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Statistical methods to detect mother–father genetic interaction effects on risk of infertility: A genome-wide approach

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Abstract

Infertility is a heterogeneous phenotype, and for many couples, the causes of fertility problems remain unknown. One understudied hypothesis is that allelic interactions between the genotypes of the two parents may influence the risk of infertility. Our aim was, therefore, to investigate how allelic interactions can be modeled using parental genotype data linked to 15,789 pregnancies selected from the Norwegian Mother, Father, and Child Cohort Study. The newborns in 1304 of these pregnancies were conceived using assisted reproductive technologies (ART), and the remainder were conceived naturally. Treating the use of ART as a proxy for infertility, different parameterizations were implemented in a genome-wide screen for interaction effects between maternal and paternal alleles at the same locus. Some of the models were more similar in the way they were parameterized, and some produced similar results when implemented on a genome-wide scale. The results showed near-significant interaction effects in genes relevant to the phenotype under study, such as Dynein axonemal heavy chain 17 (*DNAH17*) with a recognized role in male infertility. More generally, the interaction models presented here are readily adaptable to the study of other phenotypes in which maternal and paternal allelic interactions are likely to be involved.

KEYWORDS
genetic interaction model, infertility, MBRN, MoBa

1 | INTRODUCTION

An estimated 10% of couples are affected by infertility worldwide (Boivin et al., 2007). Infertility is often defined as the inability to conceive after having tried for at least a year (Zegers-Hochschild et al., 2017). The use of assisted reproductive technologies (ARTs) as fertility treatment has increased steadily in many parts of the world over the last couple of decades (Chambers et al., 2021; Wyns et al., 2022). This may be related to a growing number of couples choosing to postpone childbearing to a later age, which has been reported to negatively impact reproductive success (Balasch & Gratacós, 2012; Leridon & Slama, 2008). Although the ability to conceive decreases with parental age, a proportion of the couples who choose to postpone childbearing may have underlying causes of infertility that could undermine their ability to conceive naturally when they later try to become pregnant.

There are several recognized causes of infertility, such as sperm dysfunction and endometriosis. Additionally, environmental and lifestyle factors may also contribute to an increased risk of infertility through various pathways. Featuring prominently among these are cigarette smoking (National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health, 2014; Rockhill et al., 2019; Waylen et al., 2008), maternal alcohol consumption (Klonoff-Cohen et al., 2003; Nicolau et al., 2014; Rossi et al., 2011), and stress (Prasad et al., 2016). A handful of studies have also been published on genetic factors associated with male and female fertility. For example, a large meta-analysis utilizing different surrogates for fertility in women and men (age at first birth and number of children ever born) identified 12 loci strongly associated with fertility (Barban et al., 2016). In addition, Aston et al. (Aston, 2014) provided a comprehensive review of genome-wide studies focusing on the genetic causes of male infertility, including those underlying severe spermatogenic impairments, while other studies have investigated genetic factors related to sperm function specifically (Kosova et al., 2012; Kyrgiafini et al., 2020; Sato et al., 2018). Regarding female reproductive aging, several genes were found to be associated with age at menarche (Ong et al., 2009; Perry et al., 2009; Sulem et al., 2009) and menopause (van Asselt et al., 2004; Stolk et al., 2009). Laisk-Podar et al. (2015) reported on several single-nucleotide polymorphisms (SNPs) that were associated with ovarian function and outcomes of ovarian stimulation, while Mandon-Pépin et al. (2008) investigated the role of four meiotic genes in premature ovarian failure.

In around 30% of infertile couples, the cause of infertility is unclear and cannot be explained by commonly used diagnostic tests (Smith et al., 2003). When known individual causes cannot be identified, infertility can be regarded as a phenotype affecting the couple as a unit. One hypothesis is that susceptibility alleles in *both* parents might jointly influence the risk of infertility in the couple; that is, the effect of the maternal genotype on infertility depends on the paternal genotype, and vice versa. This can be regarded as a type of gene–gene interaction effect which, in general, can be defined as a departure from the additive effects of the maternal and paternal genotypes. Although this type of gene–gene interaction is largely understudied, other types of gene–gene interactions have been more thoroughly investigated. For example, Cordell suggested methods for the analysis of epistasis (Cordell, 2002, 2009). Sinsheimer et al. (2003) investigated combinations of alleles at the same locus in mother–child dyads and developed a maternal–fetal genotype incompatibility test using a log-linear modeling approach to case–parent triads. Methods for investigating maternal–fetal genetic interaction effects have also been described by Ainsworth et al. (2011).

With a broader perspective on disease models that involve two loci, Li and Reich systematically described all two-locus penetrance models assuming two possible alleles at each locus (Li & Reich, 2000). Given two diallelic loci (nine possible genotypes), the parameterization of a binary phenotype yields $2^9 = 512$ different models. Some of these models are zero-locus or single-locus models, while others are redundant under certain permutations. Among the remaining nonredundant two-locus models, some have a more intuitive biological interpretation than others. Moreover, some of these models can readily be parameterized according to the definition of gene–gene interaction, whereas others are more conveniently parameterized using variables that code for specific combinations of the mother's and father's genotypes. Li and Reich (2000) classified all the nonredundant two-locus models by grouping them according to similar properties when feasible, and also provided examples of types of studies where the different models could be relevant. We here aim to investigate which of these models is more reasonable to consider in a search for allelic interactions between mother–father pairs specifically. We conducted genome-wide scans for such interaction effects using different parameterization schemes inspired by the models presented by Li and Reich (2000), in addition to exploring the classic dose–response model. We used a case–control study design, treating the parental couple as the analytic unit under study, and present a detailed account of how these models can be implemented precisely and efficiently as mixed-effects models using logistic regression. Finally, we also provide interpretations of the models and compare them to each other, both with regard to implementation and results.

2 | STATISTICAL MODELS FOR SINGLE-VARIANT ANALYSIS

We studied mother–father pairs linked via their common pregnancies, and classified them as cases or controls depending on whether or not they used ART to conceive (see Figure 1). Further, we modeled interactions between maternal and paternal alleles at the same locus across the genome. Consider a diallelic SNP with major allele A and minor allele a. The role of "reference" or "effect" allele can be assigned to either A and a. The number of effect alleles at a specific locus defines the dosage of the SNP, that is, the maternal/paternal dose at a given locus is either 0, 1 (single-dose), or 2 (double-dose). An interaction effect would be apparent if the effect of the maternal dose on the couple's infertility depends on the paternal dose (and vice versa). Table 1a shows the complete parameterization of the maternal/paternal SNP-wise interaction model with no constraints imposed on the parameters. Here, x_m and x_f represent the maternal and paternal genotype, respectively, α is the baseline effect of the mother and father carrying no effect alleles (0–0 parental dose combination), M_1, F_1, M_2, F_2 are the effect parameters of the mother/father carrying 1 or 2 effect alleles, and γ_{ij} is the effect of the interaction between the mother and the father carrying i and j effect alleles, respectively.

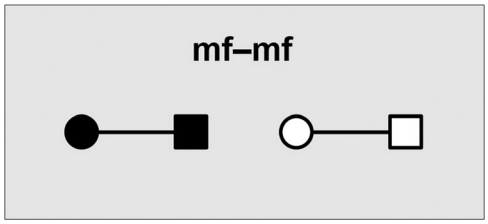

FIGURE 1 The case–control study design to assess parental allelic interaction effects. Here, a "case" is defined as a couple (mother–father, mf) who used ART to conceive (black shapes), and a "control" is defined as a couple who conceived naturally (white shapes). The mother is represented by a circle; the father by a square. The genotype of the couple's child is not included in the current genetic analyses, but the child's conception status is used to categorize the couple as belonging to a case or control group.

TABLE 1 Parameterization of (a) the full interaction model and (b) the multiplicative dose-response (*mdr*) model.

(a)

		x_f		
		0	**1**	**2**
x_m	**0**	α	αF_1	αF_2
	1	αM_1	$\alpha M_1 F_1 \gamma_{11}$	$\alpha M_1 F_2 \gamma_{12}$
	2	αM_2	$\alpha M_2 F_1 \gamma_{21}$	$\alpha M_2 F_2 \gamma_{22}$

(b)

		x_f		
		0	**1**	**2**
x_m	**0**	α	αF	αF^2
	1	αM	$\alpha MF\gamma$	$\alpha MF^2\gamma^2$
	2	αM^2	$\alpha M^2 F\gamma^2$	$\alpha M^2 F^2 \gamma^4$

2.1 | The multiplicative dose–response model

Table 1a displays nine distinct parameters to be estimated. Assuming a multiplicative dose–response (*mdr*) effect, where a double-dose effect is equal to the square of the single-dose effect, the number of parameters can be reduced as shown in Table 1b. With ART use as a dichotomous outcome (yes/no), we can apply a logistic regression model for the probability p of ART use. A two-locus *mdr* logistic model with an interaction term is given by

$$\log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_m x_m + \beta_f x_f + \beta_{mf} x_m x_f, \quad (1)$$

where the log odds is modeled as a linear function with intercept β_0 and regression coefficients β_m, β_f, and β_{mf}.

Using the above parameterization, the definition of which allele is the reference or effect allele is arbitrary when estimating the interaction effect. If we switch between reference and effect allele, we can re-parameterize the model using $\tilde{x}_m = 2 - x_m$ and $\tilde{x}_f = 2 - x_f$, and the model in (1) can be expressed as

$$\begin{aligned}\log\left(\frac{p}{1-p}\right) &= \beta_0 + \beta_m(2-\tilde{x}_m) + \beta_f(2-\tilde{x}_f) + \beta_{mf}\\ &\quad (2-\tilde{x}_m)(2-\tilde{x}_f)\\ &= (\beta_0 + 2\beta_m + 2\beta_f + 4\beta_{mf})\\ &\quad - (\beta_m + 2\beta_{mf})\tilde{x}_m - (\beta_f + 2\beta_{mf})\tilde{x}_f\\ &\quad + \beta_{mf}\tilde{x}_m\tilde{x}_f\\ &= \tilde{\beta}_0 + \tilde{\beta}_m\tilde{x}_m + \tilde{\beta}_f\tilde{x}_f + \tilde{\beta}_{mf}\tilde{x}_m\tilde{x}_f,\end{aligned}$$

with $\tilde{\beta}_0 = \beta_0 + 2\beta_m + 2\beta_f + 4\beta_{mf}$, $\tilde{\beta}_m = -\beta_m - 2\beta_{mf}$, $\tilde{\beta}_f = -\beta_f - 2\beta_{mf}$, and $\tilde{\beta}_{mf} = \beta_{mf}$. Thus, for the *mdr* model,

defining A or a as the reference allele does not change the effect size of the interaction.

2.2 | The jointly dominant-dominant model and the jointly recessive-recessive model

The jointly dominant-dominant (*jdd*) model describes the situation where the risk of expressing the phenotype changes if there is at least one copy of the effect allele in both parents. We assume that there is no difference in effect between a single and double dose of the effect allele (which contrasts with the *mdr* model). Analogously, in the jointly recessive-recessive (*jrr*) model, we assume that the presence of two copies of the effect allele in both parents influences the risk of the phenotype being expressed; here, we do not expect a difference in effect between a null and a single dose of the effect allele. Table 2a and 2b show the parameterizations of these two models. For the *jdd* and *jrr* models, the maternal and paternal genotypes (x_m and x_f, respectively) are expressed as binary variables (Table 3).

Although the choice of reference allele is not arbitrary in the *jdd* and *jrr* models, the two models with opposite choices of reference and effect alleles produce equivalent interaction effect estimates. Let x_{1m} and x_{2m} be dummy variables for the first and second allele of the mother, that is, $x_{1m} = 0$ or 1 if the first allele is A or a, respectively, and likewise for x_{2m}. As already mentioned, the order of the alleles is arbitrary. Let x_{1f} and x_{2f} be defined similarly for the alleles of the father. Note that

$$x_m = x_{1m} + x_{2m} - x_{1m}x_{2m},$$
$$x_f = x_{1f} + x_{2f} - x_{1f}x_{2f},$$

that is, x_m is 0 for the maternal genotype AA and 1 otherwise, and similarly for x_f. Thus, a dominant model can be considered as having a fixed negative interaction between the two alleles. Let $\tilde{x}_{im} = 1 - x_{im}$, $i = 1, 2$, and similarly for $\tilde{x}_{if}$, be the dummy variables with opposite reference, that is, with a as reference allele and A as effect allele. Defining $\tilde{x}_m = 1 - x_m$ results in $\tilde{x}_m = \tilde{x}_{1m}\tilde{x}_{2m}$, which is still an interaction between the two alleles, but this time positive and corresponding to a recessive model with a as reference allele. In simpler terms, x_m codes AA, Aa, aa as 0, 1, 1, whereas $\tilde{x}_m$ codes AA, Aa, aa as 1, 0, 0.

TABLE 2 Parameterization of models as described by Li and Reich (2000).

(a) Jointly dominant-dominant model

		x_f		
		0	**1**	**2**
	0	0	0	0
x_m	**1**	0	1	1
	2	0	1	1

(b) Jointly recessive-recessive model

		x_f		
		0	**1**	**2**
	0	0	0	0
x_m	**1**	0	0	0
	2	0	0	1

(c) Threshold model with $k = 2$

		x_f		
		0	**1**	**2**
	0	0	0	1
x_m	**1**	0	1	1
	2	1	1	1

(d) Threshold model with $k = 3$

		x_f		
		0	**1**	**2**
	0	0	0	0
x_m	**1**	0	0	1
	2	0	1	1

(e) Complementary model

		x_f		
		0	**1**	**2**
	0	0	0	1
x_m	**1**	0	0	0
	2	1	0	0

The *jdd* model can be written equivalently to the *mdr* model in (1), where β_{mf} measures the interaction effect, that is, how much the risk of ART use deviates from what would be expected from a (logit-)additive combination of dominant main effects in the mother and the father. If we switch between the reference and effect allele in both parents in (1), we obtain

TABLE 3 The most significant SNPs as identified in at least one of the models.

Model[a]	SNP	Gene	Chr	A1[b]	MAF	OR (95% CI)	*p* Value[c]
mdr	rs12749926		1	t	0.290	0.68 (0.58, 0.80)	1.85e−06
mdr	rs35116709		1	a	0.147	1.74 (1.38, 2.20)	3.70e−06
mdr	rs12068519		1	g	0.290	0.69 (0.59, 0.81)	3.84e−06
jdd (*mdr*)	rs7595213	*SLC8A1*	2	t	0.169	0.43 (0.31, 0.58)	6.62e−08
jdd (*mdr*)	rs9458273	*PRKN*	6	g	0.225	1.91 (1.47, 2.48)	1.14e−06
jdd	rs1511189	*CTNNA2*	2	g	0.458	0.45 (0.32, 0.62)	1.24e−06
jdd	rs5769008	*GRAMD4*	22	g	0.357	0.53 (0.40, 0.68)	1.36e−06
jdd	rs28709384		4	g	0.306	1.85 (1.43, 2.38)	2.02e−06
jdd	rs6048699		20	t	0.292	1.84 (1.43, 2.37)	2.07e−06
jdd	rs7590554	*SLC8A1*	2	g	0.176	0.49 (0.36, 0.66)	2.97e−06
jdd	rs61741523	*DNAH17*	17	c	0.166	1.98 (1.48, 2.65)	3.61e−06
jdd	rs10830104		10	a	0.230	1.85 (1.43, 2.41)	3.64e−06
jdd	rs1030056		12	c	0.475	0.45 (0.32, 0.63)	3.79e−06
th2 (*jdd*)	rs117676661	*WDR11-AS1*	10	g	0.056	3.92 (2.32, 6.62)	3.07e−07
th2 (*jdd*)	rs71563087	*LOC105377865*	6	a	0.135	2.34 (1.68, 3.26)	5.58e−07
th2 (*jdd*)	rs77350860	*LINC01250*	2	a	0.017	26.84 (7.27, 99.04)	7.87e−07
th2 (*jdd*)	rs9448278	*LOC105377865*	6	a	0.136	2.31 (1.66, 3.22)	8.09e−07
th2	rs10165666	*LINC01818*	2	c	0.055	3.89 (2.21, 6.84)	2.45e−06
th2	rs77163843		15	g	0.047	4.45 (2.38, 8.35)	3.16e−06
th2	rs6775526		3	c	0.145	2.16 (1.56, 2.98)	3.17e−06
th2 (*jdd*)	rs140258428	*LINC01250*	2	t	0.018	18.52 (5.42, 63.28)	3.23e−06
th2	rs72659743		1	a	0.177	1.98 (1.48, 2.65)	4.46e−06
th2	rs7738036		5	c	0.103	2.35 (1.63, 3.39)	4.99e−06
jrr	rs2490678	*LOC105376387*	10	a	0.463	2.46 (1.74, 3.47)	3.20e−07
jrr	rs7979964		12	t	0.491	2.19 (1.59, 3.03)	1.67e−06
jrr	rs62126784	*SLC27A1*	19	a	0.162	22.88 (6.19, 84.6)	2.71e−06
jrr	rs62126782	*SLC27A1*	19	c	0.172	19.66 (5.53, 69.9)	4.15e−06
th3	rs2434981	*KIRREL3*	11	a	0.137	6.49 (3.17, 13.3)	3.29e−07
th3	rs4595560	*KIRREL3*	11	c	0.128	6.31 (2.92, 13.6)	2.72e−06
th3	rs2974279	*CNBD1*	8	t	0.334	0.50 (0.37, 0.67)	3.93e−06
comp	rs72643197	*GPC6*	13	g	0.033	13.08 (5.08, 33.67)	9.96e−08
comp	rs72679697		8	a	0.106	2.43 (1.70, 3.46)	1.09e−06
comp	rs7995652	*GPC6*	13	a	0.034	9.94 (3.92, 25.22)	1.34e−06
comp	rs72777466	*LOC107985854*	2	c	0.045	4.81 (2.53, 9.16)	1.74e−06
comp	rs12947814		17	c	0.036	7.52 (3.28, 17.25)	1.93e−06
comp	rs56386425	*GPC6*	13	t	0.034	11.13 (4.12, 30.08)	2.03e−06
comp	rs6603815	*PRKCZ*	1	t	0.100	2.49 (1.71, 3.63)	2.17e−06
comp	rs113222322		6	g	0.061	4.16 (2.30, 7.56)	2.69e−06

(Continues)

TABLE 3 (Continued)

Model[a]	SNP	Gene	Chr	A1[b]	MAF	OR (95% CI)	*p* Value[c]
comp	rs12500393		4	a	0.221	1.72 (1.37, 2.15)	3.12e−06
comp	rs72643104	*GPC6*	13	c	0.034	10.22 (3.84, 27.19)	3.22e−06
comp	rs6683011	*PRKCZ*	1	g	0.101	2.45 (1.68, 3.56)	3.24e−06
comp	rs8065586		17	a	0.038	6.53 (2.96, 14.40)	3.28e−06
comp	rs7960561	*LOC105369611*	12	g	0.046	4.94 (2.50, 9.76)	4.09e−06
comp	rs116917650		22	c	0.038	6.58 (2.95, 14.68)	4.30e−06
comp	rs77698947		8	a	0.055	3.94 (2.20, 7.08)	4.32e−06
comp	rs10497270		2	c	0.101	2.48 (1.68, 3.66)	4.53e−06

Abbreviations: CI, confidence interval; *comp*, complementary model; *jdd*, jointly dominant-dominant model; *jrr*, jointly recessive-recessive model; MAF, minor allele frequency; *mdr*, multiplicative dose-response model; OR, odds ratio; SNP, single-nucleotide polymorphism; *th2*, threshold model with $k = 2$; *th3*, threshold model with $k = 3$.

[a]The model with the lowest *p* value (and any additional models that returned a *p* value less than 5×10^{-6}).

[b]The minor allele.

[c]The lowest *p* value from the six models.

$$\begin{aligned}\log\left(\frac{p}{1-p}\right) &= \beta_0 + \beta_m(1-\tilde{x}_m) + \beta_f(1-\tilde{x}_f) \\ &\quad + \beta_{mf}(1-\tilde{x}_m)(1-\tilde{x}_f) \\ &= (\beta_0 + \beta_m + \beta_f + \beta_{mf}) - (\beta_m + \beta_{mf}) \\ &\quad \tilde{x}_m - (\beta_f + \beta_{mf})\tilde{x}_f \\ &\quad + \beta_{mf}\tilde{x}_m\tilde{x}_f \\ &= \tilde{\beta}_0 + \tilde{\beta}_m\tilde{x}_m + \tilde{\beta}_f\tilde{x}_f + \tilde{\beta}_{mf}\tilde{x}_m\tilde{x}_f,\end{aligned}$$

where $\tilde{\beta}_0 = \beta_0 + \beta_m + \beta_f + \beta_{mf}, \tilde{\beta}_m = -(\beta_m + \beta_{mf}), \tilde{\beta}_f = -(\beta_f + \beta_{mf})$, and $\tilde{\beta}_{mf} = \beta_{mf}$. That is, β_{mf} also measures the interaction in a *jrr* model with the opposite reference alleles.

2.3 | The threshold model

The premise of the threshold model is an assumed change in the risk of the phenotype being expressed if the sum of the maternal and paternal dose is equal to or exceeds a defined threshold, k. Presented in Table 2c and 2d are the parameterizations of the threshold model with $k = 2$ (*th2* model) and $k = 3$ (*th3* model). Based on the choice of k, we define a binary threshold variable $x_{thr,k}$ corresponding to whether the sum of the parental doses satisfies the threshold. This threshold variable can be expressed in the following way: We define a set of dummy variables for the maternal and paternal genotypes. Let $x_{mat0} = 1$ for the maternal genotype AA and 0 otherwise, $x_{mat1} = 1$ for the maternal genotype Aa and 0 otherwise, and $x_{mat2} = 1$ for the maternal genotype aa and 0 otherwise. The dummy variables x_{pat0}, x_{pat1} and x_{pat2} are defined similarly for the father. The threshold variable $x_{thr,2}$ can then be written as

$$\begin{aligned}x_{thr,2} = x_{mat0}x_{pat2} + x_{mat1}x_{pat2} + x_{mat2}x_{pat0} \\ + x_{mat2}x_{pat1} + x_{mat1}x_{pat1} + x_{mat2}x_{pat2}.\end{aligned}$$

Similarly, $x_{thr,3}$ can be written as

$$x_{thr,3} = x_{mat1}x_{pat2} + x_{mat2}x_{pat1} + x_{mat2}x_{pat2}.$$

To better identify the effect of the threshold variable, we also included the main maternal and paternal effects in the model:

$$\begin{aligned}\log\left(\frac{p}{1-p}\right) &= \beta_0 + \beta_m x_m + \beta_{12m}x_{1m}x_{2m} + \beta_f x_f \\ &\quad + \beta_{12f}x_{1f}x_{2f} + \beta_{thr,k}x_{thr,k}.\end{aligned} \quad (2)$$

Here, x_{1m}, x_{2m}, x_{1f}, and x_{2f} represent each of the maternal and paternal alleles, respectively, and the interactions $x_{1m}x_{2m}$ and $x_{1f}x_{2f}$ are included to allow for a potential non-multiplicative effect of the maternal and paternal dose.

Assuming $k = 2$ in the model given in (2), we can define an alternative model with $k = 3$ and a as the reference allele. We then have $\tilde{x}_m = 2 - x_m, \tilde{x}_{1m} = 1 - x_{1m}, \tilde{x}_{2m} = 1 - x_{2m}, \tilde{x}_f = 2 - x_f, \tilde{x}_{1f} = 1 - x_{1f}, \tilde{x}_{2f} = 1 - x_{2f}$, and $\tilde{x}_{thr,3} = 1 - x_{thr,2}$, and the model in (2) can be written as

$$
\begin{aligned}
\log\left(\frac{p}{1-p}\right) &= \beta_0 + \beta_m(2-\tilde{x}_m) + \beta_{12m}(1-\tilde{x}_{1m})\\
&\quad (1-\tilde{x}_{2m}) + \beta_f(2-\tilde{x}_f)\\
&\quad + \beta_{12f}(1-\tilde{x}_{1f})(1-\tilde{x}_{2f})\\
&\quad + \beta_{thr,2}(1-\tilde{x}_{thr,3})\\
&= (\beta_0 + 2\beta_m + \beta_{12m} + 2\beta_f + \beta_{12f} + \beta thr)\\
&\quad - \beta_m\tilde{x}_m - \beta_{12m}\tilde{x}_{1m} - \beta_{12m}\tilde{x}_{2m}\\
&\quad + \beta_{12m}\tilde{x}_{1m}\tilde{x}_{2m}\\
&\quad - \beta_f\tilde{x}_f - \beta_{12f}\tilde{x}_{1f} - \beta_{12f}\tilde{x}_{2f} + \beta_{12f}\tilde{x}_{1f}\tilde{x}_{2f}\\
&\quad - \beta_{thr,2}\tilde{x}_{thr,3}\\
&= \tilde{\beta}_0 + \tilde{\beta}_m\tilde{x}_m + \tilde{\beta}_{1m}\tilde{x}_{1m} + \tilde{\beta}_{2m}\tilde{x}_{2m}\\
&\quad + \tilde{\beta}_{12m}\tilde{x}_{1m}\tilde{x}_{2m}\\
&\quad + \tilde{\beta}_f\tilde{x}_f + \tilde{\beta}_{1f}\tilde{x}_{1f} + \tilde{\beta}_{2f}\tilde{x}_{2f} + \tilde{\beta}_{12f}\tilde{x}_{1f}\tilde{x}_{2f}\\
&\quad + \tilde{\beta}_{thr,3}\tilde{x}_{thr,3}.
\end{aligned}
$$

Hence, switching from $k = 2$ to $k = 3$ while also switching reference and effect alleles results in a negation of the threshold coefficient, as $\tilde{\beta}_{thr,3} = -\beta_{thr,2}$.

2.4 | The complementary model

The 'complementary' (*comp*) model is obtained by assuming a change in the risk of ART use if both parents are homozygous for opposite alleles. This model is referred to as the interference model in Li and Reich (2000), and its parameterization is presented in Table 2e. Under this parameterization, we coded a binary variable x_{comp} to identify the 0–2 and 2–0 parental dose combinations and implemented a modified version of the model in (1):

$$\log\left(\frac{p}{1-p}\right) = \alpha + \beta_{comp}x_{comp}. \tag{3}$$

As the model in (3) consists of only one main effect, it cannot be categorized as an interaction model based on the characteristics described previously. It nevertheless fits into the overall purpose of investigating specific combinations of maternal and paternal genotypes that may influence fertility. A feature that distinguishes the *comp* model from the other models presented above is that it pinpoints the parental dose-combinations at a given locus that would, at conception, always produce an offspring that is heterozygous at the same locus, and, thus, genetically different from both parents at that locus. In the *comp* model, the choice of reference allele is completely arbitrary; switching the choice of reference and effect allele produces the same parameterization.

3 | DATA

We used data from the Norwegian Mother, Father, and Child Cohort Study (MoBa) in which pregnant women were recruited from all over Norway from 1999 through 2008 (Magnus et al., 2016). Of the women invited, 41% consented to participation. Fathers were invited from 2001, and the cohort now includes approximately 114,500 children, 95,200 mothers, and 75,200 fathers. Blood samples were drawn from the parents at around 18 weeks of gestation, and from the mother and the umbilical cord after delivery (Paltiel et al., 2014). The study participants have been followed up at regular intervals via self-administered questionnaires. The establishment of MoBa and initial data collection were based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is currently regulated by the Norwegian Health Registry Act.

The genetic data currently available consist of genotypes from more than 200,000 individuals, including approximately 79,000 children, 76,000 mothers, and 52,000 fathers. Genotyping was performed in several waves using different genome-wide genotyping arrays. The post-imputation quality control (QC) was conducted by Corfield et al. (2022), and included a thorough mapping of genetic relatedness within the cohort using a combination of known pedigree information, genetically estimated kinship coefficients, and the estimated proportion of the genome shared identical-by-descent. Principal component (PC) analysis was also done as a part of the QC.

For this study specifically, we identified mother-father dyads via their common pregnancies in MoBa. We treated ART use as a proxy for infertility and categorized parental dyads as cases (ART) or controls (non-ART), as illustrated in Figure 1. Information on whether a pregnancy involved ART use was retrieved from the Medical Birth Registry of Norway (MBRN), a national health registry containing information about all births in Norway (Irgens, 2000). We identified 1304 ART and 52,153 non-ART pregnancies with genotype data available from both parents. We randomly sampled non-ART pregnancies as controls in a case-control ratio of approximately 1:10, resulting in 14,485 control pregnancies for the current analyses. The data selection is illustrated in Figure 2. Among the selected cases and

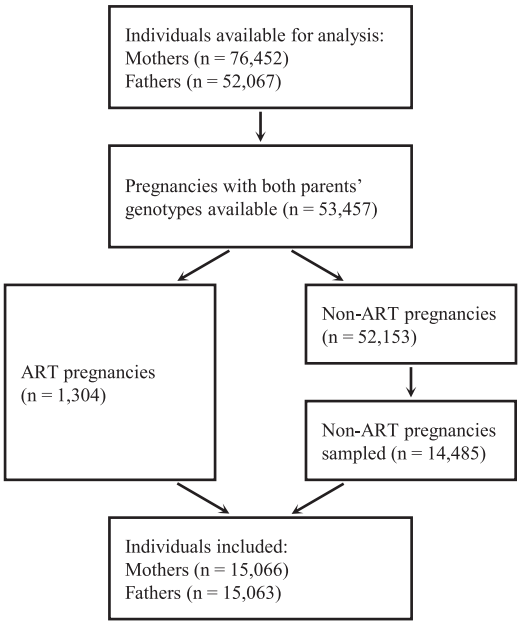

FIGURE 2 Overview of the sampling scheme. Note that some couples had multiple pregnancies, and a few of the parents were linked to multiple partners.

controls, 716 couples were registered with more than one pregnancy, 31 of whom contributed to both ART and non-ART pregnancies. We also used information on age, parity, body mass index (BMI), and smoking habits as covariates in some analyses. These variables were retrieved from either MBRN or the MoBa questionnaires administered to both parents around week 15 of gestation.

4 | IMPLEMENTATION

The data were processed and analyzed using PLINK v1.90 (Purcell et al., 2007) and R v4.0.4 (R Core Team, 2022). We implemented the parameterization schemes described in Sections 2.1–2.4 in a generalized linear mixed-effects model using the logit `link` function in the R-package lme4 (Bates et al., 2015). All figures were created using the R packages qqman (Turner, 2018), Haplin (Gjessing & Lie, 2006), and ggplot2 (Wickham, 2016).

Familial relationships between participants were determined in the initial QC conducted by Corfield et al. (2022). To roughly account for close relatedness, we constructed a family group identity variable, u, which was included as a random intercept term in the model. For instance, couples where mothers were sisters would have the same random intercept. Similarly, we accounted for repeated measurements by adding a random intercept at the "couple" level for those couples who contributed more than one pregnancy to the cohort. Thus, for a couple v belonging to family group u, the probability of ART use is

$$\log\left(\frac{p}{1-p}\right) = M + u + v, \tag{4}$$

where M represents the right-hand side of Equations (1)–(3). There were five individuals who had pregnancies with two different partners. These were coded with the same value of u, but different values of v.

We also included the covariates maternal age and parity in the genome-wide analyses using the parameterizations described above. Maternal age and parity are not confounders per se, but both are considered strong determinants of ART use. Since different genetic subpopulations of our sample might have different age and family size structures, adjusting for age and parity may be prudent. As we do not necessarily assume a linear relationship between age and infertility, maternal age was included as a categorical variable with the following intervals: <25, 25–30, 30–35, and >35 years. Parity was included as a numeric variable with values between 0 and 4, with 4 representing "4 or more" children. We also adjusted for the first three genetic PCs for both parents to account for possible confounding due to population stratification.

Furthermore, we performed multiple analyses on a subset of SNPs, adjusting for different combinations of the additional covariates: maternal BMI, maternal smoking, and imputation batch. The maternal BMI was included as a categorical variable (<18.5, 18.5–25, 25–30, 30–35, and >35 kg/m^2) to avoid assuming a linear relationship between maternal BMI and infertility. Maternal smoking was categorized according to whether the mother had ever smoked or smoked in the last couple of years before giving birth. As genotyping the entire MoBa cohort took several years and was performed through multiple different research projects, the imputation of unobserved genotypes was also performed in different batches (Corfield et al., 2022). To account for possible imputation batch effects, we included a variable for this as a random intercept term in the model.

4.1 | Allele frequencies

Handling SNPs with low minor allele frequencies (MAFs) is particularly challenging in genetic association studies due to sample size issues, especially when studying multi-loci interactions. Assuming Hardy-Weinberg equilibrium and independence of the parental genotypes, the expected proportion of couples where

both parents carry at least one copy of the minor allele is only 0.04% for a SNP with a MAF of 1%. When preparing the data in PLINK, we initially set a MAF threshold of 1% before performing the genome-wide analyses. SNPs with exceedingly low MAFs resulted in very low numbers of ART pregnancies with the relevant parental dose-combinations (depending on the choice of model) and the model estimates were therefore unreliable. These SNPs were easily identifiable due to a heavy inflation of the standard errors (SEs) of the interaction term. Consequently, we excluded SNPs based on this evident inflation of standard errors.

4.2 | SNPs at different loci

Although we mainly applied the models presented in Section 2 to SNPs at the same locus, it can be argued that it would be more favorable to use a more agnostic approach where one would search through all possible pairwise combinations of SNPs over the whole genome (Evans et al., 2006). Such a strategy would, however, be underpowered due to small sample sizes. Moreover, it may incur a large computational burden. Due to these limitations, we searched for significant interaction effects between all pairwise SNP combinations only for a small subset of SNPs.

5 | RESULTS

After preparing the genetic data in PLINK, we had approximately 2,400,000 SNPs available for analyses. We first applied the *mdr* parameterization given in (1) together with the model specified in (4) and performed a genome-wide scan. Initially, we included both the couple variable v and the family variable u in the analyses. Although there is a degree of relatedness within the total MoBa cohort that needs to be considered in analyses, we found that there was less relatedness within our sampled data. As a result, v and u were highly correlated, causing the model to not converge. Consequently, we omitted u from the final model. As covariates in the model, we added maternal age, parity, the three first PCs for mothers, and the three first PCs for fathers:

$$\log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_m x_m + \beta_f x_f + \beta_{mf} x_m x_f + v + age + parity + PCs,$$

where *age*, *parity*, and *PCs* represent appropriately coded regression terms for the respective variables.

For some SNPs with MAF close to 1%, there was only a small number of cases in the dose-combinations relevant for this model, and, therefore, the model did not converge. Furthermore, we excluded SNPs with a heavily inflated standard error for the interaction estimate, as described in Section 4.1. These SNPs were easily identifiable because there was a clear divide between the normal-range standard errors and the inflated standard errors (see Supporting Information: Figure S1). Inspecting the estimated MAFs for the excluded SNPs, we found that the maximum MAF was just above 5%. In other words, setting an initial MAF threshold of 5% instead of 1% would have also led to the exclusion of the majority of these SNPs from the analyses. For more details on the number of SNPs excluded and their MAFs, see Supporting Information: Table S1.

The Manhattan and quantile-quantile (QQ) plots of the results from the *mdr* model are presented in Figure 3a,b, respectively. We performed follow-up analyses on a subset of the index SNPs, that is, the SNPs with the most significantly associated interaction term, and applied additional adjustments for maternal BMI, maternal smoking, and imputation batch. Including different combinations of these covariates had only a minor effect on the p values for the interaction term, and the changes in effect estimates were negligible.

We repeated the genome-wide scans by applying the *jdd*, *jrr*, *th2*, *th3*, and *comp* models. Again, for some SNPs, the models did not converge, and SNPs with a heavy inflation of the interaction standard error were easily identified and subsequently excluded (see Supporting Information: Table S1). For the *jdd* and *th2* models, the maximum estimated MAF for the excluded SNPs was just above 5%, similar to the *mdr* model. For the *jrr*, *th3* and *comp* models, the ranges of estimated MAFs for the excluded SNPs were wider, with a maximum MAF of 29.1%, 13.1%, and 6.7%, respectively. The Manhattan and QQ plots in Figure 3c–f present the results of the *jdd* and *th2* models. Similar plots for the *jrr*, *th3* and *comp* models are presented in Supporting Information: Figure S2.

We estimated the odds ratios (ORs) for the maternal, paternal, and interaction effects. The OR can be interpreted as an approximation of the relative risk, given that the prevalence of ART use in the population is relatively low (i.e., applying the rare disease assumption). Figures 4 and 5 show the estimated ORs for a subset of the index SNPs from each model. The results of the *mdr*, *jdd*, and *th2* models showed some overlap in index SNPs, as shown in Figure 4. For the majority of these SNPs, the estimated marginal effects were opposite of the estimated interaction effect. That is, when the marginal maternal and paternal effects showed an estimated decrease in the

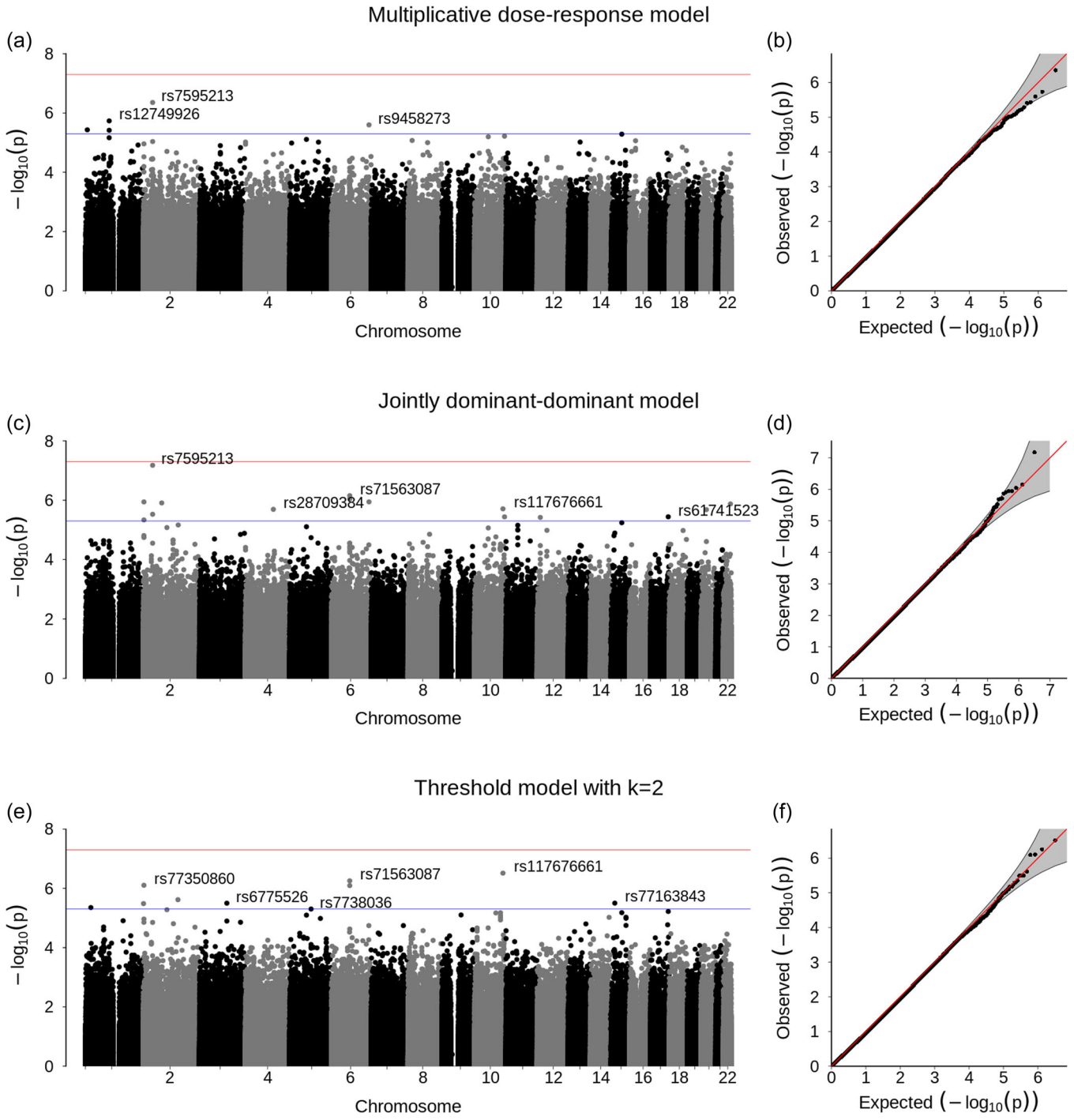

FIGURE 3 Manhattan and quantile-quantile (QQ) plots of the $-\log_{10}$-transformed p values of the interaction term for the multiplicative dose-response model (a) and (b), the jointly dominant-dominant model (c) and (d), and the threshold model with $k = 2$ (e) and (f). The red and blue horizontal lines in each Manhattan plot indicate the significance thresholds 5×10^{-8} and 5×10^{-6}, respectively. The shaded area in the QQ-plots represents the 95% confidence interval.

risk of ART use, the estimated interaction effect indicated an increased risk, and vice versa. Comparing individual OR estimates from the *mdr*, *jdd*, and *th2* models, we found that they indicated similar maternal, paternal, and interaction effects for the same SNPs. The confidence intervals were generally wider for the estimates from the *jdd* and *th2* models compared with those from the *mdr* model.

Overall, the *jrr* and *th3* models identified fewer index SNPs than the other models. Further, there was no overlap in index SNPs from these two models. The *comp* model also identified index SNPs that were distinct from

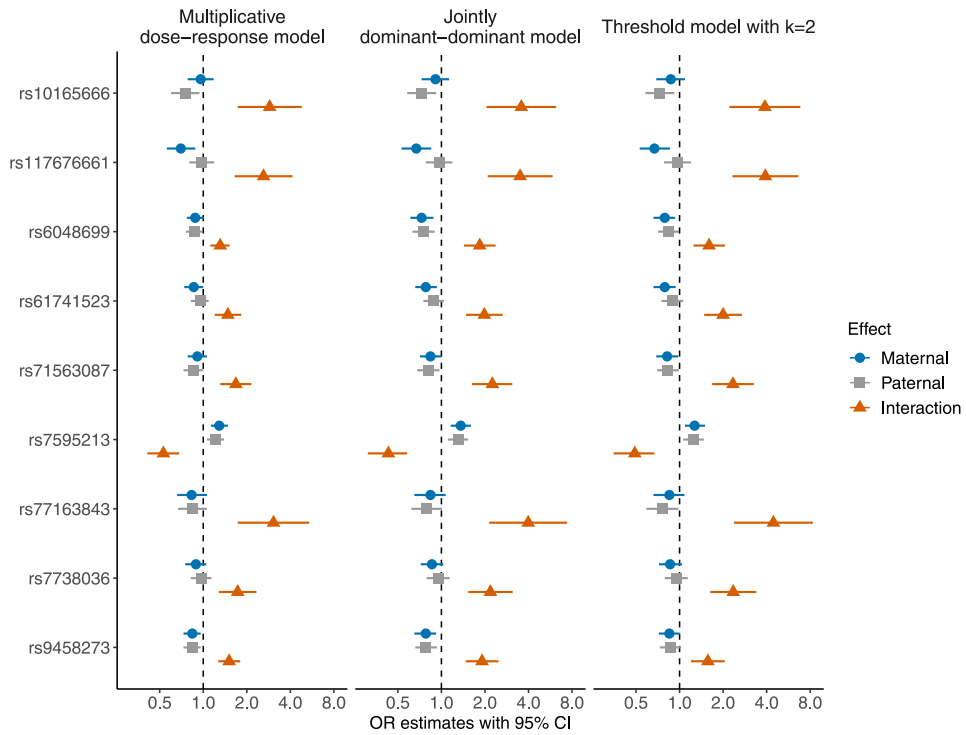

FIGURE 4 Estimates of the maternal, paternal, and interaction odds ratios (ORs) for the same single-nucleotide polymorphisms from the multiplicative dose–response model, the jointly dominant-dominant model, and the threshold model with $k = 2$. The maternal and paternal ORs are derived from the β_m- and β_f-estimates, respectively. CI, confidence interval.

those found by the other models, but, overall, the *comp* model identified the largest number of SNPs with a p value below 5×10^{-6}. Note that the *comp* model included only one main effect, that is, only one OR estimate per SNP, as shown in Figure 5. All of the index SNPs identified in the *comp* model were associated with an increased risk of ART use. Some of the OR estimates were unrealistically large. This was especially the case for the interaction OR estimate for the SNP rs62126784 in the *jrr* model, and several SNPs in the *comp* model.

A synopsis of the main gene findings is provided in Supporting Information: Table S2. Several of the identified loci were noncoding RNAs. The gene with the strongest link to infertility is Dynein axonemal heavy chain 17 (*DNAH17*), which has a recognized role in male fertility. Two other genes, Cyclic nucleotide binding domain containing 1 (*CNBD1*) and Parkin RBR E3 ubiquitin protein ligase (*PRKN*), are both relevant candidates for infertility based on the tissue in which they are involved and/or are expressed. For instance, *CNBD1* is expressed exclusively in the testis.

As a proof-of-principle, we performed an additional interaction analysis for all pairwise combinations of a subset of the index SNPs from our main analyses. We selected a representative subset of 151 SNPs from the 22 autosomal chromosomes, which resulted in a total of 22,650 possible combinations of SNPs at different loci. This included looking at both combinations of "SNP and parent" pairs, that is, SNP1 in the mother and SNP2 in the father, as well as SNP2 in the mother and SNP1 in the father. The results showed that the only borderline significant interactions were for those SNPs that were near each other (Figure 6). In other words, this limited analysis did not provide additional insights compared to the previously described genome-wide analysis performed on SNPs at the same locus.

6 | DISCUSSION

Of the six models presented here, the *mdr*, *jdd* and *th2* models had many index SNPs in common. In addition, most of the estimates from these models were relatively similar in magnitude. This finding is as expected, because a true underlying *mdr* effect would most likely also be identifiable in a *jdd* and *th2* setting, and vice versa. The confidence intervals were generally wider for the index SNPs identified by the *jdd* and *th2* models than the *mdr* model. The majority of the index SNPs in our analyses were identified by the *comp*, *jdd*, and *th2* models. As different SNPs may not necessarily belong to the same underlying effect model, we cannot conclude that one

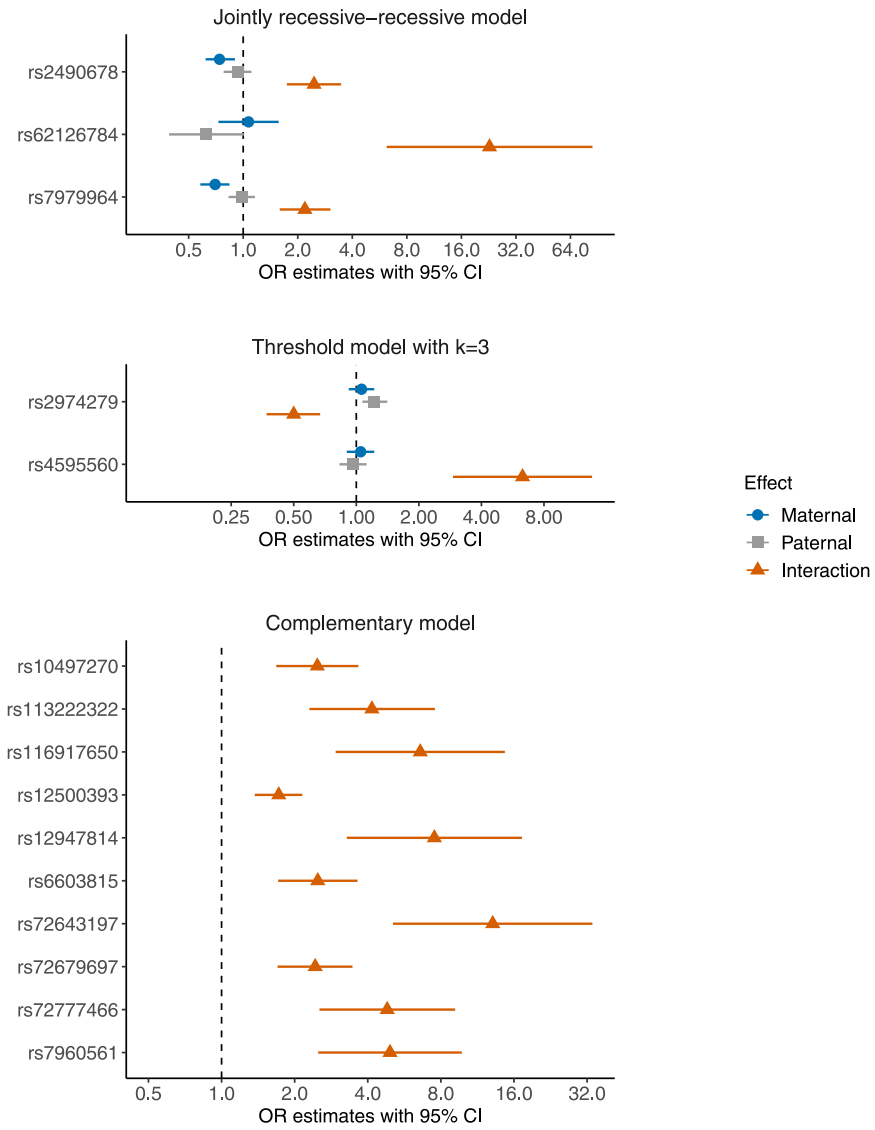

FIGURE 5 Odds ratio (OR) estimates for a subset of the index single-nucleotide polymorphisms identified by the jointly recessive-recessive model, the threshold model with $k = 3$, and the complementary model. The maternal and paternal ORs are derived from the β_m- and β_f-estimates, respectively. CI, confidence interval.

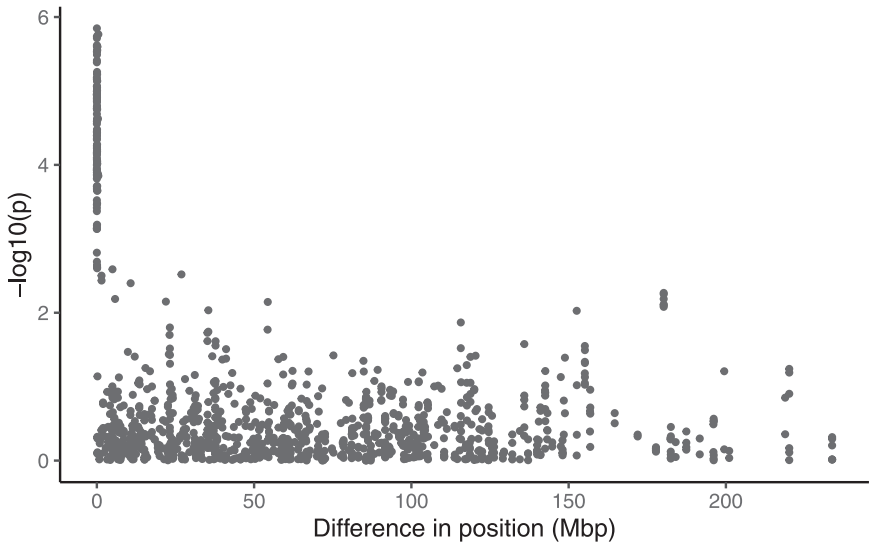

FIGURE 6 Pairwise interaction analyses of a selection of single-nucleotide polymorphisms (SNPs) from all autosomal chromosomes. Shown here are only SNP pairs from the same chromosome. The negative log10 of the p values are plotted against the difference in base-pair position.

particular model fits better overall. The OR estimates were unreasonably high for several SNPs, which may be due to the "winner's curse"—a concept based on auction theory (Lohmueller et al., 2003; Xiao & Boehnke, 2009) often used in genetic studies to explain an upward bias in effect estimates. However, although some ORs may be overestimated, they may still represent true positive associations between the SNPs and ART use.

Certain pairs of the models are in a sense "symmetric" with regard to the interaction effect when switching the choice of reference and effect allele. When only considering the interaction term, we showed that the results from the *jrr* model are equivalent to those from the *jdd* model with the opposite choice of reference/effect allele. We also observed a fixed relationship between the interaction estimates from the *th2* and *th3* models for the opposite choice of reference/effect allele. These properties are interesting in terms of their biological implications. For instance, if a common "risk" allele in the *jdd* model is associated with an increased risk of infertility, it would likely be subject to a large population selection pressure. This is because the *jdd* model assumes increased risk for four different combinations of the parental genotypes and thus a large total risk of infertility. The *jrr* model, on the other hand, imparts an increased risk only in the extreme situation where both parents are homozygous for the risk allele, thus imposing a lower selection pressure.

In this study, the *mdr*, *jdd*, and *jrr* models were implemented with both parental main effects and the interaction effect included. The *th2* and *th3* models could have been defined using only one threshold variable, coding the specific parental dose-combinations that satisfy the given threshold, but that would mean ignoring possible correlations between the main maternal and paternal effects and the threshold effect, rendering the threshold effect more difficult to interpret. We, therefore, chose to include the main effects in the threshold models to better distinguish the threshold effect. In contrast to the other models, our *comp* model is the only model that does not include separate parental main effect components. The *comp* model is based on parents who are homozygous for the opposite allele, which can be interpreted in several ways. First, if the interaction term indicates an *increased* risk of ART use, the *comp* model can be regarded as an antagonistic model in which there is an incompatibility between the two alleles of the mother and those of the father, leading to an increased infertility. On the other hand, if the interaction term indicates a *decreased* risk of ART use, the alleles of the mother and father may complement each other and enhance fertility.

The *comp* model brings up another issue: if both parental genotypes are homozygous for opposite alleles, any successful conception will produce a fetus that is heterozygous at that locus. If heterozygosity means reduced embryonic or fetal viability, the mother may experience an early pregnancy loss. If this occurs very early in embryogenesis, the mother may not even be aware of her pregnancy. It is impossible to accurately determine the number of clinically undetected early pregnancy losses, but studies suggest estimates ranging from 13% to 22% of all pregnancies (Wilcox et al., 1988; Zinaman et al., 1996). Such early losses may be erroneously interpreted as infertility, regardless of whether they are known or not, and thus increase the chances of ART use. Under this scenario, any SNP associated with an increased risk of ART in the *comp* model may alternatively be considered one of which fetal heterozygosity increases the risk of early pregnancy loss. Similarly, in most interaction models, both parental main effects and interaction terms will likely correlate with the genotype of the fetus—once conception has occurred. Therefore, we cannot rule out the possibility that SNPs identified in the interaction models may imply reduced fetal viability rather than parental genotype incompatibility. This is further exacerbated by our study being based on successfully established ART pregnancies, not only attempted pregnancies. In a planned follow-up study, we will include genotypes of the children and investigate models that incorporate both parental interactions and effects of alleles transferred to the child. This can potentially help clarify which combinations of alleles influence early fetal viability.

In the analyses presented here, we included a random intercept in a generalized linear mixed effects model to account for multiple pregnancies by the same couple. An alternative strategy is to include only one, randomly sampled pregnancy per couple, and analyze the data using a generalized linear model with logistic regression. When applying the multiplicative dose–response parameterization in a generalized linear model, and including the same covariates as in the *mdr* model, we identified a set of index SNPs, that is, SNPs with a p-value below 5×10^{-6}, that included all those identified in the *mdr* model. The run-time was approximately one fourth of the run-time for the *mdr* model. The distribution of *p* values was, however, heavily inflated (see Supporting Information: Figure S3a). This is due to some SNPs having low frequencies of ART-pregnancies for some of the parental dose combinations. We experimented with excluding SNPs that had a low frequency of ART-pregnancies for the 1–1, 1–2, 2–1 and 2–2 parental dose-combinations, and found that setting a cutoff of minimum 50 resulted in a QQ plot that indicated a more well-calibrated model (see Supporting

Information: Figure S3b). As described previously, the mixed effects model fails to converge when analyzing SNPs with low ART frequencies, leading to heavily inflated standard errors which make these SNPs easy to identify without having to choose a specific cutoff. An advantage with the mixed effects model is that we can utilize all of the available case data (1304 ART pregnancies, vs. 1218 when we sample one pregnancy per couple).

We also implemented a generalized linear mixed effects model with all parameters from Table 1a estimated, including four parameters for the interaction, applying the same covariates as in the previous models. Using a likelihood ratio test, we tested for significance of the interaction effect by comparing this model to a reduced model without interaction terms (a 4-*df* interaction test). After removing SNPs with heavily inflated interaction term standard errors, the remaining index SNPs overlapped to a large degree with those identified in the *mdr*, *jdd*, *jrr*, *th2* or *th3* models (see in the Supporting Information: Table S3). This is as expected, because this approach detects interaction effects in some of (or all) the same parental dose-combinations as these five models. The *comp* model, on the other hand, is based on the 0–2 and 2–0 parental dose-combinations, and the results from the likelihood ratio test do not align with those from the *comp* model (results not shown).

Considering the 512 parameterizations presented by Li and Reich (2000), there are still many possible models that could be investigated. As mentioned previously, some of these models may be biologically more relevant than others. One example is the "exclusive–OR" model where the parental doses 0–2, 1–2, 2–0 and 2–1 are assumed to have an effect on the probability of ART use. This model has previously been useful when analyzing the genetics of handedness (Levy & Nagylaki, 1972). Another possible extension of our analyses would be to consider all possible pairwise interactions between *different* SNPs from the mother and father. Exhaustive pairwise interaction testing, as well as approaches to reduce the computational burden, have frequently been considered in the context of within-person epistatic effects (Evans et al., 2006). We performed a truncated search for such pairwise interaction effects by restricting our analyses to only a subset of the index SNPs identified in our main analyses. Despite this simplification, there were still 22,650 pairwise combinations in total. These analyses did not reveal any new significant results other than what might be expected from index SNPs being in linkage disequilibrium with one another. However, future work may involve a more comprehensive approach where all SNP pairs across the genome are considered agnostically.

Our logistic regression models compare ART pregnancies with non-ART pregnancies. However, under certain assumptions, it might be sufficient to only look at ART pregnancies. It is well known from gene–environment interaction analyses that, by assuming independence between genes and environment in the background population, case–only analyses can be performed to detect gene–environment interactions (Umbach & Weinberg, 2000). If a statistical dependence between genes and environment is observed among cases, this would point to an interaction effect (Mukherjee et al., 2008). Similarly, in our design, we might assume that maternal and paternal genotypes are independent of each other in the background population. Observing a statistical dependence between maternal and paternal genotypes in ART pregnancies would then suggest an interaction between the two parental genotypes, because ART use must be more (or less) likely given specific combinations of maternal and paternal alleles. To detect an interaction, one might thus compare the combined genotype distribution of mothers and fathers with what would be expected from independence. However, population genetic effects such as inbreeding, assortative mating, and population stratification may give rise to correlations between parental alleles in the general population. Although inbreeding may affect several loci simultaneously, assortative mating would normally only affect selected loci, such as those driving body height. Correlations in the background population may be detected by checking non-ART parents, though this would again require the use of non-ART pregnancies. Interesting ways to combine the use of controls and the assumption of independence have been explored in the gene–environment interaction setting (Mukherjee et al., 2008). Although such ideas can be implemented in our model setup, we have restricted our current models to a standard interaction setup.

Regarding the genes and loci showing the most significant allelic interaction effects in our analyses, two genes in particular are worth mentioning here because of their association with tissues that are relevant for infertility. Firstly, age-related increased expression of the gene for Parkin RBR E3 ubiquitin protein ligase (*PRKN*) contributes to defects in meiosis and the accumulation of damaged mitochondria in germinal vesicle oocytes (Jin et al., 2022). The second gene, cyclic nucleotide binding domain containing 1 (*CNBD1*), is expressed almost exclusively in testis. Despite an extensive literature search, however, we were unable to find any previous reports linking infertility with these genes, nor with any of the other genes/loci listed in in the Supporting Information: Table S2. One exception is Dynein axonemal heavy chain 17 (*DNAH17*). This gene

has an established role in male fertility, specifically through asthenozoospermia (Whitfield et al., 2019). Furthermore, exome sequencing identified *DNAH17* as one of several genes associated with male infertility (Sudhakar et al., 2023). Experiments in rats showed that *DNAH17* is essential for spermatogenesis and fertility (Chen et al., 2021). Finally, differentially methylated CpGs in *DNAH17* and many other genes were reported to be associated with male infertility (Sarkar et al., 2019).

In summary, we found that several models are relevant for analyzing parental allelic interactions. Although we investigated infertility as our main phenotype, using ART as a proxy, other fertility-related measures can also be applied. Our results showed that the *mdr*, *jdd* and *th2* models were more robust to low ART frequencies. The most significant SNPs were identified by at least one of these three models or by the *comp* model. One particularly relevant finding was in a gene that has an established role in male fertility. Furthermore, we identified several issues that should be addressed when implementing these models, for example, if and how parental main effects should be included and how the results should be interpreted in light of the choice of model. Our models can all be analyzed using standard statistical software such as R.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Norwegian Institute of Public Health (NIPH), but restrictions apply regarding the availability of these data, which were originally used under specific approvals for the current study and are therefore not publicly available. The individual level data are available under restricted access due to regulations and access can only be given after approval by the Norwegian Ethical committees under the provision that the applications are consistent with the consent provided. Access can be obtained by application to the Norwegian Institute of Public Health using a form available on the English language portion of its website at https://www.fhi.no/en/studies/moba/. The R code used for the current analyses is available on https://github.com/siriskodvin/MF-interactions-and-infertility/tree/main/Code.

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