Letters to the Editor

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Narrowing the Candidate Region for Congenital Diaphragmatic Hernia in Chromosome 15q26: Contradictory Results

To the Editor:

Subtelomeric screening and FISH analysis of a 13-year-old girl with severe mental retardation, intrauterine growth retardation, microcephaly, facial dysmorphisms, hypoplastic kidney, and short hands and feet but without congenital diaphragmatic hernia (CDH [MIM 142340]) allowed us to find a de novo deletion in 15q26.1–26.2.

That region, as shown by Klaassens et al. (2005) in a study published in the May issue of the *Journal*, contains a candidate region for CDH, a condition that occurrs in ~1 of 3,000 newborns and is associated with a 30%–60% mortality rate, with significant morbidity among survivors (Harrison et al. 1994; Nobuhara et al. 1996). The etiology of this condition is barely known and, in most cases, is considered idiopathic, whereas ~15% of patients with CDH show chromosomal abnormalities. Recently, Biggio et al. (2004) reported on a child with a 15q26.1 deletion showing CDH, coarctation of the aorta, and dysmorphic features, suggesting this region as the possible candidate locus for CDH. Furthermore, the authors proposed myocyte-specific enhancer factor–2A (*MEF2A* [MIM 600660]) as a candidate gene for CDH, coding for a protein playing a critical role in the control of muscle differentiation and development. Klaassens et al. (2005) found 7% numerical and 5% structural chromosome abnormalities in 200 CDH patients. The most frequent chromosome abnormality was 15q deletion. Eventually, they determined the size of the deletions in seven patients with CDH. They incorporated data from two patients with terminal 15q deletions without CDH, and data from one patient with a small 15q interstitial deletion and CDH. A minimal deletion region, spanning ~5 Mb at chromosome bands 15q26.1–15q26.2, has been suggested by these authors. Two of the known genes of this region, namely, *NR2F2* (MIM 107773) and *CDH2* (MIM 602119), were considered to be the best candidates for CDH.

To better define the deletion in our patient, FISH experiments were carried out with a set of linearly ordered BACs selected by human NCBI Map Viewer (build 35.1) and provided by the Sanger Institute. This analysis showed that the BAC RP11-386M24, localized to chromosome band 15q26.1 (~9.0 Mb from the end of the chromosome), was the closest to the telomere that hybridized on both chromosomes in all examined metaphases. The immediately more centromeric CTD—2313J17 BAC showed signals of different intensities on the 15q telomeres, suggesting that the breakpoint lay within this BAC, whereas the overlapping RP11-437B10 BAC and all the distally placed BACs showed no hybridization signal (data not shown).

We thus compared our results with the most significant previously characterized 15q deletions, including ring chromosome 15, unbalanced translocations, and pure 15qter monosomies, either associated with the CDH phenotype or not. As shown in figure 1, no clear critical region can be drawn from these data, essentially because case 12 with CDH carried a ring (15) resulting in a smaller deletion than cases 1, 2, and 3, without CDH. At least two hypotheses can be made to explain these contradictory data. First, it is possible that haploinsufficiency of the CDH locus has a reduced penetrance and that data from patients without CDH could be useless in establishing the critical region. If this is true, the candidate region is restricted to ~3.5 Mb (fig. 1) and includes the *NR2F2* gene, but its telomeric limit is more distal than that defined by Klaassens et al. (2005), which was derived from a deletion of a patient without CDH (fig. 1, case 13). On the other hand, drawing genotype-phenotype relationships may be difficult in ring carriers because of the potential instability of ring chromosomes that can be associated with gain or loss of genetic material in other tissues (Tümer et al. 2004). If we omit ring cases from the analysis, then the critical region would be narrowed to a 0.7-Mb genomic portion (fig. 1). The *NR2F2* gene, in this case, would be located outside this putative critical region.

The ST8 alpha-N-acetyl-neuraminide alpha-2, 8-sialyltransferase 2 gene (*ST8SIA2* [MIM 602546]) is the unique known gene in this region and encodes for a type II membrane protein that catalyzes the transfer of sialic acid from CMP-sialic acid to the neural cell adhesion molecules (NCAMs) (Ong et al. 1998). The *ST8SIA2* gene is expressed in many tissues during development (Angata et al. 1997). Evidence suggests that polysialy-

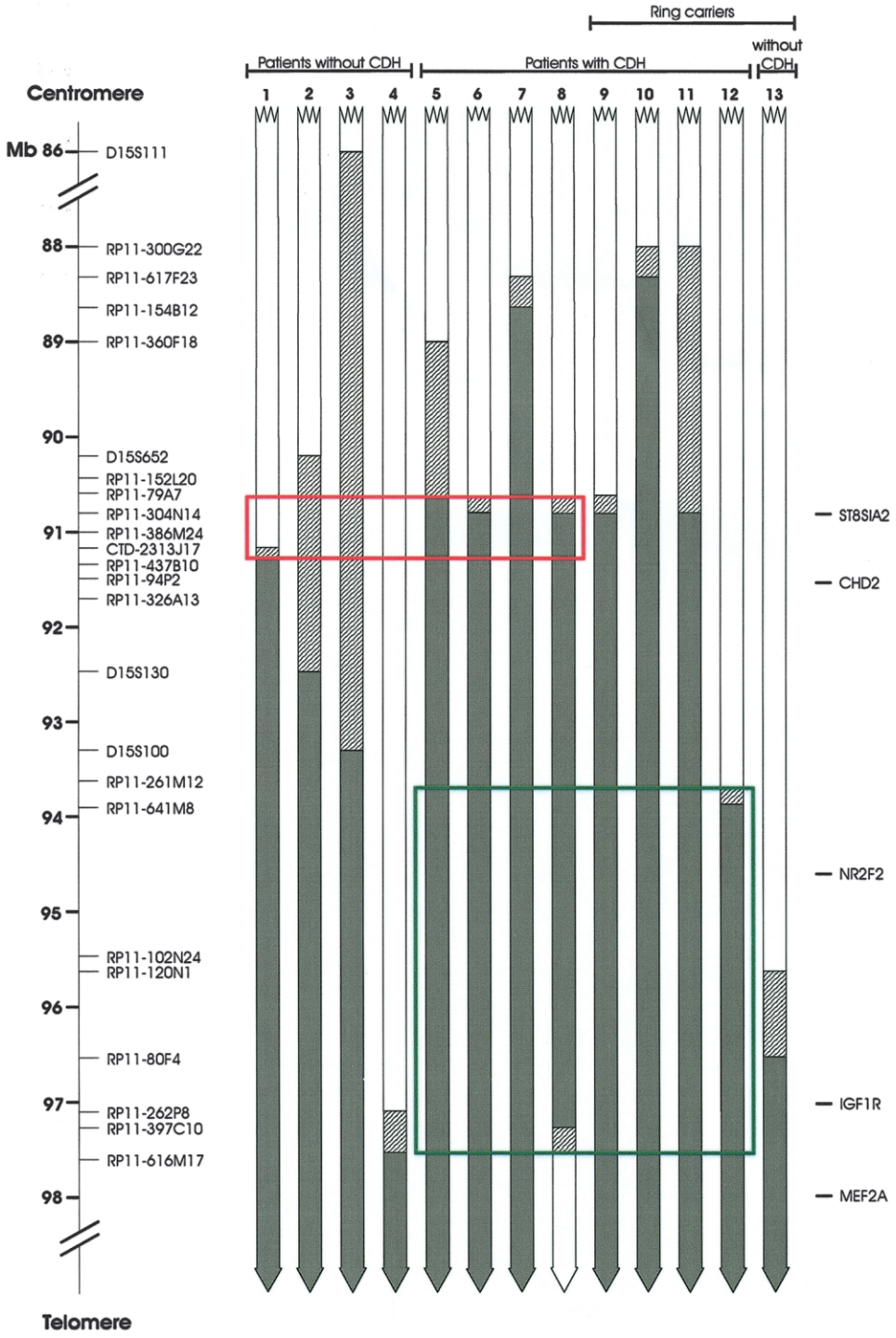

Figure 1 Graphical representation of 15q deletions in patients with and without CDH. The most significant BAC clones analyzed are shown on the left. Solid boxes represent deleted regions, hatched boxes indicate the uncertainty of the breakpoints, and open boxes reveal the normal chromosomal regions. The green rectangle includes the narrowed candidate CDH region, taking into account only cases with CDH, whereas the red rectangle indicates the critical region resulting when the ring cases are omitted from the analysis. Patient 1, our case; patient 2, Tönnies et al. 2001; patient 3, Rogan et al. 1996 (patient K); patients 4–11 and 13, Klaassens et al. 2005 (cases 9, 7, 6, 4, 1, 2, 3, 5, and 8, respectively); patient 12, Tümer et al. 2004 (case 1).

lated NCAMs promote cell migration and, thus, they are thought to play a critical role in development. More specifically, it has been shown that, during diaphragmatic morphogenesis, the expression of polysialylated NCAMs is tightly modulated along each stage of myogenesis (Allan and Greer 1998).

Finally, although it is less likely, we cannot exclude the possibility that both the mentioned hypotheses are true. In this case, the critical region would be represented by the extent of the deletion in patient 8 (fig. 1).

Additional findings are needed to refine the search for a CDH gene in 15q chromosome. However, it seems likely that *NR2F2* and *ST8SIA2* are the best candidates.

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Web Resources

URLs for data presented herein are as follows:

NCBI Map Viewer, http://www.ncbi.nlm.nih.gov/mapview/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

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Reply to Castiglia et al.

To the Editor:

In response to our article in the May issue of the *Journal* (Klaassens et al. 2005), Castiglia et al. (2005 [in this issue]) address the strategy of including patients with a 15q deletion but without congenital diaphragmatic hernia (CDH). They defined a deletion on 15q26.1–26.2 in a girl with multiple congenital anomalies but without CDH. Combining data from this patient with previously published data from two patients with a 15q deletion but without CDH (Rogan et al. 1996; Tonnies et al. 2001), Castiliglia et al. (2005) found a discrepancy between our data and the CDH locus that they determined. Of the two hypotheses postulated to explain these contradictory results, we support the first one, which suggests that including patients without CDH in the analysis might be inappropriate because of the possibility that heterozygous deletion of a part of 15q (which results in haploinsufficiency for this locus) might not be completely penetrant. Incomplete penetrance could also explain, in part, the variability in phenotype of patients with CDH and a 15q deletion.

Since the publication of our article, we have been able to more precisely define the deletions in our patients with CDH. With CDH patients only, a 4-Mb common CDH region would be located between BAC clones RP11–

44A22 (overlapping with RP11–641M8 and RP11–261M12; see fig. 1 in Castiglia et al. [2005]) and RP11–616M17 (data not shown), with the telomeric boundary determined by the interstitial deletion of patient 1 (patient 8 in fig. 1 in Castiglia et al. [2005]). We, therefore, excluded some genes from the region, including the *SIAT8B* gene (MIM 602546) suggested by Castiglia et al. (2005) as one of the candidate genes for CDH. The remaining region still contains *NR2F2, IGF1R,* and three hypothetical genes. We are in the process of screening, with mutation analysis, all genes in this deleted region in a large group of CDH patients and screening with FISH for deletions. Of the genes located in this region, we still consider *NR2F2* to be the most likely candidate. The recent report by Tümer et al. (2004) supports this hypothesis. They analyzed three ring carriers, one of which had a different phenotype than the other two patients (Tümer et al. 2004). This third patient had CDH and other anomalies, and the deletion included the same genes as in the other two patients, except for the *NR2F2* gene, which was deleted only in the patient with CDH. In contrast to the opinion of Castiglia et al. (2005), we believe ring carriers can provide valuable clues in the search for chromosomal loci that could be involved in the etiology of congenital anomalies. Although ring chromosomes can be unstable, we have not observed gain or loss of other genetic material. In addition, the new chromosomal telomeric DNA of derivative chromosomes in unbalanced translocations could be of influence.

In conclusion, we hypothesize that 15q26.1–26.2, a gene-poor region, plays an important role in the etiology of CDH. Haploinsufficiency of this region might not be completely penetrant. We still propose *NR2F2* to be the most likely candidate, but disruption of a regulatory element or other gene in this region cannot be excluded as a cause of CDH.

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