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Cytogenetic and Clinical Features in Children Suspected With Congenital Abnormalities in 1 Medical Center of Zhejiang Province From 2011 to 2014

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Abstract: This study aimed to investigate the detection rate of chromosome abnormalities in children suspected with congenital disorders in 1 single center, identify any differences according to different classification criteria, and try to enlighten the medical professionals what clinical features should be transferred for cytogenetic analysis.

From January 1, 2011 to March 31, 2014, children who were suspected with chromosomal disorders were included. All the cytogenetic analyses were performed in the Medical Biology and Genetic Department Laboratory in Zhejiang DIAN Diagnostics. We evaluated the variants of clinical indications, and incidence and types of chromosomal abnormalities among groups.

During the study period, 4129 samples were collected and analyzed. Among them, 769 children were detected with chromosome abnormalities, accounting for 18.62% of all referral cases. The ratio of sex-linked chromosomal abnormalities to autosomal ones was 1:3.2. The detection rates were 19.66% (365/1857) for boys and 17.78% (404/2272) for girls. Most of trisomy 21 were found before the age of 1 year old, while most of children with Turner syndrome were found after 6 years old. The group presenting with specific clinical stigmata had highest detection rate of 59.1%.

We demonstrated the detection rates of chromosome abnormalities in children who were suspected with chromosomal disorders. Combined with previous report, we established a database of common chromosomal anomalies and the clinical features that could be useful for genetic counseling and remind the medical professionals what kind of patients should be transferred to genetic analysis.

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Abbreviations: aCGH = array comparative genomic hybridization, FISH = fluorescence in situ hybridization, GTG = G-banding using trypsin and Giemsa, GU = genitourinary, TS = Turner syndrome.

INTRODUCTION

Chromosomal abnormalities affect about 0.5% of living newborns, and are associated with congenital malformation, cognitive defects, learning disabilities, seizures, etc.[1–4] Cytogenetic techniques can diagnose chromosomal abnormalities, and investigate the possible etiology of birth defects. It is important to know the clinical data of chromosome abnormalities in order to explore the corresponding relationships between the phenotypes and certain chromosome abnormalities, and increase the evidences of initial clinical indications of these types of disorders in different ages. Furthermore, the cytogenetic outcomes can guide medical professionals the optimal treatment, social function training, and predicting the possible prognosis.[5]

Our tertiary care referral center previously reported the results of cytogenetic survey from 1996 to 2010, which allowed us to closely gain insight into the incidence and distribution of the cytogenetic abnormalities in outpatient children suspected with congenital disorders.[5] The purpose of the present study was to collect data among children who were suspected with chromosomal disorders from January 1, 2011 to March 31, 2014 in the Children's Hospital, Zhejiang University, and tried to establish and update our previous database of common chromosomal anomalies that could be useful for genetic counseling and reminding the medical professionals which kind of patients should be transferred to genetic analysis.

MATERIALS AND METHODS

Sample Collection

We collected children who were suspected with chromosomal disorders from January 1, 2011 to March 31, 2014 since this study was an update to the previous report by the same team in the Children's Hospital, Zhejiang University. The informed consents were obtained from children's parents/guardians or other legally authorized representatives before the chromosome analysis preparation, including clinical interview of the medical histories and blood sample collections. The protocol details were described elsewhere.[5] The clinical features were recorded and the blood sample were collected, and then the blood samples were sent to the Medical Biology and Genetic Department Laboratory for cytogenetic analysis at Zhejiang DIAN Diagnostics, which is an independent third-party medical diagnostic service institution. According to the reasons for referral for cytogenetic analysis, we divided them into 4 groups: Group 1, who presented with specific clinical stigmata (such as up slanting palpebral fissure, prominent

TABLE 1. Rate of Chromosome Abnormalities in Boys and Girls in Different Age Groups

	−28 d (n = 803)	−1 yr (n = 868)	−3 yr (n = 629)	−6 yr (n = 432)	−12 yr (n = 1198)	−13 yr (n = 199)	Total
Chromosomal abnormalities in boys, n (%)	119 (25.2)	134 (25.6)	52 (14.3)	27 (17.2)	27 (9.6)	7 (11.7)	365 (19.7)
Chromosomal abnormalities in girls, n (%)	87 (26.3)	95 (27.5)	45 (17.0)	33 (12.0)	102 (11.1)	41 (29.5)	404 (17.8)
Sex-linked chromosome abnormalities in the affected, n (%)	20 (9.7)	23 (10.4)	15 (15.5)	18 (30.0)	63 (48.8)	44 (91.7)	183 (23.8)
Chromosomal abnormalities, n (%)	206 (25.7)	229 (26.4)	97 (15.6)	60 (13.9)	129 (10.8)	48 (24.1)	769 (18.6)

epicantic folds, micrognathia, etc.); Group 2, who had speech or motor developmental delay, or both, or learning disabilities; Group 3, who presented with congenital genitourinary defects (including ambiguous genitalia, abnormality of male external genitalia, concealed penis, cryptorchidism, shield chest, widely spaced nipples and amenorrhoea, etc.); and Group 4 (miscellaneous group, including obesity, congenital heart diseases, primary seizures and other indications not listed in the above three groups). For those who presented with both specific clinical stigmata and genitourinary defects we would put them into 1 group according to the main complains of their main problems.

Cytogenetic Analysis

For routine cytogenetic analysis, 0.5 to 1.0 mL peripheral blood samples were collected from the patients and stored into heparinized test tubes. The karyotypes were determined by G-banding using trypsin and Giemsa (GTG).[6] At least 30 cells were routinely analyzed; in cases of mosaicism, this number was increased to approximately 100 metaphases. The method was described elsewhere. The karyotypic descriptions were reported according to the International System for Human Cytogenetic Nomenclature recommendations (ISCN, 1995).

Statistical Analysis

The percentage of abnormal cases in each group and the distribution of the numerical and structural abnormalities were determined. We used the Chi-squared test to evaluate the detection rates and types of chromosomal anomalies among groups according to different classification criteria.

RESULTS

There were totally 4129 children referred to cytogenetic analysis from January 1, 2011 to March 31, 2014, including 1857 boys and 2272 girls. The average age was 51.7 months, median age was 33 months, and age ranged from 1 day to 18 years and 11 months old. The ratios between cases referred for cytogenetic analyses and total outpatient visits were 1:1607 (1036/1,665,048) in 2011, 1:1364 (1328/1,812,521) in 2012, 1:1318 (1448/1,908,152) in 2013, and 1:1329 (317/421,532) in first quarter of 2014, respectively. There was no statistical difference between the referral ratios in these years by Chi-squared test ($\chi^2 = 0.03, P = 0.99$). But compared to previous report, the referral ratios were higher than that in 2010 ($\chi^2 = 448$, $P < 0.001$).

There were 769 children who had chromosome abnormalities, accounting for 18.62% of all referral cases. The ratio of sex-linked chromosomal abnormalities to autosomal ones was

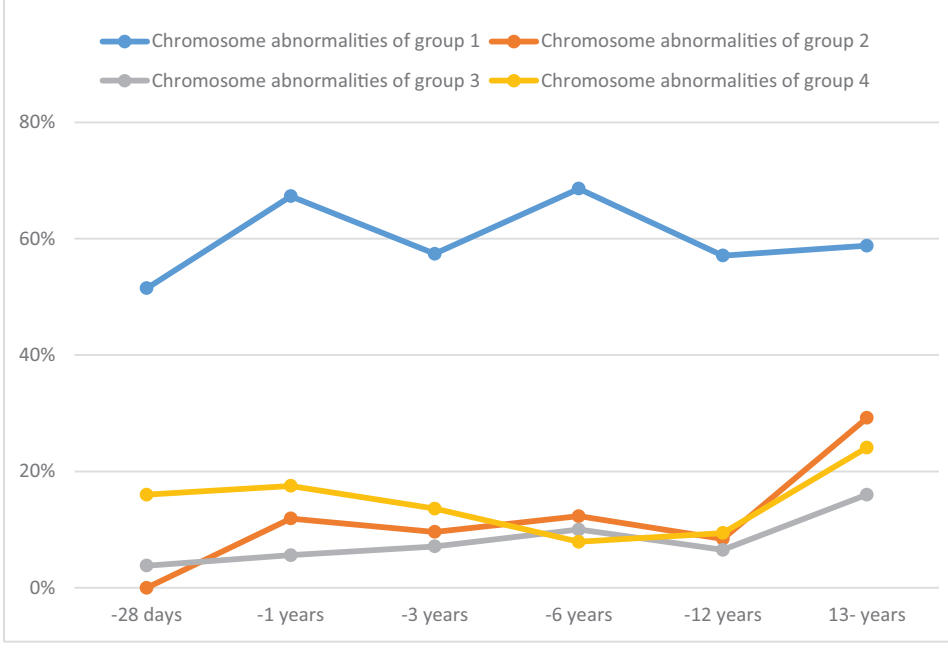

FIGURE 1. Trend of detection rate in different groups.

TABLE 2. Types of Chromosomal Abnormalities

	−28 d (n = 803)	−1 yr (n = 868)	−3 yr (n = 629)	−6 yr (n = 432)	−12 yr (n = 1198)	−13 yr (n = 199)	Total
Autosomal chromosomal abnormalities							
Numerical abnormalities							
Trisomy 21	120	156	49	23	10	1	359
Trisomy 20	1						1
Trisomy 18	7						7
Trisomy 15							
Trisomy 13	1						1
Numerical and structural abnormalities	5	2	1		2		10
Structural abnormalities							
Translocation-reciprocal	7	6	5	1	7	1	27
Translocation-Robertsonian			1				1
Inversion	5	8	6	6	17	1	43
Addition	6	4	5	5	8		28
Derivative	4	7	4		1		16
Duplication	5	2			6		13
Insertion		1			1		2
Marker	1		2	2	2		7
Deletion	9	12	1	2	2		26
h	4	3		1	6	1	15
i				1	1		2
Ring 18	3						3
Ring 15			1		1		2
Ring 13	1						1
Ring 14		1					1
Ring 9		1					1
Ring 5			1		1		2
Mosaicism							
Numerical	6	3	5	1	1		16
Structural	1		1				2
Subtotal	186	206	82	42	66	4	586
Sex-linked chromosome abnormalities							
Numerical abnormalities							
45, X		4	2	4	14	17	41
45, X Mosaicism	2	1	2	4	17	14	40
47, XXY	1	2	2		4	2	11
47, XXX	2	3	1	1	1		8
47, XYY	1	3			1	1	6
48, XYY, +21	2						2
48, XXYY		2					2
48, XXXY						1	1
Mosaicism	1	1		1		3	6
Structural abnormalities	11	7	8	8	26	6	66
Subtotal	20	23	15	18	63	44	183
Total	206	229	97	60	129	48	769

1:3.2. The detection rates of abnormalities were 19.66% (365/1857) for boys and 17.78% (404/2272) for girls ($\chi^2 = 2.34$, $P = 0.13$). The detection rate of autosomal anomalies was higher in boys (16.6% vs 12.2%, $\chi^2 = 16.6$, $P < 0.001$), but the detection rate of sex-linked chromosomal anomalies was higher in girls than that in boys (5.6% vs 3.0%, $\chi^2 = 17.2$, $P < 0.001$). The data in details was shown in Table 1.

According to referral causes, there were 4 groups set. The percentages of chromosome abnormalities for Groups 1, 2, 3, and 4 were 59.1%, 10.6%, 7.9%, and 13.1%, respectively. In Group 1, in which children presented with specific clinical stigmata, the detection rate of chromosome abnormalities was highest. While for Group 3, in which children presented with congenital genitourinary defects, the detection rate of chromosome abnormalities was lowest (Fig. 1).

Totally, 359 cases were detected with Down syndrome, 76.9% (276 cases) of which were found before their age of 1 year old, and 90.5% (325 cases) were found before their ages of 3 years old. There were 81 children with Turner syndrome (TS), 76.5% of which (62/81) were diagnosed after 6 years old. Almost half of the children with TS had the karyotypes of mosaicism (Table 2).

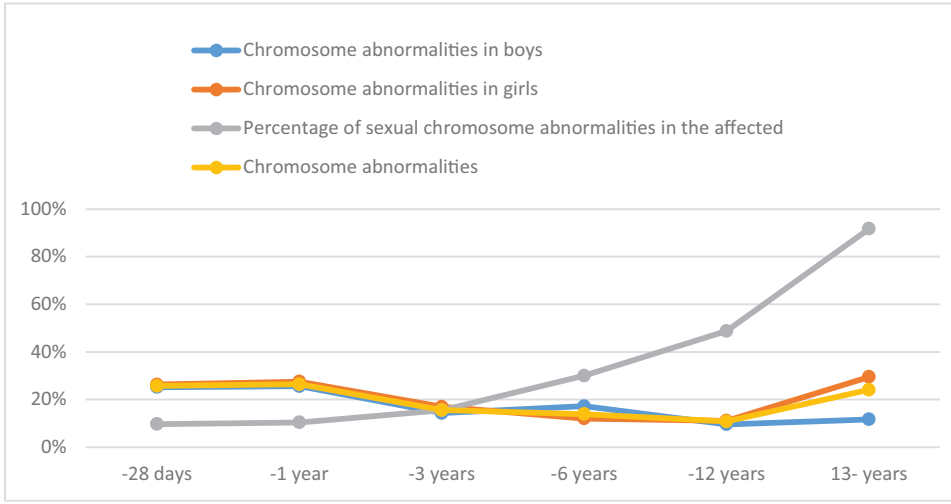

FIGURE 2. Trend of detection rate in different age groups.

According to age when children were transferred to do cytogenetic analysis, the detection rates were higher in neonates and infants than that in others (Fig. 2). Further analysis showed that most of chromosome abnormalities were trisomy 21, accounting for almost half of all the abnormalities (Fig. 3). In addition, we presented the cytogenetic results of 769 cases in detail (Table 2).

There were 2059 children with primary diagnosis and 2070 with unknown caused diseases. Four hundred two (19.5%) were confirmed by cytogenetic analysis and their results were consistent with primary diagnosis. In the other 2070 children with unknown causes, 326 (15.7%) were diagnosed by cytogenetic test.

DISCUSSION

This investigation showed the total detection rate of chromosome abnormalities was 18.62% in the cases suspected with congenital disorders, which was lower than that in the previous report undertaken by the same study group from 1996 to 2010. In recent 2 decades, the diseases spectrum are changing and diversely.[7] Cytogenetic analysis is becoming important in diagnosing these type diseases.[8] In the present report, the detection rate did not mean the incidence of specific disease. It could only indicate the occurrence rate of 1 disease in the group of suspected with 1 type of disease in 1 center.

The data from these patients were compared with that from our previous published group of children (1996–2010) from the same children's hospital.[5] We found that the ratios between cases referred for cytogenetic analyses and total outpatient visits were increased from 2011 to 2014. Compared to the previous report, these present ratios were higher than that before 2006, and equal to that post-2007. This result indicated that the professionals had more awareness of transferring the children with clinical manifestations of unknown causes to do

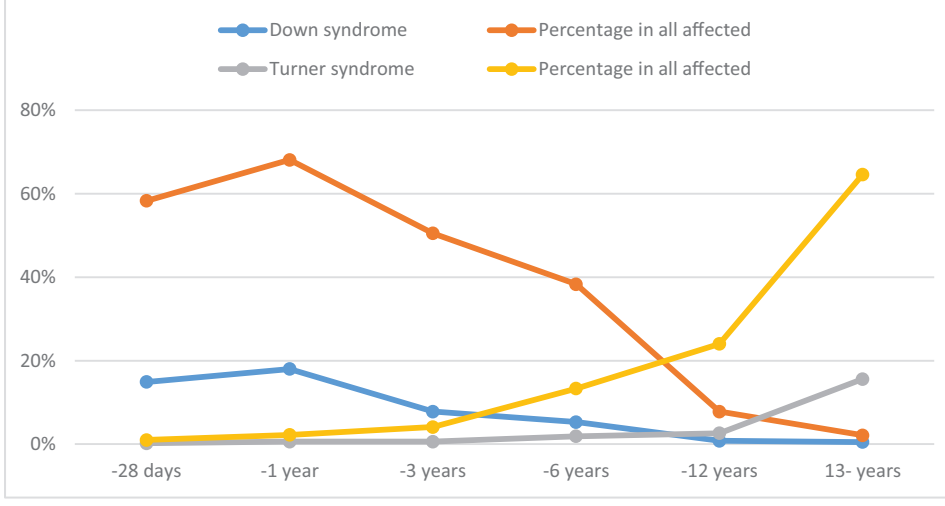

FIGURE 3. Trend of detection rate in different age groups.

cytogenetic analysis. The most notable feature of these types of disorders is diagnostic difficulty since they present diverse clinical pictures. Physical examination, radiographic studies, and histologic investigation may prove to be equivocal. Cytogenetic analyses have become a more common tool to diagnose the unknown diseases.

The data from Table 1 showed that the detection rate for the children <1 year old was higher than that for other older age groups. The detection rate was decreased gradually as their ages increased. In addition, we should take it into consideration that the percentage of trisomy 21 was 76.9% in total chromosome abnormalities in 0 to 1 year group, and 90.5% in 0 to 3 years group. These data indicated that professionals had the experiences to identify this common syndrome, even when patients were very young. However, for the most common sex-linked chromosome abnormality of TS, only 5 children were diagnosed in 1 year old, accounting for 6.2% of total diagnosed cases (n = 81). More than 2/3 of them (31/81) were identified until children had more symptoms in their adolescent stages.[9–12] This indicates the clinical doctors were not familiar with TS, especially for those who were very young. TS was the most common sex-linked chromosome abnormality in children and adolescent.[13] If we found the patients in their early stages, we could treat them timely, which would greatly improve their prognosis, including final stature and sexual development.[14] Therefore, it was significant to do more training with the diverse features of these type of chromosome disorders, which would contribute to finding them early, and being treated timely.

If we compared the detection rate according to the reasons for referral for cytogenetic analysis, we found that the Group 1 which presented with specific clinical stigmata had highest detection rate of 59.1%. Among them, 91.9% (330/359) of Down syndromes, 25.0% of TSs were found. We speculated that, as mentioned above, our professionals were familiar with Down syndrome, but were not with TS. Group 3 presented with congenital genitourinary defects had lowest detection rate of 7.9%. We should take the factor of detection resolution into consideration that might lead to low detection rate. Normal development of the genitourinary (GU) tract is a complex process that frequently goes abnormal. In male children, the most frequent congenital GU anomalies are cryptorchidism (1–4%), hypospadias (1%), and micropenis (0.35%). Current evidences suggested that monogenetic changes contributed to the congenital genitourinary defects, which could be detected at DNA levels or fluorescence in situ hybridization (FISH) or array comparative genomic hybridization (aCGH) rather than chromosomal analysis.[15,16] The professionals could transfer these children to do Next-Generation Sequencing or microarray and promote the detection rate.

The percentage of sex-linked chromosome abnormalities in all abnormalities was about 1/4. Most of them were transferred to do cytogenetic analysis because they were found with abnormal appearance of the external genitalia, or without second sexual features in their ages of more than 12 years.[17,18] Thus, the percentages of sex-linked chromosome abnormalities in children older than 6 years were higher than those younger than 6 years (10.9% vs 52.7%).

There were several limitations in our study. Firstly, we only used chromosome test in our report. Presently, most professional organizations have recommended more higher resolution tests for children suspected with chromosome abnormalities, as aCGH testing. But the aCGH testing is not popularized among general hospitals in China, and which is more expensive. Few consents can be obtained from the parents. Therefore, chromosome test was employed in this report. Secondly, we did not establish an interaction between a specific type of chromosome abnormality and clinical features. Actually, because of the miscellaneous information, the clear relationship is not established. As an alternative designation, we divided these clinical features into 4 groups to define an extensive association of clinical features with chromosome abnormalities.

CONCLUSION

This study demonstrated the detection rates of chromosome abnormalities in children who were suspected with chromosomal disorders. There were 769 children who had chromosome abnormalities, accounting for 18.62% of all referral cases. Among the affected, the percentage of sex-linked chromosomal abnormalities in all was 23.8%. The detection rates of abnormalities were 19.66% for boys and 17.78% for girls. Combined with previous report, we established a database of common chromosomal anomalies and the clinical features and remind the medical professionals what kind of patients should be transferred to genetic analysis.

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