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Original Research Article

Killer cell immunoglobulin-like receptor gene association with cryptorchidism

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ABSTRACT

Cryptorchidism is a condition where a testis persists in the abdominal cavity. Thus, due to elevated temperature we may expect induction of aberrant immune reactions depending on genetic constitution of individual. This may be reflected by development of anti-sperm antibodies (ASA) in cryptorchid males. Also, natural killer (NK) cells which belong to innate immunity may control adaptive immunity. Therefore, the gene system encoding polymorphic NK cell immunoglobulin receptors (KIRs) has been studied. 109 prepubertal boys with cryptorchidism and 136 ethnically matched young male donors were selected to study NK cell KIRs. DNA was isolated using automatic Maxwell® system from the peripheral venous blood drawn onto anticoagulant. Olerup SSP KIR Genotyping kit including Taq polymerase was used for detection of KIR genes. Human leukocyte antigen-C (HLA-C) groups, C1 and C2 were established using a Olerup SSP KIR HLA Ligand kit. KIR2DL2 (killer immunoglobulin-like receptor two-domain long 2) and KIR2DS2 (killer immunoglobulin-like receptor two-domain short 2) genes were less frequent in patients than in control individuals (corrected *p* values: 0.0110 and 0.0383, respectively). However, no significant differences were observed between ASA-positive and ASA-negative patients, or between bilateral or unilateral cryptorchidism. No association between KIR

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ligands C1 and C2, alone or together with KIR2DL2, was found. However, the results suggest that KIR2DL2+/KIR2DS2+ genotype may be, to some extent, protective against cryptorchidism.

1. Introduction

Cryptorchidism is a condition in which a testis, that should normally descend to the bottom of the scrotum, persists in the abdominal cavity. It is one of the most frequent pathologies (1–2%) in early male childhood [1]. Although the blood–testis barrier prevents autoimmunization [2] and many sperm proteins are not produced before the start of spermatogenesis at puberty, we and others showed that sera of prepubertal boys may contain anti-sperm antibodies (ASA), and their frequency in cryptorchidism is increased [3]. Another mechanism protecting from autoimmunization with spermatozoa is active immunosuppression by components of seminal plasma and by cytotoxic/regulatory function of T lymphocytes [2,4]. Indeed, decreased numbers of activated T lymphocytes, as compared with normal testes, were observed in cryptorchid epididymis and vas deferens, suggesting insufficient control of autoimmunity [5]. However, the role of natural killer (NK) cells, an important element of innate immunity and regulator of adaptive immunity, was not examined in cryptorchidism so far.

NK cells express a variety of activating and inhibitory cell surface receptors which enable them to kill abnormal (virally infected or malignant) cells and prevent them from attacking normal cells of the body [6]. The most polymorphic of these receptors are killer cell immunoglobulin-like receptors (KIRs) which recognize relatively polymorphic epitopes on human leukocyte antigen (HLA) class I molecules. KIR proteins have either two (KIR2D) or three (KIR3D) extracellular immunoglobulin domains and long (KIR2DL, KIR3DL) or short (KIR2DS, KIR3DS) intracellular tail. KIRs with long cytoplasmic tail transduce an inhibitory signal to the cell upon ligand binding. In contrast, those with short cytoplasmic tail transduce an activating signal upon ligand binding. Known ligands of KIRs, particularly inhibitory ones, are HLA class I molecules [7,8].

A peculiarity of the KIR genetic system is high polymorphism, both allelic (high numbers of allelic variants) and haplotypic (different numbers of *KIR* genes for activating and inhibitory receptors on individual chromosomes) [9]. This results in individual variation of susceptibility to many diseases, from autoimmune to malignant disorders [8,10]. As cryptorchidism increases the risk of testicular cancer [1], we attempted to examine whether boys with cryptorchidism do not differ in their repertoire of *KIR* and KIR ligand genes from healthy individuals.

2. Materials and methods

2.1. Study subjects

One hundred and nine pre-pubertal boys with cryptorchidism (mean age 5.4 ± 4.2 years) and 136 control boys or young male blood donors (mean age 24.4 ± 3.5 years) were clinically found in good health condition and were not under any vaccination regime when blood samples were drawn. The characteristics of one or the patients are shown in Table 1. The term cryptorchidism referred to the presence of both testes in abdominal cavity ($n = 34$) as well as in inguinal canal ($n = 75$). Therefore, altogether 109 cryptorhid individuals were investigated (Tables 2 and 5) unless otherwise stated. For example, uni- versus bilateral cryptorchidism was known for 108 patients (Table 3) while ASA and Tanner stage data were available for 101 patients only (Tables 1 and 4). Also, in 25% of cases, blood was drawn retrospectively after orchidopexia (intrascrotal fixation of testis to internal muscular membrane).

2.2. DNA isolation

The isolation of DNA from peripheral venous blood of patients and control males was described earlier [3]. The remaining samples were treated as follows. The whole blood samples

Table 1 – Characteristics of patients with cryptorchidism.

Characteristic	Total	ASA negative		ASA 1–10%		ASA >10%	
		n	%	*n*	%	*n*	%
Age (years)							
<1	9	6	66.7	3	33.3	0	0
1–4	46	32	69.6	10	21.7	4	8.7
5–9	28	20	71.4	4	14.3	4	14.3
10–14	16	10	62.5	4	25.0	2	12.5
16–20	2	2	100.0	0	0	0	0
Tanner stage							
P1	83	60	72.2	14	16.9	9	10.8
P2	10	5	50.0	4	40.0	1	10
P3	3	1	33.3	2	66.6	0	0
P4	3	2	66.6	1	33.3	0	0
P5	2	2	100	0	0	0	0

ASA, anti-sperm antibodies; $n = 101$ patients only, as no clinical data were available for 8 patients.