DE GRUYTER OPEN

The EuroBiotech Journal

TECHNICAL REPORT
EBTNA UTILITY GENE TEST

Genetic testing for cystic hygroma

Paolo Enrico Maltese[1]*, Yeltay Rakhmanov[1], Alessandra Zulian[2], Angelantonio Notarangelo[3] and Matteo Bertelli[1,2]

Abstract

Cystic hygroma (CH) is characterized by abnormal accumulation of fluid in the region of the fetal neck and is a major anomaly associated with aneuploidy. Morphologically characterized by failure of the lymphatic system to communicate with the venous system in the neck, the clinical manifestations of CH depend on its size and location. Incidence is estimated at one case per 6000-16,000 live births. CH has autosomal dominant or autosomal recessive inheritance. This Utility Gene Test was developed on the basis of an analysis of the literature and existing diagnostic protocols. It is useful for confirming diagnosis, as well as for differential diagnosis, couple risk assessment and access to clinical trials.

Keywords: Cystic hygroma, EBTNA UTILITY GENE TEST

[1]MAGI's Lab, Rovereto, Italy

[2]MAGI Euregio, Bolzano, Italy

[3]Division of Medical Genetics, IRCCS Casa Sollievo della Sofferenza San Giovanni Rotondo, Italy

*Corresponding author: P. E. Maltese
E-mail: paolo.maltese@assomagi.org

DOI: 10.2478/ebtj-2018-0029

Cystic hygroma

(Other synonyms: Cystic hygroma of the neck)

General information about the disease

Cystic hygroma (CH) or hygroma colli is characterized by abnormal accumulation of fluid in the region of the fetal neck and is one of the major anomalies associated with aneuploidy (1, 2). In contrast with simple increased nuchal translucency, CH is considered a possible cause of perinatal disability (3).

CH presents as a single or multiloculated fluid-filled cavity, usually in the neck. Morphologically characterized by failure of the lymphatic system to communicate with the venous system in the neck, it often leads to hydrops and fetal death (4).

Clinical manifestation depends on cyst size and location. CH usually causes functional impairment of nearby structures and organs, as well as disfigurement of affected areas (5, 6). When identified postnatally, it usually appears as a soft bulge under the skin (7). The size of the cavities may increase due to infection or bleeding within the cyst. The cyst may exert pressure on other structures, causing swallowing difficulties, obstructive sleep apnea, airway obstruction, hemorrhage, infection and deformation of surrounding bony structures and teeth (if left untreated) (8).

Prenatal diagnosis of CH via ultrasound is based on demonstration of a bilateral, largely symmetrical, septate or non septate cystic structure in the occipitocervical region (9).

Ultrasonographic diagnosis of septate CH is relatively easy, and chromosome analysis by chorion villus sampling or amniocentesis is generally accepted as a sequential step in its management (3).

The incidence of CH is estimated at one case per 6000-16,000 live births (10). CH can be part of a chromosome disorder or a monogenic syndrome or can manifest as an isolated trait (11). Mendelian CH has autosomal dominant (12, 13) or autosomal recessive

inheritance (14, 15).

Differential diagnosis should consider Meige disease, yellow nail syndrome and capillary, arteriovenous malformations, especially RASopathies.

Autosomal dominant syndromic CH

- Achondrogenesis type II (ACG2, OMIM disease 200610) - *COL2A1* (OMIM gene 120140) (16)
- Cornelia de Lange syndrome-1 (CDLS1, OMIM disease 122470) – *NIPBL* (OMIM gene 608667) (17)
- Lymphedema-distichiasis syndrome (OMIM disease 153400) - *FOXC2* (OMIM gene 602402) (13);
- Noonan syndrome-1 (NS1, OMIM disease 163950) - *PTPN11* (OMIM gene 176876) (18);
- Noonan syndrome-3 (NS3, OMIM disease 609942) - *KRAS* (OMIM gene 190070) (18);
- Noonan syndrome-5 (NS5, OMIM disease 611553) - *RAF1* (OMIM gene 164760) (18).

Autosomal recessive syndromic CH

- Carpenter syndrome-1 (CRPT1, OMIM disease 201000) - *RAB23* (OMIM gene 606144) (19)
- Congenital erythropoietic porphyria (CEP, OMIM disease 263700) – *UROS* (OMIM gene 606938) (20)
- Multiple pterygium syndrome, lethal type (LMPS, OMIM disease 253290) - *CHRNA1* (OMIM gene 100690), *CHRND* (OMIM gene 100720), *CHRNG* (OMIM gene 100730) and *NEB* (OMIM gene 161650) (21, 22)

Since a link may exist between CH and genes involved in angiogenesis and lymphangiogenesis, these genes can be also evaluated (13, 23).

Other likely genes

ADAMTS3 (OMIM gene 605011), AKT1 (OMIM gene 164730), BRAF (OMIM gene 164757), CBL (OMIM gene 165360), CCBE1 (OMIM gene 612753), CELSR1 (OMIM gene 604523), EPHB4 (OMIM gene 600011), FAT4 (OMIM gene 612411), FLT4 (OMIM gene 136352), GATA2 (OMIM gene 137295), GJA1 (OMIM gene 121014), GJC2 (OMIM gene 608803), HGF (OMIM gene 142409), HRAS (OMIM gene 190020), IKBKG (OMIM gene 300248), ITGA9 (OMIM gene 603963), KIF11 (OMIM gene 148760), MAP2K1 (OMIM gene 176872), MAP2K2 (OMIM gene 601263), NRAS (OMIM gene 164790), PIEZO1 (OMIM gene 611184), PIK3CA (OMIM gene 171834), PTPN14 (OMIM gene 603155), RASA1 (OMIM gene 139150), RIT1 (OMIM gene 609591), SHOC2 (OMIM gene 602775), SOS1 (OMIM gene 182530), VEGFC (OMIM gene 601528).

Pathogenic variants may include missense, nonsense, splicing, small insertions, small deletions, small indels, gross insertions, duplications and complex rearrangements.

Aims of the test

- To determine the gene defect responsible for the disease;
- To confirm clinical diagnosis;
- To assess the recurrence risk and perform genetic counselling for at-risk/affected individuals.

Test characteristics

Expert centers/ Published Guidelines

The test is listed in the GTR database, offered by 1 accredited medical genetic laboratories in the US.

Guidelines for clinical use of the test are described in Genetics Home Reference (ghr.nlm.nih.gov).

Test strategy

A multi-gene next generation sequencing panel is used for the detection of nucleotide variations in coding exons and flanking introns of the above genes.

Potentially causative variants and regions with low coverage are Sanger-sequenced. Sanger sequencing is also used for family segregation studies.

Multiplex Ligation Probe Amplification (MLPA) may be used for detection of duplications and deletions in *COL2A1, NIPBL, FOXC2, KRAS, UROS*

To perform molecular diagnosis, a single sample of biological material is normally sufficient. This may be 1 ml peripheral blood in a sterile tube with 0.5 ml K_3EDTA or 1 ml saliva in a sterile tube with 0.5 ml ethanol 95%. Sampling rarely has to be repeated.

Gene-disease associations and the interpretation of genetic variants are rapidly developing fields. It is therefore possible that the genes mentioned in this note may change as new scientific data is acquired. It is also possible that genetic variants today defined as of "unknown or uncertain significance" may acquire clinical importance.

Genetic test results

Positive

Identification of pathogenic variants in the above genes confirms the clinical diagnosis and is an indication for family studies.

A pathogenic variant is known to be causative for a given genetic disorder based on previous reports, or predicted to be causative based on loss of protein function or expected significant damage to proteins or protein/protein interactions. In this way it is possible to obtain a molecular diagnosis in new/other subjects, establish the risk of recurrence in family members and plan preventive and/or therapeutic measures.

Inconclusive

Detection of a variant of unknown or uncertain significance (*VUS*): a new variation without any evident pathogenic significance or a known variation with insufficient evidence (or with conflicting evidence) to indicate it is *likely benign* or *likely pathogenic* for a given genetic disorder. In these cases, it is advisable to extend testing to the patient's relatives to assess variant segregation and clarify its contribution. In some cases, it could be necessary to perform further examinations/tests or to do a clinical reassessment of pathological signs.

Negative

The absence of variations in the genomic regions investigated does not exclude a clinical diagnosis but suggests the possibility of:

- alterations that cannot be identified by sequencing, such as large rearrangements that cause loss (deletion) or gain (duplication) of extended gene fragments;
- sequence variations in gene regions not investigated by this test, such as regulatory regions (5' and 3' UTR) and deep intronic regions;
- variations in other genes not investigated by the present test.

Unexpected

Unexpected results may emerge from the test, for example information regarding consanguinity, absence of family correlation or other genetically-based diseases.

Risk for progeny

If the identified pathogenic variant has autosomal dominant transmission, the probability that an affected carrier transmit the disease variant to his/her children is 50% in any pregnancy, irrespective of the sex of the child conceived.

In autosomal recessive mutations, both parents are usually healthy carriers. In this case, the probability of transmitting the disorder to the offspring is 25% in any pregnancy of the couple, irrespective of the sex of the child. An affected individual generates healthy carrier sons and daughters in all cases, except in pregnancies with a healthy carrier partner. In these cases, the risk of an affected son or daughter is 50%.

Limits of the test

The test is limited by current scientific knowledge regarding the genes and diseases.

Analytical sensitivity (proportion of positive tests when the genotype is truly present) **and specificity** (proportion of negative tests when the genotype is not present)

NGS Analytical sensitivity >99.99%, with a minimum coverage of 10X; Analytical specificity 99.99%.

SANGER Analytical sensitivity >99.99%; Analytical specificity 99.99%.

MLPA Analytical sensitivity >99.99%; Analytical specificity 99.99%.

Clinical sensitivity (proportion of positive tests if the disease is present) **and clinical specificity** (proportion of negative tests if the disease is not present)

Clinical sensitivity: data not available.

Clinical specificity: data not available.

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for CH;

b) the sensitivity of the test is greater than or equal to that of tests described in the literature.

Clinical utility

Clinical management	Utility
Confirmation of clinical diagnosis	Yes
Differential diagnosis	Yes
Couple risk assessment	Yes
Availability of clinical trials can be checked on-line at https://clinicaltrials.gov/	

References

1. Chen CP, Chien SC. Prenatal sonographic features of Turner syndrome. J Med Ultrasound 2007; 15: 251-257.
2. De Vigan C, Baena N, Cariati E, Clementi M, Stoll C. EUROSCAN Working Group. Contribution of ultrasonographic examination to the prenatal detection of chromosomal abnormalities in 19 centres across Europe. Ann Genet. 2001; 44(4): 209-17.
3. Sanhal CY, Mendilcioglu I, Ozekinci M, Yakut S, Merdun Z, Simsek M, Luleci G. Prenatal management, pregnancy and pediatric outcomes in fetuses with septated cystic hygroma. Brazilian Journal of Medical and Biological Research 2014; 47(9): 799-803.
4. Chervenak FA, Isaacson G, Blakemore KJ, Breg WR, Hobbins JC, Berkowitz RL, Tortora M, Mayden K, Mahoney MJ. Fetal cystic hygroma. Cause and natural history. N Engl J Med. 1983; 309(14): 822-5.
5. Cystic hygroma. Genetic and Rare Diseases Information Center (GARD). 2017 Retrieved from: https://rarediseases.info.nih.gov/diseases/6234/cystic-hygroma#ref_13704
6. Lymphatic Malformations. NORD. (2016); Retrieved from: https://rarediseases.org/rare-diseases/lymphatic-malformations/.
7. Cystic hygroma. MedlinePlus. (2015) Retrieved from: http://www.nlm.nih.gov/medlineplus/ency/article/000148.htm.
8. Acevedo JL, Lymphatic Malformation (Cystic Hygroma). (2016) © 1994-2017 by WebMD LLC. Retrivied from: https://emedicine.medscape.com/article/994055-overview
9. Bianca S, Bartoloni G, Boemi G, Barrano B, Barone C, Cataliotti A, Indaco L, Ettore G. Familial nuchal cystic hygroma without fetal effects: Genetic counselling and further evidence for an autosomal recessive subtype. Congenit Anom (Kyoto) 2010; 50(2): 139-40.
10. Kamble V, Bhatia T, Patil Sh. Cystic hygroma with hydrops fetalis: a rare case report. Int J Reprod Contracept Obstet Gynecol 2017; 3(3): 847-50.
11. Tricoire J, Sarramon MF, Rolland M, Lefort G. Familial cystic hygroma. Report of 8 cases in 3 families. Genet Couns 1993; 4(4): 265-9.
12. Rotmensch S, Celentano C, Sadan O, Liberati M, Lev D, Glezerman M. Familial occurrence of isolated nonseptated nuchal cystic hygromata in midtrimester of pregnancy. Prenat Diagn 2004; 24: 260-264.
13. Ghalamkarpour A, Debauche C, Haan E, Van Regemorter N, Sznajer Y, Thomas D, Revencu N, Gillerot Y, Boon LM, Vikkula M. Sporadic in utero generalized edema caused by mutations in the lymphangiogenic genes VEGFR3 and FOXC2. J Pediatr 2009; 155 (1): 90-93.
14. Teague KE, Eggleston MK, Muffley PE, Gherman RB. Recurrent fetal cystic hygroma with normal chromosomes: case report and review of the literature. J Matern Fetal Med 2000; 9(6): 366-369.
15. Baxi L, Brown S, Desai K, Thaker H. Recurrent cystic hygroma with hydrops. Fetal Diagn Ther 2009; 25(1): 127-129.

16. Forzano F, Lituania M, Viassolo A, Superti-Furga V, Wildhardt G, Zabel B, Faravelli F. A familial case of achondrogenesis type II caused by a dominant COL2A1 mutation and „patchy" expression in the mosaic father. Am J Med Genet A 2007; 143A(23): 2815-20.
17. Wilmink FA, Papatsonis DN, Grijseels EW, Wessels MW. Cornelia de lange syndrome: a recognizable fetal phenotype. Fetal Diagn Ther 2009; 26(1): 50-3.
18. Croonen EA, Nillesen WM, Stuurman KE, Oudesluijs G, van de Laar IMBM, Martens L, Ockeloen C, Mathijssen IB, Schepens M, Ruiterkamp-Versteeg M, Scheffer H, Faas BHW, van der Burgt I, Yntema HG. Prenatal diagnostic testing of the Noonan syndrome genes in fetuses with abnormal ultrasound findings. Eur J Hum Genet 2013 Sep; 21(9): 936–42.
19. Haye D, Collet C, Sembely-Taveau C, Haddad G, Denis C, Soulé N, Suc AL, Listrat A, Toutain A. Prenatal findings in carpenter syndrome and a novel mutation in RAB23. Am J Med Genet A. 2014; 164A(11): 2926-30.
20. Pannier E, Viot G, Aubry MC, Grange G, Tantau J, Fallet-Bianco C, Muller F, Cabrol D. Congenital erythropoietic porphyria (Günther's disease): two cases with very early prenatal manifestation and cystic hygroma. Prenat Diagn 2003; 23(1): 25-30.
21. Chen CP. Prenatal diagnosis and genetic analysis of fetal akinesia deformation sequence and multiple pterygium syndrome associated with neuromuscular junction disorders: a review. Taiwan J Obstet Gynecol 2012; 51(1): 12-7.
22. Abdalla E, Ravenscroft G, Zayed L, Beecroft SJ, Laing NG. Lethal multiple pterygium syndrome: A severe phenotype associated with a novel mutation in the nebulin gene. Neuromuscul Disord 2017; 27(6): 537-41.
23. Garabedian MJ, Wallerstein D, Medina N, Byrne J, Wallerstein RJ. Prenatal Diagnosis of Cystic Hygroma related to a Deletion of 16q24.1 with Haploinsufficiency of FOXF1 and FOXC2 Genes. Case Rep Genet 2012; 2012: 490408.
24. ClinicalTrials.gov® [Internet]. U.S. National Library of Medicine.