CLINICAL REPORT

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Fulminant Hepatic Failure Requiring Liver Transplantation in 22q13.3 Deletion Syndrome

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We report on a 4-year-old girl with severe developmental delay, absent speech, and chromosome 22q13.3 deletion (Phelan–McDermid syndrome), karyotype 46,XX.ish del(22)(q13.31qter) (ARSA-,N85A-,SHANK3-). At the age of 3 years, she needed an emergency liver transplantation because of fulminant hepatic failure, most likely caused by hyperacute autoimmune hepatitis triggered by a viral infection. This is the second report of a patient with 22q13.3 deletion and fulminant liver failure. By array-CGH we identified in this patient a 5.675 Mb terminal deletion (22q13.31 → qter; including ~55 genes; from *NUP50* to *RABL2B*) and in the previous patient a 1.535 Mb deletion (22q13.32 → qter; including ~39 genes; from *BRD1* to *RABL2B*). *PIM3* is a prime candidate gene for the fulminant hepatic failure in the two patients; *SHANK3*/*PROSAP2* could be another candidate gene. We recommend liver function tests and array-CGH in the management of patients with Phelan–McDermid syndrome. This patient showed a developmental catch-up following the liver transplantation, possibly suggesting that chronic hepatic disease could contribute to the developmental delay in a subset of these patients.

Key words: 22q13.3 deletion syndrome; fulminant hepatic failure; liver transplantation; *PIM3*; *SHANK3*/*PROSAP2*; *PPARA*

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INTRODUCTION

The 22q13.3 deletion syndrome or Phelan–McDermid syndrome (OMIM 606232) is characterized by absent or severely delayed expressive speech, hypotonia, global developmental delay, mildly abnormal craniofacial features, normal or advanced growth, and autistic behavioral traits [Nesslinger et al., 1994; Phelan et al., 2001; Havens et al., 2004; Cohen et al., 2005]. The deletions vary widely in size from 100 kb to >9 Mb [Anderlid et al., 2002; Wilson et al., 2003] and are predominantly of paternal origin (69–74%) [Luciani et al., 2003; Wilson et al., 2003]. The minimum deletion region includes the most distal genes on 22q, *SHANK3*/*PROSAP2*, *ACR*, and *RABL2B* [Luciani et al., 2003]. A recent case report of autoimmune hepatitis (AIH) in a girl with a 22q13 deletion suggested a link with the development of autoimmune disorders [Tufano et al., 2009]. Meanwhile 21 months after liver transplantation under maintenance therapy with tacrolimus (blood levels 3–5 ng/ml) and low doses of prednisone, this girl had a relapse of AIH with increased aminotransferase serum levels (AST 167 U/L; ALT 232 U/L) and antibodies against liver–kidney microsomal type 1 (LKM1). Liver biopsy of graft showed interface hepatitis, perivenular cell necrosis, bridging fibrosis, and collapse without the typical changes of an acute or chronic rejection. Treatment with high doses of prednisone (2 mg/kg/day) and azathioprine (1.5 mg/kg/day) led to prompt normalization of liver enzymes.

Here, we report on a second girl with 22q13.3 deletion who needed a split liver transplantation at the age of 3 years after a viral and/or AIH and fulminant hepatic failure. The deletion comprised the terminal 5.675 Mb of 22q, including the genes from *NUP50* to *RABL2B*. The report confirms that a subset of patients with 22q13.3 deletion are at risk of hyperacute liver failure. Therefore, we also

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determined the deletion interval in the previous patient [Tufano et al., 2009] and identified *PIM3* as a prime candidate gene for the fulminant hepatic failure in both patients.

CLINICAL REPORT

The proposita is the first child of healthy, non-consanguineous German parents with a healthy younger daughter and uninformative family history. Following an uneventful pregnancy, she was born at term with normal measurements [weight 3,170 g, length 53 cm, and occipitofrontal circumference (OFC) 33 cm]. Apgar scores were 9 and 10 at 1 and 5 min. Postnatal adaptation was normal, but developmental milestones were delayed. She could crawl at the age of 1 year and walk at $1^{3}/_{12}$ years. At $2^{3}/_{12}$ years muscular hypotonia, a positive Galant reflex, broad-based gait, deficient fine motor and coordination skills, and absent speech were noted. She avoided eye contact and was easily irritated and anxious. An electroencephalogram (EEG) was normal. At $3^{3}/_{12}$ years, findings included moderate-to-severe developmental retardation, severely disturbed sensory perception, and reduced sensitivity to pain. She still had no speech, but could duplicate syllables. A diagnosis of Rett syndrome was considered but rejected, and parents did not wish genetic testing at that time. Laboratory findings, especially liver enzymes and blood counts were always normal.

At the age of $3^{10}/_{12}$ years, she suffered from fulminantly progressive liver failure requiring split liver transplantation (at University of Heidelberg, Germany). A week before, she had an upper airway infection with subfebrile temperatures, fatigue, and scleric icterus. Laboratory results at emergency admission to the pediatric intensive care unit were as follows: ALT > 1,000 U/L, AST > 1,000 U/L, total bilirubin 11 mg/dl, Quick 20%, and NH_3 79 µmol/L. Following rapid deterioration, increasing coagulation failure despite infusion of fresh frozen plasma, edema, floppiness, severe gum bleeding, and beginning hepatic encephalopathy, she was referred within hours for emergency liver transplantation (split segments 2 and 3). Laboratory results before transplantation included INR 2, AT III 20%, activated partial thromboplastin time >50 sec, and ANA 1/640. Serologic tests for hepatitis A, B, C, D, and E viruses, Epstein–Barr virus, HHV-6, varicella virus, enterovirus, and cytomegalovirus were negative. There was no history of drug intake or of metabolic disorders; ceruloplasmin and copper serum levels were normal. Liver biopsy was not performed because of the severe coagulopathy. Based on the clinical and laboratory findings, ANA-associated (type 1) AIH was suspected. Histologic evaluation of the explant liver showed subacute liver dystrophy with near-total loss of liver tissue, lobular necroses, chronic inflammatory reaction, and already considerable fibrosis. Only sparse hepatocytes remained, most of them ballooned and filling less than 5% of the organ. Fulminant hepatic failure in the course of non-A–non-E hepatitis was diagnosed. According to the criteria of the International Autoimmune Hepatitis Group the diagnosis of AIH was probable (score 14), specifically type-1 AIH [Alvarez et al., 1999]. Furthermore, on the basis of the recent history of upper airway infection and postoperative detection of HSV-I and influenza A virus in tracheal secretion, it is likely that the hepatitis could have been triggered by a viral infection.

Following the transplantation and initiation of immunosuppressive treatment, the patient returned home healthy with normal liver function. When referred for genetic testing at the age of $4^{3}/_{12}$ years, her height was 97 cm (3rd centile), weight 15 kg (25th centile), and OFC 49.8 cm (25th–50th centile). She had normal facial morphology, mild immunosuppression-derived hirsutism, non-irritant scars on her abdomen, and normal female genitalia. Her extremities were normal and fully mobile, but her big toes and the left little toe exhibited dystrophic and discolored toenails. Speech was absent. She showed reduced fine motor skills, slightly diminished coordination, and anxiety, preferring familiar activities. The parents noted global developmental catch-up of their daughter beginning immediately after the transplantation, and despite immunosuppressive treatment. Findings included marked improvement particularly in social interaction, sequential planning, and imitation of complex movements, and were confirmed by her Pediatric Neurologist, who also reported febrile seizures and generalized epilepsy from age 5 years, which was controlled by levitiracetam.

CYTOGENETIC STUDIES AND RESULTS

Cytogenetic Study, Fluorescence In Situ Hybridization (FISH) and Multiplex Ligation-Dependent Probe Amplification (MLPA)

Chromosomal analysis using GTG banding showed a normal 46,XX karyotype at 450 band resolution. Deletion of the subtelomeric region of 22q was detected by MLPA with the P036 and P070 subtelomere screening kits (MRC Holland, Amsterdam, The Netherlands), and confirmed by two-color FISH with probes for 22q11.2 and 22q13.3 (Cytocell, Cambridge, UK; not shown).

Array Comparative Genomic Hybridization (Array-CGH)

Molecular chromosome analysis of this patient was first performed using an oligonucleotide-based array platform (244K chip, Agilent Technologies, Santa Clara, CA), which has an 8.9 kb median probe spatial resolution. Results were visualized with Agilent's Feature Extraction 10.1 Image Analysis software and the CGH PRO software (Max Planck Institute for Molecular Genetics, Berlin, Germany). We then studied both this and the previously published patient [Tufano et al., 2009] using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA) with ~1,850,000 probes and an average distance of 1.3 kb between neighboring probes. Analyses were performed with the GeneChip Genome Wide SNP Sty Assay Kit 5.0/6.0 (Affymetrix) and the Genotyping Console 3.0.1 (Affymetrix). Results indicated deletion of the terminal 5.675 Mb (22q13.31 → qter; including ~55 genes; *NUP50* to *RABL2B*) in this patient (Fig. 1A), and of 1.535 Mb (22q13.32 → qter; including ~39 genes; *BRD1* to *RABL2B*) in the previously published patient [Tufano et al., 2009] (Fig. 1B).

DISCUSSION

The clinical, cytogenetic and molecular studies indicate that the proposita had a 22q13.3 deletion syndrome with typical physical

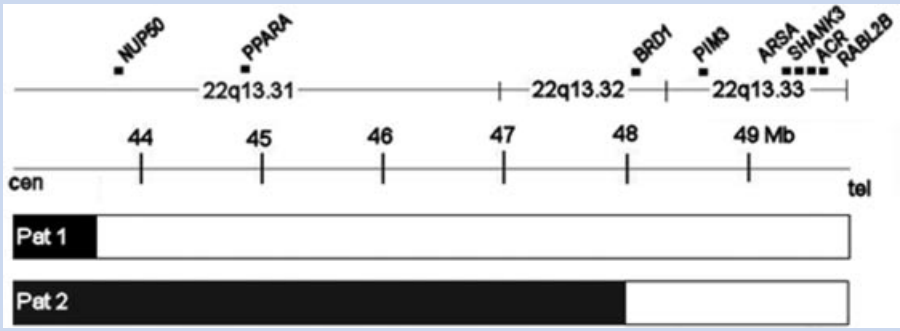

FIG. 1. Schematic representation of the chromosome 22 deletion intervals in the two patients. The deleted regions extend (A) in this patient from 43,906,513 bp to the telomere (22q13.31 → qter) and (B) in the previous patient from 48,046,496 bp to the telomere (22q13.32 → qter). The area of deletion overlap comprises the terminal 1.535 Mb of 22q13.3.

and behavioral features. Sudden liver failure occurred approximately 1 week after a common cold, most likely due to a severe AIH promoted by a HSV-I and/or influenza A virus infection. It is well known that these viruses can cause acute hepatitis and liver failure [Eron et al., 1976; Meerbach et al., 2006; Whitworth et al., 2006]. This is the second case of 22q13.3 deletion syndrome associated with fulminant AIH [Tufano et al., 2009].

This report including the recent recurrence of AIH in the previously published patient provides additional evidence for a predisposition to AIH and liver failure in a subset of these patients. The Phelan–McDermid syndrome has been associated with frequent respiratory infections and a susceptibility to viral infections [Havens et al., 2004], but it is yet unknown if and how this could be related to sudden liver failure.

This and the previously published patient [Tufano et al., 2009] share a deletion region of 1.535 Mb (Fig. 1) containing 39 genes, from *BRD1* to *RABL2B*. Of the deleted genes notably *PIM3* has been associated with liver disease before; in particular with the repair of hepatocyte injury [Liu et al., 2010]. *PIM3* is a member of the proto-oncogene Pim family encoding serine/threonine kinases and was reported to be aberrantly expressed in human and mouse hepatoma but not in normal liver [Fujii et al., 2005]. Downregulation of *PIM3* by RNA interference in human hepatoma cell lines was shown to result in decreased cell proliferation and increased apoptosis rate [Fujii et al., 2005]. In mice, liver-specific transgenic expression of *Pim3* was recently demonstrated to accelerate hepatocellular carcinoma development [Wu et al., 2010]. Most interestingly, another recent study concluded that the *Pim3* gene could protect rats from fulminant hepatic failure by inhibiting liver apoptosis and improving inflammatory response of liver tissues [Liu et al., 2010]. In addition, mice deficient for PIM kinases including *Pim3* showed reduced body size and impaired responses to hematopoietic growth factors [Mikkers et al., 2004]. Based on these reports, we suggest that *PIM3* haploinsufficiency contributed to the fulminant liver failure in these 22q13.3 deletion patients by bringing forward hepatitis (viral or autoimmune), mainly by prolonging or preventing recovery from hepatocyte injury.

Our findings could also support a role of SHANK3/PROSAP2 in the development of the fulminant hepatic failure in some patients with 22q13.3 deletion. Deletion of SHANK3/PROSAP2 appears to be the major cause of the neurological signs, speech defect, and autism spectrum disorders in Phelan–McDermid syndrome [Wilson et al. 2003; Havens et al., 2004; Durand et al., 2007]. Furthermore, the ProSAP/Shank proteins are expressed in thymocytes and in lymphoid organs, and may also function in the coordination of membrane receptor-dependent signal transduction in immune cells [Redecker et al., 2006]. Tufano et al. [2009] proposed that SHANK3/PROSAP2 belongs to a group of genes outside the major histocompatibility complex (MHC) which, if mutated, may lead to increased risk of immunodeficiency and autoimmune phenomena. However, the pathogenic links remain to be clarified.

Interestingly, the parents of this patient and her Pediatric Neurologist noted a marked global developmental catch-up after the liver transplantation, and despite the immunosuppressive treatment. A possible explanation could be that chronic hepatic disease had contributed to the developmental delay in this patient and was alleviated by the liver transplantation.

PPARA, which is deleted in this but not in the previous patient with AIH (Fig. 1), encodes the peroxisome proliferator-activated receptor alpha, which is important in hepatic and cardiac metabolism [Djouadi et al., 1998]. *Ppara*$^{-/-}$ knockout mice demonstrated hypoglycemia and massive lipid accumulation in liver and heart [Djouadi et al., 1998], and polymorphisms in *PPARA* have been associated with hyperapobetalipoproteinemia [Vohl et al., 2000] and hepatocellular carcinoma after HBV or HCV infection [Akkiz, 2008]. Further studies are needed to determine precisely how *PIM3*, SHANK3/PROSAP2, *PPARA*, and other genes lead to the developmental delay and the other clinical features in 22q13.3 deletion syndrome.

We recommend liver function tests in patients with deletion of 22q13.3, and we recommend the use of array-CGH in this disorder and other genomic disorders for predicting complications and improving management.

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