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Monozygotic Twins with Variable Expression of Van der Woude Syndrome

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To the Editor:

Van der Woude Syndrome [VWS] is a Mendelian disorder with an autosomal dominant inheritance pattern. It is a common cause of syndromic orofacial clefting, accounting for 2% of cleft lip and palate (CL/P) cases [Schutte et al., 1996]. Lower lip pits, usually bilateral, are the most common congenital malformation in VWS [Cervenka et al., 1967]. It is one of the few syndromes where it is common to find a mixture of cleft lip (CL) and cleft palate (CP) phenotypes in the same family. We present a case of confirmed monozygotic (MZ) female twins both affected with VWS due to an *IRF6* mutation, but with markedly different phenotypes. To our knowledge, a case such as this has not previously been reported, and contributes further evidence for modifying factors in the expression of this condition.

A G1P0 woman, pregnant with monochorionic, diamniotic female twins, presented to the Prenatal Genetics Clinic due to the finding of bilateral cleft lip and palate in Twin A on the 20-week antenatal ultrasound. Twin B appeared to be unaffected, and the ultrasound for both was otherwise normal. Family history was positive for bilateral lower lip pits in the father, as well as numerous members of the paternal family with cleft lip and palate, cleft palate, cleft lip and/or lip pits (Fig. 1). The twins' father was subsequently examined and found to have bilateral paramedian lower lip pits, but no orofacial clefting. Given this information, a probable diagnosis of VWS was made for Twin A. The family was counseled that Twin B might also have features of this condition, since the likelihood of monozygosity was high, given the monochorionicity.

The mother had pre-existing hypertension and took labetalol for the first two months of pregnancy, after which her blood pressure normalized and the medication was discontinued until after delivery. She did not smoke or drink alcohol during the pregnancy. The pregnancy was monitored closely, and the twins grew well until the 36th week, when an ultrasound showed oligohydramnios and poor interval growth. For these reasons, the twins were delivered by caesarean at 36 and 3/7 weeks. At delivery, Twin A weighed 1820g (3rd centile), and Twin B 1955g (5th centile). Both twins experienced transient tachypnea of the newborn, for which Twin A required oxygen. Both were otherwise well and were discharged home at 3 days of age.

Features of VWS were observed in both twins. Twin A was found to have bilateral cleft lip and cleft palate, as well as bilateral lower lip pits. Twin B showed no evidence of cleft lip or palate, but did show bilateral lower lip pits (Fig 2a,b). Neither twin was found to have other anomalies and both were otherwise healthy and developing normally. Based on their features and the family history, both twins were diagnosed with VWS.

Templates for sequencing of all exons of *IRF6* were generated by polymerase chain reaction (PCR) in an Applied Biosystem Gene Amp PCR System 9700 (Applied Biosystem, Foster City, CA). Sequencing was then performed at Functional Biosciences, Inc, Madison, WI following in-house protocols. A missense mutation in exon 4 of the *IRF6* gene (p.Y97C:c.290A>G) was found in both the twins and their father. Although this mutation has not been previously reported in VWS [de Lima et al., 2009] it is predicted to be damaging by Polyphen. The tyrosine at this position is completely conserved in mammals, frogs and chickens,

Zygosity was established in the molecular diagnostic laboratory of the Alberta Children's Hospital in Calgary, using a total of 15 microsatellite markers from both the AmpFISTR Profiler Plus kit (Applied Biosystems) and additional microsatellite markers developed in house, as per the laboratory protocol. The zygosity testing showed that alleles were shared at all 15 loci, giving a > 0.9999 probability of monozygosity.

There have been several reported cases of VWS in MZ twins, summarized in Table I. Ours is not the first reported case of discordant presentation in twins with VWS; however, it appears to be the first reported case of markedly discordant MZ twins where both are shown to have inherited the same missense mutation. The twins reported by Kondo et al. [2002] were fully discordant for the syndrome due to a new mutation in one of the twins. The cases of MZ twins with discordant presentations of VWS included Cervenka et al. [1967], where zygosity was determined by physical examination and blood group, and Tokat et al. [2005], where no details regarding how zygosity was determined are provided. In the Cervenka et al. case, both twins had lip pits and one had a cleft lip. The case reported by Tokat et al. was of twins who both had lip pits, one had a cleft lip and the other a cleft palate. Phenotype concordance in MZ twins has been reported by Hersh and Verdi [1992] and Vignale et al. [1998].

Intrafamilial variability is a recognized characteristic of VWS [Rizos and Spyropoulos, 2004]. Although concordant features are observed in some MZ twin pairs, the possibility of discordant phenotypes in MZ twins has been suggested by some reports. To our knowledge, we are reporting the first set of MZ twins, confirmed by zygosity testing, who both have VWS due to an *IRF6* gene mutation and who have strikingly different phenotypes of this condition. They provide evidence that MZ twins with VWS may have discordant phenotypes and that this is likely due to modifying factors.

Potential causes for discordant presentations of single gene disorders include genetic/epigenetic factors, those relating to the intrauterine environment, and stochastic effects. There has been little research into the causes of variable expressivity in VWS. Several studies have assessed potential genetic causes for intrafamilial variability. Moghe et al. [2010] looked at the relationship between an increase of heterochromatin on chromosome 9q and different phenotypes in VWS, and found there to be no connection. Sertie et al. [1999] suggested a locus predisposing to cleft palate in VWS at 17p11.2-17p11.1 in their linkage analysis study. Subsequently, another linkage study by Wong et al. [2001] excluded 17p11.2-17p11.1 as a modifying locus in their study of five Finnish families.

In summary, we present confirmed MZ twins with markedly variable expression of VWS. Both twins showed a missense mutation in *IRF6*. Lip pits were present in both twins;

however, CL/P was present in only one twin. This demonstrates the complexity of the expression of the VWS phenotype, and reinforces the need for further studies of modifier genes, epigenetics and environmental factors.

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Figure 1.
 A five generation pedigree showing multiple affected individuals in multiple generations.
 The * indicates individuals who were examined in our clinic.

Figure 2.

- a. Twin A at 14 days showing bilateral paramedian lower lip pits and bilateral cleft lip.
- b. Twin B at 11 months showing bilateral paramedian lower lip pits

TABLE I

Cases of Likely Monozygotic Twins with Van der Woude Syndrome in the Literature

	Zygosity/ Zygosity Testing	Presentation
Cervenka et al, 1967	Monozygotic/ Zygosity determined by examination of features and blood group	Lip pits in both, CL in one
Hersh and Verdi, 1992	Monozygotic/ DNA linkage analysis	Unilateral CL/P, lip pits and natal tooth in both
Kondo et al., 2002	Monozygotic/ Tested with microsatellite markers	One twin with the condition due to a new mutation, one twin without the mutation
Tokat et al., 2005	Monozygotic/ No information on testing	Lip pits in both, CL in one, CP in one
Vignale et al., 1998	Unknown	Unilateral CL and lip pits in both