The *COL27A1* Gene Polymorphism in Children with Tic Disorder in Southern China and Its Clinical Association

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Abstract

Background: The three single nucleotide polymorphisms(SNPs) (rs4979356, rs4979357 and rs7868992) of COL27A1 has been reported to correlate with Tourette Syndrome (TS) patients in European countries as well as northern Chinese population, though there are lack of relevant studies in southern Chinese population. In this study we aimed to evaluate the distribution of *COL27A1*(rs4979356, rs4979357 and rs7868992) gene polymorphism and its clinical relationship in children with Tic disorder (TD) in southern China.

Methods: The children with TD between November 2018 to August 2019 from Department of Neurology, Guangzhou Women and Children's Medical Center were recruited and followed up. The control children from normal primary school were also recruited. The SNP of *COL27A1* (rs4979356, rs4979357 and rs7868992) was detected in child from each groups by polymerase chain reaction(PCR) and Sanger sequencing.

Results: A total of 114 children with TD and 100 healthy control children were included. 111 out of 114 TD's children were followed up more than one year, including 32 with transient tic disorder (TTD) (Age:7.58±2.402;), 45 with chronic tic disorder (CTD) (Age:: 8.72±2.312;) and 34 with Tourette Syndrome (TS)(Age:: 8.85±2.720) respectively. The genotype frequency and gene frequency of rs4979356, rs4979357 and rs7868992 from *COL27A1* showed no statistically significant difference ($P > 0.05$) between the both groups□The distribution of genotype CG of *COL27A1* rs4979356 in TS group was significantly higher than that in TTD+CTD group(P=0.002) and in control group (P=0.001)□there was no significant difference in allele frequency among each groups (*P*□0.05)□Genotype TC of *COL27A1* rs4979357 was significantly more distributed in TS group than in TTD+CTD group (P=0.004) and control group (P=0.001)□There was no significant difference in allele frequency among three groups (*P*□ 0.05);Genotype GA of *COL27A1* rs7868992 was significantly more distributed in TS group than in TTD+CTD group (P=0.018) and control group (P=0.035). The distribution of G allele frequency in CTD group was significantly higher than that in TS group (*P*=0.025), while the difference of other allele frequency between the groups was not statistically significant(*P*□0.05).

Conclusion: The genotype CG of rs4979356, the genotype TC of rs4979357 and the genotype GA of rs7868992 might serve as the high risk factors in children with TS in southern China.

Background

Tic disorder (TD) is a common neuropsychiatric disease in childhood, and the prevalence of TD in China is 6.1% [1]. According to the symptoms and duration, TD can be divided into transient TD (TTD), chronic TD (CTD) and Tourette syndrome (TS), with TS incidence rate of 0.3-1.0% and refractory cases increasing, which has caused distress to children and their families.

At present, the pathogenesis of TD is not clear, and the research on genetic factors has attracted much attention. In recent years, genome-wide association studies (GWAS) have discovered the genetic

association recognition of many common complex traits. In 2013, Scharf and colleagues conducted the first GWAS of TS in 1285 cases and 4964 ancestry-matched controls of European ancestry, including two European-derived population isolates, Ashkenazi Jews from North America and Israel and French Canadians from Quebec, Canada. They found the strongest correlation with the single nucleotide polymorphism (SNP) rs7868992 in collagen XXVII type α 1 gene (*COL27A1*) in European samples [2]. *COL27A1* is located on chromosome 9q32-33, approximately 156 KB in length, and has 61 exons, encoding a long trihelical domain, a carboxy-terminal propeptide and a large spherical amino-terminal propeptide. *COL27A1* is strongly expressed in developing cartilage and is currently mainly related to iron and steel syndrome[3, 4]. It is weakly expressed in many other tissue types such as skin, stomach, gonad and brain[5]. Some studies have found that microRNA-455-3P encoded by *COL27A1* gene is considered as a potential biological and/or therapeutic target for Alzheimer's disease (AD)[6]. However, little is known about the role of *COL27A1* in neural development, particularly in neural circuits.

In Northern China, Shiguo Liu et al. applied haplotype relative risk (HRR) and transmission imbalance test (TDT), amplified *COL27A1* (rs4979356, rs4979357 and rs7868992) by PCR (Polymerase Chain Reaction) and then sequenced to understand the genetic distribution of the three SNPS in TS[7]. Their results indicated that: The TDT test showed that rs4979356 had significant transmission imbalance, suggesting that rs4979356 was related to the etiology of TS development. HRR and Haplotype-based haplotype relative risk(HHRR) analysis of rs4979356 showed that allele G and genotype G(+) were risk factors for TS. Similarly, allele C and genotype C(+) of rs4979357 are also risk factors for TS, providing evidence that *COL27A1* is involved in the occurrence of TS. Preliminary meta-analysis of GWAS data showed that RS7868992 had the highest correlation.

However, TDT results of rs7868992 in this study did not show statistically significant allele transfer. The results of linkage disequilibrium test confirmed that the three SNPs were inherited independently. Therefore, the difference of genetic spectrum between Chinese and European people may be the reason for the insignificant TDT results of rs7868992. Larger data sets from different populations are now needed to validate these results. Up to date,there are lack of relevant studies in southern Chinese population.

Therefore,in this study, the SNPs distribution of *COL27A1* (rs4979356, rs4979357 and rs7868992) polymorphism in children with TD in southern China was studied to reveal the genetic heterogeneity and its clinical relationship.

Methods

Subjects

TD patients: Children with TD between November 2018 to July 2019 from department of Neurology,Guangzhou Women and Children's Medical Center were included. This study was approved by the ethics committee of Guangzhou Women and Children's Medical Center. The parents/guardians of included subjects were provided written consent form for publication.

Inclusion criteria

Diagnostic Criteria based on **Diagnostic and Statistical Manual of Mental Disorders 5th Edition(DSM-5)**[8]

The diagnostic criteria of TD based on DSM-5 as follows: TTD: ①One or more motor twitch and/or vocal twitch; ②The course of disease is shorter than 1 year; ③Onset before the age of 18;④exclude certain drugs or medical diseases caused by; ⑤Do not meet the diagnostic criteria for chronic TD or TS; CTD: ①One or more motor twitch or vocal twitch, only one twitch form appears in the course of the disease; ②Since the first twitch, the frequency of twitch can be increased or decreased, the course of disease is more than 1 year; ③Onset before the age of 18; ④exclude certain drugs or medical diseases caused by; ⑤Does not meet the diagnostic criteria of TS; TS: ①multiple motor twitches and one or more vocal twitches, but not necessarily both; ②After the first twitch, the frequency of twitch can be increased or decreased, the course of disease is more than 1 year; ③Onset before the age of 18; ④exclude some drugs or medical diseases caused by.

Exclusion criteria: ①The patients with intracranial infection, epilepsy, intracranial hemorrhage, hepatolenticular degeneration, rheumatic chorea,or with the inflammation of eyes, ears and throat were excluded.

Control population:

The children from normal primary school children were recruited as the control group.

Detection of COL27A1 (rs4979356, rs4979357 and rs7868992)

TD patients' and Control children's whole blood samples were collected and stored at -20℃ until analysis. Genomic DNA was prepared from peripheral blood by using the whole blood genomic DNA mini kit (SIMGEN, Hangzhou, China). The *COL27A1* gene sequence fragment including the SNP locus was amplified by PCR, and then sent to the sequencing company for Sanger sequencing and then analyzed to determine the genotype. Primer sequences were as follows: upstream primer 5'-AGACAGGCTGCCTAGTGT-3' and downstream primer 5'-GATAGCGTCATTGAACTCC-3'. Polymerase chain reactions were performed in a total volume of 30 μl, containing 50–100 ng of genomic DNA, 0.6 μl of each 10 μM primer (Table 1) and 12.5 μl Premix Taq™ (TaKaRa Taq™ Version 2.0) (TaKaRa, Dalian City, China). Polymerase chain reaction amplification was performed in a programmable thermal cycler system (Eppendorf Master cycler nexus, USA). Cycling conditions for polymerase chain reactions were set as follows: one cycle at 94 °C for 5 minutes, 35 cycles at 94 °C for 30 seconds, 62 °C for 30 seconds, and 72 °C for 40 seconds, and one final cycle for extension at 72 °C for 10 minutes.

Table 1
clinical manifestations of 111 children with TD in different groups

			TTD	CTD	TS
gender	male		27	33	30
	female		5	12	4
age			7.58 ± 2.402	8.72 ± 2.312	8.85 ± 2.720
newly diagnosed			30	37	22
Manifestations	head	shake head	1	1	2
Motor Tic					
		nod/ raising head	3	7	3
		crooked head	2	8	1
	face	shrug eyebrow	0	1	3
		frown	4	3	2
		blink hard	16	19	12
		squint	0	1	0
		strabismus	0	1	0
		roll one's eye	2	3	1
		stare	0	1	0
		shrug nose	4	12	17
		deep breathing	0	0	1
		facial twitch	3	6	5
		grin	0	3	5
		twitch/crooked mouth	5	15	4
		lip twitching/sipping	1	0	2

[A]1 case with CTD underwent cranial MR examination, and the results were normal, and so on;

[B] There were 5 TS children with VEEG, among which 1 had abnormal results (a small amount of medium-high wave amplitude and slow wave emission in the right central area and the back head at each waking and sleeping stage), and the other 4 had normal results.

			TTD	CTD	TS
		lip biting /licking	1	0	0
		open mouth	1	4	2
		pout	0	3	1
		loll tongue	0	1	0
		breathe in/out hard	2	2	1
	neck	necking down	0	1	1
		crane	0	4	0
		neck muscle twitch	0	1	0
	shoulder	shrug	6	5	10
	trunk	chest twitch	0	1	2
		abdominal twitch	0	5	3
		twist the waist	0	1	0
	limb	upper limb twitch	3	4	6
		hand twitch/wrist rotation	0	1	2
		lower limb twitch	1	0	1
		limbs twitch	3	1	2
		body twitch	0	0	1

[A]1 case with CTD underwent cranial MR examination, and the results were normal, and so on;

[B] There were 5 TS children with VEEG, among which 1 had abnormal results (a small amount of medium-high wave amplitude and slow wave emission in the right central area and the back head at each waking and sleeping stage), and the other 4 had normal results.

			TTD	CTD	TS
Vocal Tic	twitch compulsive behavior	clap the table	1	0	0
		sitting on the ground	1	0	0
		clearing throat	3	2	15
		say "um" and "ah"	1	0	1
		irregular laryngeal	0	1	4
		cough after deep	0	0	1
		hoose	0	1	3
	cranial imaging and electroencephalogram examination	cranial MR	0	1(normal)[A]	3(normal)
		VEEG	2(all were normal)	5(all were normal)	5(1 case was abnormal)[B]

[A]1 case with CTD underwent cranial MR examination, and the results were normal, and so on;

[B] There were 5 TS children with VEEG, among which 1 had abnormal results (a small amount of medium-high wave amplitude and slow wave emission in the right central area and the back head at each waking and sleeping stage), and the other 4 had normal results.

Table 2
Comparison of genotype frequency and gene frequency of *COL27A1* rs4979356, rs4979357 and rs7868992 between TD children and the control group

SNPs	genotype/allele		case (TD) group(n)	control group(n)	χ^2value	*P* value
rs4979356	genotype	CC	29	31	3.057	0.217
		CG	64	46		
		GG	18	23		
	allele	C	122	108	0.039	0.844
		G	100	92		
rs4979357	genotype	TT	27	31	3.894	0.143
		TC	66	46		
		CC	18	23		
	allele	T	120	108	0.000	0.991
		C	102	92		
rs7868992	genotype	GG	46	42	1.538	0.464
		GA	56	45		
		AA	9	13		
	allele	G	148	129	0.219	0.640
		A	74	71		

Statistical Analysis

SPSS21.0 software package was used to manage all the data, and the measurement data was expressed as mean standard deviation(`x ± s). Comparisons between two or more population rates were tested by χ^2. The level of statistical significance was set at $p < 0.05$.

Results

General information of case group

111 of the 114 TD patients were followed up, with a male to female ratio of 4.3:1, of which 89 were newly diagnosed. Among the 111 children, 32were consistent with TTD, 45 with CTD, and 34with TS, among which 16 were 3–5 years old, 59 were 6–8 years old, 27 were 9–11 years old, 8 were 12–14 years old, and 1 was 15 years old. The composition of the three groups of TD children in different age groups was shown in Fig. 1. Children in the TTD group ranged in age from 3 to 12 years old, with an average age of 7.58 ±

2.402 years old and a male to female ratio of 5.4:1. In the CTD group, the children ranged in age from 5 to 14 years old, with an average age of 8.72 ± 2.312 years old and a male to female ratio of 2.75:1. The age range of children in TS group was 4–15 years old, with an average age of 8.85 ± 2.720 years old, and the ratio of male to female was 7.5:1.

Clinical manifestations of three groups of children

The clinical manifestations of 111 TD children in this group are diverse, with more than 30 kinds of muscle twitch. There were 47 cases of forced blinking (TTD 16 cases, CTD 19 cases and TS 12 cases), There were 47 cases of forced blinking (TTD 16 cases, CTD 19 cases and TS 12 cases), 33 cases of shrug nose (TTD 4 cases, CTD 12 cases and TS 17 cases), 24 cases of mouth twitch/crooked mouth (TTD 5 cases, CTD 15 cases and TS 4 cases), and 21 cases of shrug (TTD 6 cases, CTD 5 cases and TS 10 cases). 1 case of CTD and 3 cases of TS underwent cranial MR examination, and the results were normal. There were 12 TD children with video EEG, among which 1 had abnormal results (a small amount of medium-high amplitude and slow wave emission in the right central area and the back head during waking and sleeping), and the rest were normal. The clinical manifestations of 111 children with TD in different groups are shown in Table 1.

Comparison of genotype frequency and gene frequency of COL27A1 rs4979356, rs4979357 and rs7868992 between TD children and control group

The genotype frequency and gene frequency of COL27A1 rs4979356, rs4979357 and rs7868992 in the case group and the control group showed no statistically significant difference ($P > 0.05$). See Table 1 for details.

Comparison of genotype frequency and gene frequency of COL27A1 rs4979356, rs4979357 and rs7868992 in three sub-groups of children with TD

Genotype frequency and gene frequency of COL27A1 **rs4979356 were compared among three groups of children with TD and healthy control group**

In TTD group, CC genotype accounted for 8 cases, CG genotype accounted for 17 cases, GG genotype accounted for 7 cases, allele frequency C33 (51.6%), G31 (48.4%); CTD group: CC genotype accounted for 18 cases, CG genotype accounted for 19 cases, GG genotype accounted for 8 cases, allele frequency C55 (61.1%), G35 (38.9%); TS group: CC genotype accounted for 3 cases, CG genotype accounted for 28 cases, GG genotype accounted for 3 cases, allele frequency C34 (5 In healthy control group, CC genotype accounted for 31 cases, CG genotype accounted for 46 cases, GG genotype accounted for 23 cases, allele frequency C108 (54.0%), G92 (46.0%). The distribution of genotype CG in TS group was higher than that in TTD + CTD group ($\chi^2 = 12.452$, $P = 0.002$) and control group ($\chi^2 = 13.618$, $P = 0.001$), and the difference was statistically significant ($P < 0.05$). There was no significant difference in allele frequency between groups ($P > 0.05$). See Tables 3 and 4 for details.

Table 3
Comparison of genotype frequency and gene frequency of *COL27A1* rs4979356, rs4979357 and rs7868992 between TTD + CTD group and TS group

SNPs	genotype/allele		TTD + CTD group(n)	TS group(n)	χ^2value	*P* value
rs4979356	genotype	CC	26	3	12.452	0.002
		CG	36	28		
		GG	15	3		
	allele	C	88	34	0.972	0.324
		G	66	34		
rs4979357	genotype	TT	24	3	10.814	0.004
		TC	38	28		
		CC	15	3		
	allele	T	88	34	0.649	0.421
		C	68	34		
rs7868992	genotype	GG	38	8	8.034	0.018
		GA	32	24		
		AA	7	2		
	allele	G	108	40	2.714	0.100
		A	46	28		

Table 4
Comparison of genotype frequency and gene frequency of *COL27A1* rs4979356, rs4979357 and rs7868992 between TS group and control group

SNPs	genotype/allele		TS group(n)	control group(n)	χ^2value	*P* value
rs4979356	genotype	CC	3	31	13.618	0.001
		CG	28	46		
		GG	3	23		
	allele	C	34	108	0.326	0.568
		G	34	92		
rs4979357	genotype	TT	3	31	13.618	0.001
		TC	28	46		
		CC	3	23		
	allele	T	34	108	0.326	0.568
		C	34	92		
rs7868992	genotype	GG	8	42	6.695	0.035
		GA	24	45		
		AA	2	13	0.702	0.402
	allele	G	40	129		
		A	28	71		

Genotype frequency and gene frequency of COL27A1 **rs4979357 were compared among three groups of children with TD and healthy control group**

In TTD group: TT genotype accounted for 8 cases, TC genotype accounted for 17 cases, CC genotype accounted for 7 cases, allele frequency T33 (51.6%), C31 (48.4%). In the CTD group, TT genotype accounted for 16 cases, TC genotype for 21 cases, CC genotype for 8 cases, allele frequency T53 (58.9%), C37 (41.1%). In TS group, TT genotype accounted for 3 cases, TC genotype for 28 cases, CC genotype for 3 cases, allele frequency T34 (50.0%), C34 (50.0%). In the healthy control group, TT genotype accounted for 31 cases, TC genotype for 46 cases, CC genotype for 23 cases, allele frequency T108 (54.0%), C92 (46.0%). Genotype TC distribution in TS group was higher than that in TTD + CTD group (χ^2 = 10.814, *P* = 0.004) and control group (χ^2 = 13.618, *P* = 0.001), with statistically significant difference (*P* < 0.05). There was no significant difference in allele frequency between groups (*P* > 0.05). See Table 3 and Table 4 for details.

Genotype frequency and gene frequency of COL27A1**rs7868992were compared among three groups of children with TD and healthy control group**

In TTD group: GG genotype accounted for 12 cases, GA genotype accounted for 16 cases, AA genotype accounted for 4 cases, allele frequency G40 (62.5%), A24 (37.5%); CTD group: GG genotype accounted for 26 cases, GA genotype accounted for 16 cases, AA genotype accounted for 3 cases, allele frequency g68 (75.6%), A22 (24.4%); ts group: GG genotype accounted for 8 cases, GA genotype accounted for 24 cases, AA genotype accounted for 2 cases, allele frequency G40(In healthy control group, GG genotype accounted for 42 cases, GA genotype accounted for 45 cases, AA genotype accounted for 13 cases, allele frequency G129 (64.5%), a71 (35.5%); genotype GA in TS group was higher than that in TTD group + CTD group (χ^2 = 8.034, P = 0.018) and control group χ^2 = 6.695, P = 0.035), the difference was statistically significant (P < 0.05). The distribution of G allele frequency in CTD group was higher than that in TS group, the difference was statistically significant (χ^2 = 5.013, P = 0.025), and there was no significant difference in other allele frequencies between the two groups (P > 0.05). See Tables 3 and 4 for details.

Discussion

Tic disorders (TD) is a common neuropsychiatric disorder in childhood. The clinical manifestations of tic disorders are involuntary, aimless, rapid and rigid muscle contraction. Among 114 TD patients, 111 children were followed up with a male to female ratio of 4.3:1, including 89 newly diagnosed children. After one and a half years of follow-up, 32 cases were TTD, 45 cases were CTD and 34cases were TS. their ages were between 3–15 years old, including 16 cases of 3–5 years old, 59 cases of 6–8 years old, 27 cases of 9–11 years old, 8 cases of 12–14 years old, and 1 case of 15 years old. One case of CTD and three cases of TS underwent head MR examination, and the results were normal; 12 cases of TD children with video EEG examination, the results were abnormal (a small amount of moderate to high amplitude slow wave emission in the right central area and the back head in each stage of sleep), and the rest were normal. The clinical manifestations of this group of children were basically consistent with the literature reports[9].

The pathogenesis of TD is unknown, which is believed to be closely related to genetic factors, neurobiochemical and metabolic factors, and environmental factors, among which the study of genetic factors in TS has attracted much attention. Studies have found that most of TS patients had a family history of tic, and most of TS patients were from both parents, and their first-degree relatives had a significantly higher risk of disease than the normal population[10–12]. Other studies suggested that the prevalence rate of identical twins was 53% ~ 56%, compared with 8% for fraternal twins[13]. In recent years, studies on TS genetic characteristics mainly include genome-wide linkage studies, candidate associated genes studies, chromosome aberration studies, copy number variation, epigenetic studies of TS, and total exome sequencing studies, but the exact etiology of TD is still unclear[14, 15].

In recent years, genome-wide association studies (GWAS) have discovered the genetic association recognition of many common complex traits[16]. This non-modular genetic testing approach has not only

improved people's understanding of the pathophysiology of many diseases, but also improved drug treatment strategies[17]. The first GWAS was performed in TS cases by Scharf and colleagues in 2013[2]. They found the strongest association with rs7868992 in collagen XXVII typeα1 gene (*COL27A1*) in european-sourced TS patient samples. In Shandong province of China, Shiguo Liu et al applied haplotype relative risk (HRR) and transmission imbalance test (TDT), and used PCR-guided sequencing of *COL27A1* (rs4979356, rs4979357 and rs7868992) to understand the genetic distribution of the three SNPS in TS[7]. The results suggested that rs4979356 was associated with the etiology of TS development, and allele C and genotype C(+) of rs4979357 were also risk factors for TS, while TDT results of rs7868992 showed no statistically significant significance. The results of linkage disequilibrium test confirmed that the three SNPS were inherited independently. Therefore, the difference of genetic spectrum between Chinese and European people may be the reason for the insignificant TDT results of rs7868992.

In this study, 111 children with TD showed no significant difference in genotype frequency and gene frequency of rs4979356, rs4979357 and rs78689923 SNPS in *COL27A1* compared with children in the control group. The results showed that the distribution of genotype CG, genotype TC and genotype GA of rs4979356, rs7868992 were significantly higher in the TS group than in the TTD + CTD group and the control group. These results suggest that genotype CG of rs4979356, genotype TC of rs4979357 and genotype GA of rs7868992 are high risk factors for TS in children in Guangdong province. The results of this study were consistent with the strong correlation of *COL27A1* rs7868992 in TS in the European population reported by Scharf, and slightly different from the results of Shiguo Liu et al[2, 7]. In addition, the allele frequency of rs7868992 was significantly higher in the CTD group than in the TS group, and there was no statistically significant difference between the other allele frequency groups. The G allele frequency of rs7868992 is only higher in CTD, and its results and significance need to be verified and observed in a larger sample size.

Conclusion

The genotype frequency of rs4979356, rs4979357 and rs7868992 of *COL27A1* gene was related to the onset of TS. Whether the three SNPS of *COL27A1* gene can be included in routine screening to assist TD therapy and assess prognosis, and the role of *COL27A1* in the pathogenesis of TS still needed to be verified by expanding the sample size and expanding the ethnic range.

Abbreviations

TD: Tic disorder; SNP: single nucleotide polymorphism; PCR: polymerase chain reaction; TTD: transient tic disorder; CTD: chronic tic disorder; TS: Tourette Syndrome; GWAS: genome-wide association studies; AD: Alzheimer's disease; HRR: haplotype relative risk; HHRR: Haplotype-based haplotype relative risk; DSM-5: Diagnostic and Statistical Manual of Mental Disorders 5th Edition; DNA: deoxyribonucleic acid;

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Guangzhou Women and Children's Medical Center. The parents/guardians of included subjects were provided written consent form for publication.

Consent for publication

Not applicable.

Availability of data and material

The data that support the findings of this study are available on request from the first author (email contact: gzhslin@163.com) and ethical approval. The data are not publicly available due to restrictions as they contain information that could compromise the privacy of research participants.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

H.S.L. participated in the design, analyzed data and contributed to the writing of the manuscript. B.X.L., W.X.C., and G.S.L. participated in the design of the study, interpreted the results and helped to revise the manuscript. Z.H.Z, J.J.W., S.Y.Y, and L.W. participated in the design and conducted the study. All the authors have read and approved the final version. B.X.L. and W.X.C. is the guarantor.

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Figures

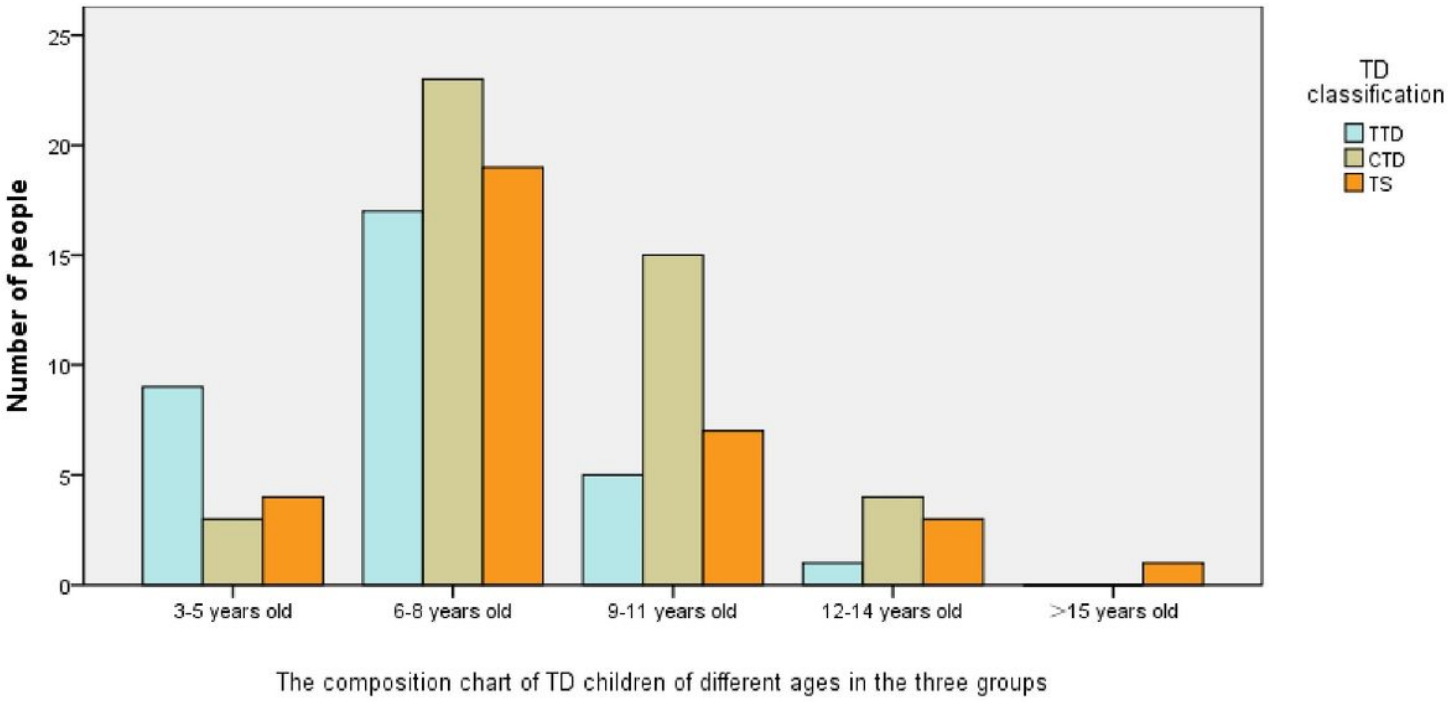

Figure 1

Composition chart of TD children at different ages in the three groups