### Title

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Short Communication

# Impact of IVS8-(TG)m(T)n on IRT and sweat chloride levels in newborns identified by California CF newborn screening☆

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**Abstract**

We examined the relation between the number of (TG) repeats at the (IVS8)-(TG)m(T)5 locus of the CFTR gene with neonatal serum immunoreactive trypsinogen (IRT) and sweat chloride (SC) concentrations in hypertrypsinogenemic infants with genotype ΔF508-9T/5T identified by California cystic fibrosis newborn screening. SC and IRT distributions increased with increasing (TG) repeats.


*Keywords:* Newborn screening; CFTR mutation; Diagnosis; Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mutation; Poly T

## 1. Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene [1–3]. While over 1800 CFTR mutations have been identified [4], few are well-described as disease-causing. Two polymorphic tracts ((TG)m(T)n) in intron 8 (IVS8) have been shown to affect exon 9 splicing efficiency. Lower numbers of (T) (5 vs. 7 or 9) and higher numbers of (TG) (13 or 12 vs. 11) repeats result in fewer copies of the full transcript and decreased synthesis of functional CFTR protein [5,6]. CF [7], CFTR-related disorders [7], and CFTR-related metabolic syndrome (CRMS) [8] are reportedly more common among those carrying 5T with (TG)13 or (TG)12 compared to (TG)11.

Few have investigated the relation of these haplotypes with CF diagnostic (sweat chloride (SC)) [9,10] and newborn screening (CFNBS) (immunoreactive trypsinogen (IRT)) [9,11–14] tests. We sought to determine the relation of number of (TG) repeats at the (TG)m(T)5 locus of CFNBS-identified hypertrypsinogenemic (HT) infants with genotype ΔF508-9T/5T (ΔF508 in *trans* with only a 5T allele and no other CFTR mutation) with IRT and SC. We also explored the relation of IRT levels with (T) length in *trans* with ΔF508-9T.

## 2. Methods

Subjects were identified during the first 2.5 years of California (CA) CFNBS by the CA 4-Step method: Step 1: IRT quantified by AutoDELFIA® Neonatal IRT L (PerkinElmer) in all newborn blood spot specimens. Step 2: CFTR mutation analysis (29–40 mutations; Asuragen Signature® CF 2.0 ASR) on specimens with IRT ≥ 62 ng/mL (highest 1.5%). Step 3: CFTR full gene sequence analysis utilizing scanning–sequencing technology (Ambry Test®:CF) [15] for specimens with only one mutation detected in Step 2. Exon 9 is sequenced for all specimens, allowing for analysis of IVS8 (T) and (TG) [2]. Step 4: SC testing, per national guidelines [16], and follow up

☆ A version of this material with a smaller population was presented as a poster at the North America Cystic Fibrosis Conference in Minneapolis, Minnesota on October 15–17, 2009 and published as an abstract in *Pediatric Pulmonology Supplement* (2009, Suppl 32: 276).

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by CF Care Centers for infants with two or more mutations, including 5T. When SC results were available from both arms, the higher SC value was recorded. Multiple SC tests were performed over time per CRMS follow-up guidelines [8].

Subjects were included in the ΔF508-9T/5T cohort if they had one copy of ΔF508 detected during Step 2 and only the IVS8 9T/5T genotype identified during Step 3. Because ΔF508 is almost always in *cis* with 9T [17], ΔF508 was considered to be in *trans* with 5T and 7T alleles.

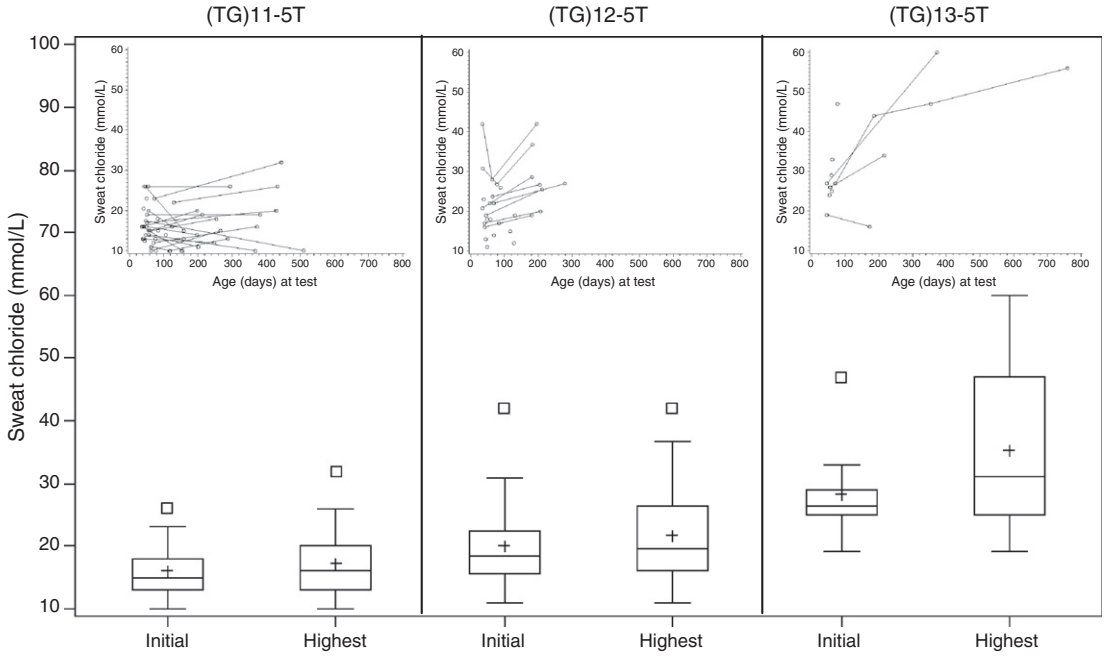
The distributions of IRT, initial SC (occurring at age (days), median: 57, range 35–159), and highest SC were analyzed by (TG) tract length among those in the ΔF508-9T/5T cohort. In a separate analysis, we compared IRT among HT infants with genotypes ΔF508-9T/5T, ΔF508-9T/7T and ΔF508-9T/9T.

Univariate statistics, box plots, and scatter plots were generated using SAS version 9.1 (Cary, NC). Differences in distributions of IRT and SC were tested using the Kruskal–Wallis and Mann–Whitney U tests.

## 3. Results

Among HT infants identified between 7/16/2007 and 1/15/2010, 75 met the inclusion criteria for the ΔF508-9T/5T cohort. (TG)11 was the most common allele (54%; n=41), followed by (TG)12 (31%; n=23), and (TG)13 (15%; n=11). Twelve (16%) subjects did not have SC results available due to: death unrelated to CF (n=1), insufficient quantity (n=5), and missed appointment (n=6). Among subjects with SC results, 49% (n=31) had one, 43% (n=27) had two, 6% (n=4) had three, and 2% (n=1) had four successfully completed tests.

Initial and highest SC increased with (TG) tract length (Fig. 1). For both initial and highest SC, differences in distributions between all (TG) subgroups reached statistical significance at the $\alpha$=0.05 level and, when combined together, the difference in SC between groups (TG)12 and (TG)13 compared to group (TG)11 was even less likely due to chance alone ($p<0.001$). Among subjects with (TG)11, none had the highest SC ≥ 40 mmol/L. Among subjects with (TG)12, 5% (n=1) had




| | (TG)11-5T | | (TG)12-5T | | (TG)13-5T | |
|---|---|---|---|---|---|---|
| | Initial | Highest | Initial | Highest | Initial | Highest |
| N | 33 | 33 | 20 | 20 | 10 | 10 |
| Mean | 16.0 | 17.1 | 20.0 | 21.7 | 28.3 | 35.3 |
| Median | 15.0 | 16.0 | 18.5 | 19.5 | 26.5 | 31.0 |
| Minimum | 10 | 10 | 11 | 11 | 19 | 19 |
| Maximum | 26 | 32 | 42 | 42 | 47 | 60 |

Fig. 1. Initial and highest SC (mmol/L) measurements among subjects with genotype ΔF508-9T/5T by 5T-(TG) group. Fig. 1 displays box and whisker plots of the initial and highest SC (mmol/L) measurements of study subjects with genotypes ΔF508-9T/5T-(TG)11, ΔF508-9T/5T-(TG)12 and ΔF508-9T/5T-(TG)13. [Lower and upper "whiskers" represent the lowest and highest values, respectively, within the lower and upper fences (1.5 times the interquartile range (IQR)). The lower edge, midline, and upper edge of the boxes represent the 25th, 50th, and 75th percentiles and the plus sign designates the mean. Outliers beyond 1.5 times the IQR are shown as boxes.] The values for the mean, median, minimum and maximum SC are presented in the accompanying table. Inset graphs display the change in SC measurements with age at sweat test for groups (TG)11-5T, (TG)12-5T, and (TG)13-5T, respectively. Solid lines connect repeated SC measurements for each subject with ≥2 SC results.

the highest SC ≥ 40 mmol/L. Among subjects with (TG)13, 30% (n = 3) had the highest SC ≥ 40 mmol/L (one subject had SC = 60 mmol/L).

SC concentration remained relatively constant with age in the (TG)11 group while the (TG)12 group showed more variability (Fig. 1 inset). All but one (TG)13 subject with multiple SC results demonstrated an increase over time (most increasing to ≥ 40 mmol/L).

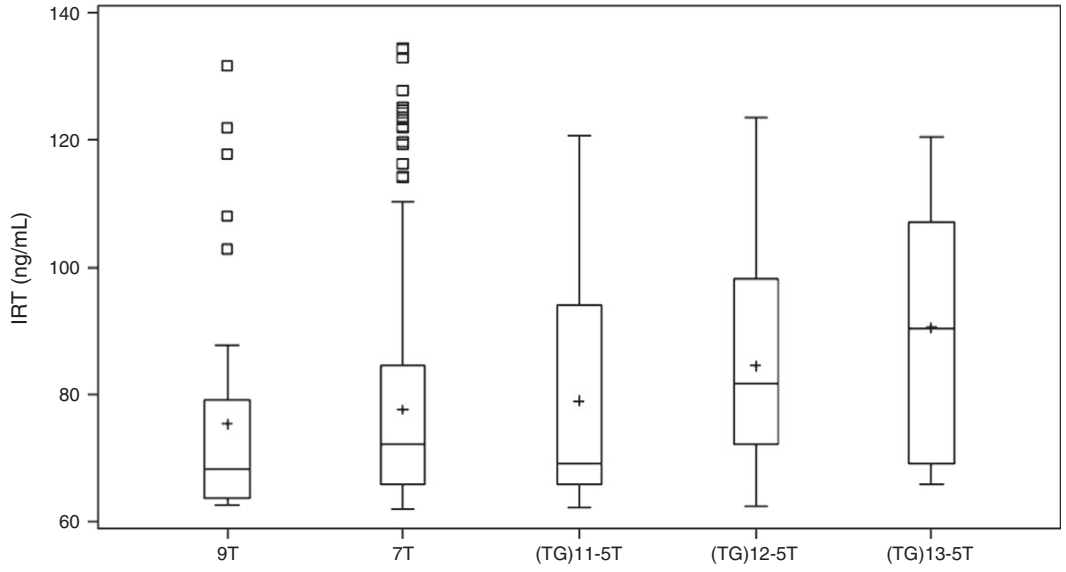
IRT increased with decreasing (T) length. Among subjects with 5T, IRT increased with increasing (TG) length (Fig. 2).The distribution of IRT did not differ significantly between the 9T and 7T groups. Subjects with the 5T allele, as a group, had higher IRT values (median = 80.5 ng/mL) than the group of subjects with the 9T or 7T allele ($p = 0.01$). Among those with 5T, the trend of increasing IRT levels with increasing number of (TG) repeats did not reach statistical significance ($p = 0.18$).

## 4. Discussion

This is the first study to prospectively assess IRT and SC by (TG) number in a relatively large cohort of HT ΔF508-9T/5T infants systematically identified by CFNBS. Thorough genotyping was performed on all subjects, minimizing the likelihood that IRT and SC results were confounded by undetected CFTR mutations. While this study was restricted to individuals with 5T in *trans* with ΔF508, we believe these results are generalizable to other disease-causing CF mutations in *trans* with 5T.

The observed increased SC in association with more (TG) repeats supports the hypothesis that the 5T allele phenotype can be modified by (TG) tract length [7]. In addition, the significant proportion of infants with SC in the indeterminate or abnormal range (>40 mmol/L) suggests that (TG)12-5T and (TG)13-5T may act as CF disease-causing mutations. This hypothesis is supported by reports of children and adults with (TG)12-5T or (TG)13-5T *trans* to a known CF disease-causing mutation who have elevated SC and symptoms consistent with CF [18–20]. SC increases during the first year after birth in genotype ΔF508/(TG)13-5T. Therefore, CFNBS algorithms relying on a single SC measurement to confirm a positive CFNBS result may improperly rule out CF in infants with this genotype.

Previous studies relating IRT to (T) length have found a higher prevalence of the 5T allele among newborns with elevated neonatal IRT [9,12]. In a large Massachusetts CFNBS cohort, a 3-fold increase in 5T allele frequency was seen in infants with IRT above the 90th percentile relative to below it [13]. Our data indicate that even among infants with very elevated IRT (≥ 98.5th percentile), IRT increases with decreasing




| | 9T | 7T | (TG)11-5T | (TG)12-5T | (TG)13-5T |
|---|---|---|---|---|---|
| N | 45 | 393 | 41 | 23 | 11 |
| Mean | 77.1 | 80.1 | 85.2 | 84.4 | 96.2 |
| Median | 68.5 | 72.6 | 72.7 | 81.7 | 92.1 |
| Minimum | 62.5 | 62.0 | 62.2 | 62.5 | 65.9 |
| Maximum | 155.8 | 301.4 | 203.4 | 123.6 | 153.6 |

Fig. 2. Distribution of neonatal IRT (ng/mL) among subjects with genotypes ΔF508-9T/9T, ΔF508-9T/7T, and ΔF508-9T/5T-(TG)m. Fig. 2 displays box and whisker plots and summary statistics of the distribution of neonatal IRT (ng/mL) in subjects with genotypes ΔF508-9T/9T, ΔF508-9T/7T, ΔF508-9T/5T-(TG)11, ΔF508-9T/5T-(TG)12, and ΔF508-9T/5T-(TG)13. [Lower and upper "whiskers" represent the lowest and highest values, respectively, within the lower and upper fences (1.5 times the IQR). The lower edge, midline, and upper edge of the boxes represent the 25th, 50th, and 75th percentiles and the plus sign designates the mean. Outliers beyond 1.5 times the IQR are shown as boxes.] Fourteen outliers above 140 ng/mL were excluded from the drawing of the box plots but included in the accompanying table: one with 9T, nine with 7T, three with (TG)11-5T, and one with (TG)13-5T. The values for the mean, median, minimum and maximum IRT are presented in the accompanying table.

(T) length. Our data also indicate that IRT may increase with increasing (TG) length. As a result, studies of the association between IRT and the 5T allele should account for (TG) length in *cis* with 5T.

As previous studies have shown, the 5T allele can be a CF disease-causing mutation. In order to better understand the significance of a 5T allele, the length of the accompanying (TG) tract must be determined. When evaluating an infant with a positive CFNBS result for whom a CF diagnosis is unclear, it is important to fully assess the (TG)m(T)n loci.

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