Increased prevalence of imprinting defects in patients with Angelman syndrome born to subfertile couples

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Recent case reports have suggested that infertility treatment with intracytoplasmic sperm injection (ICSI) may increase the risk of imprinting defects leading to Angelman syndrome (AS). Although imprinting defects account for only 4% of patients with AS, we have found four cases among 16 AS patients born to subfertile couples, who conceived with or without infertility treatment (25%; relative risk (RR) 6.25; 95% confidence interval (CI) 1.68 to 16.00). The risk in untreated couples with time to pregnancy (TTP) exceeding 2 years was identical to that of those treated by ICSI or by hormonal stimulation alone (RR 6.25; 95% CI 0.70 to 22.57). It was twice as high in couples who had received treatment and also had TTP >2 years (RR 12.5; 95% CI 1.40 to 45.13). Our findings suggest that imprinting defects and subfertility may have a common cause, and that superovulation rather than ICSI may further increase the risk of conceiving a child with an imprinting defect.

Intracytoplasmic sperm injection (ICSI) was introduced in the early 1990s to treat cases of severe male factor infertility.1 From the very beginning there has been concern about increased health risks in children conceived by ICSI. Large follow up studies have shown that the risk of major malformations, low birth weight, and pregnancy complications is increased after in vitro fertilisation, but independent of the use of ICSI.2–5

Recent case reports on three children who were conceived by ICSI and have Angelman syndrome (AS) as the result of an imprinting defect on chromosome 15 have suggested that artificial reproduction may increase the risk of imprinting defects.6–7 Similar observations have been made for children with Beckwith-Wiedemann syndrome.8–10 AS is a rare neurogenetic syndrome characterised by microcephalus, jerky movements, absence of speech, abnormal EEG pattern, severe mental retardation, and frequent laughing (incidence, 1 in 15 000 newborns). It is caused by the loss of function of the maternal UBE3A allele on chromosome 15. UBE3A is subject to genomic imprinting and, in brain, is expressed on the maternal chromosome only. Approximately 70% of patients have a deletion on the maternal chromosome, 10% have a mutation in the UBE3A gene, 4–7% have two paternal copies of chromosome 15 (uniparental disomy), and 3–4% have an imprinting defect (ID) that silences the maternal UBE3A allele. In the remaining cases, the patients have some other, hitherto unknown defect.11 The relative frequencies of the different genetic defects do not vary between different ethnic groups.

To investigate a possible correlation between infertility treatment and imprinting defects, we performed a cohort study in Germany using data from the German Angelman Syndrome Support Group.

METHODS
All members of the German Angelman Syndrome Support Group were contacted by a letter and asked to provide, on a voluntary basis, anamnestic data and also information on the method of conception and the time to pregnancy (TTP). The study was approved by the ethics review board of the Arztgemeinschaft Hamburg. Patients gave written informed consent. Parents who stated that TTP exceeded 2 years and/or who had undergone infertility treatment were asked to provide a blood sample (10 ml EDTA) or buccal smear from themselves and the child.

The methylation status of the SNURF-SNRPN gene was determined by bisulphite treatment of genomic DNA and methylation specific PCR.12 For segregation analysis of microsatellite loci along chromosome 15, fluorescence tagged PCR products were analysed on an ABI Prism 3100 Genetic Analyzer, using GeneScan and Genotyper software (ABI, Foster City, CA, USA). In accordance with established diagnostic criteria, patients carrying an unmethylated maternal SNURF-SNRPN allele were classified as having an ID. In all patients with an ID, a familial imprinting centre (IC) deletion was excluded by quantitative real time PCR analysis of the critical IC elements (Buiting and Horsthemke, unpublished).

RESULTS
Of 270 members, 82 (30%) replied. One child was adopted; two other parents refused to give detailed information. Thus, 79 valid questionnaires were returned. Sixteen children (20%) were born to subfertile couples (defined as having had a TTP >2 years and/or infertility treatment). This is a higher rate than that expected in the general population (~10–15%) and indicates a reporting bias. However, this bias

Abbreviations: AS, Angelman syndrome; ICSI, intracytoplasmic sperm injection; IC, imprinting centre; ID, imprinting defect; TTP, time to pregnancy
who had also undergone infertility treatment (n = 4). This maturation of “poor quality” oocytes that would not have undergone stimulation, which is also used for ICSI, may lead to the necessitated infertility treatment. Alternatively, hormonal suffering from a more severe form of subfertility that included ID. One possible explanation is that these couples are further ovulated without treatment, or that a too rapid maturation provoked by the hormonal stimulation procedure disturbs the process of DNA methylation in the oocyte. A lower oocyte quality following ovarian stimulation procedures has already been suggested from studies in the mouse.14

As our cohort of patients does not contain children conceived by conventional in vitro fertilisation without ICSI or intrauterine insemination, we do not know whether gamete and embryo culture or embryo manipulation increases the risk for an ID, as has been shown in animal studies.15

In summary, we have demonstrated for the first time that the prevalence of imprinting defects in patients with AS born to subfertile couples is significantly increased. Our data suggest that imprinting defects and subfertility may have a common cause, and that superovulation, rather than ICSI, may further increase the risk of conceiving a child with an imprinting defect. However, the absolute risk remains small.

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REFERENCES