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AXIN2, Orofacial Clefts and Positive Family History for Cancer

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Abstract

Background—Cancer and congenital malformations may occasionally have a common etiology. We investigated if families segregating orofacial clefts (CL/P) presented increased cancer incidence when compared to control families.

Methods—We assessed 75 CL/P families and 93 control families of Caucasian ethnicity from Pittsburgh regarding positive history of cancer. Chi-square and Fisher exact tests were used to determine significant differences. Then, we performed molecular studies with genes in which mutations have been independently associated with both cancer and craniofacial anomalies.

Results—CL/P families reported positive family history of cancer more often than control families (p=0.0002), and had higher rates of specific cancer types: colon (p=0.0009), brain (p=0.003), leukemia (p=0.005), breast (p=0.009), prostate (p=0.01), skin (p=0.01), lung (p=0.02), and liver (0.02). Overtransmission of *AXIN2* was detected in CL/P probands (p=0.003).

Conclusion—Families segregating CL/P may have an increased susceptibility for cancer, notably colon cancer. Further, *AXIN2*, a gene that when mutated increases susceptibility to colon cancer, is also associated with CL/P.

Clinical Implications—Individuals detected at a higher risk for disease predisposition will be able to adopt a better lifestyle avoiding exposure to other risk factors that may interact with the individual's genotype.

Keywords

Orofacial Clefts; Family history for cancer; *AXIN2*

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Introduction

Non-syndromic oral clefts are considered multifactorial in origin, with the possibility of genetic and environmental components interacting. [1] It has been proposed that cancer and congenital malformations such as cleft lip and palate may occasionally have a common etiology. The underlying concept is that the same genes can act in normal and also malignant development. Individuals born with orofacial clefts have a shorter lifespan, and cancer has been suggested as one of the factors reducing the life expectancy of individuals born with a facial cleft. [2–5] A higher cancer risk in parents of children born with oral clefts was reported, [6] and increased occurrence of cancer in individuals born with both cleft lip and cleft palate was demonstrated in a large population-based study. [7]

Recent evidences from genetic studies have also supported the hypothesis that some genes are simultaneously associated with cancer and craniofacial disorders. *FGF* signaling pathway genes have been associated with various types of cancer [8–11] and might contribute to approximately 3% of nonsyndromic cleft lip and palate cases (CL/P).[12] Mutations in *E-cadherin* (*CDH1*), a cell-cell adhesive molecule expressed in epithelial cell types, were reported in two families segregating hereditary diffuse gastric cancer and oral clefts.[13] Also, mutations in *AXIS inhibition protein 2* (*AXIN2*) were found to cause oligodontia (lack of six or more permanent teeth) and increased susceptibility to colorectal cancer. [14]

With the goal of investigating if families segregating isolated cleft lip and palate have increased risk for cancer, we compared the incidence of cancer in cleft families and control families without history of clefts. Furthermore, we performed candidate genes studies, selecting genes that were independently associated with both cancer and craniofacial anomalies.

Subjects and Methods

After proper IRB approval (University of Pittsburgh, IRB number – 0607057), self- reported family history of cancer was collected through a structured questionnaire from 168 families (75 CL/P families and 93 control families) of Caucasian ethnicity (individuals that did not report Native American, African, or Asian ancestry) from Pittsburgh. Participants were asked to describe the precise relationship with the reported affected relative and the type of cancer he/she presented. Of the 75 families segregating CL/P, 66 families had two or more individuals affected by CL/P. A total of 558 participants, 309 females (223 adults, 86 children) and 249 males (163 adults, 86 children) answered the proposed questionnaire. The age range for females was 4 months- old to age 85 (average age 30 years) and 6 months-old to age 86 (average age 28 years) for males. Parents were responsible for answering the questions for their respective children. Incomplete information about cancer history was not accounted for in the analysis. We used chi-square and Fisher exact tests to determine statistically significant differences between both cleft and control families with an alpha of 0.05.

We also investigated the role of genes in which mutations have been associated, independently or in the same study, with both cancer and clefts or other craniofacial anomalies. In addition to the 75 cleft families previously investigated, we added 36 additional families of Caucasian origin from Pittsburgh and St. Louis to improve statistical power. Genomic DNA samples were obtained from saliva, blood, mouthwash or buccal swabs from 90 cleft families from Pittsburgh (which included the 75 families segregating CL/P that answered the questionnaires) and from an additional 21 cleft families from St. Louis, comprising a total of 427 individuals of which 131 were affected by CL/P. Genotyping was performed by the Taqman method [15], using a 7900 automatic instrument and pre-designed probes (Applied Biosystems, CA, US). The Family Based Association Test (FBAT) 16 was used to detect transmission distortions in the families segregating CL/P. For *AXIN2* and *CDH1* genes, we used the approach proposed by

Carlson et al. (2004) [17], to select a subset of SNPs that maximally represent the linkage disequilibrium structure of a given region (HapMap European derived block structures - http://www.hapmap.org). All *FGF* and *FGFR* SNPs were derived from previous studies that showed association with clefts and/or cancer. [9,10,12] Table 3 summarizes the genes/SNPs used in this study.

Results

Based on a direct interview/questionnaire with participants of Caucasian ethnicity from Pittsburgh (75 CL/P families and 93 control families), we observed that cleft families reported more often positive family history of cancer when compared to families without history of oral clefts (p=0.0002). The expected number of families with cancer in the control group was 75.3. However, 66 had occurred. In the oral cleft families the expected number was 60.7 and 70 had occurred (Table 1). In addition, families segregating CL/P more commonly reported history of multiple types of cancer (three or 20 more) compared to the control families (p=0.00001) (Table 2). CL/P families presented increased rates of colon cancer (p=0.0009), brain cancer (p=0.003), leukemia (p=0.005), female breast cancer (p=0.009), prostate cancer (p=0.01), skin cancer (p=0.01), lung cancer (p=0.02), and liver cancer (p=0.02), in comparison to control families.

An association between *AXIN2* and CL/P (p=0.003) was also observed (Table 3).

Discussion

Childhood cancers accompany many congenital malformations. In that context, investigating the relationship between malformations and malignancies is important, as it is speculated that they might have common causes.

In the present study, families segregating CL/P reported an increased familiar history of cancer compared to families without history of oral clefts. These observations provided us the starting point to investigate genes in which mutations have been associated, independently or in the same study, with both cancer and clefts or other craniofacial anomalies, namely, *AXIN2*, *CDH1*, and members of the *FGF* gene family. Some of these genes are effectors of cell-cell adhesion and cell motility functions and/or play critical roles during embryonic development, and in turn may lead us to believe that variations in these genes could contribute to the occurrence of craniofacial disorders (CL/P, microtia, profound congenital deafness, tooth agenesis, microdontia) and cancer.

An association between *AXIN2* and CL/P (p=0.003) was observed. The protein product of *AXIN2* is a negative regulator of the Wnt-signaling pathway. [19, 20] Moreover, previous evidence showed that germline mutations in the Wnt pathway component gene *AXIN2* have been associated with tooth agenesis-colorectal cancer syndrome. 14 This fact is at least interesting once tooth agenesis is a common finding in individuals affected by oral clefts, and has been recently proposed to be used to subphenotype clefts. [18] Furthermore, the involvement of Wnt-signaling genes in carcinogenesis is well established (regulating cell growth, motility and differentiation), although relatively little is known about the connection between them and congenital malformations in humans. Wnt signaling has been implicated in regulation of diverse developmental events, as well as in aberrations of cell homeostasis that may lead to cancer, [21–23] which could in part explain the results observed here.

In our population, we did not observe associations between *CDH1* or *FGF* pathway genes with CL/P, however, these genes were previously related to CL/P, [12, 13] and further studies should consider them as candidate genes for oral clefts since they are responsible for cell-cell adhesion and various developmental steps.

We compared self-reported family history of cancer in families segregating CL/P versus families without history of CL/P; however, the cancer types reported may not be all related to the same etiologies. Furthermore, we did not have access to specific data regarding the age of onset of cancer, which makes it impossible to determine if family members of an individual born with CL/P develop cancer at earlier ages than the general population. In addition, only three individuals born with a CL/P also developed cancer (one child presented leukemia and two adults had colon cancer and skin cancer, respectively). Although we do not have enough information to test if individuals born with CL/P have increased susceptibility of cancer themselves, a previous population. based study [7] suggests that this is the case. We are increasing our sample size to be able to replicate our findings and test for association between genetic variants and specific types of cancer segregating in the families.

Our results indicate that families segregating CL/P may have an increased susceptibility for cancer, notably colon cancer. Further, *AXIN2*, a gene that when mutated increases the susceptibility to colon cancer, is also associated with CL/P.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

CL/P	Cleft lip with or without cleft palate
SNP	Single nucleotide polymorphism
AXIN2	AXIS inhibition protein 2 (conductin, axil)
CDH-1	E-cadherin
FGF	Fibroblast growth factor
FBAT	Family Based Association Test

REFERENCES

1. Murray JC. Gene/environment causes of cleft lip and/or palate. Clin Genet 2002;61:248–256. [PubMed: 12030886]
2. Windham GC, Bjerkedal T, Langmark F. A population-based study of cancer incidence in twins and in children with congenital malformations or low birth weight, Norway, 1967–1980. Am J Epidemiol 1985;121:49–56. [PubMed: 3155484]
3. Mili F, Khoury MJ, Flanders WD, Greenberg RS. Risk of childhood cancer for infants with birth defects. I. A record-linkage study, Atlanta, Georgia, 1968–1988. Am J Epidemiol 1993;137:629–638. [PubMed: 8470664]
4. Narod SA, Hawkins MM, Robertson CM, Stiller CA. Congenital anomalies and childhood cancer in Great Britain. Am J Hum Genet 1997;60:474–485. [PubMed: 9042906]
5. Nishi M, Miyake H, Takeda T, Hatae Y. Congenital malformations and childhood cancer. Med Pediatr Oncol 2000;34:250–254. [PubMed: 10742060]
6. Zhu JL, Basso O, Hasle H, Winther JF, Olsen JH, Olsen J. Do parents of children with congenital malformations have a higher cancer risk? A nationwide study in Denmark. Br J Cancer 2002;87:524–528. [PubMed: 12189550]
7. Bille C, Winther JF, Bautz A, Murray JC, Olsen J, Christensen K. Cancer risk in persons with oral cleft--a population-based study of 8,093 cases. Epidemiol 2005;161:1047–1055.
8. Jang JH, Shin KH, Park JG. Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers. Cancer Res 2001;61:3541–3543. [PubMed: 11325814]

9. Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007;447:1087–1093. [PubMed: 17529967]
10. Hunter DJ, Kraft P, Jacobs KB, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 2007;39:870–874. [PubMed: 17529973]
11. Sonvilla G, Allerstorfer S, Stättner S, et al. FGF18 in colorectal tumour cells: autocrine and paracrine effects. Carcinogenesis 2008;29:15–24. [PubMed: 17890768]
12. Riley BM, Mansilla MA, Ma J, et al. Impaired FGF signaling contributes to cleft lip and palate. Proc Natl Acad Sci U S A 2007;104:4512–4517. [PubMed: 17360555]
13. Frebourg T, Oliveira C, Hochain P, et al. Cleft lip/palate and CDH1/E-cadherin mutations in families with hereditary diffuse gastric cancer. J Med Genet 2006;43:138–142. [PubMed: 15831593]
14. Lammi L, Arte S, Somer M, et al. Mutations in *AXIN2* cause familial tooth agenesis and predispose to colorectal cancer. Am J Hum Genet 2004;74:1043–1050. [PubMed: 15042511]
15. Ranade K, Chang MS, Ting CT, et al. High-throughput genotyping with single nucleotide polymorphisms. Genome Res 2001;11:1262–1268. [PubMed: 11435409]
16. Harvath S, Xu X, Laird N. The family based association test method: strategies for studying general genotype-phenotype associations. Eur J Hum Gen 2001;9:301–306.
17. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004;74:106–120. [PubMed: 14681826]
18. Letra A, Menezes R, Granjeiro JM, Vieira AR. Defining cleft subphenotypes based on dental development. J Dent Res 2007;86:986–991. [PubMed: 17890676]
19. Jho EH, Zhang T, Domon C, Joo CK, Freund JN, Costantini F. Wnt/β-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. Mol Cell Biol 2002;22:1172–1183. [PubMed: 11809808]
20. Leung JY, Kolligs FT, Wu R, et al. Activation of AXIN2 expression by β-catenin-T cell factor: a feedback repressor pathway regulating Wnt signaling. J Biol Chem 2002;277:21657–21665. [PubMed: 11940574]
21. Huelsken J, Birchmeier W. New aspects of Wnt signaling pathways in higher vertebrates. Curr Opin Genet Dev 2001;11:547–553. [PubMed: 11532397]
22. Lustig B, Behrens J. The Wnt signaling pathway and its role in tumor development. J Cancer Res Clin Oncol 2003;129:199–221. [PubMed: 12707770]
23. Giles RH, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. Biochim Biophys Acta 2003;1653:1–24. [PubMed: 12781368]

Table 1

Observed and expected numbers of families with positive history of cancer among oral cleft cases and controls.

Group	No		Yes		Total	p value
	Obs	*Exp*	*Obs*	*Exp*		
Control	27	17.7	66	75.3	93	
Case	5	14.3	70	60.7	75	0.0002
Total	32	---	136	---	168	

obs= observed values ; exp= expected values derived from the control group.

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Table 2

Observed and expected numbers of cancer occurences among families segregating CL/P.

Families	No Cancer		One type of cancer		Two types of cancer		Three or more		Total
	Obs	*Exp*	*Obs*	*Exp*	*Obs*	*Exp*	*Obs*	*Exp*	
Controls	27	17.7	35	26.6	14	13.8	17	34.9	93
Cases	5	14.3	13	21.4	11	11.2	46	28.1	75
Total	32	---	48	---	25	---	63	---	168
p-value	---		---		---		0.0001		

*obs= observed values; exp= expected values derived from the control group

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Table 3

Summary of candidate genes/SNPs studied and results observed.

Gene	locus	SNP	Base Change	Base pair position$^{\delta}$	Applied Biosystems SNP Identification Number	SNP Type	Reason to be selected as candidate [reference]	Minor Allele frequencies (HapMap) **	Minor Allele frequecies observed	P value (FBAT)
FGF10	5p13–p12	rs1448037	**T**/C	44,388,101	C_8291276_10	Intron	Association with cleft lip with or without cleft palate [17]	0.328	0.389	0.88
FGF18	5q34	rs4073716	**C**/T	170,796,844	C_27537611_10	Intron	Association with cleft lip with or without cleft palate and colon cancer [17,18]	0.483	0.469	0.3
FGFR1	8p11.2-p11.1	rs13317	**C**/T	38,388,671	C_1358324_10	3’ UTR	Association with cleft lip with or without cleft palate [17]	0.258	0.227	0.66
FGF3	11q13	rs4631909	**C**/T	69,324,028	C_3256529_20	Intergenic	Association with cleft lip with or without cleft palate [17]	0.383	0.459	0.29
		rs4980700	**A**/G	69,328,598	C_27947621_10	Intergenic		0.5	0.451	0.22
FGF7	15q15-q21.1	rs2413958	**C**/T	47,547,838	C_15798093_10	Intron	Association with cleft lip with or without cleft palate [17]	0.267	0.302	0.4
FGFR2	10q26	rs1219648	**A**/G	123,336,180	C_2917314_20	Intron	Association with postmenopausal breast cancer [21]	0.417	0.384	0.95
		rs2981582	**C**/T	123,342,307	C_2917302_10	Intron	Association with breast cancer [22]	0.417	0.356	0.75
CDH1	16q22.1	rs11642413	A/**G**	67,347,895	C_2847356_10	Intron	Association with cleft lip with or without cleft palate in	0.375	0.438	0.09

Gene	locus	SNP	Base Change	Base pair position$^{\delta}$	Applied Biosystems SNP Identification Number	SNP Type	Reason to be selected as candidate [reference]	Minor Allele frequencies (HapMap) **	Minor Allele frequecies observed	P value (FBAT)
							two families with hereditary diffuse gastric cancer [9]			
		rs9929218	**A**/G	67,378,447	C_11509221_10	Intron		0.258	0.348	0.9
AXIN2	17q23-q24	rs7591	**A**/T	60,955,544	C_11421230_10	3' UTR	Tooth agenesis and colorectal neoplasia segregating with dominant inheritance [10]	0.425	0.432	0.68
		rs11867417	C/**T**	60,968,360	C_30669103_10	Intron		0.302	0.336	0.61
		rs2240308	A/**G**	60,985,053	C_2577354_1	Missense Mutation (P50S)		0.475	0.537	**0.003**

* letters in bold indicate the ancestral allele

** According to the HapMap Project, population with ancestry from northern and western Europe.

$^{\delta}$ According to ABI SNP browser software – version 3.5

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