Neurodevelopmental outcome of children born following assisted reproductive technology

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Part III - Follow-up of children born after IVF with Preimplantation Genetic Screening

7

Neurological condition of infants born after IVF with preimplantation genetic screening

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ABSTRACT

Objective: Aim of this study was to evaluate the effect of preimplantation genetic screening (PGS) on neurodevelopmental outcome in children.
Methods: We conducted a prospective follow-up study of children born to women randomly assigned to in vitro fertilisation with or without PGS. Primary outcome was adverse neurological outcome at 18 months; secondary outcomes were types of minor neurological dysfunction (MND), neurological outcome before 18 months, neonatal intensive care admission, and congenital malformations.
Results: Twenty women in the PGS group participated with 25 children and 26 women in the control group participated with 31 children. Five PGS pregnancies (25%) and four control pregnancies (15%) resulted in birth of at least one child with an adverse neurological outcome (adjusted odds ratio: 2.3 [0.4–12.0]). Dysfunction in fine motor abilities and posture and muscle tone dysregulation tended to be present more frequently after PGS. Neurological outcome before 18 months, neonatal intensive care admission, and prevalence of congenital malformations were similar in study and control pregnancies. Nevertheless, at child level, rates of adverse outcome were higher after PGS.
Discussion: In conclusion, outcome in pregnancies after in vitro fertilisation (IVF) with and without PGS was similar. The small sample size precludes the conclusion that PGS is not associated with less favourable neurological outcome. Safety of new assisted reproductive techniques should be evaluated before large-scale implementation.

INTRODUCTION

Children born after assisted reproduction represent a sizeable part of the population; therefore, their health is of general concern. Nowadays, in Europe and the United States, 1–4% of children are born after assisted reproduction, and the numbers are still increasing (Andersen *et al.*, 2008;Wright *et al.*, 2008).

One of the major goals for new reproductive techniques is to enhance efficiency of assisted reproduction, and newer methods are regularly introduced to achieve this goal. One of these methods is in vitro fertilisation (IVF) with preimplantation genetic screening (PGS) for aneuploidy. In this procedure, embryos obtained with IVF are biopsied, which implies that a hole is made in the zona pellucida with laser or by chemical means. One or two blastomeres are aspirated, so that copy numbers of several sets of chromosomes can be determined. The concept behind this procedure is to identify and discard embryos with an abnormal chromosomal constitution because these might have a lower implantation potential or eventually lead to miscarriage (Wilton, 2002). Higher ongoing pregnancy rates were expected due to selection of the most viable embryos but have not been demonstrated in recent randomised controlled trials (RCTs) (Staessen *et al.*, 2004;Jansen *et al.*, 2008;Schoolcraft *et al.*, 2008;Staessen *et al.*, 2008). In fact, two trials have shown a reduction in ongoing pregnancies (Mastenbroek *et al.*, 2007;Hardarson *et al.*, 2008).

Besides efficacy, another important issue in assisted reproduction is the safety of the procedure for the developing foetus. Despite the invasiveness of PGS, information on developmental outcome after this procedure is scarce (Nekkebroeck *et al.*, 2008a;Nekkebroeck *et al.*, 2008b;Banerjee *et al.*, 2008), and information on neurological outcome is completely lacking. Therefore, we conducted a prospective, assessor-blinded follow-up study of children born to women randomly assigned to IVF with or without PGS. The aim of our study was to investigate the effect of PGS on the child's neurodevelopmental outcome. The primary outcome measure of this study was adverse neurological outcome at the age of 18 months. Secondary outcome measures were specific types of minor neurological dysfunction (MND) at 18 months, neurological outcome before 18 months, admission to a neonatal intensive care unit, and congenital malformations.

METHODOLOGY

Eligible for this follow-up study were children of women participating in one centre (University Medical Center Groningen) of a multicenter trial on the efficiency of PGS to establish ongoing pregnancies (Mastenbroek *et al.*, 2007). In the trial, exclusion criteria for women were age of IVF candidate younger than 35 or older than 41 year, previously failed IVF cycles, and objections against a possible double-embryo transfer. Randomisation of women was performed centrally with minimization for age (35–37 and 38–41 year) and reproductive technique (IVF and intracytoplasmic sperm injection), with stratification according to study centre before the start of the IVF procedures. Information concerning IVF treatment procedures has been reported previously (Mastenbroek *et al.*, 2007). For this study, women who conceived naturally during the trial were excluded from the follow-up study because the aim of our study was to investigate the effect of PGS on child outcome in IVF treatment. The protocol of the follow-up study was approved by the Medical Ethics Committee of the University Medical Center Groningen.

Couples were invited for the follow-up study during the third trimester of pregnancy. After written informed consent, inclusion took place during the first 2 weeks after birth. At the first appointment, demographic information (including e.g. parity, gestational age, birth weight, neonatal intensive care unit admission, parental age, and educational level) was collected on standardised charts. Time to pregnancy was retrieved from fertility charts. Information on child health up to 18 months was collected by history taking during assessments. Major congenital malformations were classified as malformations that generally cause functional impairment or require surgical correction (Bonduelle *et al.*, 2002). All other malformations were classified as minor.

Neurodevelopmental assessments

Follow-up consisted of standardised, age-specific neurological assessments at the ages of 2 weeks and 3, 4, 10, and 18 months post term. Age-specific testing is necessary in children due to abundant structural and functional changes in the nervous system, which induce changes in expression and prevalence of neurological dysfunction (Hadders-Algra, 2005). At 2 weeks and 3 months, quality of general movements (GMs) was assessed. Four classes of GM quality can be distinguished, being normal-optimal, normal-suboptimal, mildly abnormal, and definitely abnormal GMs (Hadders-Algra *et al.*, 2004). At 4 and 10 months, the Touwen Infant Neurological Examination was used, which resulted in the classification of normal, normal-suboptimal, MND, and definitely abnormal (Touwen, 1976).

The neurological examination according to Hempel was used at 18 months

(Hempel, 1993). In this assessment, children perform various motor tasks while playing in a standardised free-field situation. Functionality is tested in five different domains: fine motor function, gross motor function, posture and muscle tone, reflexes, and visuomotor function. Multiple signs in a domain result in a dysfunctional cluster. Children are classified as neurologically normal, simple MND, complex MND, or major neurological dysfunction based on the number of dysfunctional clusters (Hadders-Algra, 2003). Neurologically normal implies the presence of no dysfunctional clusters or only the presence of the cluster reflexes. Simple MND means the presence of one cluster of dysfunction, i.e. the isolated presence of fine motor, gross motor, visuomotor dysfunction, or mild dysregulation of posture and muscle tone. It is considered to reflect a normal, but non optimal form of brain function. Complex MND denotes the presence of two or more dysfunctional clusters; it is the form of MND with clinical relevance due to its clear association with learning and behavioural disorders (Hadders-Algra, 2002;Batstra *et al.*, 2003). Major neurological dysfunction implies the presence of a distinct neurological syndrome, such as cerebral palsy (Hadders-Algra, 2003). We considered complex MND and major neurological dysfunction as an adverse neurological outcome. Specific types of dysfunction were mild fine motor dysfunction, mild gross motor dysfunction, mild visuomotor dysfunction, or a mild dysfunction in posture and muscle tone. At all ages, children were assessed by K.J.M. under supervision of M.H.-A., who were blind to mode of conception.

Statistical analysis

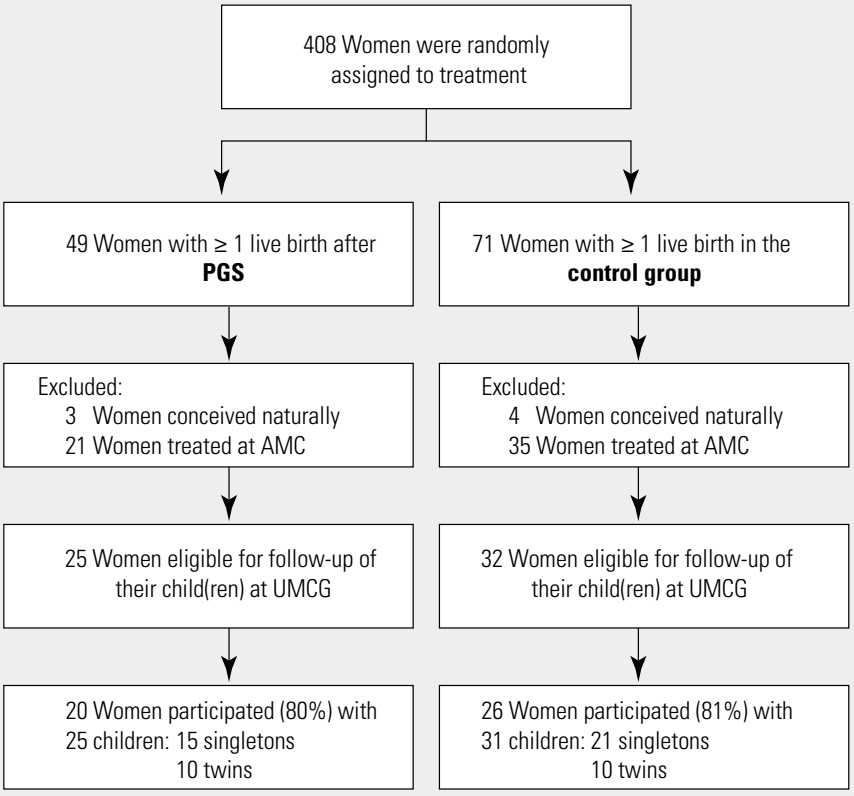
In the preceding study on the efficiency of PGS to establish ongoing pregnancies (Mastenbroek *et al.*, 2007), women were randomised into IVF with or without PGS. In this study, we continued to use women (or their pregnancies) as unit of analysis for the primary statistical analyses. In case of twins, a pregnancy was considered to have an adverse outcome when at least one of the children born had an adverse outcome. For descriptive purposes and secondary analyses, we also analysed data at the child level, under the untested assumption that lack of independence among twins is negligible.

We used t test to analyze differences of means in continuous data. For categorical data, Fisher's exact test or Pearson's χ^2 test was used when appropriate. Because group sizes were small and randomisation had occurred at the level of the women before pregnancy and not on infant level, we *a priori* decided to adjust for factors known to affect neurodevelopmental outcome, i.e. gestational age, twins, and maternal age by means of logistic regression analysis. The p values of 5% and lower were considered statistically significant. Statistical analyses were performed with the use of SPSS for Windows, version 14.0.

RESULTS

Between March 2004 and January 2006, the multicenter trial resulted in live birth of at least one child in 49 women after IVF treatment with PGS and 71 control women (Mastenbroek *et al.*, 2007). Of these women, 25 and 32 gave birth after treatment at the University Medical Center Groningen. Twenty (80%) and 26 (81%) couples agreed to participate in the follow-up study with their child or children (figure 1). Reasons for nonparticipation were logistic (three study and four control couples), unwillingness to participate in a developmental study (two study and one control couples), or resistance against hospital visits due to fertility history (one control couple). The 20 couples of the PGS group participated with 25 children and the 26 couples of the control group with 31 children (table I). Couples with

Figure 1 - Eligibility and participation of women



PGS = Preimplantation genetic screening, UMCG = University Medical Center Groningen, AMC = Academic Medical Center Amsterdam

Table I. Demographic characteristics of parents and children.

Characteristics	Women with ≥ 1 live birth after PGS *(n = 20)*	Children born following PGS *(n = 25)*	Control-women *(n = 26)*	Control-children *(n = 31)*	*P*-values
Parental characteristics:					
Maternal age at conception, mean ± SD	37.6 ± 1.6		38.2 ±1.4		0.24
Primiparity	8 (40)		14 (54)		0.53
Education level mother (high[a]), n (%)	7 (35)		12 (46)		0.65
Education level father (high[a]), n (%)	8 (40)		10 (39)		1.00
Fertility factors:					
Intracytoplasmic sperm injection, n (%)	8 (40)		8 (31)		0.73
Time to pregnancy in years, mean ± SD	3.6 ±1.9		3.9 ±2.8		0.77
Cause of subfertility, n (%)[b]					
poor semen quality	7 (35)		11 (42)		0.84
tubal	8 (40)		9 (35)		0.95
unexplained	4 (20)		7 (27)		0.73
other[c]	3 (15)		3 (12)		1.00
Perinatal characteristics:					
Twins, n (%)	5 (25)		5 (19)		0.73
Gestational age in weeks, mean ± SD	39.1 ± 2.6		38.5 ± 2.0		0.40
Preterm birth (< 37 weeks), n (%)	2 (10)		4 (15)		0.68
Caesarean section, n (%)	3 (15)		7 (27)		0.48
Child chracteristics:					
Male gender, n (%)		15 (60)		15 (48)	0.39
Birth weight in grams, mean ± SD		3187 ± 750		3016 ± 658	0.37

[a] High level of education denotes university education or vocational colleges.
[b] More than one diagnosis per couple was possible
[c] Other causes of subfertility were anovulation, endometriosis and cervical factor.

twin pregnancies participated either with both or none of the children. Postnatal attrition was low. One control child and one pair of study group twins were not assessed at the ages of respectively 2 weeks and 3 and 4 months. All included children were assessed at the ages of 4, 10, and 18 months.

Demographic and perinatal characteristics of the PGS and the control group are shown in table I. Causes of subfertility were similar in the PGS and the control group. Both groups included five twin pregnancies. Testing age (corrected for preterm birth) was similar in study and control group at all follow-up ages.

Five pregnancies after IVF with PGS (25%) compared with four pregnancies in the control group (15%) resulted in the birth of at least one child with an adverse neurological outcome at the age of 18 months (adjusted odds ratio: 2.3 [0.4–12.0]; table II). Neurological outcome at child level is presented in table III. Five PGS children (20%) and four controls (13%) showed complex MND. In addition,

one child in the study group presented with a spastic diplegia. This condition resulted from myelum compression at the level of the medulla oblongata by an arteriovenous malformation diagnosed at the age of 1 year. Analysis of the specific types of dysfunction showed that PGS pregnancies tended to result more often in children with fine motor dysfunction (P = .08) and dysfunctional posture and muscle tone (P = .03; table II). Multivariate analyses were not possible because none of the children in the control group presented with these specific types of dysfunction. There were no differences in gross motor function, reflexes, and visuomotor function between the two groups. Neurodevelopmental outcome up to and including 10 months of age was similar in children born after PGS and control children (data not presented).

Four PGS pregnancies (20%) and three control pregnancies (12%) resulted in admission of at least one child to neonatal intensive care (adjusted odds ratio: 10.2 [0.4–235.2]; table II). Reasons for neonatal intensive care admission were prematurity (four PGS twin children and two control twin children), respiratory insufficiency (two PGS twin children), short-term transitional problems after

Table II - Neurological outcome, neonatal intensive-care admissions and congenital malformations in PGS and control pregnancies.

	Women with ≥ 1 live birth after PGS (n=20)	Control-women (n=26)	Crude Odds-Ratio [95%CI] or p-value	Adjusted[a] Odds-Ratio [95%CI]
Outcome measure	**n (%)**	**n (%)**		
Pregnancies resulting in ≥ 1 child with an adverse neurological outcome at 18 months	5 (25)	4 (15)	1.8 [0.4-8.0]	2.3 [0.4-12.0]
Pregnancies resulting in ≥ 1 child with the following clusters of dysfunction at 18 months				
Fine motor function	3 (15)	0 (0)	P = .08[b]	-
Gross motor function	4 (20)	5 (19)	1.0 [0.2-4.6]	1.0 [0.2-5.0]
Posture and muscle tone	4 (20)	0 (0)	P = .03[b]	-
Reflexes	9 (45)	8 (31)	1.8 [0.5-6.2]	2.0 [0.6-7.6]
Visuomotor function	0 (0)	0 (0)	-	-
Pregnancies resulting in ≥ 1 child admitted to neonatal intensive-care	4 (20)	3 (12)	1.9 [0.4-9.8]	10.2 [0.4-235.2]
Pregnancies resulting in ≥ 1 child with a congenital malformation				
Major malformation	4 (20)	1 (4)	6.2 [0.6-61.1]	4.7 [0.4-51.2]
Minor and/or major malformation	7 (35)	5 (19)	2.3 [0.6-8.6]	2.1 [0.5-9.1]

[a] Adjusted for gestational age, twins, and maternal age at conception.
[b] Fisher's exact test.

TABLE III - NEUROLOGICAL OUTCOME, NEONATAL INTENSIVE-CARE ADMISSIONS AND CONGENITAL MALFORMATIONS IN CHILDREN.

Outcome measure	Children born following PGS (n=25)	Control-children (n=31)
	n (%)	*n (%)*
Neurological outcome at 18 months:		
Normal	18 (72)	26 (84)
Simple MND[a]	1 (4)	1 (3)
Complex MND[a]	5 (20)	4 (13)
Major Neurological Dysfunction	1 (4)[b]	0 (0)
Clusters of dysfunction at 18 months:		
Fine motor function	4 (16)	0 (0)
Gross motor function	5 (20)	5 (16)
Posture and muscle tone	4 (16)	0 (0)
Reflexes	11 (44)	8 (26)
Visuomotor function	0 (0)	0 (0)
Neonatal intensive-care admission	7 (28)	3 (10)
Congenital malformations:		
Children with a major malformation	5 (20)[c]	1 (3)[d]
Children with a malformation (minor and/ or major)	9 (36)	6 (19)

[a] MND = minor neurological dysfunction.
[b] Spastic diplegia due to spinal cord compression by an arteriovenous malformation diagnosed at the age of 1 year.
[c] One child with congenital cataract, one child with a congenital forefoot deformity (metatarsus adductus), one child with an arteriovenous malformation, and a pair of twins with undescended testis requiring orchidopexy.
[d] One child with bilateral inguinal hernia requiring surgery.

birth (one PGS singleton), and sepsis (one control singleton). In total, seven PGS children (28%) and three control children (10%) were admitted to neonatal intensive care (table III). As condition at birth, i.e. the requirement of neonatal intensive care, might be an important mediator of neurological condition at 18 months, we explored this relationship. Only one of the 10 children who had been admitted to neonatal intensive care presented with adverse neurological outcome at 18 months, indicating no statistically significant association (P = .67). In addition, we found no relation between neonatal intensive care admission and the number of dysfunctional clusters present (P = .84) or the presence of any specific type of MND.

In four PGS pregnancies (20%) and one control pregnancy, at least one child presented with a major malformation at the age of 18 months (adjusted odds ratio: 4.7 [0.4–51.2]; table II). As the presence of congenital malformations is associated with an increased risk of neurological dysfunction, we explored this relationship. We found an evident relation between major malformations and adverse neurological outcome at 18 months (P = .007). Children with a major

malformation showed more dysfunctional clusters at the age of 18 months (P < .001) and presented more often with fine motor impairment (P = .05), gross motor impairment (P = .007), and mild dysregulation of posture and muscle tone (P = .003). Congenital malformations of any kind (major or minor) were observed in respectively seven (35%) and five (19%) pregnancies (adjusted odds ratio: 2.1 [0.5–9.1]; table II). These malformations were not related to adverse neurological outcome (P = .11) or the presence of any specific type of MND, however, children with any kind of malformation had more clusters of dysfunctions (P = .04) than children without malformation.

DISCUSSION

In this prospective, assessor-blinded randomised follow-up study, we found similar rates of adverse neurological outcome, neonatal intensive care admission, and congenital malformations in pregnancies resulting from IVF with or without PGS. Mild fine motor dysfunction and mildly dysfunctional posture and muscle tone tended to be present more frequently after PGS pregnancies. At child level, results were slightly more unfavourable for children born after PGS than for control children. Therefore, an increased risk for a less favourable neurological outcome in children born after PGS cannot be excluded.

The main limitation of our study is the relatively small sample size, which was caused by the fact that the power analysis of the PGS study was based on the number of women needed to detect an increase in ongoing pregnancy rates and not on the number of pregnancies necessary for follow-up (Mastenbroek *et al.*, 2007). This means that this study has an explorative nature and that our findings should be interpreted with caution. From an infant developmental perspective, the fact that randomisation had occurred at the level of future mothers and not at infant level might also be regarded as a limitation of the study. We addressed the problem of minor differences in perinatal adversity between the groups by including multivariate statistics. Further minor limitations of the study are the 19–20% loss to follow-up and the relatively limited duration of follow-up. For practical reasons, outcome at the age of 18 months is frequently the end point in developmental studies. However, it should be realised that the majority of minor developmental disabilities first emerge at school age (Hadders-Algra, 2002). Therefore, long-term neurodevelopmental follow-up of children born after IVF with PGS is urgently needed.

The strengths of our study are prospective follow-up, blinding of the assessor, and application of longitudinal detailed, standardised neurological

assessments. Furthermore, randomisation of couples to IVF treatment with or without PGS contributed to the comparability of study and control group.

Many studies have investigated neurodevelopmental outcome of children born after assisted reproduction (reviewed by Sutcliffe and Ludwig, 2007;Middelburg *et al.*, 2008;Hvidtjørn *et al.*, 2009). Results from two recent systematic reviews suggest that IVF is associated with an increased risk for cerebral palsy. At least partly, this association can be explained by the increased risk of multiple births and preterm delivery after IVF (Middelburg *et al.*, 2008;Hvidtjørn *et al.*, 2009). Little is known about the association of assisted reproduction and MND (Middelburg *et al.*, 2008), although these so-called minor neurodevelopmental disorders may have a major impact on daily life of the child and his or her family (Batstra *et al.*, 2003; Gillberg and Gillberg, 1989). Even less is known on outcome of children born after PGS. So far, data on two nonrandomised controlled trials have been reported. Banerjee *et al.* reported similar developmental and behavioural scores in children born after PGS or natural conception (Banerjee *et al.*, 2008). In addition, a Belgian group found no adverse effect of embryo biopsy on growth, congenital malformations, neonatal intensive care admissions, behaviour, and mental and psychomotor development assessed with the Bayley Scales in singletons at 2 years (Nekkebroeck *et al.*, 2008a;Nekkebroeck *et al.*, 2008b;Desmyttere *et al.*, 2008).

Considerations and conclusions

Invasive assisted reproductive techniques such as embryo biopsy carry potential risks for further development of the embryo. Biologically, it is plausible that the use of laser or chemicals for opening of the zona pellucida may induce thermal, mechanical or mutagenic side effects (Kanyo and Konc, 2003). Likewise, intuition suggests that removal of up to one quarter of the embryo's cell mass might have developmental consequences. In addition, a different selection of embryos, on the basis of ploidy-status instead of morphologic criteria of the embryo, might hypothetically result in children with less favourable neurological outcome.

At the RCT level of pregnancies, no adverse effect of PGS could be demonstrated. However, at child level, PGS was associated with a higher rate of MND. Contrary to our expectations, adverse neurological outcome was not associated with neonatal intensive care admission. Adverse neurological outcome was, however, strongly associated with major congenital malformations. Previous studies that found an association between minor congenital malformations and impaired neurodevelopmental outcome, in particular impaired fine motor function, have suggested that the link points to an early ontogenetic origin of the neural dysfunctions (Largo *et al.*, 1989;Soorani-Lunsing *et al.*, 1993). The absence of a relation between neonatal intensive care admission and adverse

neurodevelopmental outcome in this study might be due to the small sample size.

It is important to continue neurological follow-up at least up to school age because children may grow into an adverse neurological outcome. This is a well-known developmental phenomenon. Developmental changes in the infant brain strongly affect the age-specific expression of dysfunction. For instance, early signs of dysfunction in infants may disappear because of restorative mechanisms in the brain, but dysfunction may also be uncovered by developmental progress. Cases in point of the latter are development of dyslexia or the time needed for a full expression of cerebral palsy (Hadders-Algra *et al.*, 2004).

Embryo biopsy is also applied in preimplantation genetic diagnosis; however, the indication for this procedure is fundamentally different from PGS (Sermon *et al.*, 2004). In preimplantation genetic diagnosis, blastomeres obtained with embryo biopsy are analysed for specific heritable disorders, such as cystic fibrosis. There is a steady rise in centres practicing preimplantation genetic diagnosis, and the technique is used for more and more indications (Goossens *et al.*, 2008). The increase in application of embryo biopsy in combination with the findings of this study demonstrates the need for determination of its safety.

In conclusion, in this prospective, assessor-blinded randomised follow-up study, we found similar rates of adverse neurological outcome, neonatal intensive care admission, and congenital malformations in PGS and control pregnancies. Because at child level, rates of adverse outcome were higher in children born after PGS than in control children, an increased risk for a less favourable neurological outcome in children born after PGS cannot be excluded by this study. Biologic plausibility, i.e. the possibility that interference with the very first steps of ontogeny may give rise to impaired neurological development, is reason for concern. The safety of new assisted reproductive techniques, such as PGS, should be carefully evaluated in follow-up studies preceding large-scale implementation of these techniques.