Mutations in a new member of the chromodomain gene family cause CHARGE syndrome

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CHARGE syndrome is a common cause of congenital anomalies affecting several tissues in a nonrandom fashion. We report a 2.3-Mb *de novo* overlapping microdeletion on chromosome 8q12 identified by array comparative genomic hybridization in two individuals with CHARGE syndrome. Sequence analysis of genes located in this region detected mutations in the gene *CHD7* in 10 of 17 individuals with CHARGE syndrome without microdeletions, accounting for the disease in most affected individuals.

CHARGE syndrome is a nonrandom pattern of congenital anomalies including choanal atresia and malformations of the heart, inner ear and retina¹ (Table 1). With an estimated birth incidence of 1:12,000, CHARGE syndrome is a common cause of congenital anomalies². Most cases of CHARGE syndrome are sporadic, but several aspects of this condition, including the existence of rare familial cases and a high concordance rate in monozygotic twins, support the involvement of a genetic factor. Cytogenetic abnormalities have been described previously, but no specific locus has been identified³. Systematic genome scans by conventional comparative genomic hybridization (CGH)⁴ or microsatellite analysis³ did not identify a common genetic anomaly; neither did sequencing of candidate genes *PAX2* (ref. 5) and *PITX2* (ref. 6).

We recently optimized array CGH⁷ for high-resolution genome-wide screening of submicroscopic copy-number changes⁸ and used this approach to identify microdeletions or duplications underlying CHARGE syndrome. We cohybridized genomic DNA from two individuals with CHARGE syndrome with normal reference DNA onto a genome-wide BAC array with 1-Mb resolution (Supplementary Methods online). The genome-wide array CGH profile from one of these individuals is shown in Figure 1a. The only clones

reproducibly deleted in this individual map to chromosomal band 8q12 and encompass a genomic interval of ~5 Mb. We confirmed the deletion by fluorescence *in situ* hybridization (FISH) analysis and proved that it occurred *de novo* (Supplementary Fig. 1 online). The second individual with CHARGE syndrome included in this pilot study had no microdeletion or microduplication.

To further characterize the deletion in the index individual and to screen additional individuals for abnormalities of chromosome 8, we established a tiling resolution chromosome 8 array containing 918 overlapping BAC clones. After hybridizing DNA from the index individual onto this array (Fig. 1b), we detected a deletion of 31 overlapping clones spanning a region of 4.8 Mb on 8q12, extending from RP11-44D19 to RP11-274C23 (Supplementary Fig. 1 online).

Notably, an individual with CHARGE syndrome with an apparently balanced chromosome 8 translocation was previously reported⁹. Hybridization of genomic DNA from this person onto the chromosome 8 BAC array detected two microdeletions overlapping with the one that we identified in the index individual (Fig. 1b and Supplementary Fig. 1 online): one encompassing 6 overlapping clones (from RP11-44D19 to RP11-661A3, ~0.8 Mb) and one encompassing 11 overlapping clones (from RP11-51L11 to RP11-113D4, ~1.5 Mb). Between these two deleted regions, 6 clones (~0.9 Mb) showed normal test-over-reference ratios. We used metaphase FISH analysis to confirm the presence of and determine the boundaries of the two distinct microdeletions in this person (Supplementary Fig. 1 online). Although we could not verify *de novo* occurrence of the microdeletions in this case, the translocation was previously shown to be *de novo*⁹.

Using data from these two individuals, we defined a shortest region of deletion overlap encompassing 2.3 Mb of genomic sequence on 8q12 (Fig. 1c). We then screened 17 additional individuals with CHARGE syndrome using the chromosome 8 tiling array and detected no additional chromosome 8 copy-number changes. Next, we sequenced the coding regions and the intron-exon boundaries of all nine annotated or predicted genes located in or just outside the shortest region of deletion overlap (Fig. 1c). We identified ten heterozygous mutations in the gene *CHD7*, including seven stop-codon mutations, two missense mutations and one mutation at an intron-exon boundary (Fig. 1d,e and Table 1). *CHD7* consists of 38 exons and has a genomic size of 188 kb. The stop-codon mutations were scattered throughout the gene: two in exon 2, one in exon 3, one in exon 26, two in exon 30 and one in exon 35. The two *de novo* missense mutations are predicted to lead to the amino acid substitutions I1028V (in exon 12) and L1257R (in exon 15). We observed one *de novo* mutation 7 bp upstream of exon 26 that could possibly affect splicing (IVS26-7G→A).

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^aAnomalies on computed tomography scan of inner ear (semicircular canal agenesis). ^bFrom ref. 9. ^cOnly one parent available for testing, no mutation identified. F female; M, male; U, unknown.

Microdeletions encompassing the underlying gene have been reported to occur at low frequencies in single-gene disorders^{14,15}. We show that high-resolution genome-wide screening by array CGH is an effective new approach to localize such underlying genes. This approach is of particular interest for sporadic malformation syndromes that cannot be tackled by other mapping approaches because of reproductive lethality.

Figure 1 Array CGH profiles of two individuals with CHARGE syndrome, detailed genomic view of 8q12, organization of *CHD7* and mutations detected. (a) Array CGH genome-wide profile of the index individual with a copy-number deletion of three adjacent clones on 8q12 (arrow). This profile represents the result of a single hybridization experiment; analysis of the replicate experiment identified only the 8q12 clones as being reproducibly deleted. Vertical lines indicate chromosome boundaries. (b) Profile of the same individual (brown squares) on the tiling chromosome 8 BAC array with 31 clones characterizing the deletion, and the chromosome 8 profile of an individual (black circles) with an apparently balanced t(6;8) translocation that overlaps with the deletion of the index individual. (c) Clones are ordered on the x axis according to physical mapping positions; $\log_2$ -transformed test-over-reference (T/R) ratios for each clone are given on the y axis. (d) Transcript map of the deleted 8q12 genomic region. The shortest region of deletion overlap in the two individuals is shown. Genes in green were screened for mutations. Cen, centromeric; Tel, telomeric. (e) Genomic structure of *CHD7* indicating the positions of seven nonsense mutations (circles), two missense mutations (squares) and one intron-exon boundary mutation (triangle). The corresponding functional *CHD7* domains are marked (colored bars). (f) Partial electropherograms obtained by direct sequencing of PCR products showing two nonsense mutations in individual 5 (1714C→T) and individual 8 (5418C→G) and one missense mutation in individual 7 (3770T→G).

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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