

# The hyper-IgE syndrome is not caused by a microdeletion syndrome

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**Abstract** The hyper-immunoglobulin E syndrome (HIES) is a rare primary immunodeficiency characterized by recurrent infections, elevated serum IgE-levels, and involvement of the soft- and bony tissues. We speculated that this complex disease may be caused by a microdeletion syndrome. We therefore analyzed 30 sporadic HIES

patients for the presence of chromosomal imbalances using Affymetrix 50k *Xba*I and 23 of the 30 patients with the higher-resolution 250k *Sty*I SNP mapping arrays. We detected only eight different copy number alterations in six patients with the 50k approach, and seven of these presented known polymorphic regions not associated with

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Dietmar Pfeifer and Cristina Woellner contributed equally to the work and are considered aequo loco.

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disease. However, one patient showed a unique gain on chromosome 20p. 250k array analysis identified this gain as a rare polymorphism segregating in the patient's family, but not associated with the HIES phenotype. In addition, 265 known and novel copy number variants (CNVs) were identified with the 250k arrays, but no recurrent imbalances reminiscent of a microdeletion syndrome were found. We aligned the identified CNVs with loci that have been associated with HIES or phenotypically overlapping syndromes. Doing so, a 2-Mb deletion spanning the *PEPD* gene on 19q13.11 was identified on one allele of one patient. Homozygous mutations in *PEPD* are responsible for the autosomal-recessive prolidase deficiency which resembles HIES in some aspects. Sequencing of the healthy allele, however, revealed a wild-type sequence. In summary, our results suggest that HIES is not likely to be a microdeletion syndrome.

**Keywords** Hyper-IgE syndrome · Microdeletion syndrome · Primary Immunodeficiency · SNP-chip analysis · *PEPD* · prolidase D

## Introduction

The hyper-immunoglobulin E syndrome (HIES) [also called Job's syndrome; (OMIM) nos. 147060 and 243700] is a rare primary immunodeficiency (prevalence <1:1 million), characterized by the clinical triad of recurrent staphylococcal skin abscesses, recurrent pneumonia with pneumatocele formation, and serum IgE levels of >2,000 IU/ml. HIES usually manifests in childhood and has a highly variable expressivity (Grimbacher et al. 2005).

Both autosomal dominant (AD) and autosomal recessive (AR) inheritance have been described; however, most cases are sporadic. The AD form of HIES and most sporadic cases have extra-immune manifestations, defining HIES as a multisystem disease. Skeletal abnormalities such as hyperextensibility of the joints, scoliosis, and multiple bone fractures can be variably seen in patients with HIES (Grimbacher et al. 1999b). In addition, many patients show a typical facial appearance and have retained primary teeth. Importantly, the clinical phenotype of individual patients is highly variable.

In contrast to this syndromic character of AD HIES, the AR variant is characterized by recurrent viral infections, extreme eosinophilia, and neurological complications, whereas skeletal and dental abnormalities are absent (Renner et al. 2004). Minegishi et al. (2006) recently reported the first monogenetic defect in a single patient from consanguineous parents with clinical features of AR HIES. The patient had a homozygous defect in the receptor-associated cytoplasmatic tyrosine kinase *TYK2*, indicating

**Fig. 1** **a** Copy number profiles from chromosome 20 (*left panel*) and chromosome 19 (*right panel*) of patient no. 30 and his first-degree relatives. Each panel consists of eight subpanels (*top down*: Father, mother, patient, and five healthy siblings) with the plots of the hidden Markov model-derived copy number states. The numbering on the left gives the copy number states (1 Deletion, 2 normal diploid copy number, 3 gain). The x-axis shows the physical position (*numbering from left (p-arm) to right (q-arm)*) and an ideogram of the respective chromosome. **b** Physical map of the 19q13 deletion in patient no. 30. Copy number states from the CNAT test analysis module were loaded into the UCSC Genome browser (<http://genome.ucsc.edu/>) and displayed in the context of genes and known structural variations. The section shown corresponds to the predicted size of the deletion as analyzed by GEMCA (see Table 1). Note the difference in predicted deletion size of GEMCA (outer boundaries) and the CNAT program (drop in copy number state from 2 to 1) as well as the overlap with a known structural variation

that some AR HIES cases may be due to a disorder of the T lymphocyte/IL-12/interferon axis. Even more recently, the same group has identified mutations in *STAT3* in 8 of 18 patients with HIES (Minegishi et al. 2007). Our research consortium on HIES could confirm this finding (Holland et al. 2007).

Previously, a sporadic HIES patient with an AD phenotype presenting an additional mental retardation was found to carry an interstitial deletion of 15–20 cM on chromosome 4q (Grimbacher et al. 1999a). Linkage analysis of 19 AD HIES families demonstrated linkage to markers on chromosome 4. However, six of these 19 families did not demonstrate linkage to this region, suggesting genetic heterogeneity (Grimbacher et al. 1999c).

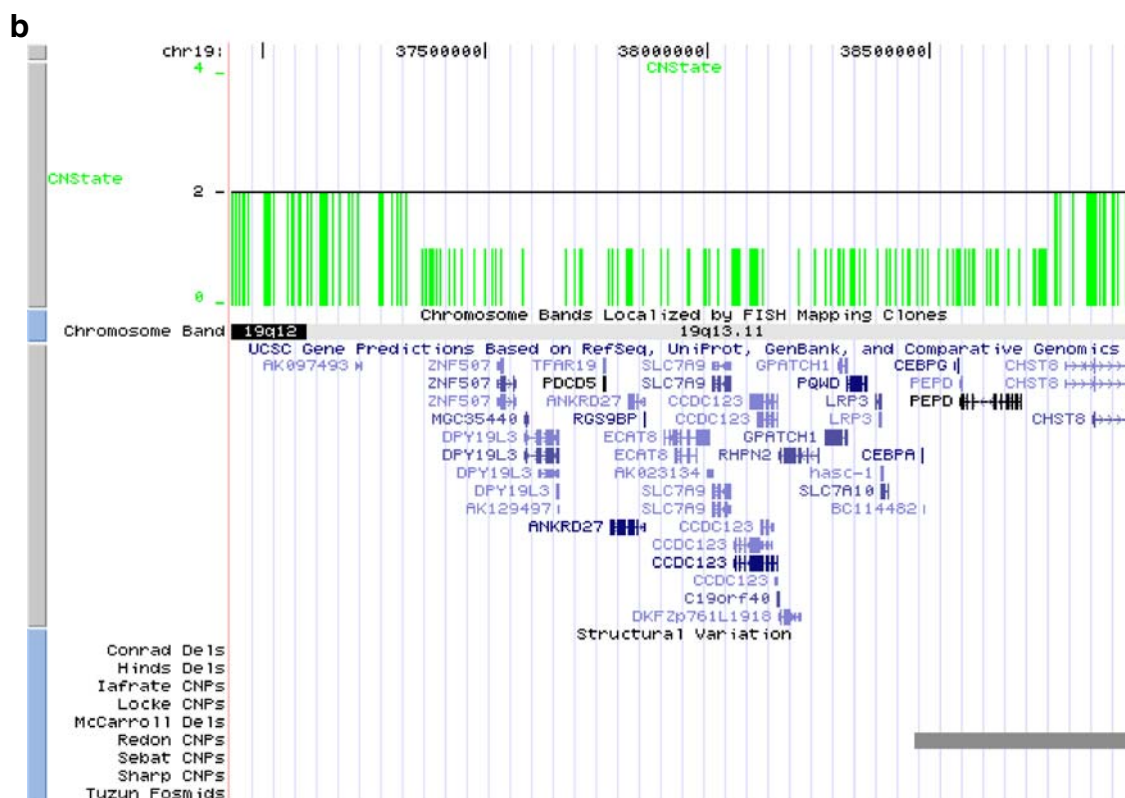
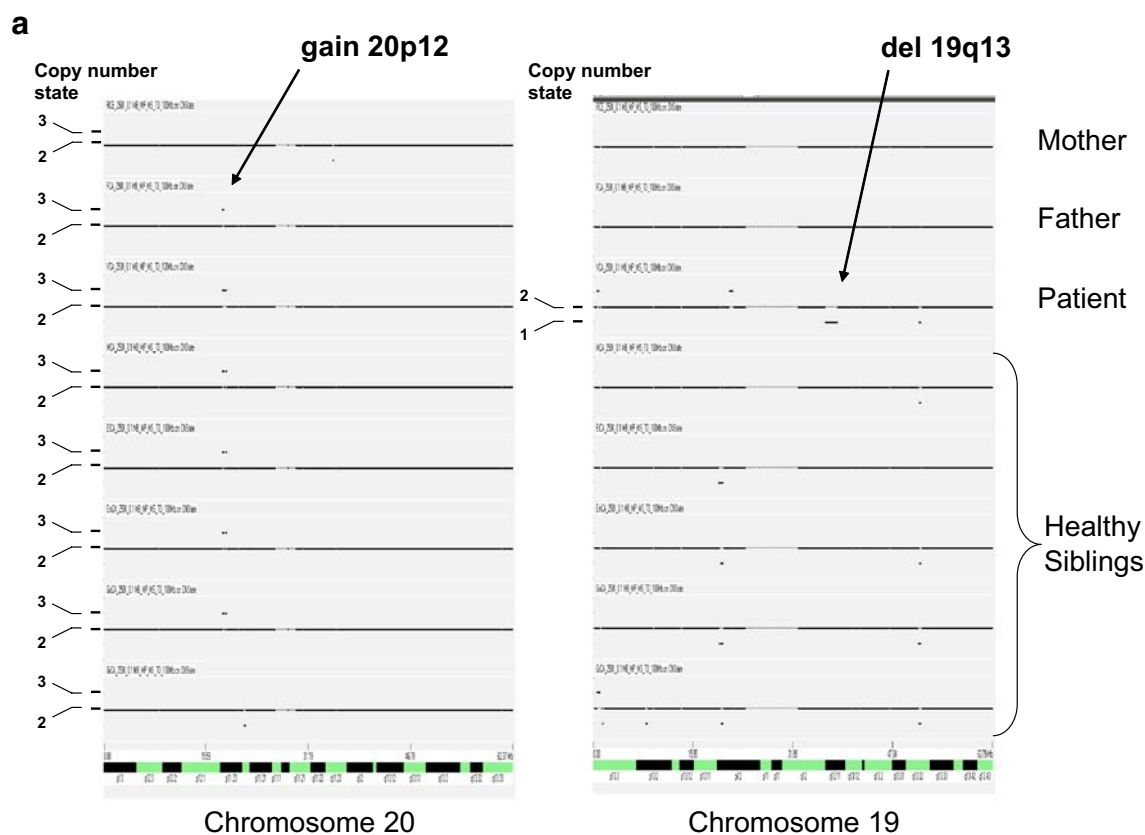
Here, we hypothesized that HIES might be associated with a genetic disorder such as a contiguous gene deletion or an amplification of a small chromosomal region. Several immunodeficiency syndromes and the 22q11.2 deletion syndrome or the Williams syndrome were shown to be caused by microdeletions. To investigate, whether sporadic HIES also might be due to genetic alterations such as contiguous gene deletion, we analyzed a group of 30 HIES patients for the presence of chromosomal gains and losses on a genome-wide level by using a single nucleotide polymorphism (SNP) chip approach.

## Patients and methods

### DNA samples

Thirty sporadic HIES patients were diagnosed according to published criteria (Grimbacher et al. 1999c). Seventeen of these patients originate from Europe, eight from Columbia and five from Iran. Twenty-five showed HIES scores [6] above 40 and five patients had scores between 20 and 40, indicating that these patients may have a variant of HIES.

The genomic DNA of the patients was extracted from peripheral blood leukocytes using standard protocols.



## SNP mapping

SNP mapping was performed using Affymetrix 50k *Xba*I and the 250k *Sly*I SNP mapping arrays containing probes for the detection of around  $5 \times 10^4$  and  $2.38 \times 10^5$  single nucleotide polymorphisms (SNPs), respectively. Their genome-wide distribution allows for a large-scale genotype and copy number analysis (Bignell et al. 2004; Huang et al. 2004). The median Inter-SNP-distance is 63 kb for the 50k *Xba*I and 5 kb for the 250k *Sly*I SNP array. The two arrays have only 6,617 SNPs in common. The SNP mapping assays were performed according to the protocol of the manufacturer (Affymetrix; Santa Clara, CA). The mean genotype call rates were 93.53% for the 50k array and 94.77% for the 250k arrays.

## Copy number analysis

Copy number analysis was done by using the Affymetrix copy number analysis Tool (CNAT 4.1.0.26, Batch analysis module 1.0.03) for 50k arrays and the GEMCA software (genotyping microarray-based copy number variant (CNV) analysis) for the 250k arrays (Komura et al. 2006; Zhao et al. 2004). The CNAT batch analysis module derives copy numbers by comparing the SNP intensities of the sample arrays against the intensity distribution of a reference set of more than 100 individuals of different ethnicity. Copy number profiles were generated in CNAT using a genomic smoothing lengths of 0.5 and 0.05 Mb. Copy number profiles were then searched for regions with smoothed copy numbers smaller than 1.3 for putative deletions and larger than 2.9 for putative gains and region with more than eight consecutive SNPs scored (Pfeifer et al. 2007).

250k *Sly*I arrays were analyzed using GEMCA software with the reference set copy number variation (CNV) analysis option. CNV calling parameters were used as described in Komura et al. (2006). Briefly, signal intensity ratio thresholds from at least four SNPs on three restriction fragments were 1.12 and 0.89 for insertions and deletions, respectively. Significance was met when a *p* value threshold <0.01 after 5,000 permutations was reached. Only CNVs that were called in at least 10% of all comparisons were scored. Male and female samples were analyzed separately in a pairwise fashion against a reference set consisting of 30 male and 20 female samples of different ethnicity (subset of the HapMap collection).

For the purpose of visualization (see Fig. 1a), 250k array data of selected patients were reanalyzed with the CNAT test analysis module 4.0.1.3 against the same reference samples used with the GEMCA software. We used the default values for the hidden Markov model (HMM) parameters and a genomic smoothing value of 0.1 Mb. The transition decay was set to 10 Mb, and the array normalization option was set to median scaling.

## Results

In a first set of experiments, we analyzed the samples of 30 sporadic HIES patients with 50k SNP arrays for the presence of overlapping gains or losses. In a subsequent analysis, 23 of these samples were reanalyzed with 250k arrays to improve the resolution of the analysis.

We detected eight different copy number alterations in six of our 30 sporadic patients with a size range from 70 to 340 kb (three gains and five deletions) using the lower resolution 50k arrays, and all eight were confirmed using the 250k system. Seven of these eight genetic alterations overlapped with one or more copy number polymorphisms that have been found also in normal individuals [database of genomic variants (DGV), Genome Build 35, <http://projects.tcag.ca/variation/>] (Iafrate et al. 2004) and thus most likely are not associated with disease. However, patient no. 30 showed a 340-kb gain of the chromosomal region 20p12 (Physical Position 18,287,493–18,623,480) that matched no entry in the database. In addition to the lack of this CNV in the 270 HapMap individuals (International HapMap Consortium 2005), we did not detect this gain in a group of 130 chronic lymphocytic leukemia patients of predominantly Caucasian origin (Pfeifer et al. 2007). The patient presented with the typical phenotype of AD HIES suffering from recurrent pneumonia with formation of pneumatocoles, several skin abscesses, and severe eczema. Reanalysis of this patient and his healthy first degree relatives with the 250k SNP chip (see below) identified the described gain as a private rare genetic copy number polymorphism that segregates within the family according to Mendel (data not shown). Thus, there was no association of this gain with the disease phenotype (Fig. 1a).

To rule out the possibility that HIES is caused by sub-megabase deletions that have been missed with the 50k system, we set out to repeat the analysis with 250k *Sly*I arrays. Due to the limited availability of sample DNA, only 23 of the 30 HIES patients were reanalyzed with 250k *Sly*I arrays using improved algorithms for CNV detection (Komura et al. 2006). These algorithms have been recently established to comprehensively analyze CNVs in 270 HapMap samples (Redon et al. 2006). In total, 265 CNVs (mean 11.5 per patient) with a size distribution from 0.63 up to 2031 kb (mean 388 kb) were identified in our 23 HIES patients by pairwise comparisons to a sex-matched set of reference samples (Table 1). Of these, 186 had a match to corresponding entries in DGV or were found in other non-HIES individuals. CNVs that were present in the samples with a frequency higher than 50% were found on chromosomes 10, 14, 15, 17, and X (Table 1). All these overlapped with already known polymorphic regions. Eleven CNVs had matches to entries that were considerably

**Table 1** Results of the copy number analysis from 23 HIES patients using GEMCA software and 250k *Sty* I SNP mapping arrays

| Sample ID | Sex    | Chromosome | Start(10)   | End(10)     | Size (kb) | Number of probes | Overlap in DGV <sup>a</sup> | Copy number <sup>b</sup> | Max density <sup>c</sup> | Number of reference samples |
|-----------|--------|------------|-------------|-------------|-----------|------------------|-----------------------------|--------------------------|--------------------------|-----------------------------|
| 11        | Male   | 1          | 2,805,750   | 3,535,498   | 729.748   | 71               | Spans smaller CNV           | 3                        | 0.366667                 | 30                          |
| 18        | Female | 1          | 3,767,418   | 3,983,437   | 216.019   | 11               | Spans tiny CNV              | 3.5                      | 0.21                     | 19                          |
| 2         | Female | 1          | 9,632,528   | 10,322,054  | 689.526   | 23               | Spans smaller CNV           | 1.5                      | 0.3                      | 20                          |
| 29        | male   | 1          | 14,253,855  | 14,393,561  | 139.706   | 12               | None                        | 1.5                      | 0.133333                 | 30                          |
| 8         | male   | 1          | 30,408,227  | 30,408,889  | 0.662     | 4                | Full                        | 1                        | 0.111111                 | 27                          |
| 2         | Female | 1          | 31,046,836  | 31,405,216  | 358.38    | 27               | Spans smaller CNV           | 1.5                      | 0.5                      | 20                          |
| 3         | Female | 1          | 33,801,947  | 33,889,474  | 87.5271   | 5                | None                        | 1.5                      | 0.15                     | 20                          |
| 8         | Male   | 1          | 87,423,641  | 87,424,277  | 0.636     | 4                | None                        | 3                        | 0.105263                 | 19                          |
| 13        | Female | 1          | 107,192,934 | 107,283,286 | 90.352    | 5                | Full                        | 0                        | 1.000                    | 20                          |
| 27        | Female | 1          | 141,484,300 | 142,855,739 | 1371.439  | 16               | Full                        | 3                        | 0.222                    | 18                          |
| 8         | Male   | 1          | 149,853,238 | 149,996,052 | 142.814   | 15               | None                        | 3                        | 0.666667                 | 30                          |
| 12        | Male   | 1          | 176,479,881 | 176,642,563 | 162.682   | 20               | Present in other sample     | 1.5                      | 0.233333                 | 30                          |
| 5         | Male   | 1          | 178,828,683 | 178,913,744 | 85.0611   | 6                | None                        | 1.5                      | 0.266667                 | 30                          |
| 7         | Female | 1          | 205,634,254 | 205,675,762 | 41.508    | 8                | None                        | 1.5                      | 0.15                     | 20                          |
| 2         | Female | 1          | 212,623,469 | 213,046,243 | 422.774   | 21               | None                        | 3                        | 0.35                     | 20                          |
| 29        | Male   | 2          | 165,407     | 439,787     | 274.38    | 28               | Full                        | 3                        | 0.166                    | 30                          |
| 3         | Female | 2          | 2,692,559   | 2,797,642   | 105.083   | 20               | None                        | 2.5                      | 0.15                     | 20                          |
| 3         | Female | 2          | 16,010,957  | 16,325,178  | 314.221   | 17               | None                        | 2.5                      | 0.15                     | 20                          |
| 3         | Female | 2          | 18,682,418  | 18,821,304  | 138.886   | 9                | None                        | 1                        | 0.7                      | 20                          |
| 25        | Female | 2          | 24,825,776  | 24,870,800  | 45.024    | 7                | None                        | 0                        | 1.000                    | 20                          |
| 25        | Female | 2          | 35,741,985  | 36,021,585  | 279.6     | 23               | Full                        | 1.5                      | 1.000                    | 20                          |
| 2         | Female | 2          | 42,272,482  | 42,812,642  | 540.16    | 22               | Present in other sample     | 1.5                      | 0.25                     | 20                          |
| 25        | Female | 2          | 74,757,796  | 74,923,982  | 166.186   | 19               | None                        | 1.5                      | 0.25                     | 20                          |
| 25        | Female | 2          | 84,945,704  | 85,228,543  | 282.839   | 17               | None                        | 1.5                      | 0.35                     | 20                          |
| 26        | Female | 2          | 109,487,816 | 111,163,677 | 1675.861  | 18               | Spans smaller CNV           | 2.5                      | 0.45                     | 20                          |
| 27        | Female | 2          | 109,500,812 | 111,168,047 | 1667.235  | 16               | Spans smaller CNV           | 3                        | 0.3                      | 20                          |
| 27        | Female | 2          | 117,447,367 | 117,638,535 | 191.168   | 10               | Spans smaller CNV           | 3                        | 0.4                      | 20                          |
| 13        | Female | 2          | 140,062,850 | 141,595,697 | 1532.847  | 66               | None                        | 3                        | 1.000                    | 20                          |
| 29        | Male   | 2          | 154,037,659 | 154,550,307 | 512.648   | 26               | Spans smaller CNV           | 2.5                      | 0.133333                 | 30                          |
| 20        | Male   | 2          | 210,507,750 | 210,724,538 | 216.788   | 11               | Present in other sample     | 0                        | 1.000                    | 30                          |
| 21        | Female | 2          | 210,507,750 | 210,724,538 | 216.788   | 9                | Present in other sample     | 0                        | 1.000                    | 20                          |
| 18        | Female | 2          | 210,540,680 | 210,724,538 | 183.858   | 8                | Present in other sample     | 0                        | 1.000                    | 20                          |
| 18        | Female | 2          | 218,088,246 | 218,224,540 | 136.294   | 32               | None                        | 2.5                      | 0.15                     | 20                          |
| 2         | Female | 3          | 1,394,395   | 1,490,394   | 95.999    | 18               | Full                        | 2.5                      | 0.2                      | 20                          |
| 8         | Male   | 3          | 2,599,938   | 2,672,187   | 72.249    | 9                | Full                        | 3                        | 0.266667                 | 30                          |
| 25        | Female | 3          | 4,111,544   | 4,148,839   | 37.295    | 8                | Full                        | 1                        | 0.15                     | 20                          |
| 24        | Female | 3          | 64,186,645  | 65,052,516  | 865.871   | 97               | Spans smaller CNV           | 2.5                      | 0.2                      | 20                          |
| 20        | Male   | 3          | 78,960,199  | 80,209,195  | 1248.996  | 41               | Spans smaller CNV           | 2.5                      | 0.133333                 | 30                          |
| 18        | Female | 3          | 148,711,343 | 149,025,453 | 314.11    | 16               | None                        | 3.5                      | 0.65                     | 20                          |
| 27        | Female | 4          | 5,814,455   | 5,872,616   | 58.161    | 16               | None                        | 2.5                      | 0.15                     | 20                          |

**Table 1** (continued)

| Sample ID | Sex    | Chromosome | Start(10)   | End(10)     | Size (kb) | Number of probes | Overlap in DGV <sup>a</sup> | Copy number <sup>b</sup> | Max density <sup>c</sup> | Number of reference samples |
|-----------|--------|------------|-------------|-------------|-----------|------------------|-----------------------------|--------------------------|--------------------------|-----------------------------|
| 22        | Female | 4          | 5,814,455   | 5,867,334   | 52.879    | 15               | None                        | 2.5                      | 0.15                     | 20                          |
| 25        | Female | 4          | 5,814,455   | 5,872,616   | 58.161    | 16               | None                        | 3                        | 0.15                     | 20                          |
| 2         | Female | 4          | 9,780,939   | 9,868,377   | 87.438    | 44               | Full                        | 2.5                      | 0.4                      | 20                          |
| 3         | Female | 4          | 136,638,092 | 137,360,314 | 722.222   | 25               | None                        | 2.5                      | 0.15                     | 20                          |
| 9         | Male   | 4          | 144,981,932 | 145,350,927 | 368.995   | 21               | Full                        | 1.5                      | 0.241379                 | 29                          |
| 8         | Male   | 4          | 156,264,761 | 156,280,635 | 15.874    | 5                | None                        | 1                        | 0.166667                 | 30                          |
| 24        | Female | 4          | 180,926,303 | 181,681,796 | 755.493   | 50               | Spans smaller CNV           | 2.5                      | 0.15                     | 20                          |
| 2         | Female | 4          | 182,774,844 | 183,128,673 | 353.829   | 41               | Present in other sample     | 3                        | 0.35                     | 20                          |
| 12        | Male   | 4          | 182,865,323 | 183,128,376 | 263.053   | 33               | Present in other sample     | 2.5                      | 0.56                     | 25                          |
| 22        | Female | 4          | 182,865,323 | 183,177,854 | 312.531   | 37               | Present in other sample     | 2.5                      | 0.15                     | 20                          |
| 12        | Male   | 5          | 672,333     | 875,123     | 202.79    | 11               | Full                        | 1.5                      | 0.166667                 | 18                          |
| 8         | Male   | 5          | 752,190     | 916,797     | 164.607   | 8                | Full                        | 1.5                      | 0.111111                 | 18                          |
| 26        | Female | 5          | 55,547,151  | 55,601,032  | 53.881    | 13               | None                        | 1                        | 0.95                     | 20                          |
| 8         | Male   | 5          | 177,806,609 | 177,897,050 | 90.441    | 14               | None                        | 3                        | 0.533333                 | 30                          |
| 5         | Male   | 6          | 166,146     | 329,546     | 163.4     | 10               | Full                        | 1.5                      | 0.111111                 | 18                          |
| 12        | Male   | 6          | 214,735     | 332,659     | 117.924   | 10               | Full                        | 1.5                      | 0.111111                 | 18                          |
| 3         | Female | 6          | 29,984,938  | 30,006,876  | 21.938    | 4                | Full                        | 0                        | 1.000                    | 14                          |
| 2         | Female | 6          | 30,559,883  | 30,895,154  | 335.271   | 44               | Spans smaller CNV           | 1.5                      | 0.5                      | 20                          |
| 7         | Female | 6          | 31,222,314  | 31,284,581  | 62.267    | 13               | Full                        | 3                        | 0.15                     | 20                          |
| 26        | Female | 6          | 32,325,070  | 32,889,532  | 564.462   | 87               | Present in other sample     | 1.5                      | 0.15                     | 20                          |
| 22        | Female | 6          | 32,443,325  | 32,867,275  | 423.95    | 58               | Present in other sample     | 1.5                      | 0.15                     | 20                          |
| 29        | Male   | 6          | 32,504,484  | 32,792,007  | 287.523   | 29               | Present in other sample     | 1.5                      | 0.133333                 | 30                          |
| 27        | Female | 6          | 84,483,165  | 84,684,198  | 201.033   | 12               | None                        | 3                        | 0.15                     | 20                          |
| 26        | Female | 6          | 92,001,319  | 92,378,064  | 376.745   | 18               | Spans smaller CNV           | 2.5                      | 0.15                     | 20                          |
| 18        | Female | 6          | 168,057,168 | 168,414,475 | 357.307   | 19               | Full                        | 3                        | 0.4                      | 20                          |
| 23        | Female | 7          | 35,382,544  | 35,875,899  | 493.355   | 24               | Spans smaller CNV           | 1.5                      | 0.3                      | 20                          |
| 8         | Male   | 7          | 38,096,725  | 38,131,968  | 35.243    | 10               | Full                        | 1.5                      | 0.933333                 | 30                          |
| 12        | Male   | 7          | 38,096,725  | 38,176,607  | 79.882    | 12               | Full                        | 1                        | 0.7                      | 30                          |
| 30        | Male   | 7          | 38,096,725  | 38,176,607  | 79.882    | 11               | Full                        | 1                        | 0.2                      | 30                          |
| 25        | Female | 7          | 53,249,866  | 53,361,989  | 112.123   | 11               | Spans smaller CNV           | 1                        | 0.95                     | 20                          |
| 14        | Male   | 7          | 83,318,167  | 84,089,522  | 771.355   | 25               | None                        | 3                        | 0.166                    | 30                          |
| 9         | Male   | 7          | 100,656,922 | 101,084,080 | 427.158   | 21               | Spans smaller CNV           | 1.5                      | 0.333333                 | 30                          |
| 30        | Male   | 7          | 141,607,918 | 141,962,328 | 354.41    | 46               | Full                        | 1.5                      | 0.551724                 | 29                          |
| 12        | Male   | 7          | 141,607,918 | 142,075,187 | 467.269   | 48               | Full                        | 1.5                      | 0.241379                 | 29                          |
| 18        | Female | 8          | 3,657,789   | 3,863,869   | 206.08    | 47               | Full                        | 3                        | 0.15                     | 20                          |
| 8         | Male   | 8          | 12,039,387  | 12,537,556  | 498.169   | 6                | Full                        | 3                        | 0.347826                 | 23                          |
| 9         | Male   | 8          | 18,136,585  | 18,287,748  | 151.163   | 19               | Spans smaller CNV           | 1.5                      | 0.2                      | 30                          |
| 9         | Male   | 8          | 75,135,676  | 75,247,824  | 112.148   | 16               | None                        | 1                        | 0.2                      | 30                          |
| 9         | Male   | 8          | 96,342,431  | 96,547,939  | 205.508   | 26               | Spans smaller CNV           | 1.5                      | 0.433333                 | 30                          |
| 3         | Female | 8          | 143,020,060 | 143,188,118 | 168.058   | 18               | None                        | 2.5                      | 0.2                      | 20                          |

**Table 1** (continued)

| Sample ID | Sex    | Chromosome | Start(10)   | End(10)     | Size (kb) | Number of probes | Overlap in DGV <sup>a</sup> | Copy number <sup>b</sup> | Max density <sup>c</sup> | Number of reference samples |
|-----------|--------|------------|-------------|-------------|-----------|------------------|-----------------------------|--------------------------|--------------------------|-----------------------------|
| 9         | Male   | 9          | 293,727     | 679,340     | 385.613   | 55               | Spans smaller CNV           | 0                        | 2.000                    | 30                          |
| 10        | Male   | 9          | 1,534,755   | 1,673,781   | 139.026   | 31               | None                        | 3                        | 0.166                    | 30                          |
| 3         | Female | 9          | 484,9872    | 581,8435    | 968.563   | 87               | Spans smaller CNV           | 1.5                      | 0.105263                 | 19                          |
| 22        | Female | 9          | 4,943,916   | 5,858,289   | 914.373   | 68               | Spans smaller CNV           | 1.5                      | 0.157895                 | 19                          |
| 26        | Female | 9          | 129,708,231 | 129,757,016 | 48.785    | 10               | None                        | 1.5                      | 0.2                      | 20                          |
| 26        | Female | 10         | 2,312,896   | 2,347,121   | 34.225    | 12               | None                        | 1                        | 0.25                     | 20                          |
| 26        | Female | 10         | 3,450,926   | 3,576,724   | 125.798   | 19               | None                        | 1.5                      | 0.25                     | 20                          |
| 2         | Female | 10         | 11,742,195  | 12,455,154  | 712.959   | 60               | None                        | 1.5                      | 0.45                     | 20                          |
| 7         | Female | 10         | 27,642,965  | 27,746,607  | 103.642   | 7                | None                        | 1                        | 0.5                      | 20                          |
| 3         | Female | 10         | 42,799,941  | 42,914,459  | 114.518   | 17               | Spans smaller CNV           | 2.5                      | 0.15                     | 20                          |
| 26        | Female | 10         | 44,446,980  | 44,729,250  | 282.27    | 37               | Spans smaller CNV           | 3                        | 1.000                    | 19                          |
| 27        | Female | 10         | 45,367,462  | 47,212,082  | 1844.62   | 35               | Full                        | 3                        | 1.000                    | 14                          |
| 29        | Male   | 10         | 45,367,462  | 47,212,082  | 1844.62   | 33               | Full                        | 3                        | 1.000                    | 17                          |
| 7         | Female | 10         | 45,536,421  | 47,212,082  | 1675.661  | 29               | Full                        | 3                        | 1.000                    | 14                          |
| 30        | Male   | 10         | 47,063,957  | 48,062,084  | 998.127   | 26               | Full                        | 3                        | 0.588                    | 17                          |
| 3         | Female | 10         | 47,063,957  | 47,143,807  | 79.85     | 15               | Full                        | 1.5                      | 1.000                    | 14                          |
| 7         | Female | 10         | 55,623,019  | 55,801,795  | 178.776   | 12               | None                        | 3                        | 0.2                      | 20                          |
| 3         | Female | 10         | 59,385,719  | 59,510,689  | 124.97    | 19               | Spans smaller CNV           | 2.5                      | 0.105263                 | 19                          |
| 2         | Female | 10         | 69,516,802  | 70,685,976  | 1169.174  | 84               | Spans smaller CNV           | 1.5                      | 0.45                     | 20                          |
| 13        | Female | 10         | 72,380,953  | 72,389,048  | 8.095     | 6                | Present in other sample     | 0                        | 1.000                    | 20                          |
| 11        | Male   | 10         | 72,384,286  | 72,389,048  | 4.762     | 6                | Present in other sample     | 0                        | 1.000                    | 30                          |
| 23        | Female | 10         | 75,631,142  | 76,238,780  | 607.638   | 18               | None                        | 1.5                      | 0.55                     | 20                          |
| 9         | Male   | 10         | 89,908,985  | 90,176,103  | 267.118   | 18               | None                        | 1.5                      | 0.166667                 | 30                          |
| 9         | Male   | 10         | 104,234,129 | 104,360,652 | 126.523   | 16               | None                        | 1.5                      | 0.133333                 | 30                          |
| 26        | Female | 10         | 111,933,510 | 112,062,492 | 128.982   | 11               | None                        | 1.5                      | 0.105263                 | 19                          |
| 14        | Male   | 10         | 115,688,018 | 115,727,353 | 39.335    | 16               | None                        | 1.5                      | 0.333333                 | 30                          |
| 26        | Female | 10         | 123,421,836 | 123,484,634 | 62.798    | 13               | Spans smaller CNV           | 1                        | 1.000                    | 20                          |
| 2         | Female | 10         | 131,457,178 | 131,615,303 | 158.125   | 25               | Spans smaller CNV           | 3                        | 0.15                     | 20                          |
| 29        | Male   | 10         | 134,598,099 | 135,262,316 | 664.217   | 36               | Full                        | 3                        | 0.24                     | 25                          |
| 5         | Male   | 10         | 135,240,018 | 135,323,432 | 83.414    | 14               | Full                        | 3                        | 0.4                      | 25                          |
| 12        | Male   | 11         | 8,780,964   | 8,922,274   | 141.31    | 20               | Full                        | 1.5                      | 0.2                      | 30                          |
| 18        | Female | 11         | 12,250,939  | 12,461,900  | 210.961   | 25               | None                        | 2.5                      | 0.263158                 | 19                          |
| 10        | Male   | 11         | 12,804,409  | 12,859,638  | 55.229    | 13               | None                        | 3                        | 0.133                    | 30                          |
| 2         | Female | 11         | 19,373,059  | 19,407,469  | 34.41     | 10               | None                        | 3                        | 0.15                     | 20                          |
| 27        | Female | 11         | 48,535,899  | 49,371,138  | 835.239   | 20               | Full                        | 3.5                      | 1.000                    | 20                          |
| 3         | Female | 11         | 61,752,884  | 61,775,118  | 22.234    | 13               | None                        | 1.5                      | 0.15                     | 20                          |
| 26        | Female | 11         | 78,401,192  | 78,462,343  | 61.151    | 17               | None                        | 3                        | 0.2                      | 20                          |
| 2         | Female | 11         | 85,150,896  | 85,483,910  | 333.014   | 29               | Spans smaller CNV           | 1.5                      | 0.15                     | 20                          |
| 10        | Male   | 11         | 91,431,323  | 91,687,315  | 255.992   | 47               | Present in other sample     | 3                        | 0.133                    | 30                          |
| 12        | Male   | 11         | 91,510,546  | 91,648,752  | 138.206   | 25               | Present in other sample     | 2.5                      | 0.166667                 | 30                          |

**Table 1** (continued)

| Sample ID | Sex    | Chromosome | Start(10)   | End(10)     | Size (kb) | Number of probes | Overlap in DGV <sup>a</sup> | Copy number <sup>b</sup> | Max density <sup>c</sup> | Number of reference samples |
|-----------|--------|------------|-------------|-------------|-----------|------------------|-----------------------------|--------------------------|--------------------------|-----------------------------|
| 9         | Male   | 11         | 91,563,799  | 91,769,195  | 205.396   | 28               | Present in other sample     | 3                        | 0.2                      | 30                          |
| 9         | Male   | 11         | 101,064,226 | 101,174,095 | 109.869   | 17               | None                        | 1                        | 0.166667                 | 30                          |
| 22        | Female | 11         | 120,642,125 | 120,839,820 | 197.695   | 27               | None                        | 1.5                      | 0.15                     | 20                          |
| 29        | Male   | 11         | 133,714,118 | 134,090,011 | 375.893   | 59               | Full                        | 3                        | 0.481481                 | 27                          |
| 11        | Male   | 11         | 133,821,432 | 134,106,222 | 284.79    | 46               | Full                        | 2.5                      | 0.37037                  | 27                          |
| 26        | Female | 11         | 133,861,143 | 134,157,118 | 295.975   | 52               | Full                        | 3                        | 0.3                      | 20                          |
| 3         | Female | 11         | 133,917,124 | 134,168,536 | 251.412   | 46               | Full                        | 3                        | 0.2                      | 20                          |
| 9         | Male   | 12         | 1,147,724   | 1,620,858   | 473.134   | 37               | Spans tiny CNV              | 1.5                      | 0.5                      | 30                          |
| 11        | Male   | 12         | 3,780,453   | 4,035,827   | 255.374   | 27               | Spans tiny CNV              | 1.5                      | 0.566667                 | 30                          |
| 2         | Female | 12         | 7,662,831   | 8,034,750   | 371.919   | 19               | Full                        | 1.5                      | 0.15                     | 20                          |
| 14        | Male   | 12         | 17,942,983  | 19,107,310  | 1164.327  | 51               | Spans smaller CNV           | 2.5                      | 0.133333                 | 30                          |
| 18        | Female | 12         | 18,524,861  | 18,917,349  | 392.488   | 23               | Spans smaller CNV           | 3                        | 0.2                      | 20                          |
| 18        | Female | 12         | 33,480,710  | 34,651,598  | 1170.888  | 38               | Full                        | 2.5                      | 0.470588                 | 17                          |
| 14        | Male   | 12         | 34,198,126  | 34,651,598  | 453.472   | 15               | Full                        | 3                        | 0.269                    | 26                          |
| 14        | Male   | 12         | 36,177,141  | 36,252,574  | 75.433    | 4                | Full                        | 2.5                      | 0.241                    | 29                          |
| 18        | Female | 12         | 36,177,141  | 36,846,337  | 669.196   | 12               | Full                        | 3                        | 0.45                     | 20                          |
| 7         | Female | 12         | 36,177,141  | 36,846,337  | 669.196   | 12               | Full                        | 3                        | 0.35                     | 20                          |
| 5         | Male   | 12         | 36,177,141  | 36,252,574  | 75.433    | 4                | Full                        | 3                        | 0.206897                 | 29                          |
| 14        | Male   | 12         | 88,142,416  | 88,798,361  | 655.945   | 42               | Present in other sample     | 1.5                      | 0.133333                 | 30                          |
| 9         | Male   | 12         | 90,240,906  | 90,619,246  | 378.34    | 22               | None                        | 1.5                      | 0.2                      | 30                          |
| 2         | Female | 12         | 121,153,309 | 121,870,227 | 716.918   | 26               | Spans tiny CNV              | 1.5                      | 0.35                     | 20                          |
| 13        | Female | 12         | 123,690,926 | 123,725,895 | 34.969    | 4                | None                        | 0                        | 1.000                    | 20                          |
| 20        | Male   | 13         | 20,765,759  | 21,246,173  | 480.414   | 42               | Spans smaller CNV           | 1.5                      | 0.266667                 | 30                          |
| 27        | Female | 13         | 36,748,678  | 36,843,045  | 94.367    | 14               | None                        | 3                        | 0.15                     | 20                          |
| 29        | Male   | 13         | 37,125,748  | 37,280,809  | 155.061   | 16               | None                        | 3                        | 0.5                      | 30                          |
| 9         | Male   | 13         | 48,418,971  | 49,449,079  | 1030.108  | 41               | Present in other sample     | 1.5                      | 0.166667                 | 30                          |
| 10        | Male   | 13         | 104,929,618 | 105,237,575 | 307.957   | 27               | Present in other sample     | 3                        | 0.133                    | 30                          |
| 23        | Female | 13         | 104,929,618 | 105,423,049 | 493.431   | 37               | Present in other sample     | 2.5                      | 0.2                      | 20                          |
| 1         | Male   | 14         | 19,272,965  | 19,595,673  | 322.708   | 14               | Full                        | 3.5                      | 0.777                    | 9                           |
| 3         | Female | 14         | 19,272,965  | 19,595,673  | 322.708   | 14               | Full                        | 1.5                      | 0.272727                 | 11                          |
| 12        | Male   | 14         | 19,272,965  | 19,492,423  | 219.458   | 10               | Full                        | 1.5                      | 0.111111                 | 9                           |
| 2         | Female | 14         | 19,272,965  | 19,492,423  | 219.458   | 10               | Full                        | 1.5                      | 0.454545                 | 11                          |
| 18        | Female | 14         | 20,863,076  | 21,462,988  | 599.912   | 44               | Spans smaller CNV           | 1.5                      | 0.3                      | 20                          |
| 12        | Male   | 14         | 21,108,365  | 22,099,182  | 990.817   | 103              | Full                        | 1.5                      | 1.000                    | 30                          |
| 22        | Female | 14         | 21,118,254  | 22,048,615  | 930.361   | 94               | Full                        | 1.5                      | 1.000                    | 20                          |
| 30        | Male   | 14         | 21,118,254  | 22,058,411  | 940.157   | 90               | Full                        | 1                        | 1.000                    | 30                          |
| 3         | Female | 14         | 21,438,691  | 22,058,411  | 619.72    | 73               | Full                        | 1.5                      | 1.000                    | 20                          |
| 1         | Male   | 14         | 21,551,291  | 22,039,606  | 488.315   | 54               | Full                        | 1.5                      | 1.000                    | 30                          |
| 7         | Female | 14         | 21,551,291  | 22,058,411  | 507.12    | 59               | Full                        | 1.5                      | 1.000                    | 20                          |
| 8         | Male   | 14         | 21,551,291  | 22,047,089  | 495.798   | 52               | Full                        | 1.5                      | 1.000                    | 30                          |
| 2         | Female | 14         | 21,567,040  | 22,099,182  | 532.142   | 68               | Full                        | 1.5                      | 0.45                     | 20                          |
| 5         | Male   | 14         | 21,607,217  | 22,058,411  | 451.194   | 48               | Full                        | 1.5                      | 1.000                    | 30                          |

**Table 1** (continued)

| Sample ID | Sex    | Chromosome | Start(10)   | End(10)     | Size (kb) | Number of probes | Overlap in DGV <sup>a</sup> | Copy number <sup>b</sup> | Max density <sup>c</sup> | Number of reference samples |
|-----------|--------|------------|-------------|-------------|-----------|------------------|-----------------------------|--------------------------|--------------------------|-----------------------------|
| 2         | Female | 14         | 25,238,076  | 25,626,474  | 388.398   | 21               | Spans smaller CNV           | 2.5                      | 0.15                     | 20                          |
| 1         | Male   | 14         | 69,679,669  | 69,776,988  | 97.319    | 20               | None                        | 1.5                      | 0.133333                 | 30                          |
| 9         | Male   | 14         | 72,826,658  | 73,465,699  | 639.041   | 33               | Present in other sample     | 1.5                      | 0.433333                 | 30                          |
| 10        | Male   | 14         | 72,867,422  | 73,418,572  | 551.15    | 29               | Present in other sample     | 1.5                      | 0.133333                 | 30                          |
| 30        | Male   | 14         | 73,006,350  | 73,418,572  | 412.222   | 30               | Present in other sample     | 1.5                      | 0.366667                 | 30                          |
| 22        | Female | 14         | 73,006,350  | 73,384,916  | 378.566   | 26               | Present in other sample     | 1.5                      | 0.105263                 | 19                          |
| 2         | Female | 14         | 73,024,250  | 73,511,098  | 486.848   | 40               | Present in other sample     | 1.5                      | 0.736842                 | 19                          |
| 2         | Female | 14         | 78,544,014  | 78,672,810  | 128.796   | 17               | None                        | 2.5                      | 0.15                     | 20                          |
| 26        | Female | 14         | 91,606,712  | 91,714,385  | 107.673   | 15               | None                        | 1.5                      | 0.15                     | 20                          |
| 7         | Female | 14         | 91,606,712  | 91,740,938  | 134.226   | 23               | None                        | 1.5                      | 0.15                     | 20                          |
| 2         | Female | 14         | 91,606,712  | 91,740,938  | 134.226   | 23               | None                        | 1.5                      | 0.25                     | 20                          |
| 27        | Female | 14         | 91,610,714  | 91,739,589  | 128.875   | 17               | None                        | 1.5                      | 0.45                     | 20                          |
| 27        | Female | 14         | 105,021,165 | 105,746,660 | 725.495   | 10               | Full                        | 4                        | 0.166                    | 12                          |
| 8         | Male   | 14         | 105,149,735 | 106,142,730 | 992.995   | 14               | Full                        | 3                        | 0.642857                 | 14                          |
| 8         | Male   | 15         | 18,451,755  | 19,403,149  | 951.394   | 15               | Full                        | 2.5                      | 0.111111                 | 9                           |
| 27        | Female | 15         | 18,451,755  | 19,403,149  | 951.394   | 16               | Full                        | 2.5                      | 0.2                      | 5                           |
| 5         | Male   | 15         | 18,451,755  | 19,831,810  | 1380.055  | 19               | Full                        | 1                        | 1.000                    | 9                           |
| 26        | Female | 15         | 18,682,601  | 20,077,554  | 1394.953  | 30               | Full                        | 1.5                      | 0.8                      | 5                           |
| 18        | Female | 15         | 18,753,439  | 20,688,804  | 1935.365  | 41               | Full                        | 1.5                      | 1.000                    | 5                           |
| 23        | Female | 15         | 18,753,439  | 20,042,468  | 1289.029  | 25               | Full                        | 1.5                      | 0.4                      | 5                           |
| 21        | Female | 15         | 18,809,644  | 19464910    | 655.266   | 12               | Full                        | 1.5                      | 0.2                      | 5                           |
| 3         | Female | 15         | 18,872,928  | 20,077,554  | 1204.626  | 26               | Full                        | 1.5                      | 1.000                    | 5                           |
| 30        | Male   | 15         | 19,464,910  | 20,077,554  | 612.644   | 18               | Full                        | 3.5                      | 0.777                    | 9                           |
| 8         | Male   | 15         | 19,856,526  | 20,343,286  | 486.76    | 13               | Full                        | 2.5                      | 0.222222                 | 9                           |
| 3         | Female | 15         | 21,942,075  | 22,098,514  | 156.439   | 11               | Full                        | 1                        | 0.2                      | 20                          |
| 11        | Male   | 15         | 24,989,155  | 25,039,127  | 49.972    | 4                | Spans smaller CNV           | 0                        | 1.000                    | 30                          |
| 9         | Male   | 15         | 49,779,718  | 49,962,828  | 183.11    | 20               | None                        | 1.5                      | 0.2                      | 30                          |
| 3         | Female | 15         | 65,060,001  | 65,083,692  | 23.691    | 9                | Spans smaller CNV           | 1.5                      | 0.15                     | 20                          |
| 9         | Male   | 15         | 83,864,137  | 84,060,543  | 196.406   | 28               | Present in other sample     | 1                        | 0.266667                 | 30                          |
| 27        | Female | 15         | 85,587,147  | 85,670,812  | 83.665    | 9                | None                        | 1                        | 1.000                    | 20                          |
| 26        | Female | 16         | 3,392,194   | 3,629,679   | 237.485   | 11               | None                        | 1.5                      | 0.4                      | 20                          |
| 9         | Male   | 16         | 11,442,884  | 11,719,576  | 276.692   | 27               | None                        | 1.5                      | 0.166667                 | 30                          |
| 3         | Female | 16         | 22,297,762  | 22,773,865  | 476.103   | 24               | Full                        | 1                        | 0.533333                 | 15                          |
| 27        | Female | 16         | 28,457,009  | 29,066,936  | 609.927   | 14               | Full                        | 1                        | 0.9                      | 20                          |
| 27        | Female | 16         | 34,246,988  | 34,614,572  | 367.584   | 18               | Full                        | 3                        | 0.312                    | 16                          |
| 7         | Female | 16         | 34,246,988  | 34,614,572  | 367.584   | 18               | Full                        | 3                        | 0.25                     | 16                          |
| 26        | Female | 16         | 64,339,519  | 64,371,857  | 32.338    | 12               | None                        | 1                        | 0.15                     | 20                          |
| 23        | Female | 16         | 64,339,519  | 64,371,857  | 32.338    | 12               | None                        | 1.5                      | 0.15                     | 20                          |
| 18        | Female | 16         | 82,335,439  | 82,342,649  | 7.21      | 13               | Spans smaller CNV           | 1                        | 0.368421                 | 19                          |
| 29        | Male   | 17         | 3,461,651   | 3,827,102   | 365.451   | 30               | Full                        | 1.5                      | 0.241379                 | 29                          |
| 8         | Male   | 17         | 21,242,033  | 21,657,532  | 415.499   | 15               | Full                        | 3                        | 0.758621                 | 29                          |
| 26        | Female | 17         | 27,963,258  | 28,214,729  | 251.471   | 14               | Present in other sample     | 1.5                      | 0.15                     | 20                          |
| 9         | Male   | 17         | 31,416,126  | 32,076,780  | 660.654   | 27               | Full                        | 1.5                      | 0.5                      | 8                           |
| 27        | Female | 17         | 31,454,449  | 31,895,171  | 440.722   | 12               | Full                        | 3.5                      | 0.75                     | 4                           |

**Table 1** (continued)

| Sample ID | Sex    | Chromosome | Start(10)  | End(10)    | Size (kb) | Number of probes | Overlap in DGV <sup>a</sup> | Copy number <sup>b</sup> | Max density <sup>c</sup> | Number of reference samples |
|-----------|--------|------------|------------|------------|-----------|------------------|-----------------------------|--------------------------|--------------------------|-----------------------------|
| 8         | Male   | 17         | 31,454,563 | 31,488,153 | 33.59     | 9                | Full                        | 1.5                      | 0.5                      | 8                           |
| 1         | Male   | 17         | 31,463,252 | 31,488,153 | 24.901    | 8                | Full                        | 1.5                      | 0.125                    | 8                           |
| 21        | Female | 17         | 40,504,036 | 41,090,087 | 586.051   | 37               | Present in other sample     | 1.5                      | 0.125                    | 8                           |
| 24        | Female | 17         | 40,564,152 | 41,112,752 | 548.6     | 31               | Present in other sample     | 1                        | 1.000                    | 8                           |
| 18        | Female | 17         | 40,825,939 | 41,109,882 | 283.943   | 21               | Present in other sample     | 1                        | 0.625                    | 8                           |
| 25        | Female | 17         | 41,006,823 | 41,022,689 | 15.866    | 9                | Full                        | 3                        | 0.125                    | 8                           |
| 8         | Male   | 17         | 41,007,016 | 41,049,321 | 42.305    | 8                | Full                        | 4                        | 0.9                      | 20                          |
| 5         | Male   | 17         | 41,007,016 | 41,049,321 | 42.305    | 9                | Full                        | 4                        | 0.35                     | 20                          |
| 3         | Female | 17         | 41,426,926 | 41,569,489 | 142.563   | 26               | Full                        | 1.5                      | 0.384615                 | 13                          |
| 26        | Female | 17         | 41,476,292 | 41,618,727 | 142.435   | 26               | Full                        | 1.5                      | 0.230769                 | 13                          |
| 25        | Female | 17         | 41,500,209 | 41,711,411 | 211.202   | 35               | Full                        | 3                        | 1.000                    | 13                          |
| 5         | Male   | 17         | 41,522,318 | 41,711,411 | 189.093   | 34               | Full                        | 4                        | 1.000                    | 26                          |
| 8         | Male   | 17         | 41,522,318 | 41,711,411 | 189.093   | 34               | Full                        | 1.5                      | 1.000                    | 26                          |
| 29        | Male   | 17         | 41,522,318 | 41,708,649 | 186.331   | 31               | Full                        | 4                        | 0.615385                 | 26                          |
| 7         | Female | 17         | 41,522,318 | 41,644,356 | 122.038   | 26               | Full                        | 1.5                      | 0.769231                 | 13                          |
| 22        | Female | 17         | 41,522,318 | 41,630,337 | 108.019   | 27               | Full                        | 1.5                      | 0.538462                 | 13                          |
| 10        | Male   | 17         | 41,522,422 | 42,223,353 | 700.931   | 36               | Full                        | 3                        | 0.615                    | 26                          |
| 1         | Male   | 17         | 41,522,422 | 42,151,961 | 629.539   | 33               | Full                        | 3.5                      | 1.000                    | 26                          |
| 23        | Female | 17         | 41,522,422 | 41,711,411 | 188.989   | 32               | Full                        | 3                        | 1.000                    | 13                          |
| 27        | Female | 17         | 41,560,151 | 42,166,078 | 605.927   | 34               | Full                        | 3.5                      | 1.000                    | 13                          |
| 13        | Female | 17         | 41,589,553 | 41,711,411 | 121.858   | 22               | Full                        | 3                        | 0.23                     | 13                          |
| 3         | Female | 17         | 57,141,121 | 57,545,412 | 404.291   | 13               | Spans tiny CNV              | 1.5                      | 0.15                     | 20                          |
| 12        | Male   | 18         | 9,636,198  | 9,737,630  | 101.432   | 12               | Spans smaller CNV           | 1.5                      | 0.133333                 | 30                          |
| 10        | Male   | 18         | 29,023,519 | 29,459,187 | 435.668   | 18               | Spans tiny CNV              | 3                        | 0.6                      | 30                          |
| 27        | Female | 18         | 64,881,549 | 64,922,370 | 40.821    | 8                | Spans smaller CNV           | 1                        | 0.277778                 | 18                          |
| 2         | Female | 19         | 9,063,085  | 9,204,855  | 141.77    | 14               | Spans tiny CNV              | 1.5                      | 0.35                     | 20                          |
| 2         | Female | 19         | 11,088,326 | 12,964,638 | 1876.312  | 82               | Spans tiny CNV              | 1.5                      | 0.25                     | 20                          |
| 3         | Female | 19         | 11,625,040 | 12,157,284 | 532.244   | 31               | Spans tiny CNV              | 1.5                      | 0.2                      | 20                          |
| 26        | Female | 19         | 15,564,927 | 15,616,442 | 51.515    | 13               | None                        | 1.5                      | 0.15                     | 20                          |
| 11        | Male   | 19         | 16,002,989 | 16,839,008 | 836.019   | 37               | Spans tiny CNV              | 1.5                      | 0.133333                 | 30                          |
| 30        | Male   | 19         | 36,930,988 | 38,962,919 | 2031.931  | 178              | Spans smaller CNV           | 1.5                      | 1.000                    | 30                          |
| 2         | Female | 19         | 47,932,219 | 48,461,673 | 529.454   | 19               | Full                        | 3                        | 0.272727                 | 11                          |
| 25        | Female | 19         | 49,671,550 | 49,867,156 | 195.606   | 16               | Spans smaller CNV           | 1.5                      | 0.25                     | 20                          |
| 30        | Male   | 20         | 18,061,486 | 18,383,181 | 321.695   | 31               | Present in other sample     | 2.5                      | 0.166667                 | 30                          |
| 30        | Male   | 20         | 18,563,063 | 18,677,271 | 114.208   | 20               | Present in other sample     | 3                        | 0.8                      | 30                          |
| 9         | Male   | 20         | 22,608,020 | 22,675,548 | 67.528    | 12               | Spans smaller CNV           | 2.5                      | 0.166667                 | 30                          |
| 24        | Female | 20         | 44,174,860 | 45,061,166 | 886.306   | 69               | Spans smaller CNV           | 1.5                      | 0.2                      | 20                          |
| 21        | Female | 20         | 46,968,668 | 47,464,220 | 495.552   | 40               | None                        | 1.5                      | 0.15                     | 20                          |

**Table 1** (continued)

| Sample ID | Sex    | Chromosome | Start(10)   | End(10)     | Size (kb) | Number of probes | Overlap in DGV <sup>a</sup> | Copy number <sup>b</sup> | Max density <sup>c</sup> | Number of reference samples |
|-----------|--------|------------|-------------|-------------|-----------|------------------|-----------------------------|--------------------------|--------------------------|-----------------------------|
| 9         | Male   | 20         | 47,552,837  | 47,880,560  | 327.723   | 30               | None                        | 1.5                      | 0.2                      | 30                          |
| 7         | Female | 20         | 58,879,012  | 58,928,466  | 49.454    | 9                | None                        | 3.5                      | 0.35                     | 20                          |
| 26        | Female | 20         | 62,301,246  | 62,374,173  | 72.927    | 12               | Full                        | 3                        | 0.15                     | 20                          |
| 9         | Male   | 21         | 25,557,670  | 25,841,440  | 283.77    | 18               | None                        | 1.5                      | 0.266667                 | 30                          |
| 11        | Male   | 21         | 32,533,765  | 33,141,602  | 607.837   | 34               | Spans tiny CNV              | 1.5                      | 0.133333                 | 30                          |
| 27        | Female | 22         | 15,252,977  | 15,549,842  | 296.865   | 13               | Full                        | 3                        | 0.411765                 | 17                          |
| 3         | Female | 22         | 16,800,853  | 17,279,671  | 478.818   | 42               | Spans smaller CNV           | 3                        | 1.000                    | 19                          |
| 26        | Female | 22         | 23,983,115  | 24,244,331  | 261.216   | 26               | Full                        | 3                        | 1.000                    | 18                          |
| 3         | Female | 22         | 35,044,924  | 35,447,115  | 402.191   | 43               | Spans smaller CNV           | 1.5                      | 0.45                     | 20                          |
| 11        | Male   | X          | 2,673,310   | 2,687,601   | 14.291    | 6                | Full                        | 1.5                      | 0.416667                 | 12                          |
| 24        | Female | X          | 2,673,310   | 2,682,320   | 9.01      | 6                | Full                        | 1.5                      | 0.636364                 | 11                          |
| 1         | Male   | X          | 2,673,310   | 2,682,320   | 9.01      | 6                | Full                        | 1                        | 0.75                     | 12                          |
| 14        | Male   | X          | 2,673,310   | 2,687,601   | 14.291    | 8                | Full                        | 1.5                      | 0.5                      | 12                          |
| 2         | Female | X          | 2,682,320   | 2,687,601   | 5.281     | 4                | Full                        | 1.5                      | 0.545455                 | 11                          |
| 12        | Male   | X          | 2,682,320   | 2,687,601   | 5.281     | 4                | Full                        | 1.5                      | 0.166667                 | 12                          |
| 29        | Male   | X          | 2,682,320   | 2,687,601   | 5.281     | 4                | Full                        | 1                        | 0.583333                 | 12                          |
| 14        | Male   | X          | 3,872,433   | 4,015,795   | 143.362   | 12               | Spans smaller CNV           | 2                        | 0.166                    | 30                          |
| 24        | Female | X          | 5,519,920   | 6,450,497   | 930.577   | 31               | Spans smaller CNV           | 1.5                      | 0.105263                 | 19                          |
| 22        | Female | X          | 11,348,425  | 11,904,509  | 556.084   | 26               | Spans tiny CNV              | 1.5                      | 0.25                     | 20                          |
| 2         | Female | X          | 51,210,792  | 51,663,987  | 453.195   | 8                | None                        | 4                        | 0.2                      | 20                          |
| 12        | Male   | X          | 125,728,657 | 126,183,384 | 454.727   | 11               | None                        | 4                        | 0.3                      | 30                          |

Columns “start(10)” and “end(10)” indicate the 10% density CNV borders and “Number of probes (10)” is the number of markers within the 10% density border. The SNPs at the border of the polymorphic region has retained 10% of the maximum density, which is defined as the largest density value for any SNP in that CNV region after comparison with the diploid reference samples. “Density” is defined as the fraction of times that the region is called as a CNV when compared with reference samples.

<sup>a</sup> Overlap in DGV: spans smaller CNV (predicted CNV is larger than the region in DGV); spans tiny CNV (the CNV in DGV is less than a third in size then the predicted CNV); present in other sample (was found in normal individuals or CLL samples but so far not present in DGV)

<sup>b</sup> Copy numbers in the GEMCA output were ordered in bins according to threshold values of the median ratios derived from hybridizations of samples with varying number of X chromosomes (1–5) to samples with 2 X chromosomes (see supplementary data to Komura et al. at [www.genome.org](http://www.genome.org) for a table with copy numbers and the corresponding median ratio threshold values). Decimal numbers reflect less certain copy numbers.

<sup>c</sup> Max density: a max density of 1 would indicate that in all pairwise comparisons against the reference set, the region was called a CNV.

smaller than the size of the predicted region in the HIES samples, leaving 69 regions that were not present in DGV, presenting as either novel CNVs or false-positives.

The great majority of these CNVs with no match in the database were so-called singletons (Redon et al. 2006) that were found only in one sample. A rather small CNV on chromosome 16 was present in two samples but does not span any known genes and is thus unlikely to be responsible for the HIES phenotype.

Additional CNVs on chromosome 4 and 14 were found in three and four patients, respectively. The gain on chromosome 4 contains only one known gene (*EVC*) which is associated with the Ellis van Creveld syndrome. The deletion on 14q32.12 however, spans three genes (*ATXN3*, *NDUFB1*, and *CPSF2*). So, we were interested if this

heterozygous deletion which was observed in patient nos. 2, 7, 26, and 27 may cause the HIES phenotype in these four individuals. At that time, our research consortium on HIES had just found mutations in exons 11–21 in *STAT3* being responsible for HIES (Holland et al. 2007). We therefore sequenced exons 11–21 in these patients and found *STAT3* mutations in three of them (nos. 7, 26, and 27). Hence, the heterozygous deletion on the chromosome is not causing HIES in these four patients.

We screened the remaining singleton CNVs for overlaps with regions that have been associated with HIES like chromosome 4q around D4S428 region (Grimbacher et al. 1999c) or the *TYK2* locus on 19p13.2 but did not identify overlapping CNVs. In addition, regions that are associated with diseases that show a phenotypic resemblance to HIES

were also aligned with the predicted CNVs from Table 1. Webb et al. (2007) performed a quantitative linkage genome scan for atopy in 690 independent Caucasian families, using serum IgE levels and percentage of positive skin prick test (SPT) as clinical discriminators and found LOD scores  $>2$  for regions on chromosomes 1, 3, and 8q near markers D1S1653 (position chromosome 1: 154,745,744 bp), D3S2322 (position chromosome 3: 134,168,906 bp), D3S1764 (position chromosome 3: 140,670,986), and D8S2324, respectively. As indicated in Table 1, no CNV could be found near the markers on chromosomes 1 and 3, but we identified a deletion 750 kb telomeric to marker D8S2324 in patient no. 9. This 112-kb deletion starts around 32 kb on the telomeric side of the *LY96* gene (physical position chromosome 8: 75,135,676–75,247,824 bp) and spans three ESTs. In the atopy linkage study by Webb et al., the 5q region (known to be associated with atopy) gave a LOD score of 1.96 (SPT) and 1.77 (IgE) at or near marker D5S1480 (position chromosome 5: 144,123,841 bp). In our HIES cohort, however, no CNV could be detected nearby marker D5S1480, as the closest CNV is more than 30 Mb telomeric to marker D5S1480.

Prolidase deficiency (PD, OMIM no. 170100) overlaps phenotypically with HIES in symptoms like elevated IgE and chronic infections and skin lesions. This autosomal recessive disease is caused by homozygous mutations in the *PEPD* gene on 19q13.11. We found a large deletion of 2,031 kb in patient no. 30 that is not present in his first-degree relatives (Fig. 1a,b). Sequencing of the patients genomic DNA, however, revealed a wild-type sequence, thus ruling out a compound heterozygote *PEPD* mutation.

In summary, with both 50k and 250k arrays, no recurrent microdeletions in a larger fraction of our samples could be identified, which makes it unlikely that HIES is a microdeletion syndrome. Nevertheless, sporadic AD HIES patients could present with nonrecurrent gains or losses as part of a mutational spectrum.

## Discussion

Several clinically defined syndromes, such as the 22q11.2 deletion syndrome (also known as DiGeorge/velo-cardio-facial syndrome) (Wurdak et al. 2006); the Williams syndrome, caused by hemizygous microdeletion of about 28 genes on chromosome 7q11.23 (Meyer-Lindenberg et al. 2006) and the Smith–Magenis syndrome, are mostly caused by interstitial deletions that may not be detected by high-resolution karyotyping. Recently, Girirajan et al. (2006) described that the severity and the variability of the clinical features of patients affected by Smith–Magenis syndrome depend on the deleted region.

In the present study, we have chosen a 50k *XbaI* followed by a 250k *StyI* SNP mapping array approach to detect possible chromosomal deletions or gains associated with the pathogenesis of the HIES in 30 patients with an HIES phenotype. Using the 50k *XbaI* array, no large aberrations could be detected using a genomic smoothing length of 0.5 Mb in CNAT. With a setting of 0.05 Mb, eight different copy number alterations below 1 Mb were identified in six patients using stringent criteria (see [Patients and methods](#) section). As seven of these alterations were also found in healthy individuals, an impact of these on the evolvement of HIES is highly unlikely.

The gain on chromosome 20p12, which we identified in 1 of the 30 patients, was not found in more than 400 control samples. However, this gain turned out to be an incidental private polymorphism with a perfect segregation in the patient's family (Fig. 1a).

The lack of large recurrent aberrations led us to switch to the higher resolution 250k *StyI* array. With an improved algorithm for the detection of copy number variations, a mean number of 11.5 CNVs per individual has been found in our HIES cohort, which is in line with the finding of Redon et al. (2006) who described a mean of 24 CNVs per individual in the HapMap samples with 500k array resolution (both *NspI* and *StyI* arrays). The median inter-SNP-distance of the 250 k array is 5 kb. GEMCA identified known CNVs with just four SNPs. Thus, in theory, a 20-kb deletion should be identified by GEMCA. As shown in Table 1, GEMCA predicted CNVs as small as 600 bp with only four SNPs, and some of them were already known to be polymorphic. However, albeit their huge number, SNPs are not evenly spaced, and one cannot completely rule out the possibility that smaller deletions have been missed.

Around 52% of the predicted CNVs were found to overlap with known entries in DGV or CNVs in non-HIES samples, and 21% spanned known CNVs to a varying degree, making them thus more likely to be extended versions of the known polymorphic regions. The remaining 69 CNVs with no match in DGV constitute a pool of potential CNVs that are either rare polymorphisms, false-positives or disease-associated regions. The huge number of these gains and losses makes it difficult to discern the true nature of the predicted region. We thus concentrated first on recurrent CNVs. The gain of chromosome 4 in three patients is unlikely to be associated with HIES as the underlying gene is associated with Ellis van Creveld Syndrome (OMIM no. 225500) and Weyers acrofacial dysostosis (OMIM no. 193530).

In addition, we have screened singleton CNVs for potential overlaps to regions that have been associated with HIES. We have identified a deletion 750-kb telomeric to the marker D8S2324 on chromosome 8 in one HIES patient. This marker had a LOD score of 2.25 for IgE in the study

of Webb et al. (2007) describing linkage of several chromosomal regions to atopy and asthma in a large collection of Caucasian families. Although this deletion does not span a known gene, it could be worthwhile to further analyze this CNV, as it is close to the *LY96* gene which cooperates with *TLR4* in the innate immune response to bacterial lipopolysaccharide (LPS), and with *TLR2* in the response to cell wall components from Gram-positive and Gram-negative bacteria.

The large deletion including the *PEPD* locus on 19q13.11 could merely present an extended version of a CNV present in non-HIES samples, as it spans smaller entries in DGV. Given the overlap in the phenotypic spectrum of HIES and prolidase deficiency, this patient could be compound heterozygote with the deletion being on one allele and a so far unknown mutation in *PEPD* on the second allele.

However, this was ruled out by direct sequencing of the *PEPD* gene where no pathogenic mutation could be identified. Although the *PEPD* gene was a strong candidate in this region, the deletion removes several other genes which may be further analyzed regarding their potential contribution to the HIES phenotype.

In summary, we have screened a cohort of 30 sporadic patients with a typical phenotype of AD HIES for submicroscopic insertions or deletions. Using 50k and 250k SNP mapping arrays, we were able to rule out the presence of overlapping deletions in these patients, making it unlikely that a common microdeletion, such as seen in the 22q11.2 deletion and other syndromes, accounts for the majority of cases with HIES. In addition, Minegishi et al. and Holland et al. described mutations in *STAT3* in patients with HIES, while this manuscript was under review. We are currently analyzing which of our patients carry mutations in *STAT3*.

Nevertheless, this project has identified numerous novel CNVs. Some of them may be further analyzed to verify or falsify their contribution to the HIES phenotype in selected sporadic patients with an HIES-like phenotype.

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