EDITORIAL

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New Bacterial Artificial Chromosome and Commercial FISH Probes for the 22q11.2 Region in Patients with Congenital Heart Defect and with Phenotype Resembling DiGeorge and Velocardiofacial Syndromes*

Badania FISH z użyciem nowych sztucznych chromosomów bakteryjnych oraz komercyjnych sond dla regionu 22q11.2 u pacjentów z wrodzoną wadą serca oraz fenotypem zespołu DiGeorge'a oraz zespołu podniebienno-sercowo-twarzowego

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Abstract

Background. The majority of patients with DiGeorge syndrome (DGS) and velocardiofacial syndrome (VCFS) have a microdeletion in 22q11.2. The minimal DiGeorge critical region (MDGCR) has been narrowed down to 250 kb using FISH analysis. The construction of bacterial artificial contigs is an essential step towards the identification of deletions of smaller size.

Objectives. A set of bacterial artificial chromosomes (BACs) was used in a FISH assay in patients with congenital heart defect and phenotype resembling DGS/VCFS to determine new, specific, deleted regions not encompassed by commercial probes.

Material and Methods. The study group comprised 69 patients with congenital malformations, including heart defects and dysmorphic features. The patients were divided into three subgroups. Group I comprised patients diagnosed with DGS/VCF syndrome by the detection of a 22q11.2 deletion using FISH applying the commercial probes TUPLE1 and N25. All the patients in this group also had a FISH study with seven BAC probes (115F6, 678G6, 770H11, 201C11, 919E7, 219G6, 431E9) comprising the critical region 22q11.2. Group II was made up of patients with clinical features of DGS/VCFS but without a deletion detected by FISH using the commercial FISH probes. FISH with BAC probes was also performed in this group. Group III was patients with clinical features suggesting DGS/VCFS with no deletion detected by the TUPLE1 and N25 probes. FISH with the BAC probes was not performed in the group.

Results. Within group I, deletions in the regions for BACs 770H11, 201C11, 919E7, 219G6, and 431E9 were detected in all 14 children with DGS/VCFS. FISH study with the 115F6 and 678G6 probes revealed the correct two signals in all patients of group I. No deletions were detected by any of the seven BACs tested in group II involving the patients with clinical DGS/VCFS features, nor was a deletion detected by the commercial probes. The clinical symptoms of the patients of the three clinically heterogeneous groups with diagnosed and suspected DGS/VCFS were compared. Palate insufficiency, hypocalcemia, and recurrent infections were significantly more frequent in patients with a 22q11.2 microdeletion confirmed cytogenetically by FISH.

Conclusions. These results strongly suggest that strict diagnostic criteria for DGS/VCFS are needed (**Adv Clin Exp Med 2007, 16, 6, 717–723**).

Key words: DiGeorge syndrome, velocardiofacial syndrome, phenotype, FISH, microdeletion.

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Streszczenie

Wprowadzenie. Delecja 22q11.2 jest jedną z najczęstszych mikrodelecji u człowieka i jest przyczyną kilku zespołów genetycznych, m.in.: zespołu DiGeorge'a (DGS) oraz podniebienno-sercowo-twarzowego (VCFS – *velo-cardio-facial syndrome*). Rutynowa diagnostyka cytogenetyczna zespołów obejmuje fluorescencyjną hybrydyzację *in situ* (FISH) z użyciem sond charakterystycznych dla obszaru krytycznego. Ostatnie doniesienia wykazały, że w jego obrębie mogą występować submikrodelecje obejmujące znacznie mniejsze obszary.
Cel. Analiza submikrodelecji w obszarze 22q11.2 metodą FISH z użyciem sztucznych chromosomów bakteryjnych (BAC) u pacjentów z wrodzonymi wadami serca i cechami dysmorficznymi.
Materiał i metody. Badania obejmowały 69 pacjentów z wadami wrodzonymi serca oraz cechami dysmorficznymi sugerującymi zespoły mikrodelecji 22q11.2. Pacjentów podzielono na 3 grupy: grupa I – pacjenci ze zdiagnozowanym zespołem DG/VCF za pomocą badania FISH z użyciem sond komercyjnych (TUPLE1, N25). W tej grupie wykonano ponadto badanie FISH z użyciem siedmiu sond bakteryjnych (BAC – 115F6, 678G6, 770H11, 201C11, 919E7, 219G6, 431E9), obejmujących krytyczny region 22q11.2; grupa II – pacjenci z klinicznymi cechami sugerującymi DGS/VCFS, ale bez zdiagnozowanej mikrodelecji sondami komercyjnymi. W tej grupie również wykonano badanie FISH z użyciem sond bakteryjnych, charakterystycznych dla regionu 22q11.2, izolowanych ze sztucznych chromosomów bakterii; grupa III – pacjenci z cechami klinicznymi sugerującymi DGS/VCFS, bez zdiagnozowanej mikrodelecji sondami komercyjnymi, u których nie wykonano badania FISH z użyciem sond bakteryjnych.
Wyniki. W grupie pierwszej u wszystkich pacjentów ze stwierdzoną mikrodelecją 22q11.2 wykazano również delecję w regionie wyznaczonym przez sondy bakteryjne (770H11, 201C11, 919E7, 219G6, 431E9). Badanie FISH, wykonane z sondami 115F6, 678G6, nie wykazało nieprawidłowości (brak delecji). W drugiej grupie u żadnego pacjenta nie stwierdzono mikrodelecji za pomocą sond bakteryjnych. Potwierdziło to swoistość sond komercyjnych. W pracy przeprowadzono ponadto analizę porównawczą danych klinicznych pacjentów z trzech grup ze zdiagnozowanym DGS/VCFS oraz z podejrzeniem ww. zespołów mikrodelecji. Wykazano istotne różnice w częstości występowania niewydolności podniebienno-gardłowej, zmniejszonego stężenia wapnia w surowicy krwi oraz nawracających zakażeń związanych z zaburzeniami odporności komórkowej u pacjentów z potwierdzonym zespołem mikrodelecji 22q11.2 w porównaniu z grupą II i III bez wykazanej delecji.
Wnioski. Powyższe dane sugerują, aby podejrzewając DGS/VCFS, kierować się pewnymi kryteriami diagnostycznymi (**Adv Clin Exp Med 2007, 16, 6, 717–723**).

Słowa kluczowe: zespół DiGeorge'a, zespół podniebienno-twarzowo-sercowy, fenotyp, FISH, mikrodelecja.

DiGeorge syndrome (DGS) is a developmental anomaly of the derivatives of the third and fourth pharyngeal pouches. It is associated with a spectrum of malformations, including absence or hypoplasia of the thymus and parathyroid glands, cardiovascular anomalies, and mild facial dysmorphism. It has been proposed that the primary defect in DGS is the failure of cephalic neural crest cells to migrate properly during early embryonic development [1, 2]. Previously, cytogenetic studies demonstrated that 20% of patients with DGS have chromosomal abnormalities, with the majority of these chromosomal rearrangements involving the loss of the proximal long arm of chromosome 22 [3]. Subsequently, more detailed cytogenetic studies demonstrated that microdeletions of one copy of the region 22q11.2 are involved in the etiology of DGS [4].

Velocardiofacial syndrome (VCFS) is a common autosomal dominant disorder characterized by cleft palate, cardiac anomaly, characteristic facial features, and learning disability. Due to the phenotypic overlap between VCFS and DGS, it was postulated that both diseases might share a common pathogenesis or be etiologically related [5]. Using DNA markers for the region of 22q11.2 deleted in patients with DGS, it was possible to demonstrate that the majority of VCFS patients are hemizygous for the same region [6]. Currently, over 85% of the patients with a clinical diagnosis of VCFS are diagnosed with microdeletions of the 22q11.2 region [7]. These findings indicate that haplo-insufficiency of the critical region is a major factor in the development of this disorder [4].

The majority of DGS/VCFS patients (about 90%) have a large deletion which includes a common set of markers in 22q11.2 [6, 8]. The size of the "commonly deleted region" (DGCR, the DiGeorge critical region) has been estimated to be about 3 Mb (megabases) and encompasses approximately 30 genes [4, 9]. About 8% of patients, however, have a smaller deletion of 1.5 Mb which encompasses 24 genes [6, 9]. Moreover, individual patients can have deletion or low copy repeat sites which flank either proximally or distally the "commonly deleted region" [6]. Analyzing the translocation breakpoints and applying fluorescence *in situ* hybridization (FISH) analysis, the region critical to DGS/VCFS has been narrowed down to a 250-kb (kilobase) area in the proximal fragment of the commonly deleted region (MDGCR, minimal DG critical region) [9, 10, 11]. This region includes two markers, D22S75 (N25) and TUPLE1 (Fig. 1), which are the most consistently deleted markers in DGS/VCFS patients [4, 6, 9–11]. However, only in a part of the patients clinically diagnosed as DGS/VCFS were microdeletion of the critical region confirmed by FISH assay [12, 13].

It is now suggested that DGS/VCFS is caused by alternations of more genes in the critical deleted region, with different extents of the deletions [14]. Thus the identification of new cytogenetic and/or molecular markers is an essential step towards the diagnosis of cases which present the typical clinical features of DGS/VCFS but without the critical 22q11.2 microdeletion diagnosed by FISH analysis with commercial probes (N25 and TUPLE1). In the present study a set of bacterial artificial chromosomes (BACs) for the FISH assay was used in patients with congenital heart defect and phenotype resembling DGS/VCFS to determine new, specific, deleted regions not encompassed by commercial probes.

Table 1. List of bacterial artificial chromosomes (BACs) for the FISH assay used in this study

Tabela 1. Lista sztucznych chromosomów bakteryjnych (BAC) do badania FISH użytych w pracy

Name of BAC (Nazwa BAC)	Size (kb) (Rozmiar)	Locus (Lokus)	Distance from 22q (Odległość od 22q)
DD05-115F6	194	D22S420	16,233
DD06-678G6	125	D22S181	16,050
DD02-770H11	140	D22S111	17,850
DD09-201C11	189	D22S553	17,690
DD11-919E7	95	D22S941	17,784
DD18-431E9	130	D22S163	19,458
DD19-219G6	220	HCF2	19,466

Material and Methods

Patients

The study group comprised 69 patients with congenital malformations, including heart defects and dysmorphic features. The patients were divided into three subgroups. The first group (group I) consisted of 14 children diagnosed with DGS/VCFS by the detection of 22q11.2 deletion using FISH applying the commercial probes TUPLE1 and N25. The second group (group II) encompassed 33 children with clinical features of DGS/VCFS but with no deletion detected in FISH by the commercial FISH probes TUPLE1 and N25. Based on their clinical features, the children of groups I and II were selected for further investigation and FISH with seven BAC probes (115F6, 678G6, 770H11, 201C11, 919E7, 219G6, and 431E9) (Fig. 1). The third group (group III) included 22 children with clinical features suggesting DGS/VCFS but showing no deletion detected by the TUPLE22 and N25 probes. This group was used as a control group for clinical features.

Table 2. Phenotype of patients (cardiac defects) with DGS/VCFS in the three groups

Tabela 2. Fenotyp pacjentów (wada serca) z zespołem DG/VCF w trzech grupach

Cardiac defect (Wada serca)	Number of patients (%) (Liczba pacjentów – %)		
	Group I (Grupa I)	Group II (Grupa II)	Group III (Grupa III)
ToF	10 (71.43)	8 (24.3)	4 (18.2)
VSD	2 (57.2)	7 (21.2)	9 (40.9)
ASD	1 (7.14)	1 (3)	3 (13.6)
TGA	0	5 (15.2)	0
HLHS	0	3 (9.1)	1 (4.6)
AVC	0	4 (12.1)	0
CoA	0	3 (9.1)	0
PDA	0	1 (3)	0
DORV	0	1 (3)	0
PFO	0	0	4 (18.2)
TA	0	0	1 (4.6)
No cardiac defect (Bez wady serca)	1 (7.14)	0	0
Total = 69 (Razem = 69)	14	33	22

ToF – Fallot tetralogy, VSD – ventricular septal defect, ASD – atrial septal defect, TGA - transposition of great arteries, HLHS – hypoplastic left heart syndrome, AVC – atrio-ventricular septal defect, CoA – coarctation of aorta, PDA – patent ductus arteriosus, DORV – double outlet right ventricle, PFO - patent foramen ovale, TA – tricuspidal atresia.

ToF – tetralogia Fallota, VSD – ubytek w przegrodzie międzykomorowej, ASD – ubytek w przegrodzie międzyprzedsionkowej, TGA – przełożenie dużych tętnic, HLHS – hipoplazja lewego serca, AVC – wspólny kanał przedsionkowo-komorowy, CoA – koarktacja aorty, PDA – drożny przewód tętniczy, DORV – podwójny odpływ komory prawej, PFO - drożny otwór owalny, TA – zarośnięcie zastawki trójdzielnej.

Fluorescent *in situ* Hybridization (FISH) with BAC Probes

The 194-kbp 115F6, 125-kbp 678G6, 140-kbp 770H11, 189-kbp 201C11, 95-kbp 919E7, 220-kbp 219G6, and 130-kbp 431E9 BAC probes, received from the Department of Molecular Genetics, University of Tuebingen, Germany, were labeled using the CGH Nick Translation Kit (Vysis) according to the protocol (Tab. 1). Twenty µl (2 µg) of purified BAC clone and 5 µl of nick translation enzyme were used. The incubation time was four hours. The labeled BAC clones were purified by

overnight precipitation with ethanol and sodium acetate according to the standard protocol. DNA was dissolved in 28 µl of Hybridization Solution (Vysis) and 12 µl of nuclease-free water and mixed at 40°C for four hours.

FISH analysis was performed using standard protocols. Three µl of BAC probe were applied at each hybridization point on freshly prepared slides. After denaturation for 2 min at 73°C and overnight incubation at 37˚C, the slides were washed for 2 min in 0.4 SSC/0.3% NP-40 wash solution in a 73°˚C water bath and for 20 sec in 2 x SSC/0.1% NP-40 at room temperature. Ten µl of DAPI counterstain (Vysis) was applied to each hybridization

Table 3. Clinical data of the three groups of patients

Tabela 3. Dane kliniczne pacjentów w trzech grupach

Clinical data (Dane kliniczne)	Number of patients (Liczba pacjentów)			Statistics (Statystyka)
	Group I (Grupa I)	Group II (Grupa II)	Group III (Grupa III)	Statistically significance (Znaczenie statystyczne) $p < 0.05$ *
Cardiac defect (Wada serca)	13 (92.8)	33 (100)	22 (100)	> 0.005
Multiple cardiac defect (Złożona wada serca)	8 (57.2)	18 (54.5)	10 (45.5)	> 0.005
Palate insufficiency, including cleft palate (Niewydolność podniebienia, w tym rozszczep podniebienia)	11 (78.6) 2 (14.3)	11 (45.5) 4 (12.2)	2 (9.09) 0	< 0.005 > 0.005
Other congenital defect (Dodatkowe wady wrodzone)	4 (28.6)	14 (45.5)	2 (9.09)	> 0.005
Dysmorphic facial features (Cechy dysmorficzne twarzy)	14 (100)	33 (100)	22 (100)	> 0.005
Hypocalcemia (Hipokalcemia)	11 (78.6)	0	0	< 0.005
Recurrent infection, cellular immunity deficit (Nawracające infekcje, zaburzenia odporności komórkowej)	9 (64.3)	8 (24.2)	4 (19.2)	< 0.005
Psychomotor delay (Opóźnienie psychoruchowe)	6 (42.7)	7 (21.2)	5 (22.8)	> 0.005
Total = 69 patients (Razem = 69 pacjentów)	14	33	22	

* Spearman's correlation coefficient test and chi squared (χ^2) Pearson's test.
Group I – patients diagnosed with DGS/VCFS by detection of 22q11.2 deletion using FISH applying the commercial probes TUPLE1 and N25.
Group II – patients with clinical features of DGS/VCFS without deletions detected in FISH by the commercial FISH probes TUPLE1 and N25 or by the seven BAC probes 115F6, 678G6, 770H11, 201C11, 919E7, 219G6, and 431E9.
Group III – patients with clinical features suggesting the diagnosis of DGS/VCFS with no deletions detected by TUPLE1 and N25 probes, without applying FISH using the BAC probes.

* test Spearmana oraz Chi² (χ^2) Pearsona.
Grupa I – pacjenci ze zdiagnozowanym zespołem DGS/VCF przez wykrycie delecji 22q11.2 w badaniu FISH z użyciem sondy komercyjnej TUPLE1 oraz N25.
Grupa II – pacjenci z cechami klinicznymi zespołów DG/VCF bez delecji w badaniu FISH z użyciem sond komercyjnych (TUPLE1, N25) oraz bez delecji w badaniu FISH z wykorzystaniem sztucznych sond chromosomowych (115F6, 678G6, 770H11, 201C11, 919E7, 219G6, 431E9).
Grupa III – pacjenci z cechami klinicznymi sugerującymi zespół DG/VCF bez delecji w badaniu FISH z użyciem sond komercyjnych (TUPLE1, N25) oraz u których nie wykonano badania FISH z użyciem sztucznych sond chromosomowych.

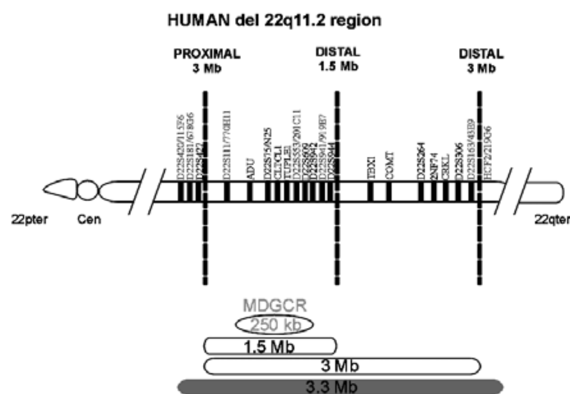

Fig. 1. Human 22q11.2 region with the sizes of the deletions described in the text

Ryc. 1. Region 22q11.2 u człowieka z obszarami delecji opisanymi w tekście

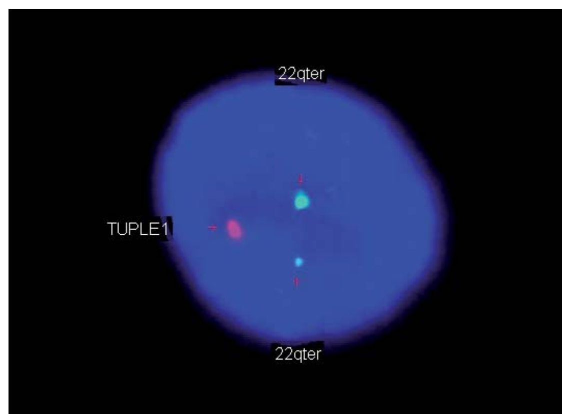

Fig. 2a. Fluorescence *in situ* hybridization (FISH) with the TUPLE1 Region Probe (22q11.2) and Control Probe (22qter). Microdeletion of 22q11.2 is diagnosed in this material. Two green signals are present representing the 22qter region and one red signal representing the TUPLE1 probe in the 22q11.2 region

Ryc. 2a. Badanie metodą fluoroscencyjnej hybrydyzacji *in situ* (FISH) z użyciem sondy DiGeorge/VFCS TUPLE1 Region Probe (22q11.2) with Control Probe (22qter) – Cytocell. W badanym materiale stwierdzono obecność mikrodelecji badanego regionu 22q11.2. Widoczne są dwa sygnały zielone odpowiadające regionom 22qter oraz jeden sygnał czerwony odpowiadający regionowi TUPLE1 (w obszarze 22q11.2)

location. A Nicon Eclipse microscope (USA) with appropriate optical filters (DAPI/FITC/TRICT) and ISIS Metasystems Software (Germany) were used for visualization and documentation (Figs. 2a and 2b). For each hybridization, 100 nuclei/metaphase were examined.

The study design was accepted by Wroclaw Medical University's Ethics Committee.

Results

Congenital heart defects within group I were diagnosed in 13 of the 14 patients (92.8%). Tetralogy of Fallot (ToF) was observed in 10 cases, ventricule septal defect (VSD) in two, and atrial septal defect (ASD) in one case (Tab. 2). Multiple congenital heart defects were detected in 8 of the 14 cases (57.2%). Palatoschisis (cleft palate) was observed in only two cases, but palate insufficiency (regurgitation) in 11 cases (together: 93%). All children presented dysmorphic facial features. Hypocalcemia (and parathyroid insufficiency) was diagnosed and treated in 11 patients (93%). Recurrent infections and deficient cellular immunity were observed in 9 patients (64.3%). An additional defect was observed in 4 cases (28.6%), these being omphalocele, cryptorchismus, eyeball defect, and liver defect, and a delay in psychomotoric and neurological development was found in 6 patients (Tab. 3).

All the patients in group II had a congenital heart defect: 9 children had ToF, 5 transposition of great arteries (TGA), 3 hypoplastic left heart syndrome (HLHS), 4 complete atrio-ventricular septal defect (AVC), 1 ASD, 7 VSD, 3 coarctation of aorta (CoA), 1 patent ductus arteriosus (PDA), and 1 with double outlet right ventricle (DORV) (Tab. 2). Multiple congenital heart defects were detected in

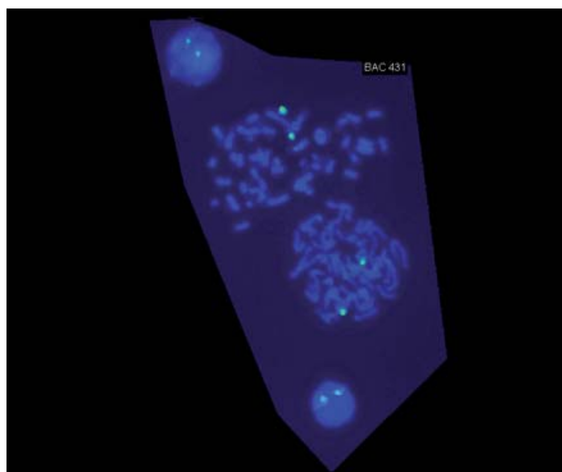

Fig. 2b. Fluorescence *in situ* hybridization (FISH) with the BAC probe 431E9. Two correct green signals are present in two metaphases and interphases

Ryc. 2b. Badanie metodą fluoroscencyjnej hybrydyzacji *in situ* (FISH) z użyciem sondy BACs 431E9. Widoczne są dwa prawidłowe sygnały w dwóch metafazach i interfazach

18 of the 33 cases (54.5%). Palatoschisis was observed in four cases and palate insufficiency (regurgitation) in 11 (together: 45.5%). All the children also presented dysmorphic facial features. No hypocalcemia (or parathyroid insufficiency) was diagnosed in this group. Recurrent infections and

deficient cellular immunity were observed in 8 patients (24.2%). In this group, a additional defect was observed in 14 cases (42.3%), such as coloboma of the retina, deficit in the retina and choroidea of both eyes, cataracta, brain defect, esophageal atresia, omphalocele, cryptorchismus, anal atresia, urethral reflux, and skeletal defect. Delayed psychomotoric development or mild learning disability was diagnosed in 7 cases (Tab. 3).

In group III the following heart defects were diagnosed: 1 had hypoplastic left heart syndrome (HLHS) with interrupted aortic arch, hypoplastic aortic valve, and aneurysm of the interventricular septum and PDA, 9 had VSD, 4 patent foramen ovale (PFO), 1 tricuspidal valve insufficiency (TA), 3 ASD, and 4 children had ToF (Tab. 2). Congenital multiple heart defects were observed in 10 cases (45.5%). Within this group, two children had a cleft palate and two presented skeletal defects (polydactyly). There was no hypocalcemia in this group. Psychomotoric delay was observed in 5 cases and recurrent infection in 4 (Tab. 3).

FISH analysis using the seven above-mentioned BAC probes was performed in the patients of the first and second groups, all together 47 patients. Within group I, deletions in the regions for BACs 770H11, 201C11, 919E7, 219G6, and 431E9 were detected in all 14 children with DGS/VCFS (Fig. 2). FISH with the remaining BACs, i.e. 115F6 and 678G6, revealed two correct signals in all the patients of group I. No deletions were detected by any of the seven BACs tested in group II, involving the patients with clinical DGS/VCFS features, nor was deletion detected by the commercial probes.

Discussion

The region of 22q11.2 deleted in the majority of patients with DGS or VCFS is greater than 1.5 Mb (DGCR). Using a limited number of patients with smaller deletions, it has been possible to narrow down the critical region to 250 kb (MDGCR) [9–12, 14]. Although several genes, such as *TUPLE1*, *COMT*, and *ZNF74*, have been described within the commonly deleted region for DGS/VCFS, more genes beyond the MDGCR were recently identified [6, 9, 14]. The construction of a detailed deletion map of chromosome 22q11.2 is particularly important in DGS/VCFS because a number of patients present the phenotypic features and defects characteristic of DGS/VCFS, but no detectable deletion at the MDGCR. This observation suggests that the size of the deletion varies among patients. This was a FISH study in patients with the DGS/VCFS phenotype performed using seven different bacterial artificial chromosomes, i.e. 115F6, 678G6, 770H11, 201C11, 919E7, 431E9, and 219G6 (Tab. 1). Owing to their small size, BACs have remained a diagnostic challenge. Seven bacterial chromosomal markers used in this study have a molecular size between 95 kb and 220 kb and flank the minimal commonly deleted region (MDGCR) either proximally or distally (Fig. 1). The complete chromosomal size encompassing all seven BAC regions was 3.230 Mb and was located at a distance from 16.230 Mb to 19.460 Mb from the 22-pter region.

The FISH analyses with the BACs were performed in 47 patients from the first and second groups. Monosomy 22q11.2 was disclosed for four BACs representing the DGCR (770H11, 201C11, 919E7, 431E9) in all patients with clinical and cytogenetic diagnoses of DGS/VCFS (microdeletion diagnosed in FISH assay with the probes N25 and TUPLE1). The specific region for these BACs extends to 3 Mb. One marker, 219G6, located distally to DGCR, was also deleted in all patients of the first group (Fig. 1). A similar finding in one multigenerational family was obtained by Rauch et al. [15]. This means that the critical region for DGS/VCFS deleted in our patients is considerably larger than the MDGCR diagnosed by commercial markers. Most DGS/VCFS deletions extend over a distance of more than 2 Mb, including a chromosomal fragment from the D22S111 to D22S163 position, as was shown in our patients (Fig. 1). This suggestion requires additional investigation.

There was no deletion identified in these patients for two other BAC markers, 115F6 and 678G6, located proximally to the centromere of chromosome 22 and on the border of DGCR (Fig. 1). Moreover, there was also no deletion detected by any of the BACs in 22 patients of the second group. This suggests that FISH results showing no deletion at MDGCR sites in patients with clinical features of DGS/VCFS (assessed by the commercial markers TUPLE1 and N25) were reliable.

Clinical conclusion to compare the clinical symptoms of patients of three clinically heterogeneous groups with diagnosed and suspected DGS/VCFS, the Spearman correlation coefficient test and Chi squared (χ^2) Pearson's test were used. There were no statistical correlations regarding the frequency and type of cardiac defect, additional associated congenital defects, dysmorphic features, or cleft palate among the patients of the three groups. However, palate insufficiency, hypocalcemia, and recurrent infections were significantly more frequent in patients with a 22q11.2 microdeletion confirmed cytogenetically by FISH assay. These results confirm the conclusions of other authors and strongly suggest that strict diagnostic

criteria for DGS/VCFS are needed [16, 17]. Each additional study concerning the etiology of DGS/VCFS, especially regarding non-deleted DGS/VCFS patients, presented in the literature will contribute to a better understanding of the phenotype of patients with 22q11.2 microdeletion. These additional studies will also be necessary to determine how the length of the deletion and the kinds of the deleted chromosomal regions contribute to the various phenotypic abnormalities associated with these disorders.

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