Published in final edited form as:

Ophthalmic Genet. 2016 March ; 37(1): 76–80. doi:10.3109/13816810.2014.889169.

Hereditary Benign Intraepithelial Dyskeratosis: Report of a Case and Reexamination of the Evidence for Locus Heterogeneity

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Abstract

Background—Hereditary benign intraepithelial dyskeratosis (HBID) is a rare autosomal-dominant disorder of the conjunctiva and oral mucosa first described in and predominantly affecting descendants of Haliwa-Saponi Native Americans. We report a spontaneous case of histopathologically-confirmed HBID affecting an individual not of Native American ancestry.

Materials and Methods—Report of a case with histopathologic examination of an excised conjunctival specimen as well as molecular and cytogenetic analysis.

Results—A Caucasian boy with a history of oral lesions and conjunctival injection from birth developed bilateral corneal opacities at age 5 and underwent penetrating keratoplasty, with recurrence of the corneal opacification shortly after surgery. Examination of a conjunctival biopsy specimen revealed features consistent with HBID. Copy number variant (CNV) analysis revealed a de novo 4q35 duplication that overlapped the duplication previously associated with HBID, although no genes were identified in the common interval. *NLRP1* gene sequencing failed to reveal a presumed pathogenic variant.

Conclusions—HBID may develop de novo in individuals who are not of Native American ancestry. The absence of coding regions in a duplicated region of 4q35 common to both the individual that we report and previously associated with HBID raises questions regarding the significance of this CNV in the pathogenesis of HBID.

Keywords

Hereditary benign intraepithelial dyskeratosis; *NLRP1*; segment duplication

INTRODUCTION

Hereditary benign intraepithelial dyskeratosis (HBID) is a disorder of the bulbar conjunctiva and oral mucosa associated with epithelial hyperplasia and hyperkeratosis. Clinical features

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

include white plaques on the bulbar conjunctiva and oral mucosa that may present at birth and progress over time. In cases in which the conjunctival epithelial abnormalities extend onto the cornea, vision may be affected and corneal transplantation may be required.^{1, 2} While HBID was originally described in members of the Haliwa-Saponi Native American tribe located in North Carolina³, and the majority of the reports of HBID have been in this population,^{4–11} six other pedigrees without Native American ancestry, consisting of between 1 and 4 affected individuals in each, have been reported.^{1, 2, 12–15}

Over 40 years after the initial clinical description of HBID was published, investigators reported the linkage of HBID to chromosome 4 (4q35).⁸ Haplotype analysis of individuals in the two families used in the linkage analysis demonstrated evidence of a duplication in the linked interval that segregated with the affected phenotype, leading investigators to conclude that the duplication was involved in the pathogenesis of HBID. Although the investigators commented on candidate genes in the duplicated region, no further studies investigating the genetic basis of HBID in these pedigrees have been published. Recently, another group has reported a Caucasian French family with histopathologically-confirmed HBID in whom quantitative PCR (qPCR) excluded a 4q35 duplication.² Next generation sequencing identified an unreported, novel missense mutation in the *NLRP1* gene on chromosome 17 in the affected proband and his affected mother that was predicted to destabilize the encoded protein. However, screening of *NLRP1* in Native Americans from 3 families with HBID failed to reveal a pathogenic mutation. The association of HBID with both 4q35 in Native Americans and 17p13 in a Caucasian French family raises the possibility that the phenotypically similar entities described in the different populations are in fact genetically distinct. As a second test for locus heterogeneity in HBID, we performed both copy number variant analysis and whole exome sequencing in a previously unreported Caucasian American individual with histopathologically-confirmed HBID.

MATERIALS AND METHODS

The authors followed the tenets of the Declaration of Helsinki in the treatment of the subjects reported herein. Study approval was obtained from the Institutional Review Board at The University of California, Los Angeles (UCLA IRB # 94-07-243-23) and the Intramural IRB for NHGRI at the NIH in Bethesda MD (97HG -0238).

***NLRP1* screening**

DNA was isolated from a peripheral blood sample from the proband using the FlexiGene DNA kit (Qiagen, Valencia, CA). PCR amplification of each of the 17 exons and intron/exon boundaries of *NLRP1* was performed via standard molecular biology techniques using previously described primers and conditions.² Automated sequencing was performed on a ABI-3130 Genetic Analyzer (Applied Biosystems; Grand Island, NY) and generated sequences were compared to the reference GenBank sequences (NM_033004.3 and NM_001033305.2).

Copy number variant analysis

Genomic DNA obtained from the proband and his parents underwent copy number variant analysis at the National Human Genome Research Institute using the Illumina OmniQuad 1.0 array (Illumina Corp; San Diego, CA). Subsequently, genomic DNA from the proband was sent to the UCLA Clinical Microarray Core for copy number variation analysis, where it was performed using the CytoScan HD array (Affymetrix; Santa Clara, CA) and analyzed using the Partek Genomic Suite software (Partek Inc; St Louis, MO). Previously reported duplications in the region of 4q35 were identified using the Database of Genomic Variants (dgvbeta.tcag.ca).

Whole exome sequencing

Agilent 38MB exome capture was performed on DNA derived from whole blood and 76bp paired end whole exome sequencing on an Illumina GAIIX (Illumina Corp) by standard methodology.¹⁶ Alignment was performed initially by the NIH Intramural Sequencing Center (NISC) as described previously for hg18 and recently realigned using Novocraft Novoalign (Novocraft Corp; Jakarta, Indonesia) to hg19. Variant calls were determined by MPG caller and viewed and analyzed in the VarSifter GUI.¹⁷ BAM files were inspected by visualization in the Integrated Genome Viewer (Broad Institute; Cambridge, MA).

RESULTS

Clinical features and course

A Caucasian boy, the youngest of seven children, was evaluated independently by two of the authors (TCM and AJA) for chronic conjunctival injection and photophobia since infancy (Figure 1). The patient was diagnosed at birth with patches of oral leukoplakia that extended into the esophagus and were diagnosed as consistent with HBID. However, both of the patient's parents denied Native American ancestry. The patient also had a history of congenital bilateral sensorineural hearing loss, for which he began wearing hearing aids at 1 year of age. At 5 years of age, sufficient corneal opacification had developed in the patient's left eye that he underwent a penetrating keratoplasty. As corneal opacification recurred only several months after surgery, no further corneal procedures were performed for management of the corneal opacification in each eye (Figure 1). No other immediate family members, including his 6 siblings, had a history of ocular, oral or auditory disorders.

Examination by one of the authors at age 6 (AJA) demonstrated the patient's visual acuity to be count fingers at 1 foot in the right eye and hand motion in his left. Diffuse bulbar conjunctival injection was noted, associated with elevated foci of epithelial thickening and keratinization overlying the limbus (Figure 1). Both corneas demonstrated diffuse superficial vascularization and scarring, with stromal opacification involving the penetrating keratoplasty in the patient's left eye. Ocular ultrasonography revealed a normal axial length and unremarkable posterior segment of each eye. A biopsy of the elevated limbal lesion in the right eye revealed marked thickening of the epithelium associated with dyskeratosis, hyperkeratosis and apparent apoptosis (Figure 2).

***NLRP1* screening**

Sequencing of *NLRP1* in the proband revealed only a single synonymous coding variant (c. 114G>C; p.(Ser38Ser)), a known SNP (rs884367), present in the heterozygous state. Segregation analysis from SNP data shows that at least one unaffected sibling inherited the same alleles from both parents in the region that flanks the *NLRP1* locus, excluding the possibility of an inherited autosomal recessive deleterious change even outside the Sanger sequenced areas of this locus.

Copy number variant analysis

Copy number variation analysis in the proband revealed seven regions of CNV across the genome, none of which involved chromosome 17 but 3 of which involved chromosome 4: a 263 Kb deletion in 4q12 and a 189 Kb and a 354 Kb duplication in 4q35. Neither of the 4q35 duplications were identified in the proband's parents. While the 354 Kbp duplication overlaps the previously reported duplication associated with HBID⁸, no open reading frames are found in the common interval and the nearest functional gene (*FRG1*) is located 580 Kbp from the telomeric border of the common interval (Figure 3). In addition to *FRG1*, two other genes (*TUBB4Q* and *FRG2*) are located in the 189 Kb duplication, although dysregulation of these genes is known to be associated with muscular dystrophy, and has not been associated with epithelial pathology.¹⁸ Several duplications in 4q35 were identified in the Database of Genomic Variants in unaffected individuals, with frequencies ranging between 0.06 – 11.1% (Figure 3).

Whole exome sequencing

No open reading frames or identified genes were found in the 354 Kb duplicated region of 4q. Additionally, an exonic sequence variant that resulted in a change in the encoded protein sequence was not identified in the *NLRP1* coding region when aligned to hg18 (all exonic segments had adequate depth of coverage). Realignment of the original data to hg19 showed no difference for mapped reads in these exons and no new variants were discovered.

DISCUSSION

We report ocular and oral lesions consistent with HBID in an individual unrelated to the Haliwa-Saponi Native American tribe. Of the six reported affected probands that do not have Native American ancestry, four demonstrated conjunctival and corneal changes that were diagnosed by an ophthalmologist as consistent with HBID, with the diagnosis confirmed by histopathologic examination of conjunctival and corneal biopsy specimens.^{1, 2, 13, 15} However, two of the reported probands demonstrated only oral manifestations characteristic of HBID, with minor actinic-appearing bulbar conjunctival changes in one proband and in 3 relatives of the other proband, consistent with pingueculae. These conjunctival lesions, photographs of which appear in the reports, appear to have been misinterpreted by the dermatologists, oral surgeons and pathologists who reported these cases as ocular manifestations of HBID.^{12, 14} The authors of one report indicated that the proband was not referred to an ophthalmologist as her eyes were unaffected and a conjunctival biopsy was not performed in the other proband.¹² Therefore, these cases do not meet the diagnostic criteria for HBID and should probably be classified as one of the closely

related disorders that affect only the oral mucosa.¹⁹ Thus, this report represents only the fifth non Native American proband with confirmed HBID, and serves as a reminder to ophthalmologists to consider the diagnosis of HBID, even in the absence of Native American ancestry and a family history.

Although HBID has been associated with both a duplication in 4q35 in two Haliwa-Saponi Native American families⁸ and a missense mutation in *NLRP1* on 17p13 in a Caucasian French family², copy number variant analysis and whole exome sequencing in a previously unreported Caucasian American individual failed to identify the genetic basis of HBID. The linkage of HBID to 4q35 with a multipoint HLOD score of 11.58 is very strong evidence that the genetic basis of HBID lies within this region. However, the absence of any genes present in the overlapping duplicated regions of 4q35 in both the proband we report and in the Native American families effectively excludes altered gene dosage as a shared cause of HBID, assuming that gene annotation is complete in this region.

While the absence of a protein-altering *NLRP1* coding region variant in the Caucasian American individual we report does not definitively exclude a role for *NLRP1* in the pathogenesis of HBID in this individual, it does make it unlikely. Even in the Caucasian French family in which the *NLRP1* missense mutation was identified, questions remain about the pathogenicity of the identified variant, which was identified in just two affected individuals and absent in five unaffected individuals that were screened. In addition, the 4q35 locus was not definitively excluded in this family as qPCR amplification of only a single polymorphic marker in 4q35 to demonstrate a normal copy number was performed. While whole exome sequencing was performed, the extensive filtering of identified variants that was required (given that DNA from only two affected and one unaffected individuals from this single family were used) increases the chances of the pathogenic mutation being filtered out. Given these considerations, and in the absence of functional studies, the identified *NLRP1* variant can only be considered as potentially pathogenic.

The absence of a *NLRP1* coding region mutation and even a predicted gene in the region of 4q35 duplicated in both the Caucasian American individual we report and in two Haliwa-Saponi Native American families is evidence of a distinct genetic locus for HBID. It is possible that locus heterogeneity exists for HBID, as is the case for many genetic phenotypes given a single medical diagnostic label, such as Meesmann corneal dystrophy.^{20, 21} As the genetic basis of HBID is likely shared amongst most, if not all of the affected Haliwa-Saponi Native American families, the genetic basis of HBID will most likely be identified with further analysis of HBID in this population using targeted exome sequencing and cytogenetic analyses of the 4q35 candidate region. Subsequently, screening of non-Native American affected individuals, such as the individual that we report, will reveal whether locus heterogeneity does in fact exist for HBID.

ACKNOWLEDGEMENTS

Support provided by National Eye Institute grants 1R01 EY022082 (A.J.A.) and P30 EY000331 (core grant), and an unrestricted grant from Research to Prevent Blindness (A.J.A.). Funding for the Undiagnosed Diseases program is through the NIH Interinstitute Common Fund, Office of the Director, NIH.

REFERENCES

1. Cai R, Zhang C, Chen R, et al. Clinicopathological features of a suspected case of hereditary benign intraepithelial dyskeratosis with bilateral corneas involved: a case report and mini review. *Cornea*. 2011; 30:1481–1484. [PubMed: 21993456]
2. Soler VJ, Tran-Viet KN, Galiacy SD, et al. Whole exome sequencing identifies a mutation for a novel form of corneal intraepithelial dyskeratosis. *J Med Genet*. 2013; 50:246–254. [PubMed: 23349227]
3. von Sallmann L, Paton D. Hereditary Dyskeratosis of the Perilimbal Conjunctiva. *Trans Am Ophthalmol Soc*. 1959; 57:53–62. [PubMed: 16693585]
4. Yanoff M. Hereditary benign intraepithelial dyskeratosis. *Arch Ophthalmol*. 1968; 79:291–293. [PubMed: 5640851]
5. Reed JW, Cashwell F, Klintworth GK. Corneal manifestations of hereditary benign intraepithelial dyskeratosis. *Arch Ophthalmol*. 1979; 97:297–300. [PubMed: 550801]
6. Haisley-Royster CA, Allingham RR, Klintworth GK, Prose NS. Hereditary benign intraepithelial dyskeratosis: Report of two cases with prominent oral lesions. *J Am Acad Dermatol*. 2001; 45:634–636. [PubMed: 11568764]
7. Cummings TJ, Dodd LG, Eedes CR, Klintworth GK. Hereditary benign intraepithelial dyskeratosis: an evaluation of diagnostic cytology. *Arch Pathol Lab Med*. 2008; 132:1325–1328. [PubMed: 18684035]
8. Allingham RR, Seo B, Rampersaud E, et al. A duplication in chromosome 4q35 is associated with hereditary benign intraepithelial dyskeratosis. *Am J Hum Genet*. 2001; 68:491–494. [PubMed: 11170897]
9. Witkop CJ Jr, Shankle CH, Graham JB, et al. Hereditary benign intraepithelial dyskeratosis. II. Oral manifestations and hereditary transmission. *Arch Pathol*. 1960; 70:696–711. [PubMed: 13786059]
10. Pollitzer WS, Menegaz-Bock RM, Renwick JH, Witkop CJ. Hereditary Benign Intraepithelial Dyskeratosis: A Linkage Study. *Am J Hum Genet*. 1965; 17:104–108. [PubMed: 14262127]
11. Shields CL, Shields JA, Eagle RC Jr. Hereditary benign intraepithelial dyskeratosis. *Arch Ophthalmol*. 1987; 105:422–423. [PubMed: 3827721]
12. Baroni A, Palla M, Aiello FS, et al. Hereditary benign intraepithelial dyskeratosis: case report. *Int J Dermatol*. 2009; 48:627–629. [PubMed: 19538374]
13. Dithmar S, Stulting RD, Grossniklaus HE. [Hereditary benign intraepithelial dyskeratosis]. *Ophthalmologe*. 1998; 95:684–686. [PubMed: 9828633]
14. Jham BC, Mesquita RA, Aguiar MC, Carmo MA. Hereditary benign intraepithelial dyskeratosis: a new case? *J Oral Pathol Med*. 2007; 36:55–57. [PubMed: 17181743]
15. McLean IW, Riddle PJ, Schruggs JH, Jones DB. Hereditary benign intraepithelial dyskeratosis. A report of two cases from Texas. *Ophthalmology*. 1981; 88:164–168. [PubMed: 7231902]
16. Adams DR, Sincan M, Fuentes Fajardo K, et al. Analysis of DNA sequence variants detected by high-throughput sequencing. *Hum Mutat*. 2012; 33:599–608. [PubMed: 22290882]
17. Teer JK, Green ED, Mullikin JC, Biesecker LG. VarSifter: visualizing and analyzing exome-scale sequence variation data on a desktop computer. *Bioinformatics*. 2012; 28:599–600. [PubMed: 22210868]
18. van Geel M, Heather LJ, Lyle R, et al. The FSHD region on human chromosome 4q35 contains potential coding regions among pseudogenes and a high density of repeat elements. *Genomics*. 1999; 61:55–65. [PubMed: 10512680]
19. Witkop CJ Jr, Gorlin RJ. Four hereditary mucosal syndromes: comparative histology and exfoliative cytology of Darier-White's disease, hereditary benign intraepithelial dyskeratosis, white sponge nevus, and pachyonychia congenita. *Arch Dermatol*. 1961; 84:762–771. [PubMed: 14007732]
20. Nishida K, Honma Y, Dota A, et al. Isolation and chromosomal localization of a cornea-specific human keratin 12 gene and detection of four mutations in Meesmann corneal epithelial dystrophy. *Am J Hum Genet*. 1997; 61:1268–1275. [PubMed: 9399908]

21. Irvine AD, Corden LD, Swensson O, et al. Mutations in cornea-specific keratin K3 or K12 genes cause Meesmann's corneal dystrophy. *Nat Genet.* 1997; 16:184–187. [PubMed: 9171831]

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Figure 1.

A. Pedigree of a family with HBID. Filled symbols indicates affected individual; open symbols indicate unaffected individuals; arrowhead indicates proband. B and C. External photographs of 6-year old boy with clinical and histopathologic changes consistent with HBID. Diffuse conjunctival injection, an elevated lesion overlying the limbus and diffuse superficial corneal vascularization and opacification are noted OD (B). Significant inferior conjunctival injection, and corneal vascularization and opacification of the previous penetrating keratoplasty are noted OS (C).

Figure 2.

Conjunctival biopsy specimen from an individual with HBID. A. A moderate chronic inflammatory infiltrate resides in the superficial substantia propria, above which the conjunctival squamous epithelium is hyperplastic and acanthotic. B. Brightly eosinophilic cytoplasmic material is present in the superficial epithelial cells (arrows), presumably representing keratin, and anucleate squamous cells are evident on the epithelial surface (arrowheads). C. Individual epithelial cells show margination and fragmentation of nuclear material with eosinophilic cytoplasm and apoptotic changes (hematoxylin and eosin stain).

Figure 3.

Schematic of chromosome 4q35 indicating location of duplications previously identified in Haliwa-Saponi Native American tribe (hatched rectangle) and 6-year old boy with HBID (shaded rectangles). The location and names of genes in the duplicated regions are shown. Genomic base position is depicted above the schematic. The location of previously reported duplications and incidence of each in control populations (Database of Genomic Variants) are depicted underneath the schematic.