ONLINE MUTATION REPORT

No live individual homozygous for a novel *endoglin* mutation was found in a consanguineous Arab family with hereditary haemorrhagic telangiectasia

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Hereditary haemorrhagic telangiectasia (HHT or Rendu-Osler-Weber syndrome; MIM 187300) is characterised by vascular dysplasia and is inherited in an autosomal dominant manner. HHT occurs among many ethnic groups over a wide geographical area. Recent epidemiological studies have revealed an incidence for this disease of 1 in 5000–8000.[1 2] In most cases, the manifestations of HHT are not present at birth, but develop with age; epistaxis is usually the earliest sign, often occurring in childhood, while mucocutaneous and gastrointestinal telangiectases develop progressively with age.[3] Arteriovenous malformations (AVMs) in the pulmonary, cerebral, or hepatic circulations account for some of the most devastating clinical complications of HHT and are due to direct connections between arteries and veins.[4] The shunting of blood through these lesions can lead to serious complications such as hypoxemia, stroke, brain abscess, heart failure, and fatal haemorrhage.[4 5] Pulmonary and cerebral AVMs can occur in children, while hepatic complications increase with age.

HHT1 is associated with a higher prevalence of pulmonary and cerebral AVMs than HHT2.[6] HHT1 is due to mutations in the *Endoglin* gene (*ENG*; MIM 131195),[7] which codes for a homodimeric integral membrane glycoprotein expressed predominantly on the vascular endothelium. A total of 112 distinct *ENG* mutations distributed throughout the gene have been reported.[8–11]

Mutations in the *ALK*-1 gene (*ACVRL1*; MIM 601284), coding for an activin-like kinase receptor type I of the TGF-β superfamily predominantly expressed in endothelial cells,[6 12–14] are responsible for HHT2. A total of 80 mutations of different types have been identified to date.[10 11]

The underlying mechanism of HHT1 (and probably HHT2) is haploinsufficiency, which implies that a reduction in the amount of protein to half normal levels predisposes to disease and that mutation type or position does not affect the clinical outcome.[15–17] Mice engineered to express a single copy of *Endoglin* ($Eng^{+/-}$) can develop signs of disease including nose and ear bleeds, telangiectases, and cerebral AVMs, as well as serious complications such as internal haemorrhage and stroke.[18–21] However, *Endoglin* null ($Eng^{-/-}$) mice die at embryonic day E10.5, with severe impairment in the development of blood vessels and heart.[18] Mice heterozygous for *Alk-1* can also develop signs of HHT,[22] while *Alk-1* null mice die at mid-gestation of vascular defects.[23] These results demonstrate that both genes responsible for HHT when expressed as single alleles are embryonically lethal in the homozygous state.

There is currently no report of a genetically confirmed case of homozygosity in human HHT. Snyder and Doan[24] reported a newborn with generalised telangiectasia who died at 11 weeks of age due to internal organ haemorrhage as was demonstrated in post mortem examination. Both parents were found to have multiple telangiectasia but no evidence of bleeding or visceral involvement. Muller *et al*[25] later described a large Arab family with 87 affected individuals in six generations and known consanguinity. One individual who had 13 affected children was predicted by statistical analysis to be homozygous for the disease. These two reports were published long before the discovery of causative genes and therefore were not confirmed by mutation analysis. As the clinical diagnosis of HHT is often confounding, one can speculate that it was not definite in all clinically diagnosed individuals. For example, someone with nosebleeds or skin telangiectasia might have been given a positive diagnosis because of the well known family history. We now report the analysis of a second large Arab family with a history of HHT and known consanguinity. We identified a novel *ENG* missense mutation that segregates with the HHT phenotype. No live child homozygous for the mutant allele was found in a marriage between first cousins, supporting the embryonic lethality observed for this phenotype in the mouse model.

Key points

- Mutation analysis was performed in a large Arab family with a known history of hereditary haemorrhagic telangiectasia (HHT) and consanguinity.
- A novel exon 7 missense mutation (c.932T→G) in the *Endoglin* (*ENG*) gene was found in the proband, suggesting HHT1.
- The mutation was present as a single allele in ten relatives with clinical signs of disease but was absent from 21 unaffected family members, indicating that the mutation segregates with the phenotype.
- Marriage between two affected first cousins yielded one normal and four affected children and two miscarriages at 6–8 weeks of gestation.
- We propose that these fetuses were homozygous for the mutant allele, and died in utero at a time when endoglin is essential for cardiovascular development.

METHODS

Patient samples

Informed consent was obtained from all the individuals participating in the study. All procedures were reviewed and approved by the Research Ethics Board of the Research Institute at the Hospital for Sick Children. Positive clinical diagnosis and family history were provided by physicians in Israel and were reviewed by a single geneticist. Confirmed

Abbreviations: AVMs, arteriovenous malformations; HHT, hereditary haemorrhagic telangiectasia

clinical diagnosis was based on the presence of at least three established criteria: epistaxis, telangiectasia, visceral manifestations such as pulmonary, cerebral or hepatic AVMs, gastrointestinal bleeding, and a family history.[26] A number was assigned to each patient and the molecular analysis was performed on 33 members from this family.

Mutation analysis

Genomic DNA was sent from Israel and analysed by exon sequencing as described previously.[27] Sequencing of all exons in the proband revealed a single missense mutation in exon 7. This exon was then sequenced for the 33 family members tested. Products were run on a MicroGene Blaster Sequencer and sequences were automatically analysed using Gene Objects DNA analysis software (Visible Genetics, Toronto, ON, Canada) as described previously.[9] The mutation was identified using the cDNA sequence GenBank: the accession number is AH006911. The mutation has been submitted to the official Hereditary Hemorrhagic Telangiectasia (HHT) Mutation Database at the following address: http://137.195.14.43/genisysDR/NVC/198/Display/index.htm.

RESULTS AND DISCUSSION

A large Israeli-Arab family living in a village in the north of Israel, with known consanguinity, was clinically and molecularly analysed for HHT. Consanguinity is high among Israeli-Arabs as shown in a study reporting 39% consanguinity in the rural Arab population in the north of Israel.[28] The most common type of consanguineous marriage is between paternal first cousins. The pedigree of this family is illustrated in fig 1 and the clinical profiles of affected individuals are summarised in table 1.

Due to the disease severity in this family and the high incidence of cerebral and pulmonary AVMs, we first searched for a mutation in the *ENG* gene. All 15 exons were sequenced for patient 1573. A novel missense mutation (c.932T→G) was found in exon 7 in this patient as well as in nine clinically affected individuals, a 5 year old boy with developmental delay but without HHT manifestations, and a 1 year old asymptomatic boy. The observed segregation of the HHT phenotype with the mutation suggests that this substitution is the disease causing mutation. Such a variant has not been found in more than 200 normal individuals nor has it been reported previously. The mutation leads to the conversion of valine 311 into glycine. This valine residue is conserved in human, pig, and mouse suggesting that it is important for the proper folding of the protein. Mutation to glycine reduces hydrophobicity and size and may destabilise the structure. Our previous studies predict that most endoglin mutations lead to structural alterations as the mutant proteins are not generally expressed.[9 15 27] Missense mutants are present as intracellular precursors and not as mature cell surface functional glycoproteins.[17] Such findings are in agreement with a haploinsufficiency model for HHT1, where reduction in the amount of endoglin is responsible for the disease rather than interference by the mutant with the normal protein.

Table 1 The clinical profiles of individuals from a large Israeli-Arab family with the *ENG* mutation c.932T→G

Patient	Age	Clinical profile
1573	34	E, T, P, C, amnesia, hemiplegia
1576	5	Developmental delay, asymptomatic for HHT manifestations
1628	54	E, T, P, C
1629	6	E
1632	8	E, T
1633	32	E, T, C, G, liver abscess
1637	7	E
1642	56	E, T
1644	31	E, T
1648	33	E, T, C
2698	36	E, T, P, brain abscess
2700	1	Asymptomatic

C, cerebral AVM; E, epistaxis; G, gastrointestinal bleeds; P, pulmonary AVM; T, telangiectases.

One of the severely affected individuals in this family is patient 1628. Now age 54, she has had daily nosebleeds for more than 20 years and was diagnosed with both pulmonary and cerebral AVMs. These were successfully embolised 10 years ago and no major neurological sequelae have been documented. She inherited this disorder from her mother who was reported to have frequent nosebleeds and telangiectasia but no clinically evident cerebral or pulmonary AVMs and who died at age 75.

The two sisters of patient 1628 had a long history of epistaxis but their DNA was not available for molecular analysis. One of them died several years ago at the age of 50 because of intracranial bleeding most likely related to cerebral AVMs and another sister died at the age of 40 subsequent to labour complications. The mutation was identified in two children (1573, 1648) of one of the deceased sisters, demonstrating that she had passed on the familial mutation. In addition, one of her sons also died of HHT cerebral complications at the age of 29. His DNA was not available for analysis, but his medical history revealed telangiectases and monthly episodes of nosebleeds.

Patient 1642 suffers from daily nosebleeds and has skin telangiectases. He has 11 children, but only one son (2698) with documented HHT and one daughter with frequent nosebleeds suspected of HHT, but not tested for the mutation. The son, now 36, had pulmonary AVMs (complicated by a brain abscess 15 years ago), that were successfully embolised 10 years ago. He also has telangiectatic lesions in the oral cavity and infrequent nosebleeds. No clinical signs have been reported for his children, and two (2701 and 1631) were shown not to carry the mutation.

Individual 1628 has two affected daughters. The oldest daughter (1633) has infrequent episodes of epistaxis. She presented 5 years ago with cerebral AVMs and intracranial bleeding which were surgically treated. In addition she had signs of gastrointestinal bleeds and a liver abscess that required repeated surgical drainages.

The other affected daughter (1644) has nosebleeds several times a month and telangiectases. She is treated with iron because of anaemia, as are the other family members who suffer from frequent blood loss. She is married to her first cousin (1648) who also has epistaxis and telangiectases. He presented 4 years ago with cerebral AVMs and was treated surgically. Both parents therefore are clinically affected and carry the mutation. They have five live children, all of them under the age of 8, and a total of seven recorded pregnancies. The two miscarriages occurred in the middle of the first trimester (around 6–8 weeks of gestation) and one of them involved twins. Four out of the five children (1629, 1637, 1632, and 2700) tested positive for the mutation. Three out of the four children carrying the mutation have nosebleeds while the 1 year old boy (2700) has no signs of disease yet. The presence of the mutant allele was observed for all four boys confirming that they were heterozygous while a normal sequence was observed for the 2 year old girl (2699). We performed identity testing on this family by finger printing with eight microsatellite markers, and confirmed that 1644 and 1648 were indeed the parents of the children and that no mistakes had occurred in sample handling.

Although the causes of the miscarriages were unknown and no DNA was available, we propose that *ENG* homozygous

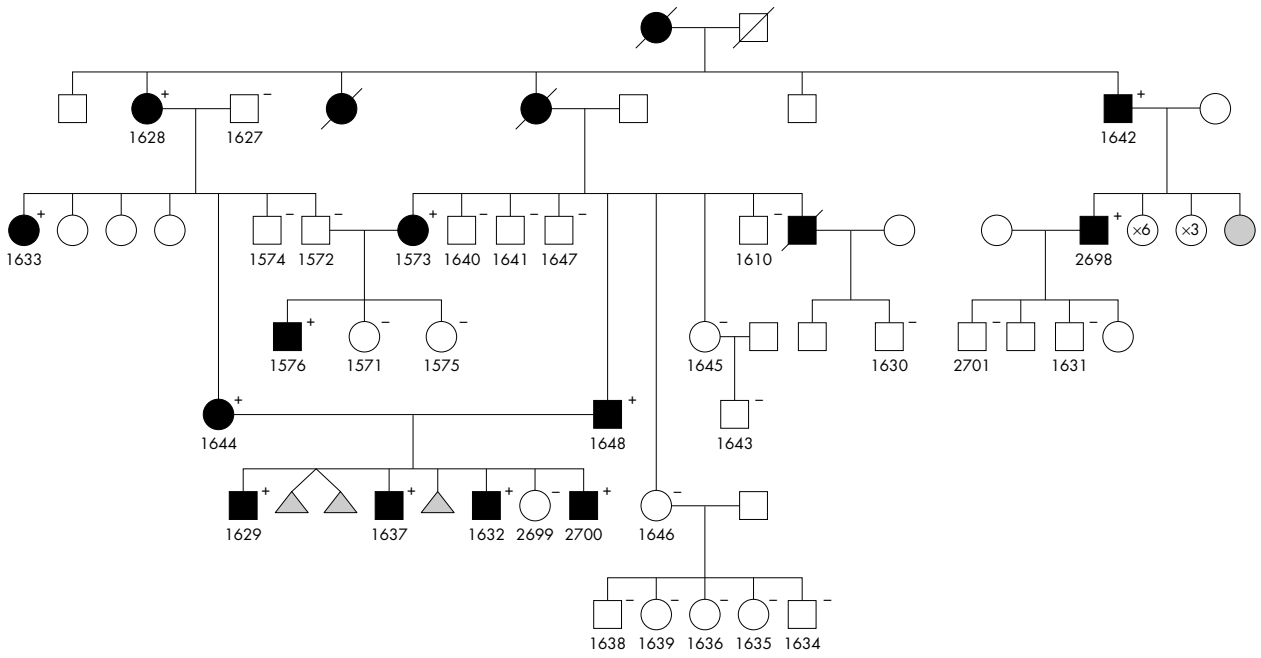

Figure 1 Pedigree of a large Israeli-Arab family with HHT. Phenotype was based on clinical examination and/or molecular screening of all the numbered individuals. Patients diagnosed with HHT are indicated by black symbols, while those suspected of HHT are indicated in grey. Positive signs indicate the presence of the familial mutation and negative signs demonstrate its absence in tested individuals. Squares represent males, circles represent females and triangles represent miscarriages. Deceased patients are indicated with a slash.

mutant human embryos die at 6–8 weeks of gestation. This corresponds to the period when high and transient endoglin expression on mesenchymal cells of cushion tissues of the developing heart atrioventricular and semilunar valves was observed.[29] Fusion of cushion tissues to form the septum intermedium necessary for definition of the heart chambers also occurs at 6–7 weeks of gestation and is associated with high levels of endoglin.[29] Analysis of $Eng^{-/-}$ embryos indicated that endocardial-mesenchymal transformation, which leads to valve formation and heart septation, did not occur; at day E10.5, heart development was completely arrested and extensive necrosis was observed.[18] The murine E10–10.5 stages of heart development parallel those observed in human embryos at 6–8 weeks of gestation. There are also visible defects in yolk sac and embryonic vessel development in the $Eng^{-/-}$ mice that could contribute to lethality at E10.5. Endoglin has been observed on all embryonic human vessels (except for placental vessels) from 4 weeks of gestation[29–31] but it is not well understood when it becomes critical for vascular development. Therefore, lethality could be due to impaired vascular and/or cardiac development.

There is another first cousin union illustrated on the pedigree, between individuals 1572 (brother of 1644) and 1573 (sister of 1648), who have three children. The father (1572) has no signs of disease and does not carry the mutation. His wife has daily nosebleeds and presented with both pulmonary and cerebral AVMs. She suffers from severe neurologic sequelae, including amnesia and hemiplegia. Only one of their three children (1576) carries the mutation, and has developmental delay; metabolic/genetic evaluation, including head imaging, failed to explain the defect. However at the age of 5, he still remains asymptomatic in terms of HHT.

Other siblings of patient 1573, including 1640, 1641, 1647, 1646, 1645, and 1610, all appear not to have the mutation and are free of clinical symptoms. In addition all five children of 1646 and a child of 1645 tested negative for the mutation and are asymptomatic.

Valuable information pertinent to the care of a large family was gained by performing molecular analysis. A mutation was identified in 12 cases that either confirmed the diagnosis inferred from the symptoms or indicated a requirement for future screening for visceral manifestations of disease. We also showed that 21 individuals do not need to be followed further clinically as they do not carry the mutation and therefore do not have HHT. In the case where both parents had the disease, we confirmed that they had the same *ENG* mutation and that four of their five children inherited a single mutated allele. In the case of the nine siblings whose mother had died of disease sequelae, we showed that six children and seven grandchildren were free of mutation and would not have to fear the complications of this disorder. For individual 1642 who has 11 children and only one confirmed with HHT, it would be most informative to test the other siblings molecularly, so that future clinical screening and medical interventions are targeted to those in need.

ELECTRONIC-DATABASE INFORMATION

The URL of the Hereditary Hemorrhagic Telangiectasia (HHT) Mutation Database is http://137.195.14.43/genisysDR/NVC/198/Display/index.htm.

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