Missense mutation in a patient with X-linked dyskeratosis congenita

We report the case of a 40-year-old male patient with dyskeratosis congenita (DKC). Sequencing of the DKC1 gene revealed an inherited missense mutation in base 1050 (GC), changing methionine to isoleucine. This is the third description of a mutation in codon 350 (exon 11), changing a very well conserved amino acid in the pseudouridine synthase (PUA) domain of dyskerin.

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Dyskeratosis congenita is a rare inherited disease characterized by the triad of abnormal skin pigmentation, nail dystrophy and mucosal leucoplakia. Further symptoms of DKC include gastrointestinal, genitourinary, neurological, dental, ophthalmic, pulmonary and skeletal abnormalities. A major cause of death is bone marrow failure [1]. Since the disease affects rapidly dividing somatic tissues in the adult, it is characterized by features of a premature ageing syndrome [2].

The mainly X-chromosomal (Xq28) inherited disease is caused by mutations of the DKC1 gene, which codes for dyskerin. The protein of 514 amino acids is highly conserved and homologous to rat Nap57 and Saccharomyces cerevisae Cbf5p [3]. Dyskerin is the pseudouridine synthase component of the box H/ACA small nucleolar RNAs [4] and appears to play a role in pseudouridination of rRNA [4, 5]. In humans, dyskerin is functionally associated with the RNA component of the telomerase complex [4]. Patients with DKC show shortening of their telomeres in different organs [6, 7]. Since telomere shortening may contribute to the physiological decline that occurs with ageing, symptoms of DKC may be explained by this telomere shortening [2]. The precise function of dyskerin with respect to telomerase activity is still unknown [8].

Several mutations have been described, causing single amino acid substitutions in the encoded protein, dyskerin. The most frequent mutation causes the substitution of alanine to valine at position 353 (A353V) [1, 9].

We describe a 40-year-old male who was referred for evaluation of thrombocytopenia, which had first been observed three years earlier. His family history was unremarkable. On physical examination, the patient appeared older than his stated age. Reticulated and speckled hyper- and hypopigmentation of the skin was seen especially at the upper trunk. His hair was sparse and most finger- and toenails were missing or dystrophic. Examination of the oropharynx revealed buccal and tongue leucoplakias and loss of almost all teeth. Furthermore atresia of lacrimal duct was noted.

Laboratory investigations showed tricytopenia with leukopenia (2.9 x 10^9/l), anaemia (haemoglobin 11.0 g/dl), thrombopenia (75 x 10^9/l). Differential blood count revealed 2% myelocytes, 2% band forms, 58% neutrophils, 2% eosinophils, 20% lymphocytes and 14% monocytes. A bone marrow biopsy showed reduction of haematopoiesis. No chromosomal aberrations were detected. We diagnosed dyskeratosis congenita. To show the X-chromosomal trait and to confirm diagnosis we sequenced the DKC1 gene.

Genomic DNA was extracted from peripheral blood of the patient, the patients mother and an unrelated female control using standard procedures. All fifteen exons and the flanking intronic regions constituting the coding region of human DKC1 gene were amplified with 30 sense- and antisense primers as described [9]. All PCR products were subcloned (Topo TA cloning kit, Invitrogen, Groningen, Netherlands) and sequenced from 3- and 5-site using M13-reverse and T7-promoter primers. Sequencing was performed using the ABI PRISM genetic analyser (PE Applied Biosystems, Foster City, CA, USA). PCR products of exon 11 were amplified by at least three different polymerase chain reactions and beside subcloning PCR products were directly sequenced using sense and antisense amplification primers.

Gene analysis revealed a missense mutation in exon 11 (1050 GC) in patient DNA resulting in the substitution of methionine to isoleucine at position 350 (M350I). Sequence analysis of the mother showed a heterozygous mutation at the same position. The female control showed no mutation (Figure1.).

To our knowledge, this is the first description of a GC mutation in base pair 1050 of the DKC1 gene. Two other missense mutations in codon 350 (1050 GA and 1049 TC) have already been analysed and published [9]. The mutations lead to an exchange of the very well conserved amino acid methionine at this position (Figure 2.).

Beside the common A353V mutation, mutations in codon 350 appear to be the second most frequent in DKC. Both of these mutations of exon 11 localize to the PUA domain (PseudoUridine synthase and Archaeosine-specific transglycosylase) of the protein [1]. Missense mutations in this domain may affect the pseudouridine synthase function of dyskerin which finally results in

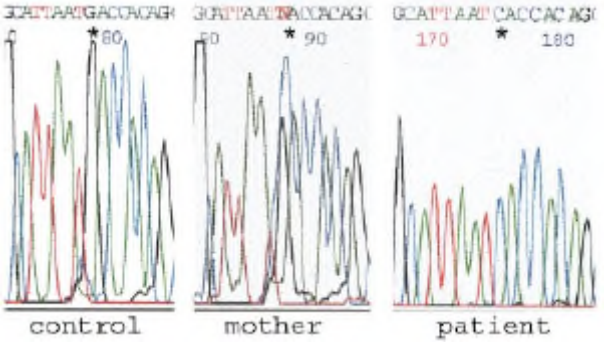

Figure 1. Sequence of PCR-products. The asterisks indicate the position of the mutated base (1050) in a female control (a), the patients mother (b) and the patient (c).

Figure 2. Prosite analysis of PUA-domain (PS50890). Aminoacid 350, methionine, (gray box) is very well conserved in different species (from top to bottom: Aspergillus fumigatus, Candida albicans, Emericella nidulans, Kluyveromyces lactis, Schizosaccaromyces pombe, Saccharomyces cerevisiae, Homo sapiens and Rattus norvegicus).

DKC. These data are in agreement with recent data of hypomorphic Dkc1 mutant mice, which were impaired in ribosomal RNA pseudouridylation before the onset of DKC [10].

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References

1. Dokal I. Dyskeratosis congenita in all its forms. Br J Haematol 2000; 110:768-79.
2. Marciniak RA, Johnson FB, Guarente L. Dyskeratosis congenita, telomeres and human ageing. Trends Genet 2000; 16:193-5.
3. Heiss NS, Knight SW, Vulliamy TJ, Klauck SM, Wiemann S, Mason PJ, et al. X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. Nat Genet 1998; 19:32-8.
4. Mitchell JR, Cheng J, Collins K. A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3' end. Mol Cell Biol 1999; 19:567-76.
5. Wang C, Query CC, Meier UT. Immunopurified small nucleolar ribonucleoprotein particles pseudouridylate rRNA independently of their association with phosphorylated Nopp140. Mol Cell Biol 2002; 22:8457-66.
6. Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. Nature 1999; 402:551-5.
7. Vulliamy TJ, Knight SW, Mason PJ, Dokal I. Very short telomeres in the peripheral blood of patients with X-linked and autosomal dyskeratosis congenita. Blood Cells Mol Dis 2001; 27:353-7.
8. Collins K, Mitchell JR. Telomerase in the human organism. Oncogene. 2002; 21:564-79.
9. Knight SW, Heiss NS, Vulliamy TJ, Greschner S, Stavrides G, Pai GS, Lestringant G, Varma N, Mason PJ, Dokal I, Poustka A. X-linked dyskeratosis congenita is predominantly caused by missense mutations in the DKC1 gene. Am J Hum Genet. 1999; 65:50-8.
10. Ruggero D, Grisendi S, Piazza F, Rego E, Mari F, Rao PH, Cordon-Cardo C, Pandolfi PP. Dyskeratosis congenita and cancer in mice deficient in ribosomal RNA modification. Science. 2003; 299:259-62.