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[原著] Two novel mutations of the FGD1 gene in Japanese patients with Aarskog Scott syndrome

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| メタデータ | 言語:<br>出版者: 琉球医学会<br>公開日: 2010-07-02<br>キーワード (Ja):<br>キーワード (En): Aarskog-Scott syndrome, Faciogenital dysplasia 1 (FGD1) gene, novel mutations, allele-specific PCR, PCR-RFLP<br>作成者: Yanagi, Kumiko, Kaname, Tadashi, Chinen, Yasutsugu, Naritomi, Kenji<br>メールアドレス:<br>所属: |
| URL   | <a href="http://hdl.handle.net/20.500.12000/0002016197">http://hdl.handle.net/20.500.12000/0002016197</a>  |

## Two novel mutations of the *FGDI* gene in Japanese patients with Aarskog-Scott syndrome

Kumiko Yanagi<sup>1)</sup>, Tadashi Kaname<sup>1)</sup>, Yasutsugu Chinen<sup>2)</sup> and Kenji Naritomi<sup>1)</sup>

<sup>1)</sup>Department of Medical Genetics,

<sup>2)</sup>Department of Pediatrics, University of the Ryukyus Graduate School of Medicine

(Received on November 19, 2004, accepted on January 14, 2005)

### ABSTRACT

Faciogenital dysplasia 1 (*FGDI*) gene has been identified as a responsible gene for Aarskog-Scott syndrome (AAS). We characterized two novel point mutations in two Japanese families with AAS, a missense mutation in exon 11 (1906C>T, R636W) and a nucleotide transition at the first position of the 5' splice donor site of intron 14 (IVS14+1G>A). The missense mutation probably results in reduced *FGDI* function and the mutation at the splice donor site decreases *FGDI* gene expression. These mutations were identified by sequencing and were confirmed by allele-specific polymerase chain reaction (AS-PCR) or by PCR-restriction fragment length polymorphism (PCR-RFLP). The mutations were absent in twenty-five Japanese control subjects, which supports the notion that these mutations result in AAS. This study represents the first mutational analysis of *FGDI* in Japanese AAS patients. *Ryukyu Med. J.*, 23(4) 143~148, 2004

Key words: Aarskog-Scott syndrome, Faciogenital dysplasia 1 (*FGDI*) gene, novel mutations, allele-specific PCR, PCR-RFLP

### INTRODUCTION

Aarskog-Scott Syndrome (AAS), also known as faciogenital syndrome or faciodigitogenital syndrome (MIM #305400), is characterised by craniofacial dysmorphism, brachydactyly, urogenital abnormalities, and short stature<sup>1,2)</sup>. The syndrome results from mutations in the faciogenital dysplasia 1 (*FGDI*) gene, which were first described using positional cloning in a family in which the phenotype was associated with balanced X-autosomal translocation<sup>3)</sup>. Twelve different mutations of the *FGDI* gene have been reported in AAS patients, including two insertions, five missense mutations and five deletions<sup>3-6)</sup>. However, there has been no mutational analysis of Japanese patients with AAS.

Thus, the goal of the present study was to perform a mutational analysis of Japanese patients with AAS to determine if novel mutations were represented in this subpopulation. Further, we de-

scribe a simple method for detection of the mutations in the *FGDI* gene.

### PATIENTS

#### Family 1 (UR03A01)

The proband (UR03A01-1) was a 10-month-old Japanese boy born to healthy nonconsanguineous parents. The child's developmental milestones were delayed, and cerebral palsy was suspected at 9 months of age. At his initial visit to our institution at 10 months of age, all growth parameters were three standard deviations below average levels. Physical examinations revealed fine and relatively sparse scalp hairs and eyebrows; high and bossed forehead; downward slanting of the palpebral fissures; blepharoptosis of the right eye; mild divergent strabismus; epicanthal folds; short nose with anteverted nares; long philtrum; linear curve depression below the lower lip; broad hands and feet;

Table 1 Primers and condition for amplification of *FGDI* gene

| Exon                           | forward primer                | reverse primer                 | Tm(°C)                     | MgCl <sub>2</sub> (mM) | size(bp) |     |
|--------------------------------|-------------------------------|--------------------------------|----------------------------|------------------------|----------|-----|
| 1                              | 5' GCTTGAGTCTCTGCAGTGGG 3'    | 5' AGAGGTTGAAAGGACTGGACAT 3'   | 60                         | 2.5                    | 644      | FA  |
| 2                              | 5' AGTCAGTTACTTCACACCCAT 3'   | 5' GGCTCCCTATCCTTCTAACA 3'     | 55                         | 1.25                   | 319      |     |
| 3                              | 5' ATCCAGCTCACTGATGTCTC 3'    | 5' AAGCAGGGTATGAGCTTGAC 3'     | 60                         | 1.25                   | 326      |     |
| 4                              | 5' CAGGAAGGGATAGTCAGGAG 3'    | 5' GGAAGAATCAAGCACAAAAG 3'     | 60                         | 1.25                   | 591      |     |
| 5                              | 5' GGGCTTGGGTGAGGGTTACGAT 3'  | 5' GGCCCTATCACTGCCTCCTTGAAA 3' | 60                         | 1.875                  | 225      |     |
| 6                              | 5' CTCAGTCTCAAGACCAATGCT 3'   | 5' TCGGCAGGCAGGTGGACAGG 3'     | 60                         | 1.25                   | 323      |     |
| 7                              | 5' CCACCCAGGGACCGCTAT 3'      | 5' ACACTCATTGGGCATCCTTGCT 3'   | 60                         | 1.25                   | 414      |     |
| 8                              | 5' CTGGAAGGAGCAGACTTGGG 3'    | 5' CAGAACCTCAATGTGGGCCCT 3'    | 60                         | 1.25                   | 400      |     |
| 9                              | 5' TCTCTGCTAGTCCCCATCTGA 3'   | 5' CCTCCTCGCCCCCTAACA 3'       | 60                         | 1.875                  | 297      |     |
| 10                             | 5' CGTGCCTTTTGTTCCTGTCTTTT 3' | 5' GGGCATGACCCACCCACAAT 3'     | 60                         | 1.875                  | 214      |     |
| 11                             | 5' ACATCCCCACTAGGCCCTCTGC 3'  | 5' TTCCTCCCAACACCAATGC 3'      | 60                         | 1.25                   | 203      |     |
| 12                             | 5' CCTCACCATGCCCTTTCTGC 3'    | 5' TCTGGGCCTGGAATGCCTCAG 3'    | 60                         | 1.25                   | 190      |     |
| 13                             | 5' CCCAGGTCTTTCTGACTCCA 3'    | 5' CTCACCTTATACACCCTCAG 3'     | 60                         | 1.25                   | 364      |     |
| 14                             | 5' ACGAAGGTGAGGCAGGGGTAGA 3'  | 5' GGTGAGGTGGGCATTTGGAAGT 3'   | 60                         | 1.875                  | 245      | PM  |
| 15                             | 5' CCCCATGATAATCCAAGCGT 3'    | 5' TCTTCCCTCAGCATACCAACTCC 3'  | 60                         | 1.875                  | 356      |     |
| 16                             | 5' AAGTCTGCTGTGGGAGTTGG 3'    | 5' GTCTCACTGGGTAGAGTTGG 3'     | 60                         | 1.25                   | 306      |     |
| 17                             | 5' GGGTGGCACTGGACAAATCA 3'    | 5' TTCCAAGGCCAAGGAGAGGT 3'     | 60                         | 1.25                   | 376      |     |
| 18                             | 5' AAGGTGGCCCCAGCTCTGT 3'     | 5' CCCCCTGTTTCCTGTCTCTG 3'     | 62                         | 1.25                   | 908      | PM  |
| allele specific forward primer |                               |                                |                            |                        |          |     |
| normal allele                  | 5' TGGCCAGAAGTTTAGCGTACG 3'   |                                | 60                         | 1.25                   | 108      |     |
| mutant allele                  | 5' TGGCCAGAAGTTTAGCGTATG 3'   |                                |                            |                        |          |     |
| primers for RT-PCR             |                               |                                |                            |                        |          |     |
| TK03001                        | 5' ACTGAGAAGGCCTACGTTTC 3'    | TK03002                        | 5' TGAGGTATCGGTCTTGAGTG 3' | 58                     | 1.25     | 688 |
| G3PDH                          | 5' AATCCCATCACCATCTTCCA 3'    |                                | 5' CCAGGGTCTTACTCCTTG 3'   | 60                     | 1.875    | 790 |

Mismatched A in allele-specific forward primers are underlined. The mutation in family 1 (1906C>T) is indicated as bold. FA, formamide; PM, Perfect Match PCR enhancer.

short fingers; interdigital webbing; and shawl scrotum. Based on these features, AAS was considered appropriate for diagnosis.

The second patient (UR03A01-2) was a 5-month-old younger brother of the proband. The child was referred to our institution because he possessed features and complaints similar to his brother. His growth parameters were also three standard deviations below average levels, and he displayed nearly the same phenotype as the proband. Thus, this patient was also diagnosed with AAS.

#### Family 2 (UR03A02)

The proband (UR03A02-1) was an 8-year-old Japanese boy born to healthy nonconsanguineous parents. Bilateral camptodactylies of the fourth and fifth fingers were noted at birth. The patient underwent operative correction of blepharoptosis at 2 years of age. Upon his initial visit to our institution at 8 years of age, the patient's height was 113 cm (−2.6 SD), weight was 21.2 kg (−1.1 SD), and occipital-frontal circumference (OFC) was 48.5 cm (−2.2 SD). Physical examinations revealed multiple dysmorphic features, including hypertelorism, blepharoptosis, epicanthal folds, prominent ears,

downward slanting of the eyebrows, wide nasal bridge with anteverted nares, linear curve depression below lower lip, webbed neck, interdigital webbing, mild syndactyly, broad hands and feet, bulbous toes, and shawl scrotum. Based on these features, a diagnosis of AAS was made.

The second patient (UR03A02-2) was a 1-year-old younger brother of the proband. He displayed virtually the same phenotype as his brother except for the absence of camptodactyly and syndactyly. Instead, he had characteristic hyperextensible proximal interphalangeal joints and flexion of the distal interphalangeal joints.

## MATERIALS AND METHODS

### Blood samples

The study protocol was performed in accordance with the standards for informed consent of the Ethics Committee of University of the Ryukyus Graduate School of Medicine (Okinawa, Japan). Peripheral blood samples were obtained and analyzed from the two Japanese families (UR03A01 and UR03A02) and twenty-five healthy Japanese subjects.

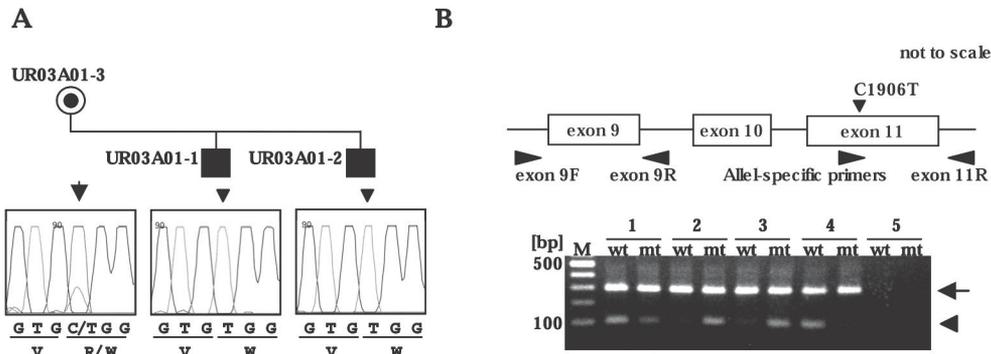


Fig. 1 The 1906C>T mutation in exon 11 of the *FGD1* in family 1 (UR03A01).

A, DNA sequences and predicted amino acid residues of exon 11 in the mother (UR03A01-3) and affected patients (UR03A01-1 and UR03A01-2). The point mutations are indicated by arrowheads (patients) and arrow (mother). B, AS-PCR for detection of the mutation 1906C>T. The schematic diagram of the intron/exon boundary around exon 11 and the primers are indicated in the upper panel. A product of internal control using a primer pair for exon 9 is indicated with an arrow, and the allele-specific band is indicated with an arrowhead. M, 100 bp ladder; lane 1, UR03A01-3; lane 2, UR03A01-1; lane 3, UR03A01-2; lane 4, normal control; lane 5, negative control (dH<sub>2</sub>O); wt, primers for the normal allele; mt, primers for the mutant allele.

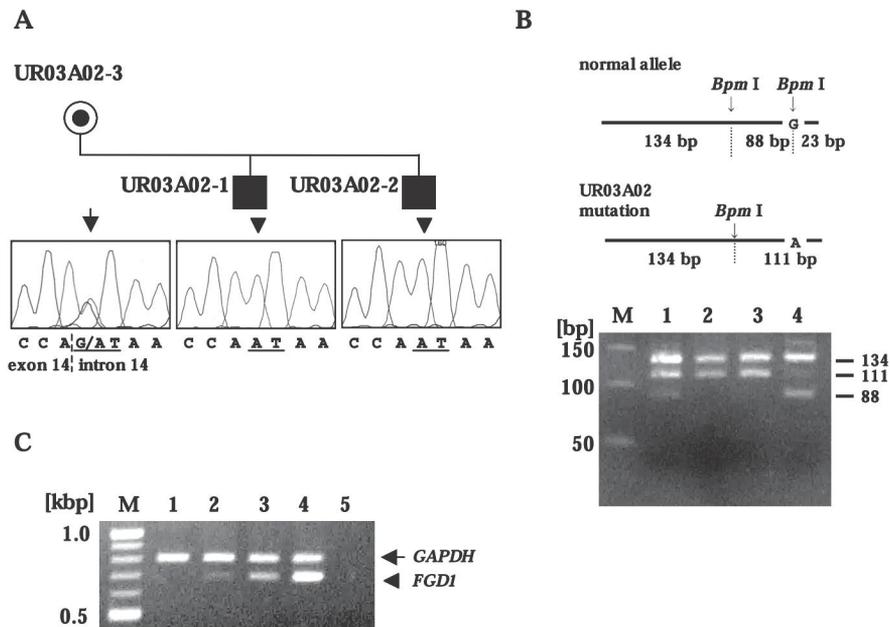


Fig. 2 The point mutation at the splice donor site in intron 14 (IVS14+1G>A) in family 2 (UR03A02).

A, DNA sequences at the splice junction in intron 14. The G to A transitions are indicated by an arrowhead (patients, UR03A02-1 and UR03A02-2) and an arrow (mother, UR03A02-3). B, Schematic representation and agarose gel showing the feasibility of the PCR-RFLP for detection of the mutation. Digestion with *Bpm* I yields three fragments (134, 88 and 23 bp) in the normal allele (lane 4) and yields two fragments (134 and 111 bp) in the mutant allele (lane 1 for UR03A02-3, lane 2 for UR03A02-1, lane 3 for UR03A02-2). M, 50 bp ladder C, RT-PCR for *FGD1* expression in the patients. *GAPDH* expression was separately investigated as an internal control. Both products were loaded on the same gel. Lane 1, UR03A02-1; lane 2, UR03A02-2; lane 3, UR03A02-3; lane 4, normal control; lane 5, negative control (dH<sub>2</sub>O).

DNA isolation and sequence analysis

Genomic DNA was extracted from peripheral blood using a standard protocol<sup>7)</sup>. The intron/exon

boundary regions of the *FGD1* gene were amplified by PCR using the primers described in Table 1. PCR reactions were performed in 10  $\mu$ l of final volume

containing 25 ng of genomic DNA, 1xPCR buffer (TAKARA), 0.25 mM dNTPs, 0.25 units Ex-Taq polymerase (TAKARA) and 1  $\mu$ M of each primer. The concentration of  $MgCl_2$  and annealing temperature were optimized for each reaction (Table 1). Exon 1 was amplified in a reaction containing 2 % formamide. One unit of Perfect Mach PCR enhancer (Stratagene, CA, USA) was added in the reactions to increase the specificity of exon 14 and 18 amplification. Thirty-two cycles were performed, and the PCR products were directly analyzed by the cycle sequencing method using a DyeTerminator cycle sequencing kit (ABI, CA, USA) and an ABI 310 sequencer (ABI, CA, USA).

#### Allele-specific polymerase chain reaction (AS-PCR)

The mutation characterized in family 1 (UR03A01) was confirmed by AS-PCR. Allele-specific primers were designed as forward primers. To increase the specificity of the AS-PCR primers, an additional mismatch (G to A) was deliberately introduced at the 3rd base from the 3' ends of each primer (Table 1)<sup>8)</sup>. Amplification was performed with 25 ng genomic DNA, 1xPCR buffer supplemented with 0.125 mM dNTPs, 1.25 mM  $MgCl_2$ , 0.5 units Ex-Taq polymerase (TAKARA), and 0.5  $\mu$ M of each primer. A primer pair (0.25  $\mu$ M) was added for exon 9 as an internal control.

#### PCR-restriction fragment length polymorphisms (PCR-RFLP)

PCR-RFLP was performed to detect the mutation characterized in family 2 (UR03A02). PCR products were digested with restriction enzyme, *Bpm* I (NEB, Hertfordshire, UK) and then run on a 3 % NuSieve agarose gel (TAKARA) with ethidium bromide.

#### RT-PCR

Total RNA was extracted from peripheral blood, and cDNA was synthesized using the SUPERScript II preamplification system (Invitrogen, CA, USA). PCR was performed with cDNA generated from 15 ng of total RNA and with the TK03001 and TK03002 primer pair (Table 1). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was amplified as an internal control.

## RESULTS AND DISCUSSION

Two different mutations were identified in the

two Japanese families with AAS.

In family 1 (UR03A01), a missense mutation was found in exon 11 (1906C>T) of *FGDI*, resulting in substitution of tryptophan for arginine at position 636 (R636W) (Fig. 1A, arrowhead). The affected patients' mother was heterozygous for the mutation (Fig. 1A, arrow). The arginine affected by the mutation is located in the pleckstrin homology (PH) domain that is essential for guanine nucleotide exchange factor (GEF) activity<sup>9-11)</sup>. The missense mutation could result in reduced GEF activity.

Allele-specific PCR was performed to confirm the 1906C>T mutation (Fig. 1B). The control primer pair targeted to exon 9 generated a 295-bp PCR product, while the allele-specific primer pair (wt for the normal allele and mt for the mutant allele) generated a 111-bp product. The allele-specific primers for the normal allele did not generate PCR products (Fig. 1B, lane 2 and lane 3, wt) but did generate the products for the mutant allele in AAS patients (Fig. 1B, lane 2 and lane 3, mt). Both pairs of primers generated PCR products in the mother (Fig. 1B, lane 1, wt and mt). In contrast, only PCR products for the normal allele were detected in normal control (Fig. 1B, lane 4).

In family 2 (UR03A02), a nucleotide transition was identified at the first position of the splice donor site in intron 14 (IVS14+1G>A) (Fig. 2A, arrowhead). As a result, the intron 14 in the mutant allele possessed a 5'-AT as a splice donor site (Fig. 2A, underline) and an AG-3' as a splice acceptor site (data not shown). Because the IVS14+1G>A transition was predicted to result in destruction of the *Bpm* I site, PCR-RFLP analysis was used to detect the mutation (Fig. 2B). In the normal allele, two *Bpm* I sites were present in the PCR product amplified with the primer pair for exon 14, yielding three fragments (134, 88 and 23 bp). The 23 bp fragment was too faint to be detected by ethidium bromide staining. In contrast, in the mutant allele, two fragments (134 and 111 bp) were generated. Digestion of the PCR product with *Bpm* I showed patterns of both the normal and mutant allele in the mother (Fig. 2B, lane 1).

A G to A transition at the first nucleotide of splice donor site inhibits splicing in vitro<sup>12,13)</sup>. This type of mutation in the human  $\beta$ -globin gene and the myosin VII A gene inhibits normal splicing and causes  $\beta$ -thalassemia<sup>14)</sup> and Usher syndrome<sup>15,16)</sup>,

respectively. RT-PCR for the *FGDI* gene also showed markedly reduced expression in affected patients (Fig. 2C). This is the first study to demonstrate a mutation detected at a splicing donor site of the *FGDI* gene.

AS-PCR and PCR-RFLP were also performed using genomic DNA obtained from normal Japanese subjects. The two point mutations were not present in twenty-five normal control subjects. We conclude that the two novel mutations characterized in the present study result in loss of function of the *FGDI* gene, similar to the 12 previously characterized mutations of the *FGDI* gene<sup>6)</sup>.

### ACKNOWLEDGEMENTS

We thank Takako Kouchi for excellent technical assistance. This work was supported in part by grants from the Ministry of Education, Science, Technology and Culture of Japan.

### REFERENCES

- 1) Aarskog D.: A familial syndrome of short stature associated with facial dysplasia and genital anomalies. *J. Pediatrics*. 77: 856-861, 1970.
- 2) Scott C.I.: Unusual faces, joint hypermobility, genital anomaly and short stature: a new dysmorphic syndrome. *Birth Defects Orig. Artic. Ser.* 7: 240-246, 1971.
- 3) Pasteris N.G., Cadle A., Logie L.J., Porteous M.E., Schwartz C.E., Stevenson R.E., Glover T.W., Wilroy R.S. and Gorski J.L.: Isolation and characterization of the faciogenital dysplasia (Aarskog-Scott syndrome) gene: a putative Rho/Rac guanine nucleotide exchange factor. *Cell*. 79: 669-678, 1994.
- 4) Schwartz C.E., Gillessen-Kaesbach G., May M., Cappa M., Gorski J.L., Steindl K. and Neri G.: Two novel mutations confirm *FGDI* is responsible for the Aarskog syndrome. *Eur. J. Hum. Genet.* 8: 869-874, 2000.
- 5) Orrico A., Galli L., Falciani M., Bracci M., Cavaliere M.L., Rinaldi M.M., Musacchio A. and Sorrentino V.: A mutation in the pleckstrin homology (PH) domain of the *FGDI* gene in an Italian family with faciogenital dysplasia (Aarskog-Scott syndrome). *FEBS Lett.* 478: 216-220, 2000.
- 6) Orrico A., Galli L., Cavaliere M.L., Garavelli L., Fryns J.P., Crushell E., Rinaldi M.M., Medeira A. and Sorrentino V.: Phenotypic and molecular characterisation of the Aarskog-Scott syndrome: a survey of the clinical variability in light of *FGDI* mutation analysis in 46 patients. *Eur. J. Hum. Genet.* 12: 16-23, 2004.
- 7) Kaname T., Matsubara S., Murata F., Yamamura K., Miyata K. and Muramatsu T.: The upstream sequence of a new growth/differentiation factor midkine (MK), mediates developmentally regulated LacZ. *Develop. Growth. Differ.* 36:231-238, 1994.
- 8) Newton C.R., Graham A., Heptinstall L.E., Powell S.J., Summers C., Kalsheker N., Smith J.C. and Markham A.F.: Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res.* 17: 2503-2516, 1989.
- 9) Obaishi H., Nakanishi H., Mandai K., Satoh K., Satoh A., Takahashi K., Miyahara M., Nishioka H., Takaishi K. and Takai Y.: Frabin, a novel *FGDI*-related actin filament-binding protein capable of changing cell shape and activating c-Jun N-terminal kinase. *J. Biol. Chem.* 273: 18697-18700, 1998.
- 10) Pasteris N.G., Gorski J.L.: Isolation, characterization, and mapping of the mouse and human *Fgd2* genes, faciogenital dysplasia (*FGDI*; Aarskog syndrome) gene homologues. *Genomics*. 60: 57-66, 1999.
- 11) Pasteris N.G., Nagata K., Hall A. and Gorski J.L.: Isolation, characterization, and mapping of the mouse *Fgd3* gene, a new Faciogenital Dysplasia (*FGDI*; Aarskog Syndrome) gene homologue. *Gene*. 242: 237-247, 2000.
- 12) Parker R. and Siliciano P.G.: Evidence for an essential non-Watson-Crick interaction between the first and last nucleotides of a nuclear pre-mRNA intron. *Nature*. 361: 660-602, 1993.
- 13) Deirdre A., Scadden J. and Smith C.W.: Interactions between the terminal bases of mammalian introns are retained in inosine-containing pre-mRNAs. *EMBO J.* 14: 3236-3246, 1995.
- 14) Treisman R., Orkin S.H. and Maniatis T.: Specific transcription and RNA splicing defects in five cloned  $\beta$ -thalassaemia genes. *Nature*. 302: 591-596, 1983.
- 15) Adato A., Weil D., Kalinski H., Pel-Or Y., Ayadi H., Petit C., Korostishevsky M. and Bonne-Tamir B.: Mutation profile of all 49

- exons of the human myosin VIIA gene, and haplotype analysis, in Usher 1B families from diverse origins. *Am. J. Hum. Genet.* 61: 813-821, 1997.
- 16) Kelley P.M., Weston M.D., Chen Z.Y., Orten D.J., Hasson T., Overbeck L.D., Pinnt J., Talmadge C.B., Ing P., Mooseker M.S., Corey D., Sumegi J. and Kimberling W.J.: The genomic structure of the gene defective in Usher syndrome type I? (*MYO7A*). *Genomics.* 40: 73-79, 1997.