

Steven Keiles¹, Ruth Koepke², Richard Parad³, Martin Kharrazi², California CF Newborn Screening Consortium

¹Ambry Genetics, Aliso Viejo, California, ²California Department of Public Health, Richmond, California; ³Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

Background and Objectives

The diminishing splicing efficiency that is associated with increasing numbers (>11) of TG repeats in the CFTR IVS8-(TG)m(T)n haplotype results in decreasing production of functional CFTR protein.¹ We sought to determine whether the number of TG repeats at the (TG)m(T)5 locus of hypertrypsinogenemic (HT) infants with genotype ΔF508/5T (ΔF508 in *trans* with only a 5T allele and no other known CFTR mutation) correlated with biochemical [immunoreactive trypsinogen (IRT)] and physiological [sweat chloride (SC)] measurements. In addition, we explored the correlation between IRT and the IVS8 Poly T length (5T, 7T or 9T) when in *trans* with ΔF508-9T.

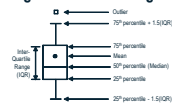
Methods

Subjects were identified during the first two years of CF newborn screening in California (CA) by the CA 4-step method:

- IRT (≥62 ng/mL, top 1.5%)
- CA-specific (29-40) CFTR mutation panel
- CFTR full gene sequence analysis utilizing scanning-sequencing technology for specimens found to have only 1 mutation detected in Step 2
- SC testing and follow up by CF Care Centers for infants with 2 or more mutations/variants, including 5T

Subjects were included in the ΔF508/5T cohort if they had one copy of ΔF508 detected during Step 2 and the IVS8 9T/5T genotype identified during Step 3. Because ΔF508 is almost always in *cis* with 9T,² ΔF508 was assumed to be in *trans* with the 5T and 7T alleles. The distributions of IRT, initial SC and highest SC were analyzed by (TG) length [(TG)11, (TG)12, (TG)13]. In a separate analysis, we compared IRT among HT infants with genotypes ΔF508-9T/5T, ΔF508-9T/7T and ΔF508-9T/9T. Univariate statistics (including means, medians, minimums and maximums), box plots (see legend, Figure 1), and scatter plots were generated using SAS statistical software. Multiple subgroup comparisons were first tested using the Kruskal-Wallis test. Post hoc two-way comparisons were tested using the Wilcoxon-Mann-Whitney U test.

Figure 1: Box Plot Legend



Acknowledgements

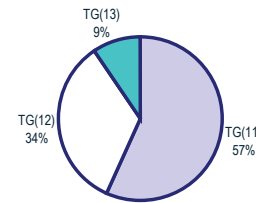
We gratefully acknowledge the dedication and hard work of the directors and staff of all of the California CF Specialty Care Centers and Newborn Screening Area Service Centers. We also thank the Stanford University Molecular Pathology, Ambry Genetics, and Genetic Disease Screening Program Newborn and Prenatal Screening Laboratories.

References

1. Chu CS, Trapnell BC, Curnutt S, Cutting GR, Crystal RG. Genetic Basis of Variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nat Genet* 1993; 3:151-156
2. Krasovec S, Maszki M Jr, Davis C, Curnutt SM, Chu CS, Graham C, Shrimpton AC, Cashman SM, Tsai LC, Mickle J. A mutation in CFTR produces different phenotypes depending on chromosomal background. *Nat Genet* 1993; 5:274-278

Results

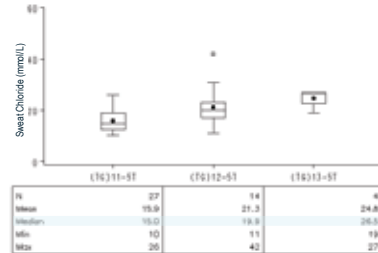
Figure 2: Number of Subjects in the ΔF508/5T Cohort, by (TG)m-5T Tract N=53



- Among the HT infants identified between 7/2007-7/2009, 53 met the inclusion criteria for the ΔF508/5T cohort.
- In this cohort, (TG)11 was the most common allele (N=30) followed by (TG)12 (N=18), and (TG)13 (N=5).

Sweat Chloride by (TG) Tract

Figure 3: Distribution of Initial SC (mmol/L) by (TG) Tract



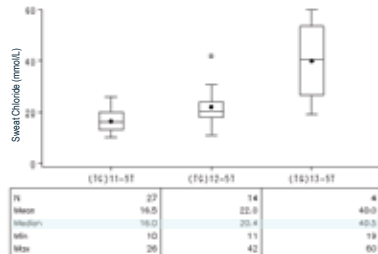
Kruskal-Wallis: p<0.01; Wilcoxon-Mann-Whitney U test: (TG)11 vs. (TG)12: p=0.01; (TG)12 vs. (TG)13: p=0.13; (TG)11 vs. (TG)13: p<0.01; (TG)11 vs. (TG)12 and (TG)13: p<0.01

- 8 subjects did not have SC results available either due to death not related to CF (N=1), repeated insufficient quantity (N=3), or scheduling difficulties (N=4).

- Most (58%) subjects had only 1 SC result of sufficient quantity available, 38% had 2 SC results available, and 4% had 3 SC results available.

- Initial SC increased with (TG) tract length (Figure 3).

Figure 4: Distribution of Highest SC (mmol/L) by (TG) Tract



Kruskal-Wallis: p<0.01; Wilcoxon-Mann-Whitney U test: (TG)11 vs. (TG)12: p=0.01; (TG)12 vs. (TG)13: p=0.06; (TG)11 vs. (TG)13: p<0.01; (TG)11 vs. (TG)12 and (TG)13: p<0.01

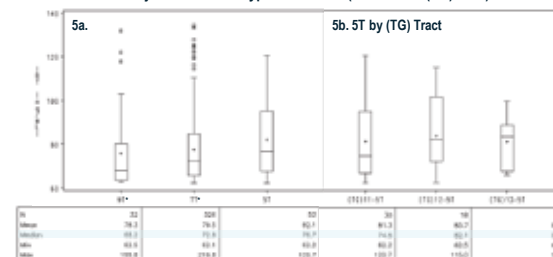
- Highest SC increased with (TG) tract length (Figure 4).

- (TG)12: 7% (N=1) had highest SC levels ≥40 mmol/L

- (TG)13: 50% (N=2) had highest SC levels ≥40 mmol/L (1 subject had SC = 60 mmol/L).

IRT by Poly T and (TG) Tract

Figure 5a-b: Distribution of IRT (ng/mL) by Poly T and (TG) Tract among Subjects with Genotype ΔF508-9T / (7T or 9T or (TG)m-5T)



- Although median IRT increased with decreasing Poly T length, the trend was not statistically significant (Figure 5a).

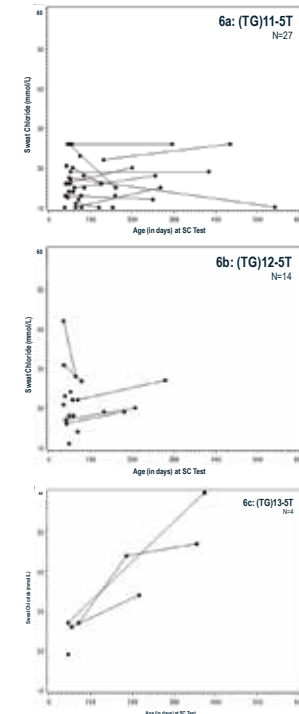
- Among subjects with 5T, median IRT increased with increasing (TG) tract, but the trend was not statistically significant (Figure 5b).

*IRT values over >140 ng/mL, 0 in the 5T group and 0 in the 7T group were excluded from the drawing of the Figure in order to show more detail of the middle of the distribution. Kruskal-Wallis (9T, 7T, 5T): p=0.06; Kruskal-Wallis (TG11, (TG)12, (TG)13): p=0.73; Kruskal-Wallis (9T, 7T, (TG)11-5T, (TG)12-5T, (TG)13-5T): p=0.22

Sweat Chloride by Age and (TG) Tract

- SC concentration remained relatively constant with age in the (TG)11 group (Figure 6a). The (TG)12 group showed more variability (Figure 6b).
- All subjects with (TG)13 who had multiple SC measurements showed a noteworthy increase after repeat testing. All but one increased to ≥40 mmol/L (Figure 6c).

Figure 6a-c: SC (mmol/L) by Age at SC Test and (TG) Tract in the ΔF508/5T Cohort



Conclusions

- Increased SC in association with more (TG) repeats supports the hypothesis that the 5T allele phenotype can be modified by (TG) length and that (TG)12-5T and (TG)13-5T may act as disease-causing mutations.
- Considerable change in SC observed during the first year of life among (TG)13-5T subjects has significant implications for newborn screening of these infants.
- IRT may increase with decreasing Poly T length. The trend did not reach statistical significance in this study due to a high degree of variability.
- Further study is warranted to determine the importance of assessing (TG)m(T)n loci when CF diagnosis is unclear and in evaluating infants with positive CF newborn screens.