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Prevalence and non-random distribution of exonic mutations in Interferon Regulatory Factor 6 (*IRF6*) in 307 families with Van der Woude syndrome and 37 families with popliteal pterygium syndrome

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Abstract

Purpose—Interferon Regulatory Factor 6 (*IRF6*) encodes a member of the IRF family of transcription factors. Mutations in *IRF6* cause Van der Woude (VWS) and popliteal pterygium syndromes (PPS), two related orofacial clefting disorders. Here, we compared and contrasted the frequency and distribution of exonic mutations in *IRF6* between two large geographically distinct collections of families with VWS and between one collection of families with PPS.

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Conflict of Interest

The authors have no conflict of interest with the information presented in any submitted manuscript

Methods—We performed direct sequence analysis of *IRF6* exons on samples from three collections, two with VWS and one with PPS.

Results—We identified mutations in *IRF6* exons in 68% of families in both VWS collections and in 97% of families with PPS. In sum, 106 novel disease-causing variants were found. The distribution of mutations in the *IRF6* exons in each collection was not random; exons 3, 4, 7, and 9 accounted for 80%. In the VWS collections, the mutations were evenly divided between protein truncation and missense, whereas most mutations identified in the PPS collection were missense. Further, the missense mutations associated with PPS were localized significantly to exon 4, at residues that are predicted to bind directly to DNA.

Conclusion—The non-random distribution of mutations in the *IRF6* exons suggests a two-tier approach for efficient mutation screens for *IRF6*. The type and distribution of mutations are consistent with the hypothesis that VWS is caused by haploinsufficiency of *IRF6*. On the other hand, the distribution of PPS-associated mutations suggests a different, though not mutually exclusive, effect on IRF6 function.

Keywords

Cleft lip and palate; mutation; haploinsufficiency; dominant negative; cryptic splice site; CpG

Introduction

The prevalence of orofacial clefting varies from 1 in 500 to 1 in 2500 births, depending on geographic origin, race and socioeconomic background [1-4]. About 70% of orofacial clefts occur as isolated cases and the remainder can be attributed to chromosomal abnormalities, maternal exposure to teratogens and syndromes where the phenotype includes other developmental or morphological abnormalities [5].

Van der Woude syndrome (VWS, OMIM 119300) is one of the most common oral cleft syndromes and accounts for ~2% of all cleft lip and palate cases. VWS is clinically characterized by congenital lower lip pits, cleft lip (CL), cleft lip with or without cleft palate (CLP), cleft palate only (CPO) and hypodontia. Other, less common, features include syndactyly of the fingers, syngnathia and ankyloblepheron [6]. VWS is inherited as an autosomal dominant trait with high penetrance (96.7%), but variable expression [7]. The phenotype of the lower lip varies from a single barely evident depression to bilateral fistulae of the lower lip, and the orofacial cleft varies from a bifid uvula to a complete cleft lip and palate [6]. These facial anomalies are also seen in individuals with popliteal pterygium syndrome (PPS, OMIM 119500), a disorder that includes other physical signs, including bilateral popliteal webs, syndactyly, genital anomalies, ankyloblepharon, oral synechiae and nail abnormalities.

The genetic localization for VWS was assigned by linkage analysis [8] and through chromosome abnormalities involving chromosome 1q32-q41 [9-11]. Overall, there is little evidence for genetic heterogeneity, although evidence for a second potential VWS locus was reported for chromosome 1p36-p32 [12]. Sertie *et al*. [13] suggested that a gene at chromosome 17p11.2-p11.1, together with the VWS gene, enhances the probability of CP in an individual carrying two risk alleles.

Previously, we described a nonsense mutation in the Interferon Regulatory Factor 6 (*IRF6*) gene in the affected sib of two monozygotic twins discordant for VWS, suggesting *IRF6* as a candidate for VWS [14]. This hypothesis was confirmed in the same study by the detection of *IRF6* mutations in 45 additional unrelated families with VWS. In addition, a unique set of mutations in *IRF6* was discovered in 13 families with PPS, demonstrating that VWS and PPS

are allelic, as previously suggested [15]. Subsequently, mutations in *IRF6* were identified in 56 additional families with VWS and three with PPS [16-36].

The objectives of this paper are to determine the prevalence and distribution of mutations in the exons of *IRF6* in families with VWS and PPS. We describe the complete sequence analysis of *IRF6* exons in two large VWS collections and one PPS collection. Despite geographical diversity between the two VWS collections, the likelihood of finding an exonic mutation in *IRF6* was similar as was their distribution. The type and distribution in location of PPS mutations differ significantly from the VWS mutations, but are not mutually exclusive. The results provide the foundation to identify genotype-phenotype correlations in disorders caused by mutations in *IRF6* and to determine structure-function relationships in the IRF family of transcription factors.

Materials and Methods

Populations

Each proband was examined by a clinical geneticist or genetic counselor. Two collections of unrelated families affected with VWS were obtained, one from Brazil (N=110) and one of mixed geographic origin (N=197). The collection from Brazil has not been described previously. The geographic origin of the mixed collection is primarily northern Europe, and includes families from the United States (152), Belgium (31), Germany (7), United Kingdom (3), Thailand (2), Phillipines (1) and Brazil (1). Many of these families (175) were described previously [14, 16, 21, 23] and were included in this study to provide a comprehensive analysis of the complete collections of families with VWS and PPS. Diagnostic criteria for individuals to be considered affected with VWS included CLP or CPO, and at least one affected individual in the family with an anomaly in the lower lip, generally bilateral pits.

In addition, a single collection of unrelated families affected with PPS (N=37) was obtained. The geographic origin of the PPS families was mainly northern Europe, but included one family from Brazil. Diagnostic criteria for individuals affected with PPS included the VWS criteria listed above along with the presence of bilateral popliteal webs or a combination of syndactyly, genital anomalies, ankyloblepharon, oral synechiae and nail abnormalities from one or more members in a family. Sample collection and processing was performed as described previously [37]. We obtained written informed consent from all subjects and approval for all protocols from the Institutional Review Boards at the University of Iowa, the University of Manchester, the University of São Paulo State and CONEP/Brazil, the Université catholique de Louvain, and Zentrum fur Gynäkologische Endokrinologie, Reproduktionsmedizin und Humangenetik, Regensburg, Germany.

PCR

Exons 1-8 and part of exon 9 of *IRF6* were amplified by standard PCR using the primers shown in Table 1. PCR experiments for exons 1-8 were performed in a 10μl total volume mixture containing 20 ng of genomic DNA, 0.5μM each primer, 200μM dNTPs, 0.25% DMSO, 0.2 unit Bio-X-Act Taq polymerase (Bioline, Reno, NV), and 1X PCR buffer supplied by the manufacturer. PCR conditions are as follows: initial denaturation 3 min at 94°C, followed by 35 cycles of denaturation at 94°C for 15 sec, annealing at 57°C for 30 sec, elongation at 68°C for 1min, and final elongation at 68°C for 3 min. Conditions for PCR experiments for exon 9 were performed as above except 0.3μM each primer, Biolase Taq polymerase (Bioline) and initial denaturation 5 min at 94°C, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 57°C for 45 sec, elongation at 72°C for 45 sec, and final elongation at 72°C for 3 min.

DNA sequence analysis

The amplified products were sequenced directly using Big Dye sequencing kit (Perkin-Elmer, Foster City, CA) as recommended. Sequence samples were purified with magnetic beads and run on an automated sequencer model ABI Prism 3700 (Perkin-Elmer). DNA sequences were aligned and analyzed using the software PHRED/PHRAP/CONSED [38]. Reference sequences for *IRF6* cDNA, genomic DNA and protein were NM_006147.2, RP3-434o14 (Genbank AL022398) and NP_006138, respectively. DNA sequence variants were confirmed by sequencing the opposite strand in the proband and, if possible, in at least one other affected family member. To identify non-etiologic polymorphisms, DNA sequence analysis was performed for all *IRF6* exons on a minimum of 200 unaffected control samples derived from geographically diverse populations [39].

Splice site prediction

The effect of mutations on splicing activity was modeled using Genscan [40]. Wild type and mutant sequences were compared using default settings.

Statistical analysis

Frequency tables showed population specific frequency distribution of mutations across the nine exons. The 2 by 9 tables were analyzed using the Chi-square statistic or Fisher's exact test when appropriate (e.g. when the expected cell count was less than 5 for at least 20% of the cells).

Results and Discussion

Prevalence of Exonic Mutations in IRF6

DNA samples were derived from two distinct VWS collections, one from Brazil (N = 110) and one of mixed origin that was primarily from northern Europe (N = 197). In addition, we screened a PPS collection of mixed geographical origin (N = 37). The mutation screen used in the current study was modified slightly from the screen described previously by Kondo et al[14]. PCR primers for exon 9 were redesigned (Table 1), and the new primers amplified this region more robustly and generated DNA sequence more reliably. In the VWS collections, we identified *IRF6* exonic mutations in 77 of 110 (71%) families from Brazil and identified 132 of 197 (67%) families from the mixed collection (Table 2). The likelihoods for finding exonic mutations in *IRF6* between these two diverse VWS collections are not statistically different (p=0.61) and are consistent with common mutation mechanisms.

Mutations located in the exons of *IRF6* have been identified for only 68% of families with VWS analyzed to date. Several possibilities exist to explain the remaining 32%. *IRF6* may have gross deletions that are not detected by our DNA sequencing strategy. Etiologic mutations may exist within *IRF6*, but located outside the exons. Finally, some proportion of the remaining families may be due to mutations located in some other gene. To date, deletions have been found in only six families with VWS [10, 11, 24 29]. In general, these have been large deletions and further studies with more sensitive methods are needed to screen for kilobase-sized deletions. Despite the lack of linkage evidence for locus heterogeneity in VWS, it is also possible that VWS-causing mutations may be found in other genes. For example, a polygenic mechanism might contribute to some cases of VWS, but would be difficult to detect in the previous linkage studies. The number and size of families that lack an exonic mutation in *IRF6* should be sufficient to test for genetic heterogeneity in the VWS collection.

In the PPS collection, we identified exonic mutations in *IRF6* in 36 of 37 unrelated families, demonstrating that *IRF6* is the principal gene involved in this disorder. When combined with the VWS mutation studies, *IRF6* exonic mutations were identified in 249 unrelated families,

representing 170 total and 106 novel alleles (Table 3: Supplemental data online only). None of these mutations were observed in our control samples (see Methods), suggesting that they are etiologic. However, we identified 41 DNA sequence variants from our mutation screen, including four non-synonymous polymorphisms, Asp19Asn, Ala61Pro, Thr224Ser, Val274Ile (Table 4). As these variants were detected in control cases, they are not etiologic for VWS nor PPS. However, Val274Ile is highly associated with isolated cleft lip and palate [41-46], and functional studies must be performed to test Val274Ile and other alleles as potential susceptibility alleles.

Non-random Distribution of IRF6 Exonic Mutations in VWS Collections

The distribution of all exonic mutations in *IRF6* in the VWS collections is not random ($p<0.0001$; Table 5, row A). More mutations were located in exons 3, 4, 7, and 9 than expected, suggesting a multi-tier approach for mutation screening of *IRF6* in VWS cases. This pattern was observed in both the Brazilian (Figure 1A) and mixed origin (Figure 1B) VWS collections, suggesting that the mutation mechanisms for *IRF6* are independent of origin of the population.

Protein truncation mutations (nonsense and frameshifts) were observed in all exons prior to the endogenous stop codon in exon 9. Interestingly, we identified point mutations in six families in exons 1 and 2 that create new start codons in the 5′ untranslated region. These new start sites should not make IRF6 protein as they are in the wrong reading frame, but may not prevent initiation at the native site. The protein truncation mutations are evenly distributed across the gene, except for exon 9 (Table 5, row B). The spike in protein truncation mutations in exon 9 appears to be due to one of five mutational hotspots in *IRF6* (see below). Overall, the high prevalence of protein truncation mutations in families with VWS (80 of 207), in addition to the six known *IRF6* deletions[10, 11, 14, 24, 29], provides further support that VWS can be caused by haploinsufficiency of *IRF6*.

Nearly all of the 117 mutations that do not truncate the protein (missense and in-frame insertions and deletions) are localized to regions encoding the DNA binding domain (64 families) and the protein binding domain (45 families). The significant over-representation of missense mutations in the DNA binding (exons 3 and 4) and protein binding (exons 7-9) domains (Table 5, row C) reinforces the importance of these domains for IRF6 function.

Non-random Distribution of IRF6 Exonic Mutations in the PPS Collection

The location of mutations identified in families with PPS is non-random (Table 5, row E). In 34 of 36 families with PPS, the mutation is located in exons 3, 4 or 9 (Figure 1C). Like VWS, these observations suggest a multi-tier approach for efficient mutation screens for PPS. However, the distribution of mutations among the exons for the PPS collection differs significantly from the VWS collections ($p < 0.0001$; Table 5, row A versus E). Another difference is the low frequency of protein truncation mutations in the PPS versus VWS collections (5/36 vs 80/207; $p = 0.036$), and the high frequency of missense mutations in exon 4 in the PPS versus VWS collections (26/36 vs 42/207; $p < 0.0001$). In addition, the distribution of missense mutations within the DNA binding domain (exons 3 and 4) is non-random for the PPS collection (Figure 2). Specifically, the missense mutations in the PPS collection are more likely to be located at residues that are predicted to contact DNA, when compared with random chance ($P \leq 7\times10^{-9}$) and when compared with missense mutations in the VWS collection ($P \leq 1\times10^{-6}$). Based on the significant differences in the frequency of the type of mutation and distribution in location of mutations found in the PPS versus the VWS collections, we conclude that the PPS-associated mutations affect IRF6 function differently than VWS-associated mutations.

How might VWS and PPS-associated mutations affect IRF6 function differently? The identification of six large deletions of *IRF6* [10, 11, 24, 29], along with the high frequency of protein truncation mutations, demonstrates that VWS can be caused by loss of function of IRF6. For families with PPS, we hypothesized previously that mutations have a dominant negative effect on IRF6 [14]. The rationale for this hypothesis is that the Arg84Cys and Arg84His mutations abrogate DNA binding [47], but are not predicted to affect protein binding. Consequently, protein dimers are predicted to form between a wild type isoform and the Arg84Cys and Arg84His isoform, but such a dimer will not be able to bind DNA. This model is supported by two main observations. First, in a previous study, mice heterozygous for a PPS-associated *Irf6* allele (Arg84Cys) had a more severe and more penetrant phenotype than mice that were heterozygous for a loss of function allele [47, 48]. Second, in the current study, we observed that mutations identified in families with PPS are much more likely to be missense mutations than in families with VWS, and that mutations are more likely to be located at residues that are predicted to directly contact the DNA. Such mutations are more likely to affect DNA binding without affecting protein stability or protein interaction. The most common examples of this class of mutations are Arg84Cys and Arg84His (Table 3: Supplemental data online only).

However, current data do not fully support a simple model whereby VWS is caused by IRF6 loss-of-function mutations and PPS is caused by IRF6 dominant negative mutations. Foremost, the same mutations were identified in patients with VWS and with PPS. For example, we identified missense mutations at Arg84 in seven families diagnosed with VWS and 21 with PPS (Table 3: Supplemental data online only). The mutations Arg84Cys and Arg84His were found in five families diagnosed with VWS. Moreover, individuals with VWS and PPS have been diagnosed in the same family [21]. These data suggest that while the association between the Arg84Cys and Arg84His mutations and PPS is strong, it is not absolute. In sum, the data is most consistent with the model that VWS is most likely caused by loss (or partial loss) of function mutations, but can also be caused by dominant negative mutations and that PPS is most likely caused by dominant negative mutations but can also be caused by loss (or partial loss) of function mutations. The range of phenotypes for VWS and PPS, including their overlap, suggests the likely contributions of stochastic events and genetic modifiers [13] for *IRF6*-related disorders.

Three other observations are relevant to the effect of VWS and PPS mutations on *IRF6* function. First, we identified a novel missense change at Arg84, Arg84Pro, in two families where affected individuals were diagnosed with VWS. In addition, Item et al., [22] identified an Arg84Gly mutation in a family where both affected individuals were diagnosed with VWS. The Arg84Pro and Arg84Gly mutations challenge the dominant negative hypothesis, since this residue is predicted to contact the DNA but these mutations are only found in individuals with VWS. However, the residue Arg84 is located in the middle of helix 3 in IRF6. The amino acids proline and glycine are known to disrupt alpha helices [49]. Consequently, the Arg84Pro and Arg84Gly mutations are predicted to disrupt the secondary and/or tertiary structure of IRF6, whereas Arg84Cys and Arg84His would not. Thus, we hypothesize that the Arg84Pro and Arg84Gly alleles cause complete loss of IRF6 function and result in VWS through haploinsufficiency of IRF6. Further biochemical and molecular studies are needed to test this hypothesis.

Secondly, the splicing mutations at the 5′ splice site of intron 3 and the protein truncation mutations in exon 9 also challenge the dominant negative hypothesis for mutations that cause PPS. To produce a dominant negative allele, a defective, but stable protein must be produced. We hypothesize that the splicing mutations at the 5′ splice site of intron 3 activate a cryptic splice site that produces a mutant IRF6 allele that is stable, but unable to bind DNA. To test this hypothesis, we used Genscan [40], a program that predicts splice sites, to model the effect of the four splicing mutations at intron 3. For the two mutations at the highly conserved position

+1 of intron 3, Genscan analysis predicts the loss of the endogenous splice site and the use of a cryptic splice site in the middle of exon 3 (Figure 3). Moreover, the cryptic splice site rejoins exon 4 in frame, but deletes 41 amino acids from the DNA binding region encoded in exon 3. Thus, these splicing mutations create a potentially stable protein with a mutation in the DNA binding domain and are consistent with the dominant negative model for PPS mutations. However, like the Arg84Cys and Arg84His mutations, these mutations do not always cause PPS, as one of these mutations was identified in a family with VWS. Also, for the other two splice mutations in intron 3 found in families with PPS, Genscan did not predict loss of the endogenous splice site (Figure 2).

Thirdly, protein truncation mutations in exon 9 were identified in families with either VWS or PPS. While the effect of these mutations on IRF6 function is not known, previous studies with the other members of the IRF family showed that the C terminus contains an auto-inhibitory domain [50]. Recently, we discovered that IRF6 binds to maspin, a tumor suppressor gene, and that the C terminus blocks this interaction [51]. Additional molecular and biochemical studies are needed to understand the effects of the PPS-causing mutations in exon 9.

Source of exonic mutations in *IRF6*

To date, we identified *IRF6* exonic mutations in 249 unrelated families and represent 170 different disease-causing alleles in *IRF6*. Thus, 68% of exonic mutations in *IRF6* are private and represent a wide array of potential mutational mechanisms. However, we identified five apparent hotspots. Mutations in the codons for Arg6, Arg84, Arg250, Arg400 and Arg412 were identified in 6, 26, 11, 7 and 14 unrelated families, respectively. The codon sequence for each of these residues contains a CpG dinucleotide. In humans, approximately one third of germline mutations result from loss of the CpG dinucleotide, and 90% of those are consistent with a mutation mechanism of cytosine methylation and deamination [52]. Similarly, in this report, 55 of 64 (86%) of the mutations in these CpG codons were consistent with the cytosine methylation/deamination mechanism.

This study shows that exonic mutations in *IRF6* are found in 68% of families with Van der Woude syndrome and nearly all families with popliteal pterygium syndrome. A few percent of families with VWS are caused by microdeletions of *IRF6*. Although the majority of the mutations are private, the distributions of exonic mutations suggest that future mutation searches should focus on exons 3, 4, 7 and 9 for families with VWS and on exons 3, 4 and 9 for families with PPS. In addition, since the distribution of mutations is consistent between geographically distinct populations, this multi-tier approach for mutation discovery should be widely applicable. Further, the distributions of mutations in the VWS and PPS collections suggest some limited guides for risk assessment and suggest a molecular rationale for clinical heterogeneity caused by genetic variation in *IRF6*.

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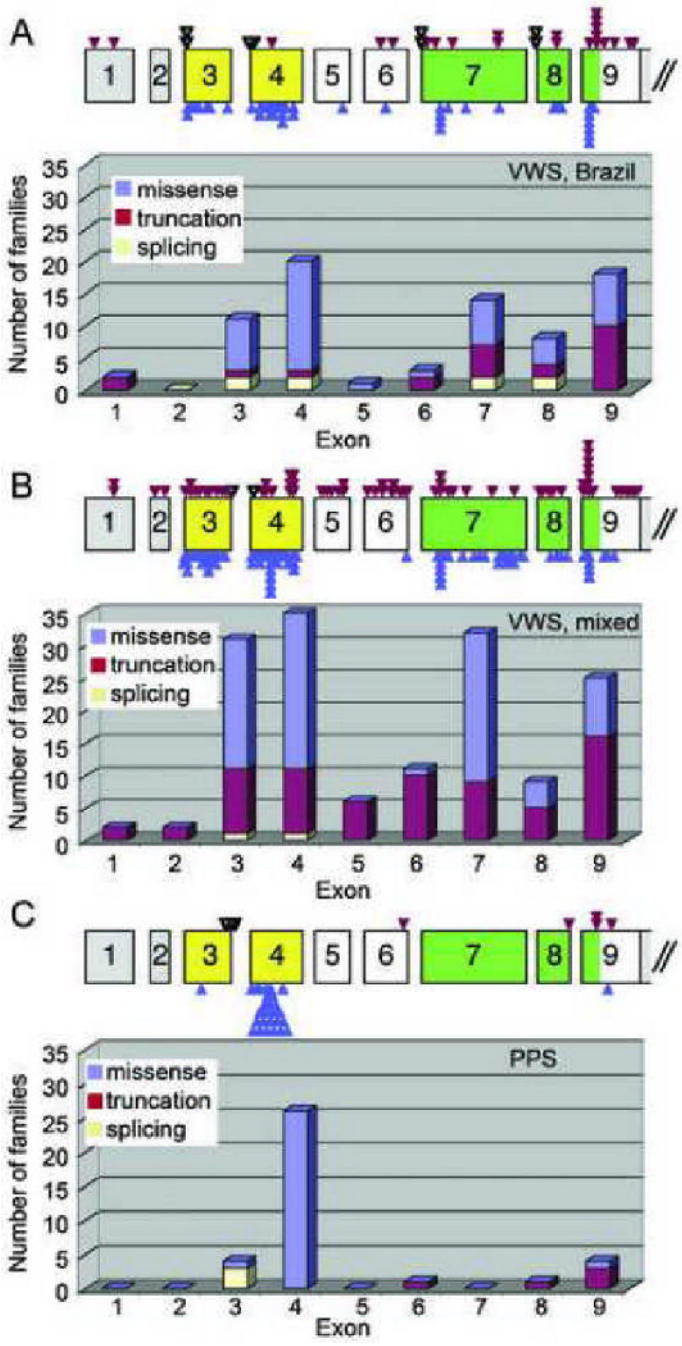

Figure 1.

Distribution of exonic mutations in *IRF6*. Each panel shows the genomic structure for *IRF6*. Exons (rectangles) are color coded as untranslated (gray), encode DNA binding domain (yellow), or encode the protein binding domain (green). The introns (space between exons) are not drawn to scale. The relative position of protein truncation mutations (red triangle), missense mutations (blue triangle) and splicing mutations (black triangle) is shown. Below each genomic structure is the distribution of missense (blue; includes in-frame deletions and insertions), protein trunctation (red; includes nonsense, frameshift and large deletions), and splicing (white) mutations in each exon for each population. A) Mutations found in VWS collection from Brazil.

B) Mutations found in VWS collection from mixed geographic origin. C) Mutations found in PPS collection.

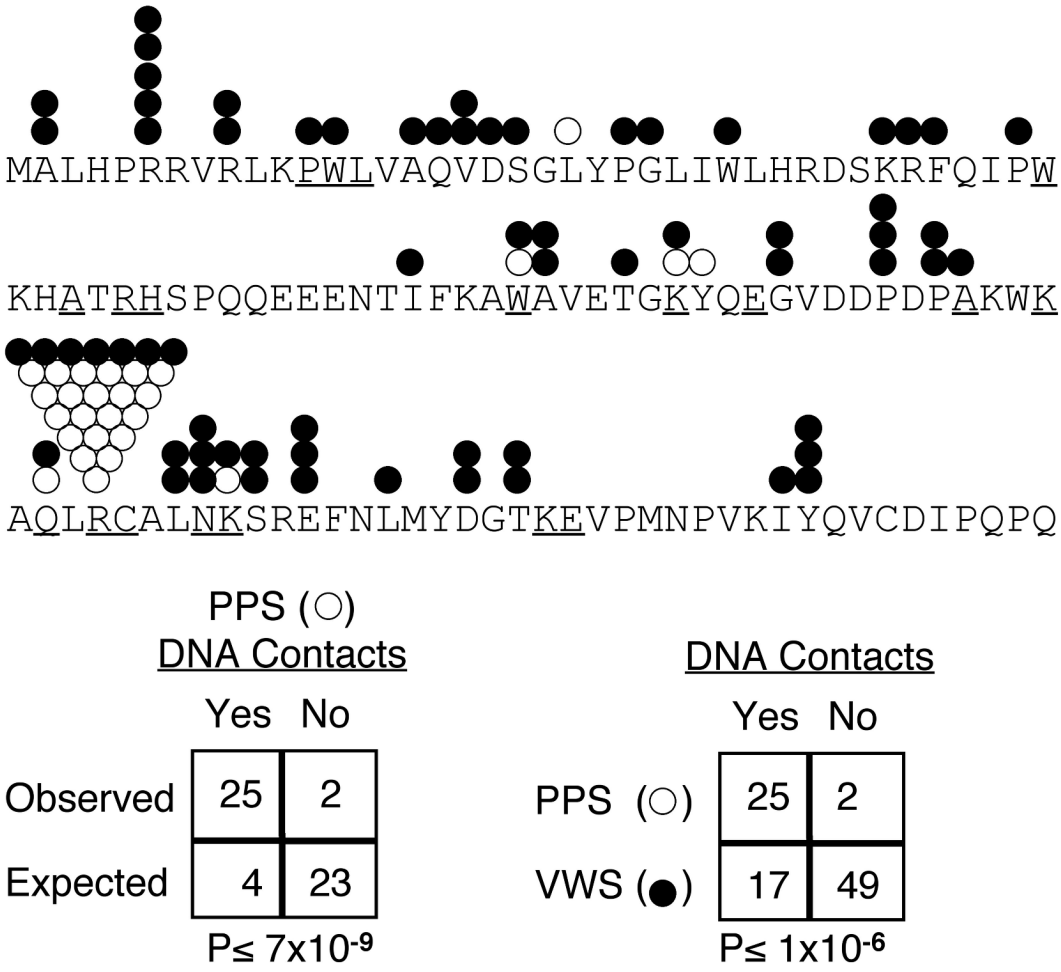

Figure 2.

Distribution of missense mutations in the DNA binding domain of IRF6. Mutations were identified in families with VWS (closed circles) and families with PPS (open circles). Amino acids predicted to directly contact DNA (underline) are based on crystal structure of IRF1 (see text). The expected number of mutations that contact DNA is based on the ratio of 17 amino acids that are predicted to contact the DNA (underlined, see text) out of 120 total amino acids in the DNA binding domain.

Allele name	DNA sequence EXON intron	Clinical Diagnosis	Genescan Prediction
Consensus splice site	MAG gtragt		wt
IRF6 i3	AAG gtaaag	Unaffected	wt
IRF6 i3 +1 g>t	AAG ttaaag	PPS	cryptic
IRF6 i3 +1 g>a	AAG ataaag	VWS	Cryptic
IRF6 x3 174 G>A	AAA gtaaag	PPS	wt
IRF6 i3 +3 a>c	AAG gtcaag	PPS	wt

DNA variant is underlined.

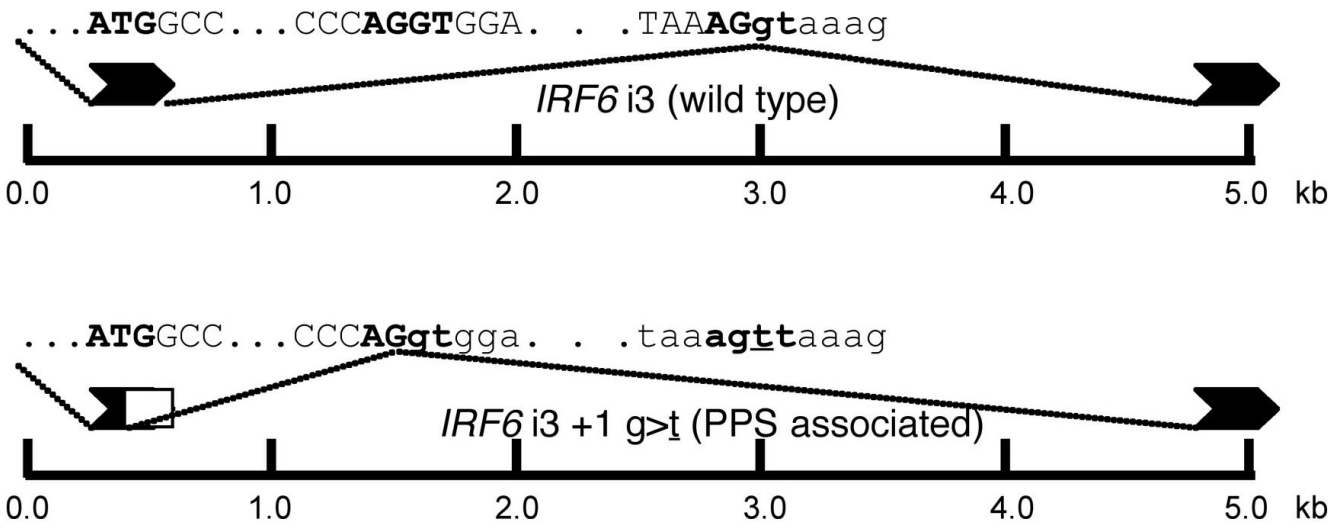

Figure 3.

Cryptic splice site in exon 3 revealed by computer modeling. The wild type (wt) and mutant sequences for the 5′ splice site for intron 3 are shown below the consensus sequence. In the consensus, M represents A or C and r represents G or A. The panel below contains the output from GENESCAN and shows the cryptic splice site in exon 3 revealed by the mutation at the endogenous site.

Table 1

PCR primers used to amplify *IRF6* exons

Exon	Domain[a]	Direction[b]	Primer sequence (5′-3′)	Product size (bp)
1	5′UTR	R	atctggaaaagggcgacagg	537
1		F	agaagcggaggagtagggtg	
2	5′UTR	R	aaagttatggaaacagcaac	382
2		F	ttattctagggcttctgagc	
3	DBD	R	catgcccccaaaagaggaat	560
3		F	ggctagagcatgaagtgtaa	
4	DBD	R	aggctttcttgctttatcca	512
4		F	gctctgggcaatgataggac	
5	Proline-rich	R	tgctttcagggcagtggtgg	425
5		F	cagtgaatctagggaggtcc	
6	Proline-rich	R	tttacttcttccctggtgac	432
6		F	cagtgtttggttcttgtcta	
7	SMIR	R	cttgacctcctccagactaa	650
7		F	agtggccttcctgaatgatg	
8	SMIR	R	gtttcagcaagactctaagg	436
8		F	aaagatggtatttgttgagt	
9	S/T-rich	R	gtcttcctcagggcctcttt	446
9		F	ggcatatttggatcacaaac	

Table 2

Likelihood for identifying *IRF6* exonic mutation in VWS and PPS populations.

	VWS			PPS			
Geographic origin	**Families**	**Families with mutation**	**fraction with mutation**	**Families**	**Families with mutation**	**fraction with mutation**	**Reference**
Mixed*	197	130	67 %	36	35	97 %	This study, [14, 21, 23,16]
Brazil	110	77	70 %	1	1	100 %	This study.
	307	207	68 %	37	36	97 %	

*In the “Mixed” collection, the previous studies account for 82 VWS and 14 PPS families with mutations.

Exonic mutations in *IRF6* in six families are not included in this table because the clinical diagnosis was not specified.

Six previously identified deletions of *IRF6* are not included in this table.

Table 3

IRF6 exonic mutations in unrelated families with VWS and PPS.

Diagnosis[a]	Mutation	nt[b]	nt change[c]	aa change[d]	exon[e]	Origin[f]	Citation
VWS	truncation	-219	C>T	0?	1	Brazil	This study
VWS	truncation	-151	G>A	0?	1	Brazil	This study
VWS	truncation	-151	G>A	0?	1	N Eur	This study
VWS	truncation	-151	G>A	0?	1	N Eur	This study
NS	truncation	-48	A>T	0?	2	NS	This study
VWS	truncation	-48	A>T	0?	2	N Eur	14
VWS	truncation	-19	C>A	0?	2	NS	This study
VWS	truncation	1	A>G	0?	3	Brazil	This study
VWS	truncation	3	G>A	0?	3	N Eur	14
VWS	missense	5	C>T	Ala2Val	3	N Eur	14
VWS	missense	16	C>T	Arg6Cys	3	Brazil	14
VWS	missense	16	C>T	Arg6Cys	3	Brazil	This study
VWS	truncation	16	del C	Arg6AlafsX3	3	NS	This study
VWS	truncation	16_17	ins C	Arg6ProfsX13	3	N Eur	This study
VWS	missense	17	G>T	Arg6Leu	3	N Eur	This study
VWS	missense	17	G>C	Arg6Pro	3	N Eur	This study
NS	missense	26	G>A	Arg9Gln	3	NS	This study
VWS	missense	26	G>A	Arg9Gln	3	Brazil	This study
VWS	missense	35	C>T	Pro12Leu	3	N Eur	This study
VWS	missense	39	G>T	Trp13Cys	3	Brazil	This study
VWS	truncation	43_44	ins ATAG	Val15AspfsX5	3	NS	This study
VWS	missense	47	C>T	Ala16Val	3	Belgium	21
VWS	truncation	49_64	del	Gln17SerfsX23	3	N Eur	14
VWS	missense	50	A>G	Gln17Arg	3	Brazil	This study
VWS	missense	52	G>A	Val18Met	3	N Eur	14
VWS	missense	53	T>C	Val18Ala	3	N Eur	14
VWS	missense	56	A>G	Asp19Gly	3	Germany	This study
VWS	missense	58	G>A	Ser20Asn	3	NS	This study
PPS	missense	65	T>C	Leu22Pro	3	Belgium	21
VWS	truncation	69	C>A	Tyr23X	3	N Eur	14
VWS	missense	71	C>T	Pro24Leu	3	N Eur	This study
VWS	missense	74	G>T	Gly25Val	3	Belgium	This study
VWS	missense	83	G>T	Trp28Leu	3	Brazil	This study
VWS	truncation	99	dup T	Lys34X	3	N Eur	This study
VWS	missense	101	A>C	Lys34Thr	3	NS	This study
VWS	missense	104	G>C	Arg35Pro	3	Brazil	This study
VWS	missense	107	T>C	Phe36Ser	3	N Eur	This study
VWS	missense	115	C>G	Pro39Ala	3	N Eur	14
VWS	truncation	136	del C	His46IlefsX15	3	N Eur	This study
VWS	truncation	154	G>T	Glu52X	3	N Eur	This study
VWS	Truncation	161	dup A	Asn54LysfsX3	3	Germany	This study
VWS	missense	167	T>C	Ile56Thr	3	Brazil	This study
PPS	splicing	174	G>A (80%>8%)	p.?	3	NS	This study
VWS	missense/splicing	175	G>T (58%>8%)	p.?	4	Brazil	This study
PPS	missense	178	T>G	Trp60Gly	4	N Eur	14
VWS	missense	180	G>T	Trp60Cys	4	Brazil	This study
VWS	missense	181	G>A	Ala61Thr	4	NS	This study
VWS	missense	182	C>G	Ala61Gly	4	N Eur	14
VWS	missense	191	T>C	Thr64Ile	4	Belgium	21
PPS	missense	197	A>C	Lys66Thr	4	N Eur	14
VWS	missense	197_199	delinsGGG	Lys66_Tyr67delinsArgAsp	4	N Eur	This study
PPS	missense	200	A>G	Tyr67Cys	4	Belgium	This study
VWS	Truncation	201	C>A	Tyr67X	4	Germany	16

Diagnosis[a]	Mutation	nt[b]	nt change[c]	aa change[d]	exon[e]	Origin[f]	Citation
VWS	truncation	202	C>T	Gln68X	4	N Eur	14
VWS	truncation	202	C>T	Gln68X	4	N Eur	14
VWS	missense	208	G>C	Gly70Arg	4	N Eur	14
VWS	missense	208	G>C	Gly70Arg	4	N Eur	14
VWS	missense	221	C>T	Pro74Leu	4	Brazil	This study
VWS	missense	221	C>T	Pro74Leu	4	Brazil	This study
VWS	missense	221	C>G	Pro74Arg	4	N Eur	This study
VWS	duplication	223_240	dup	Asp75_Lys80dup	4	N Eur	This study
VWS	missense	226	C>T	Pro76Ser	4	N Eur	14
VWS	missense	230	C>A	Ala77Asp	4	Brazil	This study
PPS	missense	244	C>A	Gln82Lys	4	N Eur	14
VWS	missense	246	G>C	Gln82His	4	Brazil	This study
PPS	missense	250	C>T	Arg84Cys	4	Belgium	This study
PPS	missense	250	C>T	Arg84Cys	4	Belgium	This study
PPS	missense	250	C>T	Arg84Cys	4	Brazil	This study
PPS	missense	250	C>T	Arg84Cys	4	NS	This study
PPS	missense	250	C>T	Arg84Cys	4	NS	This study
PPS	missense	250	C>T	Arg84Cys	4	Germany	This study
PPS	missense	250	C>T	Arg84Cys	4	N Eur	14
PPS	missense	250	C>T	Arg84Cys	4	N Eur	14
PPS	missense	250	C>T	Arg84Cys	4	N Eur	14
PPS	missense	250	C>T	Arg84Cys	4	N Eur	This study
PPS	missense	250	C>T	Arg84Cys	4	N Eur	This study
PPS	missense	250	C>T	Arg84Cys	4	N Eur	This study
PPS	missense	250	C>T	Arg84Cys	4	N Eur	This study
PPS	missense	250	C>T	Arg84Cys	4	N Eur	14
PPS	missense	250	C>T	Arg84Cys	4	N Eur	14
VWS	missense	250	C>T	Arg84Cys	4	Brazil	This study
VWS	missense	250	C>T	Arg84Cys	4	Brazil	This study
VWS	missense	250	C>T	Arg84Cys	4	NS	This study
PPS	missense	251	G>A	Arg84His	4	NS	This study
PPS	missense	251	G>A	Arg84His	4	NS	This study
PPS	missense	251	G>A	Arg84His	4	N Eur	14
PPS	missense	251	G>A	Arg84His	4	N Eur	This study
PPS	missense	251	G>A	Arg84His	4	N Eur	14
PPS	missense	251	G>A	Arg84His	4	Belgium	This study
VWS	missense	251	G>A	Arg84His	4	Belgium	This study
VWS	missense	251	G>A	Arg84His	4	N Eur	This study
VWS	missense	251	G>C	Arg84Pro	4	Belgium	This study
VWS	missense	251	G>C	Arg84Pro	4	UK	This study
VWS	missense	259	C>T	Leu87Phe	4	Brazil	This study
VWS	missense	259	C>T	Leu87Phe	4	Brazil	This study
NS	missense	262	A>T	Asn88Tyr	4	NS	This study
VWS	missense	262	A>C	Asn88His	4	Brazil	This study
VWS	missense	262	A>C	Asn88His	4	N Eur	14
VWS	missense	263	A>G	Asn88Ser	4	N Eur	This study
PPS	missense	265	A>G	Lys89Glu	4	N Eur	14
VWS	missense	265	A>G	Lys89Glu	4	Brazil	This study
VWS	missense	268	A>G	Ser90Gly	4	N Eur	14
VWS	missense	269	G>T	Ser90Ile	4	Belgium	This study
VWS	missense	274	G>A	Glu92Lys	4	Brazil	This study
VWS	missense	274	G>A	Glu92Lys	4	Brazil	This study
VWS	missense	274	G>A	Glu92Lys	4	Brazil	This study
VWS	truncation	274	G>T	Glu92X	4	Brazil	This study
VWS	truncation	274	G>T	Glu92X	4	N Eur	14
VWS	missense	284	T>C	Leu95Pro	4	N Eur	This study

Diagnosis[a]	Mutation	nt[b]	nt change[c]	aa change[d]	exon[e]	Origin[f]	Citation
VWS	missense	292	G>C	Asp98His	4	N Eur	14
VWS	missense	293	A>G	Asp98Gly	4	Brazil	This study
VWS	missense	298	A>G	Thr100Ala	4	Belgium	21
VWS	missense	299	C>T	Thr100Ile	4	N Eur	This study
VWS	missense	328	A>T	Ile110Leu	4	Belgium	This study
VWS	missense	332	A>G	Tyr111Cys	4	Brazil	This study
VWS	missense	332	A>G	Tyr111Cys	4	Brazil	This study
VWS	missense	332	A>G	Tyr111Cys	4	N Eur	This study
VWS	truncation	352	C>T	Gln118X	4	NS	This study
VWS	truncation	352	C>T	Gln118X	4	N Eur	14
VWS	missense	358	C>A	Gln120Lys	4	N Eur	This study
VWS	truncation	358	C>T	Gln120X	4	N Eur	This study
VWS	truncation	358_368	del	Gln120HisfsX	4	Thailand	23
VWS	truncation	405_406	ins TT	Asp135_Glu136LeufsX31	5	Germany	This study
VWS	truncation	427	G>T	Glu143X	5	N Eur	This study
VWS	truncation	466	dup C	His156ProfsX39	5	N Eur	14
VWS	missense	470	T>G	Val157Gly	5	Brazil	This study
VWS	truncation	494	del T	Phe165SerfsX	5	N Eur	This study
VWS	truncation	522_525	del	Ala174fsX47	6	Belgium	This study
VWS	truncation	558	C>A	Cys186X	6	N Eur	14
VWS	truncation	576	G>A	Try192X	6	N Eur	14
VWS	truncation	576	G>A	Try192X	6	Germany	This study
VWS	truncation	554_579	dup	Lys194AsnfsX23	6	Brazil	This study
VWS	missense	589	C>T	Pro197Ser	6	Brazil	This study
VWS	truncation	610	del C	Gln204ArgfsX18	6	N Eur	This study
VWS	truncation	610	C>T	Gln204X	6	Brazil	This study
VWS	truncation	610	C>T	Gln204X	6	N Eur	This study
VWS	truncation	610	C>T	Gln204X	6	N Eur	This study
VWS	truncation	634_635	ins CCAC	Ser212ThrfsX14	6	N Eur	14
PPS	truncation	651	G>A	Try217X	6	N Eur	This study
VWS	truncation	657_665	delins TA	Ser219fsX1	6	N Eur	14
VWS	missense	665	C>T	Pro222Leu	6	N Eur	This study
VWS	truncation	706	G>T	Glu236X	7	Brazil	This study
VWS	truncation	722_723	ins C	Met241IlefsX19	7	Brazil	This study
VWS	missense	743	G>C	Gly248Ala	7	N Eur	This study
VWS	truncation	744_748	del	Gly248fsX10	7	N Eur	14
VWS	missense	748	C>G	Arg250Gly	7	Brazil	This study
VWS	missense	748	C>G	Arg250Gly	7	Brazil	This study
VWS	missense	748	C>G	Arg250Gly	7	N Eur	This study
VWS	truncation	748	C>T	Arg250X	7	Belgium	This study
VWS	truncation	748	C>T	Arg250X	7	N Eur	This study
VWS	truncation	748	C>T	Arg250X	7	Belgium	This study
VWS	missense	749	G>A	Arg250Gln	7	Brazil	This study
VWS	missense	749	G>A	Arg250Gln	7	Brazil	This study
VWS	missense	749	G>A	Arg250Gln	7	N Eur	14
VWS	missense	749	G>A	Arg250Gln	7	N Eur	This study
VWS	missense	749	G>A	Arg250Gln	7	N Eur	This study
VWS	missense	755	T>C	Leu251Pro	7	Belgium	21
VWS	truncation	759	T>A	Tyr253X	7	N Eur	14
VWS	missense	775	C>T	Pro258Ser	7	Belgium	21
VWS	truncation	784	C>T	Gln262X	7	Brazil	This study
VWS	truncation	795	del C	Leu265fsX36	7	N Eur	14
VWS	missense	803	C>T	Pro268Leu	7	Brazil	This study
VWS	missense	818	A>G	Gln273Arg	7	N Eur	14
VWS	truncation	842	del A	His281LeufsX20	7	N Eur	14
VWS	deletion	870_897	del/ins A	Phe290_Asp296delinsLeu	7	N Eur	14

Diagnosis[a]	Mutation	nt[b]	nt change[c]	aa change[d]	exon[e]	Origin[f]	Citation
VWS	missense	881	T>C	Leu294Pro	7	N Eur	14
VWS	missense	889	G>A	Val297Ile	7	N Eur	14
VWS	missense	905	T>C	Leu302Pro	7	Brazil	This study
VWS	missense	953	A>C	Gln318Pro	7	N Eur	This study
VWS	missense	954	G>C	Gln318His	7	NS	This study
VWS	missense	955	T>C	Cys319Arg	7	N Eur	This study
VWS	truncation	957	C>A	Cys319X	7	Brazil	This study
VWS	truncation	957	C>A	Cys319X	7	Brazil	This study
VWS	missense	958	A>G	Lys320Glu	7	N Eur	14
VWS	missense	958	A>G	Lys320Glu	7	N Eur	14
VWS	missense	961	G>A	Val321Met	7	Belgium	This study
VWS	missense	961	G>A	Val321Met	7	N Eur	14
VWS	missense	965	A>G	Tyr322Cys	7	Brazil	This study
VWS	missense	974	G>A	Gly325Glu	7	Belgium	This study
VWS	missense	974	G>A	Gly325Glu	7	N Eur	14
VWS	Truncation	1011_1020	del	Ile337fsX22	7	Germany	This study
VWS	missense	1034	T>C	Leu345Pro	7	N Eur	14
VWS	missense	1040	G>T	Cys347Phe	7	N Eur	14
VWS	truncation	1087	del A	Ile363X	8	N Eur	This study
VWS	missense	1106	T>C	Phe369Ser	8	N Eur	This study
VWS	truncation	1107	del T	Phe369LeufsX27	8	N Eur	14
VWS	truncation	1116	C>G	Tyr372X	8	N Eur	This study
VWS	missense	1118	T>C	Leu373Ser	8	Brazil	This study
VWS	missense	1120	T>C	Cys374Arg	8	Brazil	This study
VWS	missense	1122	C>G	Cys374Try	8	N Eur	14
VWS	missense	1123	T>C	Phe375Leu	8	Brazil	This study
VWS	missense	1126	G>C	Gly376Arg	8	Brazil	This study
VWS	missense	1126	G>C	Gly376Arg	8	N Eur	This study
VWS	truncation	1136	G>A	Try379X	8	Brazil	This study
VWS	truncation	1136	G>A	Try379X	8	Brazil	This study
VWS	missense	1162	A>G	Lys388Glu	8	N Eur	14
PPS	truncation	1177	C>T	Gln393X	8	N Eur	14
VWS	missense	1190	T>C	Val397Ala	9	NS	This study
VWS	missense	1198	C>T	Arg400Try	9	Belgium	This study
VWS	missense	1198	C>T	Arg400Try	9	Brazil	This study
VWS	missense	1198	C>T	Arg400Try	9	Brazil	This study
VWS	missense	1198	C>T	Arg400Try	9	Brazil	This study
VWS	missense	1198	C>T	Arg400Try	9	Brazil	This study
VWS	missense	1198	C>T	Arg400Try	9	Brazil	This study
VWS	missense	1199	G>A	Arg400Gln	9	Brazil	This study
VWS	missense	1210	G>A	Glu404Lys	9	Brazil	This study
VWS	missense	1212_1226	del	Glu404_Gly408delAsp	9	Brazil	This study
VWS	truncation	1219	del T	Ser407LeufsX28	9	Brazil	This study
NS	truncation	1234	C>T	Arg412X	9	N Eur	This study
NS	truncation	1234	C>T	Arg412X	9	N Eur	This study
PPS	truncation	1234	C>T	Arg412X	9	N Eur	This study
PPS	truncation	1234	C>T	Arg412X	9	N Eur	This study
VWS	truncation	1234	C>T	Arg412X	9	Brazil	This study
VWS	truncation	1234	C>T	Arg412X	9	Brazil	This study
VWS	truncation	1234	C>T	Arg412X	9	Brazil	This study
VWS	truncation	1234	C>T	Arg412X	9	Brazil	This study
VWS	truncation	1234	C>T	Arg412X	9	Brazil	This study
VWS	truncation	1234	C>T	Arg412X	9	NS	This study
VWS	truncation	1234	C>T	Arg412X	9	NS	This study
VWS	truncation	1234	C>T	Arg412X	9	N Eur	This study
VWS	truncation	1234	C>T	Arg412X	9	N Eur	14

Diagnosis[a]	Mutation	nt[b]	nt change[c]	aa change[d]	exon[e]	Origin[f]	Citation
VWS	truncation	1234	C>T	Arg412X	9	N Eur	This study
VWS	missense	1262	T>G	Leu421Arg	9	N Eur	This study
VWS	truncation	1264	C>T	Gln422X	9	Brazil	This study
VWS	missense	1268	T>C	Ile423Thr	9	NS	This study
PPS	missense	1288	G>A	Asp430Asn	9	N Eur	14
PPS	truncation	1291_1311	del/ins GTAGAGGCTAAACTCCCTGGAA	Asn431ValfsX70	9	N Eur	This study
VWS	missense	1296_97	delins TT	Val433Phe	9	Germany	This study
VWS	truncation	1303_1307	del	Gln435GlufsX64	9	Brazil	This study
VWS	truncation	1327	del C	Leu443PhefsX46	9	N Eur	This study
VWS	truncation	1357_1368	del	QPTP Gln453fsX43	9	N Eur	This study
VWS	truncation	1368	dup C	Ser457GlnfsX43	9	N Eur	This study
VWS	truncation	1369_1370	ins TGCAGCCCAT	Ser457MetfsX27	9	N Eur	This study
VWS	truncation	1372	dup A	Met458AsnfsX43	9	Brazil	This study
VWS	truncation	1385	del C	Pro462LeufsX27	9	Brazil	This study
VWS	truncation	1385	dup C	Pro462fsX39	9	N Eur	14
VWS	truncation	1386_1387	ins C	Pro462_Ala463ArgfsX38	9	N Eur	This study
NS	splicing	-2	A>G (100%>0%)	p.?	IVS2	NS	This study
VWS	splicing	-2	A>G (100%>0%)	p.?	IVS2	Brazil	This study
VWS	splicing	-2	A>G (100%>0%)	p.?	IVS2	Brazil	This study
VWS	splicing	-1	G>A (100%>0%)	p.?	IVS3	Brazil	This study
VWS	splicing	-1	G>T (100%>0%)	p.?	IVS3	N Eur	This study
PPS	splicing	1	G>T (100%>0%)	Val18_Lys58del	IVS3	Belgium	This study
VWS	splicing	1	G>A (100%>0%)	Val18_Lys58del	IVS3	Belgium	This study
PPS	splicing	3	A>C (71%>2%)	Val18_Lys58del	IVS3	N Eur	This study
VWS	splicing	-5	T>G (22%>5%)	p.?	IVS6	Brazil	This study
VWS	splicing	-1	G>A (100%>0%)	p.?	IVS6	Brazil	This study
VWS	splicing	-3	C>G (55%>1%)	p.?	IVS7	Brazil	This study
VWS	splicing	-2	A>G (100%>0%)	p.?	IVS7	Brazil	This study

[a] In six cases, the phenotype was not specified (NS). Table does not include six cases with microdeletions of *IRF6* (see text).

[b] Nucleotide (nt) counted from start codon if mutation is located in an exon or counted from nearest exon if located in an intron. Reference sequences for cDNA and genomic DNA were NM_006147.2 and RP3-434o14 (Genbank AL022398), respectively.

[c] All protein and exon splicing changes are deduced and not experimentally verified. Reference protein sequence is NP_006138. Frameshift mutations (fs) are predicted to add the indicated number of amino acids beyond the mutation (fsXnumber). Residues located in the DNA binding domain (yellow) or protein binding domain (green) are indicated. For splicing mutations, the values represent the change in probability of finding the indicated nucleotide at a splice junction (Zhang et al., 1998). Predicted protein for splicing mutation is based on computational analysis shown in Figure 3.

[d] Exon and intron (IVS) numbering are based on ENSG00000117595 (www.ensembl.org).

[e] Geographic origins of pedigrees include the indicated countries, or Northern Europe (N Eur) if proband is white, but country is not known or origins not specified (NS).

Table 4

DNA variants in *IRF6* that do not cause VWS or PPS.

Location[a]	SNP	amino acid	MAF[b] (%)	Sequence
promoter	-156 G>A		35	AGGGTGGGAC**R**CTGGACGGAC
promoter	-134 G>C		36	CCGCTGGGCC**S**GGCAGCCCAG
promoter	-50 T>A		18	CTGGGAGGCG**W**GGCCGGGCGG
promoter	-39 A>T		18	GGCCGGGCGG**W**TGCGAAGGCT
1i	-4 A>G		2	TTTTCTCCAT**R**CAGAATCTTT
2e	-73 T>C		46	CCATACAGAA**Y**CTTTGAGCGG
2i	+102 T>C		1	CCTTTAGTTG**Y**CTTGTTTAAA
2i	-73 G>C		2	AGATGGGAAA**S**GTGGCTGGGA
3e	9 C>T	L3L	3	TCATGGCCCT**Y**CACCCCCGCA
3e	55 G>A	D19N	1	GGCCCAGGTG**R**ATAGTGGCCT
3i	+36 TT>T		24	CCTTTCTGGA**T**TTTTTTTTTT
3i	-138 G>C		12	TGATGGGGCA**S**TCATGCAAAA
3i	-84 GTGT>GT		12	GTGTGTGTGT**GT**TTGTGTCTA
3i	-5 C>G		49	GTTTCTTGTT**S**TCAGGCCTGG
4e	181 C>G	A61P	5	TCAGGCCTGG**S**CTGTAGAGAC
4e	339 G>T	V113V	2	TATATCAAGT**K**TGTGACATCC
4i	-174 A>G		1	AGGTCCTTCC**R**TGAGAGAAGT
4i	-155 C>T		9	GTGTTCATTC**Y**CTTGATTCTC
4i	-106 C>T		1	TGTACTGAAC**Y**TGAGGAGCCT
4i	-102 G>A		1	CTGAACCTGA**R**GAGCCTCTGG
5e	459 G>T	S153S	25	TGGATCAGTC**K**CAGCACCATG
5i	+55 A>C		32	AGGAGTTTTG**M**CCTTGGGACT
6i	+27 C>G		40	CTTTCTTGCT**S**GGTCTTCTGC
7e	671 C>G	T224S	2	CTTGCAGTGA**S**TGACCTGGAC
7e	711 C>T	Y237Y	1	GGAAGGAGTA**Y**GGGCAGACCA
7e	726 C>T	T242T	2	AGACCATGAC**Y**GTGAGCAACC
7e	820 G>A	V274I	13	CCTGGAGCAG**R**TCAAATTCCC
7i	+37 C>T		21	GTGGGAATCA**Y**TCTCTGGAAG
7i	-75 A>T		47	TGTAATGGAC**W**GCATAAAAGA
8e	1153 T>C	L385L	5	TGGGAAACCA**Y**TGGAAAGGAA
8i	+34 T>C		2	CAACTCTTCA**Y**CTTTTTGCCA
8i	+42 A>C		1	CATCTTTTTG**M**CAATGCTTAA
8i	+93 G>T		3	GCATCCATCA**K**CCCATGTAGG
9e	1608 C>T		2	TTCAAATCTC**Y**TAATGGTAGT
9e	1692 A>T		4	CTTTGCTTCC**A**ATGTGACCTT
9e	1703 G>A		2	ATGTGACCTT**R**AACAAGTCCT
9e	1751 A>T		5	TTATAAAGTG**W**AGAGATTGGA
9e	1757 T>C		1	AGTGAAGAGA**Y**TGGAGTAGTG
9e	1855 A>G		11	ATCCTTCTGC**R**TTGTTCTTGT
9e	1922 C>T		1	TGTCCAGGAT**Y**GAGCTCTGTT
9e	1962 C>T		4	AGTAAGCTGG**Y**TCCCTGATGG

[a] Mutations are located upstream of exon 1 (promoter) or in the indicated intron (i) and exon (e).

[b] Minor Allele Frequency (MAF) is based on CEPH diversity panel.

Table 5

Distribution of mutations in *IRF6* exons in VWS and PPS collections.

		********** EXONS *********											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Row Total[a]**	**Row Average[b]**	**p-value[c]**
VWS Total	A	4	2	36	54	5	13	44	15	34	207	23	<0.0001
VWS Trunc	B	4	2	11	9	4	11	13	5	21	80	9	0.05
VWS Miss	C	0	0	22	42	1	2	29	8	13	117	13	<0.0001
VWS Splice	D	0	0	3	3	0	0	2	2	0	10	1	0.45
PPS-Total	E	0	0	4	26	0	1	0	1	4	36	4	<0.0001
PPS-Trunc	F	0	0	0	0	0	1	0	1	3	5	≤1	0.73
PPS-Miss	G	0	0	1	26	0	0	0	0	1	28	4	<0.0001
PPS-Splice	H	0	0	3	0	0	0	0	0	0	3	≤1	0.79

[a] Total number of mutations in each row.

[b] Number of mutations expected in each exon if distributed randomly.

[c] p-value comparing observed to expected distribution of mutations in each exon.