LETTER TO JMG

Confirmation of CHD7 as a cause of CHARGE association identified by mapping a balanced chromosome translocation in affected monozygotic twins

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Background: CHARGE syndrome has an estimated prevalence of 1/10 000. Most cases are sporadic which led to hypotheses of a non-genetic aetiology. However, there was also evidence for a genetic cause with reports of multiplex families with presumed autosomal dominant, possible autosomal recessive inheritance and concordant twin pairs. We identified a monozygotic twin pair with CHARGE syndrome and a de novo balanced chromosome rearrangement t(8;13)(q11.2;q22).

Methods: Fluorescence in situ hybridisation was performed with BAC and PAC probes to characterise the translocation breakpoints. The breakpoint on chromosome 8 was further refined using 10 kb probes we designed and produced using sequence data for clone RP11 33111, the Primer3 website, and a long range PCR kit.

Results: BAC and PAC probe hybridisation redefined the breakpoints to 8q12.2 and 13q31.1. Probe RP11 33111 spanned the breakpoint on chromosome 8. Using our 10 kb probes we demonstrated that the chromodomain gene CHD7 was disrupted by the translocation between exons 3 and 8.

Discussion: Identifying that the translocation breakpoint in our patients occurred between exons 3 and 8 of CHD7 suggests that disruption of this gene is the cause of CHARGE syndrome in the twins and independently confirms the role of CHD7 in CHARGE syndrome.

diagnosis of CHARGE association to a greater or lesser degree. These include trisomy 18,⁸ der(9)t(9;13), der(6)t(4;6),⁹ and a case with a balanced translocation t(6;8)(6p8p;6q8q).¹⁰ The finding of many different chromosomal rearrangements suggested that the condition may be heterogeneous with a number of different genetic loci involved. We identified a monozygotic twin pair with CHARGE association and a de novo chromosomal rearrangement t(8;13)(q11.2;q22). Both girls fully meet the diagnostic criteria for CHARGE association/syndrome.¹¹ A de novo translocation in association with a syndrome suggests it may be causative. In addition, unlike many of the other chromosomal abnormalities described in association with CHARGE, which are unique, this rearrangement shared a common breakpoint with a previously reported case.¹⁰

Mapping the chromosome breakpoints in affected individuals with balanced translocations has been a successful strategy for many years in identifying genes responsible for a variety of inherited disorders, from Duchenne muscular dystrophy^{12–13} to rare sporadic genetic syndromes which are difficult to identify through conventional linkage analysis. This strategy usually involves only one or two cases, making the clinical diagnosis paramount. The diagnosis was secure in the twins described here and in the reported case with the common chromosome 8 breakpoint.¹⁰

CASE REPORT

The twins were born at 31+6 weeks gestation by normal delivery to healthy unrelated parents. There was no family history of congenital malformations. Birth weights were 1390 g (10th centile) and 1450 g (10th centile) for twins 1 and 2, respectively. They were both intubated at birth for respiratory distress. Attempts to pass nasogastric tubes were unsuccessful. Choanal atresia was presumed and they were managed with oral airways and gastric tubes. Once the twins were extubated, CT scans confirmed bilateral bony and membranous occlusion. The choanae were successfully repaired at 2 months.

Both girls were found to have bilateral colobomata of the iris and fundi, with significant visual impairment due to retinal and macular involvement.

On examination they both had external ear abnormalities and were subsequently found to have sensorineural deafness. Twin 2 was found to have profound sensorineural deafness on the left, with normal hearing on the right. High definition imaging of the ears was not performed, but CT scans for the choanal atresia also demonstrated an under-aerated, pre-

The possibility that an association between coloboma and other congenital malformations might constitute a new syndrome was first raised in 1979 by Hall.¹ The acronym CHARGE (Coloboma, Heart disease, Atresia of choanae, Retarded growth and development and/or central nervous system anomalies, Genital hypoplasia, and Ear anomalies) was suggested by Pagon *et al.*² The estimated prevalence of CHARGE association is 1/10 000.³

The cause of CHARGE association was unknown. Most cases have been sporadic leading to the hypothesis of a non-genetic aetiology, and several different pathogenic mechanisms have been suggested. Evidence supporting a genetic cause includes isolated reports of multiplex families with presumed autosomal dominant inheritance⁴ and others consistent with possible autosomal recessive inheritance.^{5–6} There are in addition several reports of concordant monozygotic twin pairs.⁷ There is phenotypic overlap with some chromosomal syndromes such as partial trisomy 13 and 4p-. A number of single cases with chromosome abnormalities have also been reported with features consistent with a

Abbreviations: CD2, chromodomain 2; PDA, patent ductus arteriosus; VSD, ventricular septal defect

Figure 1 (A) Twin 2 aged 2 months. (B) Twin 1 aged 2 years showing mildly dysmorphic features with laterally extended eyebrows with medial flare. (C) A typical CHARGE ear, low set, short, wide, protruding, simplified, and featureless. The ears were also asymmetric.

sumably contracted, middle ear cavity and possible abnormalities of the vestibular aqueducts. Twin 1 has bilateral profound sensorineural hearing loss with thresholds of 70–80 db at all frequencies on both sides. She has the same abnormalities reported on CT.

Single umbilical arteries were noted but renal scans were normal. Echocardiography revealed patent ductus arteriosus requiring surgical ligation. Twin 2 also had a ventricular septal defect (VSD), which required pulmonary banding pending closure.

The twins had normal cranial ultrasound scans.

Both girls were unable to swallow and had vomiting necessitating fundoplication. Postnatal growth continued to be poor, below the 3rd centile, and both twins had significant developmental delay.

Twin 2 died suddenly at age 8 months. A post-mortem examination could not ascertain the cause of death.

Figure 1 shows the facial features and typical external ear abnormalities.

Karyotype analysis revealed a translocation 46,XX,t(8;13)(q11.2;q22) in both girls which was not present in either of the parents.

METHODS

Parental consent and local ethics approval was obtained for the study.

Metaphase spreads were prepared from heparinised blood using standard cytogenetic techniques and fluorescence in situ hybridisation was performed with BAC and PAC probes to characterise the translocation breakpoints on both chromosomes 8 and 13. The probes were selected using the Ensembl and UCSC human genome browsers ([http://](http://www.ensembl.org/)

www.ensembl.org/ and <http://genome.ucsc.edu/cgi-bin/hgGateway>). Clones were supplied by the MRC Human Genetics Unit, Edinburgh and the Sanger Institute Mapping Core group. DNA was extracted according to the CHORI BACPAC Resources miniprep method (<http://www.chori.org/bacpac/>). FISH signals were visualised using the Cytovision image analysis system (Applied Imaging, San Clara, CA).

CHD7 gene disruption was confirmed by supplementary FISH analysis using sequential 10 kb probes. These probes were produced by long range PCR (using the LRPCR kit; Roche Diagnostics, Indianapolis, IN) with primers designed by the Primer3 website (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) using chromosome 8 breakpoint-spanning clone sequence data provided by Ensembl. Prior to primer design, repetitive sequences were masked using RepeatMasker (<http://www.repeatmasker.org/>).

RESULTS

Giemsa banding at 550 band resolution located the translocation breakpoints at 8q11.2 and 13q22. Walking the chromosome using FISH with mapped BAC and PAC probes refined the breakpoints to 8q12.2 and 13q31.1 (tables 1 and 2). The chromosome 13 breakpoint was resolved to 1.17 Mb. This region contained no obvious candidate genes. Clone RP11 33I11 was found to span the breakpoint on chromosome 8 (fig 2).

The Ensembl database identifies two genes in this region, Q7Z6C0 (Q66K35) and CHD7. Clone RP11 414L17, which includes most of the sequence for Q7Z6C0, is present only on the derivative chromosome 8. This clone contains the CHD7 exons 1 and 2 sequence. The remainder of the CHD7 sequence is present in clone RP11 33I11. Thus CHD7 was

Table 1 Hybridisation of chromosome 8 clones to twin 1

Probe name	Map location		FISH result	
	Band	Distance from p telomere (Mb)	Clone present, der(8)	Clone present, der(13)
RP11 137L15	q11.21	48.60	Yes	No
RP11 401H2	q11.22	52.20	Yes	No
RP11 56A10	q12.1	55.82	Yes	No
RP11 114M5	q12.1	59.40	Yes	No
RP11 414L17	q12.2	61.40	Yes	No
RP11 33I11	q12.2	61.80	Yes	Yes
RP11 35A5	q12.2	61.80	No	Yes
RP3 491L6	q12.2	61.83	No	Yes
RP11 317H6	q12.2	62.29	No	Yes
RP11 227F6	q12.3	62.40	No	Yes
RP11 45K10	q12.3	64.48	No	Yes
RP11 115G12	q12.3	65.34	No	Yes
RP11 366K18	q13.1	67.12	No	Yes
RP11 21C5	q13.3	69.42	No	Yes

Figure 2 Hybridisation of clone RP11 33111 to twin 1 showing signals on both derivative chromosomes 8 and 13 and the normal chromosome 8.

disrupted by the translocation; a series of 10 kb probes showed that the breakpoint was between exons 3 and 8 (fig 3). Probe 10.1 kb (which spans exon 4 and 5) localises to both the normal and the derivative chromosome 8, and probe 9.1 kb (which spans exons 6 and 7) localises to both derivative chromosomes 8 and 13. The 9.1 kb der(8) signal was not present in every cell, which would be consistent with the effect of a smaller portion of the probe sequence being present on the der(8).

DISCUSSION

The chromosome 8 breakpoint was thought to be more interesting than the chromosome 13 breakpoint since this was the region shared with the other reported case.¹⁰ In addition, the breakpoint region on chromosome 13 was found to be gene poor and contained no candidate genes. The chromosome 8 breakpoint was shown to occur between exons 3 and 8 of *CHD7*, suggesting that disruption of this gene is the likely cause of CHARGE association in the twins, and independently confirming the role of *CHD7* in CHARGE association.

Visser *et al.*¹⁵ using array comparative genome hybridisation in individuals diagnosed with CHARGE, found a deletion overlap in two affected individuals at 8q12. Sequencing each of the nine predicted genes in this region in CHARGE patients identified *CHD7* mutations in 10/17 individuals. No genotype-phenotype correlations have been found so far. The twins described here were concordant for most abnormalities although twin 1 had bilateral hearing loss and twin 2 had normal hearing on the right. Cardiac involvement was also non-concordant. Twin 1 had a patent ductus arteriosus

(PDA), and twin 2 a PDA and a VSD suggesting that penetrance is variable even with identical genetic backgrounds. Variable penetrance is recognised in monozygotic twins with both chromosomal and single gene disorders. Epigenetic factors may play a part including cytoplasmic differences which may arise as a result of the cleavage plane. It is possible that differences in copy number and mitochondrial activity may account for some of the differences observed in monozygotic twins. Hatchell¹⁴ discusses a somatic second hit hypothesis as a mechanism which may account for phenotypic variability between monozygotic twins, although this would require a very high mutation rate for the second hit. This second hit may not involve the DNA sequence directly; it may instead involve epigenetic changes which influence the expression of the gene such as DNA methylation.¹⁶

In this paper we describe our experience of breakpoint mapping as a method of identifying causative genes in rare conditions. This technique is relatively quick and inexpensive in comparison to array CGH as the critical region identified by this method is usually smaller. However, in some disorders the breakpoint has been misleading as it has been some distance from the causative gene as, for example, in campomelic dysplasia,¹⁷ or there have been chromosomal rearrangements at the site of the translocation thus complicating analysis.

CHD7 is a member of a relatively newly described family of proteins involved in the control of gene expression through chromatin modification. The proteins contain two N-terminal chromodomains, a central helicase/ATPase domain, and a DNA binding domain at the C terminus. They form part of a complex that is involved in the acetylation of histones. Acetylation and methylation of histones is important in controlling the transcriptional activity of genes through conformational changes to chromatin.^{18, 19} These alterations are made by chromatin modifying complexes. Recently, the yeast *CHD1* has been found to be part of the *SLIK* chromatin modifying complex which interacts with Lys 4 methylated histone H3. The chromodomain 2 (CD2) of *CHD1* appears to be important in recognising the substrate. PSI-Blast sequence analysis of CD2 in the Swiss-Prot database identified several proteins with significant similarity to CD2 including the second chromodomain in *CHD7*.²⁰

Genes involved in transcriptional regulation have been implicated in many malformation syndromes. The *CHD* gene family are all thought to control gene expression by chromatin modification, and hence regulate transcription. Other genes in this family are thus worth considering as candidates for other malformation syndromes. It will be of continuing interest to perform mutation analysis in other individuals with CHARGE and CHARGE-like phenotypes to assess any genotype-phenotype correlations. Identification of

Table 2 Hybridisation of chromosome 13 clones to twin 1

Probe name	Map location		FISH result	
	Band	Distance from p telomere (Mb)	Clone present, der(8)	Clone present, der(13)
RP11 370A2	q21.33	67.13	No	Yes
RP11 309H15	q22.1	71.56	No	Yes
RP11 226E21	q22.3	75.46	No	Yes
RP11 421K11	q31.1	79.62	No	Yes
RP11 533P8	q31.1	79.83	No	Yes
RP11 115N13	q31.1	81.0	Yes	No
RP11 464I4	q31.1	81.5	Yes	No
RP11 366K1	q31.1	85.3	Yes	No
RP11 275J18	q31.2	87.4	Yes	No
RP11 388D4	q31.3	88.2	Yes	No
RP11 632L2	q31.3	91.4 Mb	Yes	No

Figure 3 (A) Schematic showing mapping of chromosome 8 breakpoint. Clones to the right of the dotted line mapped to the der(13) and normal 8. (B) Schematic of CHD7 gene with position of 10.1 and 9.1 kb probes showing CHD7 is disrupted between exons 3 and 8. Short vertical lines represent exons, chromodomain (ch), SNF2 domain (SNF2), and helicase domain (H).

the genes which are up or down regulated by the actions of CHD7 will allow further clarification of the developmental pathways of the structures affected in CHARGE syndrome.

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ELECTRONIC-DATABASE INFORMATION

The URLs for this paper are as follows: UCSC Human Genome Browsers, <http://genome.ucsc.edu/cgi-bin/hgGateway>; Ensembl Human Genome Browser, <http://www.ensembl.org/>; CHORI BACPAC Resources, <http://www.chori.org/bacpac/>; Primer3 website, <http://frodo.wi.mit.edu/cgi-bin/primer3/primer3-www.cgi>; and RepeatMasker, <http://www.repeatmasker.org/>.

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