Mutations in *SCARF2* Are Responsible for Van Den Ende-Gupta Syndrome

Natascia Anastasio,[1] Tawfeg Ben-Omran,[2,3] Ahmad Teebi,[3] Kevin C.H. Ha,[1,4] Emilie Lalonde,[1,4] Rehab Ali,[2] Mariam Almureikhi,[2] Vazken M. Der Kaloustian,[1,5] Junhui Liu,[1] David S. Rosenblatt,[1,5] Jacek Majewski,[1,4,*] and Loydie A. Jerome-Majewska[1,5,*]

Van Den Ende-Gupta syndrome (VDEGS) is an extremely rare autosomal-recessive disorder characterized by distinctive craniofacial features, which include blepharophimosis, malar and/or maxillary hypoplasia, a narrow and beaked nose, and an everted lower lip. Other features are arachnodactyly, camptodactyly, peculiar skeletal abnormalities, and normal development and intelligence. We present molecular data on four VDEGS patients from three consanguineous Qatari families belonging to the same highly inbred Bedouin tribe. The patients were genotyped with SNP microarrays, and a 2.4 Mb homozygous region was found on chromosome 22q11 in an area overlapping the DiGeorge critical region. This region contained 44 genes, including *SCARF2*, a gene that is expressed during development in a number of mouse tissues relevant to the symptoms described above. Sanger sequencing identified a missense change, c.773G>A (p.C258Y), in exon 4 in the two closely related patients and a 2 bp deletion in exon 8, c.1328_1329delTG (p.V443DfsX83), in two unrelated individuals. In parallel with the candidate gene approach, complete exome sequencing was used to confirm that *SCARF2* was the gene responsible for VDEGS. SCARF2 contains putative epidermal growth factor-like domains in its extracellular domain, along with a number of positively charged residues in its intracellular domain, indicating that it may be involved in intracellular signaling. However, the function of SCARF2 has not been characterized, and this study reports that phenotypic effects can be associated with defects in the scavenger receptor F family of genes.

Van Den Ende-Gupta syndrome (VDEGS; MIM 600920) is an extremely rare autosomal-recessive disorder characterized by distinctive craniofacial and skeletal manifestations. It was first delineated by van den Ende et al. and Gupta et al.[1,2] To date, only 14 patients from nine families have been described.[1–7] Inheritance is likely autosomal recessive, which is supported by consanguinity of affected matings, the recurrence of the disorder among the offspring of unaffected couples, and equal sex involvement.[2,7] It is characterized by craniofacial abnormalities that include blepharophimosis, a flat and wide nasal bridge, malar and/or maxillary hypoplasia, prominent ears, a narrow and beaked nose, an everted lower lip, palatal abnormalities, and down-slanting eyes. Skeletal abnormalities include camptodactyly, arachnodactyly, long thumbs, and hallux valgus. Patients can have flexion contractures[5] and skeletal findings, such as slender ribs, hooked clavicles, and bowed long bones.[1–4,6,8] Respiratory problems due to laryngeal abnormalities have also been described in some patients.[4] Two patients have also been reported to have enlarged cerebella.[7]

The case for clinical heterogeneity in VDEGS has been made.[5] One mother had affected children with two different nonrelated partners. The authors postulated that in this pedigree, VDEGS was inherited in a dominant manner, possibly as a result of gonadal mosaicism.

In this paper, we are reporting on four patients. The patients reported in the present study had clinical and skeletal features consistent with VDEGS, as well as some previously unrecognized clinical findings. Patient 1 presented with scaphocephaly, blepharophimosis, a beaked nose, and maxillary hypoplasia. Her mouth was small, with an everted lower lip. She also had a high-arched and narrow palate. Both ears were posteriorly rotated, with folded helix and a small lobule. Upper limbs showed bilateral contracture of elbow joints and camptodactyly of the second to fifth fingers in both hands with long tapering fingers. There were long first toes and partial 2-3 skin syndactyly. Skeletal survey showed abnormal development of the proximal radius, with evidence of radioulnar subluxation, and no clear demonstration of osseous fusion. Her cousin, patient 2, had scaphocephaly, blepharophimosis, a beaked nose, maxillary hypoplasia, and a small mouth with an everted lower lip and limited opening. He also had a high-arched and narrow palate, abnormal teeth, and micrognathia. Both ears were posteriorly rotated, with folded helix and small lobule. Upper limbs showed bilateral contracture of elbow joints and camptodactyly of the second to fifth fingers in both hands with long tapering fingers. There were long first toes and partial 2-3 skin syndactyly. A distinguishing finding from what was seen in patient 1 was a sacral dimple. Patient 3 had similar features but also showed hypoplastic scapulae, clavicles, and ribs. Patient 4 showed similar features, but renal ultrasound showed bilateral renal pelvis dilatation.[9] All patients were the products of consanguineous marriages.

[1]Department of Human Genetics, McGill University, Montreal, Quebec H3A 1B1, Canada; [2]Section of Clinical and Metabolic Genetics, Department of Pediatrics, Hamad Medical Corporation, Doha, P.O. Box 3050, Qatar; [3]Department of Pediatrics and Genetic Medicine, Weil-Cornell Medical College, New York and Doha, P.O. Box 24144, Qatar; [4]McGill University and Genome Quebec Innovation Centre, Montreal, Quebec H3A 1A4, Canada; [5]Department of Pediatrics, McGill University, Montreal, Quebec H3H 1P3, Canada
*Correspondence: jacek.majewski@mcgill.ca (J.M.), loydie.majewska@mcgill.ca (L.A.J.-M.)
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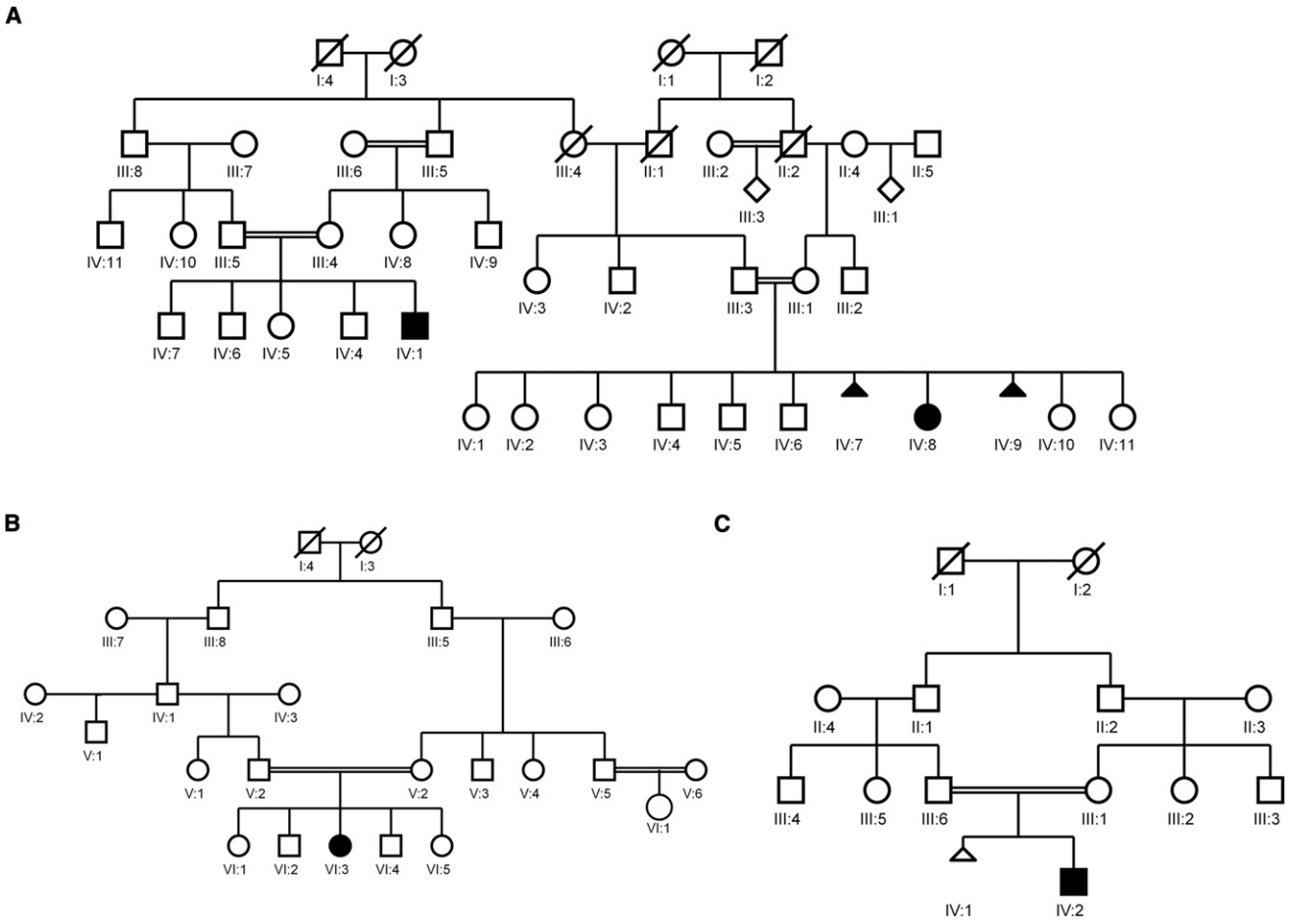

Figure 1. Pedigrees of Patients Affected with VDEGS
(A–C) The consanguinity and relationship for both patients 1 and 2 are shown in (A). The two patients are related. Information for patient 3 is shown in (B) and for patient 4 in (C). Black denotes patients affected with VDEGS.

The camptodactyly and facial features in these patients initially suggested the diagnosis of Teebi Shaltout syndrome (TSS; MIM 272950). TSS is another extremely rare autosomal-recessive syndrome, with only five cases (including three spontaneously aborted fetuses) from two families reported to date.[10,11] TSS has historically presented as a syndrome of camptodactyly, kidney anomalies, a caudal appendage, and distinctive craniofacial anomalies. The craniofacial anomalies in TSS include scaphocephaly with prominent occiput and bitemporal depression, abnormal hair, hypertelorism, ptosis and blepharophimosis or microphthalmia, a bulbous nose with hypoplastic alae nasi, a small mouth, and abnormal teeth.[10,11] Therefore, TSS seems to share some phenotypic features with our patients that have not been, as yet, linked with VDEGS.

In order to identify the genetic defect responsible for VDEGS, we utilized a homozygosity mapping or candidate gene approach, as well as a second, whole-exome capture approach. Analyses were carried out on genomic DNA from four individuals affected with the disease and a number of unaffected individuals (Figures 1A–1C). All individuals were members of the same Bedouin tribe, and two affected individuals (patients 1 and 2) were directly related (Figure 1A). This study was performed under a protocol approved by the Hamad Medical Corporation for a search for genes in consanguineous families, and consent was obtained from all participants.

In order to identify a homozygous region, Illumina Hap 550K Duo Infinium chips were used to genotype patients 1, 2, and 3, as well as one control individual, an unaffected sibling of patient 1. The program PLINK[12] with default settings was used to identify regions of homozygosity that were shared by the affected individuals but not the control. A single such segment of 2.4 Mb was found on chromosome 22q11, extending between positions 16910984 and 19296457 (hg18 reference coordinates; Figure 2). Patient 1 (0396) was homozygous across a 6.0 Mb interval, containing 1191 genotyped SNPs, delimited by rs466755 and rs8141797. Patient 2 (0946) was homozygous across a 4.0 Mb interval, containing 783 genotyped SNPs, delimited by rs2027653 and rs1108101. Patient 3 (0116) was homozygous across a 6.2 Mb interval, containing 1300 genotyped SNPs, delimited by rs9606661 and rs6003815. Patient 4 was also subsequently genotyped and was found to be homozygous for a 16.0 Mb region, delimited by rs2236639 and rs3827342. Interestingly, the haplotypes

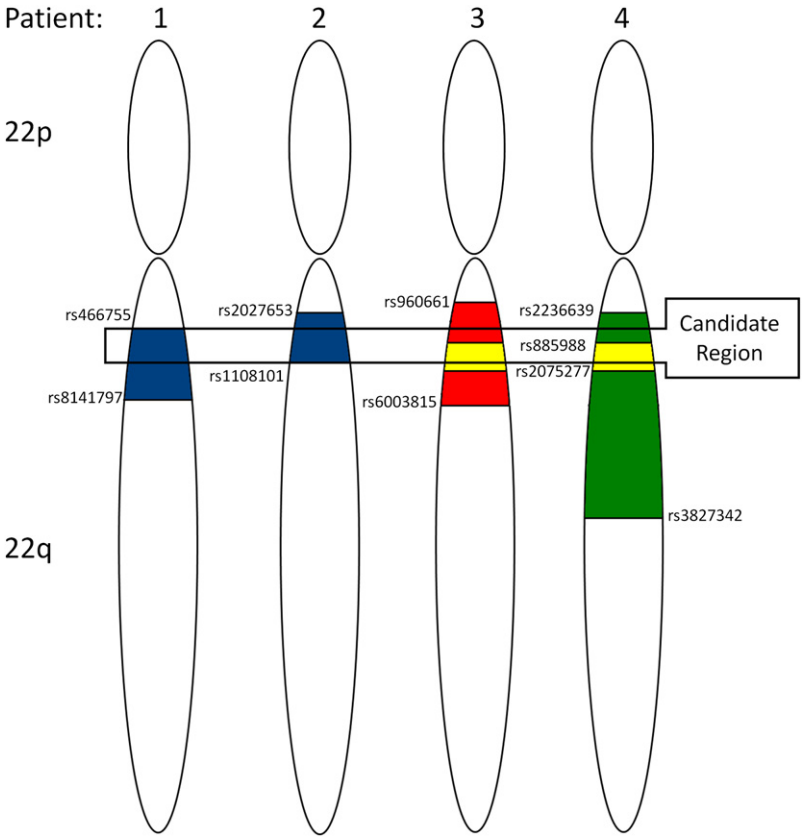

Figure 2. Homozygosity Mapping Results
Location and region delimiting SNPs of the 2.4 Mb homozygous region on chromosome 22q11 shared by the four VDEGS patients (boxed area). Blue denotes individual homozygous regions and haplotype shared by patients 1 and 2, red denotes homozygous region of patient 3, green denotes the homozygous region of patient 4, and yellow denotes the shared haplotype within the homozygous regions of patients 3 and 4. Patients 3 and 4 reflect complex levels of relatedness within the tribal population. Each patient carries a haplotypically distinct homozygous segment resulting from the most recent consanguinity. However, within these large segments, the two patients share a smaller common haplotype, most likely inherited from a more distant common ancestor of the parents.

in the region shared by the affected patients were not identical: patients 1 and 2 shared a common haplotype, whereas patients 3 and 4 were homozygous for a 1.6 Mb distinct haplotype block within their individual homozygous regions (delimited by rs885988 and rs2075277; Figure 2). This finding suggested the presence of either more than one founder mutation or a smaller shared haplotype—undetectable at the current genotyping resolution—within the candidate region. The region of shared homozygosity contained a total of 44 annotated genes.

Candidate genes were chosen via PubMed article database searches, as well as by looking at previous studies on gene expression patterns in the developing mouse fetus. Initial candidate genes were chosen based on high expression in the limb buds of developing mice. The coding regions of *DGCR8* (MIM 609030), *HIRA* (MIM 600237), *MED15* (*PCQAP* [MIM 607372]), and *MRPL40* (*NLVCF* [MIM 605089]) were all sequenced, and no causative mutations were found.

The next candidate selected was *SCARF2*. *SCARF2* (SREC-II) is a member of the scavenger receptor type F family.[13] Its 11 exons encode a protein 866 amino acids in length. It produces a membrane protein with a predicted weight of 91 kDa. The extracellular region contains a number of putative *N*-glycosylation sites and putative disulphide bridge formation sites, as well as seven epidermal growth factor (EGF)-like domains, as identified by the CXC XXXXXGXXC signature. As in the mouse, the intracellular region is rich in positively charged amino acids such as lysine and arginine and contains a number of putative phosphorylation sites, indicating that it may act as an intracellular signaling protein that may interact with other proteins within the cytoplasm to mediate intracellular signaling.[13]

Expressed sequence tag data reveal expression of *Scarf2* in branchial or pharyngeal arch and mandibular, maxillary, and urogenital ridge tissues on the tenth embryonic day.[14] It is also known to be expressed in human endothelial cells, and RNA blot analysis revealed its expression in human heart, lung, ovary, and placental tissue.[14] Because most of the clinical features of VDEGS can be explained by failure of proper branchial arch development, *SCARF2* was an excellent candidate within the region defined by homozygosity mapping.

Primers were designed, and the coding exons of *SCARF2* were sequenced from the genomic DNA of the four patients and four unaffected relatives. Sanger sequencing and analysis with Sequencher identified a homozygous 2 bp deletion in exon 8, c.1328_1329delTG (p.V443DfsX83), in patients 3 and 4 (Figure 3A). These individuals were not directly related to patients 1 and 2, and, as previously mentioned, patients 3 and 4 did not share the same haplotype as the other two affected patients in the candidate region. The deletion results in a frameshift, causing a premature stop codon that should result in the transcript's degradation via nonsense-mediated decay, resulting in absence of the protein.[15] If the mRNA transcript was able to escape degradation, the protein would lack a large portion of the intracellular region, which is thought to be involved in intracellular signaling.

A homozygous missense change, c.773G>A (p.C258Y; Figure 3B), was found in exon 4 of both patients 1 and 2. The missense mutation is predicted to eliminate the formation of the respective disulfide bridge within the putative EGF-like domain and thus affect the folding of this domain. This cysteine residue is very highly conserved and is present even in the zebrafish, whereas surrounding

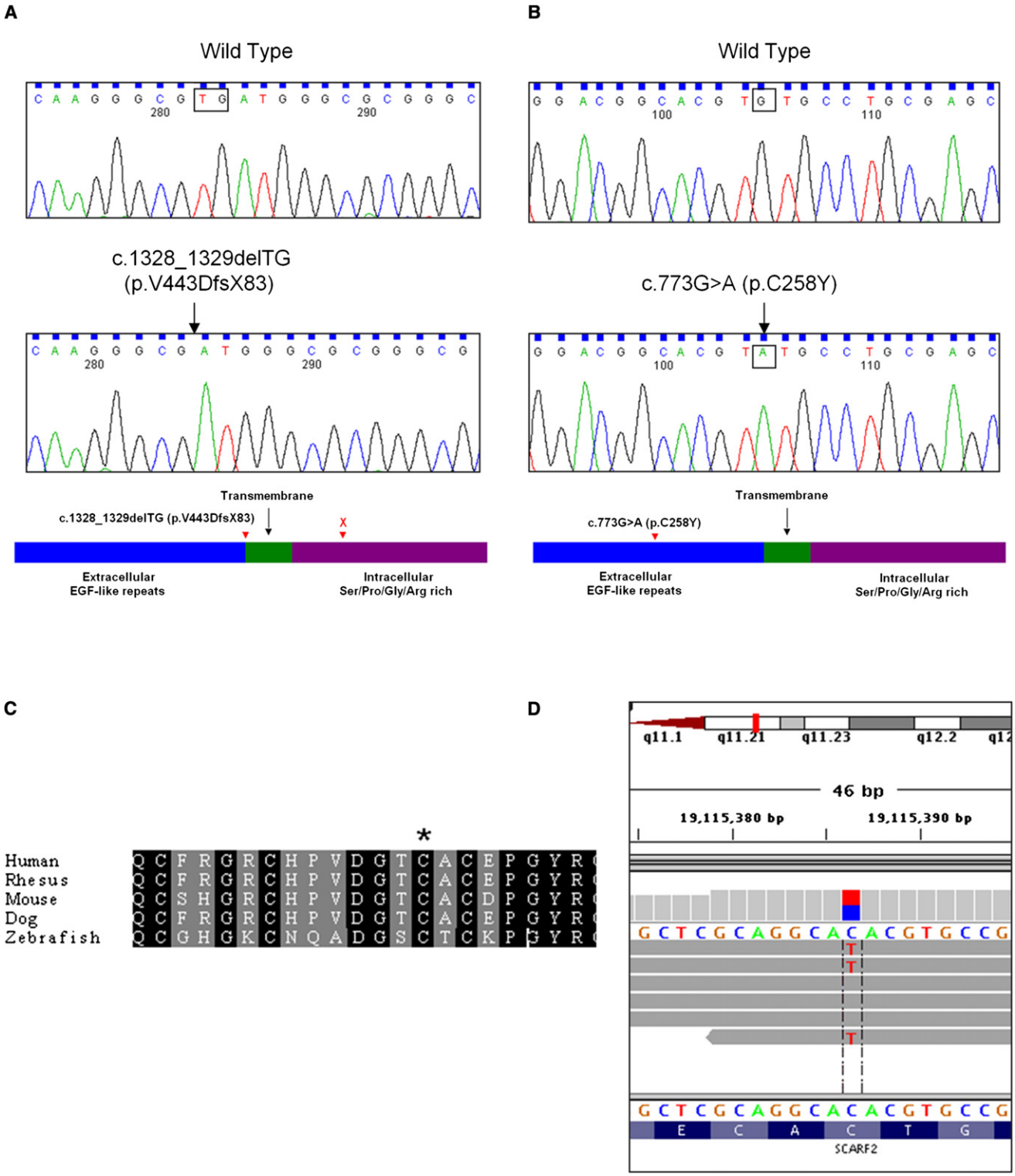

Figure 3. Sequencing and Exome Capture Results
(A) Presence and effect of c.1328_1329delTG mutation.
(B) Presence and effect of c.773G>A mutation.
(C) Conservation of residues near 773G>A (p.C258Y) mutation. Black denotes residues conserved across all species; gray denotes residues that are different in at least one species. Asterisk (*) indicates the residue changed by the mutation.
(D) Exome capture and sequencing results in the mother of patient 1. Red "T"s indicate deviation from RefSeq sequence (bottom). Sequencing shows a heterozygous state of the c.773G>A mutation.

residues are not (Figure 3C). Previous studies have also shown that mutations in the calcium-binding EGF-like domain of other proteins may result in the degradation of those products.[16]

The presence of these mutations was also confirmed via restriction endonuclease digestion with AflIII (c.773G>A) and BtgZ1 (c.1328_1329delTG). The mutations have not been reported as polymorphisms in the single nucleotide polymorphism database (dbSNP) or the 1000 Genomes Databases. We performed Sanger sequencing of the relevant regions in 196 healthy control individuals: 54 Qatar Bedouin members of the same tribe as the patients, 59 Arab, 11 Iranian, 8 Southwest Asians, and 64 Northern Europeans. The c.1328_1329delTG mutation was not found in any of the control samples. However, the c.773G>A variant was found in heterozygous state in one unaffected Bedouin. We thus estimate the frequency of this mutation to be 1/108 in this Bedouin tribe, translating to at least 1 in 12,000 incidence of the disease. Lastly, both unaffected siblings of patient 2 available in this study were homozygous for the reference allele of *SCARF2*, and the mothers of patient 2 and patient 4 were heterozygous for the missense change and the deletion, respectively. All of the above evidence strongly supports the causative role of *SCARF2* mutations in the VDEGS.

It has been previously shown that whole-exome capture technology is able to identify disease-causing genes by sequencing a single or a small number of affected individuals.[17–19] For this reason, we chose to utilize this method in parallel to homozygosity mapping and candidate gene selection. With the SureSelect Human All Exon Kit from Agilent, we were able to capture and target all coding exons—about 27.9 Mb total sequence—in the human genome. Because of a limiting amount of DNA available from the patients, we proceeded to capture and sequence the exome of the mother (person 5) of patient 1. Within the minimal candidate region, we expected to identify a number of heterozygous variants, which would then be used for validation—as homozygotes—in the patients. Using two lanes of 76 base reads of Illumina GAIIx sequencing, we obtained >4 Gb of quality-controlled reads that could be mapped to the genome. This was sufficient for an average 50× coverage of the exome. We used the Samtools[20,21] software and SIFT[22] functional annotation to identify potentially damaging SNPs within the region. Within our 44 candidate genes, we identified only two nonsynonymous variants, chr22:18156380A>G (hg18 coordinates) in *GNB1L* (MIM 610778) and chr22:19115386C>T in *SCARF2* (Figure 3D), which were not annotated as polymorphisms in dbSNP or the 1000 Genomes Database. The *SCARF2* mutation was already shown to be homozygous in patients 1 and 2, and, along with the homozygous 2 bp deletion present in patients 3 and 4, this constitutes strong evidence that *SCARF2* is the gene mutated in VDEGS. We followed up the putative involvement of *GNB1L* by sequencing this gene in our four patients. The *GNB1L* variant was found to be homozygous in patients 1 and 2, indicating that both the *GNB1L* and *SCARF2* variants are present on the same ancestral haplotype. However, neither patient 3 nor 4 carried this variant or any other mutation within *GNB1L*. Although it is possible that the *GNB1L* mutation contributes to the phenotypes observed in patients 1 and 2, its absence in patients 3 and 4 argues against its general involvement in VDEGS. Hence, we conclude that *SCARF2* is the gene mutated in VDEGS patients and is primarily responsible for the VDEGS phenotype.

The exome capture and next-generation sequencing results illustrate two points. First, this approach can indeed be used to narrow down sets of candidate genes and identify mutations, even in a heterozygote state. Second, the high-throughput sequencing results can also be used to exclude the remainder of candidate genes within the region and provide additional evidence for the causative nature of detected mutations.

Little is known about SCARF2. It is highly homologous to SCARF1 (MIM 607873), with very similar extracellular domains containing multiple EGF-like repeats, but their intracellular domains are significantly different, indicating that they may be involved in different signaling pathways within the cell.[13] Unlike SCARF1, SCARF2 has little ability to internalize modified low-density lipoprotein (LDL), such as acetylated and oxidized LDLs, although it has been demonstrated that *SCARF2* expression is upregulated in the presence of oxidized LDLs.[23] The two related proteins are thought to interact via a heterophilic *trans* interaction through their extracellular domains.[13] Evidence of a physiological relevance to the interaction between SCARF1 and SCARF2 comes from a study that demonstrated that mouse fibroblast cells showed slightly increased aggregation when either SCARF1 or SCARF2 was overexpressed on their cell surfaces and that aggregation became significantly increased when cells overexpressing SCARF1 were mixed with those expressing SCARF2. This aggregation was suppressed when modified LDLs were present.

VDEGS maps to the 22q11.2 region that contains the critical region of the velo-cardio-facial/DiGeorge syndrome (MIM 18840). Like VDEGS, DiGeorge syndrome is also characterized by craniofacial abnormalities such as micrognathia and a bulbous nose, but with the addition of cardiac defects, thyroid problems, and velopharyngeal insufficiency.[24] A majority of patients are hemizygous for a 3 Mb region of chromosome 22 or a smaller 1.5 Mb nested deletion. The major phenotypic effect in DiGeorge syndrome has been attributed to the deficiency in *TBX1* (MIM 602054),[24] but the exact roles played by other genes remain elusive. It is possible that deficiency of *SCARF2* may contribute to some of the atypical phenotypes, particularly those associated with larger deletions in patients with DiGeorge syndrome.

This study elucidates the importance of *SCARF2*, a relatively uncharacterized gene. It appears to play an important role in the proper development of a number of organs.

SCARF2's precise role requires further study, and the creation of a knockout mouse model is currently under way.

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Web Resources

The URLs for data presented herein are as follows:

Mouse Genome Informatics, http://www.informatics.jax.org/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
Sequencher 4.10.1, http://www.genecodes.com/

Accession Numbers

The *SCARF2* mRNA sequence was obtained from NCBI, accession number NM_153334.3.

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