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NOVEL GENES INVOLVED IN ETIOPATHOGENESIS OF RETINITIS PIGMENTOSA ORPHAN FORMS

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Abstract

Retinitis Pigmentosa is a rare genetic disease affecting the retina, characterized by the progressive degeneration of rods and cones photoreceptors. The disease is considered one of the main causes of visual impairment and blindness, affecting from 1/9000 to 1/750 people according to their geographical localization. More than 80 genes have been described as causative of Retinitis Pigmentosa, even if there are the so called "orphan forms" showing the same phenotipic condition of RP patients, in the absence of mutations in the already known causative genes. Since RP is a very heterogeneous disorder, it is important to clarify the possible involvement of other genes and their eventual role in the etiopathogenesis of RP orphan forms.

Key Words: Retinitis pigmentosa, retinal degeneration, eye, causative genes.

Introduction

Retinitis Pigmentosa (RP, OMIM #600105) is a rare genetic disorder affecting the retina, a light-sensitive tissue containing sensorial bodies, the photoreceptors, designated to transduce visual signals into electrical ones. RP refers to a group of retinal degenerative diseases that involve deterioration and loss of retina cells, and usually evolves over several decades, making it a long-lasting disease. In some cases, it is observed a rapid devolution followed by a slow progression [1].

RP is one of the main causes of visual impairment, affecting from 1/9000 to 1/750 people [2] variable according to geographical location.

The genes involved in the etiopathogenesis of the disease encode for proteins necessary to the proper function of the retina like those implicated in the physiology of photoreceptors. These are cells specialized to respond to light, absorbing it and converting into electrical signals, which are sent to the cells of the other layers of the retina. Finally, through the optic nerve visual information is transmitted to the brain and translated into a visual

image. Specifically, rods are the photoreceptors in the outer regions of retina, specialized to trasduce dim and dark light, while cones are mostly in central portion of the retina. They are made to transduce high light signals, allowing the vision in daylight with fine visual details and colors.

The classification of different forms of RP can be based on the age of onset, the nature of first cells affected, and the involvment of other pathological conditions unrelated to the ocular system. Therefore, it is possible to distinguish between non-syndromic Retinitis Pigmentosa and syndromic Retinitis Pigmentosa.The most common form of RP is a rod-cone dystrophy, with a primary degeneration of the rods, and subsequenta degeneration of the cones, reflecting the nature of the symptoms. In fact, the principal symptoms characterizing the early stage are night blindness, due to the early rods degeneration, followed by a visual loss of the peripherical field, evolving to ring shape scotoma and the so called "tunnel vision". During the middle stage, cones degeneration leads to lose more of the visual field. Bright lights get uncomfortable, a condition called photophobia, and the visual acuity decreases. Fundus examination reveals pigmentary deposits, attenuation of the retinal vessels and waxy pallor of the optic disc. In the end stage, the ERG is unrecordable, as a massive loss of photoreceptors is found, sometimes leading to complete blindness.

Retinitis Pigmentosa may be inherited according to following patterns:autosomal recessive (the most common kind of inheritance, with at least 43 genes associated with this form); autosomal dominant (with 24 associated) and X-linked (with at least 5 genes correlated) [3].

Today, at least 80 genes are known to be causative of RP, showing that it is a highly heterogeneous disorder [4]. These genes encode for proteins essential for rods and cones phototransduction, metabolic pathways proteins, cytoskeleton proteins, proteins involved in photoreceptor differentiation and trafficking, in the composition of extracellular matrices, as well as proteins expressed in other layers of the retina, like the RPE or in the neuronal cells composing the visual sensory pathway (amacrine cells, horizontal cells, ganglion cells)[5].Due to the high heterogeneity showed by RP patients, it is often difficult to correlate a very similar phenotipic condition to a certain causative genotype.

Following NGS sequencing data analysis, carried out on 4 exomes of patients affected by RP orphan forms, we have identified 31 new genes, not yet correlated with Retinitis Pigmentosa. Using a prioritization method, genes most likely involved in the development of RP, have been selected. In particular, this paper focus on Asp842Ala variant in IFT122 (Intraflagellar transport 122) gene, the first among selected genes.

Methods

- **ToppGene Prioritizazion**

The prioritization of the 31 candidate genes was carried out by *ToppGene* software, [https://toppgene.cchmc.org], a web-based software able to classify a small group of candidate genes from a large set of genes correlated with a pathology, giving each one a score. The score is based on the intersection of data from various databases of annotations related to cellular and physiological functions, networks of protein interaction shared between genes already known to cause the disease *(training genes)* and candidate genes *(test genes).*

- **Pathway analysis via Cytoscape, GeneMania and GeneCards**

Once identified the first genes potentially causative of Retinitis Pigmentosa by the *Cytoscape* [https://cytoscape.org] platform, it was possible to visualize the interaction network and molecular pathway between these genes, integrating the informations in output with the so-called *annotations*, which help us understand the main role of the proteins examined. Several plug-ins are available to increase the functions of Cytoscape and, among these, GeneMANIA helps to identify genes related to a list of genes provided, which are the one of our interest, based on data sources as Gene Ontology, GEO, BioGRID, EMBL-EBI, Pfam, Ensembl, NCBI, MGI, I2D, InParanoid, Pathway Commons, and crossing information between co-expression, physical interaction, genetic interaction, shared protein domains, co-localization, pathway and predicted functional relationships among the genes.

Finally, to better understand the structure, role, functions, expression, localization and genomics of a protein, GeneCards [https://www.genecards.org] was a great source of comprehensive information.

- **Structure prediction via RaptorX**

Finally, RaptorX server [http://raptorx.uchicago.edu] was used to highlight third structure aspects of IFT122-mutated and wild-type predicted proteins.

Results

Table 1 shows the results obtained after setting the training genes and the test genes, accordingly to the subsequent variables: GO: Molecular Function, GO: Biological Process, GO: Cellular Component, Human Phenotype, Domain, Pathway, Gene Family, Coexpressiom, Disease.

We have thus decided to deepen our knowledge on the Asp842Ala variant identified in the IFT122 gene. Cytoskape's app *GeneMania* results have shown that IFT122 gene is part of a complex molecular network leading to cytoskeletal activity and stability, as it appears to be involved in microtubule-mediated movement. The pathways in which it is involved include the organelle biogenesis and maintenance, the intraflagellar transport, Hedgehog off' state and signaling by GPCR.By GeneCards, we've been able to find some of IFT122 interactors, which appear to be included into the Hedgehog pathway (such as GLI1, GLI2 and SUFU) and the intraflagellar transport (such as IFT140, IFT22, IFT80, IFT81, WDR35, WDR19 and so on), thus confirming the results given by Cytoscape (Fig.1).

Structure prediction of wild-type and mutated protein by RaptorX has shown significant difference due to aminoacidic substitution Asp>Ala in position 842 of IFT122 protein (Fig. 2 and Fig.3).

We then analyzed the total number of contacts and clashes on the software *Chimera,* revealing that the wild type form of protein had a greater number of contacts than the muted one, demonstrating its higher stability. In particular, the wild type form had 3018 contacts, while the muted one had 2275 contacts. Surprisingly, the number of clashes didn't follow the same lead, in fact the wild-type form had 28 clashes, while the muted one had only 6 clashes.

Discussion

Photoreceptors have a highly specialized sensory edge, which acts as a connective structure. It binds the basal body of the receptor with the apical segment, a structure dedicated to phototransduction, called *sensory cilium* [6]. It regulates the transport of numerous molecules such as ions and proteins synthesized at the level of the basic body, and transports them towards their compartment of action, through movements in anterograde (mediated by the kinesin-2 motor protein)and retrograde sense (mediated by the dynein motor protein).

The importance of this molecular transport derives from the lack of a biosynthetic machinery in the external portion of the photoreceptors, needing the arrival of functional molecules. Molecular transport is facilitated by macromolecular protein complexes not surrounded by membrane, called Intraflagellar Transport Protein.

Intra-balloon transport is a bidirectional process that moves molecules along the microtubular path and plays a fundamental role in assembling and maintaining the structure of the eyelashes [7-9].

The IFT particles are localized along microtubules that constitute the internal scaffolding of the sensory cilium and include two protein subassemblies: IFT-A and IFT-B, which bind and transfer the charge from one part of the cell to another [10].Today, many genes coding for proteins of the ciliary system may be causative of non-syndromic forms of Retinitis Pigmentosa [11].

IFT-A and IFT-B are two distinct biochemical sub-complexes that are highly conserved among organisms and consist of distinct protein subunits, 6 IFT-A and 15 IFT-B, respectively.

IFT-B is implicated in anterograde transport, while IFT-A in retrograde transport.

One of the subunits of IFT-A sub-assembly is IFT122. IFT122 protein (Intraflagellar Transport Protein 122) is a protein of 1241 amino acids encoded by the IFT122 gene, located at the long arm of chromosome 3 (3q21.3-q22 .1) and it is longer than 80,000 bp.

IFT122 is part of the family of proteins with WD repeats, sequences conserved in eukaryotes and scaffolds for simultaneous protein interactions. IFT122 shows 7 WD domains in the N-terminal region and an AF-2 domain that recruits coregulatory molecules and transcriptional factors, which would help the whole IFT-A complex to provide the scaffold for the IFT-B complex, so that they can recruit the ciliary cargo and proteins motor. This would ascribe to IFT122 not only a role in the retrograde process, but also, indirectly, in the anterograde one.

In fact, mutations in IFT-B make it shorter or non-existent cilia [12], while mutations in IFT-A make

swollen cilia with IFT-B accumulating on the tip ciliary, disrupting the assembly of the anterograde transport [13].In addition, it was shown that, in the cilia of photoreceptors IFT122 mutants, there is accumulation of ITF88 (member of the IFT-B complex) in the outer segments, suggesting that the loss of IFT122 has consequences on retrograde transport of proteins IFT-B or, in any case, it causes difficulties in reassembling the latter. This clearly, has consequences for anterograde transport [14].

While the N-terminal region, containing the WD repeats, interacts with the IFT-A subunits IFT43:WDR35, the C-terminal region forms the trimeric sub-complex IFT122:IFT40:WDR19, where the protein is associated with TULP3. This is a protein belonging to the family of tubby-like proteins, which promotes the traffic of G protein-coupled receptors to the cilia, by its binding to IFT122, thus regulating receptors traffic. TULP3 has an effective role only when linked to the IFT122:IFT40:WDR19 complex, so much that its location depends only on the correct physiology of the "core" and not vice versa. It was also shown that the depletion of TULP3 had no effect on the assembly of IFT complexes [15].

Comparing the structures obtained from the Raptor X software, we noticed the high difference between the wild type and mutated protein. Since the correct protein folding is necessary for the correct functionality of the protein, and considering the interactions that entertains both with the other subunits forming the IFT-A complex and those forming the TULP3 binding complex, this folding modification could determine an incorrect assembly of the IFT-A complex - with consequent alteration of functionality also of IFT-B and / or an altered capacity of binding with TULP3, compromising the molecular traffic.

The presence of the variant at position 842, which determining the aminoacidic substitution Aspartate>Alanine, resulted in a modification of folding in important areas for the correct functionality of the protein, like in correspondence with the WD regions, which constitute scaffolds for simultaneous protein interactions, and above all promote the formation of protein-protein complexes. The C-terminal region, implicated in the formation of the IFT122: IFT40: WDR19 complex binding TULP3, is also highly modified in folding, and in this case it could cause a change in the formation capacity of the IFT-A complex and its bond with TULP3. Comparing the properties of aminoacids, it is known that Alanine is a small hydrophobic and apolar aminoacid, presenting the simplest side chain structure among all the aminoacids. In fact, the side chain is not very reactive, and for this reason it is rarely directly involved in protein function. Aspartate, on the other hand, is a small negatively charged aminoacid, and is therefore generally found on the surface of proteins, exposed to the aqueous environment. It is generally involved in the formation of salt bridges where it bonds with a positively charged aminoacid to create stabilizing H bridges, which may be important for protein stability. It is frequently involved in protein binding sites, and therefore its replacement with a minimally reactive amino acid, having different chemical-physical properties, could destabilize the entire protein structure. Finally, by Cupsat software prediction, which calculated the overall stability of the protein by the atomic potentials and the potential of angle torsions, it is highlighted a destabilizing and unfavorable effect on the muted protein.

Conclusions

Due to the effect of the variant in the folding of the protein and as a result of the lack of protein stability tests, we think that probably IFT122 mutated proteinhas altered binding capabilities with other subunits of IFT122 complex and/or TULP3, causing alterations in microtubular trafficking, necessary for the correct physiology of photoreceptors.

Table legend

Table 1. Gene Id and respective p value computed by ToppGene

Figures legend

Fig. 1. Interactors of IFT122 by GeneCards

Fig. 1. Third structure of wild type IFT122, predicted by RaptorX

Fig. 3. Third structure of mutated IFT122, predicted by RaptorX

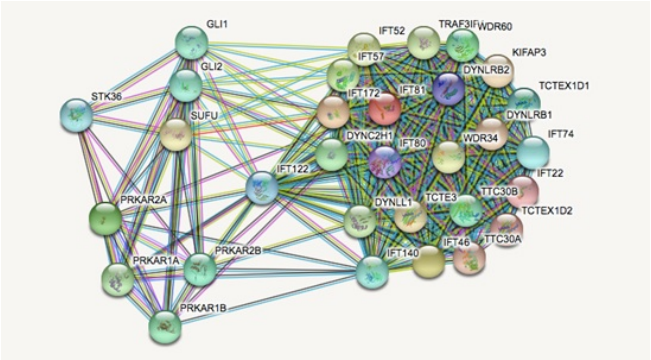

Fig. 1

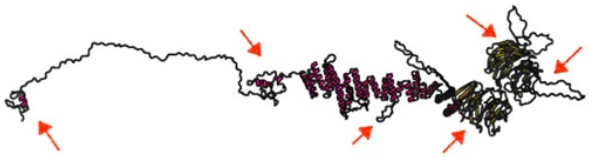

Fig. 2

Fig. 3

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Rank	GeneSymbol	Gene Id	Overall pValue
1	IFT122	55764	1,76E+10
2	CLIC5	53405	0.0014377614980135567
3	ABCC8	6833	0.030051923515502565
4	MARK2	2011	0.034607476105210755
5	SPEN	23013	0.034780757920341854
6	SPG11	80208	0.04850301326100315
7	HTR2C	3358	0.056775087709612704
8	CYP26C1	340665	0.06431811643559371
9	CDAN1	146059	0.07868772687984649
10	FSTL4	23105	0.09237710243688901
11	SLC6A2	6530	0.10529546498853315
12	ABR	29	0.12831632310169372
13	FOXI1	2299	0.13979926067522508
14	LAMB4	22798	0.14413279826038028
15	ATP2C2	9914	0.1451076056568107
16	NCKAP5L	57701	0.14577160987480242
17	ITGA1	3672	0.14685313352987983
18	SMCR8	140775	0.1764408992291817
19	MUC3A	4584	0.17794282978111609
20	ABI3BP	25890	0.19205121544732173
21	MBD1	4152	0.20448896649165416
22	RGPD8	727851	0.2091196431982565
23	BRINP1	1620	0.21090905863967624
24	BYSL	705	0.22885949333019662
25	TMEM63A	9725	0.305643837243711
26	AHNAK2	113146	0.3502737364863011
27	IRF2BPL	64207	0.35553249725991987
28	GREB1	9687	0.3783101081638127
29	ESRP2	80004	0.42310083188408387
30	ZMYM6	9204	0.48670177005309756
31	USP34	9736	0.49607217507769275