# Genetic background of congenital conotruncal heart defects – a study of 45 families

Joanna Kwiatkowska[1], Jolanta Wierzba[2], Janina Aleszewicz-Baranowska[1], Jan Ereciński[1]

[1.] Department of Pediatric Cardiology and Congenital Heart Defect, Medical University, Gdańsk, Poland
[2.] Department of Pediatrics Haematology, Oncology and Endocrynology, Medical University, Gdańsk, Poland

**Abstract**

**Introduction:** The latest achievements in molecular diagnosis create new possibilities for evaluation of congenital abnormalities.

**Aim:** To present our preliminary experience with genetic diagnosis of congenital combined conotruncal heart defects.

**Methods:** The analysis comprised 35 families with more than one member suffering from conotruncal heart defects (Group I) and 10 families (Group II) having a child with the clinical features of CATCH 22 syndrome.

All family pedigrees were performed. Each patient was investigated by echocardiography to assess the diagnosis of the cardiac defect. Anamnestic information with regard to developmental milestones, learning abilities in childhood and psychiatric disorders were recorded. All individuals were qualified for further genetic molecular diagnostic procedures such as FISH analysis for microdeletion of chromosome 22q11 using probe N25 DiGeorge Region with 22qter control Direct CP 5141-DC.

**Results**: Based on the pedigree analysis in Group I we suggest that complex heart defects are transmitted as a recessive variant. None of the members of these families has the clinical features of CATCH 22 syndrome. In Group II we did not find familial predisposition for the appearance of congenital heart defects. None of the evaluated members of the families from Group I had microdeletion of chromosome 22q11 based on FISH analysis so we decided to isolate DNA for further molecular diagnosis. In group II in 6 (60%) individuals with typical features for CATCH 22 syndrome FISH analysis confirmed microdeletion of chromosome 22q11.

**Conclusions:** 1. The huge progress in molecular genetics creates new possibilities in the diagnosis of congenital heart defects. 2. The identification of families with high risk of recurrence of conotruncal heart defects enables genetic counselling and highly specialised medical care at the proper time.

**Key words:** chromosome 22q11.2 microdeletion, congenital heart disease, conotruncal heart defect

Kardiol Pol 2007; 65: 32-37

## Introduction

Development of the heart is an extremely complicated process. It results from a cascade of molecular and morphogenetic events, in which any small mistake may lead to serious consequences [1]. Congenital heart defects arise from interactions between genetic determinants and environmental factors and may be found in isolation or as a component of multiple congenital abnormalities [2].

Conotruncal heart defects related to division of conus cordis and truncus arteriosus are the main feature of a complex of congenital abnormalities named microdeletion 22q11.2 syndrome (CATCH 22). It belongs to a group of microdeletions of greater prevalence in the population (1/4000 births). CATCH 22 includes previously described DiGeorge syndrome (DGS), velocardiofacial dyndrome (VCFS, Shprintzen syndrome) and also conotruncal anomaly facial syndrome (CTAFS, Takao syndrome) [1-3].

Loss of genes in the 22q11.2 region leads to developmental abnormalities of several organs [2]. There is observed variability of phenotypes, which is not associated directly with the extent of deletion. The

**Address for correspondence:**
Joanna Kwiatkowska MD, Klinika Kardiologii Dziecięcej i Wad Wrodzonych Serca, Akademia Medyczna, ul. Dębinki 7, 80-211 Gdańsk, tel.: +48 58 349 28 82, fax: +48 58 349 28 95, e-mail: joannak@amg.gda.pl
**Received:** 01 April 2006. **Accepted:** 11 October 2006.

DiGeorge syndrome was the first described anomaly (1965), and includes agenesis or dysfunction of thymus and parathyroid glands, neonatal hypocalcaemia, immune disorders, discrete facial dysmorphic features and coexisting congenital heart abnormalities related particularly to division of conus cordis and truncus arteriosus (conotruncal defects) [1, 2]. In the remaining previously mentioned syndromes conotruncal defects constituted the basis of diagnosis [3].

More and more frequently it is emphasised that diagnosis of conotruncal heart defect, even without evident signs of dysmorphia, should suggest searching for the presence of microdeletion in the 22q11.2 region [2, 3].

The risk of microdeletion 22q11.2 syndrome in the next child is estimated to be 0.025%, i.e. the same as in the general population, providing there is no family history of this abnormality [2, 4]. However, if either parent presents the syndrome, the risk reaches 50% [1, 2, 4]. According to the published data a positive family history of CATCH 22 syndrome can be found in 6-28% of cases [2, 3]. There are inconsistent opinions about the prevalence of 22q11.2 microdeletions in patients with isolated heart defects [2, 5].

The present study presents our preliminary experience with genetic diagnosis of congenital combined conotruncal heart defects.

## Methods

The study included two groups:

– Group I included 93 patients from 35 families, in which at least 2 subjects suffered from combined conotruncal heart defects;
– Group II included 10 families, in which only the proband presented dysmorphic features suggesting CATCH 22 syndrome.

Diagnosis of congenital heart defect was established based on history, physical examination, electrocardiography, echocardiography as well as chest X-ray. Cardiac catheterisation with cineangiography was performed in selected cases. The pedigree trees were constructed using data obtained from parents or guardians.

The presence of microdeletions in chromosome 22 was confirmed or excluded by fluorescent *in situ* hybridization (FISH) using N25 DiGeorge Region with 22qter control probe. The studies were performed in the Department of Biology and Genetics, Medical University of Gdańsk. Further molecular analysis was performed in the Division of Human Genetics and Molecular Biology, Philadelphia, USA.

## Results

Table I presents the spectrum of congenital heart defects in Group I (familial congenital combined conotruncal heart defect). Congenital heart defect was diagnosed in 74 (79%) of 93 examined patients. The most frequently detected abnormalities were tetralogy of Fallot (ToF), observed in 31 patients, pulmonary atresia with ventricular septal defect (PA+VSD), observed in 14 patients, ventricular septal defect (VSD), found in 9 patients, and also aortic arch defects. The remaining developmental abnormalities of the cardiovascular system occurred occasionally, and were diagnosed in individual cases. None of the

**Table I.** Congenital heart disease in Groups I and II

| CHD | Group I (familial conotruncal heart defect) | | Group II (symptomatic 22 microdeletion syndrome) | |
|---|---|---|---|---|
| | Number of patients with CHD | % | Number of patients with CHD | % |
| ToF | 31/93 | 33 | 3/10 | 33 |
| PA + VSD | 9/93 | 10 | 2/10 | 20 |
| VSD | 14/93 | 15 | 1/10 | 10 |
| IAA | 3/93 | 3 | – | – |
| TAC | 2/93 | 2 | 3/10 | 33 |
| DORV | 3/93 | 3 | – | – |
| DAA | 1/93 | 1 | – | – |
| ASD II | 5/93 | 5 | 1/10 | 10 |
| Other | 6/93 | 7 | – | – |
| Together | 74/93 | 79 | 10/10 | 100 |

*Abbreviations: CHD – congenital heart disease, ToF – Fallot's tetralogy, PA – pulmonary atresia, VSD – ventricular septal defect, IAA – interruption of the aortal arch, TAC – truncus arteriosus communis, DORV – double outlet right ventricle, DAA – double aortal arch, ASD II – ostium secundum atrial septal defect*

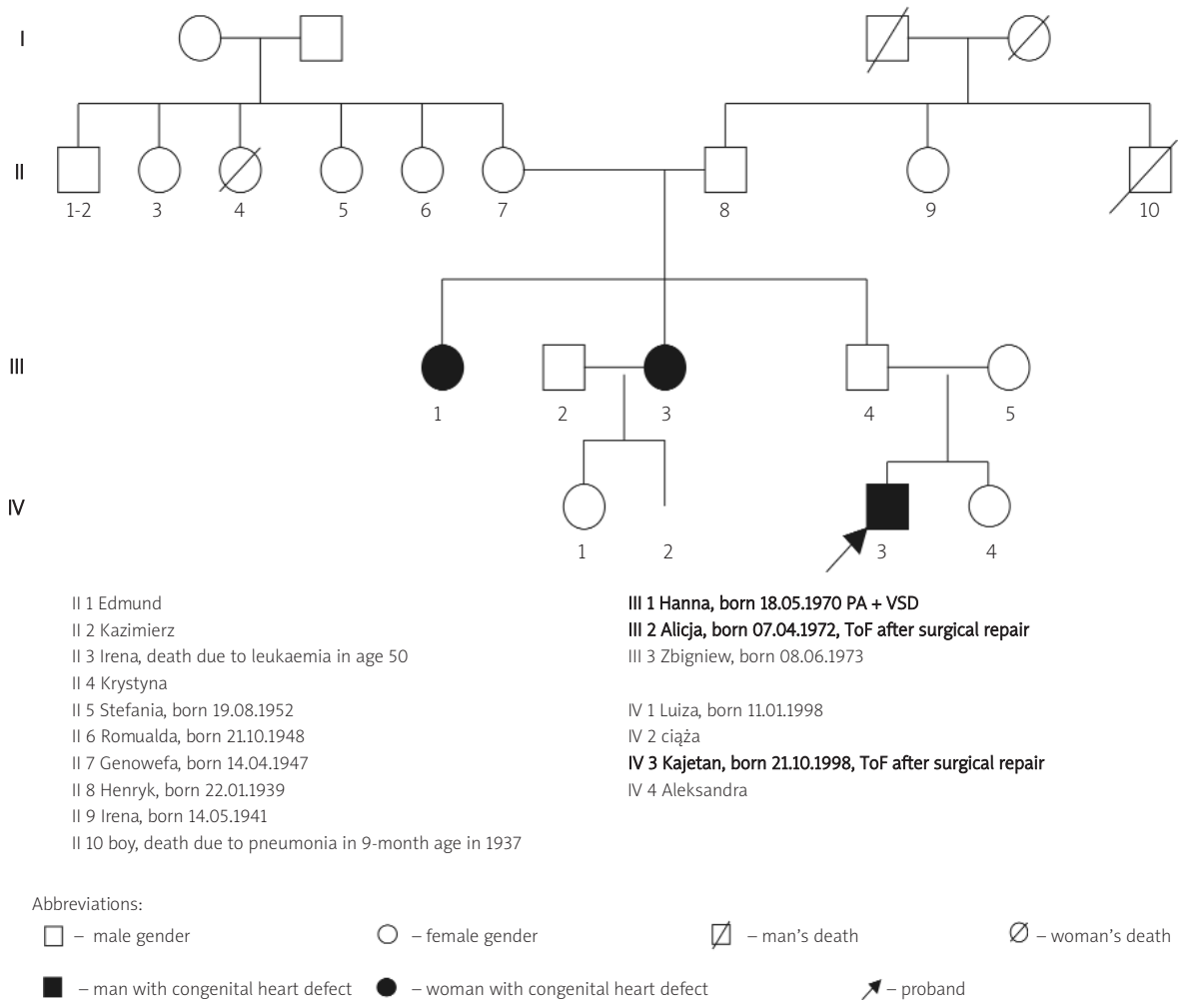


**Figure 1.** Example of family from Group I pedigree

analysed subjects presented characteristic features of clinically evident microdeletion 22q11.2 syndrome.

Figure 1 illustrates an example of pedigree of a family from Group I. The analysis revealed that in the case of familial conotruncal heart defect, the defect burden was most frequently found in siblings and cousins, as well as in siblings of parents. The pattern of inheritance, with great probability, was defined as autosomal dominant. In the present stage of the study process, the presence of microdeletions in chromosome 22 was excluded in all subjects from Group I; DNA was isolated from members of these families in order to perform further extended investigations.

In group II family predisposition to congenital heart defects was not observed. Table I shows the spectrum of diagnosed developmental abnormalities in children, whereas extracardiac features of microdeletion 22q11.2 syndrome are given in Table II. The following conotruncal heart defects were diagnosed: ToF in five (extreme ToF with pulmonary atresia in two), truncus arteriosus communis (TAC) in three, VSD in one and atrial septal defect (ASD II) in another one patient. All children from Group II demonstrated clinically evident CATCH 22 (from 4 to 7 features), and in six of them (60%) microdeletion in chromosome 22 was confirmed using the FISH method. Figure 2 present children from Group II in whom microdeletion in chromosome 22 was confirmed. Parents of examined children demonstrated no features typical for CATCH 22 and in none of them was microdeletion in chromosome 22 detected using fluorescent in situ hybridisation.

**Table II.** Developmental disorders found in children from Group II

| Parameter | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gender | M | F | M | F | F | F | M | F | M | F | |
| Facial dysmorphia | + | + | + | + | + | + | + | + | + | + | 10 |
| Cleft palate | + | + | + | – | – | + | – | – | – | – | 4 |
| Congenital heart disease | + | + | + | + | + | + | + | + | + | + | 10 |
| Learning problems | + | + | – | – | + | + | + | – | + | – | 6 |
| Skeletal anomalies | – | + | + | + | + | + | + | + | + | – | 8 |
| Immune disorders | – | + | – | + | – | + | + | – | – | + | 5 |
| Hypocalcaemia | + | + | + | + | + | + | + | + | + | + | 10 |
| Microdeletion 22 | + | + | + | + | – | + | – | – | – | + | 6 |
| Number of features | 5 | 7 | 5 | 5 | 5 | 7 | 6 | 4 | 5 | 4 | |

*Abbreviations: F – female gender, M – male gender*

**Figure 2.** Children from Group II, with confirmed 22 microdeletion

## Discussion

The nineteen eighties brought a change in studies on conotruncal heart defects. In 1981 a paper of de la Chapelle was published, and one year later Kelly demonstrated a relation between the loss of genetic material from the long arm of chromosome 22 and the DiGeorge syndrome, cited from Śmigiel et al. [2]. Further investigations demonstrated the relation between 22q1 deletion and the above-mentioned syndromes, termed presently CATCH 22.

According to the published data, the main clinical signs of 22q11.2 microdeletion syndrome include facial dysmorphia (100%), speech and learning problems (80%), neonatal hypocalcaemia (73%), immune disorders (69%), skeletal anomalies (32%), cleft palate (31%) and others. Manifestations of facial dysmorphia, i.e.: hypertelorism, epicanthus, long nose with prominent tip, microstomia, retrognathia and small, low-set ears, are very subtle in the majority of children. It is emphasised that there is an unusual variability of phenotype expression of microdeletion 22q11.2 syndrome, even within the same family, from mild forms (subclinical) to clinically advanced. These observations indicate the importance of detection of deletion not only among parents with classical syndrome, but also in parents with mild clinical form of the disease. So-called "asymptomatic" parents have to be aware of the 50% risk of deletion transmission to offspring. In evaluation of particular pedigrees one should remember the varied expression as well as genetic penetration. It is also important that the discussed microdeletion was detected in patients with isolated conotruncal heart defects.

Congenital heart defects are observed in 75-95% of patients with CATCH 22 syndrome [1, 2]. The majority of diagnosed defects are combined, and the most frequent include ToF, TAC, transposition of great arteries, double outlet right ventricle, VSD, and interrupted aortic arch type B [3]. Our observations are consistent with data from the literature [3].

The coexistence of the same or another congenital heart defect was found in at least two members of the same family in Group I. The features of facial dysmorphia or other features of 22q11.2 microdeletion syndrome were not observed in this group.

In Group II all children presented characteristic features of facial dysmorphia, congenital conotruncal heart defect and many other features of 22q11.2 microdeletion syndrome.

The achievements in genetics allowed demonstration of microdeletion in the long arms of chromosome 22 in some patients with CATCH 22 by means of the FISH method [6-9].

Some authors [10] suggested that microdeletion within chromosome 22 may be a cause of a wide

spectrum of isolated congenital conotruncal heart defects without other coexisting symptoms, observed in CATCH 22 syndrome. In group I with familial congenital conotruncal heart defect without other coexisting features typical for syndromes of developmental abnormalities caused by the presence of microdeletion 22q1.2, the loss of genetic material in the critical 22q11.2 area was not observed using the FISH technique. Our results are consistent with reports of Amati et al. [11] as well as with those of Digillo et al. [3]. They did not find deletion in 107 analysed patients with isolated ToF also, whereas microdeletion 22q11.2 was found in as many as 11 subjects of 26 with clinical symptoms of DiGeorge syndrome and with ToF. Similar data were reported by Trainer et al. [5], who showed deletion 22q11.2 in 7 of 30 patients with isolated ToF.

According to different reports [3] the incidence of analysed deletion in patients with above mentioned clinical features of the syndrome ranges from 81 to 88%. In the present study, 22q11.2 microdeletion was demonstrated in 6 of 10 children (Group II), in whom CATCH 22 syndrome was diagnosed based on clinical features. It is likely, as many authors emphasise [4, 8], that submicroscopic changes, point mutations and other molecular pathology and also interaction with environmental factors, are responsible for the described phenotypic features [1, 2, 12].

The results of studies on microdeletion 22q11.2 syndrome have varied with the use of modern diagnostic methods. Many scientific programs are focused on searching for genes located within the microdeletion area, i.e.: ZNF74, HIRA (DBR CR1), IDD (DBR CR2), COMT and TBX1 or UFD1L [3, 13]. Perhaps in the future studies will reveal gene mutations in patients in whom the test result was negative despite evident typical clinical symptoms suggesting deletion 22q11.

Scientific and technological advances have meant that the diagnosis and treatment of many congenital diseases is possible, but our understanding of their aetiology and pathogenesis is still incomplete. With the development of prenatal diagnosis and the achievements of molecular genetics the possibility of proper genetic counselling has appeared [14]. Proper analysis of familial congenital heart defects and other circulatory disorders on the basis of precise interviews may contribute to better understanding of inheritance of these diseases, and subsequently may approximate the knowledge about their aetiology [1, 3].

## Conclusions

1. The latest achievements in molecular diagnosis create new possibilities for evaluation of developmental abnormalities.
2. The presence of 22q11.2 microdeletion was not revealed in patients with isolated familial conotruncal heart defects, whereas the incidence of microdeletion was higher in the case of coexistence of these defects with typical facial dysmorphia.
3. Identification of families with genetic burden of congenital heart defects allows special counselling for family planning to be provided.

## References


1. Krajewska-Walasek M. Rozwój serca i genetyczne aspekty wad wrodzonych serca. In: Ciechanowicz A, Januszewicz A, Januszewicz W, et al. Genetyka chorób układu krążenia. *Medycyna Praktyczna,* Kraków 2002: 133-57.
2. Śmigiel R, Ślęzak R, Jagielski J, et al. Zespół CATCH 22- aspekty patogenetyczne, kliniczne i diagnostyczne. *Ped Pol* 2003; 78: 91-9.
3. Digilio MC, Marino B, Capolino R, et al. Clinical manifestations of Deletion 22q11.2 syndrome (DiGeorge/Velo-Cardio-Facial syndrome). *Images Paediatr Cardiol* 2005; 23: 23-34.
4. Digilio MC, Angioni A, De Santis M, et al. Spectrum of clinical variability in familial deletion 22q11.2: from full manifestation to extremely mild clinical anomalies. *Clin Genet* 2003; 63: 308-13.
5. Webber SA, Hatchwell E, Barber JC, et al. Importance of microdeletions of chromosomal region 22q11 as a cause of selected malformations of the ventricular outflow tracts and aortic arch: a three- year prospective study. *J Pediatr* 1996; 129: 26-32.
6. Botto LD, May K, Fernhoff P, et al. A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population. *Pediatrics* 2003; 112: 101-
7. Tobias ES, Morrison N, Whiteford ML, et al. Towards earlier diagnosis of 22q11 deletions. *Arch Dis Child* 1999; 81: 513-4.
8. Yong DE, Booth P, Baruni J, et al. Chromosome 22q11 microdeletion and congenital heart disease – a survey in a paediatric population. *Eur J Pediatr* 1999; 158: 566-70.
9. Iserin L, De Lonlay P, Viot G, et al. Prevalence of the microdeletion 22q11 in newborn infants with congenital conotruncal cardiac anomalies. *Eur J Pediatr* 1998; 157: 881-4.
10. Momma K, Kondo C, Matsuoka R. Tetralogy of Fallot with pulmonary atresia associated with chromosome 22q11 deletion. *J Am Coll Cardiol* 1996; 27: 198-202.
11. Amati F, Mari A, Digilio MC, et al. 22q11 deletions in isolated and syndromic patients with tetralogy of Fallot. *Hum Genet* 1995; 95: 479-82.
12. Burn J, Goodship J. Congenital heart disease. In: Emery AE, Rimoin DL (eds). Principles and Practise of Medical Genetics, 4th edition, *Churchill Livingstone*, Edinburgh, UK 2002, 1248-53.
13. Yagi H, Furutani Y, Hamada H, et al. Role of TBX1 in human del22q11.2 syndrome. *Lancet* 2003; 362: 1366-73.
14. Kwiatkowska J. The genetic counselling in families with the child suffering from tetralogy of Fallot. *Folia Cardiol* 2001; 8: 569-73.

# Wrodzone złożone wady serca typu *conotruncal* w aspekcie diagnostyki genetycznej

Joanna Kwiatkowska[1], Jolanta Wierzba[2], Janina Aleszewicz-Baranowska[1], Jan Ereciński[1]

[1] Klinika Kardiologii Dziecięcej i Wad Wrodzonych Serca, Akademia Medyczna, Gdańsk
[2] Klinika Pediatrii, Hematologii, Onkologii i Endokrynologii, Akademia Medyczna, Gdańsk

**Streszczenie**

**Wstęp:** Ostatnie osiągnięcia genetyki pozwalają na wczesną swoistą diagnostykę schorzeń układu krążenia.

**Cel:** Przedstawienie doświadczeń w zakresie diagnostyki cytogenetycznej i molekularnej pacjentów z wadami serca typu *conotruncal*.

**Metodyka:** Badaniami objęto dwie grupy badanych. Grupę I stanowiło 35 rodzin, w których przynajmniej u 2 osób rozpoznano wrodzoną złożoną wadą serca typu *conotruncal*. Grupę II stanowiło 10 rodzin, w których u jednego z członków wadzie typu *conotruncal* towarzyszyły cechy dysmorfii oraz wady innych narządów sugerujące zespół określany jako CATCH 22. Sporządzono rodowody wszystkich ww. rodzin.

**Wyniki:** W celu potwierdzenia lub wykluczenia mikrodelecji w zakresie chromosomu 22 przeprowadzono badania FISH, stosując sondę: N25 DiGeorge Region with 22qter control. Direct CP 5141-DC. U wszystkich osób z rodzinnie występującą wrodzoną złożoną wadą serca typu *conotruncal* (grupa I) wykluczono na obecnym etapie badań obecność mikrodelecji 22q11. Od 50 członków ww. rodzin wyizolowano DNA w celu dalszej diagnostyki molekularnej. Jak dotąd w grupie tej nie wykazano przypadków mutacji genowych. W grupie II u sześciorga dzieci (60%) potwierdzono obecność mikrodelecji chromosomu 22. We wszystkich przypadkach badania rodziców nie wykazały mikrodelecji chromosomu 22q11.

**Wnioski:** 1. U pacjentów z izolowaną, rodzinnie występującą, wrodzoną wadą serca typu *conotruncal* nie wykazano mikrodelecji 22q11.2, natomiast jeżeli wady te współistnieją z typową dysmorfią twarzy i innymi cechami fenotypowymi zespołu CATCH 22, częstość wykrywania mikrodelecji wzrasta. 2. Identyfikacja rodziny obciążonej ryzykiem genetycznym ponownego wystąpienia wrodzonej wady serca umożliwia udzielenie wiarygodnej porady genetycznej i objęcie jej specjalistyczną opieką przed planowaniem posiadania potomstwa.

**Słowa kluczowe:** mikrodelecja chromosomu 22, wrodzone wady serca, wrodzone wady serca typu *conotruncal*

Kardiol Pol 2007; 65: 32-37

**Adres do korespondencji:**
dr n. med. Joanna Kwiatkowska, Klinika Kardiologii Dziecięcej i Wad Wrodzonych Serca, Akademia Medyczna, ul. Dębinki 7, 80-211 Gdańsk, tel.: +48 58 349 28 82, faks: +48 58 349 28 95, e-mail: joannak@amg.gda.pl
**Praca wpłynęła:** 01.04.2006. **Zaakceptowana do druku:** 11.10.2006.