Antenatal Bartter syndrome with sensorineural deafness: refinement of the locus on chromosome 1p31


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Abstract


Methods. By haplotype analysis we demonstrate linkage to this locus in nine consanguineous families with antenatal Bartter syndrome associated with sensorineural deafness.

Results. The critical interval compatible with linkage was refined to 4.0 cM by two novel recombinational events with markers D1S2661 and D1S475.

Conclusion. We thereby confirmed this gene locus and distinguished this clinical subtype from other variants of Bartter syndrome as a new disease entity.

Keywords: antenatal Bartter syndrome; chromosome 1p31; deafness; haplotype analysis

Introduction

Bartter syndrome encompasses a variety of rare autosomal recessive renal tubular disorders, in which the electrolyte balance of the kidney is significantly altered due to defective solute transporters [1] (MIM #241200). The core manifestations are hypokalaemic metabolic alkalosis, elevated plasma renin, hyperaldosteronism with normal blood pressure, and increased levels of urinary chloride, sodium, and potassium [2]. So far, three distinct disease entities have been distinguished according to phenotypic characteristics. These are: (i) the antenatal variant [3–5] also termed hyperprostaglandin E syndrome; (ii) the less severe ‘classical variant’; and (iii) the Gitelman syndrome [6].

The antenatal variant shows, in addition to the above-described features, maternal polyhydramnios, premature delivery, severe polyuria, hypercalciuria, nephrocalcinosis, and elevated urinary excretion of prostaglandin E2. Patients fail to thrive and show developmental delay if not adequately treated by indomethacin, and fluid and salt supplementation. Some of the children show a typical facial appearance with prominent forehead, triangular faces, drooping mouth, and large eyes and pinnae. The antenatal variant is caused either by mutations in the Na-K-2Cl cotransporter gene (SLC12A1) [7,8] or by mutations in KCNJ1 encoding the inwardly rectifying K+ channel ROMK [9–12]. According to the two different genetic loci, these variants are also termed Bartter syndrome type 1 and 2 respectively. The classical variant is characterized by the core symptoms of Bartter syndrome, age of onset is only at early childhood, and nephrocalcinosis is usually not observed. Many of the patients with classic Bartter syndrome carry mutations in the renal chloride channel gene CLCNKB (Bartter syndrome type 3) [13,14]; however, mutations in this gene can also cause antenatal Bartter syndrome. In Gitelman syndrome, patients show hypomagnesaemia and hypocalciuria. Age of onset is in late childhood or adulthood. Typical manifestations are muscle weakness and episodes of tetany. Mutations in the Na-Cl cotransporter gene SLC12A3 are causative for the disease [15,16]. In addition to the above-described subtypes, Landau et al. [17] described an extended consanguineous Bedouin family in which an antenatal variant of Bartter was combined with sensorineural deafness. The patients of this pedigree showed electro-
lyte imbalances typical for Bartter syndrome. Deafness was diagnosed as early as 1 month of age. By applying a DNA pooling strategy and performing genome wide linkage screen, a gene locus for antenatal Bartter with deafness was mapped to human chromosome 1p31 [18].

Here we demonstrate linkage of nine consanguineous families with antenatal Bartter syndrome and deafness to human chromosome 1p31. Due to recombinations, we were able to further narrow down the critical genetic interval.

**Subjects and methods**

**Bartter kindreds**

Index cases included in this study originated from nine consanguineous families with a history of antenatal Bartter syndrome associated with deafness. Clinical details of families Marb7457, Marb7354, Marb7402, Marb7105, and Marb7246 are described elsewhere [19]. All included families were diagnosed either in paediatric nephrology departments or in antenatal intensive care units, showing polyhydramnios, neonatal salt wasting, and hypokalaemic alkalosis. These studies were approved by local ethical committees and informed consent was obtained from the patients’ parents.

**Haplotype analysis**

DNA from patients was isolated according to standard protocols from whole blood cells [20] or EBV-transformed lymphocytes [21]. Six microsatellites were taken from the Généthon human genetic linkage map spanning a total genetic distance of 4.8 cM on the sex-averaged map [22]. These markers were from telomere to centromere D1S2661, D1S417, D1S2652, D1S475, D1S200, and D1S2690. Screening was performed by radioactive PCR using 32P-labelled d-CTP in a Techne Genius Cycler in 96-well microtitre plates.

Amplification conditions were 94°C for 4 min, 1 cycle; 94°C for 30 s, 55°C for 30 s, 72°C for 60 s, 30 cycles and a final extension step at 72°C for 10 min. A 10 µl PCR reaction contained 30 ng of genomic template DNA, 1.5 mM MgCl2, 5 mM Tris–HCl (pH 9.0), 50 mM KCl, 0.1 µM of each primer, 200 µM of each dNTP, and 0.5 U Taq-polymerase. Amplified products were separated on 6% polyacrylamide gels run under denaturing conditions. The gels were blotted onto Whatman paper, dried, and analysed after autoradiography.

**Results**

In order to test whether there is genetic homogeneity in antenatal Bartter syndrome associated with deafness, nine consanguineous families with at least one affected child were investigated by haplotype analysis. Six markers that are known to localize close to the locus for antenatal Bartter syndrome and deafness on chromosome 1p31 according to the data from Brennan et al. [18], were chosen from the Généthon human genetic linkage map [22]. These markers span a genetic distance of 4.8 cM on the sex-averaged map.

Haplotypes were evaluated for potential homozygosity by descent, which would indicate linkage of antenatal Bartter with deafness with this genetic region [23]. Haplotypes of the families show that all families are homozygous at least for some of the markers tested (Figure 1). By identification of two new recombinants the critical interval compatible with linkage was refined in comparison to the data of Brennan et al. [18] (Figure 2). Marker D1S2661 is heterozygous in families Marb7457, Nij4549 and Ne13, defining this marker as a new flanking marker at the telomeric side of the locus for antenatal Bartter syndrome with deafness. This marker maps to the same position or 0.07 cM further centromeric than D1S1661, which was found to be the flanking marker on the telomeric side in the family described by Brennan et al. [18] according to the Marshfield genetic map (http://www.marshmed.org/genetics/) and the Location Database (http://cedar.genetics.soton.ac.uk/public_html/) respectively.

On the centromeric side, a recombinant was found in marker D1S475 in family Marb7246. This marker is located 0.8 cM further telomeric [22] compared to the published flanking marker D1S2690 for the Bedouin family [18]. In conclusion, we refined the critical interval compatible with linkage for the gene locus for antenatal Bartter syndrome and deafness to a sex-averaged genetic distance of less than 4.0 cM between markers D1S2661 and D1S475 according to the Généthon human genetic linkage map (Figure 2). We thereby confirmed antenatal Bartter syndrome with sensorineural deafness as a new disease entity.

**Discussion**

By haplotype analysis with consecutive microsatellite markers in nine consanguineous families with antenatal Bartter syndrome and deafness, we found linkage on chromosome 1p31 in all families as indicated by regions most probably homozygous by descent. Together with the Bedouin kindred published by Brennan et al. [18], a total of 10 families in which an antenatal variant of Bartter syndrome is associated with deafness are now shown to map to 1p31. Due to recombination events telomeric and centromeric from the disease locus, we were able to refine the interval compatible with linkage to a sex-averaged genetic distance of less than 4.0 cM. It is interesting to note that out of 25 families examined (data not shown) no family with antenatal Bartter syndrome without deafness maps to this locus. In addition, no locus for a non-syndromic or syndromic form of deafness has been assigned to this chromosomal region yet (http://dnalab-www.uia.ac.be/dnalab/hhh/). This suggests a single gene defect or at least two very closely linked genes.

Refining of the 1p31 locus indicates further genetic heterogeneity of Bartter syndrome, which can be now subdivided into four different subtypes characterized by genetic and physiological aspects. These are: (i) Bartter syndrome type 1 with a defective Na-K-2Cl
Fig. 1. Haplotype analysis in nine consanguineous families with antenatal Bartter syndrome associated with deafness. Male individuals are represented by squares, females by circles. Black symbols indicate affected persons. Microsatellite markers analysed are shown on the left side, the respective genotypes for markers are given below every individual. Haplotypes are represented by bars and were assembled to minimize recombinants. The black haplotype is assumed to be associated with the disease. Segments compatible with homozygosity by descent are encased in boxes.

cotransporter, mediating sodium and chloride reabsorption across the apical membrane of the thick ascending limb (TAL) of Henle’s loop; (ii) Bartter syndrome type 2 with mutations in the luminal ATP-regulated K⁺ channel ROMK, which is responsible for K⁺ recycling in this nephron segment; (iii) Bartter syndrome type 3 with defects in the basolateral chloride channel ClC-Kb; and (iv) antenatal Bartter syndrome
associated with sensorineural deafness and an as yet unknown gene defect.

So far, for all subtypes of Bartter and Gitelman syndrome, defective solute transporters have been identified as causative. In several syndromic and non-syndromic forms of deafness, mutations in genes encoding ion channels have shown to be responsible for these diseases. In Jervell and Lange Nielsen syndrome, the K⁺ channel KCNQ1 or the potassium channel subunit KCNE1 are mutated [24,25] and in non-syndromic autosomal dominant deafness DFNA2 KCNQ4 is involved [26]. In renal distal tubular acidosis with sensorineural deafness the β-subunit of the H⁺ ATPase is affected in a recessive fashion [26]. These findings, and the fact that in our study all families that are compatible with linkage on chromosome 1p31 exhibit both inner ear and kidney manifestations, strongly suggest that in antenatal Bartter syndrome with deafness a single gene encoding a solute transporter might be involved. For the NKCC cotransporters two isoforms have been described: NKCC2, the absorptive isoform, is expressed only in kidney while NKCC1, the secretory isoform, shows a wider expression pattern. The observation that high dosages of furosemide, which act on these cotransporters, also results in ototoxicity [28] further corroborates the assumption that either a homologue of the NKCC2 or an interplayer important in maintaining the balance of Cl⁻, K⁺ and Na⁺ across the membranes of both organs, stria vascularis, and medullary thick ascending limb of Henle’s loop in the kidney, will be responsible for antenatal Bartter syndrome with deafness. It was, in fact, recently shown by Delpire et al. [29] that a mouse model of targeted gene disruption for the secretory isoform NKCC1 exhibits deafness and imbalance but no kidney involvement. The synteny region for this gene in humans, however, is on chromosome 5q31-q33. No such candidate gene is known as yet to map to our critical interval, on which transcriptional information is very sparse. Further physical mapping and isolation of novel cDNAs will provide new positional candidates for the disease and mutational analysis will finally elucidate the disease-causing mechanisms.

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