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Loss of the 'azoospermia factor' (AZF) on Yq in man is not associated with loss of HYA

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ABSTRACT

We have typed 9 EBV cell lines from azoospermic or severely oligospermic patients for the expression of H-Y antigen, in order to test the hypothesis of the coincidence of AZF and HYA genes. Of nine patients with cytogenetically normal Y chromosomes, 7 could be tested for HYA expression and of these 6 were H-Y positive. Of the three patients showing Yq structural abnormalities, two could be tested for H-Y expression and one was negative, the other positive. These results therefore show no correlation between spermatogenic failure and the absence of HYA, thus separating the AZF locus from HYA.

INTRODUCTION

A genetic region controlling spermatogenesis in humans has been localized to the long arm of the Y chromosome [1-7], as has the gene(s) encoding the minor histocompatibility antigen, HYA [8-10]. In mice, the functional equivalent of the human azoospermia factor, AZF, is *SpY* and has been localized between *Zfy-1* and *Zfy-2* on the short arm of the Y chromosome and its translocated counterpart, *Sxr^a* [11,12]. The *Sxr^b* mutation arose by a gene fusion of *Zfy-1* and *Zfy-2*, deleting the intervening DNA in which *Hya*, as well as *SpY*, is located [13-15]. The *Sxr^b* mutation did not affect the testis determining gene *Tdy/Sry*, also present on the short arm of the murine Y chromosome [16] and on *Sxr^a* [13], thus separating *Hya* from *Sry* [13,7]. In man, HYA and TDF/SRY have also been separated by different chromosomal localization. It has been proposed that the functions of *SpY* and *Hya* in mice may be encoded by the same gene [12], although a recombinant between *Sxr^a* and *Sxr^b* challenges this hypothesis [18]. In this paper, we examine the hypothesis that, in humans, AZF and HYA are the same gene, by testing a series of azoospermic or severely oligospermic men for the expression of HYA. The results show no correlation between spermatogenic failure and the absence of HYA, either in men with apparently normal 46 XY karyotypes, or with Yq structural anomalies, thus separating AZF from HYA.

RESULTS

The chromosomal constitution, clinical details and testicular histology of 9 sterile men with cytogenetically normal Y

chromosomes and three with Yq structural anomalies are shown in Table 1, together with a summary of the H-Y phenotyping results of the 9 of them which could be typed, as they expressed the HLA-A2 or B7 allele used as the restriction molecule by our H-Y specific T cell clones. Details of the H-Y assays are shown in Table 2. Six of the seven patients with cytogenetically normal Y chromosomes who could be typed for H-Y were positive: one of the seven, KLARD, who has been previously reported to have a microdeletion in distal interval 6 of Yq [7], was H-Y negative. Another, 'JOLAR', who had a microdeletion in proximal interval 6, typed H-Y positive. Two of the three azoospermic patients with Yq structural anomalies, FRABO and BITRA, could be typed for the presence of H-Y: FRABO was positive and BITRA negative. Thus, all but two of the nine HLA A2 or B7 sterile patients, some with known deletions in the AZF region, had normal expression of HYA.

DISCUSSION

The finding of HYA expression in all but two of the nine sterile patients tested excludes the possibility that HYA and AZF are the same gene. Although they can both be localized to the same deletion interval of Yq [7,10], this still represents a large physical distance which could readily accommodate many genes. Molecular analyses of Yq deletions in 4 of the azoospermic patients reported here (JOWAL, BITRA, FRABO AND KLARD) have shown absence of a common contiguous length of DNA extending through three sub-intervals of interval 6 [7]. Of the three of these who could be typed by H-Y, one was positive, two negative, making it unlikely that HYA is located in the interval implicated for AZF. A fifth azoospermic deletion patient, 'JOLAR', showed absence of a more proximal sub-interval of interval 6 [7] which might also be implicated in spermatogenic control. He, too, typed H-Y positive. Current work is focused on a more detailed molecular analysis of the appropriate intervals of Yq, to make more precise localizations and to identify and test candidate genes, both for HYA, for which *in vitro* expression testing is available, and for AZF, which requires clinical correlates, but for which candidate genes can ultimately be tested in transgenic mice of the XOSr^b genetic constitution [12,17].

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Table 1. Genotypes and phenotypes of infertile subjects with A) Normal 46, XY karyotypes B) Yq structural anomalies

Patient Code Name	Age	Mean Sperm Count ($\times 10^6$ per ml)	Testis Vol (cc) ⁺		Hormone levels ⁺⁺			Testicular Histology	HLA Serology	H-Y phenotype
			R	L	LH	FSH	Testosterone			
A)										
'ALCHA'	38	Azoospermic	7	7	12.4	40.9	25.9	Not biopsied	A2	+
'AMGAL'	25	Azoospermic	10	10	4.2	11.3	16.1	Sertoli cells only	A2, B7	+
'BRING'	31	Azoospermic	13	13	3.2	3.6	34.0	Diminished spermatogenesis A few sperm in most tubules Prominent sloughing of cells into central lumina		Non typable
'GERTO'	29	Azoospermic	16	14	9.1	15.0	15.1	Arrested spermatogenesis between MI and MII Some tubules contain only Sertoli cells	A2	+
'INGIL'	32	Azoospermic	15	20	8.6	20.9	29.7	Diminished spermatogenesis with spermatid arrest in right testis Some atrophic tubules	B7	+
'JASTRA'	32	1.3	10	10	8.2	13.5	30.4	Sertoli cells only in most tubules Diminished spermatogenesis in remainder with some cells showing progression only to spermatocyte stage	A2	+
'JOLAR'	28	Azoospermic	10	10	6.9	11.9	26.4	Sertoli cells only	A2	+
'KLARD'	32	Azoospermic	10	10	5.2	11.1	26.4	Not biopsied	A2	-
'ROYAL'	29	<1	7	7	7.9	12.1	32.6	Greatly diminished spermatogenesis Some tubules atrophic Others show development to spermatid stage (two-thirds) or spermatozoan stage (one-third)		Non typable
B)										
'FRABO'	35	Azoospermic	12	12	12.2	21.1	36.6	Not biopsied	A2	+
46,X del(Y){pter \rightarrow q11.23}										
'JOWAL'	33	Azoospermic	'Normal range'		NT	NT	NT	Actively dividing cells in all tubules Arrested spermatogenesis between MI and MII Few spermatids, no spermatozoa (19)		non-typable
45,X/46,r(Y)										
'BITRA'	28	Azoospermic	12	12	9.3	18.6	25.3	Hyalinization of most tubules	A2B7	-
46,X del(Y){pter \rightarrow q11.23} Low numbers of spermatogonia and rare spermatocytes (2)										

+ Normal range for Caucasians 15–45cc

++ Normal range	{	LH 1–6.5n/l
		FSH 1–6n/l
		Testosterone 10–30ng/ml

NT = not tested

MATERIALS AND METHODS

Selection of patients for screening

Amongst men undergoing investigation at an infertility clinic, twelve were selected with non-obstructive oligo or azoospermia. Nine were chromosomally normal, the other three having structural anomalies of the Y chromosome (Table 1). On clinical examination, most patients had reduced testis volumes and raised levels of FSH and LH indicative of spermatogenic impairment. Nine of the patients underwent testicular biopsy in the course of their infertility investigations, the various histological findings included 'Sertoli-cell only' syndrome, spermatogenic depression and germ-cell maturation arrest.

Molecular investigations

All nine of the chromosomally normal men tested for H-Y were also investigated using interval 6 probes to detect possible microdeletions in the AZF region. Two patients, 'JOLAR' and 'KLARD', were found to have non-overlapping interruptions in their DNA, the microdeletion in 'JOLAR' being proximal in interval 6, that in 'KLARD' being more distal [7]. Both men displayed similar phenotypes of infertility (see Table 1), raising the possibility that AZF might be one very large gene or that several genes residing on the Y chromosome long arm might be important in spermatogenesis. Microdeletions were not found in the other seven individuals tested.

H-Y typing

EBV cell lines from each of the patients shown in Table 1 were serotyped for HLA class I alleles of the A and B locus by standard serotyping in the tissue typing laboratory of RPMS, by Mr Nick Davey and by FACS analysis at the CRC using the HLA A2 specific monoclonal antibody, HB82 (BB7.2) and the HLA B7 (crossreactive with B40) monoclonal antibody, HB57 (HB40.2). Expression of HLA-A2 and B7 alloantigens identified by T cells was confirmed by cytotoxic T cell lysis (CTL) experiments in which the patients' cells were also typed for H-Y, using EBV cell lines from each of the patients and normal male and female appropriate controls. The cytotoxicity was measured in a ^{51}Cr release assay as previously described [8].

The identity of the HLA molecule used as a restriction element for the detection of H-Y antigen in the CTL assays is underlined in the HLA serotyping columns. Figures underlined in the percent cytotoxicity columns are those showing significant levels of lysis at an attacker:target (A:T) ratio of 10:1, taken from regression analysis of data obtained from a titration curve using 3 or 4 A:T ratios. ND = not done.

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Table 2. Details of HLA serotyping and cytotoxic T cell phenotyping of EBV lines from patients and controls

Exp	Name	HLA		% Cytotoxicity @ 10 ¹ A T with				H-Y Typing
		A	B	A2H-Y	A2allo	B7H-Y	B7allo	
1	♂ control	<u>2</u>		<u>74</u>	<u>23</u>			+
	♀ control	<u>2</u>		2	<u>18</u>			-
	♂ control		<u>7</u>			<u>70</u>	<u>61</u>	+
	♀ control		<u>7</u>			5	<u>35</u>	-
	JOLAR	<u>1,2</u>	8,13	<u>82</u>	<u>31</u>			+
	AMGAL	<u>2,3</u>	<u>7,27</u>	<u>89</u>	<u>42</u>	<u>74</u>	<u>76</u>	+
2	♂ control	<u>2</u>		<u>76</u>	<u>8</u>			+
	♀ control	<u>2</u>		2	<u>36</u>			-
	JASTRA	<u>2</u>	12	<u>70</u>	<u>9</u>			+
	KLARD*	<u>2</u>		0	<u>38</u>			-
3	♂ control		7			<u>81</u>	<u>83</u>	+
	♀ control		<u>7</u>			7	<u>60</u>	-
	INGIL	2,3	<u>7,40</u>			<u>59</u>	<u>67</u>	+
	BITRA	2,11	<u>7,27</u>			7	<u>50</u>	-
4	♂ control	<u>2</u>		<u>94</u>	<u>96</u>			+
	♀ control	<u>2</u>		1	<u>79</u>			-
	FRABO	<u>1,2</u>	8,44	<u>93</u>	<u>94</u>			+
	GERTO	<u>2,7,19</u>	13,44	<u>93</u>	<u>100</u>			+
	ALCHA	2,3	35,44	<u>103</u>	<u>102</u>			+
Could not be typed due to inappropriate HLA-A and HLA-B alleles								
		<u>A</u>	<u>B</u>					
	BRING	29,30	18,716					
	ROYAL	1,3	17,713					
	JOWAL	1,3/11	27,715					

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