Detection of chromosomal aneuploidy by paralogous gene quantification using Pyrosequencing™

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Introduction

- Aneuploidy is currently diagnosed by either karyotyping cultured foetal cells obtained from amniotic fluid (AF) or chorionic villus sampling (CVS) or by molecular techniques such as QF-PCR. Karyotyping is capable of detecting all cytogenetic abnormalities but, in our hands, reporting of results takes an average of 7.2 days for AF and 7.9 days for CVS.
- NGRL (Wessex) have been evaluating assays developed at the University of Geneva that detect aneuploidy by paralogous gene quantification and produce results within the White Paper target guideline of three days for urgent results.

Methods

- Peripheral blood DNA from 103 normal controls and 73 trisomy 21 samples was initially tested for trisomy 21 using three Pyrosequencing assays which analyse paralogous sequences on chromosomes 5, 7 and 21 (Figure 1).
- Data from the three assays were combined and the mean percentage value for the chromosome 21 PSM (determined using the Allele Quantification function of the SNP software) was recorded (Figure 2a).
- To minimise the impact of inter assay variation a mathematical model has been developed which assigns a probability of the patient sample having trisomy 21 based on the % AQ frequency for the chromosome 21 PSM.
- 10 normal and 10 known trisomy controls are run per batch of tests and the mean and standard deviation of the two populations are normalised and used to determine the probability of a sample having trisomy 21.
- The AQ percentage value for the combined data is entered and the % risk is calculated (Figure 2b).
- 100 prospectively collected amniotic fluid samples were then analysed in parallel with our pre-natal cytogenetic laboratory (Figures 3a & 3b).
- Eight assays are currently being evaluated for the detection of trisomy 13, 18 and sex chromosome aneuploidy using 440 retrospectively collected blood and tissue samples and prospectively collected amniotic fluid samples.

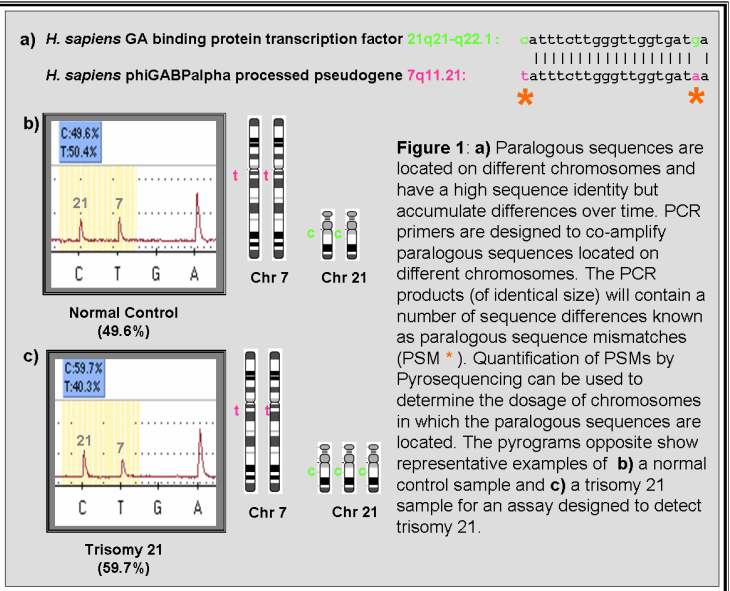

Figure 1: **a)** Paralogous sequences are located on different chromosomes and have a high sequence identity but accumulate differences over time. PCR primers are designed to co-amplify paralogous sequences located on different chromosomes. The PCR products (of identical size) will contain a number of sequence differences known as paralogous sequence mismatches (PSM *). Quantification of PSMs by Pyrosequencing can be used to determine the dosage of chromosomes in which the paralogous sequences are located. The pyrograms opposite show representative examples of **b)** a normal control sample and **c)** a trisomy 21 sample for an assay designed to detect trisomy 21.

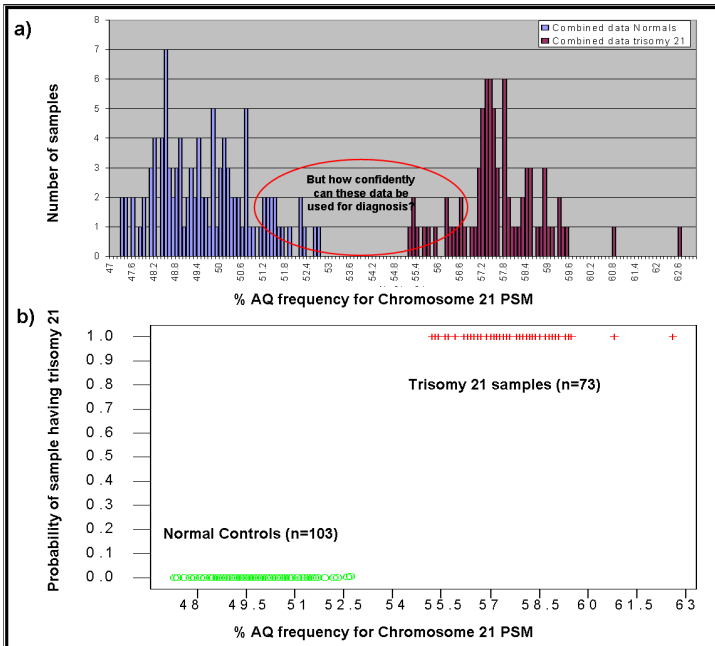

Figure 2: a) Bar chart showing the %AQ frequency for the chromosome 21 PSM for 103 normal samples (blue) and 73 trisomy 21 samples (red). The two populations have separated clearly, but in practice it would be difficult to accurately diagnose samples which fall within the red circle. **b)** Data re-plotted after analysis with the mathematical model. Here the two populations are totally independent with the probability of having trisomy 21 being < 0.005 for normal samples and >0.993 for the trisomy 21 samples.

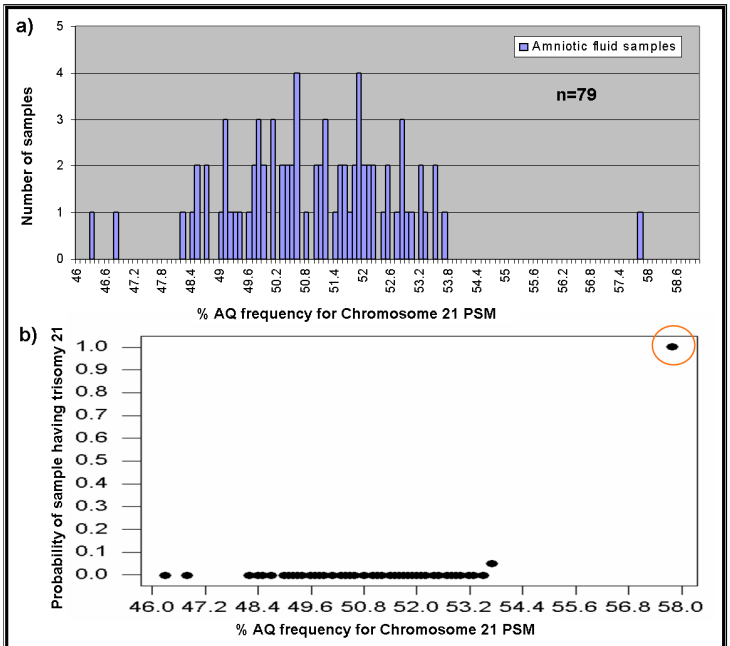

Figure 3: a) Bar chart showing the %AQ frequency for the chromosome 21 PSM for 79 prospectively collected amniotic fluid samples (blue). 21 samples were excluded from the analysis since data was only collected from 2/3 trisomy 21 assays. **b)** Data re-plotted after analysis with the mathematical model. The sample ringed in red was correctly diagnosed as having trisomy 21. All other samples were confirmed to have a normal karyotype

Conclusions

- Results from the analysis of the trisomy 21 assays are promising; the assays are robust, easy to set up and interpret.
- Use of the mathematical model alleviates problems of inter assay variation and standardises interpretation of data.
- As with MLPA, triploidy, rare chromosomal abnormalities and other structural abnormalities will not be detected.
- Analysis of assays for trisomy 13, 18 and sex chromosome aneuploidy is underway and the mathematical model will be developed further to include all data.
- These assays could represent a competitive alternative to other molecular techniques for the diagnosis of aneuploidy in routine diagnostic laboratories.