Mutations in the Chediak–Higashi syndrome gene (CHS1) indicate requirement for the complete 3801 amino acid CHS protein

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INTRODUCTION

Chediak–Higashi syndrome (CHS) [MIM #214500] is a rare autosomal recessive disorder characterized by hypopigmentation or albinism, mild bleeding tendency, progressive neurologic abnormalities and severe immunodeficiency with lack of natural killer (NK) cell activity, resulting in frequent pyogenic infections. Unless treated by bone marrow transplantation, death usually occurs in childhood from infection, hemorrhage or an unusual lymphoma-like lymphohistiocytic syndrome: the so-called ‘accelerated phase’ of the disorder; though some patients have a relatively mild clinical course and survive to adulthood with few or even no severe infections (1–5).

The hallmark of CHS is giant organelles—lysosomes, melanosomes and giant inclusion bodies—seen in virtually all granulated cells, particularly granulocytes of the peripheral blood and bone marrow. These ultrastructural abnormalities are associated with defective compartmentalization of many different protein components of lysosomes and of various types of cytoplasmic granules (6,7). Together, these findings suggest that CHS may involve either defective trafficking of specific proteins to various organelles or defective retention of these proteins by the organelles. It thus seems likely that the CHS1 gene product is in some way required for the normal genesis, structure or function of a variety of intracellular organelles: melanosomes, lysosomes and intracellular secretory granules (8–10).

Disorders similar to human CHS occur in many mammalian species, most importantly, the beige mouse (11), long considered a likely homologue to human CHS. This was supported by the mapping of the CHS1 gene to chromosome 1q43 in a segment homologous to the beige gene region of mouse chromosome 13 (12,13). Subsequently, two groups (14,15) identified partial mouse beige cDNAs; however, these cDNAs were quite different and did not overlap. Recently, we characterized full-length human CHS1 cDNA that was homologous to mouse beige (16). This 13.5 kb cDNA encompassed both of the partial mouse beige cDNAs (14,15) and encoded a predicted 3801 amino acid polypeptide. Furthermore, we identified pathologic mutations of the gene in patients with CHS (16). Similarly, Barbosa and co-workers (14) also identified a pathologic mutation in the CHS1 gene (which they termed ‘LYST’), but argued that the functional CHS1 mRNA consisted of just the 3–4 kb 5′-terminal segment.

Here we describe analysis of two of the original inbred probands we used for homozygosity mapping of the CHS1 locus to 1q42–q44 (13) and the detection of two novel homozygous CHS1 gene frameshifts in these patients. Most importantly, one of these frameshifts involves codon 3197, strongly supporting our previous assertion (16) that the predicted 3801 amino acid polypeptide encoded by the 13.5 kb cDNA represents the functional CHS protein.

RESULTS

For both of the patients studied, the parents were first-cousins; thus, each patient was homozygous by descent for a different

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pathologic mutation (Fig. 1). Patient 1 was found to be homozygous for a one base insertion (adenine) at codons Lys633/Lys634 (in a cluster of six adenine residues), resulting in a frameshift and consequent premature translational termination at codon 638. His parents were both found to be heterozygous for this mutation. Patient 2 was found be homozygous for a single base deletion (adenine) within codon Tyr3197 (TAT), resulting in a frameshift and consequent translational termination at codon 3258. His parents were both found to be heterozygous for this mutation. Neither of these mutations were identified by SSCP/heteroduplex screening of 54 unrelated individuals of Middle Eastern origin, nor were they found in 17 other unrelated patients with CHS. Thus, neither of these mutations is likely to be a non-pathologic polymorphism.

**DISCUSSION**

Our recent identification of the human CHS1 gene (16) demonstrated a 13.5 kb cDNA that corresponded closely to the predominant ~12 kb mRNA observed in many tissues (14,15). The human CHS1 cDNA contained an 11.4 kb open reading frame that predicted a 3801 amino acid CHS polypeptide, the specific function of which is as yet unknown. This conflicts with the findings of Barbosa et al. (14) who argued that the functional CHS1 mRNA (which they termed ‘LYST’) corresponded to just the 3–4 kb 5′-terminal cDNA segment.

Three different CHS1 gene mutations have been reported previously: two frameshifts, at codons 40 (14,16) and 489 (16), and one nonsense mutation at codon 1103 (16). In this report we have described two additional frameshifts, at codons 633/634 and 3197, both associated with typical, clinically severe, CHS. The codon 3197 frameshift is of particular importance, as it is located far distal to the limited coding region predicted by Barbosa et al. (14), but would truncate the 3801 amino acid CHS polypeptide predicted by Nagle et al. (16) after 84% of its length. Homozygosity for the codon 3197 frameshift results in the same fatal early-onset disease phenotype as more proximal mutations (16), proving that the complete 3801 amino acid CHS protein is required for function. Furthermore, it is of some interest that only nonsense mutations and frameshifts of the CHS1 gene have been identified so far, resulting in presumed null alleles, associated both with clinically mild and clinically severe CHS. Though this might be the result of a small sample, it might also be that missense substitutions in the CHS1 gene result in a much milder or even different clinical phenotype.

**MATERIALS AND METHODS**

**Patients**

Patient 1 was a Kuwaiti Bedouin boy with typical severe childhood CHS, with silvery hair and oculo-cutaneous albinism, recurrent pyogenic infections, cervical lymphadenopathy, hepatosplenomegaly, neutropenia, mild thrombocytopenia and low serum IgG (17). Typical cytoplasmic giant granules were seen in peripheral blood leukocytes, and a skin biopsy showed large irregular melanin granules in the melanocytes. His parents were first-cousins. Patient 2 was a Turkish boy also with typical severe childhood CHS, now deceased. His parents were first-cousins (13). Both of these patients had previously been used to assign the CHS1 locus to 1q42–q44 by homozygosity mapping (13).

**Mutation analysis**

High molecular weight genomic DNA was prepared from peripheral blood leukocytes of the CHS patients, their parents and normal controls by standard methods. The genomic DNA sequence of most, though not all, of the human CHS1 gene has been determined (our unpublished data), permitting the design of PCR primers from intronic sequences in most cases. DNA fragments spanning exonic sequences of the CHS1 gene were amplified by the PCR using various combinations of a very large number of primers derived either from the cDNA or genomic DNA sequences, and were used to carry out simultaneous single strand conformation polymorphism (SSCP)/heteroduplex screening of most, though not all, of the CHS1 coding region and splice junctions (18). PCR products exhibiting abberant SSCP patterns were either sequenced directly using the Sequitherm™ Cycle Sequencing Kit (Epicentre Technologies) or were cloned using the TA Cloning Kit (InVitrogen), and sequenced manually using the Sequenase Version 2.0 Sequencing Kit (USB). In both cases, multiple clones or replicate PCR products from the patients and unrelated normal individuals were sequenced completely on both strands. Analogous PCR products spanning the relevant mutation were also amplified from DNA of the patients’ parents and subjected to SSCP/heteroduplex screening. In addition, PCR products spanning both mutations were amplified in duplex from DNA of the two probands, 54 unrelated individuals of Middle Eastern origin and 17 additional unrelated patients with CHS, and were also subjected to SSCP/heteroduplex screening.

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Figure 1. CHS1 gene mutations and the CHS polypeptide. Diamonds represent frameshifts; the circle a nonsense mutation. Asterisks indicate the Lys633/Lys634 and Tyr3197 frameshifts described here. Green, hydrophobic motifs analogous to HEAT and ARM repeats; orange, BEACH domain; blue/violet, likely/significant WD40 repeats (after ref. 16).
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