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Homology of pendrin, sodium-iodide symporter and apical iodide transporter

Salvatore Benvenga[1,2,3], Fabrizio Guarneri[4]

[1]Department of Clinical and Experimental Medicine - Endocrinology, University of Messina, via Consolare Valeria - Gazzi, 98125 Messina, Italy, [2]Master Program on Childhood, Adolescent and Women's Endocrine Health, University of Messina, via Consolare Valeria - Gazzi, 98125 Messina, Italy, [3]Interdepartmental Program on Molecular and Clinical Endocrinology and Women's Endocrine Health, AOU Policlinico G. Martino, via Consolare Valeria - Gazzi, 98125 Messina, Italy, [4]Department of Clinical and Experimental Medicine - Dermatology, University of Messina, via Consolare Valeria - Gazzi, 98125 Messina, Italy

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1. ABSTRACT

We observed local homology between human pendrin and sodium/iodide symporter (NIS), that was absent in the NIS-homologous sodium/monocarboxylate transporter or apical iodide transporter (AIT) which, however, does not transport iodide. Thus, we analyzed the full proteins. They shared 63 identical and 66 similar residues (overall homology 14.4%, but 21% when omitting intervening sequences of 15 or more residues). Pendrin was more homologous to NIS (25%) than AIT (20%), particularly in the STAS domain (sulfate transporter and antisigma factor antagonist). Homology was concentrated in 11 segments, with 3/11 involving the STAS domain. In 9/11, homology was greater with NIS (45-58.3%) than with AIT (8.3-42.3%); in 4 of these 9 segments, homology was comparable to or greater than that between NIS and AIT (8.3-52.6%). Pendrin residues which are mutated in Pendred's syndrome are identical to those in the aligned position of NIS and AIT. Hypothyroidism-associated pendrin mutations almost always fall within 4/11 segments. These are the first data that show homology between pendrin and NIS, and topographic relationships between pendrin mutations and the hypothyroid phenotype of PDS.

2. INTRODUCTION

Pendrin is the product of the *SLC26A4* (solute carrier 26A4) gene. This 780-residue long glycoprotein is a multifunctional anion exchanger, since it functions as a coupled electroneutral iodide/chloride, iodide/bicarbonate, and chloride/bicarbonate exchanger with a 1:1 stoichiometry (1). In the thyrocyte, pendrin is expressed at the apical membrane of follicular cells and is thought to mediate iodide efflux into the follicular lumen, most likely in partnership with at least another unidentified protein (1, 2). *SLC26A4* gene mutations are involved in the Pendred's syndrome (PDS), and the nonsyndromic deafness DFNB4 associated with enlargement of the vestibular aqueduct (EVA) (1, 2). Pendred's syndrome (OMIM# 274600) is an autosomal recessive disorder characterized by sensorineural deafness, goiter, and impaired iodide organification. The incidence of Pendred's syndrome is approximately 10 in 100,000 individuals, and it is the most common form of syndromic deafness, in that it accounts for approximately 10% of all patients with hereditary deafness. Most patients are euthyroid, in that hypothyroidism develops under conditions of low nutritional iodine intake (1, 2).

Though iodide (I^-) is the preferred anion transported by pendrin and the pendrin-mediated I^- efflux has been experimentally demonstrated (2), the presence of euthyroidism (as opposed to hypothyroidism) in most, but not all, patients with PDS (3-5), and the lack of any thyroid phenotype in the pendrin knockout mice suggest that pendrin is not the sole protein to mediate I^- efflux (1). This is in contrast

with the fundamental role of the Na^+/I^- symporter (NIS) as the protein that allows the thyrocytes to internalize I^-, because NIS mutations cause I^- transport defect (absent or low thyroid I^- uptake) and associated congenital hypothyroidism (6).

In terms of secondary structure, pendrin is predicted to have an intracellular terminus of 87 residues, an intracellular tail of 273 residues (aa 508-780), and 12 transmembrane domains, which are connected by six segments extracellularly and five segments intracellularly (1). This is not much dissimilar from NIS, the product of the *SLC5A5* gene, which is localized in the basolateral plasma membrane of the thyrocyte. Indeed, the 643-residue long human NIS consists of 13 transmembrane domains, which are connected by six extracellular loops and six intracellular loops, with a 16-residue long head and a 98-residue long tail (6). Though both NIS and pendrin transport other anions, the prevalent one is I^- (1, 6). Another protein which, like NIS, is localized in the apical side of the thyrocyte, was initially thought to mediate the I^- transport between the cytosol and the follicular lumen (7). This function was inferred from its amino acid sequence homology with NIS (46% identity, 70% homology) (7). Thus, this product of the *SLC5A8* gene was initially named apical I^- transporter (AIT) but it is better termed as Na^+/monocarboxylate transporter (SMCT), because it does not transport iodide; rather, it transports a variety of monocarboxylates, including lactate, pyruvate, butyrate, and nicotinate (6). (However, the term AIT is still used, so that throughout this paper we will use AIT and SMCT interchangeably). The 610-residue long human AIT has the same secondary structure of NIS, with a short N-terminal head, a long C-terminal tail, and 13 transmembrane domains which are connected by six extracellular loops and six intracellular loops.

Pendrin contains three well defined regions, *viz* the N-terminal sulfate transporter domain (aa 69-152), the central sulfate transporter family domain (aa 203-481), and the C-terminal STAS domain (sulfate transporter and antisigma factor antagonist; aa 536-725) (8). The STAS domain of pendrin is thought to span aa 535 to 573 and residues 654 to 729, and it contains one putative protein kinase A site (RKDT at aa 714-717) (9). According to Sharma *et al* (10), the STAS domain of human pendrin encompasses aa 515-734, excluding the intervening sequence region at aa 566-653.

Although widespread among bacteria, in higher organisms the STAS domain appears restricted to SulP/SLC26 anion transporter polypeptides. The presence of disease-causing mutations in the STAS domains of SLC26A2/DTDST, SLC26A3/DRA, and SLC26A4/pendrin attests to the structural importance of the STAS domain (10). The exact role of the STAS domain has not been elucidated; it may play a role in nucleotide binding and/or interactions with other proteins (9, 10).

While recently involved in writing a review article on the inositols and the thyroid (11) and being simultaneously interested in certain characteristics of NIS, upon perusing the amino acid sequence of human NIS we realized that the segment KKPPGF at aa 599-604 reminded us the segment **RK**DTF**F** at aa 714-719 of human pendrin which contains the protein kinase A site (RKDT). The different sequence of this stretch at aa 587-592 (TDNPAF) in human AIT, which has no role in iodide transportation (see above), prompted us to explore further the amino acid sequence homology among these three proteins. Because, to the best of our knowledge, this work has not been done previously, we think it is of interest to report here.

3. MATERIALS AND METHODS

We first retrieved from the Entrez Protein database (https://www.ncbi.nlm.nih.gov/protein) the amino acid sequences of the three human proteins pendrin/SLC26A4 (accession number: NP_000432.1), NIS/SLC5A5 (accession number: AAB17378.1) and AIT/SLC5A8 (accession number: Q8N695.2). These sequences were aligned using CLUSTAL Omega (12), and the alignment was subsequently manually corrected using the Se-Al 2.0 software (http://tree.bio.ed.ac.uk/software/seal/). Manually corrected sequence alignment has been already used by other authors in the NIS setting (6). Conservative groups of amino acids were A/G (Ala/Gly), D/E/N/Q (Asp/Glu/Asn/Gln), F/Y/W (Phe/Tyr/Trp), H/K/R (His/Lys/Arg), I/L/M/V (Ile/Leu/Met/Val), S/T (Ser/Thr) (13).

4. RESULTS

4.1. Overall homology

The three proteins studied and their homologous segments are schematically represented in Figure 1, with representative alignments provided in Figure 2.

As summarized in Table 1, there are 63 positions with amino acids identical in the three proteins and 66 with amino acids of one protein being similar to those of the other two proteins, resulting in an overall homology of 14.4%. Examining the alignment in a pairwise fashion, pendrin is slightly more similar to NIS than to AIT (25.0% vs 20.0%, χ^2= 5.85, P= 0.015). The difference holds upon disregarding intervening sequences of any protein (34.2% vs 27.6%, χ^2= 5.97, P= 0.014). This approximately 5 to 7 percent point difference persists except in the STAS domain (36.5% vs 24.0%, χ^2=3.85, P= 0.05) and in the sulfate transporter family domain (23.7% vs 22.6% or 33.3% vs 31.8%) (Table 1). The region 490-611 of NIS and

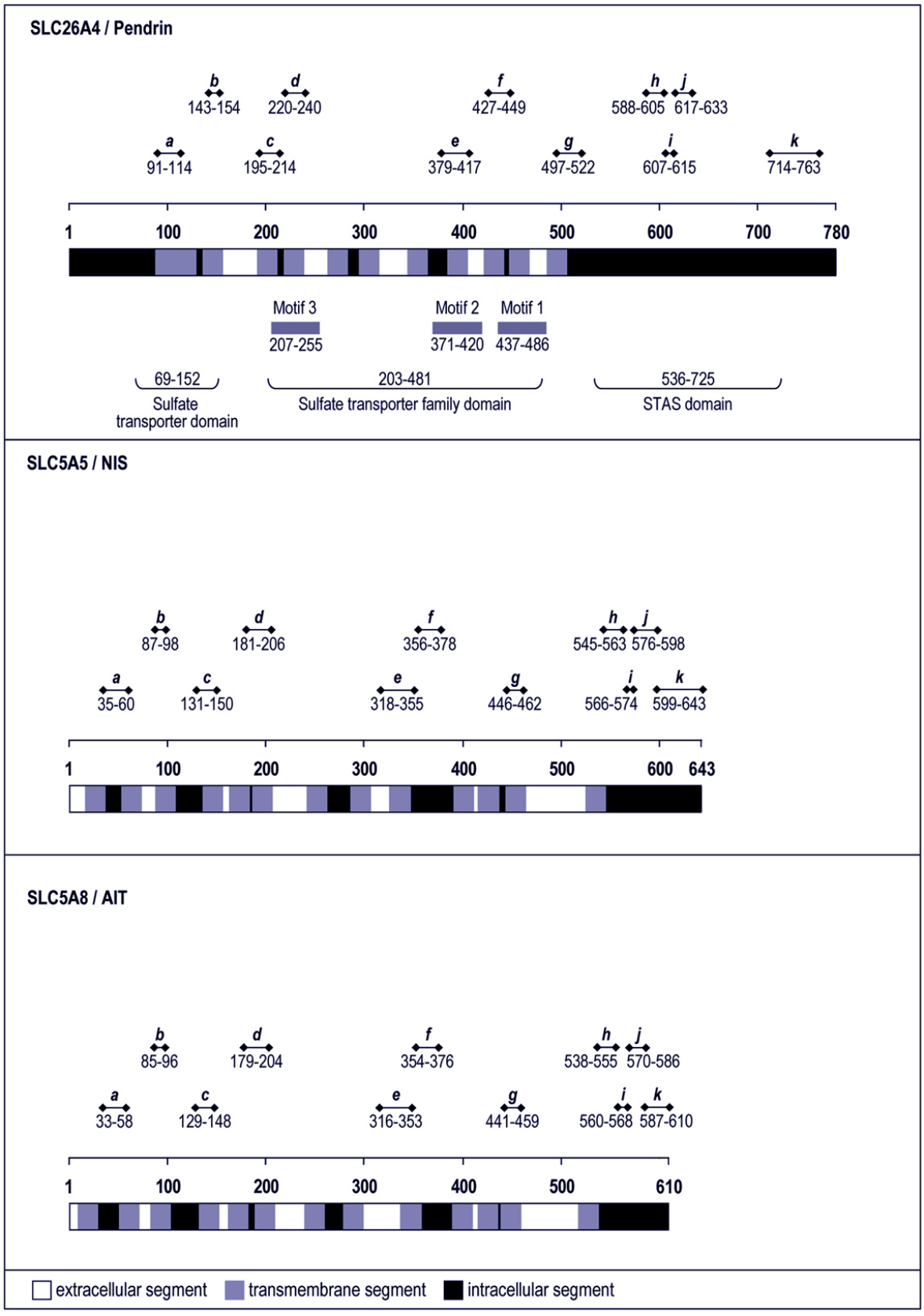
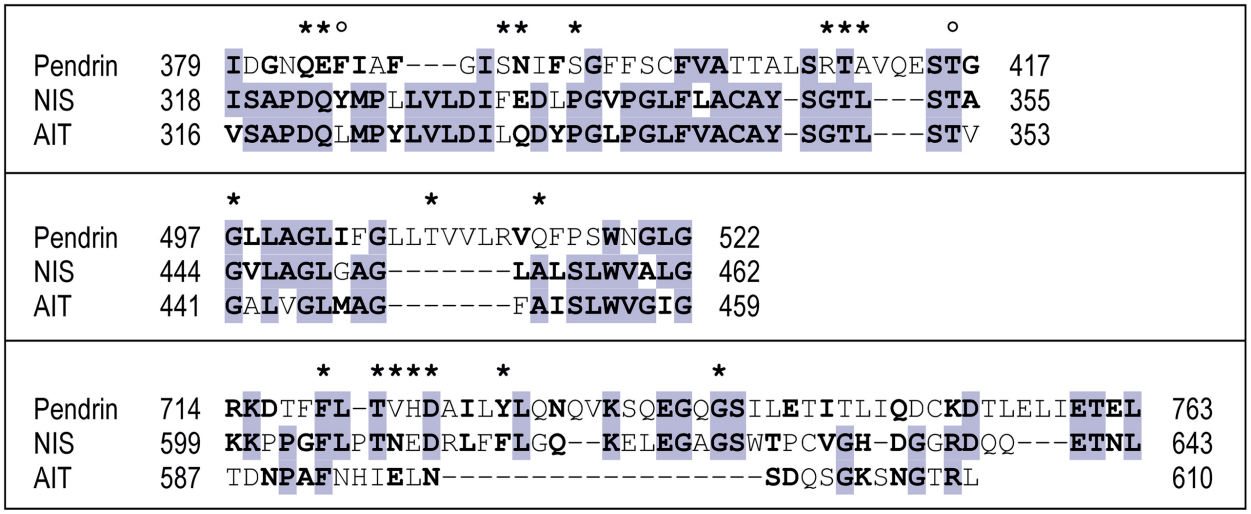


Figure 1. Homologous segments of human pendrin, NIS and AIT. These segments are indicated by the letters *a* through *k*, and are positioned relative to the membrane domains of each protein. The magnitude of the homology is provided in Table 3.



* mutations of pendrin, ° mutations of NIS
In each position, identical amino acids are typed in **boldface** on gray background, similar amino acids in **boldface** on white background.

Figure 2. Representative alignments of some segments of human pendrin, NIS and AIT. The segments are *e*, *g* and *k*, and details on their magnitude of homology are illustrated in Table 3.

Table 1. Homologies of pendrin (whole amino acid sequence or indicated domains) with NIS and/or AIT

	Pendrin, NIS & AIT	Pendrin & NIS [3]	Pendrin & AIT [3]	NIS & AIT [4]
Whole length				
Overlap	898	816	801	652
Identities	63 (7.0 %)	117 (14.3%)	90 (11.2%)	304 (46.6%)
Similarities	66 (7.3 %)	87 (10.7%)	70 (8.7%)	92 (14.1%)
Homology	129 (14.4%)	204 (**25.0**%)	160 (20.0%)	396 (60.7%)
Whole length minus intervening sequences [1]				
Overlap	600	584	569	636
Identities	60 (10.0 %)	114 (19.5%)	87 (15.3%)	304 (47.8%)
Similarities	66 (11.0%)	86 (14.7%)	70 (12.3%)	92 (14.5%)
Homology	126 (21.0%)	200 (**34.2**%)	157 (27.6%)	396 (62.3%)
Pendrin domains [2]				
Sulfate transporter				
Overlap	86	86	86	78
Identities	9 (10.5%)	18 (20.9%)	14 (16.3%)	41 (52.6%)
Similarities	10 (11.6%)	12 (14.0%)	9 (10.5%)	11 (14.1%)
Homology	19 (22.1%)	30 (**34.9**%)	23 (26.7%)	52 (66.7%)
Sulfate transporter family				
Overlap	363 [316]	363 [258]	363 [258]	305
Identities	33 (9.1%) [10.4%]	50 (13.8%) [19.4%]	43 (11.8%) [16.7%]	167 (54.8%)
Similarities	36 (9.9%) [11.4%]	36 (9.9%) [14.0%]	39 (10.7%) [15.1%]	55 (18.0%)
Homology	69 (19.0%) [21.8%]	86 (23.7%) [33.3%]	82 (22.6%) [31.8%]	222 (72.8%)
STAS				
Overlap	222 (104)	222 [104]	222 [104]	121
Identities	4 (1.8%) [3.8%]	17 (7.7%) [16.3%]	9 (4.1%) [8.7%]	29 (24.0%)
Similarities	14 (6.3%) [13.5%]	21 (9.5%) [20.2%]	16 (7.2%) [15.4%]	14 (11.6%)
Homology	18 (8.1%) [17.3%]	38 (**17.1**%) [**36.5**%]	25 (11.3%) [24.0%]	43 (35.5%)

The three pendrin domains are located at aa 69-152, 203-481 and 536-725, respectively (see text and Figure 1).

[1]: Intervening sequences of human pendrin are aa. 9-59, 331-366, 471-497, 570-587, 634-713 and 764-780, for a total of 229 residues (29.4% of the whole length of 780 residues). Intervening sequences of NIS are aa. 155-179 and 396-427, for a total of 57 residues (8.9% of the whole sequence). Intervening sequences of AIT are aa. 153-177 and 394-424, for a total of 56 residues (9.2% of the whole sequence). [2]: Numbers and percentages in square brackets are computed by disregarding the intervening sequences. [3]: An overtly higher homology value upon comparing pendrin with NIS and pendrin with AIT is typed **bold-face**. [4]: Upon double-checking in the alignment of Fig 1 of the original paper (ref. 7) and using the same group of similar residues as ours, there was an overlap of 645 residues with 296 identities (45.9%) and 99 similarities (15.3%), resulting in 395 homologous residues (61.2%).

Table 2. Homology concerning the three motifs in the Sulfate transporter family domain of pendrin [1]

Motifs	Pendrin, NIS & AIT	Pendrin & NIS [2]	Pendrin & AIT [2]	NIS & AIT
Motif 3 (aa 207-255)				
Overlap	80 [55]	80 [55]	80 [55]	76
Identities	10 (12.5%) [18.2%]	13 (16.2%) [23.6%]	13 (16.3%) [23.6%]	45 (59.2%)
Similarities	3 (3.8%) [5.5%]	3 (3.8%) [5.5%]	3 (3.8%) [5.5%]	15 (19.7%)
Homology	13 (16.3%) [23.6%]	16 (20%) [29.1%]	16 (20%) [29.1%]	60 (78.9%)
Motif 2 (aa 371-420)				
Overlap	53	53	53	36
Identities	8 (15.1%)	9 (17.0%)	9 (17.0%)	28 (77.8%)
Similarities	7 (13.2%)	7 (13.2%)	8 (15.1%)	4 (11.1%)
Homology	15 (28.3%)	16 (30.2%)	17 (32.1%)	32 (88.9%)
Motif 1 (aa 437-486)				
Overlap	95 [34]	95 [34]	95 [34]	79
Identities	4 (4.2%) [11.8%]	9 (9.5%) [26.5%]	4 (4.2%) [11.8%]	41 (51.9%)
Similarities	10 (10.5%) [29.4%]	10 (10.5%) [29.4%]	10 (10.5%) [29.4%]	15 (19.0%)
Homology	14 (14.7%) [41.2%]	19 (**20.0**%) [**55.9**%]	14 (14.7%) [41.2%]	60 (75.9%)

[1]: Numbers and percentages in square brackets are disregarding the intervening sequences. Motif 1 of pendrin corresponds to segment 143-218 of NIS and 141-216 of AIT. Motif 2 of pendrin corresponds to aa 318-355 of NIS and 316-353 of AIT, with stretch 371-378 and 417-420 corresponding to gaps in the alignment with NIS and AIT. Motif 3 of pendrin corresponds to segment 366-443 of NIS and 365-440 of AIT, with stretch 470-486 of pendrin corresponding to gaps in the alignment with NIS and AIT. [2]: Overtly higher homology values in parentheses and brackets upon comparing the pair pendrin and NIS versus the pair pendrin and AIT is typed **bold-face**.

the region 486-598 of AIT, which correspond to the STAS domain of pendrin (536-725) in the multiple alignment, display the least homology between NIS and AIT (35.5%). In contrast, the region 139-443 of NIS and the region 137-440 of AIT, which correspond to the sulfate transporter family domain in the multiple alignment, display the highest homology between NIS and AIT (72.8%) (Table 1).

4.2. Zonal homologies

As shown in Table 2, also within the sulfate transporter family domain there are zonal differences in homology. In terms of magnitude of diversity in the pendrin/NIS homology vs pendrin/AIT homology, only the C-terminal motif 1 displays some difference, though statistically insignificant (P=0.33). The stretch 318-355 of NIS and 316-353 of AIT, which correspond to motif 2 of pendrin, display the highest homology (88.9%) among the three motifs (Table 2).

There are more or less wide segments where homology between pendrin and NIS approaches or passes the 50% threshold (Table 3). Of 11 segments (termed *a* through *k*), 9 have higher values for the pendrin/NIS homology compared to the pendrin/AIT homology, and only one (corresponding to the 9-residue long pendrin stretch DAVSTNNAF at aa 607-615) features the opposite (**D**LARQTASV at aa 566-574 for NIS, and **D**FL**S**NF**D**I**F** at aa 560-568 for AIT). Concerning the homology between NIS and AIT, the last three C-terminal segments and the N-terminal segment *b* are those with minimal homology, whereas the segments corresponding to *c* and *f* (NIS 131-150 and 356-378, and AIT 129-148 and 354-376) have homology values of 90% or higher (Table 3). Representative alignments of three segments (*e*, *g* and *k*) are provided in Figure 2. In segment *k*, it should be noted that at 3 positions residues of pendrin (K715, L720, T721) matched identical residues of NIS (K600, L605, T607) but different residues of AIT, and at one position one residue of pendrin (D724) matched an identical residue of NIS (D610) and a similar residue of AIT (N608). Furthermore, the stretches A725-I742 of pendrin and R611-W626 of NIS are intervening sequences relative to AIT. In the alignment of these two stretches, there was a 33.3% identity and overall 50% homology. Another two intervening sequences were T755-L763 of pendrin and Q638-L643 of NIS, which were 44.4% homologous. The pendrin region F719-G740 should be functionally important because mutations of 7 residues are associated with disease. Of these 7 residues, 4 are identical in NIS (but only one in AIT) and 1 is similar in NIS (but missing in AIT). The correlation of homology with mutations is described more deeply in the next heading.

4.3. Correlation of zonal homology with mutations of pendrin and NIS

Known mutations of pendrin and NIS and their positions within the respective amino acid sequences are shown in Figure 3 and Table 3. There are five positions where a mutation of pendrin affects the change of an amino acid that is identical in the corresponding position of wild-type both NIS and AIT: V239, T410, S448, G497 and F719 (Table 3). In addition, there are five positions (V220, L624, T721, D724, G740) where a mutation of pendrin affects the change of an amino acid that is identical in the corresponding position of wild-type NIS only, and there are another two positions (V233 and S610) where such identity concerns AIT only. Concerning NIS, there are two positions where a mutation affects the change

Table 3. Homologies of hPendrin zones *a* through *k* with the corresponding zones of hNIS and hAIT

Zones	Pendrin, NIS, AIT	Pendrin & NIS	Pendrin & AIT	NIS & AIT	Notes [2]
***a* (91-114)**					Pendrin mutations at T94, L96, G102, A104 (matching G50 of NIS and G48 of AIT), Y105, A106. NIS mutation at **V59** (matching **V113** of pendrin and **V57** of AIT).
Overlap	26	26	26	26	
Identities	4 (15.4%)	8 (30.8%)	7 (26.9%)	13 (50.0%)	
Similarities	6 (23.1%)	4 (15.4%)	4 (15.4%)	5 (19.2%)	
Homology	10 (38.5%)	12 (**46.2**%)	11 (42.3%)	18 (69.2%)	
***b* (143-154)**					Pendrin mutation at M147. NIS mutation at **G93** (matching **G149** of pendrin).
Overlap	12	12	12	12	
Identities	0	2 (16.7%)	0	1 (8.3%)	
Similarities	0	5 (41.7%)	1 (8.3%)	0	
Homology	0	7 (**58.3**%)	1 (8.3%)	1 (8.3%)	
***c* (195-214)**					Pendrin mutation at G209. In NIS, deletion starting at M142 (matching L206 of pendrin and I140 of AIT).
Overlap	20	20	20	20	
Identities	6 (30.0%)	7 (35.0%)	6 (30.0%)	15 (75.0%)	
Similarities	1 (5.0%)	3 (15.0%)	2 (10.0%)	3 (15.0%)	
Homology	7 (35.0%)	10 (**45.0**%)	8 (40.0%)	18 (90.0%)	
***d* (220-240)**					Pendrin mutations at **V220** (matching **V181** of NIS and L179 of AIT), **V233** (matching L199 of NIS and **V197** of AIT), L236, **V239** (matching **V205** of NIS and **V203** of AIT).
Overlap	26	26	26	26	
Identities	6 (23.1%)	9 (34.6%)	6 (23.1%)	17 (65.4%)	
Similarities	3 (11.5%)	3 (11.5%)	2 (7.7%)	5 (19.2%)	
Homology	9 (34.6%)	12 (**46.2**%)	8 (30.8%)	22 (84.6%)	
***e* (379-417)** [1]					Pendrin mutations at Q383 and E384 (matching D322 and Q323 of NIS; D320 and Q321 of AIT), S391, N392 (matching E334 of NIS and Q332 of AIT), S395, R409, **T410** (matching **T351** of NIS and **T349** of AIT), A411. NIS mutations at Y324 (matching F385 of pendrin) and **T354** (matching **T416** of pendrin and **T352** of AIT).
Overlap	42	42	42	38	
Identities	8 (19.0%)	9 (21.4%)	9 (21.4%)	29 (76.3%)	
Similarities	7 (16.7%)	8 (19.0%)	8 (19.0%)	4 (10.5%)	
Homology	15 (35.7%)	17 (40.5%)	17 (40.5%)	33 (86.8%)	
***f* (427-449)**					Pendrin mutations at A429, L445, Q446, **S448** (matching **S377** of NIS and **S375** of AIT).
Overlap	23	23	23	23	
Identities	6 (26.1%)	6 (26.1%)	6 (26.1%)	20 (87.0%)	
Similarities	6 (26.1%)	6 (26.1%)	1 (4.3%)	2 (8.7%)	
Homology	12 (52.2%)	12 (**52.2**%)	7 (30.4%)	22 (95.7%)	
***g* (497-522)**					Pendrin mutations at **G497** (matching **G444** of NIS and **G441** of AIT), T508 and Q514.
Overlap	26	26	26	19	
Identities	7 (26.9%)	9 (34.6%)	8 (30.8%)	12 (63.2%)	
Similarities	2 (7.7%)	3 (11.5%)	2 (7.7%)	3 (15.8%)	
Homology	9 (34.6%)	12 (**46.2**%)	10 (38.5%)	15 (78.9%)	
***h* (588-605)**					
Overlap	20	20	20	19	
Identities	1 (5.0%)	2 (10.0%)	2 (10%)	8 (42.1%)	
Similarities	5 (25.0%)	7 (35.0%)	4 (20.0%)	2 (10.5%)	
Homology	6 (30.0%)	9 (**45.0**%)	6 (30.0%)	10 (52.6%)	
***i* (607-615)**					Pendrin mutations at V609 and **S610** (matching L562 and **S563** of AIT).
Overlap	9	9	9	9	
Identities	1 (11.1%)	1 (11.1%)	2 (22.2%)	1 (11.1%)	
Similarities	0	0	3 (33.3%)	1 (11.1%)	
Homology	1 (11.1%)	1 (11.1%)	5 (**55.5**%)	2 (22.2%)	
***j* (617-633)**					Pendrin mutations at **L624** and E625 (matching **L583** and D584 of NIS).
Overlap	23	23	17	23	
Identities	0	5 (21.7%)	0	3 (13.0%)	
Similarities	1 (4.3%)	7 (30.4%)	2 (11.8%)	1 (4.3%)	
Homology	1 (4.3%)	12 (**52.2**%)	2 (11.8%)	4 (17.4%)	
***k* (714-763)**					Pendrin mutations at **F719** (matching **F604** of NIS and **F592** of AIT), **T721** (matching **T607** of NIS), V722, H723, **D724** (matching **D610** of NIS and N598 of AIT), Y728 and **G740** (matching F614 and **G624** of NIS).
Overlap	51	51	42	40	
Identities	1 (2.0%)	15 (29.4%)	1 (2.4%)	5 (12.5%)	
Similarities	3 (5.9%)	8 (15.7%)	5 (11.9%)	6 (15.0%)	
Homology	4 (7.8%)	23 (**45.1**%)	6 (14.3%)	11 (27.5%)	

As shown in Figure 1, the 10 segments have the following span in NIS and AIT, respectively: (*a*) 35-60 and 33-58; (*b*) 87-98 and 85-97; (*c*) 131-150 and 129-148; (*d*) 181-206 and 179-204; (*e*) 318-355 and 316-353; (*f*) 356-378 and 354-376; (*g*) 444-462 and 441-459; (*h*) 545-563 and 538-555; (*i*) 566-574 and 560-568; (*j*) 576-598 and 570-586; (*k*) 599-643 and 587-610. An overtly higher homology value upon comparing NIS and AIT is typed **bold-face**. [1]: Concerning segment *e*, identities and homology are greater in the C-terminal part (aa 396-416 of pendrin, aa 338-354 of NIS and aa 334-352 of AIT). Identities and homology among all three proteins are 8 (38.1%) and 10 (47.6%). Identities and homology beween pendrin and NIS are 8 (38.1%) and 10 (47.6%); identities and homology between pendrin and AIT are 9 (42.8%) and 10 (47.6%); identities and homology between NIS and AIT are 15 (88.2%) and 17 (100%). [2:] Highlighted in bold-face print are also the mutated residues in pendrin or NIS that correspond to the same residue in the wild-type NIS, pendrin or AIT. No AIT mutation and associated thyroid syndrome has been reported for AIT thus far.

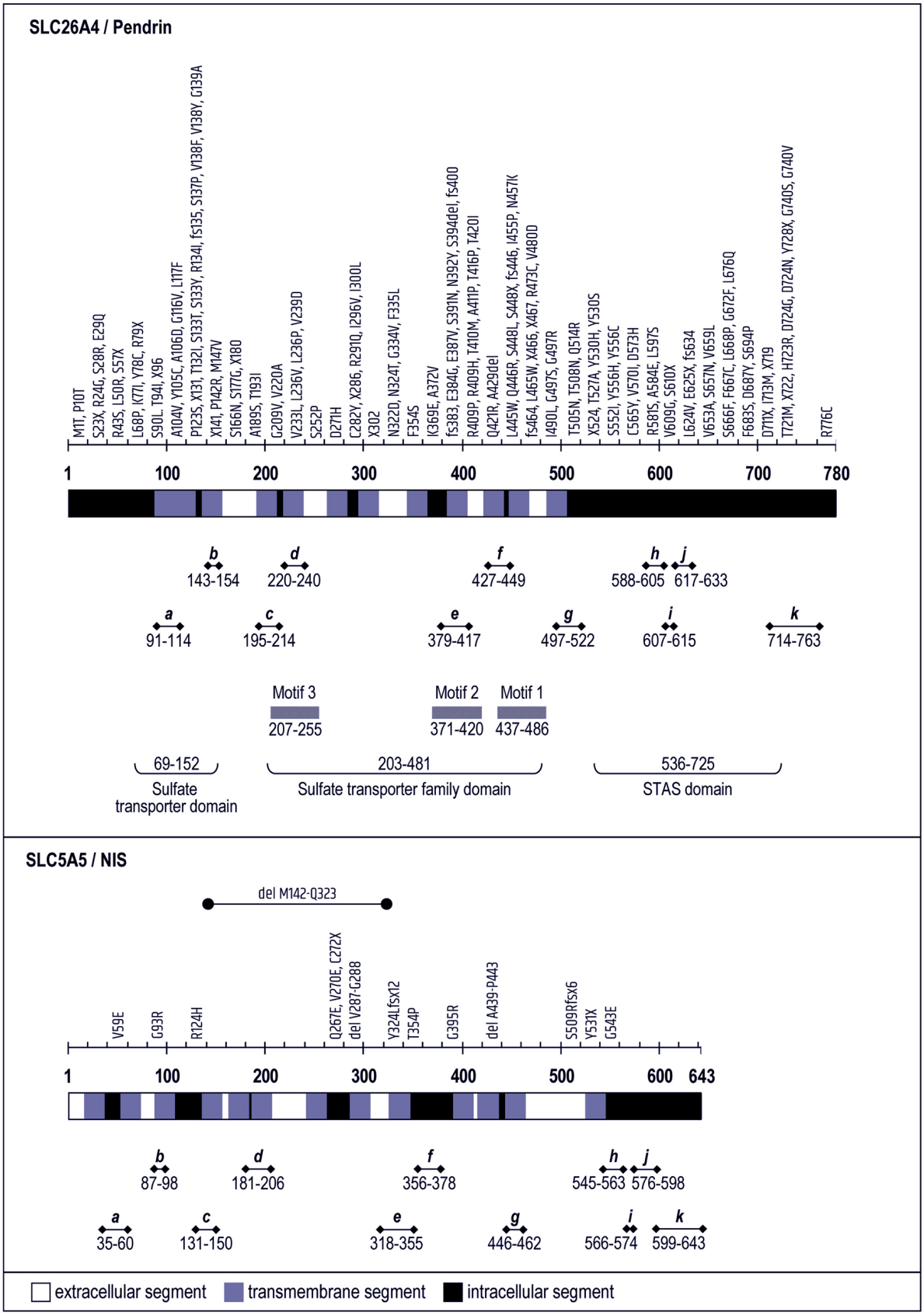


Figure 3. Known mutations of human pendrin and NIS, and their position relative to the homologous segments *a* through *k*.

of an amino acid that is identical in the corresponding position of wild-type both pendrin and AIT (V59, T354), and one involving pendrin only (G93) (Table 3).

Outside of the segments *a* through *k*, there are another nine positions where a pendrin mutation involves an amino acid identical at the corresponding position in NIS and AIT: M1 (M1 in NIS, and M1 in AIT), L117 (L63 and L61), G131 (G75 and G73), G139 (G84 and G82), I300 (I284 and I282), F401 (F343 and F341), T416 (T354 and T352), G505 (G452 and G449), and S552 (S527 and S520) (Table 3). Other mutations (n= 4) involve positions with amino acids identical in pendrin and NIS, but not AIT: D271, C466, Y556, L624 (corresponding to D233, C440, Y531, L583 in NIS). Another two mutations involve positions with amino acids identical in pendrin and AIT, but not NIS: T132 and R291 (corresponding to T74 and R273 in AIT). Concerning NIS, there is only one position where a mutation affects the change of an amino acid that is identical in the corresponding position of both pendrin and AIT: R124 (corresponding to R181 of pendrin and R122 of AIT). No additional NIS mutation concerns an amino acid that is identical in the corresponding position of solely pendrin or solely AIT. There are three NIS mutations that concern residues identical only in AIT: Q267, G395 and G543 (corresponding to Q265, G393 and G536 in AIT).

According to Bizhanova *et al*. (9) and the UniProt database (http://www.uniprot.org), 43 mutations of pendrin have been described in PDS. Eight of them abolish or severely reduce the iodide transport function and, consequently, affect thyroid function negatively: G102R, V138F, G209V, L236P, T410M, Q446R, Y556C, G672E. Noteworthy, five of these eight mutations (namely, G102R, G209V, L236P, T410M, Q446R) fall within one of the eleven segments of pendrin which are homologous to parts of NIS and AIT (Table 3), further confirming and highlighting the functional importance of these segments and the clinical consequences (hypothyroidism) which can be caused by their alterations. Table 4 reports the mutations, which are biallelic because of the recessive inheritance of PDS, that have been reported in patients with the hypothyroidism phenotype. Table 4 also shows the position of the mutated amino acid within the pendrin secondary structure, domains and local regions of homology with NIS and AIT. Furthermore, Table 4 reports the amino acid in NIS and AIT that corresponds to the mutated amino acid of pendrin. Strikingly enough, the vast majority of mutations fall in the sulfate transporter family domain or STAS domain, and within zones *e* (predominantly), *f*, *h* or *k*. In two instances (Y530 and F709), zones *g* and *k* in the STAS domains are missed by just a few positions. An obvious hot spot is evident between Cys400 and Thr416 of pendrin, with 5/17 positions (29.4%) being affected by mutation. This segment of zone *e* has the greatest concentration of identical residues across the three proteins (n= 8) and between either pendrin and NIS (n= 8) or pendrin and AIT (n= 9) (Table 3, and footnote). By comparison, the corresponding 17-residue long stretch of NIS (aa 338-354) and AIT (336-352) displays the highest homology (100%), with 15 identical residues (88%).

5. DISCUSSION

To the best of our knowledge, we are unaware of published papers that have reported amino acid sequence homology between pendrin and NIS, two membrane proteins which (i) belong to different groups of the solute carrier superfamily, (ii) are expressed at opposite sides of the thyrocyte, and (iii) are involved in transportation of iodide in opposite directions (inward from the blood into the cytosol, and outward from the cytosol into the follicular lumen). As mentioned in the Introduction, the role of NIS in the iodide uptake is unequivocal and fundamental, while the role of pendrin in the efflux is minor and in all likelihood requiring the contribution of at least one additional protein. Some data, shown herein, might indirectly support the role of pendrin in iodide transportation. First, pendrin and NIS have a level of homology slightly greater than that between pendrin and AIT/SMCT, especially in the C-terminal portion that includes the STAS domain. Second, pendrin mutations which involve positions where the amino acid is identical in the corresponding position of NIS (n=23) are only slightly more numerous than pendrin mutations where the amino acid is identical in the corresponding position of AIT/SMCT (n= 21). However, starting from position 400 (with F401 of pendrin matching F343 of NIS and F341 of AIT), there are a total of 7 positions with residues mutated in pendrin that are identical to residues present in wild-type NIS as opposed to a total of 4 positions with residues mutated in pendrin that are identical to residues present in wild-type AIT.

Although widespread among bacteria, in higher organisms the STAS domain appears restricted to sulfate permease (SulP)/SLC26 anion transporter polypeptides. The mammalian STAS domains differ from those of anti-anti-sigma proteins and from bacterial sulfate permease (SulP) polypeptides in their intervening sequences inserted into the loop between helix α1 and strand β3 (rat prestin nomenclature) (10). The largest number of distinct disease associated variants in STAS domains, as well as in holoproteins, is found in pendrin. Notably, among intervening sequences of *SLC26* human disease genes, only the intervening sequence of pendrin has been shown to date to harbor disease genes. The intervening sequences are the most divergent regions of *SLC26* STAS domains, and a Genbank search of the pendrin intervening sequence revealed no identifiably related amino acid sequences in the database

Table 4. Mutations of the pendrin gene associated with hypothyroidism in patients with Pendred's syndrome

Pendrin		NIS		AIT	
Allele 1	**Allele 2**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
Ser28Arg (IC-1, head)	Splice site	gap	N/A	gap	N/A
Val138Phe (IC-2), ST-domain	Leu445Trp (IC-6), STF-domain (zone *f*)	Tyr83 (EC-2)	Arg374 (IC-5)	Phe83 (EC-2)	Tyr372 (IC-5)
Gly334Val (EC-4), STF-domain	Noncoding exon 15 (-26T>C)	gap	N/A	gap	N/A
Glu384Gly (IC-5), STF-domain (zone *e*)	Glu384Gly (IC-5), STF-domain (zone *e*)	***Gln323 (EC-5)***	***Gln323 (EC-5)***	***Gln321 (EC-5)***	***Gln321 (EC-5)***
Glu384Gly (IC-5), STF-domain (zone *e*)	Leu445Trp (IC-6), STF-domain (zone *f*)	***Gln323 (EC-5)***	Arg374 (IC-5)	***Gln321 (EC-5)***	Tyr372 (IC-5)
Cys400Valfs*32 (TM-9), STF-domain (zone *e*)	Gln421Pro (EC-5), STF-domain	Leu342 (TM-9)	gap	Leu340 (TM-9)	gap
Arg409His (EC-5), STF-domain (zone *e*)	Arg409His (EC-5), STF-domain (zone *e*)	Gly350 (IC-5)	Gly350 (IC-5)	Gly348 (TM-9)	Gly348 (TM-9)
Arg409His (EC-5), STF-domain (zone *e*)	fsPro297 > 302X (TM-7), STF-domain	Gly350 (IC-5)	Ala281 (IC-4)	Gly348 (TM-9)	Ser279 (IC-4)
Thr410Met (EC-5), STF-domain (zone *e*)	Tyr530His (IC-7, tail)	**Thr351 (IC-5)**	Glu470 (EC-7)	**Thr349 (TM-9)**	Pro467 (EC-7)
Gln413Arg (EC-5), STF-domain (zone *e*)	Gln413Arg (EC-5), STF-domain (zone *e*)	gap	gap	gap	gap
Thr416Pro (EC-5), STF-domain (zone *e*)	His723Arg (IC-7, tail), STAS-domain (zone *k*)	gap	Glu609 (IC-7, tail)	gap	Leu597 (IC-7, tail)
Tyr530His (IC-7, tail)	Splice site	Glu470 (EC-7)	N/A	Pro467 (EC-7)	N/A
Leu597Ser (IC-7, tail), STAS-domain (zone *h*)	Leu597Ser STAS-domain (zone *h*)	Thr553 (IC-7, tail)	Thr553 (IC-7, tail)	Arg546 (IC-7, tail)	Arg546 (IC-7, tail)
Phe709Leufs*12 (IC-7, tail), STAS-domain STAS-domain	Splice site	gap	N/A	gap	N/A

N/A= not applicable. The gray background indicates that the new amino acid is identical/similar to the equivalent amino acid of either NIS or AIT. The amino acid of either NIS or AIT is typed **bold face** if identical or similar (***italics***) to the corresponding residue of wild-type pendrin. As shown in Figure 2, Tyr530 of pendrin is just C-terminal to the zone *g* (aa 497-522) and just N-terminal to the STAS domain (aa 536-725) of pendrin itself, while Phe709 is just N-terminal to zone *k* (aa 714-763).

(10). However, the exact role of the STAS domain has not been elucidated. The STAS domain may play a role in nucleotide binding and/or interactions with other proteins, including other transporters, cytoskeletal scaffolds, and with enzymes metabolizing transported anion substrates, forming putative metabolons (9, 10). As appropriately underscored by Sharma *et al*. (10), "*The yet unreported proteins hypothesized to interact with mammalian SLC26 STAS domains may similarly prove important for stabilization and structure determination of the putatively disordered intervening sequence regions of intact mammalian SLC26 STAS domains, and perhaps of the corresponding holoproteins as well. They may, in addition, provide information crucial to decoding the mechanisms by which STAS domains regulate SLC26 polypeptide trafficking, stability, and anion transport function*." (10).

In sum, the location of the highest homology between pendrin and NIS in the tail of the STAS domain (where the homology between pendrin and AIT, and the homology between NIS and AIT are the lowest) and the presence of an immediately subsequent 16-residue long segment of pendrin (aa 726-741) also having high homology with NIS (56.2%, with 50% identity; aa 612-625) but being absent in AIT, might confer to pendrin some iodide carrier/exchanger property that AIT does not have. The known function of the STAS domain to permit interaction with STAS-endowed or STAS-free domain, including at least one so far unidentified thyroid protein involved in iodide efflux into the follicular lumen, would provide the basis for such interaction with the unidentified protein(s), this being consistent with the fact that inactivating mutations of pendrin alone do not impair thyroid hormonogenesis. Finally, mutation of amino acids at certain positions in the STAS domain

and, more frequently, in the sulfate transporter family domain at least monoallelically are risky for causing thyroid failure, especially if those mutations fall in some specific regions (that is, our segments *e*, *f* or *h*).

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Send correspondence to: Fabrizio Guarneri, Department of Clinical and Experimental Medicine - Dermatology, via Consolare Valeria - Gazzi, 98125 Messina, Italy. Tel: 390902212890, Fax: 390902927691, E-mail: f.guarneri@tiscali.it