

Development of a molecular genetic diagnostic service for Cartilage-hair hypoplasia

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Introduction

Cartilage hair hypoplasia (CHH; MIM ID #250250) is a rare autosomal recessive disorder caused by mutations in the untranslated *RMRP* gene (MIM ID *157660) on chromosome 9p13-p12 (encoding the RNA component of mitochondrial RNA-processing endoribonuclease, RNase MRP).

CHH has a wide spectrum of clinical features including variable degrees of short-limbed dwarfism, hair hypoplasia and immunodeficiency. Most patients have some degree of defective cell-mediated immunity and a third of patients show abnormal humoral immunity. Incidence of CHH is estimated at 1.5 in 1000 births in the Amish and 1 in 23 000 in the Finnish population. CHH is very rare in other ethnic groups. Mutations in *RMRP* have also been shown to cause severe anauxetic dysplasia (AD; MIM ID #607095) and the milder phenotype of metaphyseal dysplasia without hypotrichosis (MDWH; MIM ID #250460).

RMRP is the only gene that has been associated with cartilage-hair hypoplasia. Extreme phenotypic variability has been observed, even within the same family. Given the variable phenotype, a definitive diagnosis relies on the molecular test.

RMRP

The *RMRP* gene is untranslated, encoding an RNA, not a protein. RMRP is a small nucleolar (sno) RNA.

RMRP encodes the RNA component of mitochondrial RNA-processing (MRP) endoribonuclease. RNase MRP encodes for structural RNA and plays a role in DNA replication and cell growth.

RMRP has been shown to form a ribonucleoprotein complex with the telomerase reverse transcriptase (TERT) catalytic subunit. This complex exhibits RNA dependent RNA polymerase activity and produces dsRNAs that can be processed to small-interfering RNAs in a Dicer-dependent manner.

Mutations in *RMRP* are found in both the transcribed region and the promoter region (from the TATA box to the transcription initiation site). A founder mutation, c.70A>G, is present in 92% of Finnish and 48% of non-Finnish patients with CHH, however >90 mutations have been identified worldwide.

Referrals

- Referrals are accepted from Consultant Immunologists and Consultant Clinical Geneticists
- Minimum criteria required for testing :
 - Short-limbed, short stature with primary metaphyseal involvement **AND**
 - Fine sparse hair **AND**
 - Malignancy **AND**
 - Cell mediated immune deficiency **OR** Humoral immune deficiency
 - **OR** Affected/carrier first degree relative

Rationale for testing

A European collaborative survey by Bordon *et al* recommended that allogeneic haematopoietic stem cell transplantation (HSCT) should be considered in all CHH patients with severe immunodeficiency/autoimmunity, before the development of severe infections, major organ damage, or malignancy might jeopardise the outcome of HSCT and the quality of life in these patients.

A molecular test is the only way to confirm CHH and allows early diagnosis and management.

Results

This assay was established to provide prenatal diagnosis for a family whose child had been found to be a compound heterozygote by Freiburg University Hospital. The mutations were confirmed, carrier testing of parents performed and subsequent prenatal analysis provided. 6 patients have since been screened by sequence analysis of the promoter region and the transcribed region of *RMRP* in two overlapping fragments.

Mutations detected

Two categories:

1. "promoter mutations" - duplications and insertions in the region between the TATA box and the TIS
 2. Mutations within the *RMRP* transcript
- Nomenclature: numbering is from the transcription initiation site (TIS), reference sequence NR_003051.2

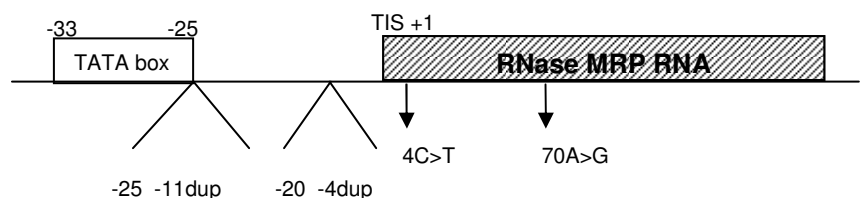


Figure 1: Mutations identified in RMRP

Conclusion

The establishment of this validated service as part of the immunodeficiency service repertoire at GOSH has enabled both prenatal diagnosis and carrier testing of potential donor siblings prior to allogeneic bone marrow transplant.

We currently offer testing for a number of other immunodeficiency syndromes, including Wiskott-Aldrich, severe combined immunodeficiency (SCID), X-linked Hyper IGM, X-linked lymphoproliferative disease and X-linked agammaglobulinaemia.

Testing is also available for the skeletal dysplasias achondroplasia, hypochondroplasia and thanatophoric dysplasia.

A gene dossier for CHH has been accepted by UKGTN.

RMRP sequence analysis: 40 working days turnaround time, £180 charge for NHS patients (index case), £125 for family mutation

References: (1) Worldwide mutation spectrum in cartilage-hair hypoplasia: ancient founder origin of the major 70A->G mutation of the untranslated RMRP, Ridanpää *et al*, Eur J Hum Genet. 2002 Jul;10(7):439-47. (2) Clinical and immunologic outcome of patients with cartilage hair hypoplasia after hematopoietic stem cell transplantation, Bordon *et al*, Blood. 2010 Jul 8;116(1):27-35