A vertical graphic on the left side of the slide, shaped like a DNA double helix. It consists of two intertwined lines, one blue and one green. Inside the loops of the helix are several small images: a newborn baby, the Wisconsin State Capitol dome, a test tube with red liquid, a classical building facade, a night scene with fireworks, and a cow's head.

# **Newborn Screening for Ornithine Transcarbamylase Deficiency using Orotic Acid**

**Patrice Held, PhD, FACMG**

**Co-Director, Wisconsin Newborn Screening &**

**Director, Biochemical Genetics Laboratory,**

**Wisconsin State Laboratory of Hygiene**

**Associate Professor, Dept of Pediatrics, University of Wisconsin-Madison**



# Ornithine Transcarbamylase Deficiency

## ❑ Most common urea cycle defect

- ❑ X-linked
- ❑ Incidence of 1 in 14,000 individuals

## ❑ Clinical Presentation

- ❑ Severe neonatal onset with hyperammonemia, encephalopathy, and respiratory alkalosis
- ❑ Later onset with neurologic impairment, developmental delay, psychiatric symptoms, liver disease
- ❑ Female carriers can also experience symptoms related to elevated ammonia levels

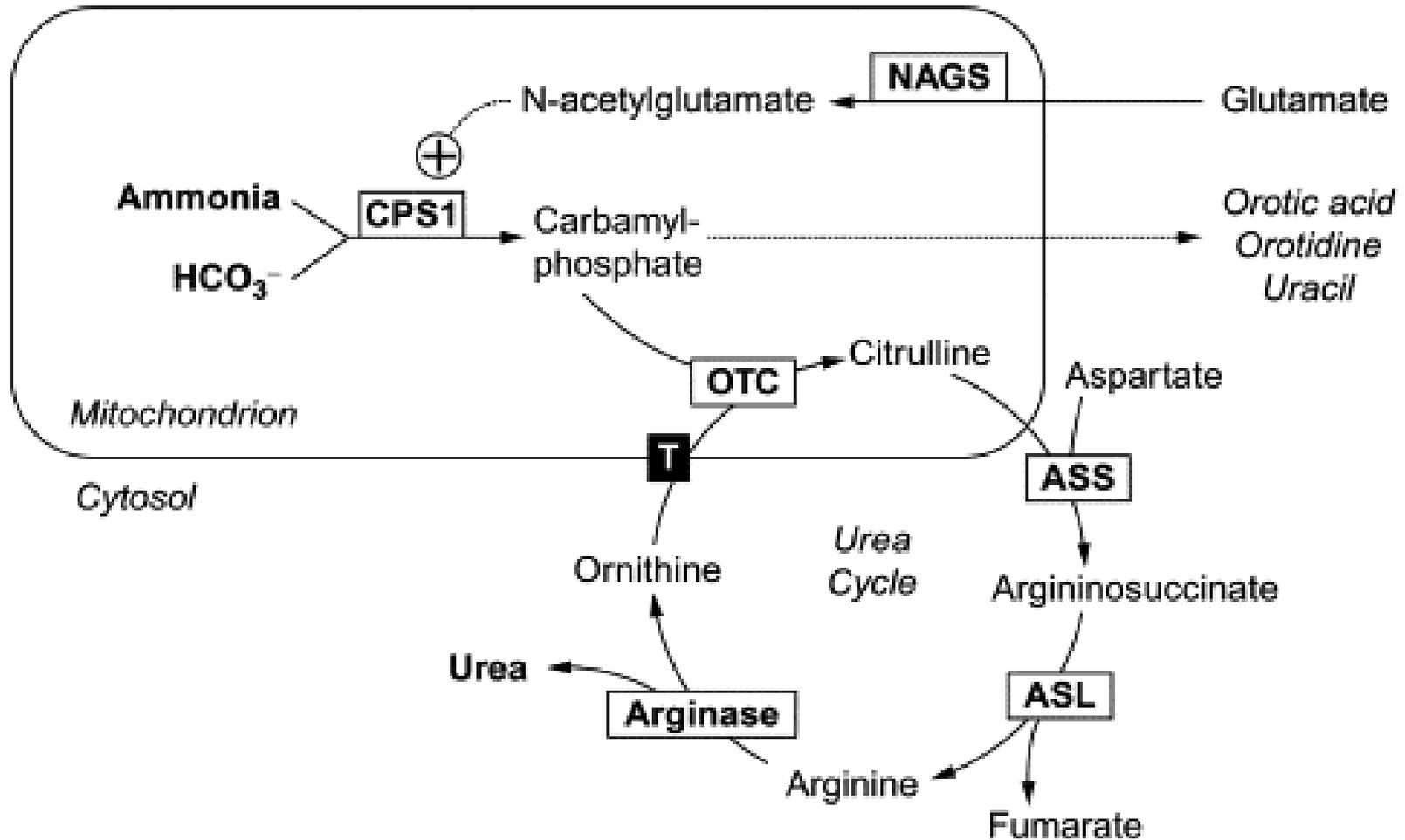
## ❑ Laboratory Indicators for OTC deficiency

- ❑ Elevated ammonia, Elevated glutamine
- ❑ Low concentrations of citrulline
- ❑ Increased excretion of orotic acid

## ❑ No single analyte has been implemented to detect cases of OTC deficiency by NBS laboratories



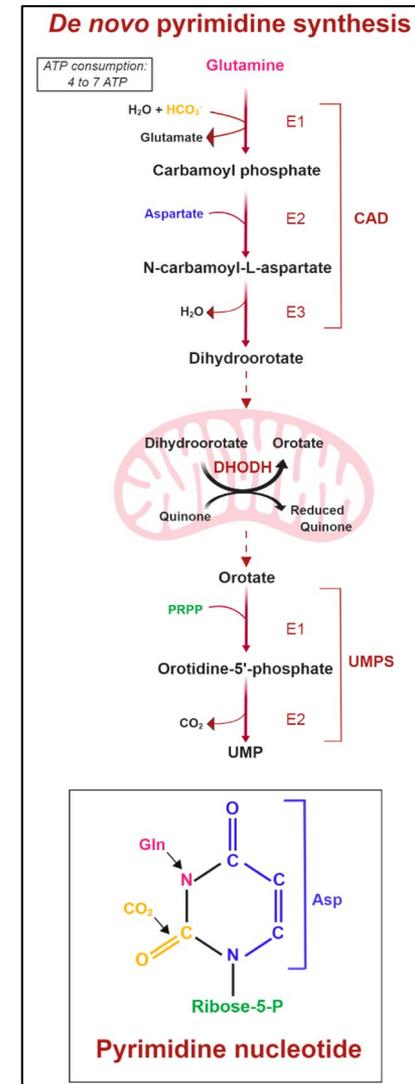
# Urea Cycle





# Orotic Acid

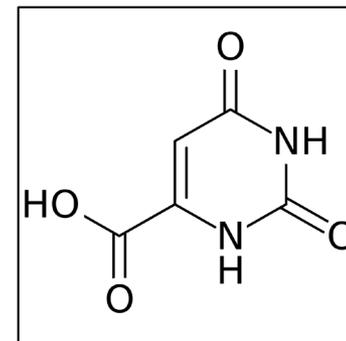
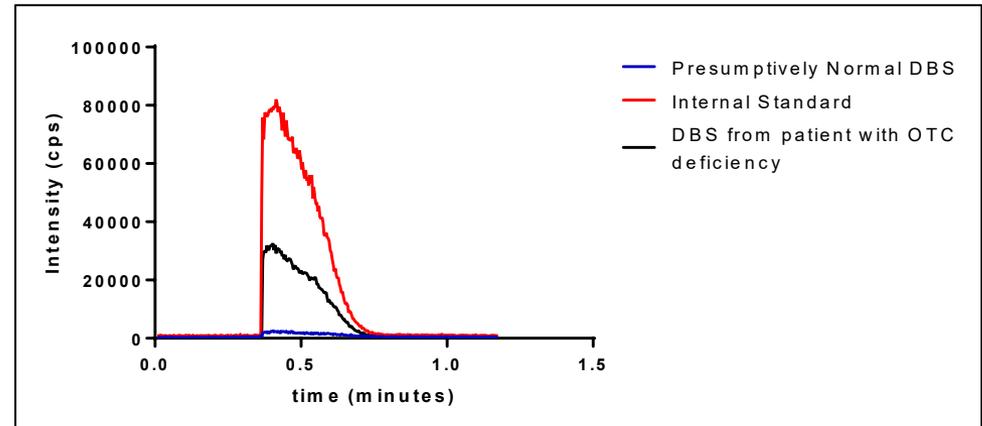
- ❑ Orotic acid is increased due to the accumulation of carbamoyl phosphate when there is a mismatch between the fluxes through carbamoyl phosphate synthetase and the urea cycle steps.
- ❑ Carbamoyl phosphate enters the pyrimidine nucleotide synthesis pathway leading to markedly increased concentrations of orotic acid
- ❑ Orotic acid can be quantified in urine or plasma using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods.





# Assay to Measure Orotic Acid

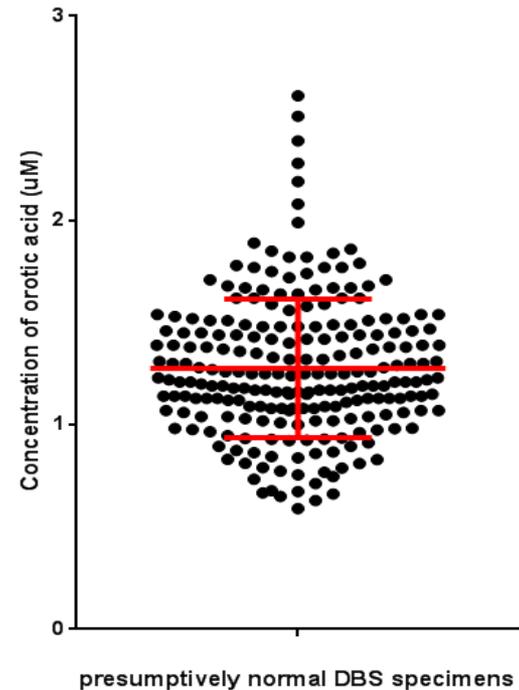
- ❑ 3mm DBS punch
- ❑ Extracted with **80:20 acetonitrile and water** containing orotic acid internal standard [1, 3-  $^{15}\text{N}_2$ ]
- ❑ **30 minute** extraction at ambient temperature
- ❑ FIA-MSMS using API4000 system in **negative mode**
- ❑ Mobile phase 80:20 acetonitrile and water containing 0.02% ammonium hydroxide
- ❑ MRM pairs 154.9→110.9 and 156.9→112.9
- ❑ **One minute analysis time**





# Assay Validation

- ❑ Precision across three control levels
  - ❑ Inter-assay CV <15%
- ❑ Limit of Quantification
  - ❑ 1 $\mu$ M with a CV of <10%
- ❑ Linear through 40 $\mu$ M enrichment
- ❑ Recovery from DBS was approximately 60%
- ❑ Evaluation of the unaffected population
  - ❑ Mean 1.28 $\mu$ M
  - ❑ standard deviation 0.34 $\mu$ M
  - ❑ cutoff 2.3 $\mu$ M





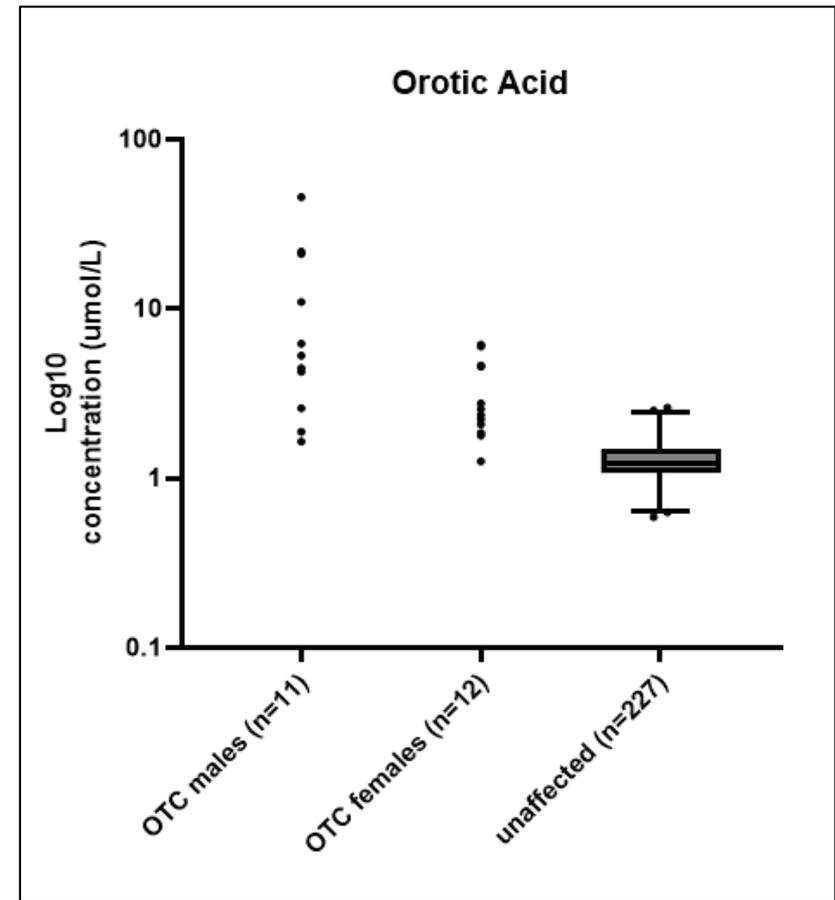
# Initial Cohort of Patients

patient	Disease	Age at Collection	Sex	citrulline ( $\mu\text{M}$ )	orotic acid ( $\mu\text{M}$ )
1	OTC	32 hours	M	5	11.00
2	OTC	52 hours	M	5	21.80
3	ASS	18 days	F	2796	71.10
4	ASS	4 days	M	1205	38.30
5	ASL	32 hours	M	130	2.58
6	ASL	6 days	M	199	7.52
7	ASL	3 days	F	107	10.60



# Additional Patients

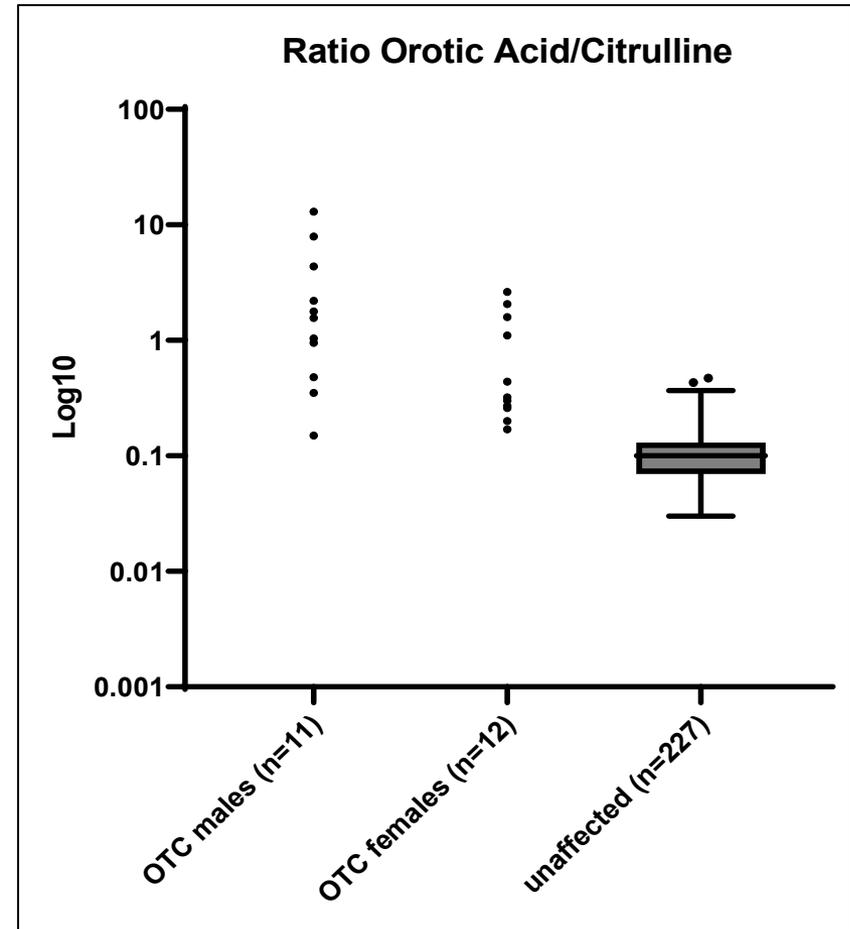
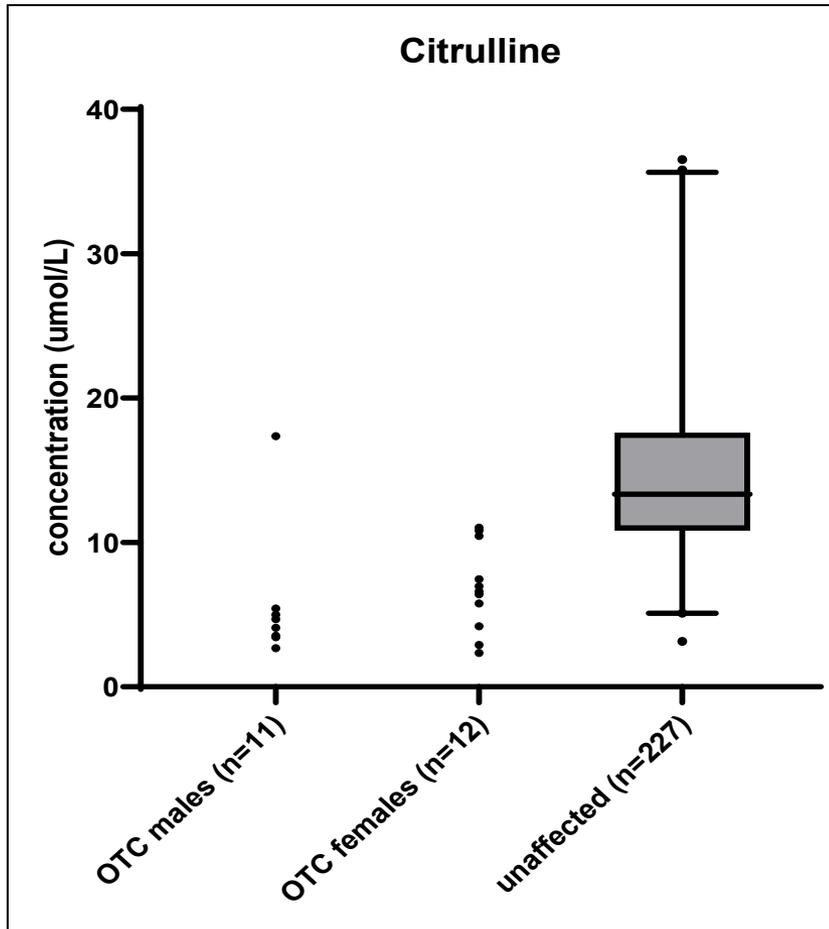
- ❑ **11 males with OTC deficiency**
  - ❑ Symptoms at birth: orotic acid ranged from 2.59 to 45.8 $\mu$ M
  - ❑ Symptoms at 8 months to 13 years: orotic acid ranged from 1.65 to 5.3 $\mu$ M
- ❑ **12 female with OTC deficiency**
  - ❑ Symptoms at birth: orotic acid 4.62 $\mu$ M
  - ❑ Symptoms at 8 months to 2 years: orotic acid ranged from 1.26 to 4.58 $\mu$ M
  - ❑ No Symptoms: orotic acid 5.99 $\mu$ M



newborn screening DBS specimens



# Additional Patients





# Conclusions

- ❑ Orotic acid can be measured in dried blood spots by LC-MSMS
- ❑ Orotic acid is ***NOT consistently*** elevated in NBS DBS collected from males with OTC deficiency, as compared to unaffected population
  - ❑ Severity of disease also does not greatly impact orotic acid concentrations
- ❑ Orotic acid is elevated in ***some, but not all*** NBS DBS collected from females with OTC deficiency



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**Wisconsin State  
Laboratory of Hygiene**  
UNIVERSITY OF WISCONSIN-MADISON