RESPIRATORY SIMILARITY IN MONOZYGOTIC TWINS

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Abstract

Twins are one of the integral members in the society. It is generally accepted that the physical characteristics of the laryngeal mechanisms are genetically determined. Since Lung volume and air flow are the source of voice production; it is vice to investigate the similarity of respiratory parameters (voice source). Slow vital capacity (SVC) and Mean air flow rate (MAFR) was investigated using Spirometer (RMS Helios 501). 17 monozygotic twins (MZ) pairs (11 F and 6 M pairs) with mean age of 22.1 yrs and age and gender matched normal subjects were participated in the study. Pearson with in pair correlation should high positive coefficient value. Only SVC showed gender differences for raw scores. No influence of age and gender on respiratory similarity. But, male showed less similarity than female. Discriminant analysis showed separation of twins group from the normal; separation was greater for absolute differences score. In conclusion present study showed similarity in SVC and MAFR of monozygotic twins. It supports the theory of genetic influence on respiratory functions.

Key Words: Vital capacity, Mean airflow rate, Discriminant analysis, Genetic.

Twins are not rare; they are one of the integral and interesting members in the society. The spontaneous rate of twinning is about 1 in 80 live births (Nylander, 1975). Monozygotic twins or identical twins come from one fertilized egg (zygote), and thus their genetic makeup would be expected to be identical. Monozygotic twins resemble each other in many aspects like aptitude, habit, taste and style that constitute what we think of as human individuality (Gedda, Fiori & Bruno, 1960). It is generally accepted that the physical characteristics of the laryngeal mechanism, such as vocal fold length and structure, size and shape of the supraglottic vocal tract, and phenotypic similarities elsewhere in the vocal mechanism are genetically determined (Sataloff, 1997). Though voice is unique to individuals, studies involving listener's perception have showed the perceptive similarity in monozygotic twins (Decoster, Van Gysel, Vercammen & Debruyne, 2001). The first attempt in this area was made by Schilling (1950) who measured the vocal range in semitones, which was found to be very similar in monozygotic twins. Also, several quantitative measures like fundamental frequency in phonation (Kalaiselvi, Santhosh & Savithri 2005), speaking fundamental frequency (Debruyne, Decoster, Van Gysel, & Vercammen 2002), formants (Forrai, & Gordos 1983) and Dysphonia Severity Index (Van Lierde, Vinck, De Ley, Clement, & Van Cauwenberge 2005) show similarity in monozygotic twins.

Van Lierde et. al. (2005) assessed vocal quality in 45 monozygotic twins (19 males and 26 females). They used Dysphonia Severity Index (DSI) to measure voice quality. The results showed that the perceptual and objective voice characteristics were similar in monozygotic twins. Likewise, Jayakumar & Savithri (2008) investigated voice source of monozygotic twins using formant based inverse filter (IF). Six female monozygotic twins and age and gender matched unrelated pairs in the age range of 19-25 years participated in the study. The acoustic pressure signals were inverse filtered (IF) using VAGHMI software (Voice and Speech Systems, India). IF were parameterized based on temporal and spectral measurements. Results indicated that the difference was less for MT for all the parameters and OQ and SQ were not significantly different in MT pairs. This showed MT had considerable similarity in voice source.

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Above recent studies shows that researchers were interested in investigating the voice similarity in twins or genetic contribution towards voice. The physiology of voice production is remarkably complex. The production of voice is dependent on three primary factors, (a) pulmonic air pressure, (b) laryngeal vibration, and (c) transfer function of vocal tract, with each of these factors having measureable parameters. It shows that respiratory system or lung volume and the airflow has great importance in voice production and the modulation of voice. Hence before going detailed investigation in to the production and the perception aspect of voice similarity in twins, it is vice to investigate the similarity of voice sources parameters in twins. Also, there is dearth of studies in respiratory functions similarity twins. Few earlier studies suggest that lung volume in terms of alveolar and airway development was majorly influenced by the genetic influences rather than environmental influences on pulmonary function (Hubert, Fabsitz, Feinleib & Gwinn, 1982; Chatterjee & Das, 1995) but some study shows till the adolescence the pulmonary functions were majorly influenced by genetic, but later on it is influenced by the environmental influence (Kawakami, Shida, Yamamoto, Yoshikawa, 1985). In this context the present study was conducted with interest in investigating lung volume and airflow similarity and influence of age and gender on similarity in monozygotic twins. Secondly, the above studies investigated monozygotic twins without any evidence of genotype similarity; Recent studies have suggested that phenotype some time misleads even in monozygotic twins (St. Clair, St. Clair, Swainson, Bamforth & Machin 1998). DNA fingerprint is a recent genotype analysis, which gives DNA sequence that is different for each individual except the monozygotic twins.

Method

Subjects: Fifty one subjects were participated in the study. Subjects were divided in to two groups. Group I consist of 17 monozygotic twins pairs (11 female and 6 male pairs) in the age range of 18 years to 26 years (mean age 22.1 year) 9 pairs were = 21 yrs and 8 pairs were > 21 yrs. Group II consist of equal number (17 subjects) of age and gender matched normal control subjects. Criteria for selecting monozygotic twins include (a) they should be same in gender, (b) Should have approximately similar height and weight. (c) Should have same blood group. (d) DNA finger print pattern should be same. Subjects having any unstable voice, voice disorder, speech disorders, neuro-motor disorder, endocrinal disorders and/or hearing disorder will be excluded from the study. A screening tool was used to rule out other disorders and ling (consisting of seven speech sounds) test will be used for hearing screening.

Procedure: Initially subjects were contacted over phone and explained about the study, roles and responsibilities of participants. Once they/ their Parents/Guardian gave their oral willingness to participate in the study, they were directly contacted with the purpose of sample collection and their written consent was obtained. The study was cleared by the AIISH ethical committee. Most of the respiratory samples were collected from the house of the participants using potable Spirometer (RMS Helios 501). Subjects were in standing position during the time of measurement and they were tested individually.

Parameters and procedure: To access the respiratory system, slow vital capacity and mean air slow rate parameters were taken

Slow vital capacity (SVC-CC): It is the total volume of air that can be exhaled after deep inhalation. Subjects were instructed to take a deep breath and blow out as long and hard as possible into the Spirometer (RMS Helios 501) mouthpiece (Awan, 2001). It was ensured that there was no air escape. Upon completion of the task, the mouth piece was closed to avoid any other environment air flow to happen which may affect the estimation. Subjects repeated the task thrice. The readings on the Spirometer were noted down.

Mean air flow rate (MAFR CC/sec): It is the average volume of air that passes through the vocal folds per unit time and depends on stiffness, sub glottal pressure, and levels of adduction of the vocal folds. Subjects were instructed to sustain vowel /a/ at habitual pitch and comfortable loudness with nasal occlusion after taking a deep inspiration (Baken, & Orlikoff, 2000) in to the mouth piece of the Spirometer (RMS Helios 501). It was ensured that there was no air escape. Upon completion of the task, the mouth piece was closed to avoid any other environment air flow to happen which may affect the estimation.

Subjects repeated the task thrice. The total amount of volume of air expired while sustaining vowel /a/ and the total duration of sustaining the vowel were recorded using Spirometer and stop watch, respectively. Mean air flow rate was calculated by using the formula MAFR = Total amount of volume of air expired (CC)/ Total duration of vowel sustained (sec)

Analysis: In each of the twin pairs (group I) first of the twin was called twin1 (T1). The co-twin was called twin2 (T2). Group II which had age and gender matched normal subjects of each twin pairs was called normal group (N). To understand the extent of the similarity or closeness between twins, and twins and non twins, the absolute differences were calculated between T1 and T2 (T1-T2). Similarly T1-N and T2-N was calculated. Using absolute differences values the influence of age and gender on respiratory similarity was analyzed..

Statistical analysis: To understand the similarity between monozygotic twin Pearson's correlation and the discriminate analysis was used. Discriminant function analysis is used to predict group membership from a set of predictors. The characteristics of predictors are related to form groups based upon similarities of distribution of dimensional space which are then compared to groups. To find significant difference between gender and age group and to know the effect of age and gender on vocal similarity, Mann Whitney U test was used.

Results

Respiratory similarity in MZ twins

(i) Mean, SD and correlation

The results indicated that SVC and MAFR values were similar in T1 and T2. But group II showed higher values than group I. Results of Pearson's correlation indicated high positive correlation between the respiratory measures of T1 and T2 (P = 0.01); low positive correlation on slow vital capacity between group I and group II (P = 0.05) and no correlation on MAFR between group I and group II. Tables 1 and 2 show the mean, SD, and correlation in group I and group II.

Table 1: *Mean, and SD of respiratory measures in group I, and group II.*

Parameters	Group I (MZ)		Group II(N)
	T1	T2	Normal subjects
	Mean (SD)	Mean(SD)	Mean(SD)
SVC (ltr)	2.25 (0.53)	2.25 (0.53)	2.83 (0.75)
MAFR (CC/sec)	139 (16.2)	137(19.2)	143(28.2)

*Table: 2: Correlation between respiratory measures of group I, and group II (** = P =0.01* = P =0.05,) .*

Parameters	(n=17)		
	T1 vs T2	T1 vs N	T2 vs N
SVC (ltr)	0.926**	0.529*	0.524*
MAFR (CC/sec)	0.925**	0.097	0.120

(ii) Discriminant function analysis for monozyg*otic*

The Respiratory data of monozygotic twins and age and gender matched normal subjects of study was subjected to discrminant function analysis. From the analysis two discrminant functions were obtained. The first function (DF1) accounts for 99% of the total variability. The second function (DF2) accounts for the remaining 1% (table 3) For the first function DF1, Wilks Lambda ? showed significance in the functions of the data that was analysed for separating twins from normals (table 4). DF2 was not found to be statistically significant for the grouping the MZ twins data. Further to interpret the first discrminant function DF1, standardized discrminant function coefficients were considered (Table 5). DF1 was found to be most heavily weighted on respiratory related measures.

Table 3: *Eigenvalues and variance value for raw score.*

Function	Eigenvalue	% of Variance
1	0.214	99.0
2	0.002	1.0

Table 4: *Wilks' Lambda value for absolute difference score.*

Test of Function	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	0.822	9.292	4	0.05
2	0.998	0.098	1	0.75

Table 5: *Function coefficient, Structure Matrix and centroids for groups for raw scores of respiratory measures.*

	Standardized canonical Discriminant function coefficient		Structure Matrix			Centroids for groups	
						Function	
	Function		Function			1	2
	1	2	1	2	T1	-.329	.053
SVC	1.067	-0.214	0.978*	0.206	T2	-.305	-.055
MAFR	-0.225	1.065	0.197	0.980*	N	.634	.001

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions

Variables ordered by absolute size of correlation within function.

* Largest absolute correlation between each variable and any discriminant function.

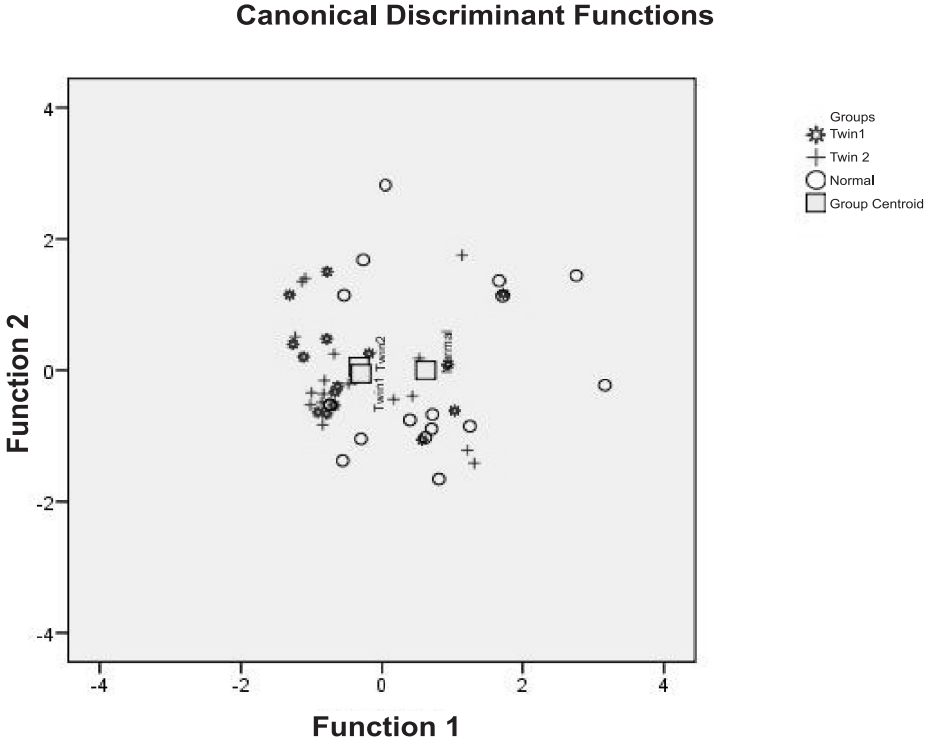

Figure 1: Canonical discriminant functions for twin1, twin2 and Normal.

Discriminate analysis showed twins1 and twin 2 is closer than normal subjects. Slow vital capacity (DF1 function) had significant importance than MAFR (DF2) in grouping these subjects. 47% of original grouped subjects correctly classified by Discriminant function using DF1 and Df2.

(Iii) Discriminant function analysis for Absolute differences value in monozygotic twins

The result indicated that first function (DF1) accounts for 100% of total variability. The second function (DF2) accounts for the remaining 0% (Table 6). DF1 is significant at $p<0.001$ and DF1 is more important than DF2.

Table 6: Eigenvalues and variance value for raw score.

Function	Eigenvalue	% of Variance
1	0.654	100
2	0.000	0

Table 7: *Wilks' Lambda value for absolute difference score.*

Test of Function	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	0.604	23.912	4	0.000
2	1.000	.008	1	0.927

Table 8: *Function coefficient, Structure Matrix and centroids for groups for absolute differences scores of respiratory measures.*

	Standardized canonical Discriminant function coefficient		Structure Matrix			Centroids for groups	
	Function		Function			Function	
	1	2	1	2		1	2
SVC	0.781	-0.693	0.907*	-0.421	T1-T2	-1.109	.001
MAFR	0.439	0.947	0.664	0.748*	T1-N	.583	.015
					T2-N	.527	-.016

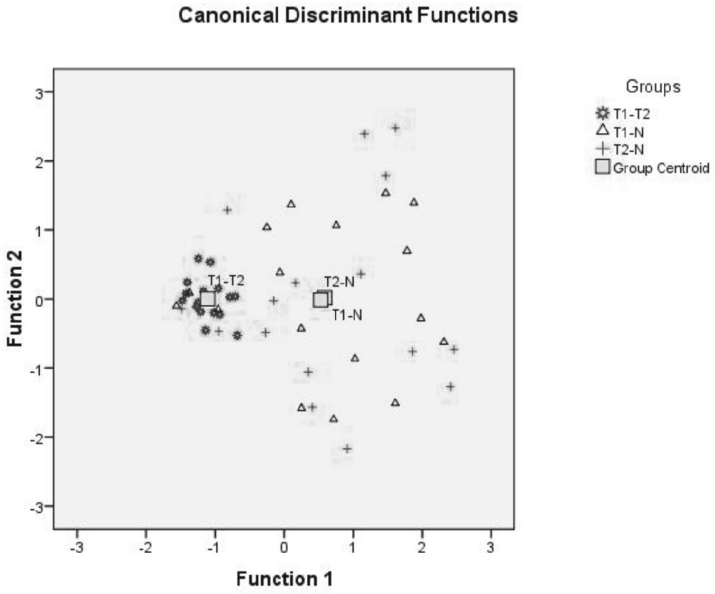

Figure 2: Canonical discriminant functions for T1-T2, T1-N, and T2-N.

Discriminant analysis showed T1-T2 was well separated from T1-N and T2-N. T1-N and T2-N was closer in comparison with T1-T2. Slow vital capacity (DF1 function) showed had importance than MAFR (DF2). 62% of original grouped subjects correctly classified by Discriminant function using DF1 and Df2.

Effect of Gender and age

(i) Gender differences

SVC showed significant difference between gender in group I and group II. Mean and SD of SVC was lower in females, and males of group I compared to those in group II. Since SVC showed gender difference, the correlation was calculated for male and female separately. Results of Pearson's correlation indicated high positive correlation between the SVC of T1 and 2 (P = 0.05) and no correlation between T1 and N, and T2 and N. Tables 9 and 10 show the mean, SD, and correlation in group I and group II.

Table 9: *Mean, SD and Z values for gender difference of respiratory measures in group I and group II using raw score. (**p<0.005 * p<0.01)*

Parameters	Group I (MZ) (Male = 6, Female =11)						Group II (Normals)		
	Male T1 Mean (SD)	Female T1 Mean (SD)	Z - Value	Male T2 Mean (SD)	Female T2 Mean (SD)	Z - Value	Male N Mean (SD)	Female N Mean (SD)	Z - Value
SVC	2.81 (0.52)	1.95 (0.16)	3.22**	2.83 (0.44)	1.94 (0.21)	3.11**	3.53 (0.42)	2.44 (0.42)	2.42*
MAFR	141 (18.6)	138 (15.6)	0.30	140 (25.3)	136 (16.3)	0.35	152 (31.6)	137 (30.0)	0.95

Table 10: *Correlation of respiratory measures between MZ-female twins and normal subjects*

Parameters	Females (n = 11)			Males (n = 6)		
	T1 vs T2	T1 vs N	T2 vs N	T1 vs T2	T1 vs N	T2 vs N
SVC (ltr)	0.638*	-0.117	-0.123	0.771*	0.029	-0.086

Result of Mann-Whitney U test significant difference between gender on MAFR for T1-N and T2-N. SVC for T1-N male showed greater absolute differences compare to female. Table 11 shows the Mean, SD and gender difference of respiratory measures using absolute differences value.

Table 11: *Mean, SD and Z values for gender difference of respiratory measures using absolute differences value. P<0.05*

Parameters	Male T1-T2 Mean (SD)	Female T1-T2 Mean (SD)	Z - Value	Male T1-N Mean (SD)	Female T1-N Mean (SD)	Z - Value	Male T2-N Mean (SD)	Female T2-N Mean (SD)	Z - Value
SVC	0.22 (0.12)	0.14 (0.07)	1.514	1.06 (0.38)	0.56 (0.36)	2.312*	0.96 (0.59)	0.60 (0.34)	1.308
MAFR	7.88 (4.74)	5.87 (3.5)	1.106	40.27 (13.6)	18.7 (15.1)	2.111*	41.7 (16)	16.3 (21.2)	2.412*

(ii) Age group differences

No significant difference was observed between age groups in group I for group II for SVC and MAFR. SVC and MAFR were lower age groups of group I compared to those in group II. Since there was no significant difference, between age groups, correlation was not calculated separately. Tables 12 show the mean, SD and Z value for age group difference between group I and group II.

Table 12: *Mean, SD and Z value for age group difference of respiratory measures in subgroup I and group II using raw scores.*

Parameters	Group I (MZ) (= 21 years = 9, > 21 years =8)						Group II (Normals)		
	= 21 y T1 Mean (SD)	> 21 y T1 Mean (SD)	Z - Value	= 21 y T2 Mean (SD)	> 21 y T2 Mean (SD)	Z - Value	= 21 y N1 Mean (SD)	> 21 y N Mean (SD)	Z - Value
SVC	2.24 (0.48)	2.27 (0.61)	0.00	2.20 (0.48)	2.31 (0.60)	0.43	3.02 (0.86)	2.61 (0.62)	0.72
MAFR	142 (15.37)	137 (17.9)	0.62	140 (17.4)	133 (21.7)	1.10	147 (28.4)	138 (34.0)	0.72

Both SVC and MAFR did not show significant difference between age groups in all the conditions. T1-T2 condition had lower mean value than other two conditions. Table 13 shows the Mean, SD and Z value for age group difference using absolute differences value.

Table 13: Mean, SD and Z value for age group difference of respiratory measures using absolute differences value using absolute difference scores.

Parameters	= 21 y T1-T2 Mean (SD)	> 21 y T1-T2 Mean (SD)	Z - Value	= 21 y T1-N Mean (SD)	> 21 y T1-N Mean (SD)	Z - Value	= 21 y T2-N Mean (SD)	> 21 y T2-N Mean (SD)	Z - Value
SVC	0.14 (0.09)	0.19 (0.11)	1.01	0.79 (0.47)	0.68 (0.42)	0.48	0.81 (0.56)	0.63 (0.32)	0.57
MAFR	6.25 (4.0)	6.95 (4.1)	0.00	24.6 (15.3)	28.2 (24.1)	0.38	21.6 (14.3)	29.3 (23.5)	0.05

Discussion

The present study was aim to investigate the respiratory similarity in monozygotic twins. Also the researchers were interested to know whether age and gender has any effect on the degree of similarity. Result showed that there is significant high positive correlation between MZ twins on SVC, MAFR. It indicates that, a good similarity between twin's respiratory parameters (table 2). Low positive correlation was noticed between twins and normal on SVC and no correlation was shown between twins and normal on MAFR. This result is inconsonance with study by Wu, Boezen, Postma, Los, Postmus, Snieder and Boomsma (2010) showed good correlation between twin pairs on forced vital capacity and its related measurements. This similarity respiratory similarity can be attributed to the genetic similarity of the monozygotic twins. Chatterjee and Das (1995) investigated the relative contributions of genetic and environmental components in the variability of lung function measurements in 30 MZ and 24 DZ twin pairs. From the Spirometer measurement (like Inspiratory capacity, slow vital capacity, forced vital capacity), within pair variance was significantly lower in MZ than DZ. The result data showed major lung function measurements are possibly influenced more by genetic than environmental factors. Genetically influenced measurements show higher levels of heritability estimates and suggest that genetic determination of lung function is possibly independent of the influence of physical characteristics. Hubert, Fabsitz, Feinleib and Gwinn, (1982) analyzed 127 MZ and 141 DZ twins in the age range of 42 to 56 yr on forced vital capacity and its related measures. Twin analyses showed significant genetic variance ($p < 0.001$) for Forced Vital Capacity. The findings of this study are also, consistent with theories of genetic influences on alveolar and airway development and argue in favor of early as well as adult environmental influences on pulmonary function. Sataloff (1997) said that the physical characteristics of the laryngeal mechanism, such as vocal fold length and structure, size and shape of the supraglottic vocal tract, and phenotypic similarities elsewhere in the vocal mechanism are genetically determined. This study shows that that similarity not restricted to vocal mechanism but even source mechanism for the vocal production.

The discriminate analysis for raw score showed the difference between twins (T1 and T2) was less compare to difference between one twin and normal subject (Figure 1 and table 5). SVC had significant important role in differentiating twin from the normal in comparison with MAFR. But this function was able to classify correctly for nearly 50% of the subjects. Similarly, The discriminate analysis for absolute differences score showed the difference between twins (T1-T2 vs T1-N, T2-N) was less compare to difference between one twin and normal subject (Figure 2 and table 8). This showed good separation compare to the raw score. Even for this functions SVC had important role in differentiating twin from the normal in comparison with MAFR. Also, this function was able to classify correctly for nearly 62% of the subjects. Discriminant analysis shows that from the respiratory parameters nearly two third of twins can be identified correctly.

Gender comparison for raw scores showed significant difference between gender for SVC and no significant difference for MAFR (table 4). This was supported by early literatures saying broad difference between male and females in terms of lung capacities especially vital capacity measurement. Gender comparison for respiratory similarity was analysed using absolute differences score; it showed no significant difference for SVC but, MAFE showed significant different for T1-N and T2-N (table 5). Overall male showed less similarity than females for both the respiratory measures. This can be attributed due to the dynamic range difference in lung volume for male and females. Since male showed greater lung capacity the possibility for variability is more. Second the same lung capacity variation might have effect in the airflow and the pressure between the vocal fold. Age group comparison for raw scores and for absolute difference score did not show any significant differences. But early study by Kawakami, Shida, Yamamoto, Yoshikawa (1985) showed age group differences in respiratory similarity in monozygotic twins. They examined in 20 adolescent MZ twins, 11 adolescent DZ twins, and 20 adult MZ twins. Within pair variability analysis indicate that small airway dynamics are influenced in larger part by genetic factors in adolescence as well as adulthood, whereas lung volumes in terms of forced vital capacity are controlled by genetics only in adolescence.

Conclusion

The present study was design to investigate the respiratory similarity in monozygotic twins using slow vital capacity and mean airflow rate. Result showed that high positive correlation between twins for SVC

and MAFR and poor correlation between twins and normal. SVC showed gender differences for raw scores. The influence of age and gender on respiratory similarity was analysed using absolute differences between twins and normal, which showed there was no effect of age and gender on respiratory similarity, but males showed less similarity in twins a well as for normal comparison, although it was not significant. Discriminant analysis showed separation of twins group from the normal; separation was greater for absolute differences score. In conclusion present study showed a similarity in monozygotic twins of respiratory measurements. It supports the theory of genetic influence on respiratory functions.

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