

SUMMARY

More than 40% of men attending fertility clinics have oligo- or azoospermia with unknown etiology. During recent years microdeletions in the AZF regions on the Y chromosome have been implicated as one of the causes of male infertility. The prevalence of microdeletions in the AZF region ranges from 0-55% in populations of azoospermic or severe oligozoospermic men. The exact relationship between the severity of male infertility and the size or position of microdeletions involved in the regulation of spermatogenesis has not been resolved.

Today, the PCR approach is a widely used method for screening for Y microdeletions, since it can detect a wide size-range of deletions by amplification of a series of short sequences referred to as sequence tagged sites (STSs) spanning the region of interest. However, there is still no standardized methodology for screening for Y microdeletions. At the time this project started, most assays used were cumbersome and the existing approaches were not suitable for analysis of a large number of samples.

In the present project as a first step we developed a new multiplex PCR combined with separation of amplified DNA fragments by agarose gel electrophoreses. Later, we improved the methodology by introducing capillary electrophoresis combined with fluorescent PCR for separation and analysis of PCR products. This method has several advantages, e.g. shorter running times, online detection, added flexibility, autosampling and allowing very small amount of PCR products to be analysed.

In the present project screening for Y microdeletions was performed in 400 ICSI candidates, 200 fertile men, and 100 men with a diagnosis of undescended testis or testicular cancer.

In 400 ICSI candidates, Y microdeletions were found in 3 severely oligo- or azoospermic men. In our study, the overall frequency of Y microdeletions was found to be lower than in previous studies of unselected ICSI candidates (0.75%), but also in specific subgroups classified on the basis of sperm concentration (2% in azoospermic and 0.6% in oligozoospermic men). All deletions comprised ≥ 2 contiguous STSs. An analysis of the literature shows similarly that the majority of deletions in infertile men are composed of larger deletions comprising at least 2 deletions in tandem, while deletion of a single STS locus is rare. Large deletions are generally associated with more severe spermatogenic defects. These data strongly indicate that a Y microdeletion has a pathogenetic effect only if at least two STS loci in tandem are deleted.

A single missing STS was found in one fertile men and his brother (sY153). Our analysis indicated that the missing signal was not due to a real deletion, but probably to a sequence variation in the primer binding site, since the presence of PCR products of this STS with expected size was demonstrated when using lower annealing temperature in the PCR. Accordingly, true Y microdeletions were not found in fertile men. A similar sequence variation in the primer binding site was demonstrated in one case of undescended testis (sY139) and testicular cancer (sY153), respectively. Since no true Y microdeletions were found in these men, it is less likely that Y microdeletions can be a significant etiological link between compromised spermatogenesis, testicular cancer and undescended testis.

At present, it is recommendable to screen infertile men with severe oligo- or azoospermia for Y microdeletions in order to look for a cause of the spermatogenic defect and to offer genetic counseling informing the couple of the likeliness to transmit the deletion to sons, who should be recommended early andrological examination. In conclusion, the rapid progress in molecular biology technologies has allowed us to improve both quality of the diagnosis and the time needed for a Y microdeletion screening. Currently, screening for Y microdeletion is used for research purposes mostly, but development of assays such as ours represent the first step towards its use in routine laboratory medicine.