Submicroscopic duplications identified during prenatal aneuploidy testing

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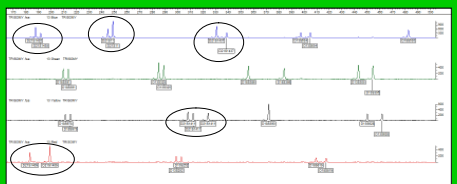

Fig. 1. QF-PCR electrophoretogram for a trisomy 21 sample with abnormal markers circled

- Rapid QF-PCR (Quantitative Fluorescence PCR) is a widely-used approach for the prenatal diagnosis of trisomy for chromosomes 13, 18 and 21 (Mann et al 2001 *Lancet* 2001; 358: 1057–61).
- Since 2000, we have tested >40,000 prenatal samples using a one-tube multiplex containing polymorphic microsatellite markers specific for chromosomes 13, 18 and 21.
- ~ 10% of samples receive an abnormal trisomy result (see fig. 1), as represented by 1:1:1, 1:2 or 2:1 peak area ratio patterns for all markers on a particular chromosome

- ~0.15% of samples exhibit a triallelic profile at a single locus, with all other loci showing normal biallelic or uninformative results indicating either benign submicroscopic duplications (SMDs), or partial chromosome imbalance with phenotypic consequences.
- In all but one case of submicroscopic imbalance, QF-PCR analysis of parental blood samples showed inheritance of the SMD, indicating benign variation (see fig. 2).
- Follow-up testing and reporting of these results can cause parental anxiety.
- UK CMGS/ACC Best Practice Guidelines now recommend that SMDs that are flanked by normal markers and have previously been associated with a normal parental phenotype are not reported. All others should be investigated by testing parental blood samples.
- Twelve recurring SMD regions have been identified in our laboratory (see fig. 3).
- The most commonly detected SMD was D13S742 ★ which has subsequently been removed from our multiplex.
- Only one case has shown a de novo SMD but inherited SMDs have also been demonstrated for this marker. ☆
- Previously unreported SMDs that are not present in the Database of Genomic Variants (http://projects.tcag.ca/variation/) present difficulties of interpretation, especially when found to be de novo, or to represent terminal or unflanked loci.
- **A list of SMDs observed via QF-PCR for aneuploidy detection is available (kathy.mann@gsts.com) comprising data from UK and European labs to aid interpretation and reporting of these results.**

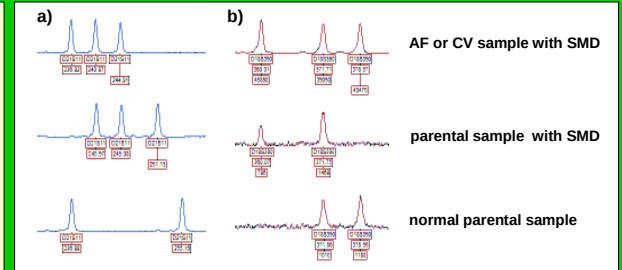

Fig. 2. Examples of markers exhibiting inherited SMDs

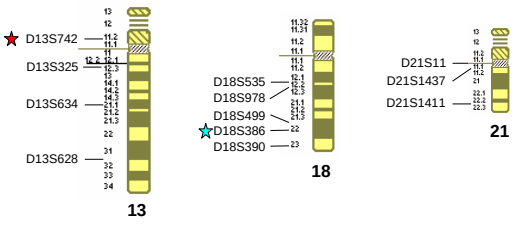

Fig. 3. Cytogenetic locations of the twelve autosomal markers exhibiting SMDs identified in our multiplex

Further investigation of the most frequent SMD – D13S742

Microsatellite analysis

- Polymorphic markers within 3 Mb of D13S742 were identified (ensembl.org) and validated for QF-PCR dosage analysis.
- None was found to be duplicated in D13S742 SMD samples, defining the maximum duplicated sequence as less than 800 kb.

Analysis of non-polymorphic sequences

- Primers were designed for amplicons flanking D13S742 at 1, 10, 50 and 80 kb intervals for incorporation into a fluorescence-based quantitative multiplex assay. Dosage quotients were calculated using MPZ exons (chr 1) as controls.
- Testing of six D13S742 SMD cases found the breakpoints of the duplicated sequence to lie between 10 and 50 kb proximal and between 10 and 80 kb distal to D13S742 (Figure 4).
- The duplicated segment is therefore between 20 and 130 kb in size.

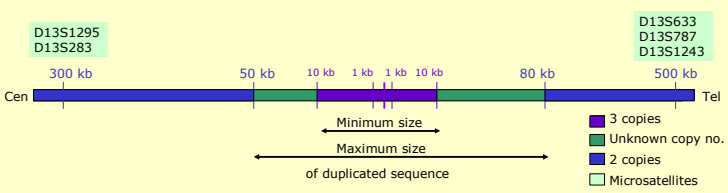

Fig. 4 Size of the D13S742 SMD

Quantitative FISH

- BAC clones (average size 180 kb) and fosmid clones (average size 40 kb) spanning the D13S742 sequence were obtained.
- D13S742 presence within the clones was confirmed by PCR.
- Analysis of metaphase chromosomes from a D13S742 SMD sample following FISH with a 180kb BAC probe showed consistently brighter hybridization on one chromosome 13 homologue than the other (see Fig. 5).

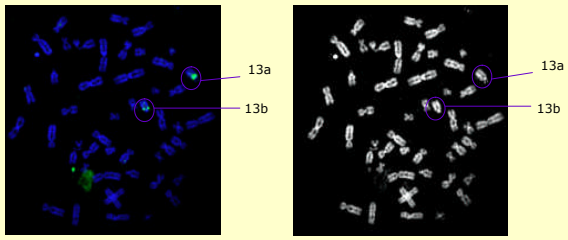

Fig. 5 Metaphase spread of D13S742 SMD case hybridised with a FITC-labelled BAC clone (left). A brighter signal can be seen on one of the homologues (13a) than the other (13b); DAPI banding only is shown on the right.

- In addition, 100 interphase nuclei from a control sample and a D13S742 SMD sample were analysed following FISH with a BAC probe.
- 89/100 nuclei of the SMD case showed only 2 signals, of different intensities, consistent with a tandem intrachromosomal duplication.
- IP lab signal intensity analysis was carried out on metaphase chromosome images to determine if the difference between the two signals was significant.
- The mean signal intensities from 50 images were 97 units for 13a and 64 units for 13b. This was found to be significant (paired t-test).
- The 1.52 13a/13b ratio indicates that the duplicated D13S742 sequence is considerably smaller than 180kb (the size of the BAC clone).

Conclusions

- The D13S42 SMD is located in 13q12.12, probably in a tandem formation and is detected in approximately 0.2% of samples.
- The duplicated segment is between 20 and 130 kb in size and appears to be of a similar size in all tested cases.
- A single gene ATP12A (ATPase H+/K+ transporting, non-gastric alpha polypeptide) is located within this region, although further refinement of the breakpoints is required to ascertain if the whole gene sequence is present.
- Allele lengths are generally different between cases suggesting a recurrent mechanism as opposed to a founder effect.
- The above evidence and the presence of duplicons in the region registered on the DGV support the case for duplicon-mediated generation of this SMD.