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# External quality assessment of prenatal diagnosis of a rare and subtle chromosomal structural abnormality

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**Abstract. – PURPOSE OF THE STUDY: To explore the misdiagnosis probability of subtle chromosomal structural abnormalities and find proper strategy to improve the accuracy of prenatal genetic diagnosis, we carried out a preliminary external quality assessment of prenatal detection of a rare case.**

**PATIENTS AND METHODS: Three karyograms of a rare case of cri du chat syndrome associated with t(11;22) translocation [46,XY, del(5)(p15.2), t(11;22)(q23;q11.2)] were chosen. The patient's information and karyograms were emailed to 21 laboratories simulating the scenarios of prenatal diagnosis. The laboratories were required to provide a report using current nomenclature.**

**RESULTS: Seven laboratories sent results for evaluation (response rate: 33.33%). For t(11;22), Two labs incorrectly reported that chromosome 22 was deletion. 5 of 7 labs reported t(11;22) translocation consistent with the actual karyotype. Among them, lab 6 suspected the abnormal 5q and lab 7 incorrectly considered chromosome 22 was deletion or reduplication. All laboratories missed to report the karyotype of del(5).**

**CONCLUSIONS: Conventional cytogenetic analysis couldn't always detect subtle chromosomal structure abnormalities correctly during prenatal diagnosis. To improve the quality of prenatal genetic diagnosis, an excellent external quality assessment (EQA) scheme is currently imperative in China.**

*Key Words:*
Cytogenetics, Prenatal diagnosis, Chromosomal abnormality, External quality assessment.

## Introduction

Prenatal cytogenetic diagnosis has become a mainstay of the repertoire of technologies available to obstetrician and geneticist since it was first carried out in the 1970s[1]. All aspects of quality control for prenatal diagnosis require particular consideration, since the procedure is associated with a risk of miscarriage and an incorrect report would result in improper management of the pregnancy. In order to maintain confidence in this technology, laboratories must implement the highest standards of quality assurance[1,2]. External quality assessment (EQA) is recognized as an essential component to monitor and improve the quality of laboratory output[2,3]. A satisfactory performance in EQA gives assurance both to patients and referring clinicians that the diagnostic laboratory is competent to produce reliable and accurate results[3,4]. Accredited laboratories are required to participate in a recognized EQA scheme for all aspects of the diagnostic service, if available[4].

Nowadays, quality control is widely carried out in different laboratories all over the world[4,5,6], since the first results of EQA scheme were reported in 1947[7]. Many different molecular cytogenetics EQA schemes have also been applied in Europe, since UK started the first one in 1981[4]. During an Internet-based external quality assessment in molecular cytogenetics, the vast majority of laboratories obtain a satisfactory performance in all their EQA rounds. However, to the best of our knowledge, although the importance has been attached to Internal Quality Control of conventional cytogenetic analysis in prenatal diagnosis and the EQA about rapid prenatal detection of aneuploidies demonstrated a high level of genotyping accuracy[1], the EQA about prenatal karyotype analysis of chromosomal structural abnormalities has rarely been reported in the literature. Based on our recent study[2], we tried to explore the misdiagnosis probability of subtle chromosomal anomalies during prenatal genetic diagnosis and find proper strategy to improve the accuracy, through a preliminary multiple images based EQA about karyotype analysis of a case with rare abnormal chromosomal structure [46,XY, del(5)(p15.2),t(11;22)(q23;q11.2)].

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## Patients and Methods

Peripheral blood was collected from a neonate with abnormal chromosomal structure. Routine G-banding karyotype analysis was processed in our laboratory. Images were taken by an automated cytogenetics platform cytovision (Leica Microsystems Co, San Jose, CA, USA). The patient's information has been published as a case report[8]. In brief, a woman with the karyotype 46, XX, t(11;22)(q23;q11.2) was pregnant after accepted an IVF/PGD procedure. Amniotic fluid karyotyping at 20 weeks revealed that the fetus carried the same translocation as the mother. The baby was found to have a high-pitched, cat-like cry after birth. Further peripheral blood karyotyping confirmed a rare case of cri du chat syndrome associated with t(11;22) translocation [46,XY, del(5)(p15.2), t(11;22)(q23;q11.2)].

Three representative images of this karyogram were chosen for EQA (Figure 1). Twenty-one prenatal diagnosis laboratories belonging to the Chinese Public Health Service were enrolled in the trial. To simulate the scenarios of prenatal diagnosis, the patient's history and karyogram images were emailed to the laboratories simultaneously except the clinical manifestations of cri du chat syndrome. The laboratories were required to provide a report using current nomenclature. The approval from the Ethics Committee and informed consent from the patients have been obtained before the study.

## Results

Twenty-one laboratories were enrolled for the prenatal cytogenetic test trial and only 7 laboratories (33.33%) sent results for evaluation. The reports from 7 laboratories were listed in Table I. Two labs incorrectly reported that chromosome (chr) 22 was deletion. 5 of 7 labs (71.43%) reported t(11;22) translocation consistent with the standard karyotype. Among them, lab 6 suspected the abnormal 5q and lab 7 incorrectly considered chr 22 was deletion or reduplication. Unfortunately, all laboratories (100%) missed to report the karyotype of del(5).

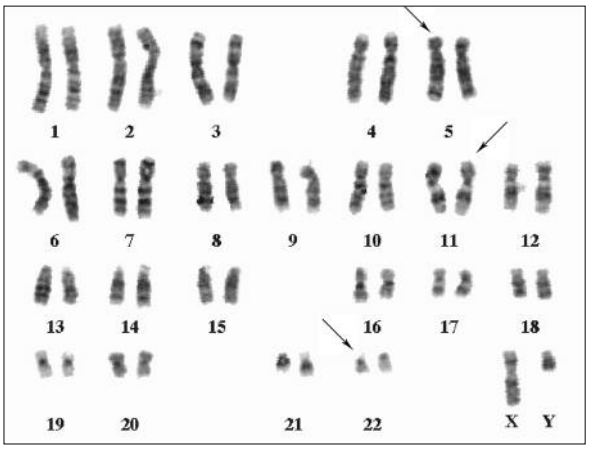


**Figure 1.** Routine G-banding karyotype analysis of the neonate with cri du chat syndrome associated with t(11;22) [46,XY, del(5)(p15.2) t(11;22)(q23;q11.2)]. Three images were emailed for EQA. One representative image is shown here. *The abnormal chromosomes are pointed with the arrows.

## Discussion

The medical genetics workforce is increasingly challenged in meeting demands of a growing referral population and a continuously evolving field. Karyotype analysis of dividing cells is routinely carried out to assess chromosome copy number and identify structural rearrangements, mainly translocations. It is also the routine method for prenatal diagnosis, because the most common reason for prenatal diagnosis remains an increased risk of having a child with Down

**Table I.** The results of karyotype analysis from 7 laboratories.

| Lab | Karyotype | Karyotype analysis description |
|---|---|---|
| 1 | 46,XY,del(22) | Chr 22 is deletion |
| 2 | 46,XY,t(11;22) | Translocation between chr 11 and 22 |
| 3 | 46,XY,del(22) | Chr 22 is deletion, no traslocation between chr 11 and 22 was found, band of chromosome16 seems abnormal |
| 4 | 46,XY,t(11;22) | Translocation between chr 11 and 22, pleased combined with clinical manifestations |
| 5 | 46,XY,t(11;22) | The number of chromosome is normal, chr 11 and 22 is translocation |
| 6 | 46,XY,der(5q) ,t(11;22) | Long arm of chr 5 is abnormal chr 11 and 22 is translocation |
| 7 | 46,XY,der(22), t(11;22) | Chr 11 and 22 is translocation, and chr 22 is deletion or reduplication, molecular genetics method is required |

syndrome[1]. Besides considerable experience is required, karotyping still might have analytical difficulties because it is not always possible to obtain an adequate number of metaphases in the process or the quality of these metaphases does not permit a detailed study of the chromosomes. Based on our preliminary inter-laboratories investigation, we found that karyotype analysis was difficult to make accurate cytogenetic diagnosis of subtle chromosomal abnormalities. The t(11;22)(q23;q11.2) is the only known recurrent, non-Robertsonian, constitutional translocation in humans. Carriers of reciprocal translocations are phenotypically normal, but they may have reproductive problems, such as birth of chromosomally abnormal children, recurrent spontaneous abortion. Usually translocation is the common indication for karyotpe analysis. However, present study revealed that only 5 of 7 labs reported the correct karyotype of translocation. To improve the accuracy of prenatal diagnosis of subtle abnormal chromosomal structures, cytogenetics training on subtle chromosome anomalies is peremptorily required. Further molecular cytogenetic techniques such as fluorescence in situ hybridization (FISH) and comparative genome hybridization (CGH) are also helpful.

Cri du chat syndrome (CDCS) is a subtle chromosome anomalies resulting from a deletion in the short arm of the chromosome 5, which is characteristic with cat like cry. The incidence varies from 1 in 15,000 to 1 in 50,000 live births[8,9]. Regarding the 5p microdeletion, it was supposed to make correct diagnosis by routine karyotype analysis. But inncreasing studies reported the inconsistency between the conventional cyotgenetics analysis and molecular genetic techniques used for detection of chromosome subtle abnormalities[10-12]. Marinescu et al[15] used FISH probes to reanalyze *de novo* terminal deletions determined by standard cytogenetic approaches. Unexpected results were found in 7 of 110 patients supposedly with CDCS. Among them, 4 patients were determined to have interstitial deletions, 1 patient had an unbalanced translocation, and no deletion could be detected in 2 patients. Kondoh et al reported one CDCS infant was diagnosed only by the FISH analysis[16]. This infant was clinically typical of CDCS. Although her karyotype was reported to be normal by the ordinary G-banding method, FISH analysis using a probe D5S23 targeting 5p15.2 demonstrated chromosomal microdeletion of 5p15.2. Mosca et al also reported[17] that standard cytogenetic analysis was interpreted as normal in a girl suspected to present a CDCS, but FISH studies revealed a microdeletion that was then confirmed by CGH-array. In the present case, the 5p deletion was overlooked during prenatal genetic diagnosis, in partly because it was a small-sized deletion and the focus of the examination was on the translocation based on clinical information. These data demonstrated that standard cytogenetic analysis was not absolutely correct for detecting a microdeletion, and FISH analysis could be used to complement for any subtle cytogenetic finding.

Up to today, although the importance has been attached to Internal Quality Control of conventional cytogenetic analysis in prenatal diagnosis in China, there is still no official EQA scheme of prenatal genetic diagnosis in China, which might be one of the reasons why only 33.33% (7/21) laboratories gave results for evaluation in present study. In our study, the qualities of the laboratories were evaluated through simulating the scenarios of prenatal diagnosis with a subtle chromosome anomalies karyotype [46, XX, t(11;22) (q23;q11.2)], none of them could report the correct karyotype of the del(5). These results revealed that such mistake was a prevalent phenomenon during prenatal diagnosis. As far as we know, CDCS associated clinical parameters indicated for prenatal diagnoses are still unknown and a majority of cases were diagnosed postnatal. Lack of experience might be another reason for missed detection of 5p deletion during prenatal diagnosis. A feasible EQA scheme of prenatal genetic diagnosis should be well designed and widely applied in China, since EQA is a good method to improve cytogenetic technique. Furthermore, feedback of the standard karotype should be given to the laboratories timely after assessment, which will extraordinarily increase the chance of the laboratories to recognize the rare subtle chromosome anomalies. Consequently, improvement would be easily achieved through setting up a proper EQA and feedback protocol for a rare subtle chromosome anomalie. Since there is still lack of efficient EQA for prenatal karyotype analysis of chromosomal structural abnormalities, here we provide an alternative EQA scheme for detection of chromosomal abnormalities through using multiple karyogram images.

## Conclusions

Taken all together, although conventional cytogenetic analysis is a gold criteria for diagnosing chromosomal structural abnormalities, the present study revealed that it was sometimes difficult to detect subtle chromosomal structural abnormali-

ties in prenatal diagnosis. Molecular genetic techniques could be used to validate such difficult cases if needed, since the results may have relevance to important lifetime decisions both for the individuals being tested and for their family. Clinical manifestations might occasionally misguide the genetic diagnosis of complex chromosomal abnormalities. Most importantly, we found karyogram images could be an effective material for EQA of prenatal cytogenetic diagnosis. EQA is not only an ideal method to assess the quality of prenatal genetic diagnosis, it also allows for other applications such as continuing professional development, competency testing, or independent learning programmes. An excellent EQA scheme is currently imperative in China. Until EQA participation becomes mandatory as a component of compulsory laboratory accreditation, the quality of prenatal genetic laboratory is unpredictable.

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### Conflict of Interest

The Authors declare that they have no conflict of interests.

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