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ORIGINAL ARTICLE

Identification of a microdeletion at Xp22.13 in a Taiwanese family presenting with Nance-Horan syndrome

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Nance-Horan syndrome (NHS) is a rare X-linked disorder characterized by congenital cataracts, dental anomalies and mental retardation. The disease has been linked to a novel gene termed *NHS* located at Xp22.13. The majority of pathogenic mutations of the disease include nonsense mutations and small deletions and insertions that lead to truncation of the NHS protein. In this study, we identified a microdeletion of ~0.92 Mb at Xp22.13 detected by array-based comparative genomic hybridization in two brothers presenting congenital cataract, dental anomalies, facial dysmorphisms and mental retardation. The deleted region encompasses the *REPS2*, *NHS*, *SCML1* and *RAI2* genes, and was transmitted from their carrier mother who presented only mild cataract. Our findings are in line with several recent case reports to indicate that genomic rearrangement involving the *NHS* gene is an important genetic etiology underlying NHS.
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INTRODUCTION

Nance-Horan syndrome (NHS) (OMIM 302350) is a rare X-linked genetic disorder characterized by congenital cataracts, dental defects, facial dysmorphism and mental retardation. The disease has been linked to a novel gene termed *NHS* that was mapped to Xp22.13.[1–3] The *NHS* gene comprises at least 10 exons that encompass approximately 650 kb of genomic DNA, and at least three isoforms of *NHS* transcripts have been generated by alternative splicing.[1,4–6] The NHS protein is a putative nuclear protein involved in the regulation of the development of eye, tooth, brain, face and skull,[1,4] although the real function of NHS protein is still not very clear. It is interesting that the most pathogenic mutations associated with NHS are nonsense mutations that result in truncation of the NHS protein.[1,3,7–10] In addition, small deletion and insertion mutations of nucleotide from one to several base pairs at the coding sequences that lead to alteration of reading frame and premature stop codon of the *NHS* gene have also been identified.[1,7,11] The allelic heterogeneity of the *NHS* gene and the clinical phenotype variability of patients with NHS make the study of genotype–phenotype correlations of NHS a challenging task. Recently, copy number variations at the *NHS* gene locus have been found to be associated with patient with NHS or X-linked congenital cataract,[6,12] suggesting that genomic rearrangement also contributes to the genetic etiology underlying the NHS.

In this report, we describe the clinical and genetic analysis of an interstitial microdeletion of ~0.92 Mb at Xp22.13 detected by array comparative genomic hybridization in two brothers presenting with congenital cataracts, dental anomalies, facial dysmorphisms and mental retardation. The microdeletion was transmitted from the carrier mother who had only mild cataract, but no dental anomalies or mental retardation.

MATERIALS AND METHODS

The Taiwanese family was ascertained through the medical genetics clinic of Veterans General Hospital-Taipei. The parents were unrelated, healthy and did not receive medical attention until they delivered two boys affected with congenital cataract and other developmental abnormalities. The etiology of the two affected boys was not verified until recently they were found to have the same interstitial microdeletion at the *NHS* locus as detected by array comparative genomic hybridization. The patients and their parents gave their written consent forms after all the details of the study were fully explained. The study was approved by the Ethics Committee of the Veterans General Hospital-Taipei.

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Array comparative genomic hybridization analysis

Affymetrix genome-wide human SNP 6.0 (Affymetrix, Santa Clara, CA, USA) that contains ~1 850 000 probes was used for copy number variation analysis of genomic DNA. The array data were analyzed and visualized using Genotyping Console 3.0.1 (http://www.affymetrix.com). The experiments were performed in the National Genotyping Center at Academia Sinica, and the experimental procedures followed the protocol provided by the manufacturer.

Fluorescence *in situ* hybridization analysis

Fluorescence *in situ* hybridization analysis was performed on the metaphase chromosome preparation of lymphocytes from the affected family members according to standard protocol. Briefly, BAC clone of RP11–80N6 at Xp22.13, RP11–115I10 at Xp22.13 and RP11–296N8 at Xq28 were grown and BAC DNA was isolated using a Qiagene plasmid isolation kit (Qiagen, Valencia, CA, USA). DNA of RP11–80N6, RP11–115I10 and RP11-379N10 were labeled by nick translation with d-UTP-FITC, d-UTP-Texas Red and d-UTP-diethylaminocoumarin, respectively. Hybridization was carried out in a humidified chamber at 37 °C for at least 16 h. After washing, chromosomes were counterstained with 4′, 6-diamidino-2-phenylindole (Kirkegaard & Perry Laboratories Gaithersburg, MD, USA). Cells were observed under a Zeiss fluorescence microscope (Carl Zeiss, Goettingen, Germany) and images were captured and analyzed using the MetaSystems ISIS workstation (MetaSystems, Altlussheim, Germany).

Real-time quantitative PCR (RT-qPCR)

RT-qPCR was performed using the SYBR Green method and implemented in an ABI Prism 7900HT Sequence Detection System according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). Fragments of the *NHS* gene at Xp22.13 and *MTX2* at 2q31.3 were PCR amplified from each individual, respectively. In brief, two primers (5′-ATTGTGCACACAAACCCA GA-3′ and 5′-CAGAAATGTTGCCAGCAGAA-3′) were used to generate an amplicon of 175 bp from the *NHS* gene, whereas the two primers (5′-AGTAT GGGACCTGTGGGTGA-3′ and 5′-AAGACTCCTGAGACTAACACATAACTC-3′) were used to generate an amplicon of 291 bp from the *MTX2* gene. The RT-qPCR experiment was carried out with the annealing temperature of 60 °C. We used a comparative ddCt method to validate the Xp interstitial deletion. The ddCt of each subject was obtained by subtracting the Ct of *NHS* by the Ct of *MTX2* of the subject, then by dCt of a normal control subject. The relative copy number was finally determined as 2^{-ddCt}. The PCR was carried out in triplicate.

RESULTS

Clinical findings

The pedigree of the family is shown in Figure 1a. The elder brother (II-1) was born at full term with normal birth weight and body length. He was noted to have had congenital cataract, microphthalmia and nystagmus of his both eyes since infancy. He received an operation at the age of 9 months. He was found to have psychomotor retardation from childhood. When he was 7 years old, he received surgical correction for bilateral talipes planus. He was also noted to have bilateral hallux valgus, prominent pads on his fingers and toes and hypotrichosis over inguinal area with incomplete cryptochidism; moreover, his right testis did not fully descend to scrotum. The Wechsler Adult Intelligence Scale-Revised, performed at the age of 17, showed that he had moderate mental retardation, with a full intelligence quotient (IQ) of 57, a verbal IQ of 62 and a performance IQ 56. Report of karyotyping analysis was normal. A brain magnetic resonance imaging study, performed when he was 19 years old, showed that he had normal brain size and parenchyma; the results of an electroencephalogram examination result were normal as well. No history of seizures was noted. He had a long face, a prominent nose and large and anteverted pinnae. He also had screwdriver-shaped incisors and widely spaced teeth.

The younger brother (II-2) was also born at full term with congenital cataracts. He received an operation at the age of 6 months. He was also noted to have psychomotor retardation from childhood. He could not sit well until he reached 2 years of age and started to walk at 2 years and 10 months. He started to speak single words at the age of 3 years and sentences at the age of 6 years. He was also found to have impaired social interactions and stereotyped behaviors, and he met the diagnostic criteria of autism spectrum disorders. Karyotyping analysis was normal. Wechsler Adult Intelligence Scale-Revised performed at the age of 15 years showed that he had moderate mental retardation, with a full IQ of 41, a verbal IQ of 55 and a performance IQ of 35. A brain magnetic resonance imaging study performed when he was 17 years old showed that he had normal brain size and parenchyma; electroencephalogram was also normal. However, he was noted to have scoliosis of T-L spine convexing to right side with a Cobb angle of about 19° from the upper endplate of T4 to the lower endplate of L1. He had bilateral hallux valgus, prominent pads on his fingers and toes and pedis planus bilateral. He also had the same facial dysmorphic features and dental anomalies as his elder brother. Neither of the brothers had an abnormal heart condition or a history of seizures.

The mother (I-2) was found to have normal intelligence and normal dental condition. However, she was found to have a localized posterior subcapsular cataract in her right eye and mild nuclear sclerosis in her left eye.

Molecular cytogenetic analyses

Oligonucleotide-based array comparative genomic hybridization analysis showed a common microdeletion at chromosome Xp in these two brothers (Figure 1c). The deletion was located from nucleotide positions 16 853 030 to 17 768 574 (Build 36.3, 2008), corresponding to cytogenetic band Xp22.13; the estimated deleted length was 915 544 bp, including the following genes: *REPS2*, *NHS*, *SCML1* and *RAI2*. The centromeric breakpoint was located between the *RBBP7* and *REPS2* genes, whereas the telomeric breakpoint was located in intron 1 of the *RAI2* gene. The deletion was inherited from the mother. The deletion was further confirmed by RTq-PCR of the genomic DNA (Figure 1b) and fluorescence *in situ* hybridization analysis (Figure 1d).

DISCUSSION

In addition to nonsense mutations, small deletion and insertion, genomic rearrangements involving the *NHS* gene locus have been reported recently in several patients presenting with symptoms of NHS. Van Esch and colleagues first reported an interstitial deletion of 2.8 Mb at Xp22 comprising *CDLK5* and *NHS* genes in a boy with bilateral congenital cataracts, tetralogy of Fallot, and severe encephalopathy.[12] Coccia and colleagues revealed several genomic rearrangements associated with patients with NHS or X-linked congenital cataracts, including a complex re-arrangement resulting in the truncation of the *NHS* gene at exon 8 in a family with NHS, but unusually mild cataracts; a deletion of ~0.9 Mb encompassing *NHS*, *SCML1*, *RAI2* and *CXorf20* genes in a family with typical features of NHS; a complex duplication–triplication event at the *NHS* locus in a family with X-linked cataract without features of NHS; and a 4.8 kb deletion within intron 1 of the *NHS* gene in a family with X-linked cataracts, but no NHS features.[6] Our findings in this study lend further support that genomic rearrangement involving the *NHS* gene is an important event that is associated with NHS. We summarized our finding and all the reported microdeletions involving the *NHS* locus in Figure 2 for comparison.

The deleted region identified in this study encompasses *REPS2*, *NHS*, *SCML1* and *RAI2* genes. The mechanism of the deletion might be because of the non-allelic homologous recombination mediated by

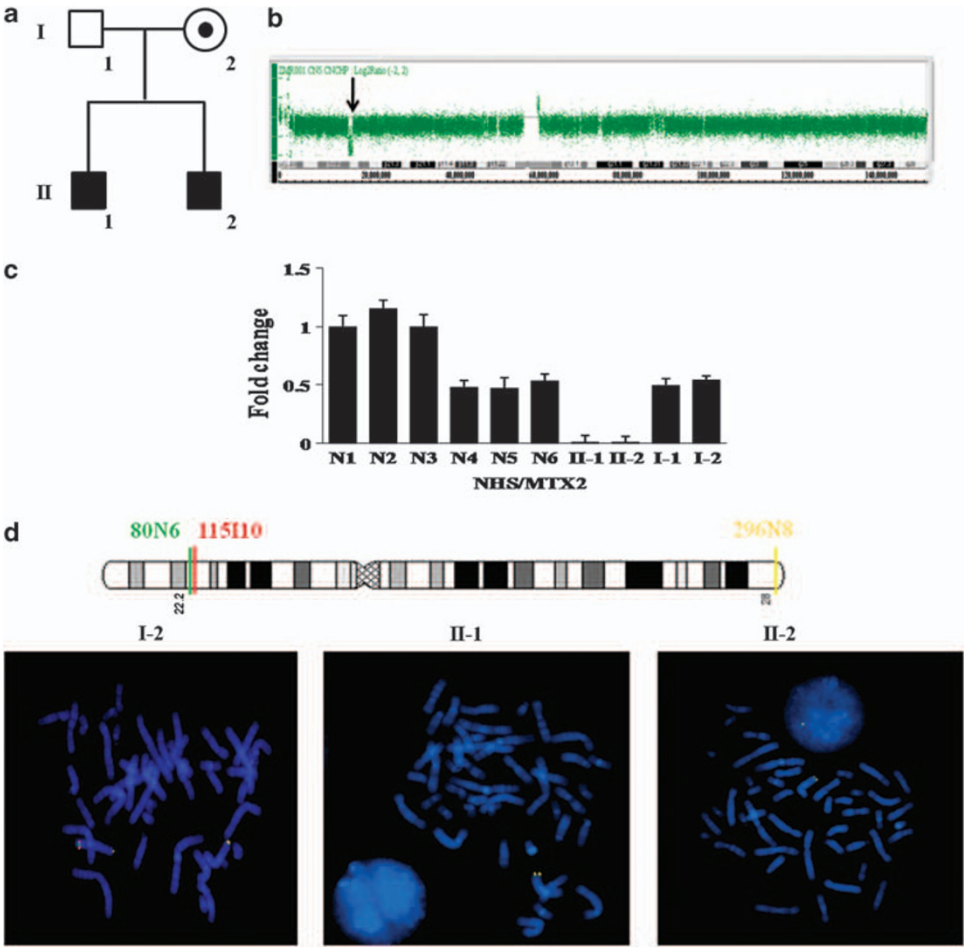

Figure 1 (**a**) The pedigree of the family with two brothers presenting with congenital cataract, dental anomalies, facial dysmorphism and mental retardation. (**b**) An interstitial microdeletion at Xp22.13 (indicated by arrow) was identified in the two brothers and their mother by Affymetrix genome-wide human SNP 6.0. (**c**) The Xp22.13 interstitial deletion in this family was further validated by RTq-PCR. The fold change of the *NHS* gene was normalized by the *MTX2* gene located at 2q31.3 and compared with a normal female control (N1). The *NHS* gene was absent in two affected brothers (II-1 and II-2). The mother (I-2) who was the carrier of the deletion had only one copy of the *NHS* gene, whereas the father (I-1) and three normal male controls (N4–6) had only one copy of the *NHS* gene. N2 and N3 were normal female controls with two copies of the *NHS* gene. (**d**) Fluorescence *in situ* hybridization analysis of metaphase chromosome confirmed the interstitial deletion at Xp22.13 in two brothers and their mother. Two probes prepared from BAC RP11-80N6 (green dots) and 115I10 (red dots) were used for detecting the Xp22.13 interstitial deletion, whereas a probe prepared from BAC RP11-296N8 (brown dots) at Xq28 was used as a reference. An intact chromosome X should have two red dots, two green dots and two brown dots. Absence of red and green dots indicates a deletion at Xp22.13.

the presence of many repetitive sequences in this region, as shown in the UCSC Genome Browser on Human Mar. 2006 (NCBI36/hg18) Assembly (http://genome.ucsc.edu/cgi-bin/hgTracks/). Although the haploinsufficiency of the *NHS* gene may account for the majority of clinical symptoms in the affected brothers, the absence of the other genes in this family may also have affected the clinical symptoms and health conditions of the affected brothers. As retinoic acid is involved in the regulation of cellular differentiation and early embryonal development, the absence of the *RAI2* gene that encodes a protein-induced by the retinoic acid may have contributed to not only foot and bone abnormalities, but also mental retardation in the affected brothers. The *SCML1* gene is preferentially expressed in the germ stem cells of testis and is likely involved in spermatogenesis.[13] The absence of this gene may predict reproduction problem in affected males. The encoded protein of the *REPS2* gene forms a complex with Ral-binding protein 1 and is involved in the regulation of ligand-dependent receptor-mediated endocytosis. Decreased expression of the *REPS2* has been shown to be associated with the loss of control of epidermal growth factor signaling during the progress of prostate cancer.[14,15] Thus, the impact of the absence of the *REPS2* gene on the pathogenesis of the prostate cancer in these two affected brothers needs long-term observation and follow-up.

Mental retardation has been observed in patients with NHS. A previous study reported a frequency of 30% of mental retardation in patients with NHS, and suggested that mental retardation is a major component of NHS.[16] The presence of mental retardation and behavioral symptoms, such as social withdrawal and stereotyped behavior in our patients are consistent with several observations to support that the *NHS* gene is essential for the development of normal intelligence.[6,8] However, not all patients who carry the pathogenic mutations of the *NHS* gene present mental retardation, even within the same family.[1,9] Hence, the role of the *NHS* gene in the pathogenesis of mental retardation remains to be clarified. In this study, the mental retardation can also be attributed to the haploinsufficiency of the *REPS2* gene. *REPS2* gene is highly expressed in the brain tissue (LSBM, http://www.lsbm.org/index.html) and is associated with

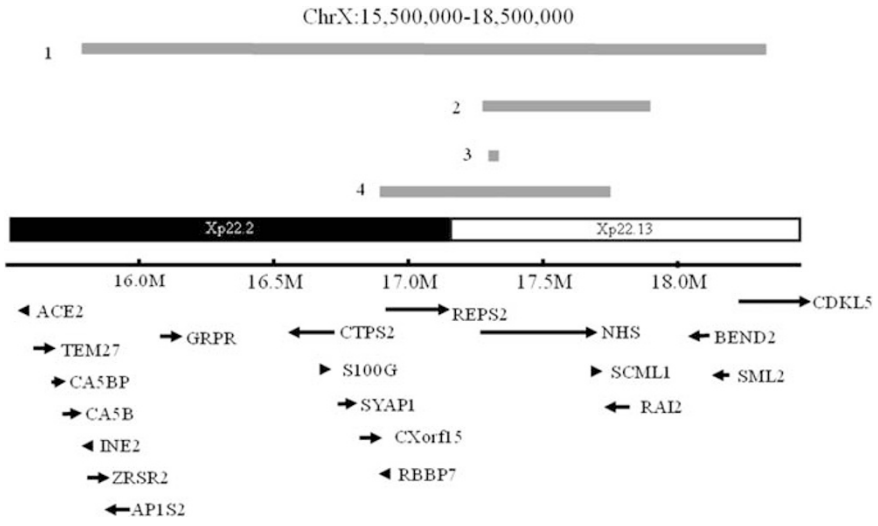

Figure 2 Schematic illustration of the locations and ranges of all the known microdeletions (gray bar) involving the NHS region as reported from Van Esch *et al.*[12] and Coccia *et al.*[6] 1 indicates a deletion of ~2.8 Mb comprising the *NHS* and other genes. 2 indicates a deletion of ~0.9Mb deletion encompassing the exon 2–8 of *NHS* gene, *SCML1*, *RAI2* and *CXorf20* genes. 3 indicates a deletion of ~4.8 kb deletion in the intron 1 of the *NHS* gene. 4 indicates the deletion of ~0.9 Mb deletion reported in the present study. 1 was reported by Van Esch *et al.*,[12] whereas the 2 and 3 were reported by Coccia *et al.*[6]

signaling pathway involving the *Rho* family of small *GTPases*. Alterations in signaling pathways involving the *Rho* family of small *GTPases* contribute to syndromic and nonsyndromic mental retardation,[17] and mutations in the small GTPase gene *RAB39B* (OMIM300774) were also identified in two patients with mental retardation.[18] In this study, magnetic resonance imaging analysis did not reveal structural abnormalities in the brain of these two brothers. Furthermore, the brothers' normal electroencephalograms and lack of history of seizures indicate that this microdeletion does not affect the development of gross brain structure and does not cause epilepsy.

Some patients with genomic rearrangement of *NHS* locus also have presented congenital heart diseases. For example, a boy with an interstitial deletion of Xp22 that contains the *CDLK5* and *NHS* genes presented tetralogy of Fallot.[12] In another family with a complex duplication–triplication at the *NHS* locus, four affected males presented with X-linked congenital cataracts and ductus arteriosus, tetralogy of Fallot, ventriculoseptal defect and stenosis of a major cardiac vessel.[6] In this study, both brothers had normal heart conditions after physical examinations, electrocardiograms, chest X-rays and heart echo studies. Hence, absence of *NHS* gene expression does not appear to result in congenital heart defects. However, our finding cannot rule out the possibility that aberrant expression of the *NHS* gene might be associated with congenital heart diseases in the two examples mentioned above.

The affected brothers had severe clinical symptoms, whereas their mother had only mild cataract and nuclear sclerosis. The mild clinical presentations of the carrier mother might be attributed to the effect of random X chromosome inactivation, or the other unidentified factors that affect the clinical penetrance of the mother.

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