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REVIEW ARTICLE

DiGeorge syndrome: an historical review of clinical and cytogenetic features

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It is now over 28 years since Dr Angelo DiGeorge[1] commented on a paper by Dr Max Cooper and colleagues[2] regarding the congenital absence of the thymus. At the 1965 Society for Pediatric Research (SPR) meeting, Dr Cooper gave a paper showing that the lymphoid system of the chicken consisted of two different components, the bursal system and the thymic system. Ablation of the bursal system caused agammaglobulinaemia but did not affect cellular immunity. However, thymectomy impaired cellular immunity. In his comment, Dr DiGeorge noted that there was a group of infants with congenital absence of the thymus who might represent a human homologue of the thymectomised chicks. DiGeorge and his co-worker, Dr James Arey, had noted three infants with congenital absence of the parathyroids who also had no evidence of thymic tissue. As DiGeorge stated, "the concurrent absence of both structures is not surprising if one recognizes that both are derived from common primordia. Furthermore, this association has been previously recorded although its physiologic significance has not been recognized."[1] Just before the 1965 SPR meeting, DiGeorge and colleagues (Drs Harold Lischner, Catherine Dacou-Voutetakis, and Hope Punnett) were in the process of studying a fourth infant with congenital hypoparathyroidism who was predicted to have absence of the thymus. In addition to the absence of the thymic shadow on chest radiograph, the infant had abnormal cellular immunity with persistent candidiasis, negative monilial skin test, and failure to reject a homologous skin graft, although the lymphocyte count, plasma cell numbers in lymph nodes, and serum immunoglobulins were normal. The infant was also noted to be 'runted' in spite of adequate control of serum calcium levels. DiGeorge suggested that all infants with congenital hypoparathyroidism should be studied for defects in cellular immunity. This was contrary to the prevailing notion that patients with absent thymus would have normal immunoglobulins and normal total peripheral blood lymphocyte counts.

As DiGeorge had mentioned, thymic aplasia was first noted by Harrington[3] in 1829 and later in association with congenital hypoparathyroidism by Lobdell[4] in 1959. In spite of those earlier papers and the lack of a published paper by DiGeorge, Dr Robert A Good dubbed this association 'DiGeorge syndrome'.[5] DiGeorge's first published paper on the subject actually did not appear until 1968 in the *British Defects: Original Article Series* with the proceedings of a Sanibel Island meeting on the development of the immune system.[6] For additional information about the early history of DGS, I refer you to DiGeorge's paper.[6]

After several additional case reports of DGS and evaluation of 18 additional cases, Dr Harold Lischner outlined the first categorisation of third and fourth branchial pouch defects in an editorial comment.[7] Lischner suggested three categories. The first is the III-IV pharyngeal pouch syndrome which he defined as "congenital malformation, hypoplasia (with normal histology), or absence of the thymus and/or parathyroid glands, including significant maldescent of those organs so that they are located in the neck or other abnormal sites." Additional anomalies were noted, especially of the great vessels, micrognathia, ear defects, oesophageal atresia, blunted nose, thyroid anomalies, and endocardial cushion. DiGeorge syndrome was defined as those cases of the III-IV pharyngeal pouch syndrome in which no thymic tissue was noted on careful postmortem examination, even in an ectopic position. The third category was partial DiGeorge syndrome in which the cases of III-IV pharyngeal pouch syndrome had defective cell mediated immunity or thymic hypoplasia by reduced thymic weight (<2 g). In addition, Lischner noted seven generalisations about partial DGS which still hold true.[7] (1) Cell mediated immunity is grossly depressed. (2) Most lymphocytes in the peripheral blood and lymph nodes will be B cells. (3) The absolute lymphocyte count will usually be slightly or moderately depressed but may be normal. (4) Responsiveness of peripheral blood lymphocytes to phytohaemagglutinin in vitro may be depressed but not consistently. (5) Lymph nodes will be grossly depleted on lymphocytes in the deep cortical areas. (6) There may be some depression of antibody responses to specific immunisation, thought to be the result of the required interaction of T lymphocytes with B lymphocytes. (7) Serum immunoglobulins will be within or near the normal range.

Although by 1979, DiGeorge had evaluated

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29 patients, the next important publication on DGS was that of Conley *et al*[8] on the delineation of the spectrum of DGS. The Conley study was a retrospective investigation of complete and partial DGS with ascertainment at necropsy or by review of clinic records from the Cardiology, Endocrinology, and Immunology services. The patients were required to have two or more of the following findings: (1) complete or partial absence of the thymus or cellular immune deficiency or both, (2) symptomatic hypocalcaemia or parathyroid hypoplasia or both, (3) congenital heart disease. They ascertained 25 patients, most of whom were diagnosed at necropsy. Two patients were ascertained prospectively in the newborn period. Sixty percent of patients (15/25) came to attention because of significant congenital heart disease within 48 hours of birth. Three patients presented with multiple congenital anomalies. One patient presented with hypocalcaemic seizures, three with heart murmurs, and one died suddenly at home at the age of 7 days. Among the 25 patients, 15 were described as having abnormal facies and four had cleft lip or palate or bifid uvula. In addition to one patient with both DGS and Zellweger syndrome, two other subgroups were found but not commented upon. The association of DGS with CHARGE association and of DGS and holoprosencephaly/arhinencephaly were both noted in Conley's series. Of great importance, Conley *et al*[8] point out the association of DGS with conotruncal cardiac defects, especially type B interrupted aortic arch (with 50% of cases having DGS) in 9/25, right sided aortic arch in 6/25, and persistent truncus arteriosus in 8/25. The association of these specific cardiac defects and DGS was substantiated by Moerman *et al*[9] and later by van Mierop and Kutsche.[10] In both reports, tetralogy of Fallot was added to the list of associated conotruncal defects.

Since most of the patients in the Conley study were ascertained postmortem, all but one patient had died. Most of the early deaths were the result of congenital heart disease. Eight died in the first week of life, 11 patients between one week and three months, and five after three months. The latter deaths were more likely to be because of chronic infection.

Before reviewing the cytogenetic and molecular aspects of DGS, I will review several other clinical and aetiological studies.

In 1988 and 1989, Mueller *et al*[11 12] reported on 16 prospectively ascertained patients with DGS. They stressed the distinction between complete and partial DGS with a higher mortality in those patients with complete DGS. They proposed a DGS index using clinical signs and laboratory tests. They gave points for clinical signs (facial dysmorphism, absence of thymic shadow, congenital heart disease), hypoparathyroidism (hypocalcaemia, hyperphosphataemia, raised alkaline phosphatase, low parathyroid hormone levels), and immunological studies (low white blood cell count, low lymphocyte count, low E rosetting cells, high IgG, low IgA, low IgM, low IgE, and low anti-BSA). Thus a total possible score would be 15. The index would be calculated and divided by 15. The three patients with complete DGS had an index of over 0·6 with a range of 0·642 to 0·750. The 13 partial DGS patients had a mean index of 0·357 with a range of 0·266 to 0·533. Although possibly useful, the findings are not weighted and some findings may be more important than others. Some findings such as those for hypoparathyroidism are not independent variables. In addition, the scores of individual patients may vary over time since thymic function may especially change with age.

Following this, Bastian *et al*[13] reported on serial immunological studies of 18 DGS patients. Of these 18 patients, 14 had normal T cell function to moderate T cell deficits. None of these patients went on to develop immunodeficiency. The remaining four patients with low CD4+ cells ($<400/\mu g$) and decreased PHA stimulation did develop immunodeficiency. Such patients, Bastian *et al* suggest, are candidates for immunological reconstitution.

From the standpoint of pathogenesis and aetiology, Lammer and Opitz[14] wrote a review of the various mechanisms in 1986. Before this, in 1980, Dr John Carey wrote a letter to the editor in the *Journal of Pediatrics* in which he discussed the heterogeneity of DGS and suggested that it was not really a syndrome since there appeared to be several aetiologies.[15] With the occurrence of DGS in some patients with isotretinoin embryopathy, Lammer and Opitz[14] discussed this aetiological heterogeneity and suggested that DiGeorge syndrome should be DiGeorge anomaly (DGA) as a developmental field defect involving the third and fourth branchial arches and pouches. Aetiologically, DGA can occur because of various chromosome abnormalities (discussed later), mendelian disorders (including velocardiofacial syndrome (VCFS) and Zellweger syndrome), teratogenic exposure (alcohol, maternal diabetes, retinoids), and other associations (CHARGE associations and with Kallmann syndrome or with holoprosencephaly) (the latter two may be a spectrum of the same defect).

Among families with mendelian inheritance, autosomal dominant, autosomal recessive, and possible X linked recessive inheritance of DGA had been reported. Since the four families with possible X linked inheritance had only affected male sibs without affected maternal uncles, autosomal recessive inheritance could still be more likely. However, even autosomal recessive inheritance may be hard to prove unless both parents are adequately examined for even mild features of DGA. The occurrence of a bifid uvula or submucous cleft could be a subclinical manifestation of DGA or VCFS, or both. In addition, in one report, subclinical hypoparathyroidism in the mother of a DGA patient was 'unmasked' by a disodium edetate (EDTA) infusion.[16] At the present time, all cases of familial DGA should be studied (or restudied) cytogenetically by fluorescence in situ hybridisation and by molecular

techniques (as described below) to clarify this inheritance issue.

In spite of this aetiological heterogeneity of DGA, there is increasing evidence that the most common cause is monosomy 22q11. This story begins with the 1981 report of a Finnish family with an autosomal translocation involving chromosomes 20 and 22 with breakpoints at 20q11 and 22q11. Four infants with unbalanced karyotypes with only the derivative chromosome 20 and loss of proximal 22q all had features of DGA.[17] In this paper, de la Chapelle *et al* suggested that DGA could be caused by a gene in band 22q11. They also mentioned a patient previously reported briefly by Rosenthal *et al*[18] in 1972 with complete monosomy 22 and DGA. In 1981, Dr Richard Kelley, who was then at the Children's Hospital of Philadelphia, found a DGA patient with an unbalanced derivative chromosome 10 with loss of 22q11. This led to the ascertainment of two previous DGS patients at St Christopher's Hospital for Children in Philadelphia with two other unbalanced rearrangements involving 22q11. (One of these DGA patients had a de novo 3q29;22q11 translocation which I personally karyotyped but was not sure what it meant at the time.) These three DGA patients were reported by Kelley *et al*.[19]

At the time of the preparation of the manuscript of Kelley *et al*[19] I had recently arrived at Baylor College of Medicine. During my first year, I saw a patient with suspected DGA who had one previous sib who died and was noted to have DGA at necropsy. Another previous sib in Mexico died in the neonatal period from a congenital heart defect but no necropsy was done. Suspecting a chromosome 22 abnormality, I sent blood to Dr David Ledbetter's laboratory for analysis with the notation "Look at chromosome 22". The following week, David called excitedly one evening to inform me that I was right. The patient had a derivative chromosome 4 with loss of of 22q11. As it turned out, his mother had the same derivative chromosome 4 but was clinically normal (so it seemed). This case was mentioned as an addendum to the Kelley *et al*[19] paper and then, subsequently, published as a separate case report.[20]

The discovery of this DGA patient started me on a prospective study of cytogenetic abnormalities in DGA patients.[21] Over a five year period, I was able to ascertain 28 patients with DGA. Comparisons between our prospective study and the retrospective study of Conley *et al*[8] are shown in tables 1 to 3. (Bear in mind that by 1988, DiGeorge had evaluated 57 patients.) We successfully obtained high resolution cytogenetic studies on 27 and, of those, five had detectable chromosome abnormalities. Three had monosomy 22q11, one had monosomy 10p13, and one had monosomy 18q21. Two of the monosomy 22q11 patients had unbalanced translocations but one had an apparent interstitial deletion of 22q11. Because we were so uncertain about the validity of this interstitial deletion, the karyotypes were reviewed in an unbiased manner by Drs Beverly Emanuel and Jim Mascarello, both of whom concurred. A subsequent patient with an interstitial deletion of 22q11 was reported by Mascarello *et al*[22] in 1989. These findings underlined the importance of monosomy 22q11 in the aetiology of DGA. By this time, the late Dr Roy Schmickel had suggested that monosomy 22q11 associated with DGA was one of the prototypic 'contiguous gene deletion syndromes'.[23]

However, a number of other cytogenetic abnormalities were also noted in association with DGA. As outlined in Greenberg *et al*,[21] there have been several cases of monosomy 10p13 associated with DGA. This region appears to contain another possible DGA locus. The other individual reports of chromosome abnormalities and DGA are isolated cases and may represent non-specific disruption of the branchial arches leading to DGA. One exception to this is the fetus reported by our group with a very large 17p13 deletion with associated DGA.[24] More recently, the nude mouse locus was mapped to the region syntenic to 17p13.[25] Thus, another possible DGA locus may be analogous to the nude mouse gene.

This now brings us to the molecular era. Since 1989, the laboratories of Beverly Emanuel in Philadelphia and Peter Scambler in London have been isolating probes from 22q11 to determine the smallest region of overlap in DGA patients with the eventual plan to clone the DGA locus in that region.[26-30] In the mean time, two important findings have changed the clinical aspects of DGA. First of all, the use of fluorescence in situ hybridisation (FISH) has dramatically increased the sensitivity of detection of interstitial deletions of

Table 1 Studies of DiGeorge anomaly.

	Conley *et al*[8]	BCM
Period	1950–75	1982–87
No of patients	25	28
Type	Retrospective	Prospective
Sex ratio	0·92	1·00
Ascertainment		
By CHD	15/25 (60%)	21/28 (75%)
By MCA	3/25 (12%)	3/28 (11%)
By HCA	1/25 (12%)	4/28 (14%)
Positive FH	0/25 (0%)	2/28 (7%)

BCM = Baylor College of Medicine.

Table 2 Cardiac defects in DGA.

Defect	Conley *et al*[8] 1950–1975	BCM 1982–1987
IAA type B	9/25 (36%)	7/28 (25%)
RAA	6/25 (24%)	7/28 (25%)
TA	8/25 (32%)	7/28 (25%)
TOF	2/25 (8%)	3/28 (11%)
Other	3/25 (12%)	5/28 (18%)
None	0/25 (0%)	3/28 (11%)

Table 3 Other associated defects.

Defect	Conley *et al*[8] 1950–1975	BCM 1982–1987
Holoprosencephaly	3/25 (12%)	1/28 (4%)
Cleft lip/palate	4/25 (16%)	3/28 (11%)
Colobomata	1/25 (4%)	2/28 (7%)
Ear anomalies	NA	13/28 (46%)
Micrognathia	NA	10/28 (36%)
Diaphragmatic defects	3/25 (12%)	0/28 (0%)
GI anomalies	6/25 (24%)	0/28 (0%)
GU anomalies	7/25 (28%)	0/28 (0%)

NA = not available.

22q11 in DGA, suggesting that as many as 90% of DGA patients have small interstitial deletions. The other important finding is the overlap between DGA and VCFS, both associated with deletion 22q11. In fact, in retrospect, the DGS patient and his mother reported with the 4;22 translocation[20] both have typical features of VCFS.

This suggests that the two disorders represent a spectrum of the same gene defect, although Schmickel's suggestion that DGA is a contiguous gene deletion syndrome has not been completely ruled out. Thus, patients with suspected or confirmed DGA should be evaluated for features of VCFS (including velopharyngeal incompetence) and for features of VCFS in the parents. Conversely, patients with VCFS should probably be evaluated for subclinical manifestations of DGA, including hypoparathyroidism and T cell defects. In addition, the recent finding of monosomy 22q11 in some patients with isolated conotruncal cardiac defects,[31] suggests that the hypothetical DGA-VCFS gene is generally involved with the development of the third and fourth branchial arches and defect in this gene may produce different manifestations.

I would like to propose that this DGA-VCFS gene will be a good model for a gene defect which is likely to be affected by other genes or environmental influences, as a true polygenic or multifactorial disorder. In this regard, other hypothetical DGA loci such as in 10p13 or 17p13 should not be neglected.

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