nature genetics

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

Lisenka E L M Vissers[1], Conny M A van Ravenswaaij[1], Ronald Admiraal[2], Jane A Hurst[3], Bert B A de Vries[1], Irene M Janssen[1], Walter A van der Vliet[1], Erik H L P G Huys[1], Pieter J de Jong[4], Ben C J Hamel[1], Eric F P M Schoenmakers[1], Han G Brunner[1], Joris A Veltman[1] & Ad Geurts van Kessel[1]

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 retina[1] (**Table 1**). With an estimated birth incidence of 1:12,000, CHARGE syndrome is a common cause of congenital anomalies[2]. 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 identified[3]. Systematic genome scans by conventional comparative genomic hybridization (CGH)[4] or microsatellite analysis[3] did not identify a common genetic anomaly; neither did sequencing of candidate genes *PAX2* (ref. 5) and *PITX2* (ref. 6).

We recently optimized array CGH[7] for high-resolution genome-wide screening of submicroscopic copy-number changes[8] 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 reported[9]. 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).

[1]Departments of Human Genetics and [2]Otorhinolaryngology, University Medical Center Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands. [3]Department of Clinical Genetics, The Churchill Hospital, Old Road, Headington, Oxford OX3 7LJ, UK. [4]Children's Hospital Oakland Research Institute, BACPAC Resources, 747 52nd Street, Oakland, California 94609-1809, USA. Correspondence should be addressed to J.A.V. (j.veltman@antrg.umcn.nl).

Published online 8 August 2004; doi:10.1038/ng1407

Table 1 Clinical characteristics of individuals with CHARGE syndrome and mutations in *CHD7*

		Main features						Associated features					
Individual	Sex	Coloboma	Heart malformation	Atresia of choanae	Retardation of growth and development	Genital hypoplasia	Ear abnormality including deafness	Vestibulo-cochlear[a]	Facial nerve palsy	Cleft lip/palate	DNA mutation	Exon	Protein consequence	*De novo*?
1	F	+	+	–	+	–	+	+	–	–	Del 8q12 region			Yes
2[b]	F	+	+	–	+	–	+	U	+	–	Del 8q12 region			U
3	F	+	+	–	+	–	+	U	–	+	469C→T	2	R157X	U
4	F	–	+	+	+	–	+	U	–	–	1078G→T	2	G360X	U[c]
5	M	–	–	–	+	+	+	+	+	+	1714C→T	3	Q572X	Yes
6	F	+	–	–	+	–	+	+	–	–	3082A→G	12	I1028V	Yes
7	F	+	–	–	+	+	+	+	–	–	3770T→G	15	L1257R	Yes
8	F	–	+	+	+	+	+	+	–	–	5418C→G	26	Y1806X	Yes
9	M	+	–	–	+	+	+	U	–	+	6051T→A	30	C2017X	U
10	F	+	+	–	+	–	+	+	+	–	6070C→T	30	R2024X	U[c]
11	F	–	+	+	+	–	+	+	–	–	7824T→A	35	Y2608X	Yes
12	M	+	+	–	+	+	+	+	–	+	IVS26-7G→A	26	Splice site	Yes
13	F	+	+	+	+	+	+	+	–	+				
14	F	+	–	+	+	–	+	U	–	+				
15	M	+	–	–	+	–	+	+	–	–				
16	F	+	+	–	+	+	+	+	–	+				
17	F	–	+	–	+	+	+	+	+	–				
18	M	+	+	–	+	+	+	U	+	+				
19	M	+	+	+	+	+	+	+	–	–				

[a]Anomalies on computed tomography scan of inner ear (semicircular canal agenesis). [b]From ref. 9. [c]Only one parent available for testing, no mutation identified. F, female; M, male; U, unknown.

cDNA clone KIAA1416 (ref. 10), encompassing the last 34 exons of *CHD7*, is a member of the chromodomain helicase DNA-binding (CHD) genes. CHD proteins belong to a superfamily of proteins that have a unique combination of functional domains, including two N-terminal chromodomains, a SNF2-like ATPase/helicase domain and a DNA-binding domain[11] (**Fig. 1d**). This class of proteins is thought to have pivotal roles in early embryonic development by affecting chromatin structure and gene expression[12]. The congenital malformations found in CHARGE syndrome have their origin in early embryonic development. *CHD7* has ubiquitous expression in several fetal and adult tissues (**Supplementary Fig. 2** online), including those affected in CHARGE syndrome.

The identification of seven heterozygous *CHD7* stop-codon mutations and two single-copy 8q12 deletions of *CHD7* indicate that haploinsufficiency of this gene could account for most cases of CHARGE syndrome. Two amino acid changes are located in one of the functional domains of CHD7, the SNF2 domain, and both affect a conserved amino acid (**Supplementary Fig. 3** online). We did not observe any overt phenotypic difference between individuals with 8q12 deletions and those with nonsense or missense mutations in *CHD7* (**Table 1**). The index individual, with a 4.8-Mb deletion, has relatively severe mental retardation, which may be due to the deletion of genes adjacent to *CHD7*. In seven of the individuals with CHARGE syndrome that we studied, we identified no *CHD7* mutations or deletions. This might be due to the presence of intronic or promoter mutations or to whole-exon deletions. CHARGE syndrome might also have a genetically heterogeneous etiology, as different genomic abnormalities have been identified in affected individuals[3,4]. Notably, monosomy with respect to 22q11.2 has been reported to occur in individuals with features of both DiGeorge syndrome and CHARGE syndrome[13]. Deletion of 22q11.2 was excluded in the individuals in this study who did not have *CHD7* deletions or mutations.

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.

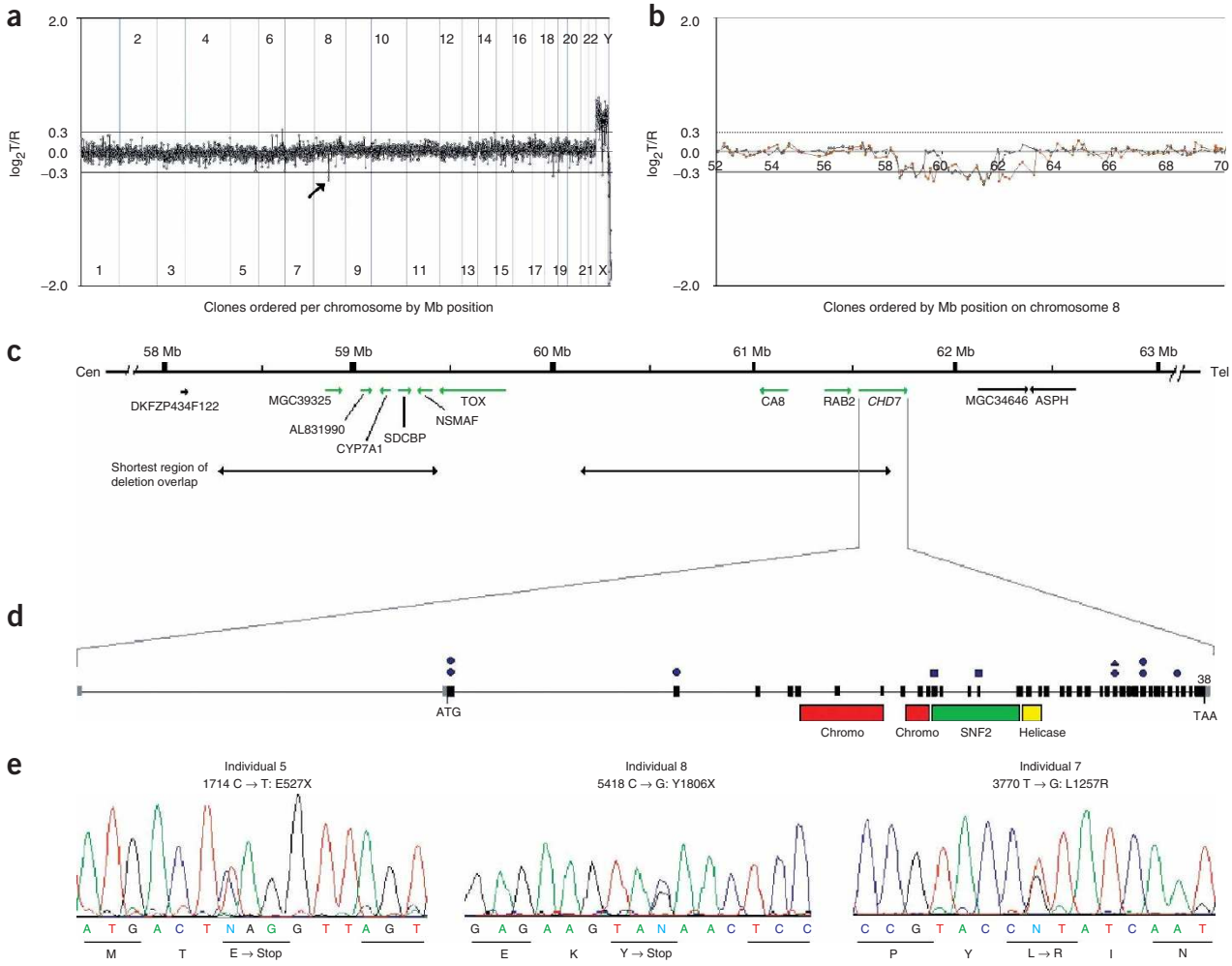

Figure 1 Array CGH profiles of two individuals with CHARGE syndrome, detailed genomic view of 8q12, organization of *CHD7* and mutations detected. (**a**) Array CGH genome-wide profile of the index individual with a copy-number deletion of three adjacent clones on 8q12 (arrow). This profile represents 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. (**a**,**b**) 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. (**c**) 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. (**d**) 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). (**e**) 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).

ACKNOWLEDGMENTS

We thank the affected individuals and their parents for their participation, H. Straatman and M. Leisink for statistical assistance, E. van Wijk and M. Sweers for technical assistance, E. Bongers for collecting samples from affected individuals, the sequence facility of our department and MACROGEN for sequencing, K. Osoegawa for participation in the production of the microarrays and M. Marra and M. Krzywinski for suggestions relating to the minimal BAC tiling set. This work was supported by grants from the Netherlands Organisation for Health Research and Development (B.B.A.d.V., H.G.B. and J.A.V.).

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 26 April; accepted 29 June 2004
Published online at http://www.nature.com/naturegenetics/

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