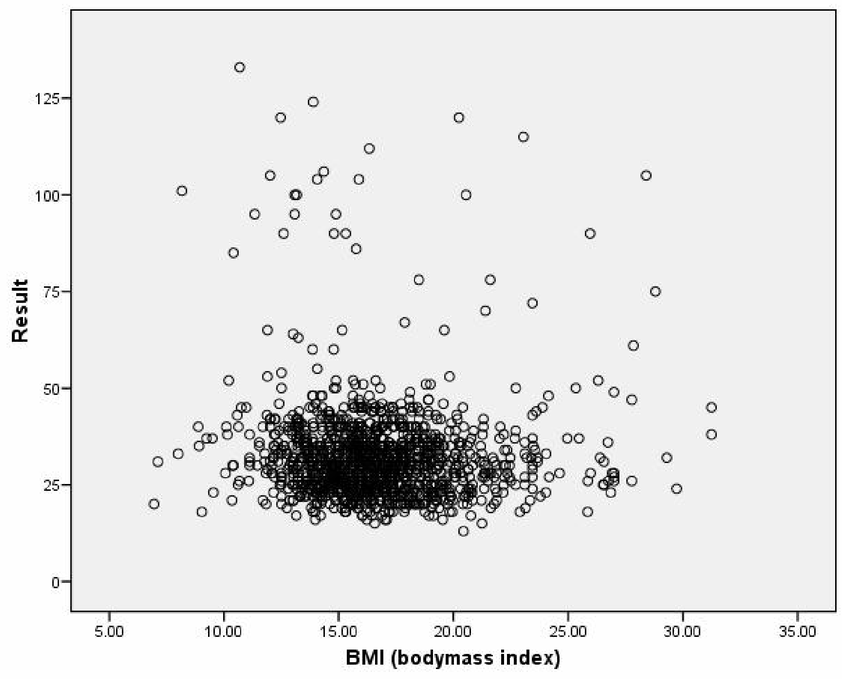
SWEAT TEST RESULTS ON A SUSPECTED CYSTIC FIBROSIS POPULATION WITH CF MUTATION ANALYSIS AND THE FREQUENCY OF BORDERLINE RESULTS

Tijen Tanyalcin[1], Gary Hoffman[2] , Ibrahim Tanyalcin[3], Philip M. Farrell[4],[1]Tanyalcin Medical Lab.Selective Newborn Screening & Metabolism, Izmir,[2] Newborn Screening laboratory Wisconsin State of laboratory Hygiene Madison, [3] Department of Molecular Biology and Genetics Bogazici University ,Istanbul ,[4] Pediatrics and Population Health Sciences UW School of Medicine and Public Health

AIM and INTRODUCTION

The aim of the study is to perform sweat test analysis from suspected population of Ege Region of Turkey , in order to define CF cases and to perform CF mutation analysis from high sweat test results.

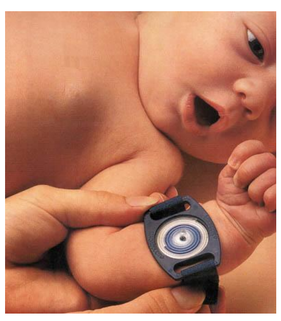
Cystic fibrosis (CF) is a life-shortening, autosomal recessive inherited disease with a frequency of 1:1500 and 1:2000 live births in Caucasian communities. Based on the cost effectiviness of the sweat test, in our country, laboratory diagnosis of CF remains largely dependent on the measurement of electrolytes in sweat. In our country for the patients over 2 months old and with suspected clinical findings with high levels of sweat test are then screened genetically for specific mutant alleles of the CTRF. However , due to the high levels of genetic mutations genetic analysis may fail to confirm the diagnosis of clinically observed CF patients even with high sweat test result.

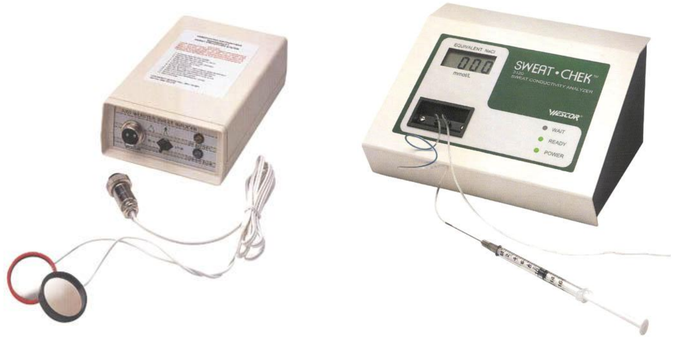


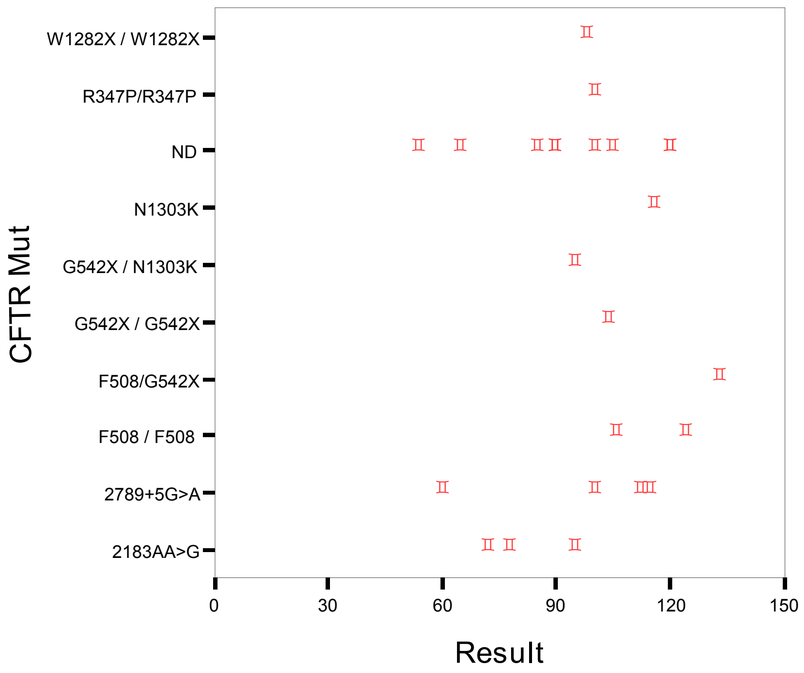
MEASUREMENT PROCEDURE

Based on the cost effectiviness of the sweat test, in our country, laboratory diagnosis of CF remains largely dependent on the measurement of electrolytes in sweat. Following induction of sweat glands by pilocarpine iontophoresis, sweat is collected by macroduct collection system within a coil of plastic tubing from which significant evaporation can not occur, then the sweat analyzed immediately by Sweat –Check analyzer (Wescor).

The conductivity means that sweat sample has an electrical conductivity that is equivalent to that of NaCl solution of the displayed mmol/L concentration . The reading in such units do not represent the actual concentration either Na or Cl in the sweat, always exceed the actual molar Na and Cl concentration due to the small contribution by ammonium as cation and lactate and bicabonate as anions. By this fact, diagnostic range in conductivity measurement is different from that of established for Cl with the proved , strong excellent correlation between chloride and conductivity proved by Hammand's regression. Conductivity up to 60 mmol/L is normal, 60-80 borderline (need to be retested), above 80 mmol/L is CF. In our laboratory protocol for the patients over 2 months old and with suspected clinical findings together with high levels of sweat test are then screened for 25 mutation in WSLH newborn screening lab with Linear array CF Gold PCR/detection (Roche) /gentra extraction .The routinely screened mutations are as follows: F508,G551D, R1162X, R347P, 1078delT, 1898+1G>A, 384+10kbC>T, 2184delA, R553X, 3659delC, G542X, R117H, G65E, A455E, R560T, N1303K, R334W, I148T, 2789+5G>A, I507, 1717-1G>AW1282X, 3120+1G>A, 621+1G>T, 711+1G>T







RESULTS

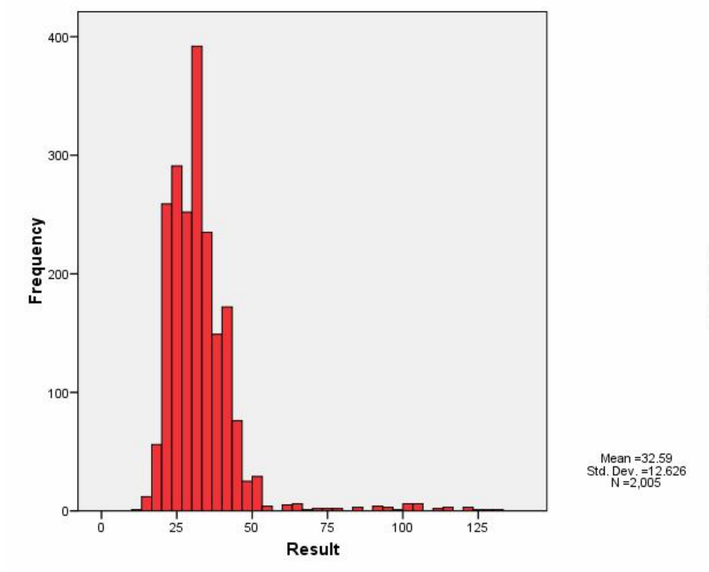
1.Sweat test results (n=2005) are not normaly distributed .Kolmogorow-Smirnow Z value 0.00 *(histogram 1)*

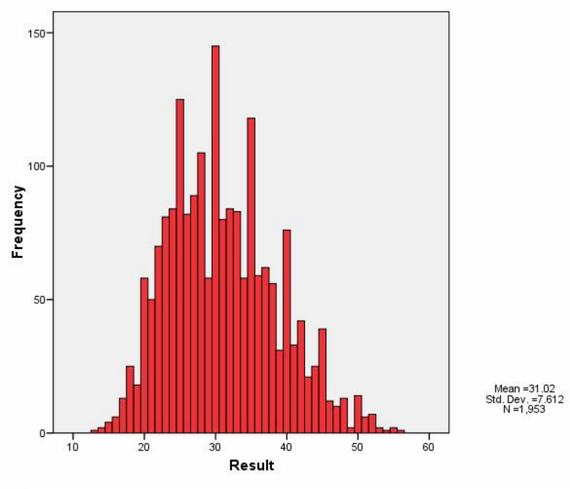
2.Sweat test values <60 , (n= 1953) are also showing bimodal distribution *(histogram 2)*

3.The rankkit plot with ln transformation also showed that the distribution is not normal *.(rankitplots)*

4.The correlation (spearman) between body mass index and sweat test results are NOT significant P= 0.352 with a correlation coefficient -0.155 *(scattegram)*

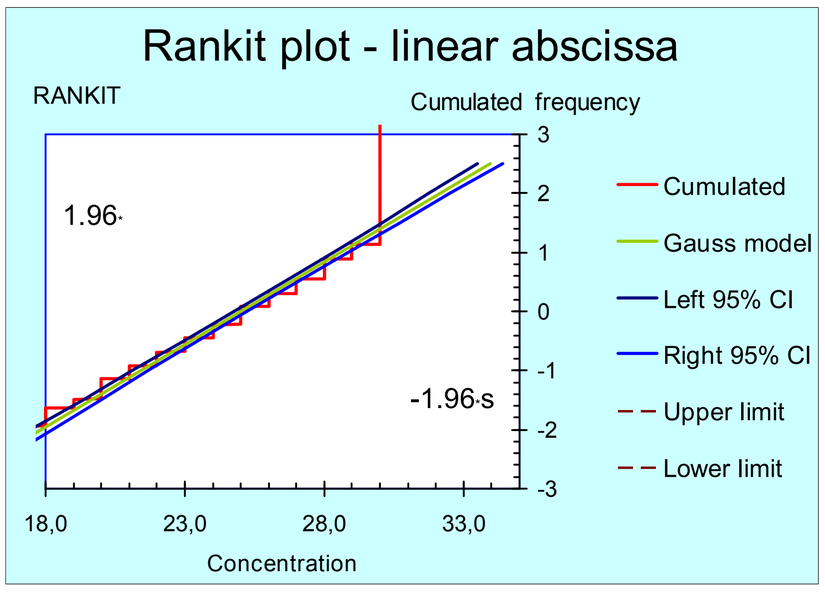
5. Among 2020 patient sweat test results ≥ 60 , 24 samples were screened for mutation. 2789+5G>A and 2183AA>G were detected more when compared to other mutations. However the second mutation was detected by luminex. The frequency of *NOT DETECTED* (ND) is very high *(frequency table)*

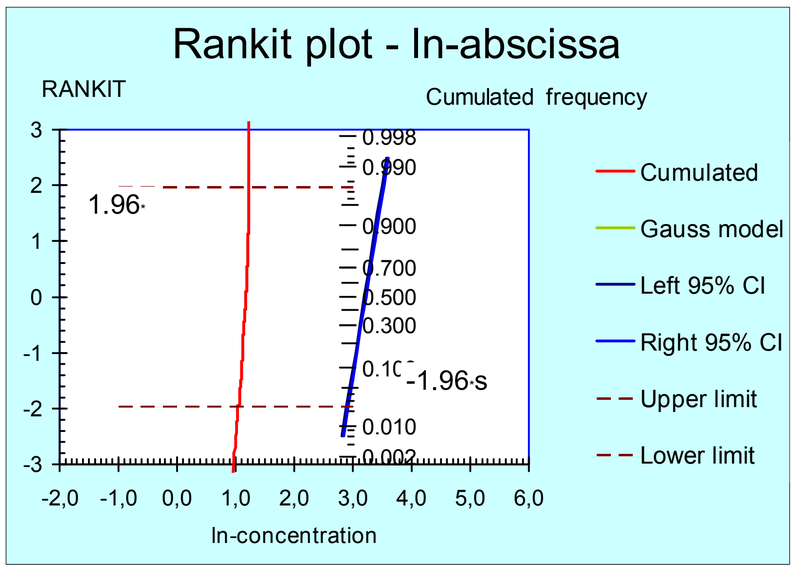




CFTR Mut

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2183AA>G	3	.1	12.5	12.5
	2789+5G>A	4	.2	16.7	29.2
	F508 / F508	2	.1	8.3	37.5
	F508/G542X	1	.0	4.2	41.7
	G542X / G542X	1	.0	4.2	45.8
	G542X / N1303K	1	.0	4.2	50.0
	N1303K	1	.0	4.2	54.2
	ND	9	.4	37.5	91.7
	R347P/R347P	1	.0	4.2	95.8
	W1282X / W1282X	1	.0	4.2	100.0
	Total	24	1.2	100.0	
Missing	1	1996	98.8		
Total		2020	100.0		





CONCLUSION

1.Identification of population specific CF mutational arrays in the assay system is very important .

2.Cost effective design and choose of the efficient/effective arrays will help to detect the mutations instead of ND results.

3. IRT measurement from blood spot cards has been added to sweat test / CF mutation analysis for the symptomatic babies younger than 4 months old.

4.This small project as a long term study with 5 years in the past and a few more in the future will help us to elucidate the type of mutations.

5.Collobaration with CF experts, their contribution and suggestions will help us to evaluate the data obtained.

REFERENCES

1.Human Mutation 19:575-606 (2002)
2.ClinChem Lab 42(7):715-724 (2004)
3.Pediatrics 115(6) (2005)