Detection of a case of Angelman syndrome caused by an imprinting error in 949 pregnancies analyzed for AS following IVF

John P. Johnson¹ · Jonathon Schoof¹ · Linda Beischel^{1,2} · Corbin Schwanke¹ · James Goldberg³ · Lauri Black⁴ · Lori Ross⁵ · Suchina Bhatt⁶

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Introduction

In vitro fertilization (IVF) is now responsible for achieving 1–2% of US pregnancies and subsequent births (www.cdc.gov/art). A number of factors have led to the steady increase in IVF, including increasing rates of infertility and delayed onset of reproduction, especially for women [1].

In the early 2000s, reports of children born with imprinting disorders following IVF appeared in the literature. At first, these included Angelman (AS) and Beckwith-Wiedemann syndromes (BWS, [2–7]), but later Russell-Silver syndrome [8] as well. These reports were first anecdotal, followed by larger retrospective registry studies [9–14].

From the above, there clearly are increased risks for imprinting syndromes following IVF/ICSI. The estimates of increased risk vary significantly but range from 0-fold to about 13-fold. However, molecular details in the above studies are mostly lacking. In addition, there are no results from prenatal series and fewer studies on the increased risk for AS following IVF.

To assess the increased risk of these disorders, we report on a consecutive series of 949 IVF pregnancies tested for imprinting disorders, AS and BWS (no case of BWS was detected),

the only indication for testing. We have found one molecularly confirmed case of Angelman syndrome caused by an imprinting defect. The expected probability of this event in the general population is approximately 1/200,000 (prevalence of Angelman syndrome ~1/10,000–20,000, using a conservative high figure of 1/10,000, with only 2–5% being due to primary imprinting disorders, using a high figure of 5% to reach this overall risk), and the probability of detecting such an event in our small series is <.0001 (chi-square (www.medcalc.org/calc/comparison_of_proportions.php)). We believe this observation provides substantial support to the assertion that IVF significantly increases the risk of fetal imprinting disorders, from this report specifically, AS.

Methods and materials

In 2003, our laboratory began to receive prenatal samples from a group in the San Francisco Bay Area providing prenatal diagnosis for couples who had conceived by IVF. In subsequent years, many centers have sent us samples (see Acknowledgments). These were collected for clinical diagnosis of imprinting disorders given the unknown increase in the risk and the interest in these centers and patients being tested.

Most of the samples were from women of advanced maternal age (Table 1). We do not have information on couple infertility but suspect this was the main indication for IVF. We also have little information on the type of IVF other than to note that about 25% of pregnancies were conceived by ICSI. Samples received included cells grown from either amniocentesis or chorionic villus sampling.

Isolated fetal DNA from either chorionic villus or amniocentesis cultured cells was subjected to Southern blot analysis with the SNRPN sequence [15] (Fig. 1a). Later, samples were additionally analyzed by a methylation polymerase chain

✉ John P. Johnson
jjpgobblue@gmail.com

¹ Medical Genetics, Shodair Children's Hospital, PO Box 5539, Helena, MT 50604-5539, USA

² Department of Public Health and Human Services, State of Montana, Helena, MT, USA

³ Counsyl, South San Francisco, CA, USA

⁴ Pacific Reproductive Genetic Counseling, Pacifica, CA, USA

⁵ Neogenomics Laboratories, Irvine, CA, USA

⁶ Illumina, Redwood City, CA, USA

Table 1 Results of molecular analysis for AS and BWS in 1065 pregnancies conceived by IVF

Total cases referred	1065
CVS	334
Amniocentesis	731
Tested for AS/BWS	922
Tested for AS only	27
Tested for BWS only	116
ICSI marked	268

reaction (PCR) [16] (msPCR; Fig. 1b), and by an assay, we developed termed restriction digest PCR (rdPCR; Fig. 1c, d), both using SNRPN sequence. Details follow as this technique has not been described elsewhere. In this procedure, sample DNA is digested either with a methylation-sensitive (not cutting methylated CpG) restriction enzyme, with an enzyme which preferentially recognizes methylated CpG, with both enzymes, or with neither. We are able to use these latter methods to estimate a methylation index (MI; [17]) because of the more quantitative properties of PCR versus Southern

blots. Expected results on all studies include roughly equal maternal and paternal quantitative PCR results indicating an MI near .5. The reason to use both the newer assays is the variability in the testing itself with either, with msPCR varying with the amount of bisulfite conversion of DNA, and for rdPCR, the extent of digestion with restriction enzymes. We obtain a better estimate of MI by comparing results from each test.

Results

The sample numbers are presented in the Table 1. As can be seen, amniocentesis samples outnumbered CVS by almost 2:1. An ICSI pregnancy was marked in about 25% of samples. We have not subdivided the sample by DNA analysis techniques as described above. Gestational age at testing was approximately 11–12 weeks for CVS and 15–16 weeks for amniocentesis. Other than an average patient age in the advanced maternal age range, we have no further details on the mothers or methods of artificial reproduction.

Fig. 1 Methods and results of fetal DNA analysis for Angelman syndrome. In the positive fetus, note absence or reduced intensity of the maternal fragment in the Southern blot (a) and in the msPCR (b). rdPCR shows that DNA digested with methylation-sensitive or methylation-dependent enzymes amplifies similarly (c; control). The two digests approximate each other because there is one copy of methylated and one copy of unmethylated DNA in normals. In an AS-affected positive control (d), the sample digested with a methylation-dependent enzyme amplifies like a control sample (undigested), whereas the sample digested

with a methylation-sensitive enzyme digests normally (the DNA is not methylated) and amplifies late like a double digest control. The Southern blot is from the first amniocentesis sample of the AS-positive twin fetus, flanked by a normal control and the co-twin sample. The msPCR is from the second confirmatory amniocentesis done at the time of twin termination. The positive RDPCR is from an AS patient. rdPCR: blue: DNA not digested, green: DNA digested with a methylation-dependent restriction enzyme, red: DNA digested with a methylation-sensitive enzyme, black: DNA double digested with both enzymes

We detected an AS case in one of identical twins in the sample number 212 of 949; the co-twin was normal. The mother in this case was 39 years of age at delivery, and the indication for the study was “IVF pregnancy” with no further detail. This fetus showed an abnormal imprinting pattern for SNRPN, with hypomethylation of the maternal allele (Fig. 1a; MI is ~ 0 on the blot). The sample was subjected to FISH testing with a fluorescent SNRPN probe, with negative results (not shown). We requested samples to test for UPD but did not receive them. Instead, we compared the two twins with multiple 15q11 markers and results were identical. This makes UPD15 less likely in the affected twin as these were apparently identical twins. This also indicates non-concordant methylation in identical twins, a situation not unusual in BWS [18]. Given that 15q11 deletion was ruled out and UPD very unlikely, the abnormal methylation of SNRPN as detected in this case on a Southern blot and methylation PCR (Fig. 1) were likely caused by a presumed imprinting error, and not by chromosome 15 microdeletion or uniparental disomy. Imprinting errors are the rarest of known causes of Angelman syndrome. We were able to obtain a sample from pregnancy termination and confirmed the results of the previous amniocentesis sample (MI is .06 on msPCR; Fig. 1b).

Angelman syndrome overall has a frequency of about 1/10,000–1/20,000. It is caused by a primary imprinting error in about 2–5% of all cases. This would mean that conservatively, using a 1/10,000 high estimate of AS frequency and a (high) 5% estimate of AS cases produced by imprinting errors, the incidence of such cases is about 1/200,000. We observed 1/949. We evaluated this discrepancy by a chi-square comparison of proportions. In comparing the expected versus observed frequency of imprinting AS in our study, the significance of our observation reaches a p value of $< .0001$ (www.medcalc.org/calc/comparison_of_proportions.php).

Discussion

We detected a case of imprinting anomaly AS in a small series of tested pregnancies ($n = 949$). The probability of chance observation of a case in our series is $< .0001$. Our results are consistent with the anecdotal and retrospective studies showing an increased incidence of AS following IVF. From our results, the increase in risk for the abnormal imprinting subtype of AS could be substantial. In a separate paper (submitted), we show that the frequency of IVF associated with BWS/omphalocele is increased almost 20-fold. There is no known increase in imprinting disorders related solely to advanced maternal age (except for maternal UPD, which is a primary chromosomal nondisjunction disorder, different from the primary imprinting disorders we are discussing).

We think that the risk for an imprinting disorder such as AS following IVF could be high enough to warrant prenatal diagnosis and at a minimum, counseling concerning the risk. However, we do not know the denominator for incidence of AS following IVF because our sample is small, and the patients likely came from a biased group, given that they received counseling for imprinting disorders, chose to have testing, and came from a limited but substantial number of prenatal centers in the USA (Acknowledgments). A similar but very large prenatal series or a large case-control study (some have been done; [10]) will be required to establish an actual risk range for imprinting disorders following IVF.

Many have speculated regarding reasons for an increased risk of imprinting disorders associated with IVF pregnancies. Different techniques and steps in the procedures have been suggested as sources of the increased risk, but there is no consensus on these reasons. Manipulation of the eggs, sperm, or zygotes in any manner which is not natural or physiological seems to increase the risk [19]. In the BWS study mentioned above, one case was conceived by in utero sperm injection, the simplest of artificial reproductive techniques. Alternatively, the frequent underlying problem, infertility, might account for an increased risk [20]. Regardless, the risk merits discussion with parents achieving a pregnancy by IVF, with consideration of prenatal diagnosis for AS and BWS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Balasch J. Investigation of the infertile couple: investigation of the infertile couple in the era of assisted reproductive technology: a time for reappraisal. *Hum Reprod.* 2000;15:2251–7.
- Cox GF, Burger J, Lip V, Mau UA, Sperling K, Wu BL, et al. Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am J Hum Genet.* 2002;71:162–4.
- DeBaun MR, Niemitz EL, Feinberg AP. Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alteration of LIT1 and H19. *Am J Hum Genet.* 2003;72:156–60.
- Maher ER, Brueton LA, Bowdin SC, Luharia A, Cooper W, Cole TR, et al. Beckwith-Wiedemann syndrome and assisted reproductive technology (ART). *J Med Genet.* 2003;40:62–4.
- Gicquel C, Gaston V, Mandelbaum J, Siffroi JP, Flahault A, le Bouc Y. In vitro fertilization may increase the risk of Beckwith-Wiedemann syndrome related to the abnormal imprinting of the KCNQ1OT gene. *Am J Hum Genet.* 2003;72:1338–41.
- Chang AS, Moley KH, Wangler M, Feinberg AP, DeBaun MR. Association between Beckwith-Wiedemann syndrome and assisted reproductive technology: a case series of 19 patients. *Fertil Steril.* 2005;83:349–54.
- Sutcliffe AG, Peters CJ, Bowdin S, Temple K, Reardon W, Wilson L, et al. Assisted reproductive therapies and imprinting disorders—a preliminary British survey. *Hum Reprod.* 2006;21:1009–11.
- Svensson J, Bjornstahl A, Ivarsson SA. Increased risk of Silver-Russell syndrome after in vitro fertilization? Correspondence section. *Acta Paediatr.* 2005;94:1163–5.
- Lidegaard O, Pinborg A, Andersen AN. Imprinting diseases and IVF: Danish National IVF cohort study. *Hum Reprod.* 2005;20:950–4.
- Halliday J, Oke K, Breheny S, Algar E, J. Amor D. Beckwith-Wiedemann syndrome and IVF: a case-control study. *Am J Hum Genet.* 2004;75:526–8.
- Källén B, Finnström O, Lindam A, Nilsson E, Nygren KG, Otterblad PO. Congenital malformations in infants born after in vitro fertilization in Sweden. *Birth Defects Res A Clin Mol Teratol.* 2010;88:137–43.
- Hiura H, Okae H, Chiba H, Miyauchi N, Sato F, Sato A, et al. Imprinting methylation errors in ART. *Reprod Med Biol.* 2014;13:193–202.
- Lazaraviciute G, Kauser M, Bhattacharya S, Haggarty P, Bhattacharya S. A systematic review and meta-analysis of DNA methylation and imprinting disorders in children conceived by IVF/ICSI compared with children conceived spontaneously. *Hum Reprod Update.* 2014;20:840–52.
- Seggers J, de Walle HE, Bergman JE, Groen H, Hadders-Algra M, Bos ME, et al. Congenital anomalies in offspring of subfertile couples: a registry-based study in the northern Netherlands. *Fertil Steril.* 2015;103:1001–10.
- Glenn CC, Porter KA, Jong MT, Nicholls RD, Driscoll DJ. Functional imprinting and epigenetic modification of the human SNRPN gene. *Hum Mol Genet.* 1993;12:2001–5.
- Kubota T, Das S, Christian SL, Baylin SB, Herman JG, Ledbetter DH. Methylation-specific PCR simplifies imprinting analysis. *Nat Genet.* 1997;16:16–7.
- Blik J, Maas SM, Ruijter JM, Hennekam RC, Alders M, Westerveld A et al. Increased tumour risk for BWS patients correlates with aberrant H19 and not KCNQ1OT1 methylation: occurrence of KCNQ1OT1 hypomethylation in familial cases of BWS. *Eur J Hum Genet.* 2008;16:328–34.
- Weksberg R, Shuman C, Caluseriu O, Smith AC, Fei YL, Nishikawa J, et al. Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. *Hum Mol Genet.* 2002;11:1317–25.
- Marchesi DE, Qiao J, Feng HL. Embryo manipulation and imprinting. *Semin Reprod Med.* 2012;30:323–34.
- Vermeiden JP, Bernardus RE. Are imprinting disorders more prevalent after human in vitro fertilization or intracytoplasmic sperm injection? *Fertil Steril.* 2013;99(3):642–51.