Genetics of cleft lip and palate revisited

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Cleft lip with or without cleft palate (CL/CP) is one of the most common structural birth defects, with treatment including multiple surgeries, speech therapy, and dental and orthodontic treatments over the first 18 years of life. Providing care for these patients and families includes educating patients and parents about the genetics of CL/CP, as well as meeting the immediate medical needs. Attempts at identifying susceptibility loci via family and case-control studies have proved inconsistent. It is likely that initial predictions of the complex interactions involved in facial development were underestimated. The candidate gene list for CL/P is getting longer and the need for an impartial, systematic screening technique, to implicate or refute the inclusion of particular loci, is apparent. So we are faced with the question "Can this complex trait be too complex?" The aim of this review is to make the dentist aware of the differences between syndromic and non-syndromic cleft as well as understanding the etiological variation in cleft lip with and without cleft palate. This will aid the dentist in diagnosis and give proper genetic counseling to parents and patients of cleft lip and palate.
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INTRODUCTION

Cleft lip (CL) and cleft palate (CP) are the single most common defects affecting the oral facial structures in man and as such, has been the subject of considerable investigations, both in terms of etiology and as a model for the understanding of other less common morphologic defects. Cleft lip and cleft palate are extremely disfiguring and particularly in the child, affect function to a considerable extent. Genetic factors undoubtedly play a role in the etiology of CL and CP. The dentist can assess from a good family history the possible extent to which genetic factors are involved in the etiology of CL + CP in a given proband, and provide genetic counseling.

Why does the mode of inheritance of CLP still remains controversial?

Although the mode of inheritance of CLP has been investigated for many years, the results appear to be controversial. The discrepancy is probably due both to the sample and the models employed. Usually, samples comprising of families from different racial and ethnic backgrounds were clubbed together. Also studies based on different cultural background could be biased. As it is CLP investigations are limited:

- Small pedigrees are usually available.
- This malformation exhibits genetic heterogeneity.
- Low penetrance leading to reduced number of affected individuals in the pedigree.
- Environmental factors may play a role in the onset of the malformation (have a different impact even in homogenous population groups).

Syndromic vs nonsyndromic cleft

CL±CP can be syndromic or nonsyndromic. More than 150 disease syndromes have been identified where cleft of the lip and palate may be an associated congenital defect.[1] A cleft is syndromic,[2] if the patient has more than one malformation involving more than one developmental field. Table I gives list of some major syndromes associated with cleft lip and / or palate.

It is nonsyndromic if there is only a single malformation, if there are multiple anomalies that are the result of a single initiating event or primary malformation or if multiple anomalies are limited to a single developmental field.

Previous estimates suggest that posterior CP is associated with other congenital anomalies in 13-50% of the cases, whereas, CL+P is syndromic in 2-13% of the cases.[3] However, Bixier found 1% of CL±P and 8% of CP cases as syndromic.[4] Although syndromic clefts comprise only a minority of all facial clefts, syndrome identification is extremely important because of the need for accurate counseling and the burden imposed on patients and the families.

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Syndromic Clefting

The known causes of syndromic clefting include the following:

Chromosomal abnormalities

Human autosomal anomalies are usually characterized by prenatal or postnatal growth failure, mental retardation and multiple dysmorphic features that indicate a widespread disturbance of early morphogenesis. There are major malformations in addition to minor anomalies therefore patients with a facial cleft and other anomalies involving more than one developmental field should have a chromosomal analysis if the pattern of anomalies is not diagnostic of a well defined syndrome, not known to be due to a chromosomal abnormality.

Single gene disorders

To determine whether a clefting syndrome is caused by a single gene mutation, one must analyze the family pedigrees of affected individuals. Vertical transmission of the syndrome, (from generation to generation) suggests dominant inheritance. Parental consanguinity and affected siblings suggests autosomal recessive inheritance and expression in males related through unaffected females suggests X-linked inheritance.

Teratogenic syndromes

Human teratogens have been delineated because they cause a recognizable pattern of anomalies. In humans we associate teratogens with a syndrome or more precisely with one or more disruptions or disruption sequences. Counseling may be difficult because of parental guilt that is associated. Recurrence risk will be based on the likelihood of exposure to the teratogen during future pregnancies. Specific drugs are known to have a terato genie effect on facial development like phenytoin[5] and retenoic acid derivatives.[6] Cigarette smoking[7,8] and alcohol uses[9] have associations with clefting. Preliminary data shows that periconceptional vitamin use may decrease the recurrence of clefting in families, which also supports environmental influences.[10] Perhaps with relative vitamins deficiencies may be contributing occurring on specific genotypic backgrounds.[11]

Syndromic clefting with unknown cause

If a patient has a facial cleft plus other malformations in different morphogenetic fields it is still appropriate to call this a syndrome even though there is no identifiable chromosomal abnormality, Mendelian inheritance pattern or teratogenic exposure e.g., mandibular - limb hypogenesis syndrome and the de Lange syndrome. One should keep in mind the likelihood of etiological heterogeneity when evaluating such patients. It is possible that some of these sporadic disorders are actually caused by new dominant mutations, currently undetectable chromosomal deletion and unidentified teratogens.

Table I. Some major syndromes associated with cleft lip and/or palate

Autosomal dominant
Van der Woude syndrome (lip pits with cleft lip/palate)
EEC syndrome (ectrodactyl, ectodermal dysplasia and clefting)
Hereditary artho-ophthalmopathy (Stickler syndrome)
Larsen syndrome (originally thought to be recessive)
Retinal detachment, myopia and cleft palate (Marshall syndrome)
Sondyloepiphyseal dysplasia congential
Autosomal recessive
Chondrodysplasia punctata (Conradi syndrome)
Diastrophic dwarfism
Smith-Lemli-Opitz syndrome
Meckel syndrome
Orofaciodigital syndrome, type II
Fryns syndrome (with diaphragmatic hernia, limb and facial anomalies)
Roberts syndrome
Velocardiofacial (Shprintzen) syndrome
X-linked
Orofaciodigital syndrome, type I (dominant, lethal in male)
Otopalatogidital syndrome
Isolated X-linked cleft palate with ankyloglossia
Chromosal
Trisomy 13
Trisomy 18
Chromosome 18 deletions
Various other autosomal abnormalities
Non-medelian
Peirre Robin sequence
Clefting with congenital heart disease
De Lange syndrome

Nonsyndromic clefting

Like many other isolated birth defects is sometimes familial, but the pattern of affected individuals seen in families usually is not consistent with Mendelian inheritance. To explain the observed pedigrees, mathematic models have been proposed that assume a continuous distribution of liability in the general population and others hold point in that liability distribution beyond which individuals are affected. Observations in human populations have tended to support these hypotheses. As a result isolated clefting is commonly viewed as "multifactorial" or "polygenic". The latter term meaning in a strict sense, that genes with small additive effect, provide the underlying genetic predisposition to clefting. Table II summarizes the popular theories, which have been propagated to explain non-syndromal clefting.

Mapping the cleft-lip genes (nonsyndromic)

Several genes are already known to be associated with CL/P and CP. There are various gene-mapping studies for CL/P, but none have shown a clear association. Table III gives summary of these studies.

Multifactorial concept - quasi continuous variants

A multifactorial trait is determined by interaction of a

Table II. Various concepts proposed to explain the genetics of Cleft lip and palate

Author (year)	Proposed Concept	Observations
#Fogh-Andersen (1942)[12]	Proposed every imaginable mode of inheritance -Dominance with reduced penetrance -Partial dominance (homozygous more effected) -Double or polyploid recessivity -Partial sex linkage - "Polymeric recessivity"	The "Polymeric recessivity" concept was highly imaginative 2 factors from upper lip (left & right) & 2 from the hard palate
#Fraser (1957)[13]	Concept of liability	
	Concept of Developmental threshold	The stage at which palatal shelf moved from vertical to horizontal prefusion Maximum amount of delay compatible with closure
#Carter (1969) **Fraser (70,89)**[14-16]	Multifactorial threshold model	Melnick et al[17] dissented this view
#Morton (1970)[18] **Elston & Stewart (1971),**[19] **Morton & Maclean (1974)**[20] **Lolovel & Morton (1981)**[21]	Complex Segregational analysis involving a combination of a major gene, a polygenic contribution, sproradic (nonrecurrent) cases a familial environmental component	Major means a big enough effect to be detectable.
#Bixler (1973)[22]	-Dominant gene (allelic restriction)	Supported by Melnick
	-Neither multifactorial threshold nor single major gene model	Melnick supports it
	-Major gene or full mixed model plus a large admixture of sporadic cases caused by polygenic inheritance or environmental agents	Supported by Marazita et al[23]
#Transler & Fraser (1977)[24]	Multifactorial inheritance	Discussed shelf movement influence by genes and environment
#Biddle, Fraser, **Juriloff (1986)**[25]	Major gene with low penetrance (5%) and modified by maternal genes	Map location of the gene is elusive
#Chung (1986) Marazita (1992) **DePalpe (1989) Ray (1993)** **Temple (1989) Hecht (1990)**[26-30]	Single major gene - autosomal recessive	But always incomplete penetrance
#Ardinger (1989)[31]	Significant association exists b/w clefting and 2 restriction fragment length polymorphs (RFLPs Taq1 and Bam H1) of transforming growth factor alpha	
#Qian (1992)[32]	TGFα/may be a modifier gene not a major gene plays a role in development of bilateral clefting	Vintinger[33] observed no evidence of linkage of TGFα
(1993)[34]	Found excess frequency of Bam H L A 1 allele in BCL/P only	
#Mitchell & Risch (1992)[35]	Compatible with either multifactorial threshold trait or with Oligogenic model	Farral & Holder[36] supported the Oligogenic model (predicted that 4-7 different genes cause clefting)
#Murray et al (1995)[37]	CL±P is heterogeneous & different chromosome regions such as 2p, 4q, 6p, 17q, 19q have been claimed to contain the clefting locus.	
#Clements et al (1995)[38]	Two locus model i.e. a major dominant locus & modifier locus	Pezzetti et al[39] proposed an interactive effect between loci mapping in 6P23 & 2P13
#Pirinan (1998)[40]	Genes MSX 1 and TGFB 3 with a gene linkage disequilibrium (LD) strategy	IOWA study initiated to extend search for non-coding parts of MS X 1

number of genes at different loci each with a small but additive effect together with environmental factors (i.e. genes are rendering the individual unduly susceptible to environmental agents). The final shapes of most of the oral facial structures in common with other morphologic structures in the body, are regulated by multiple genes rather than by a single gene.[73]

Characteristics of polygenic inheritance are as follows: 1). Gene segregation occurs at an indefinitely large number of genetic loci. 2). Mutation of one or two genes for a polygenically controlled trait produces little effect on the over all manifestation of that trait. 3). Phenotypic expression of a polygenic trait can be similar with the large variety of subtly different genotypes. 4). Phenotypic expression of polygenic traits is not only affected by gene alteration but also is susceptible to significant modification by the environment; polygenic traits are thus characterized by gene-environmental interaction. 5). Occasionally, polygenes can act as a "polygenic block" and exhibit a single gene inheritance pattern, but can be expressed as many phenotypic characters. This may be the case with some of the multifactorial syndromes.[74]

If we apply these principles to CL and CP we can postulate that palatal shelf length e.g. is polygenic and is continuously variable from very long to very short in relation, say to the width of the maxilla. With a given

Table III. Various linkage & association studies for causation of CL±P

Chromosome	Author (year)	Observations
CHROMOSOME2	***Arlinger et al (1989)[31]**	Linkage between CL±P & RFLPs (restriction fragment length polymorphs). At TGF α location on short arm of chromosome 2 region 2p13 (OFC2). Confirmed by **Pezzetti et al (1998)[39]**
	***Hecht et al (1991a)[41]**	Did not find linkage to support the role of TGF α in early stages of CL±P development. **Wyszynski et al (1997)[42] Vintiner et al (1992)[33]** & **Field et al (1994)[43]** confirmed the same.
	***Chenevix-Trench (1991,92)[44-45]**	Association with specific C2 allele of the TGF α locus Taq 1 polymorhism. Also confirmed by **Holder et al (1992)[46]** & **Sassani et al (1993)[47].** Refuted by **Stoll et al (1993)[48]** & **Jara et al (1995)[49]**
	***Farral et al (1993)36 & Feng et al (1994)[50]**	Positive linkage disequilibrium with the C2 allele (family based association study)
	***Jara et al (1995)[49]**	Association with BamHI allele.
	***Mitchell (1997)[51]**	Confirmed the association of CL±P & TGF α using a meta analysis study. **Shaw et al (1996)[52]** had earlier detected no allelic association
CHROMOSOME 4	***Beiraghi et al (1994)[53]**	Evidence of linkage between cleft & markers on long arm of chromosome 4
	***Mitchell et al (1995)[54]**	Evidence of a locus in region 4q25-4q31.3 Refuted later on by **Blanton et al (1996)**
CHROMOSOME 6	***Eiberg et al (1987)[55]**	Linkage to 6p24 at F13A locus. However his claims have been refuted by **Heeth et al (1993)56 & Blanton et al (1996)[57]**
	***Korman-Bortolotto et al (1990)[58] Donnal et al (1992)[59]**	CL±P associated with chromosomal aberrations involving short arm of chromosome 6p
	***Mehra & Verma (1991)[60]**	The HLA locus is 6p21.3. This was earlier refuted by **Van Dyke et al (1980)[61]** & **Watanable et al (1984)[62].**
	***Davies et al (1995)[63]**	3 cases with chromosomal abnormalities (2 balanced Translocations & 1 deletion). Analyzed Yeast clones & Localized the clefting locus within the 6p24.3 region
	***Carinci et al (1995)[64]**	Evidence of genetic heterogeneity & linkage to 6p23 region Confirmed by **Scapoli et al (1997)[65]**
CHROMOSOME 2 & 6	***Murray et al (1993)[37]**	Implicated a primary role for OFCI on 6p & OFC2 on 2p13 is a modifier of the clefting status
	***Pezzeti et al (1998)[39]**	Proposed an interactive effect involving two disease loci mapping in 6p23 & 2p13
CHROMOSOME 17	***Chenevix-Trench (1992)[45]**	Significant difference between non-syndromic cleft cases & Unrelated controls a the retinoic acid receptor α (RARA) Pst I RFLP located at 17q21.1 Confirmed by **Shaw et al (1993)[66]** They found a significant difference in D17S579 (micro satellite marker close to RARA)
	***Vinitner et al (1993)[67]**	Found no linkage between RARA and clefting aiso confirmed by **Stein et al (1995)[68].**
CHROMOSOME 19	***Stein et al (1993)[69]**	Found linkage with BCL3 a proto oncogene mapping in 19q13.2
	***Amos et al (1996a)[70]**	Evidence of disequilibrium between BCL3 alleles and cleft. Confirmed by **Wynszynski et al (1997b)[71]**
	***Martinelli et al (1998)[72]**	Found linkage with D19S574 a polymorhic markertightly linked to the BCL3 gene

maxillary width, progressively shorter palatal shelves will show increased difficulty in coming to a point of contact for fusion during development and at a given threshold level, they will be too short to allow fusion and a cleft palate will result. This kind of variation could also occur for maxillary width, tongue size and palatal height, all of which are probably polygenic, and it is probable that all these factors contribute to result in failure of palatal closure. This type of theoretic model for genetic involvement in CL and CP is usually referred to as "quasi-continuous" because the manifestation of the defect is all or none in its expression (i.e.) present or absent.[75] The defect is presumably present only when enough genes are present in an individual to give rise to a phenotypic expression of say "short" palatal shelves or "wide" maxilla, and so forth, such that the threshold for non fusion is reached and the defect results.

The differences in severity of clefts are good indicators of the phenotypic expression of continuous variation in a trait such that progressively shorter and shorter palatal shelves produce more severe defects. Environmental factors effective in prenatal development also affect gene expression and the production of the defect. Thus a complex interaction of genes and environment and even

Table IV. Sequence of events for diagnosis & genetic counseling

Evaluation

Gestational history: for possible teratogens
(Exposure to anticonvulsant, Alcohol, Steroids, Hyperthermia in first trimester of pregnancy)

Family history: for any relatives with similar or different congenital anomalies or developmental abnormalities.

Microforms: Presence of lip pit as in Van der Woude Syndrome[87] and autosomal dominant form of CL (P) may be absent or subtle in the new born but diagnostic when examining the parent. A clear uvula, submous cleft of soft palate or velopharygeal incompetence may have important implication for genetic counseling.

Physical examination:

⇨ Clearly describe all anatomic variation from normal
(take photographs) including: Major anomalies / Minor anomalies
⇨ Are these →a) Malformations? →b) Disruptions? →(c) Deformations?
⇨ Are these anomalies?
a) All part of the same developmental field →(single anomaly, nonsyndromic)?
b) All related to one primary defect or event →(sequence, nonsydomic)?
c) In more than one developmental field →(syndrome)?

Other Investigations indicated:

⇨ Chromosomal analysis if patient had syndrome (that is multiple anomalies in more than one developmental (field) that does not have a well defined etiologically.
⇨ Others as suggested by the patient's phoetype e.g. Skeletal radiographs for short stature, Craniofacila radiographs for other anomalies of the skulll or face and an ophthalmologic Examination if stickler syndrome is a possiblity. CNS diagnosing in cases of suspected Holoprosencephaly.
⇨ As cardiac anomalies are associated with 20% of clefting cases cardiac evalation may be useful.
⇨ Facial clefts can be diagnosed by visualization fetoscopy in utero[88]. This was recommended in case of high-risk families Where risk is more than 10%. This technique is associated with fetal loss of 4-5%. CL may also be diagnosed is utero by Ultrasound and presently this is the recommended technique[89]. However, an expert ultrasonographer is required. Ultrasound and presently this is the recommended technique89. Howeve, an expert ultrasonographer is required.

Counseling

Counseling involves helping a patient and family to deal with having a cleft Malformation or syndrome and and to understand and deal with the risk of recurrence.

Who should have an evaluation and genetic counseling???

a). Anyone who has a syndromic cleft
b). Anyone who has a familial cleft whether syndromic or nonsyndromic.
c). Anyone who has questions about etiology, pathogens or recurrence risks.

Deformations and disruptions depend on the likelihood of the severe determing factors, (Such as a known teratogen or mechanical factor)being present in future prenancy.

Counseling for cleft malformations depend on whether or not the cleft is syndromic or nonsydromic and familial or non familial.

when the optional set of genetic factors is present in a given individual, the disorder may still not result unless "something" in the environment also is present. Thus, a person at high genetic risk because of accumulation of deleterious polygenes will produce the defect in the absence of an adverse environmental influence. Empiric risk figures take both these factors into account. Tables IV an V give the genetic risk for having a cleft lip and palate child with reference to non-identifiable syndrome and risk to siblings with reference to severity of the cleft.

Table V. Diagnosis and genetic counseling in a patient with cleft lip with or without cleft palate.

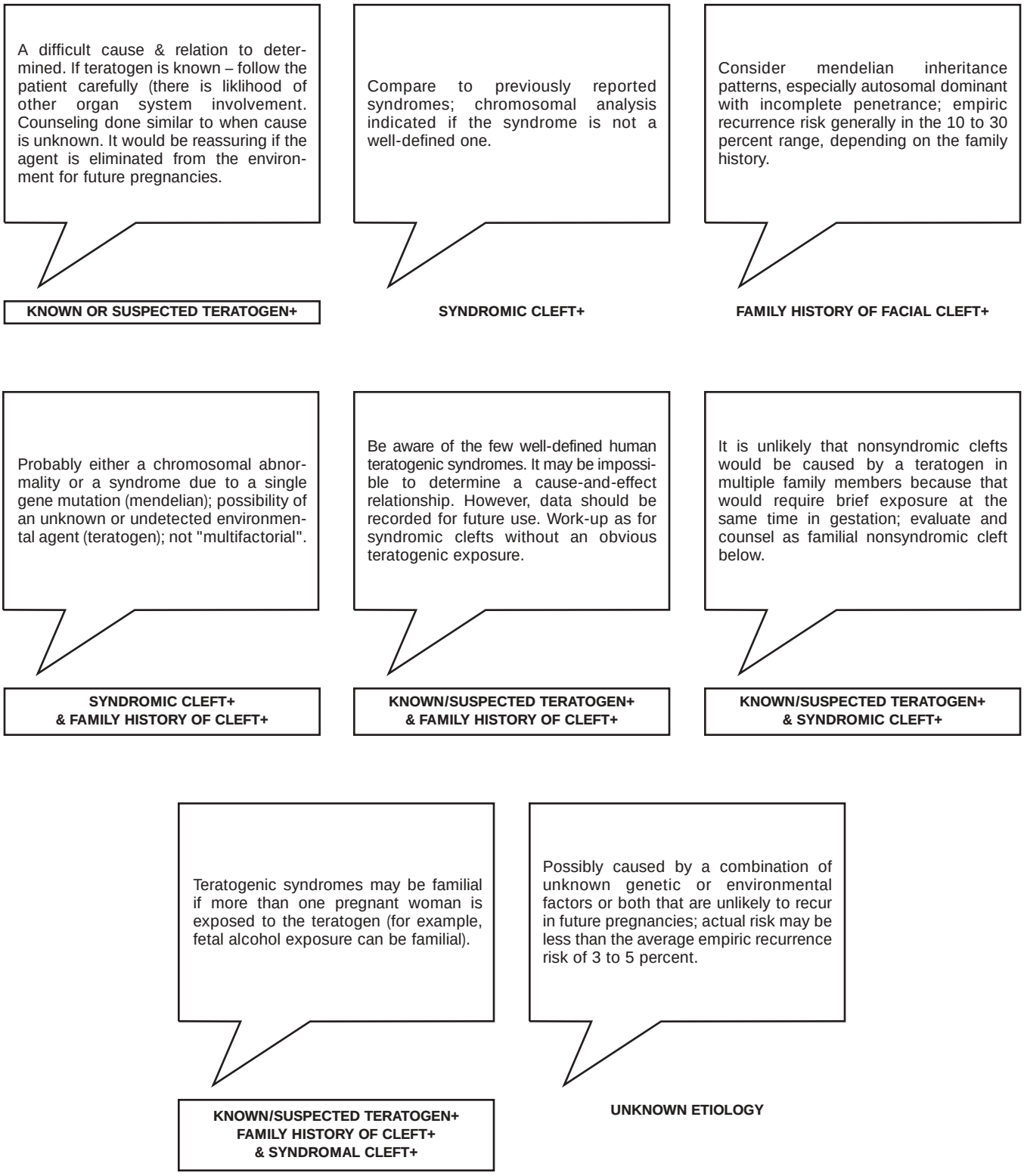

Table VIa: Cleft lip and palate - genetic risk in the absence of a defined syndrome or mendelian pattern

Relationship to index	Cleft lip + palate (%)	Isolated cleft palate (%)
Sibs (overall risk)	4.0	1.8
Sib (no other affected members)	2.2	
Sib (2 affected sibs)	10	8
Sib and affected parent	10	
Children	4.3	3
Second-degree relatives	0.6	
Third-degree relatives	0.3	
General population	0.1	0.04

Table VIb. Genetic risks in cleft lip/palate: effet of severity

Anomaly	Risk to sibs (%)
Bilateral cleft lip and palate	5.7
Unilateral cleft lip and palate	4.2
Unilateral cleft lip alone	2.5

Genetics of cleft lip with or without palate vs cleft palate only

Clefts of the human face can classified anatomically as those involving the secondary palate only (the posterior or soft palate) or the cleft palate only (CPO) and those involving the primary palate and encompass cleft of the lip with or without the palate (CL±P).[76] This distinction is biologically relevant and is supported on embryological grounds, indeed, the primary and secondary palates are formed independently. Furthermore it is unusual to find familial CPO if the index case has CL±P and vice-versa.[75]

The incidence of CL±P ranges from 1 in 700 to 1 in 1000 in Caucasians.[75,77] The nature of genetic contribution to the etiology of non-syndromic CLIP (also called as orofacial cleft) is a matter that is still greatly studied and investigated.[37,42] Earlier studies did not identified a Mendelian pattern of inheritance.[14,15] The multifactorial hypothesis became popular. Recent data based on segregation analysis suggested a model of an autosomal major gene with or without multifactorial contribution.[23,26,27,28,30] Several multigenerational studies suggested an autosomal dominant inheritance.[29,30] Re-analysis of the recurrence patterns led to the oligogenic model.[35,36] It has become evident that CLP is heterogeneous and different chromosomal regions have been claimed to control the clefting locus.[37]

CPO is less frequent than CL±P has a global birth prevalence of 6.5 incidence per 10,000 live births.[78] Mode of inheritance of CPO is unclear. Several studies have demonstrated familial aggregation and found that an oligogenic model with several interacting loci fits the data the best.[79,82] Moreover CPO involving the hard and soft palate may be etiologically distinct.[82,83]

Recently it was shown that a recessive single major gene locus with low penetrance provided a significant best fit.[38] A significant association has been found between alleles of TGFA and CP0.[84] Various other studies may be found in literature, which study the mode of inheritance of CP0.[85,86]

Genetic counseling

The steps involved in proper diagnosis and then going for genetic counseling is shown in Tables IV and V. The general guidelines for counseling are as:

- An affected female has a greater chance of having an affected offspring than an affected male, although both have 40 times greater risk than population risk.
- More severe the defect in the parent greater the risk for an affected offspring e.g. a parent with BCLP is more likely to have an affected offspring than a parent with UCLP.

  The greatest risk is for severely affected female parent.
- A 1st degree relative has the highest risk (40 times the population incidence), 2nd degree an intermediate risk (7 times the population incidence) and 3rd degree the least risk (3 times the population incidence). The risk thus decreases rapidly with decreased degree of relationship as distinct from the single gene inheritance which merely halves the risk for a second child to be affected increases rapidly if one child is already affected. This rises to 10% for one affected child (unaffected parents) to 9% for two affected children. For an affected parent with one affected child is risk is about 10%. (Tables VIa & VIb)[90,100]
- If the evaluation/investigations suggest a known syndromic/chromosomal or single gene entity counseling is to be done accordingly.

CONCLUSION

It is likely that initial predictions of the complex interactions involved in facial development were underestimated. The candidate gene list for CL/P is getting longer and the need for an impartial, systematic screening technique, to implicate or refute the inclusion of particular loci, is apparent. So we are faced with the question “Can this complex trait be too complex?” Understanding the genetics of CL/P will aid the dentist in diagnosis and give proper genetic counseling to parents and patients of cleft lip and palate.

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