Wadey *et al.*,⁵ Burn *et al.*,⁶ Holder *et al.*,⁷ and Wilson *et al.*⁸ devote considerable attention to the overlap of a number of conditions, including velocardiofacial syndrome, DiGeorge sequence, and conotruncal anomaly face syndrome. These authors do not mention other case reports which also described patients with overlapping phenotypes, including those by Strong⁹ and Sedláčková.¹⁰ As we pointed out in one of our earlier articles,¹¹ the family reported by Strong⁹ with autosomal dominant inheritance of heart anomalies clearly had velocardiofacial syndrome. In Sedláčková's series of cases,¹⁰ many, but not all, of the cases shown had phenotypic features consistent with velocardiofacial syndrome. Reviews of photographs shown in other articles also show the classic phenotype of velocardiofacial syndrome, such as cases shown in Kaplan's description of occult submucous cleft palate.¹² What all of these cases help to illustrate is the familiar parable of the five blind men and the elephant. Many clinicians with different focuses of attention have described from a variety of perspectives what may be a single class of patients. If one believes that heart anomalies are the primary defect, DiGeorge syndrome becomes the diagnostic nosology of primary significance, whereas if one studies children with craniofacial anomalies, velocardiofacial syndrome may be of prominence. The problem relative to nosology is the absence of rigorous standards for clinical description and diagnosis. Often, those who focus attention on cardiac or immunological disorders might do so at the expense of other anomalies, such as speech disorders, minor limb anomalies, or eye findings. In our series of patients with velocardiofacial syndrome, we have attempted to be as rigorous as possible in describing all of the clinical manifestations in our patients. This is, in part, an outgrowth of the interdisciplinary nature of this Center (and others like it) which calls on 26 disciplines in the evaluation process. It was obvious to us long ago that a number of aetiologically non-specific disorders such as Robin, DiGeorge, and CHARGE occurred as secondary sequences to velocardiofacial syndrome.¹³⁻¹⁵ Included in the over 40 clinical features known to be associated with velocardiofacial syndrome are findings consistent with Robin, DiGeorge, and CHARGE, as well as other more obscure disorders such as the so-called Sedláčková syndrome. As pointed out by Stevens *et al.*,¹⁶ there is little doubt that the familial cases of DiGeorge which have been reported actually represent velocardiofacial syndrome.

The importance of accurate clinical description and diagnostic identification of velocardiofacial syndrome (or any other disorder, for that matter) is clearly illustrated by the article by Driscoll *et al.*⁴ They report a prevalence of 76% 22q11 deletions in patients referred to them as velocardiofacial syndrome. In other words, the diagnosis was applied by several different clinicians without ascertaining the validity or reliability of the clinical diagnostic technique used to reach that conclusion. Therefore, this prevalence statistic is essentially meaningless. Rigorous research demands the accuracy of scientific observations, and without proper assessments of that accuracy the observations can not be accepted as true. It should be mentioned that in our own series sent for molecular analysis to Dr Scambler's laboratory, there was a 100% prevalence of 22q11

deletion.^{17,18} It should also be mentioned that not all of those cases had heart anomalies, and few met the criteria of DiGeorge. In another series analysed by Dr Driscoll's laboratory, all of the cases successfully analysed were deleted^{19,20} except for one who had not been seen by us since early infancy in 1987. On subsequent clinical examination at the age of 6 years in 1993, it became obvious that this patient did not have velocardiofacial syndrome. In fact, it was the coincidence of Robin sequence and a ventriculoseptal defect in this case which led to the diagnosis in the neonatal period. With growth and time, it became obvious that we were incorrect in our earlier diagnosis. Additional cardiac evaluation after the diagnosis showed anomalies not consistent with velocardiofacial syndrome. Therefore, in our experience, clinical application of the diagnosis of velocardiofacial syndrome by careful analysis (preferably longitudinal) of clinical phenotype has led to a 100% accurate detection of a 22q11 microdeletion in all cases.

The 83% prevalence of DiGeorge cases deleted at 22q11 as reported by Driscoll *et al.*⁴ may reflect the aetiological heterogeneity of DiGeorge syndrome. The criteria for DiGeorge syndrome are clinically more confined than the expansive phenotype of velocardiofacial syndrome so that the diagnostic label is more easily attached. Even so, 17% of Driscoll's DiGeorge cases were not deleted at 22q11. It may be that the 83% prevalence of deletions denote that the majority of DiGeorge cases actually are caused by the deletion specific to velocardiofacial syndrome. Stated another way, the 17% of DiGeorge cases not deleted may be related to some of the other known chromosomal sites to which DiGeorge has been linked (such as 4q, 10p, and 17p, among others) whereas, to date, velocardiofacial syndrome has been isolated only to 22q11.

Finally, Dr Hall's support of the new acronym CATCH 22 only serves to confuse the clinical and diagnostic picture further. Dr Hall cites Driscoll's prevalence data as if to indicate that velocardiofacial syndrome is aetiologically heterogeneous. She states that "... 68% of Shprintzen syndrome patients ... have been recognised to have deletions of 22q11." This statement is not true. It should more accurately be stated that 68% of patients sent to Dr Driscoll's laboratory identified by other clinicians as having velocardiofacial syndrome were deleted. In our sample, 100% were deleted. Is this a difference in clinical experience, expertise, criteria, or all of the above? There is simply no valid evidence to suggest that velocardiofacial syndrome is aetiologically heterogeneous. The DiGeorge anomaly is known to be so, as is CHARGE. Therefore, placing velocardiofacial syndrome, DiGeorge syndrome, and CHARGE under a single diagnostic category is an example of what used to be referred to as "lumping", which will only confuse clinicians, molecular geneticists, and, most importantly, patients and their families. If the data reported in volumes 30 (pages 801-856) point out nothing else, it is that molecular geneticists are dependent on accurate clinical detection in order to prove primary aetiology.

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Multiple origins of X chromosome tetrasomy

The extra chromosomes in all previously reported cases of X chromosome tetrasomy or pentasomy have been maternal in origin and compatible with being the product of successive meiosis I and meiosis II non-disjunctions in the mother.¹⁻⁴ This is inferred by the presence of heterozygosity for maternal alleles at all informative X loci, implying transmission of one or both chromatids from both X chromosome pairs from the mother. In our investigation of three 48,XXXX persons, molecular results for one 48,XXXX case were incompatible with a completely meiotic origin of the extra chromosomes. Another case also differed from previous reports in that there was complete absence of any paternal alleles.

Hybridisation of M27 β (DXS255) to TaqI digested DNA of 48,XXXX cases 1 and 2. Case 1 shows one paternal and three copies of a single maternal allele. Case 2 shows equal dosage of the two maternal alleles, but no paternal allele (relatively more DNA from the mother was loaded in case 2).

Molecular analysis was performed as previously reported⁴ using the probes dic56 (DXS143), 113D (DXYS15), p602 (DXYS17), pM27 β (DXS255), DP34 (DXYS1X), and F814 (DXS144E). None of the three 48,XXXX patients showed any evidence of mosaicism among at least 20 metaphases examined. Parental karyotypes were not performed to exclude the presence of an XXX cell line in the mother; however, molecular analysis always showed two alleles of equal intensity at heterozygous loci in the mothers. A maternal origin of the extra chromosomes and heterozygosity for both maternal alleles at one or more loci was found in all cases. However, one 48,XXXX case (XXXX1) showed inheritance of only a single maternal allele in three copies, plus one paternal allele, at DXS255 and DXYS1X, which were the most centromeric on the p and q arms respectively (Xp11.22 and Xq21.3) of the markers examined here (figure). This same person (case 1) showed two maternal alleles, one in double dose, at DXS143 and DXYS15, both mapping to Xp22.3 and for DXS144E (Xq28). A second 48,XXXX case (XXXX2) showed heterozygosity for maternal alleles (in equal dosage), but no paternal X allele was detected at DXYS17 or at DXS255 (figure). She therefore shows uniparental maternal tetrasomy for the X chromosome. Case 3 of the present study was similar to both previous reports of 48,XXXX,²³ whereby a single paternal allele and both maternal X alleles, one in double dose, were observed at all informative loci (data not shown).

Investigations of three 49,XXXXY cases (partial results of two have been published previously⁴) were similar to previous reports and showed that all four X chromosomes were maternal in origin with equal dosage of alleles at all heterozygous loci in the mother. These results support a mechanism of successive MI and MII meiotic non-disjunctions in the mother involving both chromatid pairs in MII. This may also explain the inheritance in case 2, which, in addition, must have had a pre- or postfertilisation loss of the paternal sex chromosome. Case 3 could have originated either from a tetrasomy X oocyte with postmeiotic loss of one maternal chromosome or from successive MI and MII non-disjunctions with involvement of only one of the X chromatid pairs in MII (resulting in transmission of three X chromosomes to the oocyte).¹²

Case 1 of the present study, however, is the

only case which cannot be explained by meiotic non-disjunction alone, since three copies of a single maternal X allele were observed for some loci. Therefore, either an extra X chromosome was present in the mother's germ cells before meiosis, or the zygote originated from a 47,XXX karyotype, with the third maternal X duplicated in the zygote or early postzygotically. In either case, both meiotic and mitotic non-disjunctions would contribute to the X chromosome polysomy. Thus, although most cases of X chromosome tetrasomy are compatible with the hypothesis of successive meiotic non-disjunction in the mother, other mechanisms may also occasionally be involved.

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Distribution of RARA allele frequencies

	Chenevix-Trench et al ^{2*}		Vintiner et al ¹		Combined	
Allele	A1	A2	A1	A2	A1	A2
CL $\pm$ P	46	172	26	96	72	268
Controls	49	101	32	88	81	189
χ^2 (p value)	6.21 (0.013)		0.95 (0.329)		6.24 (0.012)	
OR (95% CI) [†]	1.81 (1.13-2.91)		1.34 (0.74-2.43)		1.60 (1.10-2.30)	

* Excludes the single subject with an A3 allele.

[†] OR = bc/ad, 95% CI = exp [ln(OR) $\pm$ 1.96(1/a + 1/b + 1/c + 1/d)^{1/2}] where a and c refer to the number of A1 alleles in cases (that is, CL $\pm$ P subjects) and controls, respectively, and b and d refer to the number of A2 alleles in cases and controls, respectively.

Interpreting the evidence for an association between the retinoic acid receptor locus and non-syndromic cleft lip with or without cleft palate

Vintiner et al¹ recently reported the results of a negative association study for non-syndromic cleft lip with or without cleft palate (CL $\pm$ P) and the PstI polymorphism at the retinoic acid receptor (RARA) locus, and concluded that their data failed to confirm the reported association between CL $\pm$ P and RARA in Australian subjects.² Failure to reject the null hypothesis in these data does not, however, constitute evidence against an association of the magnitude detected in the Australian study.² The best estimate of the odds ratio (OR) for the association of CL $\pm$ P and RARA in Australian subjects is 1.81 (table). The British data do not constitute evidence against such an association, since they provide relatively low power: 56% under a two sided alternative and 68% under a one sided alternative, to detect an odds ratio of this magnitude at $\alpha=0.05$. In fact, the direction of the association between the A2 allele and CL $\pm$ P is consistent across studies, and the 95% confidence interval obtained in the British data includes the point estimate based on the Australian data (table). Moreover, tests of heterogeneity for the RARA allele frequencies were non-significant in both cases (p=0.28) and controls (p=0.96). Relative to the Australian data, the combined data provide slightly stronger evidence (p=0.012) for a somewhat weaker association (OR=1.60, 95% CI 1.10-2.30) between CL $\pm$ P and the A2 allele of the RARA PstI polymorphism (table). Thus, while the British data are compatible with the null hypothesis, they are also consistent with the Australian data. Confirmation of an association between CL $\pm$ P and RARA, therefore, awaits replication in study populations with sufficient power to detect an odds ratio of at least 1.60.

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