Incidence of AZF (Azoospermia Factor) Deletions and Familial Forms of Infertility Among Patients Requiring Intracytoplasmic Spermatozoa Injection (ICSI)

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Purpose: The aim of our study was to determine the incidence of AZF deletions and familial forms of infertility suggesting autosomal mutations among patients requiring intracytoplasmic sperm injection with ejaculated sperm.

Methods: Cases with obstructive pathologies were excluded; 81 patients were classified according to the numeration of spermatozoa. The distribution was as follows: 10 cases with normal numeration (greater than 20 million/ml) (group 1), 10 cases with between 10 and 20 million/ml (group 2), 6 cases with between 5 and 10 million/ml (group 3), 15 cases with between 1 and 5 million/ml (group 4), 29 cases with less than 1 million/ml (group 5), and 11 azoospermic patients (group 6). The infertility of 11 of the 81 patients might be explained by testicular ectopy.

Results: We found two deletions limited to the AZFc region among our 81 infertile patients—one deletion in group 5 and one deletion in group 4 (both groups of oligozoospermic patients)—and no deletion in the groups with normal or subnormal numerations. We found six familial forms of infertility. We did not find any AZF deletion, neither in these 6 patients nor in the 11 with testicular ectopy. The identification of these families of infertile men will allow research of autosomal genes involved in male infertilities.

Conclusions: It is important to test deletions of the AZFc region for oligozoospermic patients, and familial forms of infertility do not seem to concern the same individuals.

KEY WORDS: male infertility; molecular biology; genetic cause; Y chromosome deletion.

INTRODUCTION

The incidence of the genetic causes of male idiopathic infertility remains uncertain. Spermatogenesis requires

many gene products, and severe defects in human sperm production can be related to different causes including genetic ones. Indeed, different types of genetic causes can be distinguished. They include chromosomal abnormalities and molecular causes, either Y deletions of the region controlling spermatogenesis (AZF) or possible autosomal gene mutations. Recently several studies demonstrated that microdeletions occurring in at least three regions, AZFa, AZFb, and AZFc, were associated with male infertility (1–3). It was suggested that deletions in AZFa and AZFb result in azoospermia and that deletion in AZFc was more likely involved in severe oligozoospermic cases. Nevertheless, these correlations could not be confirmed in other similar studies; a recent study (4) reported that 7% of 200 infertile men and 2% of 200 fertile men presented a Y chromosome microdeletion. The correlation between the size and the position of the deletion with the severity of infertility was very poor. According to the various clinical studies published recently (1–11), the incidence of microdeletions of the AZF region ranges between 7 and 30% in the population of azoospermic or severe oligospermic men. Thus, the pathologic value of these Y deletions remains unclear, and the correlation between the phenotype and the genotype needs to be clarified.

The aim of our study was to determine the incidence of AZF deletions among men requiring intracytoplasmic sperm injection (ICSI). Establishing the pedigree of the patient (12), we investigated the familial forms of infertility suggesting autosomal mutations.

MATERIALS AND METHODS

We present results concerning 81 infertile men with a normal karyotype. Cases with obstructive pathologies

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were excluded. According to the numeration of spermatozoa, the distribution was as follows: 10 cases with normal numeration (greater than 20 million/ml) (group 1), 10 cases with between 10 and 20 million/ml (group 2), 6 cases with between 5 and 10 million/ml (group 3), 15 cases with between 1 and 5 million/ml (group 4), 29 cases with less than 1 million/ml (group 5), and 11 azoospermic patients (group 6). The infertility of 11 of these 81 patients might be explained by testicular ectopy. The complete series of patients was screened for microdeletions by PCR using primers (sY147/sY149) prepared as described (2) and located in the AZFc region likely to be involved in oligospermic patients.

Genomic DNA was prepared from peripheral blood lymphocytes by a standard laboratory protocol; PCR was carried out on 200 ng of genomic DNA in 50 μ l of reaction volume as described (7). PCR reaction products were separated on a 2.5% agarose gel by electrophoresis and visualized with ethidium bromide. The sY 147 primer pair gives an amplification product of 100 bp, and the sY 149 pair a product of 132 bp. When a deletion was observed, it was confirmed by two independent experiments, and one positive primer pair was added as an internal control (SY14). Every analysis contained a blood sample from a fertile woman and from a fertile man as negative and positive controls, respectively.

For the first subgroup of 50 patients from the same previous series, we completed this study performing a multiplex PCR using five primers (SY 81 and SY 182, located in region AZFa; SY 143, located in region AZFb; SY 147 and SY 157, located in region AZFc) according to the protocol described previously (7). Another PCR was performed using a simple primer pair, SY 128, located in region AZFb (2). To summarize, this second screening protocol included six primers: two were located in AZFa, two in AZFb, and two in AZFc.

RESULTS

We found two deletions in the AZFc region (SY 147, SY 149) among our 81 infertile patients—one deletion in group 5 (sperm with 80,000 spermatozoa/ml) and one deletion in group 4 (sperm with 5 million spermatozoa/ml)—and no deletions in the groups with normal or subnormal numerations. Those two deletions did not extend to the regions corresponding to the other primers tested subsequently [neither by the multiplex PCR (SY 81, 182, 143, and 157) nor by the single

PCR concerning SY 128]. The multiplex PCR results confirmed the deletion observed for SY 147 in the two previously diagnosed deleted patients. We concluded that the deletions of those two patients were limited to the AZFc region, located between SY 147 and SY 149 and excluding AZFa, AZFb, and the terminal end of AZFc. These two deletions were quite large and similar. Among the 6 patients with familial forms of infertility and the 11 patients with testicular ectopy, we did not find any AZFc deletions. The six familial forms of infertility belonged to three groups (1, 5 and 6). According to the interview, four patients had a brother with similar sperm abnormalities, and two patients had an infertile uncle. None of the 48 patients who was diagnosed normal by the first PCR (SY 147, SY 149) was found to have deletions using the more complete screening protocol (with six primers located in AZFa, AZFb, and AZFc).

DISCUSSION

These results confirm the interest in screening AZFc deletions for oligozoospermic patients requiring ICSI. We did not find any AZFc deletion among the 26 cases with a sperm numeration greater than 5 million/ml. A further analysis of the AZFb and AZFa regions is in progress for the same patients to complete this study, but the 50 first cases did not show any deletion, which was not demonstrated in the first study. Our cases of deleted patients presented either mild or severe oligospermia, probably due to similar deletions covering the AZFc region between SY 147 and SY 149. This confirms the hypothesis that suggests that the AZFc region is involved in oligospermia but the phenotype concerning spermatogenesis cannot be precisely predicted from the deletion. Great care must be taken in genetic counseling of male infertile patients. It seems quite hazardous to predict the fertility of the possible sons of those two patients presenting an AZFc microdeletion. The familial cases of infertility do not seem to concern the same individuals as the ones affected with the deletion. Familial male infertility should be related to autosomal mutations. The identification of these families and the collection of DNA from the patients are important because they will allow research of autosomal genes involved in male infertilities. This requires a detailed interview concerning the relatives of the patient and a pedigree before ICSI. Screening the Y chromosome deletions of patients requiring ICSI is possible in an infertility clinic and can be integrated into the clinical diagnosis of male infertility. This will

allow the understanding of the pathologic value of these Y deletions to progress and the identification of the genes from this Y region, which have an important role in spermatogenesis.

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