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Wound Complications Following Cleft Repair in Children with Van der Woude Syndrome

Jodi Jones, M.D.^{1,7}, John W. Canady, M.D.^{1,3}, James T. Brookes, M.D.^{1,8}, George L. Wehby, Ph.D.⁴, Brian C. Schutte, Ph.D.⁵, Jeffrey C. Murray, M.D.^{2,6}, and Martine Dunnwald, Pharm.D., Ph.D.²

¹Department of Otolaryngology, Carver College of Medicine, The University of Iowa

²Department of Pediatrics, Carver College of Medicine, The University of Iowa

³Department of Surgery, Carver College of Medicine, The University of Iowa

⁴Department of Health Management and Policy, College of Public Health, The University of Iowa

⁵Departments of Microbiology and Molecular Genetics, Pediatrics and Human Development, Michigan State University

⁶Departments of Epidemiology (College of Public Health), Biology (College of Liberal Arts and Sciences), Nursing (College of Nursing) and Dentistry (College of Dentistry), The University of Iowa

Abstract

Van der Woude Syndrome (VWS, OMIM #119300) is an autosomal dominant condition associated with clefts of the lip and/or palate and lower lip pits and is caused by mutations in *Interferon Regulatory Factor 6* (*IRF6*). The standard of practice for children born with cleft lip/palate is surgical repair, which requires proper wound healing. We tested the hypothesis that children with VWS are more likely to have wound complications following cleft repair than children with non-syndromic cleft lip/palate (NSCLP). Furthermore, we hypothesized that children with VWS have more surgical procedures. A retrospective, case-controlled study was performed. Seventeen children with VWS and 68 matched controls with NSCLP were scored for presence of wound complications following cleft repair, severity of complications and number of surgeries from ages 0–10. Of the 17 children with VWS, 8 had wound complications. Thirteen of 68 controls had wound complications ($P=0.02$). Six of eight wound complications in the VWS group were major with nine of 13 complications in the control group major ($P=0.04$). The majority of wound complications were fistulae and occurred in isolated cleft palate and bilateral cleft lip. The mean number of surgeries in the VWS group was 3.0 compared to 2.8 in the control group ($P=0.67$). Our studies suggest that children with VWS have an increased risk for wound complications following cleft repair compared to children with NSCLP. Furthermore the data supports a role for *IRF6* in wound healing.

Corresponding Author: Martine Dunnwald, Pharm.D., Ph.D., 206 MRC, Department of Pediatrics, The University of Iowa, 500 Newton Road, Iowa City, Iowa, 52242, (319) 384-4645 (Tel), (319) 335 6970 (Fax), martine-dunnwald@uiowa.edu.

⁷Current position: Department of Otolaryngology; University of Manitoba, Canada

⁸Current position: Department of Otolaryngology, University of Calgary, Canada

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Keywords

complications; Van der Woude Syndrome; wound healing; cleft; *IRF6*

INTRODUCTION

Van der Woude Syndrome (VWS; OMIM 119300) is an autosomal dominant condition associated with clefts of the lip and/or palate and lower lip pits (Van Der Woude, 1954). It is a syndrome associated with the presence of clefts, responsible for approximately 2% of all cases [1]. VWS is caused by mutations in *Interferon Regulatory Factor 6 (IRF6)*. *IRF6* mutations also cause popliteal pterygium syndrome (PPS; OMIM 119500), a syndrome that shares clinical features of VWS with additional cutaneous and oral webs [2]. Generation of mice deficient for *Irf6* confirmed the role for *Irf6* in craniofacial development, as *Irf6*-deficient mice demonstrated shorter facial skeletal structures, oral adhesion, and lack of proper palatal elevation [3,4]. In addition, these mice exhibited skeletal and cutaneous abnormalities, suggesting additional functions for *Irf6* in bone and epidermal homeostasis. The cutaneous phenotype was striking, as *Irf6*-deficient mice failed to form the two outer layers of the epidermis, and keratinocytes proliferated ectopically in the suprabasal layers. These results demonstrate that *Irf6* is necessary for epidermal proliferation and differentiation, two essential processes for the maintenance of cutaneous homeostasis.

The standard of practice for children born with cleft lip and palate is surgical repair. Among the reported post-operative complications are oropharyngeal infection, upper respiratory tract infection, airway obstruction, feeding difficulties, flap dehiscence and palatal fistula [5]. The latter two are of particular interest, as they relate to wound healing. Both flap dehiscence and palatal fistula require the reopening of the closed wound along surgical suture lines. The incidence of these post-operative complications varies: 0–63% reported for palatal fistula [6] and about 10% of cases at the University of Iowa (John Canady, personal communication). Their etiology is likely multifactorial, and among several factors, age of the patients, type of cleft, and experience of the surgeons have been proposed to influence the occurrence of post-operative wound complications [7]. It is unknown, however, if genetic variation can contribute to these post-operative complications, and in particular to wound healing.

Because of the newly identified role of *Irf6* in cutaneous homeostasis, we hypothesize that *IRF6* is critical for proper cutaneous wound healing, and that mutations in *IRF6* impair the wound healing process after cleft repair. Herein we report a retrospective chart study evaluating the post-surgical complications of 17 patients with VWS and 68 patients with isolated cleft lip/palate following their cleft repair.

METHODS

This retrospective case-control study was performed at the University of Iowa Hospitals and Clinics. After institutional review board approval was obtained, hospital records were searched from 1983 to 2007. Children with clefts of the lip and/or palate carrying a diagnosis of VWS that had previously consented to a genetic study of VWS were considered for our study. A total of 17 cases were identified. Using the cleft database developed by our cleft team, four controls were selected for each case, matched by cleft type, sex, and birth date. All controls were patients with non-syndromic cleft lip/palate (NSCLP). Due to a small number of available controls, 14 cases required the use of non-sex matched controls in order to achieve the desired number of four. The number of controls was selected based on our

power calculations for a type I error of 0.05 and the ability to detect a 75% decrease in wound complications among the non-VWS controls.

The following information was collected for each patient: date of birth, type of cleft, surgeon, age at labial repair, description of cleft at the time of surgery, outcome of labial repair, age at palatal repair, description of cleft at time of surgery, outcome of palatal repair, any additional surgeries (revisions, alveolar bone grafts), age at additional surgeries, and outcomes of additional surgeries. The total number of surgeries was also recorded. Data were collected on children from birth to 10 years of age, when the majority of surgeries related to cleft repairs have been completed. The same surgeon (JC) performed all surgeries on all patients, to ensure consistency in surgical and post-surgical care. Children were sent home on a course of oral antibiotics, arm restraints for 3 weeks in children under 2 years of age, regular bottle/breastfeeding for labial repairs, and a cup diet for palatal repairs. Patients having cleft lip repair are generally seen at two weeks post-surgery, and again at six weeks. Children with cleft palate are seen at three weeks post-surgery, then generally at four to six months.

Wound complications were determined from the descriptions available in the medical records. Since this was a retrospective chart review it was not possible to blind the reviewer to case/control status, but we used predefined categories for complications to enable standard data collection. All data was reviewed by at least two team members. Minor wound complications were defined as small wound dehiscences and pinpoint oronasal fistula, not requiring further surgical intervention. Major wound complications were defined as wound dehiscence and/or oronasal fistula requiring further surgical intervention or failure of bone grafts, requiring further surgery.

The primary outcome studied was any wound complication with major complication and type of complication as secondary measures. Fisher's exact test was used to determine statistical significance, with $P < 0.05$.

RESULTS

Wound Complications

A total of 17 patients were identified as having Van der Woude Syndrome. Characteristics of these children are summarized in Table 1. Sixty-eight patients with non-syndromic cleft lip and palate were used as controls and also reviewed. For the primary outcome of any wound complication 8 children with VWS (47%) had wound complications, while 13 control children (19%) had wound complications. This difference was statistically significant ($P = 0.02$). Of all VWS cases, 6 (75%) were considered major while the remaining 2 (25%) were minor. In the control group, 9 (69%) had major complications and 4 (31%) had minor. This difference was borderline significant ($P = 0.04$). These results are summarized in Table 2. The mean age of labial and palatal repair was not significantly different between VWS cases and controls. Of those who had wound complications in the VWS group, the majority of wound complications were oronasal fistulae (ONF) (5/8, 63%). Nine children in the control group (53%) developed ONF. Ten of the seventeen VWS cases had some degree of kin (see footnotes in Table 1), and of them, three pairs were discordant for complications, where one had complications and the other did not. Although the numbers are very small, these results are not consistent with an obvious genotype-phenotype relationship between *IRF6* and wound healing.

Number of Surgeries

The mean number of surgeries in the VWS group was 3.0 (SD 2.2). The control group had a mean of 2.8 surgeries (SD 1.6). The differences between the two groups were not statistically significant ($P=0.67$). These results are also summarized in Table 2.

DISCUSSION

The present study is the first to compare post-surgical wound complications between patients with Van der Woude (VWS) and patients with nonsyndromic cleft lip and palate (NSCLP). We found that children with VWS had an increased likelihood of post-surgical wound complications. Moreover, most complications were major, requiring additional surgery. However, the total number of surgeries was not significantly different between patients with VWS compared to patients with NSCLP.

Van der Woude Syndrome is a syndrome associated with cleft lip/palate, and mutations in *IRF6* cause VWS and popliteal pterygium syndrome (PPS) [2]. In order to elucidate the role of *IRF6* in orofacial development, Knight et al. [8] examined the expression of *Irf6* during facial development in murine and chick embryos. In the mouse, they noted strong expression of *Irf6* in the epithelial fusion zone between medial and lateral nasal processes as well as the maxillary process. Expression of *Irf6* was also highly expressed before, during and after fusion of primary and secondary palate as well as along the base of the nasal septum. As in the mouse, *Irf6* was highly expressed in chicks in the fusion zones. However, as development progressed, *Irf6* was down-regulated at the medial palatal edges as the shelves made contact but did not fuse, leaving the chick with a secondary cleft palate. These data demonstrate that *Irf6* is required for proper fusion during embryological development. Embryo morphogenesis requires building tissues and organs in orchestrated episodes that are recapitulated when the organism is wounded in order to replace missing tissue and repair the wound [9]. Therefore, it is possible that palatal fusion and wound healing share common processes and genetic pathways, and that *IRF6* is one gene likely to be involved in both clefting and wound healing.

Studies in mice deficient in *Irf6* revealed several skeletal and craniofacial anomalies including delayed ossification and cleft palate [3]. Lack of *Irf6* also affects keratinocyte differentiation and proliferation. Indeed, *Irf6*-deficient mice lack the granular and the cornified layers, leading to the absence of the cutaneous barrier. Proliferation and differentiation are essential for epidermal homeostasis and wound healing, suggesting that the *Irf6*-deficient animal may have impaired wound healing. These studies are under current investigation.

Other genes may also play a modifier role in human wound healing. For example, transforming growth factor-beta 3 is also necessary for palatogenesis in mice [10], and mutations in *TGFB3* are associated with clefting [11]. Recombinant TGFB3 is now in clinical trials for preventive scar formation [12]. Interestingly, *Tgfb3* is required for *Irf6* expression, as *Tgfb3*-deficient mice and *Tgfb3*-deficient mice lack *Irf6* expression [8,13]. *Bone morphogenetic protein 4 (BMP4)* is another gene associated both with clefting and wound healing. Evidence from a conditional inactivation of the *Bmp4* gene using the *Nestin cre* transgenic murine line demonstrate a spontaneous healing of clefting during embryogenesis [14] and mutations in *BMP4* have been identified in patients with microform clefts that appear as “healed” clefts at birth [15].

Our results suggest that patients with VWS have an increased likelihood of post-surgical complications, and in particular, major complications requiring additional surgery. *IRF6* mutations are clearly causal in VWS, but only about 70% of VWS cases have exonic

mutations in *IRF6* [16]. Many of these (deletions and nonsense mutations and some missense mutations) are gene inactivating suggesting that *IRF6* levels in affected individuals would be ~50% of baseline. In addition, children with isolated, NSCLP also can carry predisposing genetic variations in *IRF6*. Zuccheri et al [17] studied 8003 subjects from 10 different populations to determine the likelihood of cleft lip/palate derived from mutations in *IRF6*. They found that variations in *IRF6* conferred a 12% genetic contribution to CLP, as well resulting in a tripled risk of recurrent clefting in a family with one child already affected. Other similar studies [18–20] confirmed the genetic contributions of *IRF6* in NSCLP in a variety of populations. Finally, Rahimov et al [21] has shown that a particular single nucleotide polymorphism variant in an AP2 binding site is responsible for much of the observed effect in European derived populations. The effect of an allele on the “unaffected chromosome” might serve as an additional modifier of the VWS wound healing phenotype and might also affect wound healing in children with NSCLP. Studies of the role of common *IRF6* variants as well as variants in other genes suggested to play a role in wound healing as described above could contribute to a better understanding of risks for surgical complications and a potential role in anticipatory management based on genotype.

There are several limitations to the present report. It is retrospective, therefore there are inherent difficulties in obtaining complete and accurate information, particularly with regards to the description of the wound complications. We had to rely on the description of findings and subsequent treatment to make a determination of the severity of the complication. That said, the primary difference between the two groups was in absolute number of complications, making the subtype less important. There were also a modest number of patients in the VWS group limiting the power to detect differences. Finally, there were limitations with our numbers in obtaining gender-matched controls in 6 cases resulting in the control being of the opposite sex. There are no known gender predilections to *IRF6* mutations or expression, therefore this was not felt to be a major impediment to analysis.

In summary, this study provides clinical evidence to support a role for *IRF6* in wound healing. If confirmed, these findings could alter patient counseling with regards to expected outcomes from surgical repair of cleft lip/palate as we would anticipate more wound healing complications in those children with VWS. It further suggests a possible role for the *IRF6* genetic background for playing a role in isolated cleft wound healing as well, perhaps correlated to the known *IRF6* genetic predisposition to isolated clefting related to common variants associated with regulation of *IRF6* expression. Better characterization of such genetic risks could enable alterations in the timing or type of surgery as well as in postoperative management.

In conclusion, children with VWS have an increased likelihood of wound complications following surgical cleft repair than children with NSCLP in our institution. Despite having an increased risk of wound complications, children with VWS did not have more surgeries. These findings, along with the role of *Irf6* in epidermal differentiation, are consistent with a role for *IRF6* in wound healing.

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Table 1

Characteristics of Children with VWS

Patient	Cleft Type	Wound Complication	Type(s) of Complication(s)	IRF6 mutation
1	CP	No		P222L ^{a,b} , [16]
2	CP	No		F369S ^{a,c} , [2]
3	UCLP	No		Y372X [16]
4	CP	No		S212ThrfsX14 [16]
5	BCLP	Major	Wound dehiscence	P222L ^{a,b} , [16]
6	CP	Minor	ONF	G325E ^d , [2]
7	BCLP	Major	ONF	NF [16]
8	CP	Major	ONF	NT ^g
9	CL+A	Major	ONF/Failed ABG	N88S ^e , [16]
10	BCLP	Major	Wound dehiscence/ONF	Whole gene deletion [22]
11	UCLP	No		F369S ^c , [2]
12	CL+A	No		G325E ^d , [2]
13	UCLP	No		P203fs
14	BCL	No		R250Q [16]
15	UCLP	Major	Failed ABG	N88S ^{a,e} , [16]
16	BCL	No		IVS3-1G/t ^f , [16]
17	BCLP	Minor	Wound dehiscence	IVS3-1G/t ^{a,f} , [16]

CP=cleft palate only; CLP=unilateral cleft lip/palate; CL=cleft lip; CL+A=cleft lip & alveolus; U=unilateral; B=bilateral; ONF=oronasal fistula; ABG=alveolar bone graft; NF=not found; NT=not tested; fs=frame shift.

^a Mutation was found in affected family member, and patient was not screened.

^{b,e} First degree relative with known mutation

^c Seventh degree relative with known mutation

^f Second degree relative with known mutation

^g Mutations were not found in affected family member, and patient was not screened

Table 2

Comparison of Wound Complications and Surgeries in Children with VWS and NSCLP

	VWS	NSCLP	p^a
Total number of patients	17	68	
Wound Complications			
Major	6 (35 %) ^b	9 (13 %)	
Minor	2 (12 %)	4 (6 %)	
Total	8 (47 %)	13 (19 %)	P=0.02
Number of Surgeries	3 ± 2.2 ^c	2.8 ± 1.6	P=0.67

^a P<0.05 is considered significant after Fisher's exact test^b Raw number of cases (percentage of total number of patients)^c Data are means ± standard deviation