GENOTYPE-PHENOTYPE ANALYSIS OF 4q DELETION SYNDROME: PROPOSAL OF A CRITICAL REGION

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GENOTYPE-PHENOTYPE ANALYSIS OF 4q DELETION SYNDROME: PROPOSAL OF A CRITICAL REGION

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ABSTRACT

Chromosome 4q deletion syndrome (4q- syndrome) is a rare condition, with an estimated incidence of 1 in 100,000. Although variable, the clinical spectrum commonly includes craniofacial, developmental, digital, skeletal and cardiac involvement. Data on the genotype-phenotype correlation within the 4q arm is limited. We present detailed clinical and genetic information by array CGH on twenty patients with 4q deletions. We identified a patient who has only a ~ 465 kb deletion (186,770,069-187,234,800, hg18 coordinates) in 4q35.1 with all clinical features for 4q deletion syndrome except for developmental delay, suggesting that this is a critical region for this condition and a specific gene responsible for clefts and congenital heart defect resides in this region. Since the patients with terminal deletions all had cleft palate, our results provide further evidence that a gene associated with clefts is located on the terminal segment of 4q. By comparing and contrasting our patients' genetic information and clinical features, we found significant genotype-phenotype correlations at a single gene level linking specific phenotypes to individual genes. Based on these data, we constructed a hypothetical partial phenotype-genotype map for chromosome 4q which includes *BMP3, SEC31A, MAPK10, SPARCL1, DMP1, IBSP, PKD2, GRID2, PITX2, NEUROG2, ANK2, FGF2, HAND2* and *DUX4* genes.

INTRODUCTION

The 4q deletion syndrome, also called 4q- syndrome, is a rare chromosomal disorder caused by interstitial and terminal deletions of the long arm of chromosome 4, with an estimated incidence of 1 in 100,000 [Strehle et al., 2001, Strehle et al., 2003]. The majority of deletions are *de novo* but approximately 14%, of cases result from unbalanced segregation of parental reciprocal translocations [Strehle et al., 2003]. The male to female ratio is ca. 1. 4q deletion syndrome is a distinct congenital malformation syndrome associated with clinical findings affecting multiple organs and systems including developmental delay, facial and digital dysmorphology, Pierre Robin sequence, abnormalities of the cardiovascular, musculoskeletal and gastrointestinal systems. A review of 101 patients with 4q deletion syndrome revealed craniofacial anomalies in almost all individuals. Additionally, 88% had digital anomalies, 54% had skeletal anomalies, and almost half had congenital heart disease (CHD) [Strehle et al., 2003]. Autistic spectrum disorder and attention deficit hyperactivity disorder are part of the behavioral phenotype in 4q deletion syndrome [Strehle et al., 2007]. Figure 1 shows a female infant with 4q deletion syndrome.

Interstitial deletions have been associated with short limbs and small hands, Rieger syndrome and piebaldism [Strehle et al., 2003]. More distal deletions involving 4q34-q35 were associated with a lesser degree of characteristic features and cognitive impairment [Keeling et al., 2001; Kocks et al., 2002]. Satyr ears and hypoplastic fifth finger with a distinctive pointed nail were mainly found in terminal deletions involving 4q34 [Vogt et al., 2006]. Region 4q33 has been proposed as the critical region for 4q deletion syndrome [Keeling et al., 2001; Giuffre et al., 2004], containing genes responsible for development of the left ulnar ray, central nervous system and cleft lip and palate.

The first review on 4q deletions including a clinical correlation study dates back to 1981 [Mitchell et al.]. Since then, over 150 patients with 4q deletion syndrome have been reported in the literature [Lin et al., 1988; Strehle, 2011]. Many of these patients have been evaluated through traditional chromosome analysis with standard or high resolution banding only. Traditional cytogenetic studies such as high resolution chromosome banding will not detect a deletion less than 3-5 million base pairs, and exact breakpoints cannot be defined. Therefore, it is difficult to establish individual genotype-phenotype correlations. With the emergence of microarray-based comparative genomic hybridization (array CGH), the resolution for detecting deletions can reach the single-gene level.

Recently, array CGH has been used to characterize patients with 4q deletions in an attempt to elucidate genotype-phenotype correlations [Quadrelli et al., 2007b; Sensi et al., 2008; Kitsiou-Tzeli et al., 2008; Kaalund et al., 2008; Rossi et al., 2009; Hilhorst-Hofstee et al., 2009; Moreira et al., 2010; Chien et al., 2010; Al-Owain et al., 2010; Bonnet et al., 2010]. Previously published reports are limited to a small number of cases. For this study, we characterize 20 patients with 4q deletion syndrome. All patients were analyzed by array CGH. Significant correlations were found between deletions on the 4q arm and clinical signs and symptoms; they enabled us to propose genotype-phenotype correlations for a larger group of patients.

MATERIALS AND METHODS

Patient recruitment. All patients were recruited under the Institutional Review Board (IRB) protocol approved by the human subject committee of the University of California, Irvine (UCI). Individuals with a deletion in the 4q region diagnosed by array CGH as described previously [Quadrelli et al. 2007a] were eligible to enroll. One group of patients was seen by our group at the University of California Irvine Medical Center (UCIMC). After consent was obtained, the patients were evaluated by our board certified clinical geneticists. A second group of patients was referred to us by their local physicians or geneticists. Medical records were obtained and reviewed.

Array CGH. Oligonucleotide-based microarray analysis was performed on patients with a 105K-feature whole-genome microarray (SignatureChip Oligo Solution™, custom-designed by Signature Genomics Laboratories, made by Agilent Technologies, Santa Clara, CA). Microarray analysis was performed as previously described [Quadrelli et al., 2007a, Quadrelli et al., 2007b]

Fluorescence in situ hybridization (FISH). All deletions determined to be abnormal by array CGH were visualized by metaphase FISH using Bacteria Artificial Chromosome (BAC) clones, as previously described [Traylor et al., 2009]. Parental blood samples, where available, were karyotyped and/or assayed with metaphase FISH.

RESULTS

The clinical and molecular data for 20 patients were analyzed and summarized in Table I. The patients were numbered from 1 to 20 based on the location of the deletion, with number 1 closest to the centromere and number 20 closest to the telomere. Fourteen females and six males were enrolled in the study ranging from age 3 days to 33 years. Hypotonia was noted in ten patients (50%), cardiac involvement in nine (45%), developmental delay in eighteen (19%), and digital involvement in thirteen (65%) out of twenty patients. Thirty percent of patients had behavioral problems. The frequency and characteristics of clinical symptoms in our cohort was similar as previously reported [Strehle and Bantock, 2003] as illustrated in Table II, with a few new observations. Our cohort had a lower incidence of hearing and respiratory tract abnormalities (15% versus 37% and 32% respectively). A higher incidence of dentition abnormalities was reported (30% versus 18% previously). Parental chromosomes were available on nine patients and abnormal in four (44% ± 22%) which is higher than previously reported 14% ± 7% with 13 abnormal parental chromosomes in a cohort of 90 [Strehle and Bantock, 2003].

Figure 2 shows the region of the deletion for all 20 patients. The smallest deletion detected was 160 KB (3 OMIM genes) and the largest deletion was 25.7 MB (45 OMIM genes) covering a region from 4q21.1 to 4q35. The clinical features shared by patient 1 and patient 2 include short stature, brachydactyly, hypotonia, movement disorder, developmental delay, absence of speech and incontinence. The clinical feature shared by patient 2 and 3 is epilepsy; an additional unique feature of patient 3 is unilateral polycystic kidney dysplasia. Interestingly, patient 5 was found to have a micropenis while patient 7 was diagnosed with Axenfeld-Rieger syndrome and glaucoma. The deletion in patient 6 is relatively small and has no overlap with any other patients. The

phenotype of patient 6 includes developmental delay and speech delay, behavior problems, staring spells, a large tongue, a large head, asthma and possible overgrowth syndrome.

The deletions in patients 17-20 overlap with the deletions in patients 13-16. Patients 18, 19 and 20 all have abnormal teeth and finger/toe anomalies. Patients 17, 18 and 20 have a large tongue and congenital heart defects. Patients 17, 18 and 19 have developmental delay and speech delay. In addition, patients 17 and 20 both have cleft palate, upturned nose, hypotonia and gastroesophageal reflux. Patients 18 and 19 have growth deficiency or growth failure. Patients 18 and 20 both have small hands and feet. Patient 19 and 20 both have frontal bossing, hypertelorism and cleft lip.

The size of the deletion is largest in patients 13-16 and is progressively smaller in patients 17, 18, 19, and 20 with each preceding patient covering the same deletion the later ones have. Therefore one might expect that the severity of the phenotypic abnormality would be greatest in patients 13-16 and would become progressively less severe in patients 17, 18, 19, and 20 and that each preceding patient's features would include the same abnormal phenotype that the later one has. However, such correlations were not seen in our data.

Most interestingly, Patient 20, who has only two genes deleted, *PDLIM3* (PDZ and Lim Domain Protein 3) and *TLR3* (Toll-Like Receptor 3), shows a variety of abnormal features that are difficult to explain by the two genes deleted. The only phenotype that presents in all other patients but not in Patient 20 is developmental and speech delay. However, since the patient was young (3 years old) at the time of evaluation and had multiple congenital anomalies including ventriculomegaly, it is very likely that she will have developmental delay later in life. It is also interesting to see that patients 13, 15, 17 and 20 all have cleft palate. Although a specific gene,

which could be responsible for clefts or influence palate development, could not be located, it is most likely to reside in 4q33-4q35.1.

DISCUSSION

In this study, we recruited 20 individuals with 4q deletion characterized by array CGH. Clinical phenotypes were also intensively evaluated. The clinical characteristics of our patients are compatible with the 101 patients reviewed by Strehle and Bantock [2003]. The primary abnormalities in this group of patients are craniofacial malformation, developmental delay, and digital, skeletal, and congenital heart defects. Array CGH is an emerging technology for characterizing patients with 4q deletion syndrome in an attempt to elucidate genotype-phenotype correlations [Quadrelli et al., 2007b]. Recently, Li's group reported two patients with 4q deletions. In one of their cases, they found a 2.5 Mb duplication and a 12.6 Mb deletion in 4q34.1. In the other case, a patient presented with history of Pierre-Robin sequence, cardiac malformation, and learning disability. This patient had a *de novo* deletion of 16.4 Mb in 4q34.1 to 4q35.2 [Rossi et al., 2009]. The authors suggested that a 4 Mb region on chromosome 4q is harboring a candidate gene for Pierre-Robin sequence.

Molecular genetic information for patients with chromosome 4 deletions is limited due to the small number of published cases. The combination of the availability of the human genome sequence and the emergence of high-density oligonucleotide array CGH allowed us to study genotype-phenotype correlations at the single gene level in twenty patients with 4q deletion syndrome.

By comparing and contrasting the phenotypes and genotypes, the study suggests the following:

Interstitial Deletions

Among the genes deleted in patients 1 and 2, *Bone Morphogenetic Protein 3* (*BMP3)* gene is a member of the transforming growth factor beta family. Studies suggest that BMP3 regulates cartilage cell proliferation [Gamer et al., 2008]. Therefore, deletion of *BMP3* may be associated with short stature and the skeletal anomalies shared by these patients. *Sec31a* is also in this region and is a component of the Coat Protein Complex II (COPII) dependent collagen secretion. It has previously been shown to be important for normal craniofacial development [Stagg et al., 2008], therefore this gene may be associated with the abnormal craniofacial development shared by these two patients. In contrast with patient 2, patient 1 also has abnormal teeth. This suggests that *Galactokinase 2* (*GK2)* specifically deleted in patient 1 may be associated with abnormal teeth and other specific phenotypes. Similarly, the genes deleted specifically in patient 2 may be responsible for his specific phenotypes, including macrocephaly and hypoplastic suborbital region, short palpebral fissures and other craniofacial features. With this approach, we found:

Mitogen-Activated Protein Kinase 10 (MAPK10) plays an important role in neuronal apoptosis. Disruption of this gene in a *de novo* balanced translocation has been reported, associated with a patient with pharmacologically resistant epileptic encephalopathy [Shoichet et al., 2006]. Therefore, the deletion of *MAPK10* may be associated with the finding of the epilepsy in patients 2 and 3.

Polycystin-2 (*PKD2)* encodes the membrane protein polycystin 2. This protein affects renal tubule development, morphology, and function. It is able to modulate intracellular calcium and other signal transduction pathways [Wu et al., 2002]. The protein interacts with polycystin 1.

Heterozygous mutations in both *polycystin-1* and *polycystin-2* are associated with autosomal dominant polycystic kidney disease [Harris et al., 2009; Mochizuki et al., 1996]. Patient 3 has unilateral polycystic dysplasia indicating that haploinsufficiency of *PKD2* may be responsible for the cystic renal anomalies.

Dentin Matrix Acidic Phosphorprotein 1 (DMP1) is an extracellular matrix protein. Mutations of the *DMP1* gene are associated with autosomal recessive hypophosphatemia, a disease that manifests as rickets and osteomalacia [Feng et al., 2006]. Deletion of *DMP1* in mice results in decreased bone matrix development. However, carriers do not show clinical/biochemical evidence of the disease. Therefore, whether deletions of *DMP1* in patients 3 and 4 are associated with the growth deficiency in these two patients is yet undetermined.

Integrin-Binding Sialoprotein (IBSP) is also a bone matrix protein. Deletion of the *IBSP* gene could also play an important role in the differentiation of the osteoblast and development of the bone matrix [Ogata 2008]. Therefore, deletion of *IBSP* may be associated with the growth deficiency in these two patients.

Tachykinin receptor 3 (*TACR3)* is deleted in patient 5 but not in patient 4. This gene encodes a receptor for tachykinin neurokinin 3, also referred to as neurokinin B. Homozygous mutations of *TACR3* have been associated with congenital gonadotrophin deficiency and puberty failure [Topaloglu et al., 2009]. Therefore, a deletion of *TACR3* may be associated with the micropenis and small testes in patient 5.

The 160 kb deletion in patient 6 has no overlap with any other patients and covers a total of 3 genes listed in the OMIM) database: *3'-phosphoadenosine 5'-phosphosulfate synthase 1 (PAPSS1) Sphingomyelin synthase 2 (SGMS2)* and *Cytochrome P450, family 2, subfamily U,*

Polypeptide 1 (CYP2U). Haploinsufficiency of one or more of these genes may be related to the phenotype in this patient.

PAPSS 1 is the sulfate donor co-substrate for sulfotransferase (SULT) enzymes [Xu et al., 2000]. SULTs catalyze the sulfate conjugation of many endogenous and exogenous compounds, including drugs and other xenobiotics. In humans, PAPS is synthesized by two isoforms, PAPSS1 and PAPSS2. In brain and skin, PAPSS1 is the major expressed isoform [Venkatachalam, 2003]. *PAPSS1* is implicated to be a candidate hepatocellular carcinoma-susceptibility gene in hepatitis B carriers [Shih et al. 2009]. The effect of hemizygosity for *PAPSS1* on this patient's phenotype is not clear.

The protein encoded by *SGMS2* (Sphingomyelin Synthase 2) is an enzyme that catalyzes sphingomyelin (SM) biosynthesis. SM is a major component of cell and Golgi membranes. Experiments by Ding et al. [2008] indicated that SGMS2 is a key factor in the control of SM and diacylglycerol levels within the cell and thus influences lipopolysaccharide-mediated apoptosis. The effect of hemizygosity for *SGMS2* on this patient's phenotype is unclear.

Lastly, *Cytochrome P450, Family 2, Subfamily U, Polypeptide 2* (*CYP2U1)* encodes a member of the cytochrome P450 superfamily of enzymes. This enzyme is a hydroxylase that metabolizes arachidonic acid, docosahexaenoic acid, and other long chain fatty acids. Long chain fatty acids have recently emerged as critical signaling molecules in neuronal, cardiovascular and renal processes. Chuang and others postulate that CYP2U1 plays an important physiological role in fatty acid signaling processes in both cerebellum and thymus, and therefore it may play a role in brain and immune functions (Chuang et al., 2004).

The deleted regions in patients 7-10 overlap and have many genes in common. The genes that are deleted in these four patients are *Traf-interacting protein with Forkhead-associated domain*

(TIFA), Alpha-kinase 1 (*ALPK1*), Neurogenin-2 (*NEUROG2), La Ribonucleoprotein domain family, member 7* (*LARP7)* and *Ankyrin 2* (*ANK2).* There are no obvious clinical features shared by these patients except for developmental delay. Among the deleted genes, *NEUROG2* is of particular interest. *NEUROG2* is a member of the neurogenin subfamily of basic helix-loop-helix transcription factor genes that play an important role in neurogenesis from migratory neural crest cells. Heng et al. [2008] demonstrated that NEUROG2, which controls neurogenesis in the embryonic cortex, directly induces the expression of the small GTP-binding protein Rnd2 in newly generated mouse cortical neurons before they initiate migration. Thus, deletion of this gene may be associated with neurological findings in some of these patients.

ANK2 encodes a member of the ankyrin family of proteins that link the integral membrane proteins to the underlying spectrin-actin cytoskeleton. Ankyrins play key roles in activities such as cell motility, activation, proliferation, contact and the maintenance of specialized membrane domains. The protein encoded by this gene is required for targeting and stability of Na/Ca exchanger 1 in cardiomyocytes. A loss-of-function (E1425G) mutation in *ANK2* causes dominantly inherited type 4 long-QT cardiac arrhythmia in humans [Mohler et al., 2003], suggesting that the patients with deletion of *ANK2* should be examined for arrhythmia.

Several genes of interest are found in the deletions carried by patient 7, 8 and 10 but not in patient 9. *PRSS12* encodes a member of the neurotrypsin family of serine proteases. Mutations in *neurotrypsin 12* are associated with autosomal recessive mental retardation [Molinari et al., 2002]. Studies in *Drosophila* suggest that this neurotrypsin may be involved in structural reorganizations associated with learning and memory [Didelot et al., 2006]. Therefore, deletion of *PRSS12* may affect learning in these patients.

Phosphodiesterase 5A (*PDE5A)* encodes for a phosphodiesterase that specifically hydrolyzes cGMP to 5'-GMP. It is involved in the regulation of intracellular concentrations of cyclic nucleotides and is important for smooth muscle relaxation in the cardiovascular system [Sebkhi et al., 2003]. The effect of hemizygosity for *PDE5A* on these patient phenotypes is not clear, but it is possible that the deletion is relevant for the cardiovascular findings in these patients.

Lastly, the protein encoded by *FGF2* is a member of the fibroblast growth factor (FGF) family. FGF2 is a wide-spectrum mitogenic, angiogenic, and neurotrophic factor that is expressed at low levels in many tissues and cell types and reaches high concentrations in brain and pituitary. It has been implicated in diverse biological processes such as limb and nervous system development, wound healing, and tumor growth. The study of Ortega et al. showed that *FGF2* homozygous knockout mice had abnormalities in the cytoarchitecture of the neocortex, most pronounced in the frontal motor-sensory area [Ortega et al., 1998]. Dono *et al.* [1998] established in their study that FGF2 participates in controlling fates, migration, and differentiation of neuronal cells, whereas it is not essential for their proliferation. The homozygous knockout mouse model by Montero *et al.* revealed that FGF2 helps determine bone mass as well as bone formation [Montero et al., 2000]. Using FGF2-deficient and wild type cardiomyocyte precursor cells from neonatal mouse hearts, Rosenblatt-Velin and colleagues proposed that cardiogenic differentiation depends on FGF2 [Rosenblatt-Velin et al., 2005]. Although heterozygous mutations in *FGF2* have not been fully explored, deletion of *FGF2* may have an impact on some of the central nervous system, limb or cardiac abnormalities in patients 7, 8 and 10.

The features that were found only in patient 7 (but not in patients 8-10) include Axenfeld-Rieger syndrome, hearing loss/impairment, short nose and ventricular septal defect. Among the genes

deleted in patient 7 but not in Patient 8-10 is Paired-Like Homeodomain Transcription Factor 2 (*PITX2)* which encodes a member of the RIEG/PITX homeobox family. This protein is involved in the development of the eye, teeth and abdominal organs and acts as a transcriptional regulator involved in basal and hormone-regulated activity of prolactin. Mutations in this gene are associated with the Axenfeld-Rieger syndrome, iridogoniodysgenesis syndrome, and sporadic cases of Peter's anomaly. Axenfeld-Rieger syndrome results in abnormal development of the anterior segment of the eye and results in blindness from glaucoma in approximately 50% of affected individuals (Fitch et al., 1978). Deletion of *PITX2* explains the glaucoma and Axenfeld-Rieger syndrome present in patient 7.

In contrast to patient 10, patient 11 has a deletion of *PCDH10*, which belongs to the protocadherin gene family, a subfamily of the cadherin superfamily. The gene encodes a cadherin-related neuronal receptor thought to play a role in the establishment and function of specific cell-cell connections in the brain [Kim et al., 2007]. *PCDH18*, deleted next to *PCDH10*, shares a similar function. Thus, the deletion of *PCDH10* and *PCDH18* may be associated with the neurological findings in patient 11.

The deletion in patient 12 has no overlap with any of the other patients. The phenotype of patient 12 includes large head, frontal bossing, maxillary hypoplasia, short saddle nose, bilateral postaxial polydactyly, clinodactyly of toes, short stature, poor weight gain, speech delay, learning difficulties, hyperactivity, oppositional behavior, cryptorchidism, seizures and severe ichthyosis. The 2.13 Mb deletion on chromosome 4q includes eight OMIM genes. Among them, *HHIP* (hedgehog interacting protein) encodes a protein similar to the mouse hedgehog-interacting protein, a regulatory component of the hedgehog signaling pathway. Members of the hedgehog family are evolutionarily conserved proteins, which are involved in many fundamental

processes in embryonic development, including anteroposterior patterns of limbs and regulation of left-right asymmetry. It has been reported that heterozygous mutations in *Indian hedgehog* (*IHH*) result in brachydactyly type 1 [Gao et al., 2009]. Thus, the deletion of *HHIP* could potentially be associated with the digital anomalies in this patient.

Another gene of interest in patient 12 is *SMAD1*. SMAD1 mediates the signals of the bone morphogenetic proteins (BMPs), which are involved in a range of biological activities including cell growth, apoptosis, morphogenesis, development and immune responses [Tsuchida et al., 2008]. This protein can be phosphorylated and activated by the BMP receptor kinase. The phosphorylated form of this protein forms a complex with SMAD4, which is important for its function in the transcription regulation. The clinical significance of heterozygosity for *SMAD1* has not been reported. A literature review of the other deleted genes on chromosome 4q did not reveal any likely association between those genes and the specific phenotype in patient 12. This patient also has a partial deletion of the X chromosome. Among the genes deleted on chromosome X, the deletion of *STS* (Steroid sulfatase) is known to cause X-linked ichthyosis (XLI). This explains the presence of severe ichthyosis in this patient.

Terminal Deletions

Patient 15 has a terminal 4q deletion and an additional chromosome abnormality; the 7 MB duplication on chromosome 20p includes a total of 55 OMIM genes. Among them, one gene of interest is *TMC2*. The specific function of this gene is unknown; however, expression in the inner ear suggests that it may be crucial for normal auditory function. It has been reported to be associated with autosomal recessive nonsyndromic hearing impairment [Tlili et al., 2008]. The effect of duplication for *TMC2* on this patient's phenotype is not clear, but it is possible that the

duplication is relevant for the conductive hearing loss in patient 15. Literature review of the other duplicated genes does not reveal any likely association between those genes and the specific phenotype in patient 15 and patient 18.

The deleted regions in patients 13-16 have many genes in common. Patients 13-16 all have congenital cardiac defects, finger/toe anomaly, developmental delay and speech delay. Among the genes that are deleted in patients 13-16 is *HAND2*. The protein is a basic helix-loop-helix family of transcription factor and expressed in the developing ventricular chambers and plays an essential role in cardiac morphogenesis, implicating them as mediators of congenital heart disease (RefSeq, 2009; Morikawa et al., 2008]. In addition, this transcription factor may also play a role in limb and branchial arch development [Liu et al., 2009]. The deletion of *HAND2* may explain why Patients 13-16 all have congenital cardiac defects.

Vascular Endothelial Growth Factor C (VEGFC) encodes a platelet-derived growth factor/vascular endothelial growth factor, which is active in angiogenesis and endothelial cell growth. Deletion of *VEGFC* may be associated with development of the glabellar hemangioma in patients 13 and 14.

Critical Region

SORBS2 which is partially deleted in patient 20 encodes a protein containing N-terminal Sorbin and a C-terminal SH3 domain. The protein is high expressed in epithelia and cardiac muscle tissue. It has been found that the gene product interacts with ARG and c-ABL proteins (Hand et al., 2005). High expression level in cardiac tissue suggests that this gene may play an important role in heart development and may potentially contribute to congenital heart disease in patients

with 4q deletion syndrome. Molecular tests in patients with congenital heart defects for mutations in the *SORBS2* gene are in progress.

Limitations

The findings of this genotype-phenotype correlation study are interesting despite some limitations. Firstly, the sample size is relatively small and the number of patients with any specific locus deleted is limited. Our cohort included a wide range of genomic imbalances with different clinical presentations. By increasing the sample size, we should be able to fill the gaps and increase the confidence in the genotype-phenotype correlations. We hope that the genotypes and phenotypes presented here will, in combination with future findings and case reports, allow a better comparison of patients and enhanced phenotype-genotype correlations. Additionally, some of the clinical features were extracted from the clinical report of a geneticist, instead of by completion of a specifically designed checklist. Therefore, some of the clinical features may have been missed or overlooked. Furthermore, some of our patients have other chromosomal rearrangements that complicate the analysis of a genotype-phenotype correlation. In future, we plan to recruit additional patients with 4q deletion syndrome and build a fine map of deletions, through which, using the same approach, we will be able to pinpoint important genes for the phenotypes observed in 4q deletion syndrome. This information should prove useful for developing a more specific management and treatment plan for an individual with 4q deletion syndrome, based on the location and gene contact of the deletion.

Conclusions

Our findings as summarized in Table III and Figure 3 suggest that haploinsufficiency of the genes in 4q deletion syndrome is associated with specific phenotypes. In summary, haploinsuficiency of *BMP3* on 4q21.21 may be associated with short stature and other skeletal

anomalies. The loss of *SEC31A* on 4q21.22 may affect normal craniofacial development. The deletion of *MAPK10* on 4q21.3 may be an explanation for the neurological findings and epileptic activities in some 4q deletion patients. On 4q22.1, *SPARCL1* may be associated with central nervous system development, while both *DMP1* and *IBSP* genes may play an important role in growth. *PKD2* on 4q22.1 is known to be associated with abnormal renal phenotypes. *GRID2* may be associated with neurological findings and wide-based gait. On 4q25, deletion of *PITX2* is responsible for Axenfeld-Rieger syndrome, while *NEUROG2* plays an important role in neurogenesis in the embryonic cortex. The deletion of *ANK2* may be associated with cardiac arrhythmia, and the loss of *FGF2* on 4q27 may be associated with some CNS or limb anomaly. In addition, *HAND2* on terminal 4q plays an essential role in cardiac morphogenesis, and the deletion of this gene may result in congenital cardiac defects. Although none of our patients was reported to exhibit features of FSHD, *DUX4* located on 4q35.2 is known to be associated with autosomal dominant FSHD. However, the patients with 4q deletions do not show the typical clinical phenotype of this muscular dystrophy, suggesting that haploinsufficiency of *DUX4* is not the causative mechanism in FSHD. Recently, it was shown that specific single nucleotide polymorphisms (SNPs) in the chromosomal region distal to the last *D4Z4* repeat play an important role in this condition [Lemmers et al., 2010]. Finally, since four of our patients with terminal deletions all had cleft palate, it is likely that a gene associated with clefts resides on the 4q terminal region.

In summary, the 4q deletion syndrome is characterized by mild facial and digital dysmorphisms, developmental delay, learning disability, growth deficiency, skeletal and heart defects, and neurological and behavioral abnormalities. This syndrome is unusual so far as it includes deletions along the whole long arm of chromosome 4. In this phenotype-genotype study we have

been able to associate clinical findings with gene deletions by array CGH, with haploinsufficiency being the proposed underlying mechanism. In particular, the chromosome band 4q35.1 appears to harbor essential genes that contribute to this condition, if they are missing or mutated. However, considering the example of developmental delay, which is universally present in 4q deletion syndrome, and indeed in most chromosome imbalances, other causative mechanisms such as epigenetic factors and gene dosage effects should be explored.

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FIGURE AND TABLE LEGENDS

Fig. 1. Six-month-old girl with terminal deletion 4q33. The clinical features include growth deficiency, cleft palate, cardiovascular malformations (ASD, VSD). Dysmorphic features include microcephaly, rounded facies, small eyes, broad nasal bridge, upturned nose, full cheeks, small mouth and chin, short neck and Pierre-Robin sequence. She also has developmental delay and hypotonia

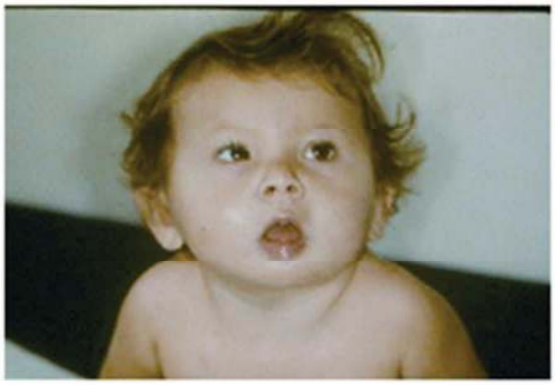
Fig. 2. Ideogram of the long arm of chromosome 4 depicting the position and size of each of the 20 deletions described in this report

Fig.3. Schematic representation of chromosome 4 with arrows indicating mapped and hypothetical genes that may contribute to the phenotype in patients with 4q deletion syndrome

Table I. Array CGH results and phenotypic characteristics of 20 patients with 4q deletion syndrome

Table II. Comparison of the clinical characteristics found in this study with those of the 101 patients reviewed by Strehle et al., 2003

Table III. Deleted genes and their functions in patients with 4q deletion syndrome



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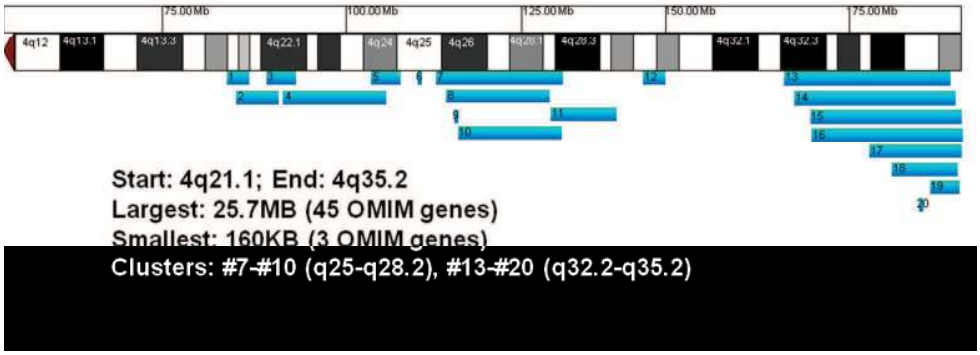

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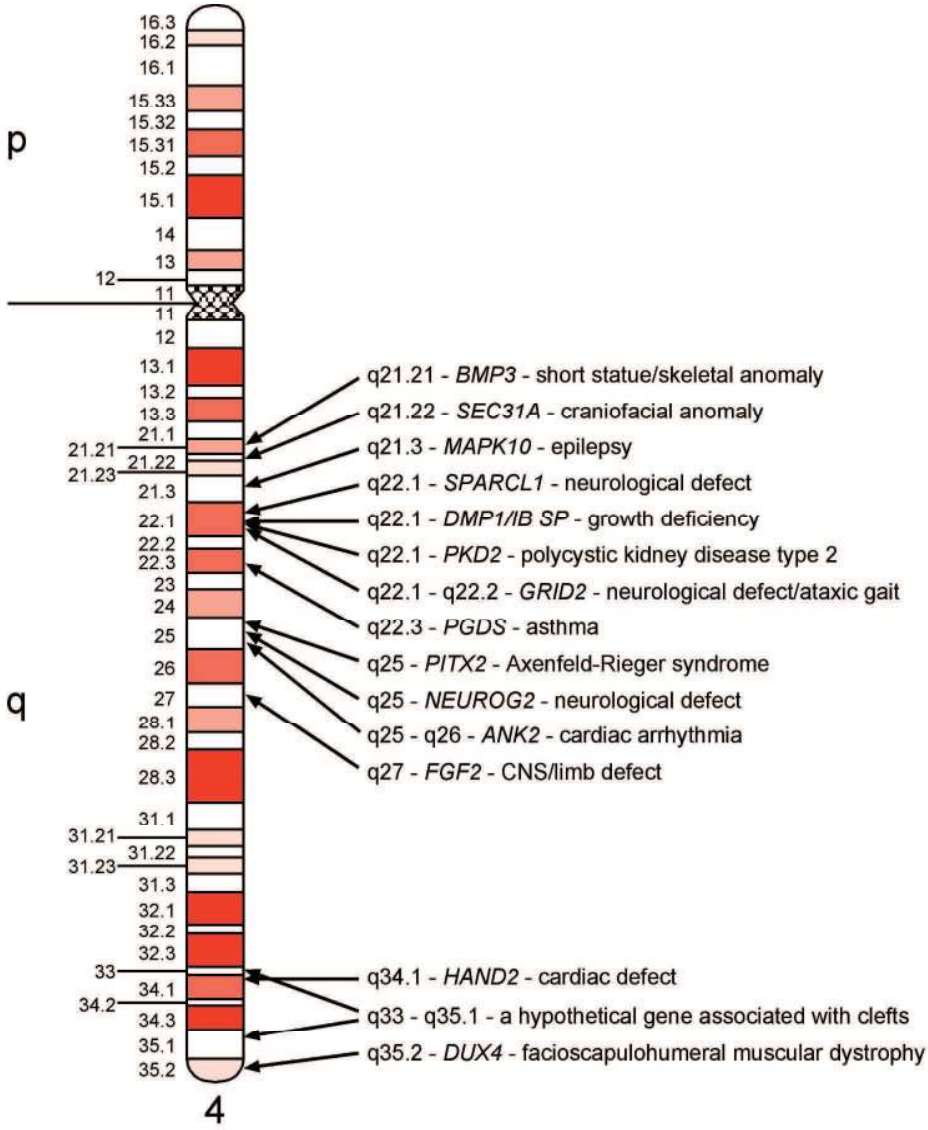

194x225mm (300 x 300 DPI)

Table I. Array CGH results and phenotypic characteristics of 20 patients with 4q deletion syndrome

No.	Deletion Start-End (hg18 coordinates)	Age (yrs), Sex	Parental Studies	Phenotype							
				Craniofacial	Digital	Central Nervous System	Cardio-vascular	Growth/ Development	Behavioral/ Psycho-logical	Gastrointestinal/ Urogenital	Other
1	79,888,401-84,257,219 (4.37 Mb)	22, F	-	Widely spaced teeth	Small hands and feet, short fingers and toes	Hypotonia	-	Severe global DD, no speech, can sit but not crawl or walk, fully dependent on caregiver, short stature	Aggressive behavior, on antipsychotic medication	Incontinence	Scoliosis
2	80,882,789-87,801,982 (6.92 Mb)	8, M	Normal	Large head, hypoplastic supraorbital ridges, low-set posteriorly rotated ears, short nose, philtrum and neck, short palpebral fissures, strabismus, lacrimal duct stenosis	Brachydactyly, club feet	Seizures, hypotonia.	VSD	DD, no speech, short stature.	Autism, severe LD	Feeding problems, gastrostomy, volvulus, eosinophilic enteropathy, incontinence	-
3	86,790,304-91,894,904 (5.10 Mb)	2, F	Father: del(4)(q21.23 q22.1)	Microcephaly, right ear has simple helix and is protuberant, nasal septum extends below the nares, strabismus	Clinodactyly of left 5th finger	VM and white matter changes on MRI, decreased volume in the gyri of both frontal lobes on CT, HIE	-	DD, speech delay, growth deficiency (wt <3%)	-	Infantile chronic vomiting, left multicystic dysplastic kidney, VUR, nephroureterectomy at 6 months of age	-
4	88,127,632-104,150,487 (16.0 Mb)	3.5, M	-	Microcephaly, arched eyebrows, blue sclerae, up slanting palpebral fissures, anteverted notched nares, long philtrum, thin upper lip	-	Hypotonia, hypoplasia of corpus callosum, possible cerebral atrophy	-	Global DD, little speech, growth deficiency, wide-based gait	Abnormal behavior	Feeding problems, GER, undescended testes, hydrocele	Asthma, recurrent infections
5	101,871,569-106,368,264 (4.50 Mb)	2.5, M	-	Epicanthus	-	Hypotonia, chorea	-	Severe DD, no speech	Autism	Feeding problems, micropenis.	Café-au-lait macules
6	108,834,399-108,994,048 (160 kb)	8.5, F	Normal	Large head, macroglossia	-	Staring spells	-	DD, speech delay, wt and ht >98%	Low IQ, behavior problems, anxiety, ADHD, sleeping difficulties	-	Asthma, streaky birthmark partially covering one arm
7	111,310,828-130,503,896 (19.2 Mb)	3 days, F	Normal	Glaucoma, low-set ears, thick ear helices, short nose with bulbous tip, natal tooth	Single transverse palmar crease	-	VSD	-	-	Excess umbilical skin	Axenfeld-Rieger syndrome, hearing impairment
8	4q deletion: 112,026,190-128,778,904 (16.8 Mb) X deletion: 9,441,237-9,757,010 (316 kb)	8, F	-	Glaucoma, hypertelorism, down slanting eyes, epicanthus, sluggish pupils, low-set small ears, cleft palate, small chin, long nose with high nasal root and down pointing tip, short webbed neck	Single transverse palmar crease, overlapping toes and hypoplastic toe nails bilaterally	Hypotonia, focal seizures	-	DD, LD, speech delay, precocious puberty	-	Feeding problems, anteriorly-positioned anus	Wide-spaced nipples
9	113,274,905-114,363,040 (1.09 Mb)	1.5, F	-	Upturned nose, small mouth	-	Hypotonia, infantile spasms	-	DD, speech delay	-	-	-

10	113,517,078-130,278,522 (16.8 Mb)	2, M	Normal	Sagittal craniosynostosis, dolichocephaly, flat supraorbital ridges, right divergent squint, asymmetrical pupils, low-set posteriorly rotated ears, prominent philtrum, double alveolar ridge, paracentral grooves in palate, micrognathia, short neck	Rhizomelia, overlapping toes	Hypertonia, intermittent extension dystonia	-	DD	Autism	-	Wide-spaced nipples
11	127,979,585-140,587,349 (12.6 Mb)	33, F	Mother: 46, XX, inv(9)(p11q13), normal phenotype	Hypotelorism, epicanthic folds, strabismus (esotropia), keratoconus, coarse trabecular iris pattern, small ears, short nose and philtrum, retrognathia, facial asymmetry	Hypoplastic 5th finger nails, small toe nails	Hypotonia.	Inter-mittent tachy-cardia and hyper-tension	Delayed motor and speech development, mild LD, growth deficiency	Emotional problems	GER, paraesophageal hiatus hernia	Graves' disease, multiple nevi, scoliosis, valgus deformity
12	4q deletion: 145,016,967-147,149,231 (2.13 Mb) 4q duplication: 186,766,425-186,893,799 (127 kb) X deletion: 6,410,891-7,686,762 (1.28 Mb)	5, M	Mother: 46, XX, del(4)(q31.21 q31.22) del(X)(p22.31 p22.31)	Macrocephaly, frontal bossing, maxillary hypoplasia, short saddle nose	Bilateral postaxial polydactyly, clinodactyly of toes	Seizures	-	Speech delay, learning difficulties, poor weight gain, short stature	Hyperactive and oppositional behavior	Cryptorchidism	Severe ichthyosis (harlequin type) on forehead, arms and legs, family history of ichthyosis
13	164,074,495-188,987,971 (24.9 Mb)	13, M	-	Facial asymmetry, glabellar hemangioma, prominent nasal root with hypoplastic alae, short nose with anteverted nares, overfolded ear helices, flat philtrum, cleft soft palate, dental crowding, fine long hair under chin	Absent left 3rd, 4th and 5th fingers	-	-	DD, speech delay, severe learning difficulties and delay in adaptive behavior, short stature	-	Recurrent UTI, left hydroureter	Absent left ulna, short curved left radius
14	164,807,106-190,490,075 (25.7 Mb) [Quadrelli et al., 2007b]	4, F	Normal	Hypoplastic supraorbital ridges, large fontanelles, upslanting and short palpebral fissures, hypertelorism, glabellar hemangioma, overfolded ear helix, microstomia and micrognathia	Overlapping fingers, clinodactyly of 5th fingers, hypoplastic 5th toe overlaps 4th toe	Occipital encephalocele, A-C mal-formation type II, neuronal migration defects, supratentorial hydrocephalus	COA, PDA, VSD	DD, growth deficiency.	-	Feeding difficulties, abnormal labia minora, prominent clitoris	Lumbo-sacral hemangioma
15	Deletion: 166,719,262-4qter (24.6 Mb) Duplication: 7,051,757-20pter (7 Mb)	5, F	Father and sister have balanced reciprocal 4q/20p translocation	Increased fetal nuchal translucency, microcephaly, broad nasal bridge, full cheeks, absent lower incisors, cleft palate, micrognathia (Pierre Robin sequence)	Bilateral pes planus, malpositioned 4th toes	Hypotonia	Tetralogy of Fallot	DD, speech delay, low birth weight and poor weight gain	-	Feeding difficulties	Long-sightedness and conductive hearing loss, genua valga
16	166,860,495-4qter (24.5 Mb)	2, F	Normal	Epicanthic folds, upturned nose, receding chin	Hypoplastic 5th finger, overlapping toes	-	VSD	DD, wt and ht normal	-	-	Prematurity, sacral dimple
17	176,754,691-4qter (14.5 Mb)	4, F	-	High forehead, facial asymmetry, almond shaped eyes, coloboma, upturned	-	Hypotonia, panhypo-pituitarism	Cardiac arrhyth-mia	DD, growth hormone deficiency, normal growth on hormone treatment	-	GER	Near-sightedness, DDH

				nose, low-set ears, cleft soft palate, macroglossia							
18	4q deletion: 180,707,438-190,490,075 (9.78 Mb) 4q duplication: 65,376,915-67,787,670 (2.41 Mb)	7, F	Father: balanced chromosome 4 translocation	Glabellar naevus flammeus , eyelid abnormality, thin lips, macroglossia, delayed tooth eruption	Small hands and feet, hypermobile finger joints, brittle finger and toe nails, overlapping toes	-	CHD (nos)	LD, speech delay, wt and ht <3%	Shyness	Feeding difficulties in infancy	Long-sightedness, hearing impairment, asthma
19	4q deletion: 186,766,425-191,025,415 (4.26 Mb) 4p deletion (Wolf-Hirschhorn Region): 62,447-671,791 (609 kb)	8.5, F	-	Microcephaly, craniosynostosis, "Greek warrior helmet" appearance, prominent glabella, frontal bossing, hypertelorism, broad beaked nose, upslanting palpebral fissures, bilateral epicanthic folds, short philtrum, cleft lip, thin upper lip, high palate, crowded teeth, slight retrognathia, small protruding ears	Tapering fingers, reduced palmar creases, right thumb anomaly, clinodactyly of the 5th fingers, syndactyly of 2nd and 3rd fingers	-	-	DD, LD, severe speech delay, growth deficiency, truncal obesity	-	Solitary kidney	Hyper-pigmented skin on neck and face, wide-spaced nipples
20	186,770,069-187,234,800 (464 kb)	3, F	-	Macrocephaly, hypertelorism, overfolded ear helix, frontal bossing, large frontanelles, broad nasal bride, upturned nose, cleft lip, submucous cleft palate, macroglossia, missing teeth, midline cleft of tongue, tongue hamartomas, multiple frenulae	Brachydactyly, clinodactyly of 2nd and 5th fingers, small hands and feet	VM, hypotonia	PVS, PDA, ASD, VSD	-	-	GER	Recurrent rhinitis

yrs, years; F, female; M, male; DD, developmental delay; VSD, ventricular septal defect; LD, learning disability; VM, ventriculomegaly; MRI, magnetic resonance imaging; CT, computed tomography; HIE, hypoxic ischemic encephalopathy; VUR, vesicoureteric reflux; wt, weight, GER, gastroesophageal reflux; ht, height; IQ, intelligence quotient; ADHD, attention deficit hyperactivity disorder; UTI, urinary tract infection; A-C, Arnold-Chiari; COA, coarctation of aorta; PDA, patent ductus arteriosus; DDH, developmental dysplasia of hips; CHD, congenital heart defect; PVS, pulmonary valve stenosis; ASD, atrial septal defect; '-', 'not present'.

Table II. Comparison of the clinical characteristics found in this study with those of the 101 patients reviewed by Strehle et al., 2003

Characteristics	Our study (% ± C.I.)	Review (% ± C.I.)
Sample size	n=20	n=101
Male/Female Ratio	0.43 (6/14)	0.91 (48/53)
Abnormal Parental Chromosomes	4 (44% ± 22%)[1]	13 (14% ± 7%)[4]
Developmental Delay	18 (95% ± 10%)[2]	77 (94% ± 5%)[5]
Growth Failure	9 (47% ± 21%)[3]	56 (60% ± 10%)[6]
Craniofacial Anomalies	20 (100% ± 0%)	100 (99% ± 2%)
Cleft Lip	2 (10% ± 13%)	N/A
Cleft Palate	5 (25% ± 19%)	37 (37% ± 9%)
Central Nervous System Defects	8 (40% ± 21%)	34 (34% ± 9%)
Ocular Defect	8 (40% ± 21%)	44 (44% ± 10%)
Hearing Defect	3 (15% ± 16%)	16 (37% ± 9%)[7]
Digital Anomalies	14 (70% ± 20%)	89 (88% ± 6%)
Skeletal and Extremity Defects	10 (50% ± 22%)	54 (54% ± 10%)
Muscular Defect	11 (55% ± 22%)	45 (45% ± 10%)
Cardiovascular Defect	10 (50% ± 22%)	50 (50% ± 10%)
Respiratory Tract Anomaly	3 (15% ± 16%)	32 (32% ± 9%)
Dental Defect	6 (30% ± 20%)	18 (18% ± 7%)
Gastrointestinal Tract Anomaly	8 (40% ± 21%)	40 (40% ± 10%)
Endocrine Defect	3 (15% ± 16%)	6 (6% ± 5%)
Renal and Urinary Anomalies	2 (10% ± 13%)	19 (19% ± 8%)
Genital Anomaly	4 (20% ± 18%)	28 (28% ± 9%)
Skin/Hair Defect	7 (35% ± 21%)	43 (43% ± 10%)
Behavior problems	6 (30% ± 20%)	N/A

[1] Parental chromosomes available on 9 cases
[2] Developmental history applicable on 19 cases
[3] Growth history applicable on 19 cases
[4] Parental chromosomes available on 90 cases
[5] Developmental history applicable on 82 cases
[6] Growth history applicable on 94 cases
[7] Hearing evaluation available on 43 cases

Table III. Deleted genes and their functions in patients with 4q deletion syndrome

Patient	Deleted gene	Relevant Function	Literature	Phenotype
1 and 2	*Bone Morphogenetic Protein 3* (*BMP3*)	Belongs to the transforming growth factor-beta superfamily of regulatory molecules.	Regulates cartilage cell proliferation [Gamer et al., 2008].	Short stature and skeletal anomalies.
1 and 2	*Sec31a*	Component of the Coat Protein Complex II (COPII) dependent collagen secretion.	Important for normal craniofacial development [Stagg et al., 2008].	Abnormal craniofacial development.
2 and 3	*Mitogen-Activated Protein Kinase 10 (MAPK10)*	Important role in neuronal apoptosis.	Disruption in a *de novo* balanced translocation was seen in a patient with pharmacologically resistant epileptic encephalopathy [Shoichet et al., 2006].	Epilepsy.
2	*Polycystin-2* (*PKD2*)	Renal tubule development, morphology and function. Encodes membrane polycystin protein which interacts with polycistine 1.	Modulates intracellular calcium and other signal transduction pathways [Wu et al., 2002]. Heterozygous mutations in *PKD1* and *PKD2* are associated with AD polycystic disease kidney [Mochizuki et al., 1996; Harris et al., 2009].	Unilateral polycystic renal dysplasia.
3 and 4	*Dentin Matrix Acidic Phosphorprotein 1 (DMP1)*	Extracellular matrix protein.	Mutations in *DMP1* are associated with AR hypophosphatemia characterized by rickets and osteomalacia [Feng et al., 2006]. Mice with a *DMP1* deletion show decreased bone matrix development. Carriers do not show evidence of disease.	Growth deficiency.
3 and 4	*Integrin-Binding Sialoprotein (IBSP)*	Bone matrix protein.	Involved in differentiation of osteoblasts and bone matrix development [Ogata, 2008].	Growth deficiency.
5	*TachykininRreceptor 3* (*TACR3)*	Encodes a receptor for tachykinin neurokinin 3.	Homozygous mutations are associated with congenital gonadotrophin deficiency and puberty failure [Topaloglu et al., 2009].	
7-10	*Neurogenin 2 (NEUROG2)*	Member of neurogenin subfamily of basic helix-loop-helix transcription factor genes. Plays an important role in neurogenesis from migratory neural crest cells.	Controls neurogenesis in the embryonic cortex and induces expression of GTP-binding protein Rnd2 in newly generated mouse cortical neurons prior to migration [Heng et al., 2008].	Neurological findings (hypotonia, seizures, dystonia, delayed motor development).
7-10	Ankyrin 2(*ANK2)*	Linking integral membrane proteins to the underlying spectrin-actin cytoskeleton. Plays key role in cell motility, activation, proliferation, contact and the maintenance of specialized membrane domains. Protein is required for targeting and stability of Na^+/Ca^{2+} exchanger in cardiomyocytes.	A loss-of-function mutation (E1425G) in *ANK2* causes dominantly inherited type 4 long-QT cardiac arrhythmia in humans [Mohler et al., 2003].	Arrhythmia (screening of patients recommended).
7, 8,10	*Protease Serine, 12 (PRSS12)*	Member of the neurotrypsin family of serine proteases.	*PRSS12 m*utations are associated with AR mental retardation [Molinari et al., 2002]. *Drosophila* studies suggest involvement in structural reorganizations associated with learning and memory [Didelot et al., 2006].	Learning disability and developmental delay.
7, 8,10	*Phosphodiesterase 5A (PDE5A)*	Hydrolyzes cGMP to 5'-GMP.	Regulation of intracellular concentrations of cyclic nucleotides, important for smooth muscle relaxation in the cardiovascular system [Sebkhi et al., 2003].	Congenital heart defects (VSD).

7, 8,10	*Fibroblast Growth Factor 2 (FGF2)*	A mitogenic, angiogenic, and neurotrophic factor expressed at low levels in many tissues and cell types; it reaches high concentrations in brain and pituitary gland. Implicated in diverse biological processes such as limb and nervous system development, wound healing, and tumor growth.	Participates in controlling fate, migration, and differentiation of neuronal cells [Dono et al., 1998]. Homozygous knockout mice had abnormalities in cytoarchitecture of the neocortex, most pronounced in the frontal motor-sensory area [Ortega et al., 1998]. *FGF2* helps to determine bone mass as well as bone formation [Montero et al., 2000]. Controls cardiogenic differentiation [Rosenblatt-Velin et al., 2005].	May have an impact on CNS, limb and cardiovascular abnormalities.
11	*Protocadherin 10* and *18* (*PCDH10, PCDH18*	Encodes a cadherin-related neuronal receptor.	Establishment and function of specific cell-cell connections in the brain [Kim et al., 2007]	Neurological findings (delayed motor development, hypotonia).
12	*Hedgehog Interacting Protein (HHIP)*	Regulatory component of the hedgehog signaling pathway. Evolutionarily conserved protein, involved in many fundamental processes in embryonic development, including anteroposterior patterns of limbs and regulation of left-right asymmetry.	Heterozygous mutations result in brachydactyly type 1 [Gao et al., 2009].	Digital anomalies (bilateral polydactyly, clinidactyly of toes).
12	*Steroid Sulfatase(STS)* (X chromosome)	Membrane-bound microsomal enzyme, hydrolyzes several 3-beta-hydroxysteroid sulfates a metabolic precursors for estrogens, androgens, and cholesterol.	Causes X-linked ichthyosis	Severe ichthyosis.
15	7 MB duplication on chromosome 20p	Unknown function.	AR nonsyndromic hearing impairment [Tlili et al., 2008].	May be relevant to conductive hearing loss.
13-16	*Heart And Neural Crest Derivatives expressed 2 (HAND2)*	Basic helix-loop-helix family of transcription factors Expressed in the developing ventricular chambers.	Essential role in cardiac morphogenesis; implicated as mediators of congenital heart disease [Morikawa and Cserjesi, 2008]. May play a role in limb and branchial arch development [Liu et al., 2009].	Congenital heart defects (VSD, coarctation of aorta, cardiomegaly, Tetralogy of Fallot).
13 and 14	*Vascular Endothelial Growth Factor C (VEGFC)*	Platelet-derived growth factor/vascular endothelial growth factor; active in angiogenesis and endothelial cell growth. Osmosensitive, hypertonicity-driven gene; intimately involved in salt-induced hypertension.	VEGFC signaling in phagocytes is a major determinant of extracellular volume and blood pressure homeostasis [Machnik et al., 2009].	Glabellar hemangioma.
13-18 and 20	*SORBS2*	Sorbin and SH3 domain containing 2 protein present in epithelial and cardiac muscle cells.	Adapter protein to assemble signaling complexes linking ABL kinases and actin cytoskeleton [Hand et al., 2005].	Clefts and congenital heart defects.

AD, autosomal dominant; AR, autosomal recessive; CNS, central nervous system; VSD, ventricular septal defect.