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SHORT REPORT

Identification of the gene for Nance-Horan syndrome (NHS)

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Background: The disease intervals for Nance-Horan syndrome (NHS [MIM 302350]) and X linked congenital cataract (CXN) overlap on Xp22.
Objective: To identify the gene or genes responsible for these diseases.
Methods: Families with NHS were ascertained. The refined locus for CXN was used to focus the search for candidate genes, which were screened by polymerase chain reaction and direct sequencing of potential exons and intron-exon splice sites. Genomic structures and homologies were determined using bioinformatics. Expression studies were undertaken using specific exonic primers to amplify human fetal cDNA and mouse RNA.
Results: A novel gene *NHS*, with no known function, was identified as causative for NHS. Protein truncating mutations were detected in all three NHS pedigrees, but no mutation was identified in a CXN family, raising the possibility that NHS and CXN may not be allelic. The *NHS* gene forms a new gene family with a closely related novel gene *NHS-Like1* (*NHSL1*). *NHS* and *NHSL1* lie in paralogous duplicated chromosomal intervals on Xp22 and 6q24, and *NHSL1* is more broadly expressed than *NHS* in human fetal tissues.
Conclusions: This study reports the independent identification of the gene causative for Nance-Horan syndrome and extends the number of mutations identified.

Nance-Horan syndrome (NHS) is a rare X linked disease characterised by severe congenital dense nuclear cataracts in hemizygous males. Distinctive dental anomalies are seen in affected males, including super-numerary incisors and crown shaped permanent teeth. Characteristic facial features are anteverted pinnae, long face, and prominent nasal bridge and nose. In addition, approximately 30% of cases have developmental delay. Carrier females display milder variable symptoms of disease with lens opacities often involving the posterior Y sutures, and on occasion dental anomalies and the characteristic facial features described.[1–5]

We have investigated a pedigree with X linked congenital cataract (CXN), associated with ventriculoseptal defect (VSD) in some affected males.[6] Our genetic mapping of this pedigree suggests that the locus may be synonymous with NHS, and was recently refined to a 3.2 Mb interval.[7] Affected males with CXN have a total nuclear opacity of the lens, requiring cataract surgery within the first few months of life. Carrier females show a sea-fan type of nuclear lens opacity. Four of the six affected males within this family were found to have VSD, but no cardiac abnormalities have been reported in patients with NHS.

We also studied pedigrees with NHS, independently identifying the *NHS* gene first reported by Burdon and colleagues,[8] and found truncating mutations in three pedigrees. After excluding four candidate genes (*PPEF1*, *RAI2*, *RBBP7*, and *TL1*),[7] we identified further candidate genes in the region of overlap between the refined NHS and CXN loci.[7 9] A novel gene was identified telomeric to *SCML1* and centromeric to microsatellite marker DXS1195 (AFM207ZD6). Numerous expressed sequence tag (EST) hits (NCBI Unigene cluster Hs.444940)—expressed in multiple tissues including fetal eye, brain, kidney, and heart—supported this gene prediction.

METHODS

Our three NHS families, ascertained after appropriate informed consent and institutional ethical committee board approval were obtained, displayed typical features of the syndrome. The youngest brother of family 1 also had bilateral cleft palate which was repaired in infancy, and the affected male in family three had severe neurological problems (table 1). Direct sequencing of polymerase chain reaction (PCR) amplicons from all predicted coding exons and splice sites was carried out (primers and conditions available on request) on samples from an affected male from the CXN family and NHS families 1, 2, and 3 (fig 1).

RESULTS AND DISCUSSION

We identified a two base pair (bp) deletion (3738–3739delTG), causing a frameshift and resulting in a premature stop codon after the addition of 15 novel amino acids (C1246-A1247fsX15), within exon 6 of NHS family 1. A 1 bp deletion (2687delA) was also identified within exon 6 of NHS family 3 (fig 1). Deletion of this one base causes a frameshift which is predicted to result in a premature stop codon following the addition of 10 novel amino acids (Q896fsX10). Our work corroborated the findings of Burdon and colleagues,[8] which revealed a novel exon 1. Subsequently, oligonucleotide primers were designed to PCR amplify and sequence exon 1 of the *NHS* gene within NHS family 2 and the CXN family (primers and conditions available on request). A 1 bp deletion was identified within exon 1 (400delC) of NHS family 2, which is predicted to cause a frameshift leading to the addition of 61 novel amino acids before a premature stop codon (R134fsX61). However, no mutation was identified in the CXN family.

All three mutations have been shown to segregate with disease and have not been detected in 200 control chromosomes screened either by direct sequencing of PCR products or by the WAVE nucleic acid fragment analysis system (Transgenomic, Omaha, Nebraska, USA; following manufacturer's instructions).

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Abbreviations: CXN, X linked congenital cataract; NHS, Nance-Horan syndrome

Table 1 Summary of mutations in the *NHS* gene

Sequence change	Exon/intron	Predicted protein	Family and phenotype
3738–3739delTG	Exon 6	C1246-A1247fsX15	Family 1 with typical NHS features and bilateral cleft palate
400delC*	Exon 1	R134fsX61	Family 2 with typical NHS features
2687delA	Exon 6	Q896fsX10	Family 3 with typical NHS features and severe mental retardation, epilepsy and hypotonia
3459delC†	Exon 6	L1154fsX28	
718insG†‡	Exon 3	E240fsX36	
IVS2-3C→G†‡	Intron 2		
2387insC†	Exon 6	S797fsX35	
1117C→T†	Exon 5	R378STOP	

*Also reported by Burdon and colleagues.
†Reported by Burdon and colleagues.
‡Identified within the same family.

An identical 1 bp deletion (400delC) in exon 1 in family 2 reported here was also seen in family 5 described by Burdon and colleagues.[8] This, however, does not reflect a mutation hotspot as subsequently these families were found to be the same (personal communication). These findings increase the total number of mutations reported in *NHS* to 7 (table 1), all of which are truncating mutations predicted to result in an aberrant protein product. Four of these lie within exon 6 (three deletions and one insertion), which is considerably larger than the remaining seven coding exons. As exons 1, 2, and 3 encode isoform A only (the larger isoform of *NHS*), mutations identified within these exons are predicted to affect expression of isoform A and not isoform B. Two families harbour such mutations (table 1); however, phenotypic differences exist between the families, so there appears to be no direct phenotype–genotype correlation with ablation of isoform A. The possibility that NHS and CXN are allelic has not been excluded by the inability to identify a mutation in the CXN pedigree. Similarly, Burdon and colleagues[8] observed that another NHS family lacks a mutation in *NHS*. Further characterisation of the NHS gene may reveal mutations in cryptic exons or regulatory sequences. Interestingly, a mouse model for X linked congenital cataract maps to the syntenic region of CXN and NHS[10]; therefore identification of the causative gene in this model will be important in determining whether CXN and NHS are allelic.

As NHS is a congenital syndrome we evaluated the developmental expression of *NHS* through PCR analysis of human fetal tissue cDNA (BD Biosciences, Palo Alto, California, USA) and murine tooth cDNA at postnatal day zero, P0, and postnatal day two, P2 (Amersham RNA extraction kit). Primers were designed to amplify a 590 bp and an 268 bp product in genomic DNA and cDNA, respectively, from exons 6 and 7 of human *NHS* (Fw [5′-TGCCTGGTACTATCAGCTATG-3′], Rev [5′-TCCTCAGTTGTT CGAGGTTTG-3′). Primers were also designed to amplify a 206 bp fragment in exon 6 of murine *Nhs* (Fw[5′-ACTGCAACCTCAGCTAGCAG-3′], Rev[5′-TGCTGTACAGTG CCATCTGG-3′]). *NHS* transcripts were detected in murine tooth cDNA at P0 but not at P2 (data not shown). *NHS* expression was also detected in human fetal brain, thymus, lung and kidney (fig 2C), confirming our initial expression studies in which a 207 bp fragment within exon 6 of human *NHS* was amplified and detected in the same samples (data not shown). These results are in accord with the expression studies in mouse, showing that the *Nhs* gene is broadly expressed throughout brain development, and in situ hybridisation studies with *Nhs* expression found in a range of mouse embryonic tissues—including the lens, brain, craniofacial mesenchyme, and dental primordial.[8] The significance of the additional expression in human fetal kidney, lung, and thymus is presently not clear.
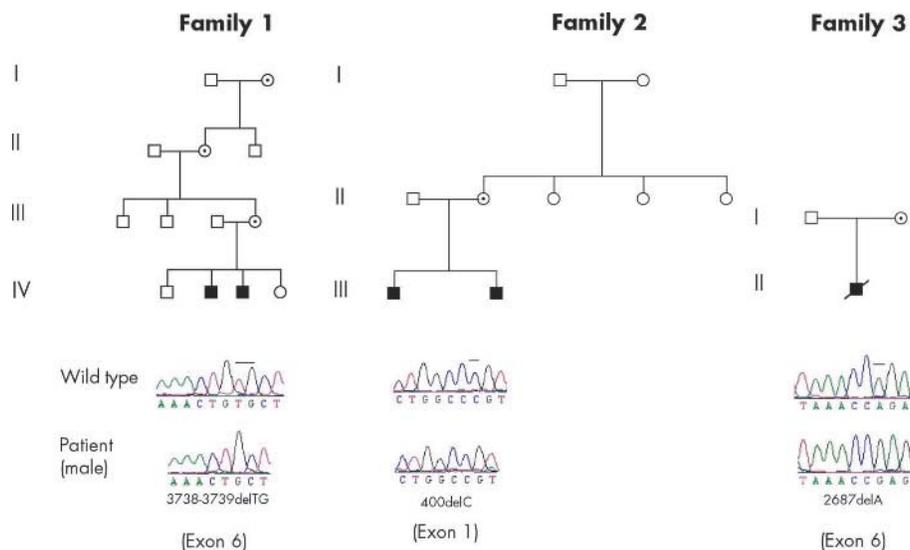

Figure 1 Pedigrees of families with NHS and corresponding sequence chromatograms highlighting identified mutations. Squares denote males and circles, females. Filled squares represent affected individuals and circles with black dots represent females with features of NHS.

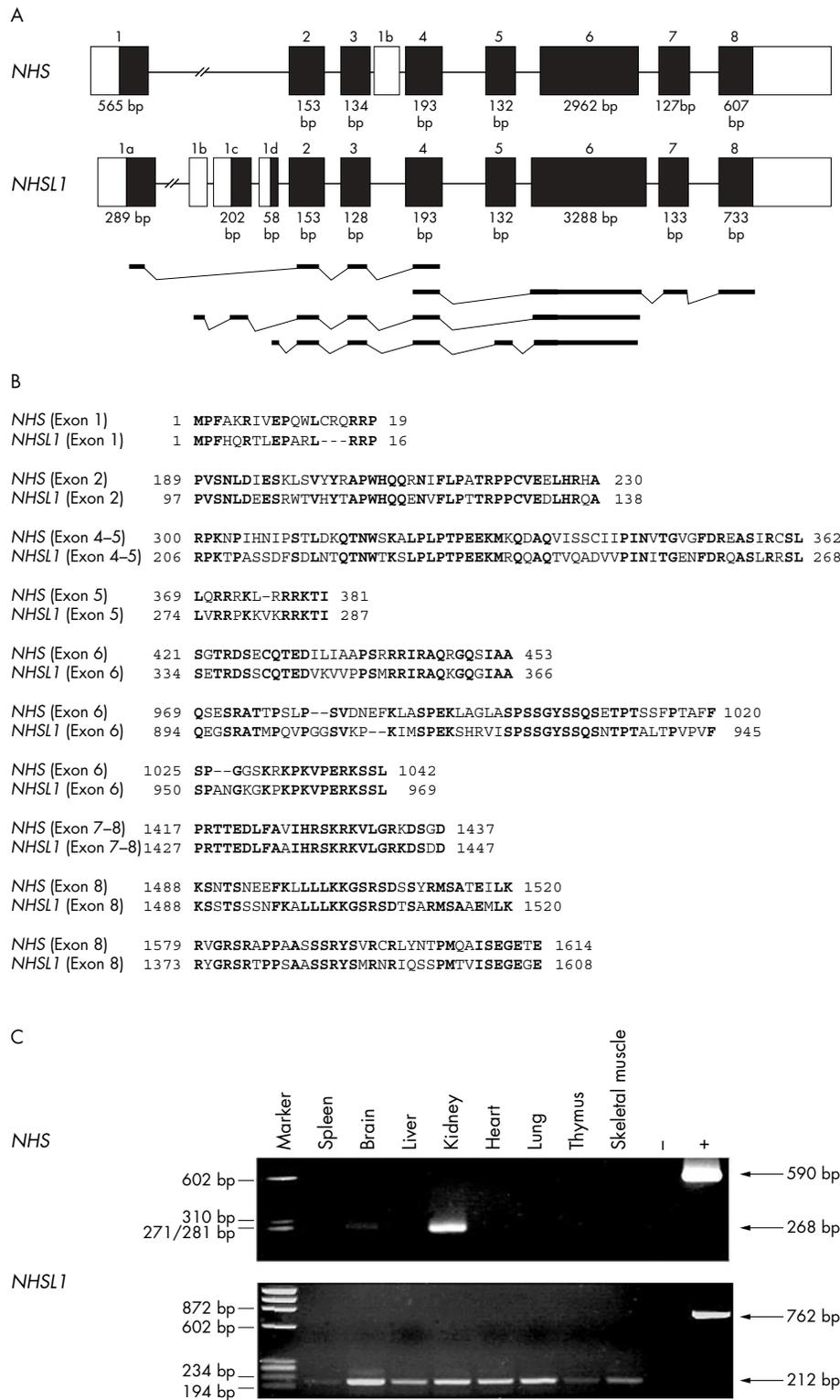**Figure 2** Comparison of *NHS* and *NHSL1* genomic structures, protein sequences, and expression. (A) Genomic structure of the *NHS* gene alongside the predicted genomic structure for *NHSL1* with supporting expression evidence. Blackened segments indicate the open reading frame (ORF) in each gene, with sizes of coding portions of exons below. Expressed sequence tags representing *NHSL1* show alternative 5′ ends and splicing out of exon 5. (B) Partial protein alignment of human NHS and NHSL1, highlighting shared domains. Conserved amino acid residues are in bold. (C) Polymerase chain reaction from human fetal cDNA using primers designed to exon 6 and 7 of the *NHS* and *NHSL1* genes; +, genomic DNA control; −, no DNA control.

As the function of NHS is currently unknown we searched for homologies that may provide functional clues. We detected significant homology to sequence KIAA1357 located on chromosome 6q24.1 (www.ncbi.nlm.nih.gov/ using BLAST algorithms). This sequence was predicted to be a novel gene by computational analysis of supporting mRNA and EST evidence (including UniGene Cluster Hs.170162) from multiple tissues, including adult and fetal brain, fetal eyes, and adult lens, kidney, liver, and intestine. Inspection of the genomic interval (http://genome.ucsc.edu) revealed the structure of this novel gene, hereafter named *NHSL1* (*NHS-Like1*), on chromosome 6, with striking resemblance to the genomic structure of the *NHS* gene (fig 2A).

NHSL1 is predicted to be alternatively spliced with at least four alternative 5′ exons (1a–1d) and a cryptic exon 5. Exon 1a lies 196 kb upstream of exon 2. Similarly, exon 1 of *NHS* is found 350 kb upstream of exon 2. In addition, exon 1a of *NHSL1* has sequence homology at the protein level to *NHS* exon 1 (fig 2B). Exons 2, 4, and 5 of the *NHS* and *NHSL1* genes are identical in size and show a high degree of protein homology (fig 2B). The terminal exons 6, 7, and 8 of both genes are very similar in size and also share protein homology; however, the large exon 6 of both genes is also divergent. Exon 3, again, is a similar size in both genes but encodes different protein sequences. The *NHS* and *NHSL1* genes thus form a new gene family with strikingly similar genomic structure and a high degree of protein homology. We propose, therefore, that *NHS* and *NHSL1* are isospecic heterofunctional paralogs. Furthermore, *REPS1* and *REPS2*, lying telomeric to *NHSL1* and *NHS*, respectively, are close paralogs which share 32% protein sequence identity. This implies that Xp22.13 and 6q24.1 are derived from a duplicated genomic sequence which has undergone significant sequence change throughout evolution but has retained sequence coding for conserved protein domains important to the function of these protein families.

To examine the expression of human *NHSL1*, primers to exon 6 and 7 were designed to amplify a 212 bp fragment in cDNA or a 762 bp fragment in genomic DNA (Fw[5′-AGCAGAGTGGAAGCCAATGTC-3′], Rev[5′- ACCTCATCATT CCCGTCTTCC-3′]). *NHSL1* transcripts were detected in all human fetal cDNA tissues tested (fig 2C). Interestingly, these corresponding exons in *NHSL1* show a more ubiquitous expression than *NHS*.

Conclusions

In summary, we report the independent identification of the gene causative for Nance-Horan syndrome, and extend the number of mutations identified. No mutations were identified in the CXN family, raising the possibility that NHS and CXN are not allelic. Interestingly we have identified a second gene (*NHSL1*) with striking similarity to *NHS*, forming a new gene family lying in paralogous duplicated chromosomal intervals on Xp22 and 6q24. *NHSL1* is more broadly expressed than *NHS*, as transcripts were detected in all fetal samples tested. We also detected additional expression of *NHS* in kidney, lung, and thymus, not reported in the original study.

Further functional characterisation of NHS is now essential to determine the pathological mechanism resulting in cataract-dental syndrome with variable additional symptoms including developmental delay.

ELECTRONIC DATABASE INFORMATION

Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/Omim/ National Centre for Biotechnology Information home page: http://www.ncbi.nlm.nih.gov/

University of California, Santa Cruz home page: http://genome.ucsc.edu

ExPASy tools: http://ca.expasy.org/tools/

Gene and protein analysis using NIX and PIX: http://www.hgmp.mrc.ac.uk/

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Conflicts of interest: none declared

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