X/X
	1	p.F508 del/p.262 263delT
	2	p.F508del/p.N1303K
	1	p.F508del/p.R1157H
	1	p.F508del/1078delT
	1	X/X
Detected	**46**	**37**
Missed by primary analyte	**1**	**10**
	1 X/X	4 p.F508del/p.F508del 2 p.F508del/p.N1303K 3 p.F508/X 1 p.R553X/X

SA PATHOLOGY

*? Better discrimination by using a product of IRT */ PAP*

Evidence that IRT and PAP are independant markers of CF

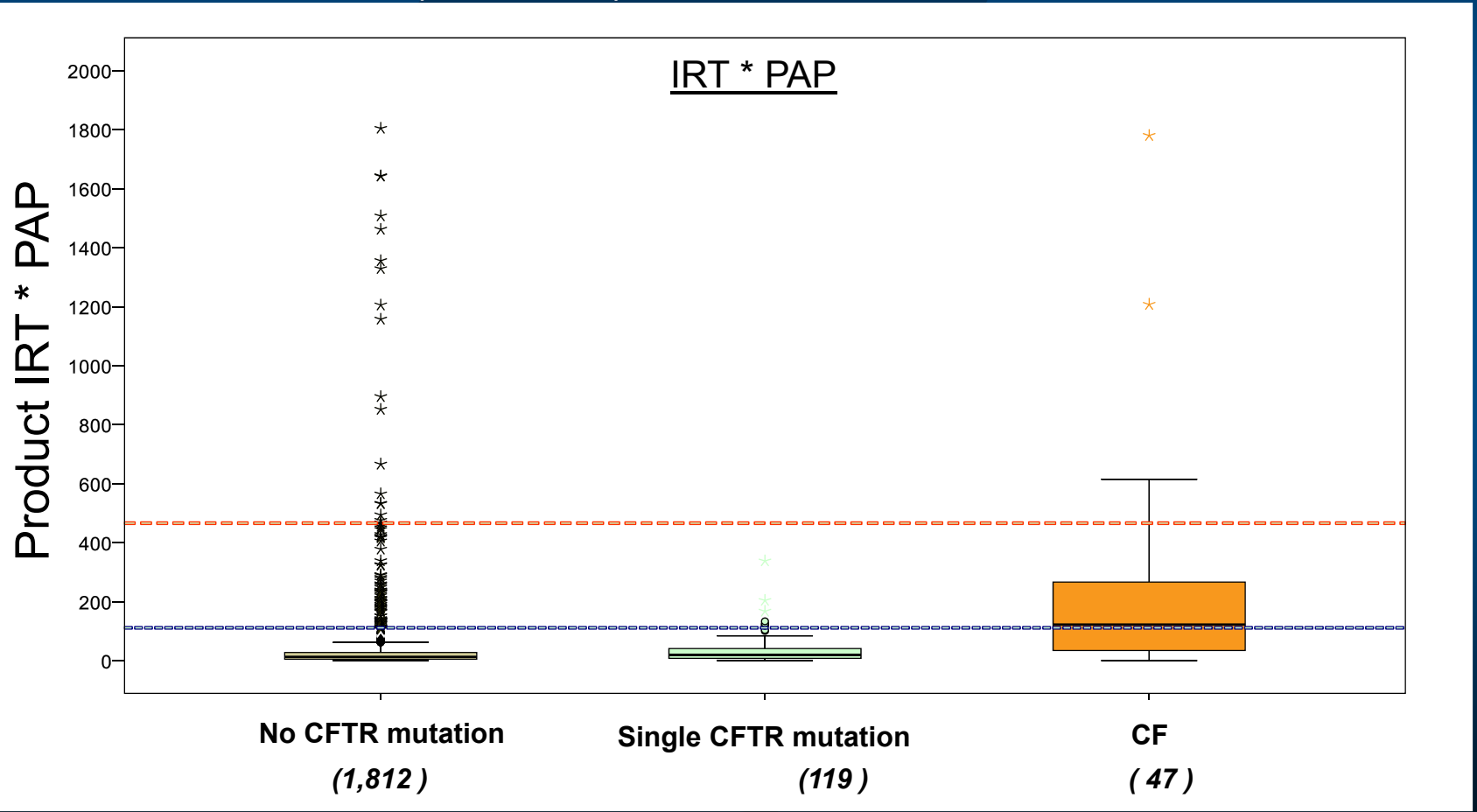
- Combination of IRT & PAP may provide better discrimination
  - IRT * PAP
  - IRT – (IRT * PAP) (Proposed by M Stopsack, Dresden Germany at the 7th ISNS, August 2011)

SA PATHOLOGY

? Better discrimination by using a product of IRT */ PAP

Given that IRT and PAP show independence as markers of CF

- Combination of IRT & PAP may provide better discrimination
  - IRT * PAP
  - IRT – (IRT * PAP) (Proposed by M Stopsack, Dresden Germany at the 7th ISNS, August 2011)



SA PATHOLOGY

Summary

- PAP in dried whole blood-spots:
  - Elevated in a percentage of sick-preterm infants.
  - Independent of IRT level in non-CF infants.
  - No discernable correlation with either birth weight or age at collection in normal (non-CF) infants.
  - Levels decline on storage at room temperature.
  - Levels appear to increase over time in infants with CF

SA PATHOLOGY

Summary

- Phase II Clinical Study…..*ongoing*
  - ✓ PAP reduces the number of infants identified as a CFTR carrier
    - ✓ For p.F508del **1 in 80** versus the **1 in 16** as seen with IRT≥99th centile.
      - ✓ Reduce the number of sweat-tests performed.
      - ✓ Likely to reduce the number with equivocal sweat test and mild “CF” disease.
      - ✓ Reduce cost of sweat-testing

SA PATHOLOGY

Summary

- Phase II Clinical Study…..*ongoing*

  - ✓ PAP reduces the number of infants identified as a CFTR carrier
    - ✓ For p.F508del **1 in 30** versus the **1 in 13** as seen with IRT≥99th centile.
      - ✓ Reduce the number of sweat-tests performed.
      - ✓ Likely to reduce the number with equivocal sweat test and mild "CF" disease.
      - ✓ Reduce cost of sweat-testing
  - ✓ Evidence that an elevation of PAP in infants with CF is independent of both the IRT & CFTR genotype.
  - X PAP is elevated in most infants with CF.
    - **BUT a significant number of infants with CF have a PAP <90th centile on samples collected at 2 days of age.**

SA PATHOLOGY

Summary

- PAP in dried blood-spots:
  - This study does ***not*** support the clinical utility of adding PAP to our single-sample IRT/DNA protocol, given our early age of sample collection (<48 hours).

SA PATHOLOGY

Summary - continued

- CF Programmes may find PAP *useful*-
  - that collect samples at a later age, ? >72 hours of age
  - that adopt a 2nd specimen screening strategy
- CF Programmes are *unlikely* to find PAP useful-
  - That collect a single sample at or near 48 hours, (optimal for MSMS screening)
- A complex algorithm would be required to develop an IRT/PAP/DNA CF screening strategy

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Acknowledgment

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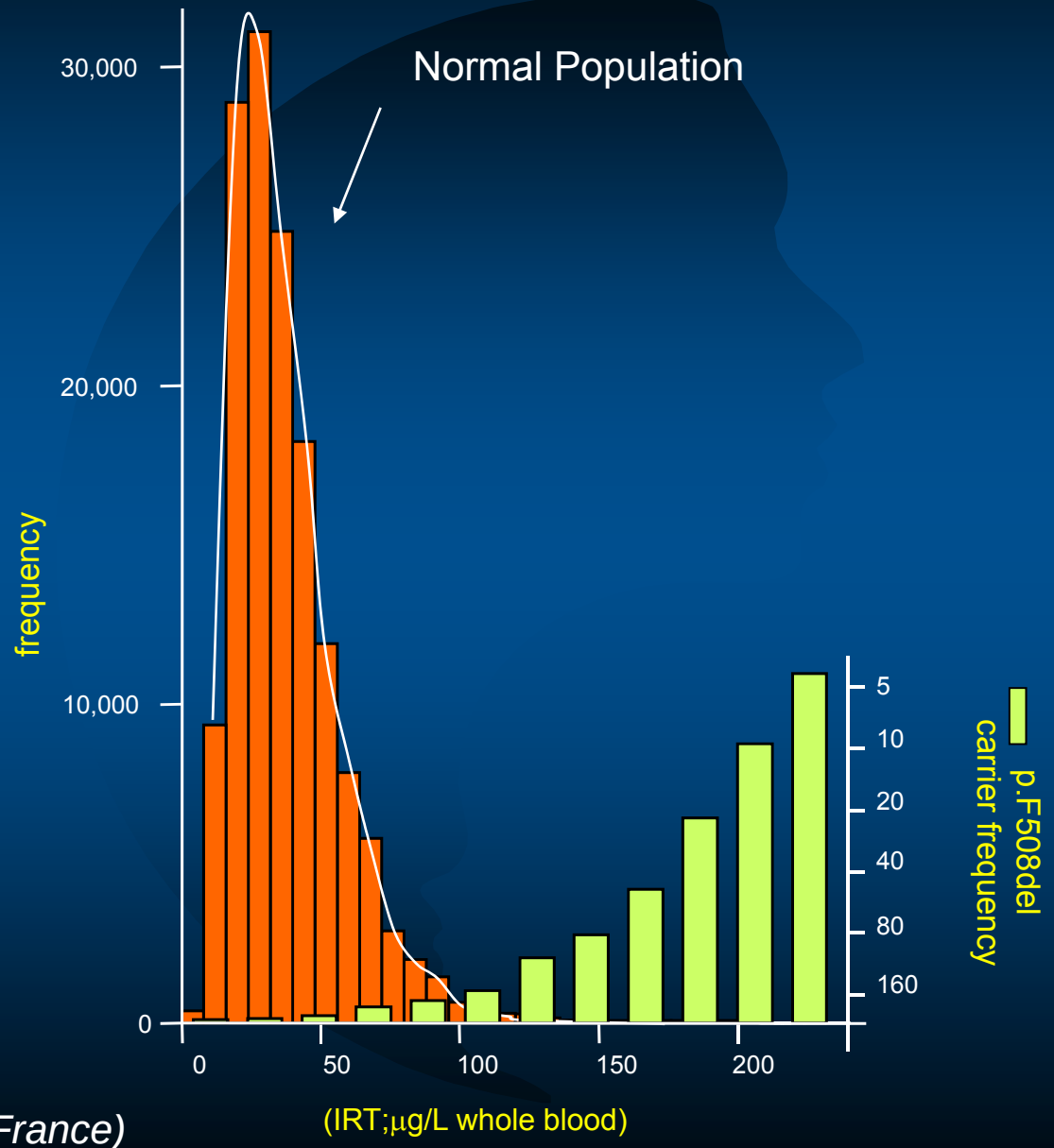
p.F508del carrier frequency in elevated IRT



(G Travert, Caen France)

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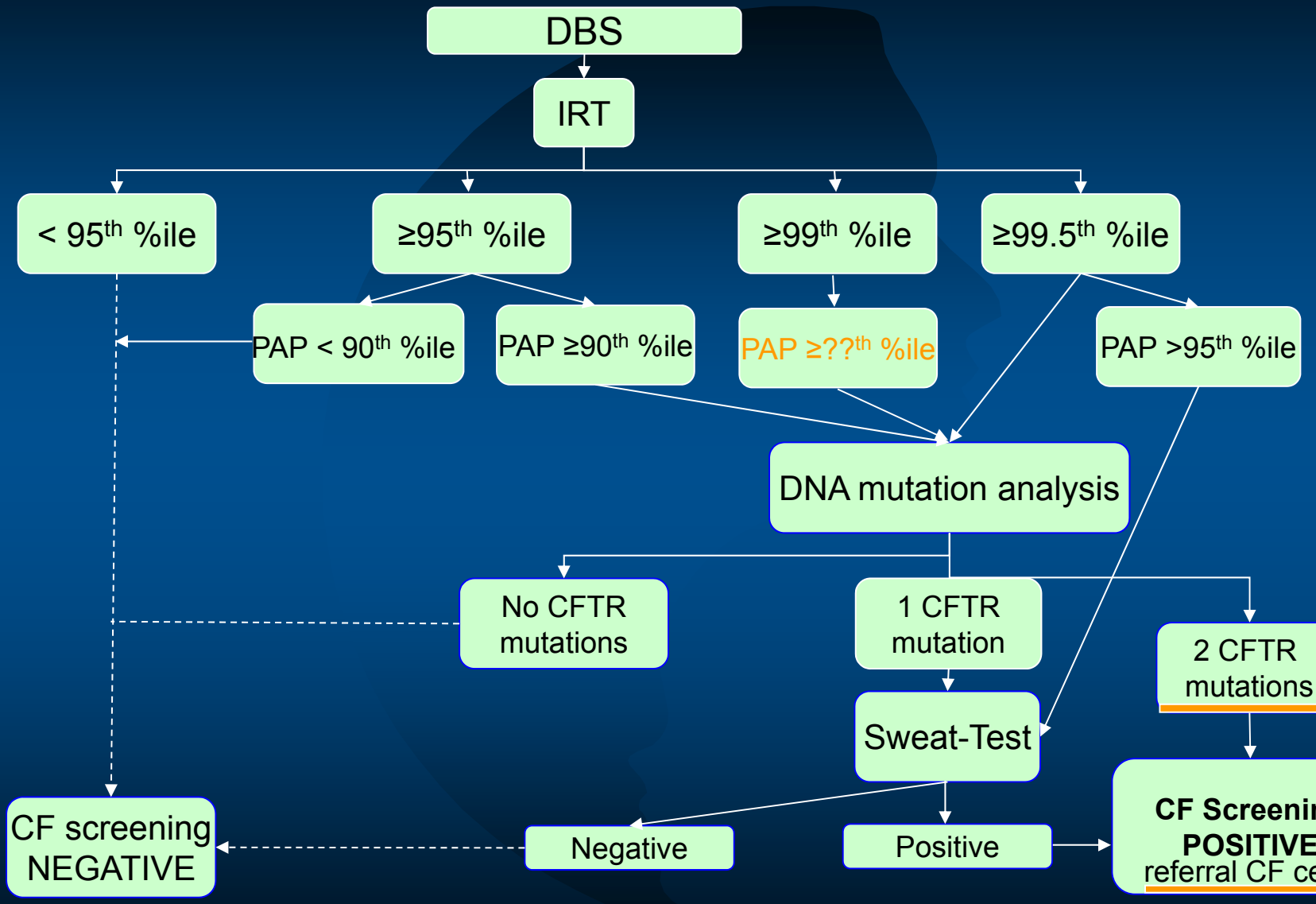
A possible IRT/PAP/DNA CF Screening Strategy?



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