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A recurrent 14q32.2 microdeletion mediated by expanded TGG repeats

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Nearly all recurrent microdeletion/duplication syndromes described to date are characterized by the presence of flanking low copy repeats that act as substrates for non-allelic homologous recombination (NAHR) leading to the loss, gain or disruption of dosage sensitive genes. We describe an identical 1.11 Mb heterozygous deletion of 14q32.2 including the *DLK1/GTL2* imprinted gene cluster in two unrelated patients. In both patients, the deleted chromosome 14 was of paternal origin, and consistent with this both exhibit clinical features compatible with uniparental disomy (UPD) (14)mat. Using a high-resolution oligonucleotide array, we mapped the breakpoints of this recurrent deletion to large flanking $(TGG)_n$ tandem repeats, each approximately 500 bp in size and sharing ≥88% homology. These expanded $(TGG)_n$ motifs share features with known fragile sites and are predicted to form strong guanine quadruplex secondary structures. We suggest that this recurrent deletion is mediated either by NAHR between the TGG repeats, or alternatively results from their inherent instability and/or strong secondary structure. Our results define a recurrent microdeletion of the 14q32.2 imprinted gene cluster mediated by flanking $(TGG)_n$ repeats, identifying a novel mechanism of recurrent genomic rearrangement. Our observation that expanded repeats can act as catalysts for genomic rearrangement extends the role of triplet repeats in human disease, raising the possibility that similar repeat structures may act as substrates for pathogenic rearrangements genome-wide.

INTRODUCTION

The recent widespread use of high resolution genome analysis by microarray technologies has led to identification of several novel microdeletion and microduplication syndromes (1–8). In most recurrent microdeletion/duplication syndromes, the rearranged genomic segments are flanked by large and highly homologous low copy repeat (LCR) structures. These LCRs are blocks of DNA, typically >10 kb in length and sharing >95% sequence homology, that act as recombination substrates for non-allelic homologous recombination (NAHR), leading to the gain or loss of dosage sensitive gene(s) in the intervening segment (9). To date, LCRs that are known to mediate recurrent genomic disorders are generally either simple structures such as the CMT1A-REP repeat that consists of two copies of a 24-kb sequence on chromosome 17p11.2-12 that mediate Charcot Marie-Tooth disease type 1A (CMT1A; MIM118220) (10) or contain highly complex arrangements of duplicated motifs, such as those that mediate the 22q11 microdeletion syndrome (11).

Uniparental disomy (UPD) describes the inheritance of two copies of a chromosome pair from only one parent (12). Many chromosome-specific UPDs have been described associated with distinct clinical phenotypes depending on the parental origin of the chromosomes (13). Paternal and maternal uniparental disomies for chromosome 14 (UPD (14)pat and UPD (14)mat) cause distinct phenotypes. UPD (14)mat has been reported to be associated with intrauterine growth retardation, followed by postnatal hypotonia, feeding problems, motor delay, short stature, small hands and feet, scoliosis, obesity,

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distinctive facial appearance and early puberty (14–17). UPD (14)pat (MIM608149) is associated with skeletal abnormalities, joint contractures, dysmorphic facial features and developmental delay/mental retardation (18).

The critical region for UPD (14) phenotypes overlaps a cluster of imprinted genes in 14q32.2 (19) including the paternally expressed genes *DLK1* and *RTL1*, and maternally expressed genes *GTL2* (alias *MEG3*), *RTL1as* (RTL1 antisense), and *MEG8*. Evidence that this imprinted gene cluster underlies the UPD (14) phenotype is reinforced by description of methylation defect in the intergenic differentially methylated region (IG-DMR) that regulates this imprinted locus (20). To date, eleven UPD (14)mat-like cases without UPD have been described. Of these, seven patients displayed a deletion in the 14q32.2 imprinted gene domain encompassing the *DLK1*/IG-DMR/*GTL2* cluster (21–26).

In the present study, we identify an identical 1.1 Mb deletion encompassing the *DLK1*/*GTL2* cluster in two unrelated patients presenting with a UPD (14)mat-like phenotype. High resolution analysis in these two cases revealed that both deletions were identical, with the breakpoints occurring in large $(TGG)_n$ tandem repeats. These data indicate a novel recurrent deletion of 14q32 that is mediated by $(TGG)_n$ repeats.

RESULTS

Patient 1 was reported with normal GTG-banded karyotype. Array-CGH analysis revealed a heterozygous 1.1 Mb deletion on chromosome 14q32 between position 99.466 and 100.572 Mb [NCBI build 36]. Patient 2 [Case 3 in Buiting *et al*. (22)] was previously reported with a loss of a 1.1 Mb segment from position 99.47 Mb and 100.57 Mb. Thus, array-CGH suggested deletion of the same segment of 14q32 in both cases. This deletion encompasses the imprinted gene cluster including *DLK1, GTL2, RTL1, RTL1as*, *MEG8*, as well as a snoRNA and part of a miRNA cluster (http://www.geneimprint.com/site/genes-by-species). Furthermore, 10 additional RefSeq non-imprinted genes (*EVL, DEGS2, YY1, SLC25A29, c14orf68, WARS, WDR25, BEGAIN, c14orf70* and the 3′ end of *EML1*) are deleted (Fig. 1). No other genomic unbalances were detected for patient 1. Patient 2 was shown to have an additional loss of 0.66 Mb in 19p13 inherited from her mother classified as benign (22,27). Array-CGH showed the 14q32.3 deletion was *de novo*, in patient 1 while the father's sample was not available in patient 2 (22). Microsatellite analysis of D14S1006 for patient 1 showed that the deleted allele was of paternal origin, consistent with the UPD (14)mat-like phenotype. Patient 2 was similarly reported to have UPD (14)mat-like phenotype and a deletion of the paternally derived allele (22).

Because of the apparent similarity in the breakpoints of these two deletions, we hypothesized that they might share a common molecular mechanism. Examination of the proximal and distal breakpoint regions (http://genome.ucsc.edu/) showed the presence of large ∼500 bp $(TGG)_n$ tandem repeat tracts at both flanks (chr14:99,463,844–99,464,347 and chr14:100,574,282–100,574,769) which share an average of 88% homology. Subsequent fine mapping of the breakpoints using a custom oligonucleotide array with a density of 1 probe per 100 bp across the breakpoint regions confirmed that the deletion in both patients was identical, with both proximal and distal breakpoints mapping to these $(TGG)_n$ repeat motifs (Fig. 1). Analysis of the guanine quadruplex potential of the two breakpoint regions showed that these $(TGG)_n$ repeats are highly enriched for motifs predicted to form G_4 DNA (40), suggesting that they have strong secondary structure.

Based on the high predicted secondary structure of these $(TGG)_n$ repeats, we hypothesized that the recurrent 14q32 deletion might result from the intrinsic instability of these sequences. To test this hypothesis, we performed an analysis of the size distribution of all triplet repeats in the reference genome sequence based on their nucleotide motif, shown in Figure 2. We observed that repeats containing repetitions of the motif TGG (including TGG, GTG, GGT and their reverse complements CCA, CAC and ACC) represent the longest types of triplet repeat in the genome. $(TGG)_n$ repeat motifs have a mean length of 146 bp, which represents more than twice the average length of all other types of triplet repeat in the genome (mean length 64 bp).

DISCUSSION

We report two individuals with an identical 1.11 Mb microdeletion which includes the imprinted *DLK1*/*GTL2* gene cluster in 14q32.3. Both breakpoints map precisely to large $(TGG)_n$ tandem repeats which mediate this recurrent deletion, thus defining a novel region of recurrent genomic rearrangement. While five other patients with UPD (14)-like phenotypes and deletions of this imprinted locus have been reported previously, none were found to carry the same deletion that we describe (21–23,25). Thus, it seems likely that this recurrent rearrangement accounts for a minority of patients with 14q32 deletions.

Both patients have clinical features compatible with the phenotype described for UPD (14)mat, including pre- and post-natal growth retardation, hypotonia, feeding difficulties, precocious puberty and developmental delay. Mental retardation is not normally associated with UPD (14)mat phenotype, but considering that these 14q32 deletions also encompass many additional non-imprinted genes, we hypothesize that the mental retardation observed in these patients is caused by haplo-insufficiency for one or more dosage sensitive genes, of which *BEGAIN* (brain-enriched guanylate kinase-associated protein) represents a good candidate based on its localization to neuronal synapses (28).

The 14q32 deletion differs from most other known sites of recurrent rearrangement in the nature of the sequences catalysing these events. Most genomic disorders described to date are characterized by the presence of flanking LCRs which show significant homology over 10–100 s of kilobases and have a nucleotide composition typical of euchromatic sequence, often including genes and common repetitive elements (9). In contrast, breakpoints of these 14q32 deletions map to large $(TGG)_n$ repeats, representing the first recurrent rearrangement described that is catalysed by a trinucleotide repeat. Expanded trinucleotide repeats are more classically associated with a number of different genetic diseases including fragile X, myotonic dystrophy and Huntington disease (29,30). In these diseases, the mechanism by which the expanded repeat

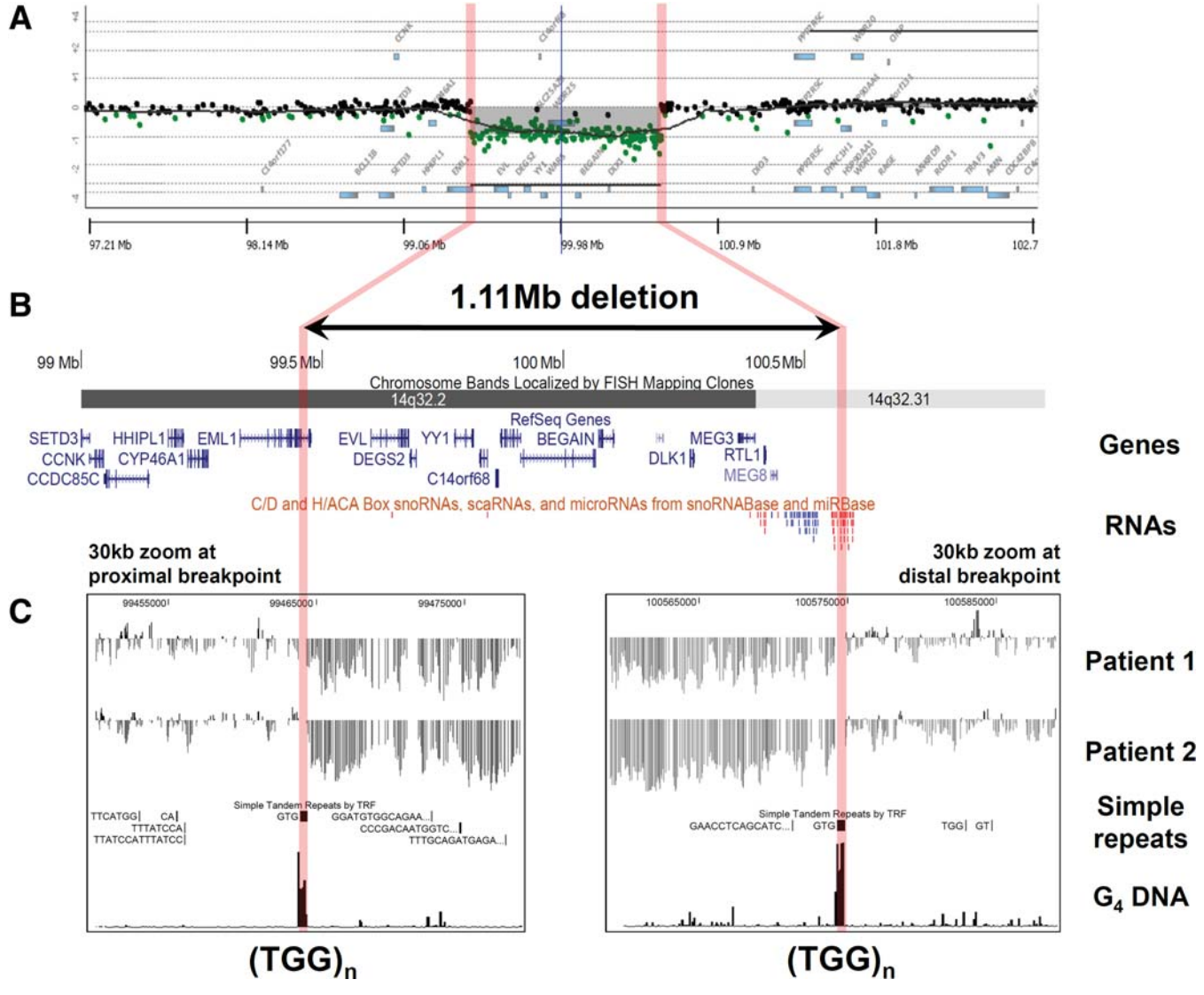

Figure 1. Fine mapping of a recurrent 1.11 Mb microdeletion in 14q32 catalysed by TGG repeat motifs. (**A**) 244 K oligonucleotide array CGH profile of the 1.1 Mb 14q32 deletion in patient 1. Patient 2 was previously reported to have a similar deletion (22, data not shown). (**B**) Enlargement of a 2 Mb region from the UCSC genome browser showing the deleted segment (black arrow), cytogenetic bands, RefSeq genes and small RNAs (pre-miRNA in red, C/D box snoRNA in blue). (**C**) Enlargement of 30 kb regions at the proximal and distal breakpoints of the deletion. Data show fine mapping of the breakpoints in patients 1 and 2 using a custom oligonucleotide array with a density of 1 probe per 100 bp across each region. Both breakpoints are apparently identical and coincide with highly homologous $(TGG)_n$ tandem repeats. Analysis of the potential of these regions to form guanine quadruplexes (G_4 DNA) was performed and counts of the total number of overlapping motifs capable of forming G_4 DNA are shown plotted in 100 bp windows. These data indicate that the TGG repeats located at both proximal and distal breakpoints have strong secondary structures. The shaded red line indicates the breakpoint regions in each figure.

sequence causes pathology varies from transcriptional inactivation to the production of toxic intracellular RNA or protein aggregates (31). Our observations that similar expanded repeats can also act as catalysts for genomic rearrangements therefore extends the role of triplet repeats in human disease.

Expanded tandem repeat motifs could sponsor increased rates of chromosomal rearrangement through two alternate mechanisms. In the first model, pairs of expanded repeats of sufficient length provide a substrate for NAHR, either *in cis* or *in trans*. Previous studies have demonstrated an exponential relationship between the frequency of NAHR and the length of uninterrupted homology between two sequence elements in mammalian cells (32,33). Thus, expanded pairs of homologous repeats, including $(TGG)_n$ tracts or alternatively other tandem repeat motifs, would provide an improved substrate for aberrant recombination by providing longer stretches of homology for NAHR. In a second alternate model, tandem repeats such as $(TGG)_n$ could sponsor double-strand chromosome breaks by their inherent instability resulting from a propensity to form secondary structures that interfere with normal DNA replication and chromosome condensation. In particular, $(TGG)_n$ repeats are known to form complex intra-molecular structures termed guanine quadruplexes or G_4 DNA (34) that progressively inhibit DNA synthesis with increasing repeat length (35).

In support of this latter hypothesis, we made two observations. First, an analysis of the $(TGG)_n$ repeats at the 14q32

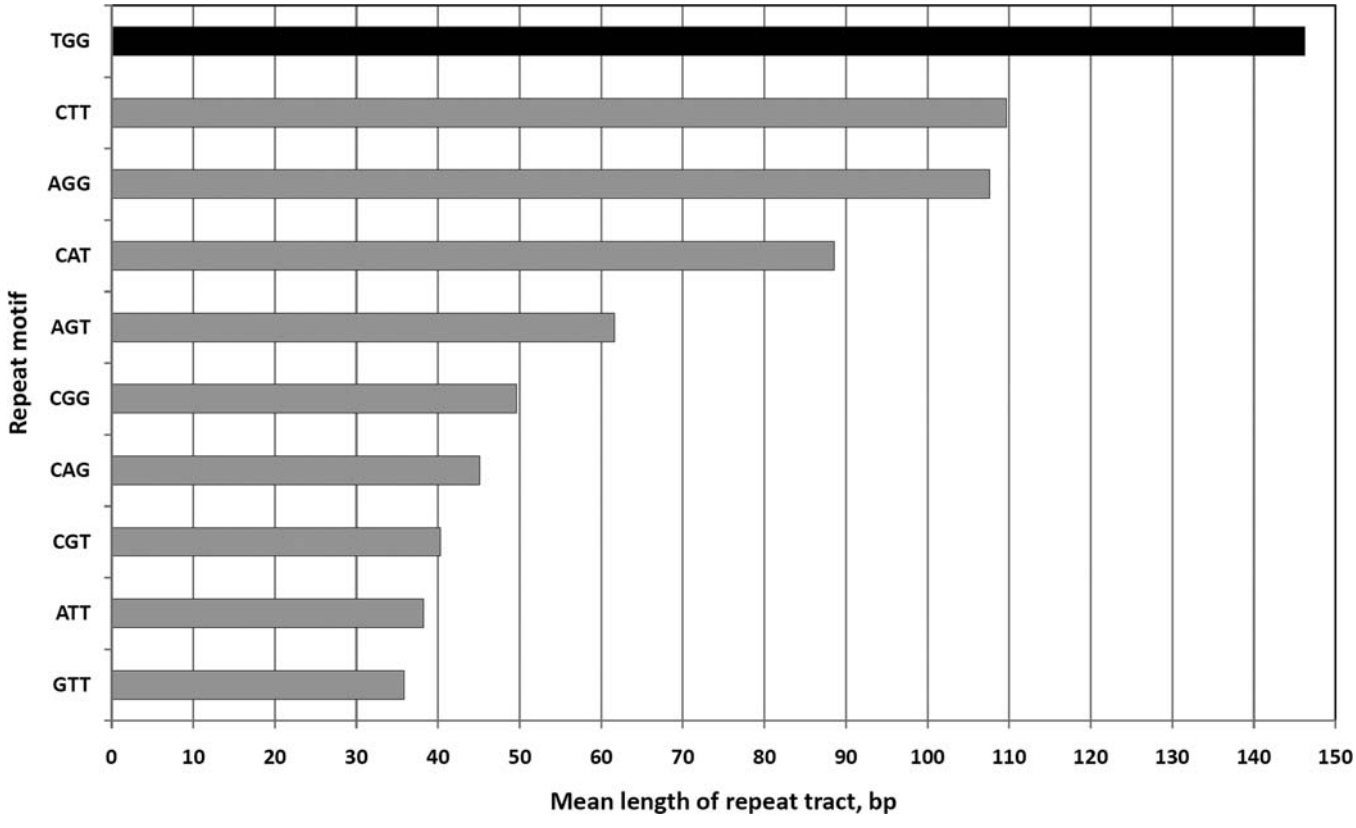

Figure 2. Size distribution of different triplet repeat types in the human genome. Repeats containing repetitions of the motif TGG (TGG, GTG, GGT and their reverse complements CCA, CAC and ACC) (black bar) show a greater mean length than all other types of triplet repeat (grey bars). This observation suggests that TGG-like repeats have a greater tendency to expand compared with other types of repeat, consistent with the idea that TGG-like repeats have a structure that is inherently unstable. The graph displays the mean size of a total of 17 370 triplet repeat tracts, divided by motif, identified by Tandem Repeats Finder in hg18 (36,40). Repeats containing repetitions of the same three nucleotides, and their reverse complements, were collapsed into single categories for display (see Materials and Methods).

deletion breakpoints showed that they are highly enriched for G_4-forming motifs (Fig. 1). Many cytogenetically visible fragile sites are caused by the presence of similar expanded CGG or AT-rich repeats (36,37), the best known of which is the CGG repeat at Xq28 associated with Fragile X syndrome. A review of these fragile sites shows that their repetitive nature results in both an unusually high DNA flexibility and a tendency to form stable secondary structures. Both of these features can induce stalling of the replication machinery during cell division and cause a reduction in nucleosome density of the local chromatin, resulting in an increased frequency of chromosomal breakage at these sites (38). Secondly, by analysing the size distribution of triplet repeats in the human reference genome, we observed a striking trend for $(TGG)_n$-like repeats to be of increased length compared with other triplet repeat motifs (Fig. 2). Repeats containing repetitions of the motif TGG represent the longest types of triplet repeat in the genome, with a mean length more than twice the average of all other triplet repeat types. Although many triplet repeats are highly polymorphic in length, their abundance in the genome (17 370 entries in hg18) means that analysis of the reference sequence still provides a meaningful sample of the length of each type of repeat in the human population. This increased mean length of $(TGG)_n$-like repeats in the genome suggest that they have a greater propensity to expand compared with other types of triplet repeat, supporting the notion that $(TGG)_n$ repeats are inherently unstable in nature.

Future studies may be able to determine the mechanism underlying recurrent deletions of 14q32. Specifically, while the process of NAHR between flanking $(TGG)_n$ repeats is predicted to create the reciprocal duplication and potentially also an inversion product, this observation is not expected from the alternate 'fragile site' model.

We note that a recent analysis of the breakpoints of ~8600 CNVs identified in the HapMap population supports the notion that many structural variants are catalysed by local sequence features (39). Consistent with our observations, both simple repeats and guanine quadruplex DNA were found to be significantly enriched at the breakpoints of CNVs identified in the normal population. In total, Conrad *et al*. (39) found that ~11% of CNV breakpoints identified were found to coincide with simple repeats, while ~8% occurred at sequences predicted to form G-quadruplexes. Taken together, these data suggest a model in which many structural genomic variations are not simply random events, but that sequences such as expanded repeats and guanine quadruplexes predispose certain loci to increased frequencies of rearrangements. We therefore predict that the breakpoints of other chromosomal

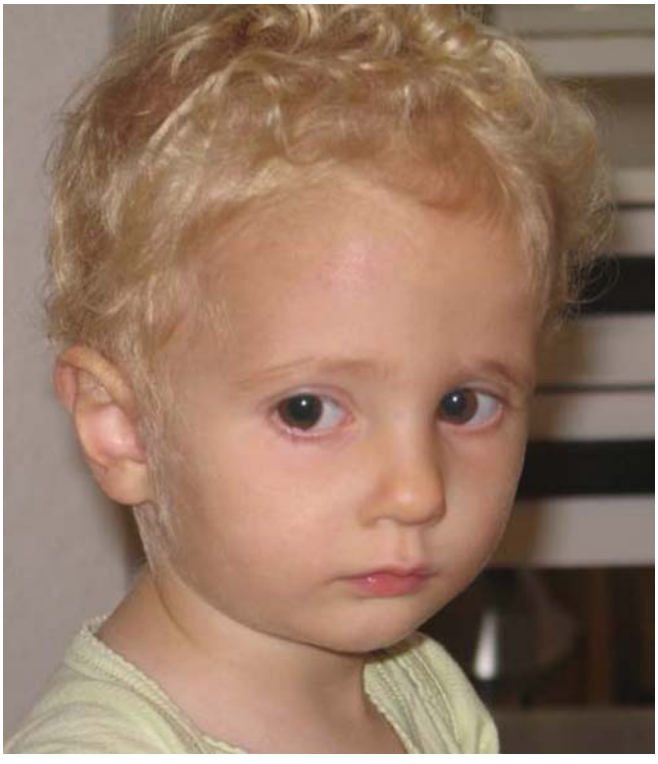

Figure 3. Photograph of patient 1 aged 4 years.

rearrangements associated with human disease will coincide with DNA elements that have similar repetitive and secondary structures.

Given their highly repetitive structure, the $(TGG)_n$ motifs that occur at the breakpoints of 14q32 deletions are likely to be highly polymorphic in the general population. However, all attempts to test this by PCR amplification of either the proximal or distal repeat regions, and efforts to amplify across the deletion breakpoints in the two probands, failed, likely because of the structural properties of these regions that make them refractory to DNA polymerases. As significant contractions or expansions of these flanking $(TGG)_n$ repeats would conceivably alter their ability to catalyse rearrangement by NAHR, it is tempting to speculate that variation in repeat length between different individuals may be associated with a significantly altered deletion frequency during meiosis.

In summary, we describe a novel recurrent microdeletion overlapping an imprinted gene cluster on 14q32 catalysed by large $(TGG)_n$ tandem repeats. Significantly, these repeats are predicted to have extremely high secondary structure, and we provide evidence suggesting that TGG repeats such as these are inherently unstable in nature. Our data are consistent with a model in which expanded tandem repeats catalyse sporadic chromosome rearrangements in the genome, either by providing an improved substrate for NAHR or alternatively resulting from their inherently fragile nature and propensity to form strong secondary structures. Future efforts to characterize the nature of sequences located at rearrangement breakpoints are warranted.

MATERIALS AND METHODS

Patients

Patient 1 is a 4-year-old girl born after 39 weeks of gestation by caesarean section due to foetal distress. Birth weight was 2130 g (−3 SD), length 46 cm (−3 SD) and head circumference 32 cm (−3 SD). The first year of life was uneventful. She could sit at 10 months and started to walk at 16 months. Our first evaluation was done at 21 months. She was very sociable and tonic. Mild facial dysmorphism was noted with a high forehead, small chin and posteriorly rotated ears (Fig. 3). She had curly blond hair and coarse voice. She presented frequent flapping of both hands and tendency to put things in her mouth. Language development was delayed with only one word, small sounds and mimics used for communication. Physical examination showed a mild hypotonia and flat feet. At $2^{8/12}$ years, she had to wear glasses because of hypermetropia. At the age of 3 years, her height was 86.5 cm (−2 SD), weight 9.35 kg (< −3 SD) and head circumference was 46 cm (−2 SD). Her motor and cognitive skills were delayed mostly due to coordination difficulties and severe speech retardation. Endocrinologic exam at $3^{7/12}$ years did not show any hormonal deficiency and bone age was concordant with her chronologic age.

Patient 2, previously reported by Mitter *et al*. (21) and Buiting *et al*. (22), is a $14^{3/12}$ year old girl presenting with pre- and post-natal growth retardation, hypotonia, feeding difficulties, precocious puberty and mental retardation.

Cytogenetic and molecular analysis

Standard GTG banding in patient 1 was performed at a resolution of 550 bands on metaphase chromosome preparations from peripheral blood lymphocyte cultures. Oligonucleotide array-CGH was performed on patient 1 using the Human Genome CGH Microarray Kit 244A (Agilent Technologies, Palo Alto, CA, USA) covering the whole genome with a resolution of ∼20 kb according to the manufacturer's protocol.

Fine mapping of the breakpoints of 14q32 microdeletions utilized a custom-designed 12plex oligonucleotide array comprising a total of 135 000 probes, which included coverage of both the proximal (chr14:99,449,000–99,479,000) and distal (chr14:100,559,000–100,589,000) breakpoint regions at a probe density of 1 per 100 bp. Array hybridizations, washing and scanning were performed according to manufacturer's recommendations using a single female reference DNA (Roche NimbleGen, Madison WI, USA).

Microsatellite analysis used fluorescent-tagged PCR amplified products analysed using an ABI3100 capillary genetic analyser and Genescan and Genotyper softwares (Applied Biosystems). Parental origin of the deleted chromosome 14 in patient 1 was determined using microsatellite markers D14S985 and D14S1006, which lie within the deleted region.

Genomic analysis of repeat content and guanine quadruplex DNA

Data were downloaded from the Simple Repeats track of the UCSC Browser, hg18 (http://genome.ucsc.edu/; 40). For the data shown in Figure 2, as the classification of repeat motifs

by Tandem Repeats Finder is arbitrary depending on the orientation and precise start and endpoints of a motif, triplet repeats containing repetitions of the same three nucleotides were collapsed into single categories. For example, the motifs CCT, CTC and TCC, and their reverse complements AGG, GAG and GGA, were grouped into a single classification, represented in Figure 2 as AGG.

Motifs capable of forming guanine quadruplexes (G_4) DNA were identified by QRGS Mapper (http://bioinformatics.ramapo.edu/QGRS/index.php; 41) using default search parameters.

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Conflict of Interest statement. A.J.S. has received reimbursement of conference and travel fees and has participated in invited seminars and webinars hosted by Roche Nimblegen.

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