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# Cohen syndrome diagnosis using whole genome arrays

Nuria Rivera-Brugués,[1] Beate Albrecht,[2] Dagmar Wieczorek,[2] Heinrich Schmidt,[3] Thomas Keller,[4] Ina Göhring,[5] Arif B Ekici,[5] Andreas Tzschach,[6] Masoud Garshasbi,[6] Kathlen Franke,[7] Norman Klopp,[8] H-Erich Wichmann,[8] Thomas Meitinger,[1,9] Tim M Strom,[1,9] Maja Hempel[1,9]

[1]Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany
[2]Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany
[3]Department of Pediatrics, Ludwig-Maximilians-Universität, Munich, Germany
[4]Department of Pediatrics, Josefinum Augsburg, Augsburg, Germany
[5]Institute of Human Genetics, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany
[6]Max Planck Institute for Molecular Genetics, Department Human Molecular Genetics, Berlin, Germany
[7]Private Clinic B.Prager & A. Junge, Dresden, Germany
[8]Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany
[9]Institute of Human Genetics, Technische Universität München, Munich, Germany

**Correspondence to**
Dr Maja Hempel, Institute of Human Genetics, Technische Universität München, Trogerstraße 32, D-81675 Munich, Germany; hempel@humangenetik.med.tu-muenchen.de

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## ABSTRACT

**Background** Cohen syndrome is a rare autosomal recessive disorder with a complex phenotype including psychomotor retardation, microcephaly, obesity with slender extremities, joint laxity, progressive chorioretinal dystrophy/myopia, intermittent isolated neutropenia, a cheerful disposition, and characteristic facial features. The *COH1* gene, which contains 62 exons, is so far the only gene known to be associated with Cohen syndrome. Point mutations, deletions and duplications have been described in this gene. Oligonucleotide arrays have reached a resolution which allows the detection of intragenic deletions and duplications, especially in large genes such as *COH1*.

**Method and results** High density oligonucleotide array data from patients with unexplained mental retardation (n=1523) and normal controls (n=1612) were analysed for copy number variation (CNV) changes. Intragenic heterozygous deletions in the *COH1* gene were detected in three patients but no such changes were detected in the controls. Subsequent sequencing of the *COH1* gene revealed point mutations in the second allele in all three patients analysed.

**Conclusion** Genome-wide CNV screening with high density arrays provides a tool to detect intragenic deletions in the *COH1* gene. This report presents an example of how microarrays can be used to identify autosomal recessive syndromes and to extend the phenotypic and mutational spectrum of recessive disorders.

## INTRODUCTION

The phenotype of Cohen syndrome (MIM 216550), a rare autosomal recessive disorder, has been described to be fairly homogeneous in Finnish patients where the founder mutation c.3348_3349delCT is detected in about 75% of mutant alleles.[1] But in non-Finnish and especially in young Cohen patients, a high genotypic and phenotypic variability occurs. Several clinical diagnostic criteria for Cohen syndrome have been introduced.[2–5] Chandler *et al* proposed that, next to significant learning disabilities, two of the following criteria should be present for Cohen syndrome diagnosis: facial gestalt, pigmentary retinopathy, and neutropenia.[3] Kohlemainen *et al* suggested Cohen syndrome in patients fulfilling at least six of the following criteria: developmental delay, microcephaly, typical facial gestalt, truncal obesity with slender extremities, overly sociable behaviour, joint hypermobility, high myopia and/or retinal dystrophy, and neutropenia.[5] El Chehadeh *et al* concluded that mutation analysis is not indicated in the absence of chorioretinal dystrophy or neutropenia.[6]

The *COH1* gene (VPS13B, MIM 607817), so far the only gene known to be associated with Cohen syndrome, is one of the largest known genes in the human genome and comprises 62 exons distributed along 864315 bp of chromosome 8. More than 96 different mutations in *COH1* gene have been detected in association with Cohen syndrome. The majority of them are terminating mutations predicted to result in a functional null allele.[5 7–9] Recently large intragenic deletions and duplications in the *COH1* gene have been identified as a cause of Cohen syndrome.[10 11] Molecular diagnosis of syndromes has been improved by the introduction of oligonucleotide arrays which have reached a resolution facilitating the detection of intragenic copy number variations (CNVs).

## ARRAY DATASETS AND METHODS

### Array datasets

Recruitment of patients (n=1523) had been part of the German Mental Retardation Network (MRNET) study (http://www.german-mrnet.de/). In the present study we focus on three patients, in which CNVs in the *COH1* gene were detected. As a control set, CNV data (n=1612) had been generated from the population based KORA study (Cooperative Health Research in the Augsburg Region).

### CNV analysis

Genome-wide screening for CNVs was performed using the Infinium Human550 Genotyping BeadChip (Illumina, SanDiego, California, USA) in patients 1 and 2 and the Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, California, USA) in patient 3. The controls have been investigated with Illumina Infinium Human550-Quad or Human610-Quad arrays.

The genomic DNA of the patients and controls was isolated from peripheral blood lymphocytes according to standard procedures and processed following the manufacturer's instructions. Arrays were scanned with the Illumina BeadArray Reader and with the Affymetrix Scanner 3000 7G. Genotypes were called with the Illumina GenomeStudio Software or using the Affymetrix Genotyping Console Software (version 3.0.2), respectively.

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Data analysis of the Illumina arrays was performed according to Wagenstaller *et al*.[12] CNV profiling of the Affymetrix array data was accomplished by using the Segment reporting Tools of the Genotyping Console Software. To determine a deletion we used as a cut-off the smoothing median of five or more adjacent single nucleotide polymorphisms (SNPs) with copy number values ≤1.5 or with log2 intensity ratios ≤−1 for Illumina and Affymetrix arrays, respectively. CNVs with copy number values ≥2.5 or with log2 intensity ratios ≥1 were suspicious for duplication. All CNVs were checked for gene content and overlap with known genetic variants as provided by the genome browser of the University of California Santa Cruz (UCSC) (http://genome.ucsc.edu, hg 18) and the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation). CNVs not annotated as structural polymorphisms and containing RefSeq genes were genotyped by quantitative PCR (qPCR). Monitoring of the PCR reaction and setting of baseline and threshold cycle values were accomplished automatically with the Sequence Detection System Version 2.3 Software (SDS 2.3, Applied Biosystems, Darmstadt, Germany). The relative quantification analysis based on the comparative $C_t$ method was performed using an in-house developed Perl script.

### Sequencing

In the patients with a partial heterozygous deletion of the *COH1* gene, direct sequencing of the entire coding region and the exon/intron boundaries of *COH1* was carried out using BigDye Ready Terminator Sequencing Kit and an 48 capillary Abi 3730 Genetic Analyzer (Applied Biosystems) in accordance with standard procedures. All identified variants were genotyped in 676 individuals of a population based cohort (KORA-cohort) via the MassARRAY system (Sequenom genotyping platform) and the iPLEX Gold chemistry. The assay design used the AssayDesign 3.1.2.2 software with default parameters. Genotype calling was performed by the SpectroTYPER 3.4 software.

### Nomenclature

Gene model NM_17890.3/NP_060360 based on UCSC browser was used to describe the detected *COH1* gene variants (http://genome.ucsc.edu hg18).

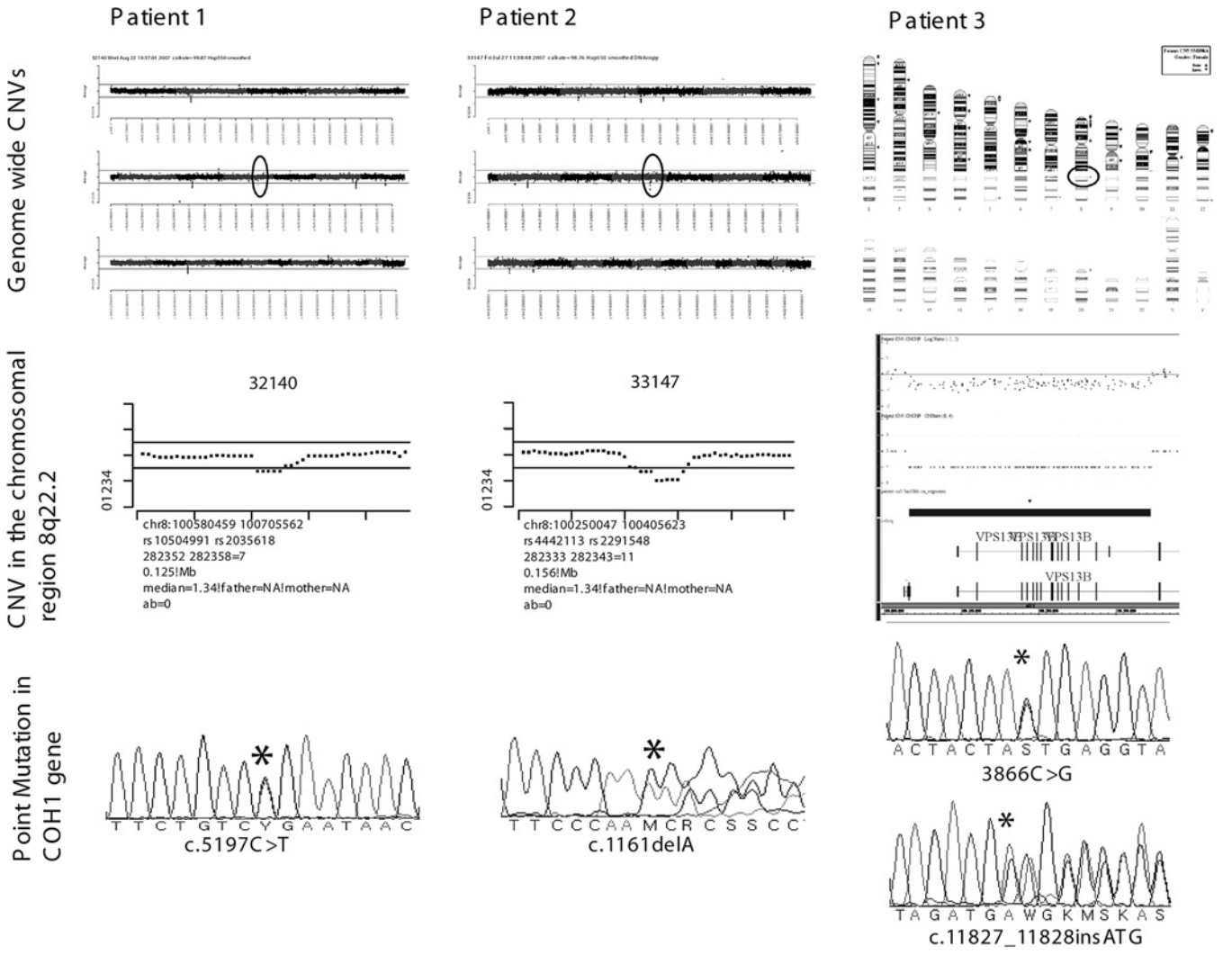


**Figure 1** Array results and electropherograms of the *COH1* mutations in patients 1 to 3. Patient 1 is affected by a 125 kb deletion encompassing exons 26 to 31 of the *COH1* gene (chr.8: 100 563 167 …100 642 020) and a missense mutation c.5197C→T of the second allele. In Patient 2 a 156 kb deletion encompassing exons 16 to 19 of the *COH1* gene (chr.8:100 250 047…100 405 623) and a one base pair deletion c.1161delA in the second allele was detected. In Patient 3 a 315 kb deletion encompassing exons 1 to 17 of the *COH1* gene (chr8:100 015 029…100 347 846) and missense mutation 3866C→G and three base pair insertion c.11827_11828insATG in the second allele were found. CNV, copy number variation.

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**Figure 2** Craniofacial phenotype of patients 1 to 3. Patient 1 at the age of 3 years with horizontal eyebrows, a broad and down turned nasal tip, a broad columella, a short philtrum, a small upper lip and everted lower lip. Patient 2 at the age of 18 months with horizontal eyebrows, almond shaped and downslanting palpebral fissures, a broad nasal root, a round nasal tip, thick columella, a short philtrum, and open appearance of mouth with prominent upper gingiva. Patient 3 at the age of $2^{8}/_{12}$ years with round and flat face, bushy eyebrows with lateral flaring, broad nasal bridge, short philtrum and microtia with overfolded helices.

## RESULTS

### Molecular findings

Analysis of SNP oligonucleotide array and subsequent qPCR discovered CNVs in the *COH1* gene in three patients: a maternal 67 kb deletion encompassing exons 26 to 31 of the *COH1* gene (chr8: 100 573 090…100 639 924; c.3871-5024del/p.G1291fsX42) in patient 1, a paternal 193 kb deletion encompassing exons 9 to 19 of the *COH1* gene (chr8: 100 216 034…100 409 167; c.1207-2824del/p.L403fsX11) in patient 2, and a maternal 315 kb deletion encompassing exon 4 of the *OSR2* gene and exons 1 to 17 of the *COH1* gene (chr8:100 015 029…100 347 846; c.1-2515del) in patient 3 (figure 1). There were no such changes in the 1612 controls.

Sequencing of the *COH1* gene identified in patient 1 a paternal missense mutation in exon 32 leading to a stop codon (c.5086C→T/p.R1696X), in patient 2 a maternal 1 bp deletion in exon 60 leading to a stop codon (c.11505delA/p.K3835fsX43), and in patient 3 a heterozygous missense mutation in exon 25 (c.3866C→G/p.T1289S) and a heterozygous three base pairs insertion in exon 62 (c.11827_11828insATG/p.D3942_G3943insD), both inherited from the father (figure 1). These mutations were not present in 676 samples from a general population cohort (KORA) and not annotated as polymorphisms in dbSNP (NCBI, http://www.ncbi.nlm.nih.gov/SNP).

### Clinical data

#### Patient 1

The boy was born after an uneventful pregnancy at term as the first child of unrelated and healthy Arabian parents. Birth weight, length and head circumference were not recorded. A delay in motor development became evident within his first year of life. Sitting started at the age of 18 months, walking at 24 months. A detailed examination at the age of 3 years showed a hypotonic boy with a height of 79 cm (−4.8 SD), a weight of 9300 g (−5.2 SD), and a head circumference of 44 cm (−4.9 SD). A delay in speech development as well as in comprehension was obvious. There was mild craniofacial dysmorphism including horizontal eyebrows, a broad and downturned nasal tip, a broad columella, a short philtrum, a thin upper lip, and an everted lower lip (figure 2). The ophthalmologic examination revealed bilateral myopia, astigmatism and a slightly increased pigmentation of the retina. There was no neutropenia.

#### Patient 2

This boy was born at term after an uneventful pregnancy as the second child of healthy unrelated German parents, with a birth weight of 2460 g (−2.4 SD), a birth length of 46.5 cm (−2.3 SD), and a head circumference of 31.5 cm (−2.9 SD). Developmental milestones were delayed with a sitting age of 12 months and a crawling age of 17 months. Examination at the age of

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18 months showed a hypotonic toddler with a weight of 10.1 kg (−1.5 SD), a height of 80 cm (−1 SD), and a severe microcephaly with an occipitofrontal circumference (OFC) of 42.5 cm (−4.8 SD). Speech development had not occurred but comprehension was nearly normal. Facial dysmorphism consisted of mild occipital flattening, horizontal eyebrows, almond shaped palpebral fissures, a broad nasal root, a round nasal tip, a thick columella, a short philtrum, an open appearance of the mouth with a prominent upper gingiva, and a large gap between the incisors (figure 2). Funduscopy and complete blood count revealed no abnormalities.

### Patient 3

The patient is the second child of a healthy non-consanguineous German/African couple. The premature birth occurred at 35 weeks of gestation with a birth weight of 2450 g (−0.1 SD), a birth length of 46 cm (−0.4 SD), and an OFC of 33.5 cm (0.6 SD). A heart defect (atrioseptal defect (ASD) II and pulmonary stenosis) and arrhythmia were diagnosed after birth. Developmental delay persisted until 2 years of age and improved after surgical correction of the heart defect. The patient started to walk at the age of 24 months and at the age of $2^3/_4$ years she showed normal body measurement with a height of 95 cm (0.4 SD), a weight of 11.5 kg (−1.4 SD), and an OFC 50 cm (0.6 SD). She had a flat face with broad and flat nasal bridge and almond shaped eyes, a short philtrum with thin vermillion border, and deep set ears with overfolded helices (figure 2). Funduscopy and complete blood count revealed normal results.

## DISCUSSION

Our report demonstrates that the diagnosis of Cohen syndrome can be reached in patients with unexplained mental retardation by applying high resolution oligonucleotide arrays. Although multiple exon deletions in the *COH1* gene have been reported in single patients,[5 7 8 13] the distribution of deletions and duplications has only been recognised recently.[10 11] The frequency of copy number alterations in the *COH1* gene is unknown. Parri *et al* disclosed that *COH1* CNVs account for 42% of *COH1* mutations.[11] Balikova *et al* reported an increase in the detection rate of 18% (88% instead of 70%) in typical Cohen patients.[10] Arrays targeted at individual exons will further increase the detection rate. We have recently identified—by molecular analysis—a homozygous 66 kb deletion comprising exons 32 and 33 of *COH1* which had escaped detection by current array comparative genomic hybridisation (CGH) analysis. The fact that we failed to detect CNVs in the *COH1* gene in 1612 controls from a general German population cohort indicates that CNVs in the *COH1* gene are rare. This is in contrast to the annotation of the *COH1* gene CNVs as benign polymorphisms in the UCSC genome browser and the DGV.

To our knowledge this is the first report on patients with Cohen syndrome diagnosed by molecular whole genome analyses but not by clinical examination. Cohen syndrome was not suspected at first instance in the patients, although all of them were examined by experienced paediatricians or clinical geneticists. This can be explained by the young age of patients (16 months, 18 months, $2^3/_4$ years). The facial appearance of the infant and young children with Cohen syndrome differs from adult patients and myopia/retinal pigmentary changes usually develop in the pre-school age.[3] The common facial characteristics in our small series of young patients with Cohen syndrome were a hypotonic facial expression, almond shaped palpebral fissures, a prominent nose, and a short philtrum. All patients were affected by mental retardation and delay in motor and speech development. Pigmentary retinopathy and neutropenia were absent in all patients. The unusual phenotype in patient 3 may due to the fact that in addition to exons 1 to 17 of the *COH1* gene, the deletion affected the neighbouring exon 4 of the *ORS2* gene. Although the gene function of *ORS2* in humans is unknown, an influence of the phenotype cannot be ruled out.

In conclusion, the phenotype of Cohen syndrome defined by *COH1* mutations is fairly unspecific, particularly in very young patients but in older children too. In addition, deletions in neighbouring genes may affect the phenotype of Cohen syndrome in the context of a contiguous gene syndrome. Nevertheless, young patients with a hypotonic facial expression, almond shaped eyes, short philtrum, mental retardation, and motor and speech delay are suspicious for Cohen syndrome. Microarrays have the potential to diagnose Cohen syndrome in very young patients and in patients with an atypical phenotype.

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**Competing interests** None.

**Patient consent** Obtained.

**Ethics approval** Approval for the study had been obtained by the ethical review boards of the participating institutions.

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# Poetry

## The battle of replication fork

Small soldiers march swiftly, the battle draws near
Helicase from the cannons split legions, no fear
Commanders ride forth with bellowing calls
Sending signals to primers, engage, pair them all!
Deoxy stands back, a path must be made
Those RNA boys bear the brunt of the trade
At last help's arrived; they've come through the trees
3-primed and ready, polymerase threes
RNA clears the field, a job nicely done
Their stations soon filled by polymerase one
"Who leads?" cries the general, he asks for a name
It's 3-prime not 5, the phosphates to blame
Fragmented and lagging are the men from Japan
Send ligase post haste, it's part of the plan
The fighting drones on, with no end in sight
Gyrase eases tensions, gets men through the night
At last the sun rises, dust settles, all clear
Polymerase checks that the win is sincere
Not a moment for rest, they're worked to the ground
For over those hills, more ori abound


### Victor Laurion

**Correspondence to** Victor Laurion, Bryn Mawr College, 900 Montgomery Avenue, Bryn Mawr, PA 19010, USA; victor.laurion@gmail.com



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