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What about gr/gr deletions and male infertility? Systematic review and meta-analysis

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TABLE OF CONTENTS

BACKGROUND: The impact of gr/gr deletions on male fertility is unclear. These partial deletions of the AZFc region of the Y chromosome have been detected more frequently in infertile patients. However, few individual studies have demonstrated a statistically significant association. This study aims to quantify the strength of association between gr/gr deletions and male infertility, and to explore potential sources of heterogeneity, including ethnicity and geographical location.

METHODS: Medline was searched up to 31 December 2009 for full articles investigating the prevalence of gr/gr deletions in infertile and control men. A pooled odds ratio (OR) was estimated by a random-effects model. Heterogeneity was assessed by the Cochran's Q test, and quantified by I^2 statistic.

RESULTS: A total of 18 case–control studies, including 6388 cases and 6011 controls, met our inclusion criteria and showed that gr/gr deletions were present in 6.86% of cases and 4.69% of controls. The association between gr/gr deletions and infertility was significant ($P < 0.001$), with a pooled random-effects OR of 1.76 (1.21–2.66) for infertile men versus normozoospermic controls (13 studies). The test for heterogeneity among studies yielded a Q test $P = 0.089$ with I^2 value of 37%, indicating moderate heterogeneity. The association between gr/gr deletions and infertility was dependent on ethnicity and geographic region.

CONCLUSIONS: Our meta-analysis comprising >12 000 men demonstrates that gr/gr deletions occur more frequently in infertile than control men. The association between gr/gr deletions and infertility varies according to ethnicity and geographic region, with an association reaching significance among Caucasian men, in Europe and the Western Pacific region.

Key words: male infertility / gr/gr deletions / meta-analysis / ethnicity / azoospermia

Introduction

The long arm of the Y chromosome contains several genes that are essential for normal spermatogenesis. The presence of Yq microdeletions is well-known. These deletions are mainly detected in three regions: AZFa, AZFb or AZFc. Among these Yq microdeletions, the frequency of deletions of the complete AZFc region is highest and cause azoospermia or severe oligozoospermia. Recently, partial deletions of the AZFc region have also been described (Repping *et al.*, 2003). Three such deletions are gr/gr, b1/b3 and b2/b3 of which the gr/gr deletions have most been investigated. These gr/gr deletions are a common nominator for deletions caused by recombination between amplicons g1/g2, r1/r3 and r2/r4 (Repping *et al.*, 2003; Vogt, 2005).

Repping *et al.* (2003) defined the gr/gr deletions as partial AZFc deletions where sY1291 is missing, but all flanking markers, including the single copy marker sY1191 and multi-copy markers sY1206 and sY1161, are present. In the case of a b1/b3 deletion, sY1191, sY1197 and sY1161 are also deleted, as well as sY1291 (Repping *et al.*, 2003, 2004). A b2/b3 deletion is characterized by the absence of only sY1191 and is presumed to be caused either by a gr/gr inversion followed by a b2/b3 deletion or by a b2/b3 inversion followed by a gr/gr deletion. Analysis of markers sY1291 and sY1191 can thus distinguish gr/gr deletions from the other partial AZFc deletions predicted to occur. Furthermore, it is well documented that the AZFc region is highly polymorphic, thus it is possible that other rearrangements may occur. In the current systematic review and meta-analysis, we consider gr/gr deletions as deletions removing sY1291, but with the other neighbouring markers being present. Recently, a further differentiation was introduced: simple gr/gr deletion, gr/gr deletion + b2/b4 duplication, gr/gr deletion + b2/b4 multiple duplication, gr/gr deletion + CDY1 and DAZ amplification. The term 'gr/gr deletion rearrangement' was introduced as a general term for all types only recently (Krausz *et al.*, 2009; Yang *et al.*, 2010). Since most studies do not further subcategorize the different types of gr/gr deletions, we did not consider this distinction for the current systematic review and meta-analysis.

The ~1.6-Mb gr/gr deletion, as defined by Repping *et al.* (2003), removes two copies of the DAZ gene (DAZ1/DAZ2), one copy of CDY1 (CDY1a) and one copy of the BPY2 gene. The most important AZFc candidate infertility genes are believed to be DAZ and CDY1 (Reijo *et al.*, 1995; Habermann *et al.*, 1998; Lahn and Page, 1999; Fernandes *et al.*, 2002; Lahn *et al.*, 2002). Therefore, the presence/absence of only these genes have been studied in detail in view of gr/gr deletions. It has been observed in several studies that other gene copies might be deleted as well. Krausz *et al.* (2009) detected four major combinations of deletions: DAZ1/DAZ2 + CDY1a, DAZ1/DAZ2 + CDY1b, DAZ3/DAZ4 + CDY1a and DAZ3/DAZ4 + CDY1b. It is presumed that the localization of the genes has been changed due to an inversion in palindrome P1 (Krausz *et al.*, 2009). However, some rare deletion patterns were also observed (Stouffs *et al.*, 2008; Krausz *et al.*, 2009; Yang *et al.*, 2010), again demonstrating that the AZFc region is highly polymorphic.

Several research groups have investigated the prevalence of gr/gr deletions in their infertile patient and control samples. Results are very different between individual studies. Tüttelmann *et al.* (2007) concluded that, altogether, more gr/gr deletions are detected in all infertile men versus all control samples analysed. This relationship became even more obvious when only patients with a few or absent spermatozoa were compared with control men with normal sperm parameters, with a reported odds ratio (OR) of 2.29 and a corresponding 95% confidence interval (CI) of 1.51–3.51.

Inconsistencies between the different studies are likely caused by the differences in the ethnic background of patient and control samples between the studies, the number of samples analysed and different inclusion criteria between patients and controls. In particular, the ethnic background of the patients is presumed to influence the outcome of the study. As shown by Repping *et al.* (2003), Lu *et al.* (2009) and Yang *et al.* (2010), gr/gr deletions are fixed on haplogroups D2b and Q1, which are frequently observed in Japan and China, and are predicted not to hamper male infertility. Furthermore, the definition of the control group deserves special attention. While it is generally accepted that men with normozoospermic sperm parameters should be used as a control group, other groups of individuals are frequently used, especially in Asian countries.

The current systematic review and meta-analysis aim to quantify the strength of the association between gr/gr deletions and male infertility, and to explore potential sources of heterogeneity including ethnicity, geographical location and the definition of infertility. A further aim is to pay attention to the kind of control groups used in the primary studies, and to explore potential differences among men with normozoospermia, men with proven fertility and men with unknown fertility status, i.e. unselected control men.

Methods

Search strategy

We searched for English articles using Medline (PubMed), with the last computerized search undertaken on 31 December 2009. To avoid missing any relevant study, we used broadly defined medical subject heading terms and text words, including the following: gr/gr deletion or partial AZFc deletion. Duplicate publications were considered only once. The computerized search was supplemented by a manual search of the bibliographies of all retrieved articles. Potentially relevant articles

were assessed for inclusion against pre-specified eligibility and exclusion criteria (Stroup *et al*., 2000).

Study eligibility

We included case–control studies published in full that evaluated the prevalence of gr/gr deletions in infertile and control men and for whom analysis was performed with at least markers sY1291 and sY1191. If a particular patient population was reported in more than one publication, we selected the article that provided the most complete data set. Reviews, letters to the editor, uncontrolled studies, i.e. studies providing data on only patient or control samples and studies providing insufficient data to permit completion of a 2 × 2 contingency table were excluded.

Data extraction

The following data were abstracted: the first author's name; the publication year; the country of origin; the total number of men included; the men's ethnicity (Caucasian versus Chinese); geographical location [country of origin and geographic region defined according to the categories for the Global Burden of Disease 2000 World Health Organization Member States project (Johnell and Kanis, 2006)]; definition of sub/infertility (azoospermia or oligozoospermia cases); origin type and recruitment of control men (men with normozoospermia, men with proven fertility or men with unknown fertility status, i.e. unselected control men); methodology used; whether the authors provided separate data according to the investigation of DAZ or CDY copies and haplogroup analysis. Data were independently extracted by two of us (P.H. and K.S.) and checked for accuracy in a second review. Consensus was achieved for all data.

Statistical analyses

Our first (primary) aim was to quantify the strength of the association between gr/gr deletions and male infertility, and to explore potential sources of heterogeneity. From each eligible study, we abstracted data into 2 × 2 tables and calculated the OR and the corresponding 95% CI. The number of cases, the number of controls and the number of men with a gr/gr deletion in each group, were retrieved as defined and reported by the authors in the original publication. One exception pertains to those studies in which the presence of AZF deletions was also investigated: here, the original number of patients was decreased by the number of patients with an AZF deletion. If a 2 × 2 table contained a cell value of 0 (zero), that study was not omitted, but 0.5 was added to each cell value of the 2 × 2 table of that study (Sutton *et al*., 2000; Deeks *et al*., 2001). The pooled summary OR was estimated by the inverse-variance fixed-effects model and the DerSimonian and Laird random-effects model (DerSimonian and Laird, 1986). As a general rule, a DerSimonian and Laird random-effects models produce wider CIs around the pooled estimates than do the fixed-effects models and are more appropriate when homogeneity is threatened.

The results were examined for heterogeneity by visually examining forest plots and using formal statistical tests for heterogeneity (Deeks *et al*., 2001). Between study heterogeneity was assessed using the chi-squared distributed Cochran's Q test, $P < 0.10$ indicating significance (Sutton *et al*., 2000; Deeks *et al*., 2001). We also formally quantified heterogeneity by calculating the I^2 statistic: values <25% indicate low heterogeneity, 25–50% moderate heterogeneity and >50% high heterogeneity (Higgins and Thompson, 2002; Higgins *et al*., 2003). An I^2 value of 75% or more indicates very high heterogeneity, and suggests that the studies are too different to combine and generate a pooled estimate (Higgins and Thompson, 2002; Higgins *et al*., 2003).

We identified, *a priori*, the following potential sources of heterogeneity: publication year, total sample size, baseline prevalence of gr/gr deletions among controls, ethnicity, country of study and geographic region. In this regard, we performed categorical and meta-regression analyses. We further postulated, also *a priori*, that the findings would be affected by predetermined subgroup characteristics. To this end, we constructed separate 2 × 2 tables for studies providing separate data pertaining to cases with azoospermia, cases with oligozoospermia and controls with normozoospermia, and conducted separate meta-analyses accordingly. All meta-regression analyses and categorical meta-analyses were planned beforehand.

To evaluate the impact of each selected study on the overall results of the meta-analysis, we performed a one-way sensitivity analysis (one study excluded at the time), also defined *a priori*. We also planned a cumulative meta-analysis by year of publication.

Potential publication bias was explored visually by the funnel plot method of Sterne and Egger (2001), and the Egger's regression intercept test (Sutton *et al*., 2000; Deeks *et al*., 2001). The potential implications for our results were assessed by the Duval and Tweedie's trim-and-fill method (Duval and Tweedie, 2000; Borenstein *et al*., 2005) and the Orwin's fail-safe N method (Rosenthal, 1979).

A further (secondary) aim of our systematic review was to focus on to the kind of controls used in the primary studies. More particularly, we explored potential differences among control men with normozoospermia, with proven fertility and with unknown fertility status (i.e. unselected men). To this end, we abstracted the number of men with a gr/gr deletion (numerator) and the number of control men (denominator) with, in turn, normozoospermia, proven fertility and controls with unknown fertility status, as defined and reported in the original papers. We calculated the proportions of men with a gr/gr deletion in each subgroup. If a study reported no gr/gr deletions (value of zero) that study was not omitted, but 0.5 was added to both numerator and denominator for that study (Sutton *et al*., 2000; Deeks *et al*., 2001). To examine heterogeneity (differences across control groups) and to calculate pooled summary proportions across all samples and for each control group, we used the logit method. In the logit method, the observed proportions are converted to logits, all analyses (fixed-effects and random-effects models) are performed on the logit, and the final results are converted back into proportions for ease of interpretation (Lipsey and Wilson, 2001).

Results

Study characteristics

Our initial search identified 70 unique publications, of which 18 case–control studies met all our inclusion criteria (Supplementary Data, Figure S1) (Repping *et al*., 2003; de Llanos *et al*., 2005; Ferlin *et al*., 2005; Hucklenbroich *et al*., 2005; Carvalho *et al*., 2006; de Carvalho *et al*., 2006; Fernando *et al*., 2006; Zhang *et al*., 2006; Imken *et al*., 2007; Lardone *et al*., 2007; Lin *et al*., 2007; Navarro-Costa *et al*., 2007; Wu *et al*., 2007; Giachini *et al*., 2008; Stouffs *et al*., 2008; Lu *et al*., 2009; Ravel *et al*., 2009; Yang *et al*., 2010). We excluded 9 narrative reviews; 37 original studies, published in full, but, not relevant to our research question; 5 duplicate studies (Giachini *et al*., 2005; Ravel *et al*., 2006; Yang *et al*., 2006, 2008; Zhang *et al*., 2007), and one study lacking information on the type of controls (Lynch *et al*., 2005). Thus 18 full papers, all published in English, were included in the current review (Tables I and II). Considered together, the 18 studies included 12 399 men: 6388 cases and 6011 controls. Only 13 of these 18 studies reported data pertaining to normozoospermic control men.

Quantitative data synthesis

Our primary meta-analysis focused on the 13 studies that reported data for all cases with infertility versus normozoospermic controls.

Table I Characteristics of the case–control studies included in the meta-analyses.

First author, and year published	Country[a]	Geographic region[b]	Ethnicity[a]	Ethnicity (recoded for categorical meta-analysis)	Analysis of		
					DAZ[a]	CDY1[a]	Haplogroup[a]
Repping et al. (2003)	The Netherlands/ USA	Europe/Americas	Mixed	Mixed	No	No	Yes
de Llanos et al. (2005)	Spain	Europe	NA	Caucasian	No	No	No
Ferlin et al. (2005)	Northern Italy	Europe	Italian	Caucasian	Yes	No	No
Hucklenbroich et al. (2005)	Germany	Europe	NA	Caucasian	No	No	Yes
Carvalho et al. (2006)	Brazil	Americas	Brazil	Mixed	No	No	Yes
de Carvalho et al. (2006)	Japan	Western Pacific	Japanese	Japanese	No	No	Yes
Fernando et al. (2006)	Sri Lanka	Southeast Asia	Sri Lanka	Indian	No	No	No
Zhang et al. (2006)	East Asia	Western Pacific	Mixed	Mixed	Yes	No	Yes
Imken et al. (2007)	Morocco	Eastern Mediterranean	Moroccan	Moroccan	No	No	Yes
Lardone et al. (2007)	Chile	Americas B	Chilean	Mixed	Yes	No	No
Lin et al. (2007)	Taiwan	Western Pacific	Han Chinese	Han Chinese	No	No	Yes
Navarro-Costa et al. (2007)	Portugal	Europe	Portuguese	Caucasian	No	No	Yes
Wu et al. (2007)	China	Western Pacific	Han Chinese	Han Chinese	Yes	Yes	No
Giachini et al. (2008)	Italy	Europe	Italian	Caucasian	Yes	Yes	Yes
Stouffs et al. (2008)	Belgium	Europe	Belgian/ Holland	Caucasian	Yes	Yes	No
Lu et al. (2009)	China	Western Pacific	Han Chinese	Han Chinese	Yes	Yes	Yes
Ravel et al. (2009)	France	Europe	Mixed	Mixed	Yes	Yes	Yes
Yang et al. (2010)	China	Western Pacific	Han Chinese	Han Chinese	Yes	Yes	Yes

[a]Data as provided by the authors in the original paper; NA, data not available.
[b]Geographic region defined according to the categories of the Global Burden of Disease (GBD) 2000 World Health Organization member states project (Johnell and Kanis, 2006).

In these 13 studies, the prevalence of gr/gr deletions varied from 2.1 to 12.5% among all cases, and from 0 to 10.2% among normozoospermic controls (Table III). In the individual studies, the OR of gr/gr deletions in infertile men (cases) compared with controls ranged from 0.65 to 13.06 (Fig. 1). The pooled fixed-effects and random-effects ORs (and corresponding 95% CI) of 1.66 (1.32–2.10) and 1.76 (1.21–2.66), respectively, indicate that gr/gr deletions are significantly more likely to occur among infertile men.

Heterogeneity was suggested by visual inspection of the forest plot (Fig. 1) showing that the OR of 10 and 3 individual studies were located to the right and left side of the vertical line of no effect, respectively. Formal testing for heterogeneity among studies yielded a Cochran's *Q* test *P*-value of 0.089 with an I^2 value of 37%, indicating moderate heterogeneity. Categorical meta-analyses indicated that gr/gr deletions and infertility were associated more particularly in some ethnic groups (Fig. 2a) and geographic regions (Fig. 2b). The pooled random-effects OR was statistically significant, i.e. with a 95% CI not including the 1 value of no effect, for Caucasian groups (3.77; 1.93–7.35). For Han Chinese, the pooled random-effects OR was 1.62 (0.99–2.64). Partially overlapping 95% CIs and formal statistical testing with a between group *Q* test *P*-value of 0.075 are inconclusive for a difference between Caucasian and Han Chinese ethnicity. Figure 2b shows the association between gr/gr deletions and infertility according to geographic region: the pooled random-effects OR was significant in Europe (3.05; 1.33–7.03) and the Western Pacific region (1.51; 1.01–1.87) with 95% CIs excluding unity, but not in the Americas (0.65; 0.06–7.37), Southeast Asia (0.98; 0.24–4.04) and the Eastern Mediterranean region (1.88; 0.38–9.27). The results of this categorical meta-analysis provide no robust evidence for between group differences given the largely overlapping 95% CIs and a between group *Q* test *P*-value of 0.58.

One-way sensitivity analyses demonstrated that the overall effect size and its statistical significance were consistent across the studies and did not depend on any single study (data not shown).

A cumulative meta-analysis by year of publication indicated that the pooled estimate of the association between gr/gr deletion and fertility status became evident in 2005 when three studies were available, but remained statistically significant whenever another additional study was published only from 2008 onwards (Fig. 3).

Visual inspection of a funnel plot of effect size versus precision suggested that publication bias may have occurred due to the absence of, or inability to find, at least two small negative studies on the left side of the summary estimate (i.e. suggesting no effect of gr/gr deletions; Fig. 4). The Egger's regression intercept test did not

Table II Characteristics of the case–control studies included in the meta-analysis.

First author, and year published	Patients in original study	Inclusion criteria	Link with haplogroups	Haplogroup analyses in cases and controls	Link with DAZ	Link with CDY	STS markers used	Patients excluded from our analyses
Repping *et al.* (2003)	473 + 237 men with spermatogenic failure	None	gr/gr deletions fixed on haplogroup D2b	Similar haplogroup distribution	NA	NA	sY1291 + sY142, sY1191, sY1197, sY1201, sY1206	Karyotype abnormality in 2 men
de Llanos *et al.* (2005)	66 azoospermic + 217 severe oligozoospermic $<5 \times 10^6$/ml	Normal karyotype, no Yq microdeletion	NA	NA	NA	NA	sY1291 + sY1161, sY1191, sY1201, sY1206	
Ferlin *et al.* (2005)	73 azoospermic + 193 severe oligozoospermic $<5 \times 10^6$/ml + 71 mild oligozoospermic $5–20 \times 10^6$/ml	Normal karyotype, no Yq microdeletion	NA	NA	DAZ1/DAZ2 more frequently deleted in patients	NA	sY1291 + sY142, sY1161, sY1191, sY1197, sY1201, sY1206, sY1258	
Hucklenbroich *et al.* (2005)	61 azoospermic + 133 severe oligozoospermic $<1 \times 10^6$/ml + 154 mild oligozoospermic $1–20 \times 10^6$/ml	Normal karyotype, no Yq microdeletion, no known causes of male infertility	No link	NA	NA	NA	sY1291 + sY1161, sY1191, sY1201, sY1206	
Carvalho *et al.* (2006)	117 azoospermic	Normal karyotype, no Yq microdeletion, no known causes of male infertility	No link	NA	NA	NA	sY1291 + sY1161, sY1191, sY1201, sY1206, sY1258	AZF deletion in 7 men
de Carvalho *et al.* (2006)	49 azoospermic + 29 unknown	No Yq microdeletion	All gr/gr deletions belong to haplogroup D2	Similar haplogroup distribution	NA	NA	sY1291 + sY1161, sY1191, sY1201, sY1206, sY1258	AZF deletion in 3 azoospermic men and 2 unknown infertile men
Fernando *et al.* (2006)	79 azoospermic + 15 severe oligozoospermic $<1 \times 10^6$/ml + 2 oligozoospermic $1–20 \times 10^6$/ml	None	NA		NA	NA	sY1291 + sY1161, sY1191, sY1201, sY1206	AZF deletion in 6 azoospermic men and oligozoospermic man
Zhang *et al.* (2006)	49 azoospermic + 16 severe oligozoospermic $<5 \times 10^6$/ml + 22 mild oligozoospermic $5–20 \times 10^6$/ml	None	In all haplogroups, but more frequent in haplogroup 0	Similar haplogroup distribution	DAZ1/DAZ2 always deleted; haplogroup K* unsure	NA	sY1291 + sY1161, sY1191, sY1201, sY1206	
Imken *et al.* (2007)	48 azoospermic + 79 severe oligozoospermic + 22 asthenozoospermia	None	All patients and controls with gr/gr deletions belong to haplogroup E3b2	NA	NA	NA	sY1291 + sY1191	AZF deletion in 4 azoospermic men

Continued

Table II *Continued*

First author, and year published	Patients in original study	Inclusion criteria	Link with haplogroups	Haplogroup analyses in cases and controls	Link with DAZ	Link with CDY	STS markers used	Patients excluded from our analyses
Lardone *et al.* (2007)	95 patients of whom 67% azoospermic	No Yq microdeletion, no known causes of male infertility	NA	NA	No link		sY1291 + sY1161, sY1191, sY1201, sY1206, sY1258	
Lin *et al.* (2007)	142 oligozoospermic $<20 \times 10^6$/ml	None	No link	NA	NA	NA	sY1291 + sY1161, sY1191, sY1201, sY1206	
Navarro-Costa *et al.* (2007)	90 azoospermic + 210 oligozoospermic $<10 \times 10^6$/ml	Normal karyotype, no Yq microdeletion, no known causes of male infertility	No link	NA	NA	NA	sY1291 + sY142, sY1191, sY1197, sY1201, sY1206	
Wu *et al.* (2007)	164 azoospermic + 78 oligozoospermic $<20 \times 10^6$/ml + 209 normozoospermic	Normal karyotype, no known causes of male infertility	NA	NA	No link	No link	sY1291 + sY142, sY1054, sY1161, sY1191, sY1197, sY1201, sY1206, sY1258	AZF deletion in 24 patients
Giachini *et al.* (2008)	72 azoospermic + 26 cryptozoospermic $<1 \times 10^6$/ml + 187 severe oligozoospermic $1–5 \times 10^6$/ml + 271 moderate oligoozospermia $5–20 \times 10^6$/ml	Normal karyotype, no Yq microdeletion, no known causes of male infertility	No link	NA	DAZ1/DAZ2 potentially correlated with infertility	CDY1a correlated significantly with infertility	sY1291 + sY142, sY1161, sY1191, sY1197, sY1201, sY1206	
Stouffs *et al.* (2008)		Normal karyotype, no Yq microdeletion, no known causes of male infertility	NA	NA	No link	No link	sY1291 + sY1191, sY1197	
Lu *et al.* (2009)	220 azoospermic + 199 oligozoospermic $<20 \times 10^6$/ml + 337 normozoospermic	Normal karyotype, no known causes of male infertility	Gr/gr deletion fixed on haplogroup Q1	Similar haplogroup distribution	No link	No link	sY1291 + sY142, sY1054, sY1161, sY1191, sY1197, sY1201, sY1206, sY1258	AZF deletion in 34 azoospermic men and 11 oligozoospermic men
Ravel *et al.* (2009)	115 azoospermic + 72 severe oligozoospermic $<1 \times 10^6$/ml + 159 moderate oligoozospermia $1–10 \times 10^6$/ml + 18 mild oligozoöspermie $10–20 \times 10^6$/ml	Normal karyotype, no Yq microdeletion, no known causes of male infertility	No link	NA	No link	No link	sY1291 + sY1191	
Yang *et al.* (2010)	1426 azoospermic + oligozoospermic men	Normal karyotype, no Yq microdeletion, no known causes of male infertility	Gr/gr deletion fixed on haplogroup Q1; haplogroups C and DE* more frequently deleted in patients	NA	DAZ1/DAZ2 correlated significantly with infertility	No link	sY1291 + sY254, sY1054, sY1125, sY1161, sY1191, sY1201, sY1206	

Table III Number of men with a gr/gr deletion among infertile men (cases) and a reference group (controls), stratified according to degree of infertility status.

First Author, And Year Published	Cases			Controls				
	Azoospermia	Oligo-zoospermia	All cases	Normo-zoospermia	Proven Fertility	Normal Biopsy	Unselected Controls	All controls
Repping *et al.* 2003			24/708 (3.4%)	0/148 (0%)			4/215 (1.9%)	4/363 (1.1%)
de Llanos *et al.* 2005	1/66 (1.5%)	11/217 (5.1%)	12/283 (4.2%)	0/34 (0%)	0/75 (0%)		0/123 (0%)	0/232 (0%)
Ferlin *et al.* 2005	3/73 (4.1%)	13/264 (4.9%)	16/337 (4.7%)	1/263 (0.4%)				1/263 (0.4%)
Hucklenbroich *et al.* 2005	2/61 (3.3%)	12/287 (4.2%)	14/348 (4.0%)	3/170 (1.8%)				3/170 (1.8%)
Carvalho *et al.* 2006	5/110 (4.5%)		5/110 (4.5%)		3/122 (0.5%)		4/118 (3.4%)	7/240 (2.9%)
de Carvalho *et al.* 2006	11/46 (23.9%)		22/73 (30.1%)				19/56 (33.9%)	19/56 (33.9%)
Fernando *et al.* 2006	3/73 (4.1%)	1/16 (6.3%)	4/89 (4.5%)	4/87 (4.6%)				4/87 (4.6%)
Zhang *et al.* 2006 *	5/49 (10.2%)	4/38 (10.5%)	9/87 (10.3%)	9/89 (10.1%)			71/886 (8.0%)	80/975 (8.2%)
Imken *et al.* 2007	3/44 (6.8%)	3/79 (3.8%)	7/145 (4.8%)	2/76 (2.6%)	5/100 (5.0%)			7/176 (4.0%)
Lardone *et al.* 2007			2/95 (2.1%)	1/31 (3.2%)	0/19 (0%)	1/27 (3.7%)		2/77 (2.6%)
Lin *et al.* 2007		10/142 (7.0%)	10/142 (7.0%)		3/107 (2.8%)		32/580 (5.5%)	35/687 (5.1%)
Navarro-Costa *et al.* 2007	5/90 (5.6%)	10/210 (4.8%)	15/300 (5.0%)		3/300 (1.0%)			3/300 (1.0%)
Wu *et al.* 2007	10/144 (6.9%)	5/74 (6.8%)	30/427 (7.0%)		19/248 (7.7%)			19/248 (7.7%)
Stouffs *et al.* 2008	1/44 (2.3%)	7/143 (4.9%)	8/187 (4.3%)	5/278 (1.8%)	4/83 (4.8%)	2/33 (6.1%)		10/394 (2.5%)
Giachini *et al.* 2008 **	0/72 (0%)	18/484 (3.7%)	18/556 (3.2%)	2/487 (0.4%)				2/487 (0.4%)
Lu *et al.* 2009	25/186 (13.4%)	29/188 (15.4%)	89/711 (12.5%)	40/391 (10.2%)°				40/391 (10.2%)
Ravel *et al.* 2009 ***	4/115 (3.5%)	11/249 (4.4%)	15/364 (4.1%)	6/109 (5.5%)	7/84 (8.3%)			13/193 (6.7%)
Yang *et al.* in press ****			138/1426 (9.7%)	33/672 (4.9%)				33/672 (4.9%)

All values represent number of men; each numerator represents the number of men with a gr/gr deletion; each denominator represents the total number of men with the characteristic as indicated in the column header (prevalence of gr/gr deletions between brackets).

°These controls were normozoospermic and proven fertile (but considered as normozoospermic in the meta-analysis).

*Part of the study population was published in Zhang *et al.* (2007).

**Part of the study population was published in Giachini *et al.* (2005).

***Part of the study population was published in Ravel *et al.* (2006).

****Part of the study population was published in Yang *et al.* (2006, 2008).

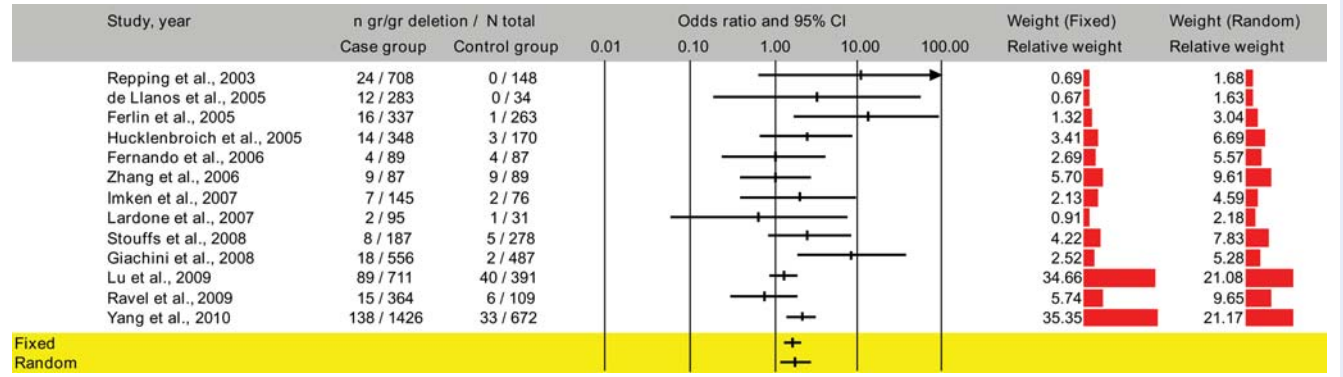

Figure 1 OR of gr/gr deletions in infertile men compared with controls with normozoospermia: fixed and random-effects meta-analysis of 13 case–control studies including controls with normozoospermia. Small vertical bars represent individual studies. Error bars represent 95% CIs. The lower panel, highlighted yellow, represents pooled diagnostic OR, with its 95% CI.

suggest a potential for publication bias (intercept, 0.616; 90% CI, −0.667 to 1.888; $P = 0.16$). This approach is limited in some important ways, mainly because the power of the method is low unless there is severe bias, and even if a statistically significant test suggests that bias exists, it does not directly address the implications of this bias. The trim-and-fill method, on the other hand, imputes missing studies and recalculates the pooled risk estimate (Fig. 4). The imputed ORs under the fixed and random-effect models are 1.60 (95% CI 1.27– 2.01) and 1.51 (95% CI 1.61–2.42), which are similar to our original risk estimates (1.66 and 1.76, respectively), suggesting that the apparent publication bias in this area is insufficient to cause a material change in our findings. The fail-safe value for our pooled analysis of 48 indicates that, for each study that we found, 3.7 missing studies would be needed to nullify the effect, i.e. to conclude that there is no significant relationship between gr/gr deletions and male sub/infertility.

The predefined subgroup meta-analysis restricted to 10 studies providing data on cases with azoospermia versus controls with normozoospermia showed an increased but statistically non-significant pooled OR of 1.33 (0.90–1.96). No between study heterogeneity was observed (Q test $P = 0.75$, $I^2 = 0\%$, Fig. 5a).

By contrast, for the predefined subgroup meta-analysis of the 10 studies providing data on cases with oligozoospermia versus controls with normozoospermia, the pooled OR of 2.03 (1.24–3.34) was statistically significant ($P = 0.005$). There was moderate heterogeneity between studies. (Q test $P = 0.16$, $I^2 = 32\%$, Fig. 5b).

A further (secondary) aim of our systematic review was to focus on to the kind of controls used in the primary studies by exploring potential differences among men with normozoospermia, men with proven fertility and men with unknown fertility status, i.e. unselected control men. According to the 18 individual studies providing information on the type of controls, the observed prevalence of gr/gr deletions among control men with normozoospermia, proven fertility and controls with unknown fertility status ranged from 0 to 10.2, 0 to 8.3 and 0 to 33.9%, respectively (Table III, right panel). Our analyses using the logit method confirmed that prevalence values of gr/gr deletions among controls were highly heterogeneous. The I^2 value was 82%, with a P-value for the overall Q statistic of <0.001, suggesting that the individual studies were too different to combine and generate a pooled estimate for prevalence. However, predefined categorical meta-analyses indicated that the pooled prevalence was lowest for controls with proven normozoospermia and highest for controls recruited from the community (random effect analysis, Table IV). The clinical relevance of this finding should be interpreted with some caution, keeping in mind both the wider CIs of the estimated prevalence and the larger P-value testing heterogeneity across the three groups of control men calculated by the random-effects model analysis.

Discussion

Summary of key findings

The results of the current systematic review and meta-analysis, which included more than 12 000 men analysed for the presence of gr/gr deletions, demonstrate that these partial AZFc deletions occur more frequently in infertile men than in controls. The association between gr/gr deletions and infertility is dependent on ethnicity and geographic region.

When comparing normozoospermic control men to azoospermic or oligozoospermic patients, a significantly higher occurrence of gr/gr deletions is observed only in patients with oligozoospermia.

Among control men, the prevalence of gr/gr deletions varies widely. Yet, as expected, the prevalence is lowest for controls with proven normozoospermia and highest for unselected controls recruited from the community.

Do the overall findings presented in our analysis have potentially important implications in standard day-to-day clinical practice? Our overall findings show that gr/gr deletions should be considered as a risk factor for male infertility. Our subgroup meta-analysis suggests that gr/gr deletions are significantly associated with oligozoospermia, but not with azoospermia, when comparing these patient groups with normozoospermic controls. This shows that in azoospermic men at least one extra factor must be involved. Unfortunately, different studies use different values to define oligozoospermia. Therefore, it is

(a)

Model	Group by ethnicity	Study, year	n gr/gr deletion / N total Case group	Control group
	Caucasian	de Llanos et al., 2005	12 / 283	0 / 34
	Caucasian	Ferlin et al., 2005	16 / 337	1 / 263
	Caucasian	Hucklenbroich et al., 2005	14 / 348	3 / 170
	Caucasian	Stouffs et al., 2008	8 / 187	5 / 278
	Caucasian	Giachini et al., 2008	18 / 556	2 / 487
	Caucasian			
	Han Chinese	Lu et al., 2009	89 / 711	40 / 391
	Han Chinese	Yang et al., 2010	138 / 1426	33 / 672
	Han Chinese			
	Indian	Fernando et al., 2006	4 / 89	4 / 87
	Indian			
	Maroccan	Imken et al., 2007	7 / 145	2 / 76
	Maroccan			
	Mixed	Repping et al., 2003	24 / 708	0 / 148
	Mixed	Zhang et al., 2006	9 / 87	9 / 89
	Mixed	Lardone et al., 2007	2 / 95	1 / 31
	Mixed	Ravel et al., 2009	15 / 364	6 / 109
	Mixed			
Random	Overall			

Odds ratio and 95% CI: 0.01 0.10 1.00 10.00 100.00

(b)

Model	Group by Geographic Region	Study, year	n gr/gr deletion / N total Case group	Control group
	Americas	Lardone et al., 2007	2 / 95	1 / 31
Random	Americas			
	Eastern Mediterranean	Imken et al., 2007	7 / 145	2 / 76
Random	Eastern Mediterranean			
	Europe	Repping et al., 2003	24 / 708	0 / 148
	Europe	de Llanos et al., 2005	12 / 283	0 / 34
	Europe	Ferlin et al., 2005	16 / 337	1 / 263
	Europe	Hucklenbroich et al., 2005	14 / 348	3 / 170
	Europe	Stouffs et al., 2008	8 / 187	5 / 278
	Europe	Giachini et al., 2008	18 / 556	2 / 487
	Europe	Ravel et al., 2009	15 / 364	6 / 109
Random	Europe			
	Southeast Asia	Fernando et al., 2006	4 / 89	4 / 87
Random	Southeast Asia			
	Western Pacific	Zhang et al., 2006	9 / 87	9 / 89
	Western Pacific	Lu et al., 2009	89 / 711	40 / 391
	Western Pacific	Yang et al., 2010	138 / 1426	33 / 672
Random	Western Pacific			
Random	Overall			

Odds ratio and 95% CI: 0.01 0.10 1.00 10.00 100.00

Figure 2 (**a**) Forest plot for the categorical meta-analysis according to ethnicity. Squares represent individual studies. Error bars represent 95% CIs. Squares are proportional to random-effects model weight. The lower panel, highlighted dark yellow, represents the overall pooled diagnostic OR, with its 95% CI, already indicated in Fig. 1. The middle panels, highlighted lemon yellow, represent the pooled diagnostic OR, with its 95% CI for a specific subgroup. (**b**) Forest plot for the categorical meta-analysis with geographic region defined according to the categories for the GBD 2000 World Health Organization member states project (Johnell and Kanis, 2006). Squares represent individual studies. Error bars represent 95% CIs. Squares are proportional to random-effects model weight. The lower panel, highlighted dark yellow, represents the overall pooled diagnostic OR, with its 95% CI, already indicated in Fig. 1. The middle panels, highlighted lemon yellow, represent the pooled diagnostic OR, with its 95% CI for a specific subgroup.

hard to make subcategories according to severe or mild oligozoospermia. It thus remains unknown whether in patients with severe oligozoospermia, other factors are also involved in the aetiology of the fertility problems. gr/gr deletions are also detected in normozoospermic men and men who have fathered at least one child without medical assistance. Having a gr/gr deletion therefore does not necessarily result in having fertility problems. Thus it should be explained to couples intending to have a child as a risk factor. However, as yet, no prognosis can be made for their sons, who will have the same deletion as their fathers. Consequently, we feel that it is too early to advise routine testing for the presence of gr/gr deletions in infertile men in a clinical setting. Especially when the male partner is suffering from azoospermia, it is hard to explain to the couple that a potential risk factor has been found, but that probably at least one extra, as yet unknown, co-factor will be involved too. Depending on the nature of this second factor, any male progeny may, or may not, inherit it and suffer from infertility problems. At present, therefore, gr/gr testing may create more questions than answers.

Our analyses among controls also show that gr/gr deletions are less frequent in normozoospermic men, compared with the proven fertility group or a randomly chosen control group. It is well known that men with proven fertility do not necessarily have normal sperm parameters. Furthermore, it is obvious that an unselected group of controls must be a mixture of fertile and infertile men, or normozoospermic and azoospermic/oligozoospermic men. Our analysis shows that, for gr/gr deletions, but also for other association studies regarding male infertility, the group with normozoospermic men is the best control group comparator.

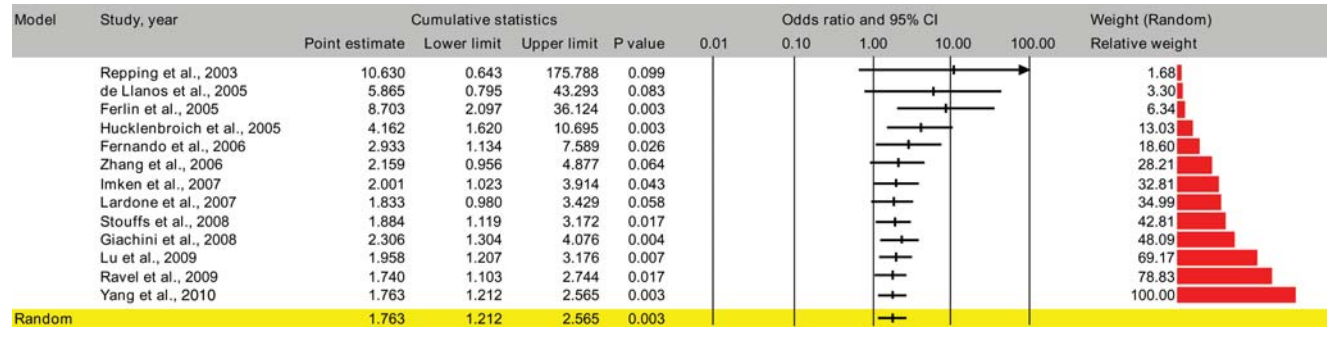

Figure 3 Cumulative meta-analysis by year of publication of 13 case–control studies including controls with normozoospermia (random-effects model). OR of gr/gr deletions in infertile men compared with controls: small vertical bars represent individual studies. Error bars represent 95% CIs.

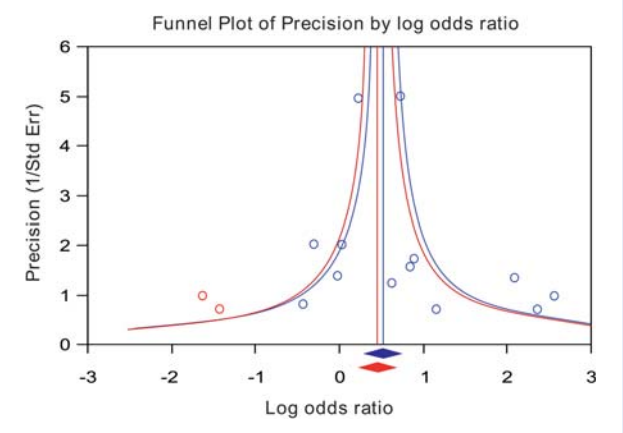

Figure 4 Publication bias and its potential impact for the relationship of gr/gr deletions in infertile men compared with controls. The blue circles represent observed individual studies, the blue lines are the funnel plot, and the blue diamond is the OR with its 95% CI for the meta-analysis. The red circles represent the imputed studies, and the red lines represent the adjusted funnel plot. The red diamond is the OR and its 95% CI for the meta-analysis, after adjusting for publication bias.

Implications for future directions and research

Our findings highlight the need to address the critically important issues related to differences in patients and controls. It is clear from this and other studies that both patients and controls may carry a gr/gr deletion. From our meta-analysis, it became evident that these partial AZFc deletions are more frequent among oligozoospermic patients. Most studies analysing the influence of a genetic modification and male infertility use a case–control design. In view of the fact that spermatogenesis is a continuous variable and that other factors influencing spermatogenesis (or sperm concentrations) may act together to cause fertility problems, cohort studies controlling for these factors would be superior in their ability to answer the question regarding the clinical impact of gr/gr deletion. Unfortunately, only one cohort study has been reported so far: among male partners of subfertile couples Visser *et al.* (2009) found that men with gr/gr deletions had a lower median sperm concentration compared with men without gr/gr deletions. This finding again supports a link between gr/gr deletions and male subfertility suggesting that gr/gr deletions should be considered to be a risk factor for male infertility.

When studying genetic alterations in the Y chromosome, such as gr/gr deletions, it is important to take into account the polymorphic nature of the Y chromosome.

One should also bear in mind that gr/gr deletions, defined by the absence of sY1291 and the presence of at least sY1191, are a common nominator for deletions resulting from recombination between amplicons g1/g2, r1/r3 or r2/r4. The differences in breakpoints might influence the fertility status of the patient (although presumably only to some extent).

In addition, duplication(s) might have followed the deletions. In this case, again at least four copies of DAZ and two copies of CDY1 are present, however they are twice the same gene or gene pair. Only a few studies have investigated the impact of this kind of rearrangements. In the study of Yang *et al.* (2010), no differences in the frequency of gr/gr deletions+b2/b4 duplications were observed between patients and controls. Krausz *et al.* (2009) concluded that a duplication after a deletion could not restore spermatogenesis, and might even worsen the spermatogenic efficiency.

The Y chromosomal background should also be taken into consideration. According to the revised Y haplogroup tree, 311 haplogroups should be distinguished (Karafet *et al.*, 2008). Yet, if haplogroup analysis was performed, most published studies considered only the major haplogroups. The most important conclusions from these analyses are that gr/gr deletions are fixed on haplogroups D2b and Q1, which are frequently found in Japan and China, respectively (Repping *et al.*, 2003; Lu *et al.*, 2009; Yang *et al.*, 2010). Yang *et al.* (2010) showed that haplogroup Q1 with the fixed gr/gr deletion was found in equal frequencies in patients as well as controls. Krausz *et al.* (2009) also suggested that this deletion probably has no pathogenic consequences, and, presumably, a still unknown compensatory mechanism is present to rescue the deleterious effect of gr/gr deletions. The presence of this neutral deletion also explains why higher frequencies of gr/gr deletions are observed in Chinese populations.

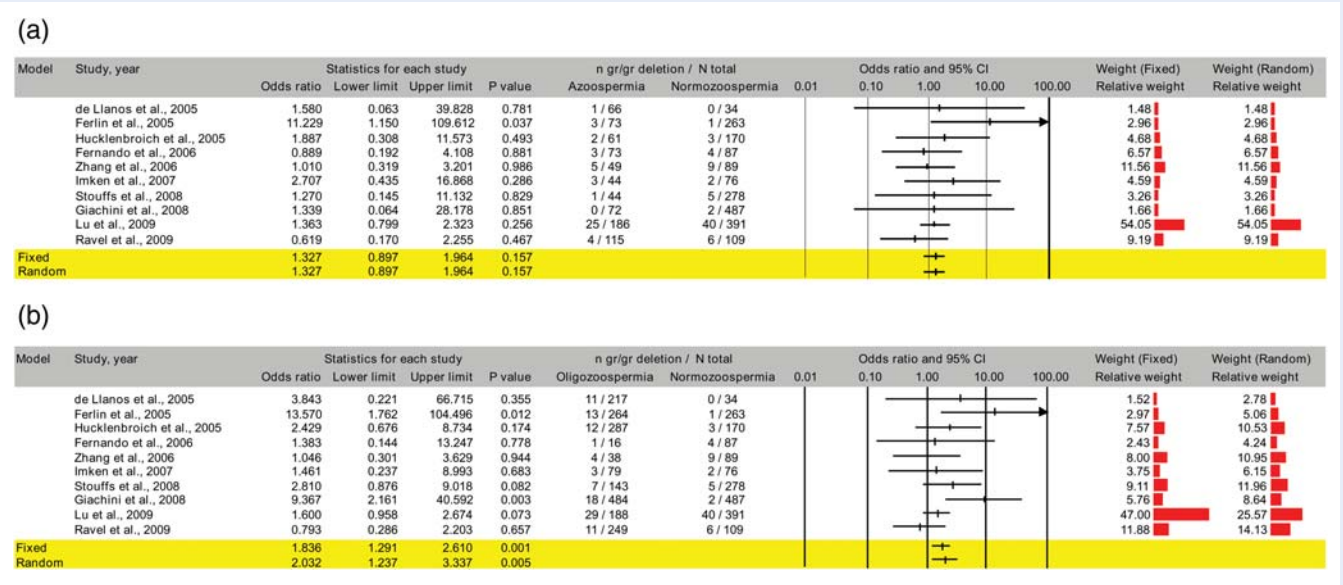

Figure 5 (**a**) Forest plot for the predefined subgroup meta-analysis including 10 studies providing data on cases with azoospermia versus controls with normozoospermia. Small vertical bars represent individual studies. Error bars represent 95% CIs. The lower panel, highlighted yellow, represents pooled diagnostic OR, with its 95% CI. (**b**) Forest plot for the predefined subgroup meta-analysis including 10 studies providing data on cases with oligozoospermia versus controls with normozoospermia. Small vertical bars represent individual studies. Error bars represent 95% CIs. The lower panel, highlighted yellow, represents pooled diagnostic OR, with its 95% CI.

Table IV Pooled prevalence of gr/gr deletions among the reference population of control men either with normozoospermia, proven fertility or unknown fertility status (unselected control men).

Fertility status of reference male population (control men)	Number of publications providing information	Fixed-effect analysis* Pooled prevalence (95% CI)***	Random-effect analysis** Pooled prevalence (95% CI)***
Normozoospermia	13	5.8% (4.8–6.9%)	3.1% (1.8–5.2%)
Proven fertility	9	5.1% (3.8–6.8%)	3.9% (2.3–6.5%)
Unselected controls	6	8.4% (7.1–9.9%)	8.1% (3.7–16.7%)

*P-value = 0.006 for testing heterogeneity across the three groups of control men (Q statistic = 10.2, df = 2).
**P-value = 0.39 for testing heterogeneity across the three groups of control men (Q statistic = 1.2, df = 2).
***95% CI, 95% confidence interval.

In our meta-analysis comparing patients and controls, we were unable to explore the potential impact of Y haplogroups. Only three individual papers provide complete Y haplogroup analyses in all cases and all controls irrespective of gr/gr deletions (Repping *et al.*, 2003; Zhang *et al.*, 2006; Lu *et al.*, 2009). Other studies investigated the presence of Y haplogroups only in men with gr/gr deletions.

Most individual reports observed no link between the Y haplogroups and male infertility or the frequency of gr/gr deletions, except for haplogroups Q1 and D2b. However, it is too early to draw robust conclusions. As mentioned previously, in the majority of studies haplogroup analysis was only performed in men with gr/gr deletions. Therefore, it is impossible to draw conclusions about the frequencies of certain haplogroups in men with or without gr/gr deletions. Imken *et al.* (2007), for instance, showed that all 14 gr/gr deleted men belonged to haplogroup E3b2. However, this is the most frequent haplogroup in Morocco, and, therefore, it is impossible to determine whether certain Y haplogroups are more prone to gr/gr deletions.

Another potential for selection bias pertains to the recruitment strategy. Large infertility centres recruit patients from all over the world, while the control group might be gathered more locally. In our own study describing patients and controls attending the Centre for Reproductive Medicine of our University Hospital, we have taken this issue into account by including only patients and controls from Belgium or the Netherlands (Stouffs *et al.*, 2008). If, and how, this issue is addressed in other studies remains unclear. This, again, shows the need to systematically test Y haplogroups both in patients and controls, irrespective of the presence or absence of gr/gr deletions. Systematic Y haplogroup analysis and information on the distribution of Y haplogroups in all patients and controls will help to address the issue of selection bias related to potential ethnic differences between patients and controls. The studies from Repping *et al.*

(2003), Zhang et al. (2006) and Lu et al. (2009) took into account the potential for selection bias by reporting a similar distribution of Y haplogroups in patients and controls. Furthermore, these studies include normozoospermic controls, although the Repping et al. (2003) and the Zhang et al. (2006) papers do not provide enough information to judge whether the patients suffer from idiopathic male infertility.

Until now, it also remains unknown whether the presence/absence of specific gene copies are different among patient and control groups. It was suggested from individual studies that the deletion of DAZ1/DAZ2 and/or CDY1a were related to male infertility (Table II). A recent multicenter study that examined the impact of the deletion of different DAZ and CDY1 gene copies in Europe (Krausz et al., 2009) concluded that the presence/absence of specific DAZ or CDY1 copies was not associated with the fertility problems of the patients. That study did show a correlation between haplogroups and the loss of either CDY1a or CDY1b, suggesting that structural rearrangements such as inversions arose independently on different Y lineages. In another study Yang et al. (2010) also reported a link between haplogroups in Han Chinese men and the loss of CDY1a or CDY1b in gr/gr deletion carriers. They also observed that both deletions of CDY1a and CDY1b are common in the patient group, and found a correlation between deletion of DAZ1/DAZ2 and spermatogenic failure. Despite the large sample size of both studies, it remains uncertain whether or not the deletion of specific gene copies is influencing the outcome of gr/gr deletions. To this end, large multicenter studies should be set up, keeping in mind the geographic or ethnical origin of patients and controls.

Conclusions

In summary, our study shows that gr/gr deletions can be considered to be a risk factor for male infertility, especially oligozoospermia in European countries. However, the underlying mechanisms of the gr/gr deletions in relation to male infertility, as well as the causes of geographic variations, remain to be elucidated.

Authors' roles

P.H. had the idea for the study. K.S. and P.H. designed the study, collected the data, had full access to all of the data, take responsibility for the integrity of the data and the accuracy of the data analysis and are the guarantors for the paper. P.H. did the statistical analysis. All authors provided advice on interpretation of the results and drafted the paper. All authors revised the paper critically and approved the final manuscript.

Supplementary data

Supplementary data are available at http://humupd.oxfordjournals.org/.

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