**VSX2 mutations in autosomal recessive microphthalmia**

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**Purpose:** To further explore the spectrum of mutations in the Visual System Homeobox 2 (VSX2/CHX10) gene previously found to be associated with autosomal recessive microphthalmia.

**Methods:** We screened 95 probands with syndromic or isolated developmental ocular conditions (including 55 with anophthalmia/microphthalmia) for mutations in VSX2.

**Results:** Homozygous mutations in VSX2 were identified in two out of five consanguineous families with isolated microphthalmia. A novel missense mutation, c.668G>C (p.G223A), was identified in a large Pakistani family with multiple sibships affected with bilateral microphthalmia. This p.G223A mutation affects the conserved CVC motif that was shown to be important for DNA binding and repression activities of VSX2. The second mutation, c.249delG (p.Leu84SerfsX57), was identified in an Iranian family with microphthalmia; this mutation has been previously reported and is predicted to generate a severely truncated mutant protein completely lacking the VSX2 homeodomain, CVC domain and COOH-terminal regions.

**Conclusions:** Mutations in VSX2 represent an important cause of autosomal recessive microphthalmia in consanguineous pedigrees. Identification of a second missense mutation in the CVC motif emphasizes the importance of this region for normal VSX2 function.

**VSX2** (Visual System homeobox 2, formerly known as CHX10) is a homeodomain containing transcription factor expressed in the developing retina in human [1], mouse [2], and zebrafish embryos [3,4]; VSX2/VSX2 deficiency results in microphthalmia with various associated ocular anomalies in all three species [1-3], suggesting that the function of this gene is evolutionarily conserved. The VSX2 homeoprotein is believed to mostly act as a repressor and, in some contexts, a weak activator, and utilizes the homeodomain and CVC domain in its interaction with DNA [5].

Mutations in VSX2 are associated with autosomal recessive anophthalmia/microphthalmia (A/M), with or without iris coloboma and other ocular anomalies; eleven families have been described with eight different VSX2 mutations [1,6-9]. In most cases, the ocular anomalies are isolated, but occasional extraocular features have been reported, including learning difficulties and hormone deficiency [9]. All affected individuals have homozygous mutations; to date, mutations have only been identified in consanguineous kindreds, primarily of West and South Asian background. Two previous studies failed to identify any VSX2 mutations in 150 probands with A/M from Scotland [10] and 50 from Mexico [11].

A/M is a heterogeneous condition with numerous known causative genes. The most common causes of A/M are associated with autosomal-dominant inheritance and include SRY-Box 2 (SOX2) [12], Orthodenticle Homeobox 2 (OTX2) [13], and Bone Morphogenetic Protein 4 (BMP4) [14] mutations. Several recessive alleles have also been reported. Homozygous and/or compound heterozygous mutations have been identified: in Forkhead Box E3 (FOXE3) in several families affected with nonsyndromic microphthalmia, often accompanied by aphakia and anterior segment anomalies [15-19]; in Retina and Anterior Neural Fold Homeobox Gene (RAX) in two probands with nonsyndromic anophthalmia [20,21]; and in Stimulated by Retinoic Acid 6 (STRA6) in syndromic A/M patients [22-24].

To further characterize the spectrum of VSX2 mutations in A/M and other eye disease, we undertook screening of this gene in a large cohort of patients with various ocular conditions.

**METHODS**

This human study was approved by the Institutional Review Board of the Children’s Hospital of Milwaukee, WI with informed consent obtained by local physicians for every subject.

Genomic DNA was isolated from whole blood or buccal samples using standard procedures. The entire coding region and exon-intron junctions of VSX2 (reference sequence NM_182894.2) were screened by direct DNA sequencing of PCR products in cases and controls, as previously described.
RESULTS

Homozygous mutations in VSX2 were identified in two probands from consanguineous kindreds.

Patient 1 is an 11-year-old Pakistani male with isolated bilateral microphthalmia. He was found to have a homozygous c.668G>C (p.Gly223Ala) mutation, not previously reported. There is an extensive family history of consanguinity and microphthalmia; the mutation cosegregates with the disease phenotype with all affected individuals homozygous for the mutation and all tested unaffected individuals either heterozygous carriers or wild type (Figure 1).

Patient 2 is a 26-year-old Iranian female with bilateral microphthalmia, ‘disorganized eye,’ and blindness. She was found to have a homozygous c.249delG mutation (p.Leu84SerfsX57), previously reported [9]. The parents are first cousins and there is a history of a similar phenotype in two siblings; the mutation cosegregates with the disease phenotype (Figure 2). An affected brother is homozygous for the mutation while the two unaffected siblings and the unaffected parents are heterozygous carriers and an unaffected maternal aunt is wild type. The other two siblings were not available for testing.

Neither mutation was observed in control samples including 96 Asians and 94 Caucasian individuals. The first mutation is predicted to change a highly conserved amino acid inside the CVC-motif while the second mutation is predicted to result in a severely truncated mutant protein lacking the homeodomain, CVC-motif, COOH-terminal region, and a portion of the NH2-terminal arm (Figure 3).

DISCUSSION

These data confirm the role of VSX2 in autosomal recessive isolated microphthalmia. Similar to previous reports, mutations were identified in consanguineous kindreds from Pakistan and Iran and no causative mutations were seen in probands with A/M from non-consanguineous kindreds. VSX2 mutations were identified in 33% (2 out of 6) of consanguineous families with isolated microphthalmia.

The novel missense mutation seen in Patient 1, c.668G>C (p.Gly223Ala), is located within the conserved CVC motif, similar to the previously reported p.Arg227Trp mutation [6, 9]. The absence of this mutation in controls and its perfect cosegregation with disease phenotype provides strong evidence that this change disrupts VSX2 function. The CVC motif is shared between the members of human VSX family, VSX1 and VSX2, as well as their numerous orthologs in other species. The glycine at position 16 of the CVC motif resulted in a mild alteration of DNA binding but severely affected its repression ability [5].

This is the second report of the c.249delG mutation, previously reported in two sisters from Iran with microphthalmia, coloboma, and no perception of light [9]. Electoretinography (ERG) was performed on both parents in the previous report and demonstrated inner retinal dysfunction in both, suggesting a possible dominant effect for this mutation [9]. Unfortunately, we were unable to obtain ERG data for the heterozygous relatives of Patient 2 and thus cannot determine whether any mild retinal dystrophy is present in this family.
The VSX2 mutations/phenotypes reported in this paper are consistent with the previously described VSX2 spectrum. The absence of mutations in syndromic A/M cases is also in agreement with previous studies and further supports an eye-specific role for this gene in humans. This is only the second report of a missense mutation predicted to affect the VSX2 CVC motif and resulting in a microphthalmia phenotype. The identification of this mutation emphasizes the importance of
Figure 2. Pedigree and VSX2 sequencing results for Patient 2 and family members. A: Patient 2 is indicated with a black arrow. VSX2 genotype is indicated for each family member tested; genotypes of affected individuals are shown in red. WT, wild type; NT, not tested. B: Mutation Surveyor view of reverse VSX2 sequencing data are shown; the position of the mutation is indicated with an arrow; the first position displaying the “phase shift” in the electropherogram trace which is characteristic of a heterozygous deletion is indicated with an asterisk.
the CVC motif for normal VSX2 function and provides opportunities for further functional dissection.

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REFERENCES

1. Ferda Percin E, Ploder LA, Yu JJ, Arici K, Horsford DJ, Rutherford A, Bapat B, Cox DW, Duncan AM, Kalnins VI, Kocak-Altintas A, Sowden JC, Traboulsi E, Sarfarazi M, McInnes RR. Human microphthalmia associated with mutations in the retinal homeobox gene CHX10. Nat Genet 2000; 25:397-401. [PMID: 10932181]

2. Burmeister M, Novak J, Liang MY, Basu S, Ploder L, Hawes NL, Vidgen D, Hoover F, Goldman D, Kalnins VI, Roderick TH, Taylor BA, Hankin MH, McInnes RR. Ocular retardation mouse caused by Chx10 homeobox null allele: impaired retinal progenitor proliferation and bipolar cell differentiation. Nat Genet 1996; 12:376-84. [PMID: 8630490]

3. Barabino SM, Spada F, Cotelli F, Boncinelli E. Inactivation of the zebrafish homologue of Chx10 by antisense oligonucleotides causes eye malformations similar to the ocular retardation phenotype. Mech Dev 1996; 12:376-84. [PMID: 8630490]

4. Asselin MA, Levine EM, Canger AK, Raymond PA, Schechter N. Vxs-1 and Vxs-2: differential expression of two paired-like homeobox genes during zebrafish and goldfish retinogenesis. J Comp Neurol 1997; 389:495-505. [PMID: 9368586]

5. Dorval KM, Bobechko BP, Ahmad K, Bremner R. Transcriptional activity of the paired-like homeodomain proteins CHX10 and VSX1. J Biol Chem 2005; 280:10100-8. [PMID: 15647262]

6. Bar-Yosef U, Abuelaish I, Harel T, Hendler N, Ofir R, Birik O. CHX10 mutations cause non-syndromic microphthalmia/anophthalmia in Arab and Jewish kindreds. Hum Genet 2004; 115:302-9. [PMID: 15257456]

7. Faiyaz-Ul-Haque M, Zaidi SH, Al-Mureikhi MS, Peltekova I, Tsui LC, Teebi AS. Mutations in the CHX10 gene in non-syndromic microphthalmia/anophthalmia patients from Qatar. Clin Genet 2007; 72:164-6. [PMID: 17661825]

8. Burmeister M, Novak J, Liang MY, Basu S, Ploder L, Hawes NL, Vidgen D, Hoover F, Goldman D, Kalnins VI, Roderick TH, Taylor BA, Hankin MH, McInnes RR. Ocular retardation mouse caused by Chx10 homeobox null allele: impaired retinal progenitor proliferation and bipolar cell differentiation. Nat Genet 1996; 12:376-84. [PMID: 8630490]

9. Iseri SU, Wyatt AW, Nurnberg G, Kluck C, Nurnberg P, Holder GE, Blair E, Salt A, Ragge NK. Use of genome-wide SNP homozygosity mapping in small pedigrees to identify new mutations in VSX2 causing recessive microphthalmia and a semidominant inner retinal dystrophy. Br J Ophthalmol 2010; 94:836-8. [PMID: 20213582]

10. Faiyaz-Ul-Haque M, Zaidi SH, Al-Mureikhi MS, Peltekova I, Tsui LC, Teebi AS. Mutations in the CHX10 gene in non-syndromic microphthalmia/anophthalmia patients from Qatar. Clin Genet 2007; 72:164-6. [PMID: 17661825]

11. Gonzalez-Rodriguez J, Pelcastre EL, Garcia-Ortiz JE, Amato-Almanza M, Villanueva-Mendoza C, Espinosa-Mattar Z, Zenteno JC. Mutational screening of CHX10, GDF6, OTX2, RAX and SOX2 genes in 50 unrelated microphthalmia-anophthalmia-coloboma (MAC) spectrum cases. Br J Ophthalmol 2010; 94:1100-4. [PMID: 2049491]

12. Faiyaz-Ul-Haque M, Zaidi SH, Al-Mureikhi MS, Peltekova I, Tsui LC, Teebi AS. Mutations in the CHX10 gene in non-syndromic microphthalmia/anophthalmia patients from Qatar. Clin Genet 2007; 72:164-6. [PMID: 17661825]

13. Faiyaz-Ul-Haque M, Zaidi SH, Al-Mureikhi MS, Peltekova I, Tsui LC, Teebi AS. Mutations in the CHX10 gene in non-syndromic microphthalmia/anophthalmia patients from Qatar. Clin Genet 2007; 72:164-6. [PMID: 17661825]

14. Faiyaz-Ul-Haque M, Zaidi SH, Al-Mureikhi MS, Peltekova I, Tsui LC, Teebi AS. Mutations in the CHX10 gene in non-syndromic microphthalmia/anophthalmia patients from Qatar. Clin Genet 2007; 72:164-6. [PMID: 17661825]

15. Faiyaz-Ul-Haque M, Zaidi SH, Al-Mureikhi MS, Peltekova I, Tsui LC, Teebi AS. Mutations in the CHX10 gene in non-syndromic microphthalmia/anophthalmia patients from Qatar. Clin Genet 2007; 72:164-6. [PMID: 17661825]

16. Figure 3. Alignment of protein sequences of human, mouse and zebrafish VSX2/Vsx2/vsx2. The homeodomain sequence is highlighted in green and the CVC motif in blue. The positions of the mutations identified in Patients 1 (P1) and 2 (P2) are marked in red.
15. Valleix S, Niel F, Nedelec B, Algros MP, Schwartz C, Delbosc B, Delpech M, Kantelip B. Homozygous nonsense mutation in the FOXE3 gene as a cause of congenital primary aphakia in humans. Am J Hum Genet 2006; 79:358-64. [PMID: 16826526]

16. Iseri SU, Osborne RJ, Farrall M, Wyatt AW, Mirza G, Nurnberg G, Kluck C, Herbert H, Martin A, Hussain MS, Collin JR, Lathrop M, Nurnberg P, Ragoussis J, Ragge NK. Seeing clearly: the dominant and recessive nature of FOXE3 in eye developmental anomalies. Hum Mutat 2009; 30:1378-86. [PMID: 19708017]

17. Reis LM, Tyler RC, Schneider A, Bardakjian T, Stoler JM, Melancon SB, Semina EV. FOXE3 plays a significant role in autosomal recessive microphthalmia. Am J Med Genet A 2010; 152A:582-90. [PMID: 20140963]

18. Ali M, Buentello-Volante B, McKibbin M, Rocha-Medina JA, Fernandez-Fuentes N, Koga-Nakamura W, Ashiq A, Khan K, Booth AP, Williams G, Raashid Y, Jafri H, Rice A, Inglehearn CF, Zenteno JC. Homozygous FOXE3 mutations cause non-syndromic, bilateral, total sclerocornea, aphakia, microphthalmia and optic disc coloboma. Mol Vis 2010; 16:1162-8. [PMID: 20664966]

19. Anjum I, Eiberg H, Baig SM, Tommerup N, Hansen L. A mutation in the FOXE3 gene causes congenital primary aphakia in an autosomal recessive consanguineous Pakistani family. Mol Vis 2010; 16:549-55. [PMID: 20361012]

20. Voronina VA, Kozhemyakina EA, O’Kernick CM, Kahn ND, Wenger SL, Linberg JV, Schneider AS, Mathers PH. Mutations in the human RAX homeobox gene in a patient with anophthalmia and sclerocornea. Hum Mol Genet 2004; 13:315-22. [PMID: 14662654]

21. Lequeux L, Rio M, Vivouroux A, Titeux M, Etchevers H, Malecaze F, Chassaing N, Calvas P. Confirmation of RAX gene involvement in human anophthalmia. Clin Genet 2008; 74:392-5. [PMID: 18783408]

22. Golzio C, Martinovic-Bouriel J, Thomas S, Mouguou-Zrelli S, Grattagliano-Bessieres B, Bonniere M, Delahaye S, Munich A, Encha-Razavi F, Lyonnet S, Vekemans M, Attie-Bitach T, Etchevers HC. Matthew-Wood syndrome is caused by truncating mutations in the retinol-binding protein receptor gene STRA6. Am J Hum Genet 2007; 80:1179-87. [PMID: 17503335]

23. White T, Lu T, Metlapally R, Katowitz J, Kherani F, Wang TY, Tran-Viet KN, Young TL. Identification of STRA6 and SKI sequence variants in patients with anophthalmia/microphthalmia. Mol Vis 2008; 14:2458-65. [PMID: 19112531]

24. Chassaing N, Golzio C, Odent S, Lequeux L, Vigouroux A, Martinovic-Bouriel J, Tiziano FD, Masini L, Piro F, Maragliano G, Delezoide AL, Attie-Bitach T, Manouvrier-Hanu S, Etchevers HC, Calvas P. Phenotypic spectrum of STRA6 mutations: from Matthew-Wood syndrome to non-lethal anophthalmia. Hum Mutat 2009; 30:E673-81. [PMID: 19309693]