BACKGROUND: The bladder exstrophy-epispadias complex represents a spectrum of urogenital anomalies in which part or all of the distal urinary tract fail to close and are exposed on the outer abdominal wall. Previous studies are suggestive of an underlying multifactorial mode of inheritance. However, no genetic or nongenetic factor has been identified so far. In this study, we sought risk loci by parametric and nonparametric linkage analysis, searching for homozygous segments, and more complex inherited loci, respectively.

METHODS: Two pedigrees, Spanish and German, each comprising two members affected with classical bladder exstrophy, were analyzed by genome-wide linkage scan.

RESULTS: Evidence for possible risk/modifying loci on chromosomes 2p22.1–p21, 2p25.2–p25.1, 4q23–q32.3, 7q21.3–q33, 7q34–q36.1, 14q31.1–q32.2, and 19q13.33–q13.43 (LOD scores >1.50) was obtained.

CONCLUSIONS: This study was the first positional approach to identify chromosomal candidate regions causally related to bladder exstrophy-epispadias complex. Our results suggest the presence of susceptibility genes in the regions identified. These regions need to be confirmed in future studies.


Key words: bladder exstrophy-epispadias complex; linkage analysis; SNP markers; genome-wide linkage scan; exstrophy; epispadias
to underlie the BEEC (Boyadjiev et al., 2004). This has been supported by an estimated recurrence risk among Caucasian siblings of 0.5–2%, a 400-fold ([s 400) increase compared to the general population (Shapiro et al., 1984; Reutter et al., 2003). Furthermore, pairwise concordance rates among monozygotic and dizygotic BEEC twin pairs of 45% (95% CI: 29–62) for monozygotic and 6% (95% CI: 0.1–27) for dizygotic twins are suggestive of genetic influence (Reutter et al., 2007). Familial occurrence is rare, with only 23 families being reported to date (Keppler-Noreuil, 2001; Boyadjiev et al., 2004; Kajbafzadeh et al., 2006). All of these families, except two, have two affected members. In these two families described, there are three affected members of both genders, which, however, show variable defects (Keppler-Noreuil, 2001; Boyadjiev et al., 2004). Most data on these familial cases suggest autosomal-dominant inheritance with reduced penetrance (Reutter et al., 2003). To our knowledge, the present study represents the first approach to identify susceptibility genes involved in the etiology of the BEEC by using a genome-wide linkage scan.

**MATERIALS AND METHODS**

**Subjects**

Blood samples were obtained from all available family members with their informed consent and the study was approved by the Ethics Committee of the University of Bonn. All family members were recruited by H. R., and because patients had undergone most of their surgical reconstructions, no photographs were taken. However, medical charts of all affected were carefully studied and the physical examination that had been performed gave a clear picture of the clinical situation.

The first family (no. 33) is a five-generation family from Germany with nine subjects, including two CBE patients (male and female) who are third-degree cousins. This family has been described before by Reutter et al. (2003). The second family (no. 83), which has not been described previously, is a consanguineous, in regards to the LOD-calculation applied, five-generation family from Spain with two CBE males, where 10 individuals could be tested. In both these families probands underwent primary bladder closure and bladder neck reconstruction.

**Genotyping**

Blood DNA was purified and the whole-genome genotyping scan was performed as described previously (Timmann et al., 2007). In brief, a high density SNP genome scan was performed using a whole-genome sampling analysis approach (Kennedy et al., 2003) with the Affymetrix GeneChip Human Mapping 10K v2 Array comprising 10,032 SNP markers with an average heterozygosity in Caucasians of 38% and a mean intermarker distance of 258 kb/0.36 cm. Mapping order and genetic distances of markers were obtained from Affymetrix (Affymetrix, NetAffx Annotation files, http://www. affymetrix.com).

**Statistical Analysis and Quality Control**

Parametric and nonparametric linkage analysis was performed with Merlin (Abecasis et al., 2002). Deceased relatives were entered as unknown, whereas alive members where no DNA was available (not tested) were entered as not affected. For a dominant model we used trait locus mutant allele frequency of 0.0001 and incomplete penetrances (0.00, 0.80, 0.80) for homozygous wild-type, heterozygous, and homozygote mutant genotypes, respectively. The recessive model was calculated with a trait locus mutant allele frequency of 0.001 and complete penetrances (0.00, 0.00, 1.00). Allele frequencies from a Caucasian population were used (NetAffx SNPannotation file).

The graphical user interface ALOHOMORA was used for Data Management and Quality Control (Rüschendorf and Nürnberg, 2005). Gender of individuals was estimated by counting heterozygote genotypes of X-linked markers for each sample, and compared to the pedigree definition. The correct pedigree structure was checked by the program Graphical Relationship Representation (Abecasis et al., 2001). All SNPs were analyzed and Mendelian errors were revealed by PedCheck (O’Connell and Weeks, 1998) and deleted in the family where they appeared. Non-Mendelian errors or unlikely genotypes were identified by Merlin (Abecasis et al., 2002) and deleted in the individual where they appeared. Given this and to avoid inflated LOD scores a total of 252 markers were excluded from the analysis. To reduce linkage disequilibrium, we also calculated with a depleted set of SNPs: ALOHOMORA allows presetting of a minimum spacing (here: 10,000 nucleotides in the case of maximum heterogeneity LOD [HILOD] calculation) between the markers that resulted in a dataset comprising 6,989 SNPs.

**RESULTS**

At least three regions yielded suggestive positive results in both families investigated (Table 1). In family 33 (Fig. 1A), two maximum LODs of 1.916 in regions 2p22.1–p21 and 14q31.1–q32.2 were found under a dominant model (Fig. 1B,D). These regions were confirmed under a recessive model with full penetrance (LODs 1.906). An additional lower double-peak on chromosome 7 (corresponding to 7q21.3-q33 and 7q34–q36.1) was observed under the dominant model, but only the first peak was also found under the recessive model (Fig. 1C). In family 83, calculations yielded three LOD score peaks of 1.45 in the dominant model in regions 2p25.2–p25, 4q23–q32.3, and 19q13.33–q13.43 (Fig. 1B). Here, the recessive model yielded LODs of 1.504 for the same regions.

Although there was no apparent overlap in the regions identified in these families a joint analysis was performed. As expected, this analysis gave no significant results (data not shown).

**DISCUSSION**

Our genome-wide scan for BEEC yielded seven loci with a parametric LOD score close to or above 1.5. Unfortunately, as recently summarized (Ludwig et al., 2005), none of the structural aberrations identified in BEEC patients so far affects one of the susceptibility loci determined here. Aside from seven BEEC cases with numerical chromosomal anomalies (47,XXX [observed twice]; 47,XY; 47,XY; 47,XX, +21; 47,[no sex reported], +18; 45,XO/46XX [mosaic]), structural aberrations...
were found in two patients with isolated epispadias (46,XY,dup[9p]; 46,Xydel[4]p–q21), in two CBE cases (46,XY,t[8;9]p11.2;q13; 46,XY,t[2;9]q13;q32), in two CE patients (46,X,der[Y]t[5;9]q11.23;q34.1;del[5][q11.2][q13.3]; Kosaki et al., 2005), and in one CE patient with Hypomelanosis Ito (diploid/tetraploid/t[1;6] mosaicism), respectively. On the other hand, possible candidate genes are located within the regions identified in the present study, which might contribute to the development of BEEC.

Table 1

<table>
<thead>
<tr>
<th>Flanking SNP</th>
<th>Markers</th>
<th>Position Cytogenetic</th>
<th>Physical (bp)*</th>
<th>Parametric LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 33</td>
<td>rs1368113</td>
<td>rs1368086</td>
<td>2p22.1–p21</td>
<td>38,585,994 – 43,876,658</td>
</tr>
<tr>
<td></td>
<td>rs1375671</td>
<td>rs1378647</td>
<td>7q21.3–q33</td>
<td>96,166,612 – 136,090,438</td>
</tr>
<tr>
<td></td>
<td>rs1986764</td>
<td>rs722933</td>
<td>7q34–q36.1</td>
<td>141,872,311 – 147,601,309</td>
</tr>
<tr>
<td></td>
<td>rs54566</td>
<td>rs1951094</td>
<td>14q31.1–q32.2</td>
<td>79,705,600 – 98,140,120</td>
</tr>
<tr>
<td>Family 83</td>
<td>rs771166</td>
<td>rs953071</td>
<td>2p25.2–p25.1</td>
<td>6,911,295 – 11,957,213</td>
</tr>
<tr>
<td></td>
<td>rs1540053</td>
<td>rs4129776</td>
<td>4q23–q32.3</td>
<td>100,439,332 – 165,639,920</td>
</tr>
<tr>
<td></td>
<td>rs3810261</td>
<td>rs1368467</td>
<td>19q13.3–q13.43</td>
<td>54,914,726 – 62,128,208</td>
</tr>
</tbody>
</table>

*Derived from the NCBI (Build 35.1).

1LOD = 1.916 for regions on chromosome 2 and 14 in a dominant model with complete penetrance and LOD = 1.906 for regions on chromosomes 2, 7, and 14 in a recessive model with complete penetrance.

2LOD = 1.45 in a dominant model with 80% penetrance.

Figure 1. (A) Pedigree of family 33 (Germany) with classical bladder exstrophy. Affected individuals are shown with blackened symbols, and samples included in the analysis as unaffected but where no DNA was available are indicated by nt (not tested). (B–D) Maximum LOD score values obtained from Merlin parametric analysis predicted under a dominant (red lining) or recessive model (blue lining) for regions on chromosomes 2, 7, and 14.

and/or its severity. These include, amongst others, PLEKHH2 on 2p21, which is involved in membrane and juxtamembrane targeting (DiNitto and Lambright, 2006). Also, the region of interest on chromosome 19 harbors the serine protease gene cluster with 13 KLK (Kalikrein-related peptidases: KLK) genes. KLKs are involved in the regulation of cell differentiation and tissue regeneration and most of these genes show a tissue-specific expression profile (e.g., skin or prostate) (Gan et al., 2000).

From the pedigree structures an autosomal dominant, intermediate, or recessive trait involving each of the risk/modifying loci identified might explain the disease, whereas an X-linked mode seems to be more unlikely, at least in the German family, where both sexes are affected. Alternatively, depending on the type of mutation, a given mutation in the same gene may be inherited in a recessive or dominant fashion influenced by mutations in (an)other gene(s) (Badano and Katsanis, 2002). Thus, according to a non-Mendelian model, patients may carry zero, one, or two mutated alleles at one locus, which, together with other risk/modifier alleles, would influence formation and strength of the BEEC.

Finally, it might be possible—though not very likely—that our data obtained are just chance findings. Because this study, to our knowledge, represents the first genome-wide scan for BEEC, further studies analyzing independent families need to be performed to replicate these data and narrow down the suggested regions.

ACKNOWLEDGMENTS

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REFERENCES


