Reviewer's report

Title: Absence of mutations in NR2E1 and SNX3 in MMEP (microcephaly, microphthalmia, ectrodactyly, and prognathism) and related phenotypes

Version: 1 Date: 7 March 2007

Reviewer: Uta Francke

Reviewer's report:

General
The authors looked for mutations in the gene NR2E1 in 5 patients with a syndrome comprised of microcephaly, microphthalmia, ectrodactyly and prognatism (abbreviated MMEP). Two of the patients had a translocation with a breakpoint in 6q21. In one patient, the translocation breakpoint had previously been mapped within the gene SNX3 (sorting nexin 3) leading to disruption of this locus. However, no SNX3 mutations had been identified in another case. Therefore, in the current manuscript, Kumar et al completed SNX3 genotyping in all other patients and excluded mutations in this gene as a cause of MMEP. Therefore, they hypothesize that mutations in NR2E1 could be responsible for MMEP because this gene could be abnormally expressed in the translocation case. In this manuscript, the authors report the absence of coding mutations in NR2E1.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The authors report that they have not found any "coding mutations" in NR2E1, presumably meaning mutations that change an amino acid. However, they should report all sequence changes that they observed. It is well known that synonymous nucleotide substitutions that do not change the amino acid code can cause disease by activating cryptic splice sites (e.g. Berg et al, Hum. Mut. 1:24-34 (1992) or by altering splice enhancer binding sites. The effect of such mutations can only be studied at the RNA level. With brain tissue not easily available from patients, transcript analysis can be done by introducing the mutation into in vitro splicing systems (Liu et al, Nature Genet. 16:328-329 (1997).

2. The authors do report a single sequence change in the NR2E1 3' UTR g.21502T>C that they call a "candidate regulatory mutation", even though the same nucleotide change was present in the unaffected mother. They report that the same sequence change was seen in the previous study (reference 9) in a patient with microcephaly, but not in a population control sample. But the previous study reports that the g.21502T>C change was also present in the unaffected father of the microcephaly patient, a fact that the authors omitted from the present manuscript. Therefore, it is most likely that g.21502T>C is an innocuous sequence variant and not a "regulatory mutation". Of note, the SNP database currently lists 62 sequence variants in NR2E1.

3. The hypothesis that NR2E1 could be involved in MMEP is weak because the gene is brain-specific, and the authors have previously excluded mutations in this gene in a large series of patients with microcephaly and other brain malformations. MMEP is distinguished from these cases by the presence of ectrodactyly, a malformation of the feet. In the absence of any evidence that NR2E1 is expressed during limb development, this is a poor candidate gene. The authors argue for NR2E1 as a candidate because it is the closest gene to SNX3, located about 22 kb downstream. However, on the other side of SNX3 there is another positional candidate, LACE1, located approximately 33 kb upstream of SNX3. This gene encodes a protein with possible ATPase function that is highly conserved in fly, yeast and bacteria. As LACE1 is more widely expressed in tissues compared to NR2E1, it would be a reasonable candidate to study in these five patients.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

4. The paragraph on transcription factor binding site analysis is redundant because it is part of the previous manuscript (ref. 9) in which this candidate g.21502T>C mutation was already included.

5. The translocation designated as "6q21;7q31.2" should be presented in the standard nomenclature which
is t(6;7)(q21;q31.2) in the text. In the reference section, the incorrect nomenclature needs to be left because this is what the authors of the original paper called it.

Discretionary Revisions (which the author can choose to ignore)
6. The authors could also consider sequencing other genes not located at 6q21, such as PAX3 known to be involved in cortical phenotypes. Their best bet, however, would be to focus on genes that are expressed during development of brain and limbs.

What next?: Reject because too small an advance to publish

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests