Endocrine Journal 2011, **58** (3), 155-159

ORIGINAL

Identification of chromosome 15q26 terminal deletion with telomere sequences and its bearing on genotype-phenotype analysis

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Abstract. We report a *de novo* heterozygous 5,013,940 bp terminal deletion of chromosome 15q26 in a 13 9/12 -year-old Japanese girl with short stature (–3.9 SD), mild mental retardation, and ventricular septal defect (VSD). This terminal deletion involved *IGF1R* but not *NR2F2*, and was associated with an addition of telomere repeat sequences (TTAGGG) at the end of the truncated chromosome. The results provide further support for the notion that terminal deletions are healed by *de novo* addition of telomere sequences essential for chromosome stability and DNA replication. Furthermore, while growth failure and mental retardation are primarily explained by loss of *IGF1R*, the occurrence of VSD might suggest the existence of a cardiac anomaly gene, other than the candidate cardiac anomaly gene *NR2F2*, in the deleted region.

Key words: 15q deletion, *IGF1R*, Telomere, Congenital heart defect

TERMINAL deletions of chromosome 15q are relatively rare chromosomal anomalies that are usually associated with variable degrees of pre- and post-natal growth failure and are sometimes accompanied by mental retardation and/or congenital anomalies such as congenital heart defect (CHD) [1]. In this regard, haploinsufficiency of the gene for insulin-like growth factor 1 receptor (*IGF1R*) at 15q26.3 is known to be relevant to the growth failure and mental retardation [2], and hemizygosity of the gene for nuclear receptor subfamily group F member 2 (*NR2F2*) at 15q26.2 has been postulated as an underlying factor for CHD [3–6].

The chromosome end is associated with 3–20 kb of tandemly repeated telomere sequences $(TTAGGG)_n$ that are extended and maintained in human germline by telomerase using an integral telomere-complementary RNA template [7–9]. The telomere sequences are essential for chromosome stability and DNA replication. Indeed, eukaryotic chromosomes with terminal deletions undergo end-fusion and degradation events in the absence of functional telomeres. Thus, truncated chromosomes must be healed by a *de novo* telomere addition, to replicate and segregate normally [10, 11].

Here, we report clinical and molecular findings in a Japanese female patient with a heterozygous *de novo* 15q terminal deletion. The data provide further support for the addition of telomere sequences to truncated chromosome ends, and imply the possible presence of a gene for CHD other than *NR2F2* on distal 15q.

Case Report

This Japanese female patient was born at 39 weeks of gestation after an uncomplicated pregnancy and delivery. At birth, her length was 47.0 cm (–0.7 SD), her weight 2.17 kg (–2.1 SD), and her head circumfer-

Received Aug. 27, 2010; Accepted Dec. 20, 2010 as K10E-251
Released online in J-STAGE as advance publication Jan. 14, 2011

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Abbreviations: CHD, congenital heart defect; IGF1, insulin-like growth factor 1; IGF1R, insulin-like growth factor 1 receptor; LINE-1, long-interspersed nuclear element-1; MLPA, multiplex ligation-dependent probe amplification; NR2F2, nuclear subfamily group F member 2; VSD, ventricular septal defect.

ence 32.5 cm (–0.5 SD). Although she was found to have ventricular septal defect (VSD), it closed spontaneously at 6 years of age. Menarche occurred at 11 years 6 months of age (menarchial age of Japanese females, 9.75–14.75 years).

At 11 years 9 months of age, she was referred to us because of short stature. Her height was 129. 4 cm (–3.2 SD), and her weight 37.5 kg (–0.6 SD). There were no dysmorphic features. She exhibited mild mental retardation and learning difficulties. Her breast development was at Tanner stage 4 and her pubic hair at Tanner stage 3. Endocrine studies showed an extremely high serum insulin-like growth factor-1 (IGF1) value (1070 ng/mL) (age- and sex-matched reference data, 206–731 ng/mL) and apparently normal serum growth hormone value (2.85 ng/mL) (reference data, 0.32–3.85 ng/mL) and IGF-binding protein-3 value (3.78 μg/mL) (reference data, 2.30–4.39 μg/mL), suggesting the presence of either bioinactive IGF1 or IGF1R resistance in this patient. Her bone age was assessed as 12 years 6 months. On the last examination at 13 years 9 months old, she measured 134.7 cm (–3.9 SD), weighed 45 kg (–0.5 SD), and manifested full pubertal development.

The non-consanguineous parents and three elder brothers were clinically normal. The father was 174 cm (+0.6 SD) tall, and the mother was 151 cm (–1.3 SD) tall.

Molecular Studies

We performed molecular studies using leukocyte DNA samples and lymphocyte metaphase spreads. This study was approved by the Institutional Review Board Committee at National Center for Child Health and Development, and performed after obtaining written informed consent.

We first performed direct sequencing of *IGF1* and *IGF1R* of this patient, identifying no mutation or variation in all the coding exons and their flanking splice sites (the primer sequences are available on request). Thus, multiplex ligation-dependent probe amplification (MLPA) was performed for *IGF1R* using a commercially available MLPA probe mix (P217) (MRC-Holland, Amsterdam), indicating heterozygous deletion of *IGF1R* (Fig. 1A). This deletion was confirmed by FISH with an RP11-262P8 BAC probe detecting a region within *IGF1R* (BACPAC Resources Center, Oakland, CA) (Fig. 1A). To examine the deletion size, oligoarray comparative genomic hybridization was carried out with 1x244K Human Genome Array (catalog No. G4411B) (Agilent Technologies, CA), showing a ~5.0 Mb deletion distal to 15q26.2 (Fig. 1B). Furthermore, to determine the precise deletion junction, we obtained long PCR products with a forward primer hybridizing to a region proximal to the deletion (5'-TAT AACAGACCAAAGCTGGAATGA-3') and a reverse primer complementary to telomere repeat sequences (5'-CTAACCCTAACCCTAACCCTAACC-3'), and examined the sequence of the PCR products with serial primers. Consequently, the breakpoints were determined by direct sequencing with the following primer (5'-GCAATACAAAGACTAGATGCCGTA-3') (Fig. 1B). According to the NCBI Database (NC_000014.7) (http://www.ncbi.nlm.nih.gov/), the deletion was 5,013,940 bp in physical size and was associated with an addition of telomere repeat sequences at the end of the truncated chromosome. The proximal deletion breakpoint was found to reside within a long-interspersed nuclear element-1 element (LINE-1) (subfamily LIME3F) by Repeatmasker (http://www.repeatmasker.org). While this deletion removed 44 genes including *IGF1R, NR2F2* was preserved (Ensemble Genome Browser, http://ww.ensemble.org/). Microsatellite analysis for *D15S120* on the deleted region showed that the deletion occurred in the paternally derived chromosome (Fig. 1C). The parents were found to have neither 15q deletion nor reciprocal translocation involving 15q.

Discussion

We identified a heterozygous *de novo* simple 15q26 terminal deletion with telomere sequences. The results provide further support for the notion that terminal truncated deletions are healed by *de novo* addition of telomere repetitive sequences indispensable for chromosome stability and DNA replication. In this regard, the telomerase activity is found in the germline as well as in most cancer cells, but it is generally undetected in normal human somatic tissues [9]. It is likely, therefore, that the chromosomal deletion and the telomere addition took place in the germline.

Phenotypic comparison between this patient and previously reported patients with simple terminal 15q26 deletions [1] provides several useful implications for genotype-phenotype correlations. In this regards, while terminal 15q26 deletions can also occur in complex chromosomal rearrangements such as ring chro-

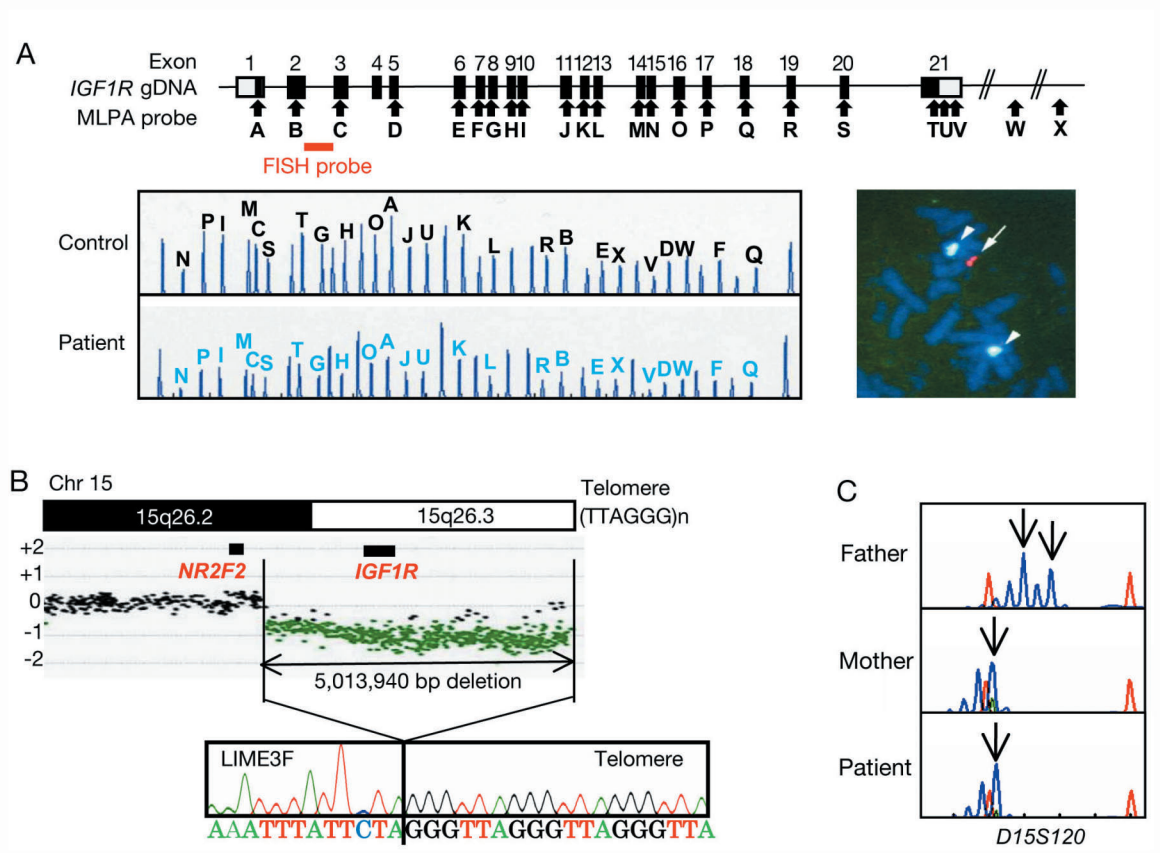

Fig. 1 Deletion analysis.
A MLPA and FISH analyses. The black and white boxes on genomic DNA (gDNA) denote the coding regions and the untranslated regions, respectively. The sites examined by MLPA probes (A–X) are indicated by arrows, and the region detected by an RP11-262P8 BAC FISH probe is shown by a thick horizontal line. In MLPA analysis, the peaks corresponding to sites within the *IGF1R* gene (A–V) and two sites at 2.0 (W) and 2.8 Mb (X) downstream of the *IGF1R* gene are reduced in the patient. In FISH analysis, the probe for *IGF1R* (RP11-262P8) detects only a single signal (an arrow), whereas the CEP 15 (D15Z4) control probe identifies two signals (arrowheads). The probe for *IGF1R* was labeled with digoxigenin and detected by rhodamine anti-digoxigenin, and the CEP 15 probe was detected according to the manufacturer's protocol (Abbott, http://www.abbottmolecular.com/).
B Oligoarray CGH analysis and direct sequencing of the deletion junction. The deletion is 5,013,940 bp in physical size and is associated with an addition of telomere repetitive sequences at the end of the truncated chromosome 15.
C Microsatellite marker analysis. For *D15S120*, one of the maternal alleles is transmitted to the patients, whereas both of the paternal alleles are not transmitted to the patient.

mosome 15 and unbalanced translocation involving 15q26 [12], genotype-phenotype analysis of 15q26 deletion is difficult in complex chromosomal rearrangements because of accompanying chromosomal aberrations.

First, this patient had short stature and mental retardation, as have been observed in most patients with simple terminal 15q26 deletions [1]. This would primarily be ascribed to loss of *IGF1R*, although other genetic and environmental factors may also have some effects. Indeed, heterozygous intragenic *IGF1R* mutations are usually associated with pre-and post-natal growth failure and sometimes accompanied by mental retardation [2, 13–16]. While the birth length of this patient remained within the normal range, this is not necessarily inconsistent with *IGF1R* deletion. It is known that *IGF1R* haploinsufficiency sometimes permit normal birth length and/or weight [1]. Similarly, while the serum IGF1 level was markedly elevated in this patient, this would basically be compatible with *IGF1R* deletion. It is known that serum IGF1 levels tend to be elevated in patients with *IGF1R* haploinsufficiency [1, 2]. To our knowledge, however, such a high serum IGF1 level has not been reported to date. Thus, although underlying factor(s) for the extremely high IGF1 level remains to be clarified, the present data indicate that serum IGF1 level can be markedly elevated in patients with *IGF1R* haploinsufficiency.

Second, she also had CHD (VSD) that is often identified in patients with simple terminal 15q26 deletions [1, 4–6]. In this regard, although previous genotype-phenotype correlations in patients with 15q26 deletions and knockout mice studies argue that loss of *NR2F2* may lead to various types of CHD with incomplete penetrance [3–6], *NR2F2* was preserved in this patient. In addition, the development of CHD is obviously inexplicable by *IGF1R* deletion [1, 2]. Thus, it might be possible that a hitherto unknown gene(s) for CHD (VSD) with variable expressivity and penetrance resides on the deleted region, although CHD (VSD) could be a co-incidental feature independent of the deletion or a non-specific feature common to various chromosomal abnormalities [17–19]. Furthermore, loss of the putative gene may also be relevant to the development of CHD in the previously reported patients with 15q26 deletions [4–6].

Lastly, she had no other clinically discernible features, while most patients with simple 15q26 terminal deletions have additional features such as diaphragmatic hernia, renal anomalies, club feet, oculocutaneous albinism, and seizure [1]. However, such features have been observed in a single or only a few patients with apparently large simple terminal 15q26 deletions. Thus, such features could be regarded as developmental insults that became recognizable by chromosome imbalance [18, 19]. This notion would be supported by the fact that such additional features are more frequently found in complex chromosomal rearrangements involving 15q26 than in simple terminal 15q deletions [1, 12], because chromosomal imbalance is more severe in complex chromosomal rearrangements. In addition, if a gene(s) for such features is present on distal 15q, it is likely that such a gene(s) was preserved in this patient with a relatively small deletion, or that loss of the gene(s) did not cause clinically discernible phenotypes in this patient because of low penetrance.

In summary, the results provide further evidence for the addition of telomere sequences to truncated chromosome ends, and suggest the possible presence of a gene for CHD other than *NR2F2* on distal 15q. Furthermore, it is recommended to examine 15q deletions in patients with clinical features indicative of *IGF1R* abnormality (growth failure with relatively to obviously high serum IGF1, and mental retardation) and other phenotypes including CHD.

Acknowledgements

This study was supported by Grants for Child Health and Development (20C-2) and Research on Children and Families (H21-005) from the Ministry of Health, Labor, and Welfare, and by Grants from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Appendix

The authors have nothing to declare.

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