Monoubiquitin Signaling in Genetic Diseases

Subjects: Cell Biology | Genetics & Heredity

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Ubiquitination is a reversible post-translational modification that controls protein function and stability. Different types of ubiquitination were described including polyubiquitination, the attachment of multiple ubiquitin residues, or monoubiquitination, the attachment of a single ubiquitin molecule. While most of the studies have described the role of polyubiquitination, recent evidences show that monoubiquitination is a key regulator of different cellular processes, including vesicular trafficking and protein complex formation and degradation. Enzymes regulating monoubiquitination such as E2 conjugating enzymes, E3 ligases or ubiquitin hydrolases, are found altered in several genetic diseases, including Parkinson's disease or Noonan syndrome.

ubiquitin system | genetic diseases | ubiquitin ligase | deubiquitinases | monoubiquitin signaling | vesicular trafficking | protein complex formation

1. Introduction

Ubiquitination is a reversible post-translational modification process during which the highly conserved 76-aminoacid protein ubiquitin is conjugated to target proteins. Ubiquitin can be conjugated to a protein substrate via distinct mechanisms. Monoubiquitination is the attachment of a single ubiquitin molecule to a single lysine residue on a substrate protein, whereas multi-monoubiquitination is the conjugation of a single ubiquitin molecule to multiple lysine residues. Polyubiquitination occurs when ubiquitin molecules are attached end-to-end to a lysine residue on a substrate protein to form a poly-ubiquitin chain. In this case, ubiquitin molecules are conjugated through one of the seven lysine residues present on the ubiquitin itself (K6, K11, K27, K29, K33, K48, and 63) or the N-terminal methionine (M1). While most of the studies have described the role of specific polyubiquitination, such as K48-linked polyubiquitination for proteasomal degradation [1][2] or K63-linked polyubiquitination for vesicular trafficking [3], emerging evidences implicate monoubiquitination and multi-monoubiquitination in controlling numerous aspects of protein function, such as degradation, subcellular localization, and protein–protein interaction. In this review, we focus on the role of monoubiquitin conjugation in normal physiology and genetic disease.

Comparing to the role of polyubiquitination in proteasomal degradation, the function of non-degradative monoubiquitination in human disease has been relatively understudied so far. Recent emerging evidences have highlighted the key function of monoubiquitination in a wide range of cellular processes. The findings listed here only represent the most characterized enzymes controlling monoubiquitination. Nonetheless, it reflects the high prevalence of alterations of the monoubiquitin pathway in such a broad array of genetic disorders. This suggests

that disruption of the monoubiquitin pathway may be a major force driving the pathogenic phenotypes of such diseases.

Table 1. Genetic diseases associated with genes regulating monoubiquitination. Short list of substrates modified by the indicated E2 conjugating enzymes, E3 ligases, and ubiquitin hydrolases (DUBs) are shown, together with the indication of the modulated cellular functions and the type of mutations detected in patients.

Disease	Gene	Type of Enzyme	Monoubiquitinated Substrate	Cellular Function	Disease-Associated Mutations
X-linked syndromic mental retardation	UBE2A	Ubiquitin-conjugating enzyme E2 A	PCNA [4]; Histone H2B [5]	DNA damage tolerance pathway [6][7][8]; epigenetic regulation [5]	Loss of function: missense mutations, microdeletions, larger deletions [9] [10]
Autosomal recessive juvenile parkinsonism	Parkin or PARK2	RBR E3 ubiquitin ligase	VDAC1 [11][12]	Mitophagy, apoptosis [13][14]	Loss of function: missense mutations, deletions [15]
Fanconi Anemia	UBE2T	Ubiquitin-conjugating enzyme E2 T	FANCD2/FANCI [16] [17]	Cross-linked DNA repair [18] [19]	Loss of function: missense mutations [20]
	FANCL	PHD FINGER E3 ubiquitin ligase			Loss of function: missense, frameshift mutations [21]
	BRCA1	RING E3 ubiquitin ligase	FANCD2/FANCI [22]		Loss of function: missense frameshift mutations, deletions [23]
Charcot-Marie-Tooth disease	LRSAM1	RING E3 ubiquitin ligase	TSG101 [24]	Endosomal sorting [25]	Loss of function: missense, frameshift mutations [26]
Cushing disease	USP8	Ubiquitin specific peptidase 8	EGFR [27][28]; CHMP1B [29]	Endosomal sorting [30][31]	Gain of function: missense mutations [32][33]
Noonan Syndrome	LZTR1	BTB-Kelch ubiquitin	RAS [34][35]; CHMP1B [36]	RAS localization and signaling [34] [35]; VEGFR	Loss of function: missense,

Disease	Gene	Type of Enzyme	Monoubiquitinated Substrate	Cellular Function	Disease-Associated Mutations
		ligase adaptor		trafficking and signaling [36]	frameshift mutations [37][38]
	CBL	RING E3 ubiquitin ligase	SH3KBP1 [39]	EGFR trafficking and signaling [40]	Loss of function: missense mutations [41][42][43]
Autoimmune disorder associated to facial dysmorphism	ITCH	HECT E3 ubiquitin ligase	TIEG1 [44]; SMN [45]	Nuclear translocation of FOXP3 [44], translocation of SMN to Cajal body [45]	Loss of function: frameshift mutations [46]

The abundance of the ubiquitin-related enzymes mutated in genetic disorders indicates that targeting the ubiquitin pathway might be of therapeutic use for a range of genetic diseases. However, at present, we lack a detailed knowledge on how monoubiquitin signals are generated and how they are decoded by the cell. This is challenged by the diversity and complexity of the ubiquitin pathway. Moreover, monoubiquitinated proteins might not have been accurately identified, because polyubiquitinated conjugates are recognized more efficiently by anti-ubiquitin specific antibodies. This leads to the underestimation of the pool of monoubiquitinated proteins present in the cell and challenges their characterization. The development of novel tools to purify monoubiquitinated proteins using high-affinity ubiquitin-binding domains and synthetic biology approaches to efficiently generate monoubiquitinated proteins can overcome these issues.

2. Development

It is also worth noting that when looking at the few drugs that were developed to target the ubiquitin pathway, most are meant only to inhibit its functioning. Several inhibitors targeting the ubiquitinating enzymes described in this review have been reported. Ubiquitin variants that block the E2-ubiquitin binding surface of the RING domain of CBL were shown to specifically inhibit the activity of CBL [47][48]. A high-throughput screening to identify ITCH inhibitors discovered that clomipramine, a common antidepressant drug, blocks ITCH autoubiquitination and affects the ability of ITCH to ubiquitinate its substrates [49]. Screening for the inhibitors of UBE2T/FANCL identified two compounds that sensitize cells to DNA crosslinking [50]. Pharmacological inhibition of USP8 was shown to effectively suppress ACTH synthesis in vitro without causing any significant cytotoxicity, indicating its potential for the management of ACTH hypersecretion in Cushing's disease [51]. However, considering that the disease-associated alterations of the ubiquitin ligases and hydrolases are mostly loss of function, inhibitors targeting these enzymes would not be beneficial. This indicates that there is a need to develop novel strategies for targeted therapies of genetic diseases [52]. Several screens identified compounds activating PARKIN ubiquitin ligase activity [53]and enhancing mitophagy [54], such as the compound described in patent WO2018023029. While no in vivo

validation is available for this compound yet, this demonstrates the feasibility of identification of E3 ligase activators, opening novel therapeutic options for patients with genetic disorders.

The analysis of the alterations of the ubiquitin system associated to genetic diseases generated evidence that monoubiquitination is a key process underlying the development of such diseases; highlighting the need for further research to identify new monoubiquitination-dependent signaling pathways as novel targets suitable for therapeutic approach of genetic diseases.

References

1. Julia S. Thrower; Laura Hoffman; Martin Rechsteiner; Cecile M. Pickart; Recognition of the polyubiquitin proteolytic signal.. *The EMBO Journal* **2000**, *19*, 94-102, 10.1093/emboj/19.1.94.
2. Mary E. Matyskiela; Andreas Martin; Design Principles of a Universal Protein Degradation Machine. *Journal of Molecular Biology* **2013**, *425*, 199-213, 10.1016/j.jmb.2012.11.001.
3. Zoi Erpapazoglou; Olivier Walker; Rosine Haguenauer-Tsapis; Versatile Roles of K63-Linked Ubiquitin Chains in Trafficking. *Cells* **2014**, *3*, 1027-1088, 10.3390/cells3041027.
4. Zhang, H.; Yu, D.S. One stone, two birds: CDK9-directed activation of UBE2A regulates monoubiquitination of both H2B and PCNA. Cell Cycle 2012, 11, 2418.
5. Jaehoon Kim; Mohamed Guermah; Robert K. McGinty; Jung-Shin Lee; Zhanyun Tang; Thomas A. Milne; Ali Shilatifard; Tom W. Muir; Robert G. Roeder; RAD6-Mediated Transcription-Coupled H2B Ubiquitylation Directly Stimulates H3K4 Methylation in Human Cells. *Cell* **2009**, *137*, 459-71, 10.1016/j.cell.2009.02.027.
6. Linlin Zhao; M Todd Washington; Translesion Synthesis: Insights into the Selection and Switching of DNA Polymerases. *Genes* **2017**, *8*, 24, 10.3390/genes8010024.
7. Roman Alpatov; Bluma J. Lesch; Mika Nakamoto-Kinoshita; Andres Blanco; Shuzhen Chen; Alexandra Stützer; Karim J. Armache; Matthew D. Simon; Chao Xu; Muzaffar Ali; Jernej Murn; Sladjana Prisic; Tatiana Kutateladze; Christopher R Vakoc; Jinrong Min; Robert E. Kingston; Wolfgang Fischle; Stephen T. Warren; David C. Page; Yang Shi; A chromatin-dependent role of the fragile X mental retardation protein FMRP in the DNA damage response.. *Cell* **2014**, *157*, 869-81, 10.1016/j.cell.2014.03.040.
8. Szilvia Juhász; Amira Elbakry; Arthur Mathes; Markus Löbrich; ATRX Promotes DNA Repair Synthesis and Sister Chromatid Exchange during Homologous Recombination. *Molecular Cell* **2018**, *71*, 11-24.e7, 10.1016/j.molcel.2018.05.014.
9. Bartlomiej Budny; Magdalena Badura-Stronka; A Materna-Kiryluk; A Tzschach; Martine Raynaud Md; A Latos-Bielenska; Hh Ropers; Novel missense mutations in the ubiquitination-related gene

UBE2A cause a recognizable X-linked mental retardation syndrome. *Clinical Genetics* **2010**, *77*, 541-551, 10.1111/j.1399-0004.2010.01429.x.

10. Dingyuan Ma; Jianxin Tan; Jing Zhou; Jingjing Zhang; Jian Cheng; Chunyu Luo; Gang Liu; Yuguo Wang; Zhengfeng Xu; A novel splice site mutation in the UBE2A gene leads to aberrant mRNA splicing in a Chinese patient with X-linked intellectual disability type Nascimento. *Molecular Genetics & Genomic Medicine* **2019**, *7*, e976, 10.1002/mgg3.976.
11. Hampe, C.; Ardila-Osorio, H.; Fournier, M.; Brice, A.; Corti, O. Biochemical analysis of Parkinson's disease-causing variants of Parkin, an E3 ubiquitin - Protein ligase with monoubiquitylation capacity. Hum. Mol. Genet. 2006, 15, 2059–2075.
12. Su Jin Ham; Daewon Lee; Heesuk Yoo; Kyoungho Jun; Heejin Shin; Jongkyeong Chung; Decision between mitophagy and apoptosis by Parkin via VDAC1 ubiquitination. *Proceedings of the National Academy of Sciences* **2020**, *117*, 4281-4291, 10.1073/pnas.1909814117.
13. J. Wade Harper; A. Ordureau; Jin-Mi Heo; Building and decoding ubiquitin chains for mitophagy. *Nature Reviews Molecular Cell Biology* **2018**, *19*, 93-108, 10.1038/nrm.2017.129.
14. Brent Ryan; Selim Hoek; Edward A. Fon; Richard Wade-Martins; Mitochondrial dysfunction and mitophagy in Parkinson's: from familial to sporadic disease. *Trends in Biochemical Sciences* **2015**, *40*, 200-210, 10.1016/j.tibs.2015.02.003.
15. Hideki Shimura; Nobutaka Hattori; Shin-Ichiro Kubo; Yoshikuni Mizuno; Shuichi Asakawa; Shinsei Minoshima; Nobuyoshi Shimizu; Kazuhiro Iwai; Tomoki Chiba; Keiji Tanaka; Toshiaki Suzuki; Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nature Genetics* **2000**, *25*, 302-305, 10.1038/77060.
16. Molly C. Kottemann; Agata Smogorzewska; Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature* **2013**, *493*, 356-363, 10.1038/nature11863.
17. Pablo Alcón; Shabih Shakeel; Zhuo A. Chen; Juri Rappsilber; Ketan J. Patel; Lori A. Passmore; FANCD2–FANCI is a clamp stabilized on DNA by monoubiquitination of FANCD2 during DNA repair. *Nature Structural & Molecular Biology* **2020**, *27*, 240-248, 10.1038/s41594-020-0380-1.
18. Jasti, V.P.; Sharma, S.; Althaus, I.; Weinstein, I.; Ramani, S. Repairing interstrand DNA crosslinks (ICL): Characterization of an ICL incision/lesion bypass polymerase complex regulated by the fanconi anemia pathway. FASEB J. 2017, 31.
19. Jones, N.; Makki, F.; Davis, G.; Marriott, A.; Wilson, J.; Kupfer, G. The Fanconi anaemia pathway and the repair of DNA lesions resulting from endogenous aldehydes and chemotherapeutic agents. Mutagenesis 2014, 29, 80.
20. Asuka Hira; Kenichi Yoshida; Koichi Sato; Yusuke Okuno; Yuichi Shiraishi; Kenichi Chiba; Hiroko Tanaka; Satoru Miyano; Akira Shimamoto; Hidetoshi Tahara; Etsuro Ito; Seiji Kojima; Hitoshi Kurumizaka; Seishi Ogawa; Minoru Takata; Hiromasa Yabe; Miharu Yabe; Mutations in the Gene

Encoding the E2 Conjugating Enzyme UBE2T Cause Fanconi Anemia. *The American Journal of Human Genetics* **2015**, *96*, 1001-1007, 10.1016/j.ajhg.2015.04.022.

21. Amom Ruhikanta Meetei; Johan P De Winter; Annette L Medhurst; Michael Wallisch; Quinten Waisfisz; Henri J Van De Vrugt; Anneke B Oostra; Zhijiang Yan; Chen Ling; Colin E Bishop; Maureen E Hoatlin; Hans Joenje; Dongyi Xu; A novel ubiquitin ligase is deficient in Fanconi anemia. *Nature Genetics* **2003**, *35*, 165-170, 10.1038/ng1241.
22. Irene Garcia-Higuera; Toshiyasu Taniguchi; Shridar Ganesan; M.Stephen Meyn; Cynthia Timmers; James Hejna; Markus Grompe; Alan D D'andrea; Interaction of the Fanconi Anemia Proteins and BRCA1 in a Common Pathway. *Molecular Cell* **2001**, *7*, 249-262, 10.1016/s1097-2765(01)00173-3.
23. Betsy Hirsch; Akiko Shimamura; Lisa Moreau; Shari Baldinger; Maha Hag-Alshiekh; Bruce Bostrom; Susan Sencer; Alan D. D'andrea; Association of biallelic BRCA2/FANCD1 mutations with spontaneous chromosomal instability and solid tumors of childhood. *Blood* **2004**, *103*, 2554-2559, 10.1182/blood-2003-06-1970.
24. Ido Amit; Liat Yakir; Menachem Katz; Yaara Zwang; Mina D. Marmor; Ami Citri; Keren Shtiegman; Iris Alroy; Shmuel Tuvia; Yuval Reiss; Eli Roubini; Maya Cohen; Ron Wides; Eran Bacharach; Ulrich S. Schubert; Yosef Yarden; Tal, a Tsg101-specific E3 ubiquitin ligase, regulates receptor endocytosis and retrovirus budding. *Genome Research* **2004**, *18*, 1737-1752, 10.1101/gad.294904.
25. Alexander M Rossor; James M. Polke; Henry Houlden; Mary M. Reilly; Clinical implications of genetic advances in Charcot–Marie–Tooth disease. *Nature Reviews Neurology* **2013**, *9*, 562-571, 10.1038/nrneurol.2013.179.
26. Paschalis Nicolaou; Carlo Cianchetti; Anna Minaidou; Giovanni Marrosu; Eleni Zamba-Papanicolaou; Lefkos Middleton; Kyproula Christodoulou; A novel LRSAM1 mutation is associated with autosomal dominant axonal Charcot-Marie-Tooth disease. *European Journal of Human Genetics* **2012**, *21*, 190-194, 10.1038/ejhg.2012.146.
27. Martin Reincke; Silviu Sbiera; Akira Hayakawa; Marily Theodoropoulou; Andrea Osswald; Felix Beuschlein; Thomas Meitinger; Emi Mizuno-Yamasaki; Kohei Kawaguchi; Yasushi Saeki; Keiji Tanaka; Thomas Wieland; Elisabeth Graf; Wolfgang Saeger; Cristina L. Ronchi; Bruno Allolio; Michael Buchfelder; Tim M Strom; Martin Fassnacht; Masayuki Komada; Mutations in the deubiquitinase gene USP8 cause Cushing's disease. *Nature Genetics* **2014**, *47*, 31-38, 10.1038/ng.3166.
28. Zeng-Yi Ma; Zhi-Jian Song; Jian-Hua Chen; Yong-Fei Wang; Shi-Qi Li; Liang-Fu Zhou; Ying Mao; Yi-Ming Li; Rong-Gui Hu; Zhao-Yun Zhang; Hong-Ying Ye; Ming Shen; Xue-Fei Shou; Zhiqiang Li; Hong Peng; Qing-Zhong Wang; Dai-Zhan Zhou; Xiao-Lan Qin; Jue Ji; Jie Zheng; Hong Chen; Yin Wang; Dao-Ying Geng; Wei-Jun Tang; Chaowei Fu; Zhi-Feng Shi; Zhao Ye; Wen-Qiang He; Qi-

Lin Zhang; Qi-Sheng Tang; Rong Xie; Jia-Wei Shen; Zu-Jia Wen; Juan Zhou; Tao Wang; Shan Huang; Hui-Jia Qiu; Nidan Qiao; Li Pan; Wei-Min Bao; Ying-Chao Liu; Chuan-Xin Huang; Yongyong Shi; Yao Zhao; Yi-Chao Zhang; Yi Zhang; Recurrent gain-of-function USP8 mutations in Cushing's disease.. *Cell Research* **2015**, *25*, 306-17, 10.1038/cr.2015.20.

29. Xènia Crespo-Yàñez; Carmen Aguilar Gurrieri; Anne-Claire Jacomin; Agnes Journet; Magda Mortier; Emmanuel Taillebourg; Emmanuelle Soleilhac; Winfríed Weissenhorn; Marie-Odile Fauvarque; CHMP1B is a target of USP8/UBPY regulated by ubiquitin during endocytosis. *PLOS Genetics* **2018**, *14*, e1007456, 10.1371/journal.pgen.1007456.
30. Günter Stalla; Ulrich Renner; Ballmann C; Thiel A; Korah He; Reis Ac; Saeger W; Stepanow S; Köhrer K; Reifenberger G; Knobbe-Thomsen Cb; Knappe Uj; Scholl Ui; Faculty Opinions recommendation of USP8 Mutations in Pituitary Cushing Adenomas-Targeted Analysis by Next-Generation Sequencing.. *Faculty Opinions – Post-Publication Peer Review of the Biomedical Literature* **2018**, *2*, , 10.3410/f.733389597.793549878.
31. Paula E. Row; Han Liu; Sebastian Hayes; Rebecca Welchman; Panagoula Charalabous; Kay Hofmann; Michael J. Clague; Christopher M. Sanderson; Sylvie Urbe; The MIT Domain of UBPY Constitutes a CHMP Binding and Endosomal Localization Signal Required for Efficient Epidermal Growth Factor Receptor Degradation. *Journal of Biological Chemistry* **2007**, *282*, 30929-30937, 10.1074/jbc.m704009200.
32. Marily Theodoropoulou; Martin Reincke; Martin Fassnacht; Masayuki Komada; Decoding the genetic basis of Cushing's disease: USP8 in the spotlight. *European Journal of Endocrinology* **2015**, *173*, M73-M83, 10.1530/eje-15-0320.
33. Dufner, A.; Knobeloch, K.P. Ubiquitin-specific protease 8 (USP8/UBPy): A prototypic multidomain deubiquitinating enzyme with pleiotropic functions. Biochem. Soc. Trans. 2019, 1867–1879.
34. M. Steklov; S. Pandolfi; M. F. Baietti; A. Batiuk; P. Carai; P. Najm; M. Zhang; Hyunbum Jang; F. Renzi; Y. Cai; L. Abbasi Asbagh; T. Pastor; M. De Troyer; Michal Simicek; Enrico Radaelli; Hilde Brems; Eric Legius; J. Tavernier; Kris Gevaert; Francis Impens; L. Messiaen; Ruth Nussinov; S. Heymans; S. Eyckerman; Anna A. Sablina; Mutations in LZTR1 drive human disease by dysregulating RAS ubiquitination. *Science* **2018**, *362*, 1177-1182, 10.1126/science.aap7607.
35. Johannes W. Bigenzahn; Giovanna M. Collu; Felix Kartnig; Melanie Pieraks; Gregory I. Vladimer; Leonhard X. Heinz; Vitaly Sedlyarov; Fiorella Schischlik; Astrid Fauster; Manuele Rebsamen; Katja Parapatics; Vincent A. Blomen; André C. Müller; Georg E. Winter; Robert Kralovics; Thijn R Brummelkamp; Marek Mlodzik; Giulio Superti-Furga; LZTR1 is a regulator of RAS ubiquitination and signaling. *Science* **2018**, *362*, 1171-1177, 10.1126/science.aap8210.
36. Raj Nayan Sewduth; Silvia Pandolfi; Mikhail Steklov; Aidana Sheryazdanova; Peihua Zhao; Nathan Criem; Maria Francesca Baietti; Benoit Lechat; Rozenn Quarck; Francis Impens; et al.Anna A. Sablina The Noonan Syndrome Gene Lztr1 Controls Cardiovascular Function by

Regulating Vesicular Trafficking. *Circulation Research* **2020**, *126*, 1379-1393, 10.1161/circresaha.119.315730.

37. Jennifer J. Johnston; Members Of The Undiagnosed Diseases Network; Jasper J. Van Der Smagt; Jill A. Rosenfeld; Alistair T. Pagnamenta; Abdulrahman Alswaid; Eva H. Baker; Edward Blair; Guntram Borck; Julia Brinkmann; William Craigen; Vu Chi Dung; Lisa Emrick; David B. Everman; Koen Van Gassen; Suleyman Gulsuner; Margaret H. Harr; Mahim Jain; Alma Kuechler; Kathleen A. Leppig; nna M. McDonald-McGinn; Ngoc Thi Bich Can; Amir Peleg; Elizabeth R. Roeder; R. Curtis Rogers; Lena Sagi-Dain; Julie C. Sapp; Alejandro A. Schäffer; Denny Schanze; Helen Stewart; Jenny C. Taylor; Nienke E. Verbeek; Magdalena A. Walkiewicz; Elaine H. Zackai; Christiane Zweier; Martin Zenker; Brendan Lee; Leslie G. Biesecker; Can Thi Bich Ngoc; Autosomal recessive Noonan syndrome associated with biallelic LZTR1 variants. *Genetics in Medicine* **2018**, *20*, 1175-1185, 10.1038/gim.2017.249.
38. Adeline Jacquinet; Adeline Bonnard; Yline Capri; Didier Martin; Bernard Sadzot; Elettra Bianchi; Laurent Servais; Jean-Paul Sacré; Hélène Cavé; Alain Verloes; Oligo-astrocytoma in LZTR1-related Noonan syndrome. *European Journal of Medical Genetics* **2020**, *63*, 103617, 10.1016/j.ejmg.2019.01.007.
39. Gayathri Swaminathan; A Y Tsygankov; The Cbl family proteins: Ring leaders in regulation of cell signaling. *Journal of Cellular Physiology* **2006**, *209*, 21-43, 10.1002/jcp.20694.
40. Kaisa Haglund; Noriaki Shimokawa; Iwona Szymkiewicz; Ivan Dikic; Cbl-directed monoubiquitination of CIN85 is involved in regulation of ligand-induced degradation of EGF receptors. *Proceedings of the National Academy of Sciences* **2002**, *99*, 12191-12196, 10.1073/pnas.192462299.
41. Noonan's syndrome. *The Lancet* **1992**, *340*, 22-23, 10.1016/0140-6736(92)92429-j.
42. C. Niemeyer; Michelle W. Kang; Danielle H. Shin; Ingrid Furlan; Miriam Erlacher; Nancy J Bunin; Severa Bunda; Jerry Z. Finklestein; Thomas Gorr; Parinda Mehta; Irene Schmid; Gabriele Kropshofer; Selim Corbacioglu; Peter J Lang; Christoph Klein; Paul-Gerhard Schlegel; Andrea Heinzmann; Michaela Schneider; Jan Starý; Marry M. Van Den Heuvel-Eibrink; H. Hasle; Franco Locatelli; Debbie Sakai; Sophie Archambeault; Leslie Chen; Ryan C. Russell; Stephanie S. Sybingco; Michael Ohh; Benjamin S. Braun; Christian Flotho; Mignon L. Loh; Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia.. *Nature Genetics* **2010**, *42*, 794-800, 10.1038/ng.641.
43. Anand Pathak; NCI DCEG Cancer Genomics Research Laboratory; Alexander Pemov; Mary L. McMaster; Ramita Dewan; Sarangan Ravichandran; Evgenia Pak; Amalia Dutra; Hyo Jung Lee; Aurelie Vogt; Xijun Zhang; Meredith Yeager; Stacie Anderson; Martha Kirby; Neil Caporaso; Mark H Greene; Lynn R. Goldin; Douglas R Stewart; NCI DCEG Cancer Sequencing Working Group;

Juvenile myelomonocytic leukemia due to a germline CBL Y371C mutation: 35-year follow-up of a large family. *Quality of Life Research* **2015**, *134*, 775-787, 10.1007/s00439-015-1550-9.

44. K Venuprasad; Haining Huang; Yousuke Harada; Chris Elly; Malayannan Subramaniam; Thomas Spelsberg; Jin Su; Yun-Cai Liu; The E3 ubiquitin ligase Itch regulates expression of transcription factor Foxp3 and airway inflammation by enhancing the function of transcription factor TIEG1. *Nature Immunology* **2008**, *9*, 245-253, 10.1038/niXXXX.
45. Ke-Jun Han; Daniel Foster; Edward W. Harhaj; Monika Dzieciatkowska; Kirk Hansen; Chang-Wei Liu; Monoubiquitination of survival motor neuron regulates its cellular localization and Cajal body integrity.. *Human Molecular Genetics* **2016**, *25*, 1392-405, 10.1093/hmg/ddw021.
46. Naomi J. Lohr; Jean P. Molleston; Kevin A. Strauss; Wilfredo Torres-Martinez; Erin Sherman; Robert H. Squires; Nicholas L. Rider; Kudakwashe R. Chikwava; Oscar W. Cummings; D. Holmes Morton; Erik Puffenberger; Human ITCH E3 Ubiquitin Ligase Deficiency Causes Syndromic Multisystem Autoimmune Disease. *The American Journal of Human Genetics* **2010**, *86*, 447-453, 10.1016/j.ajhg.2010.01.028.
47. Jichun Wang; Li Li; Hong Jiang; c-Cbl inhibition: A novel therapeutic approach for attenuating myocardial ischemia and reperfusion injury. *International Journal of Cardiology* **2015**, *186*, 50-51, 10.1016/j.ijcard.2015.03.254.
48. Mads Gabrielsen; Lori Buetow; Mark A. Nakasone; Syed Feroj Ahmed; Gary J. Sibbet; Brian O. Smith; Wei Zhang; Sachdev S. Sidhu; Danny T. Huang; A General Strategy for Discovery of Inhibitors and Activators of RING and U-box E3 Ligases with Ubiquitin Variants.. *Molecular Cell* **2017**, *68*, 456-470.e10, 10.1016/j.molcel.2017.09.027.
49. Mario Rossi; Barak Rotblat; K Ansell; Ivano Amelio; Michele Caraglia; Gabriella Misso; F Bernassola; Claudio N. Cavasotto; R A Knight; A Ciechanover; Gerry Melino; High throughput screening for inhibitors of the HECT ubiquitin E3 ligase ITCH identifies antidepressant drugs as regulators of autophagy.. *Cell Death & Disease* **2014**, *5*, e1203-e1203, 10.1038/cddis.2014.113.
50. Matthew J. Cornwell; Graeme J. Thomson; Julia Coates; Rimma Belotserkovskaya; Ian D. Waddell; Stephen P. Jackson; Yaron Galanty; Small-Molecule Inhibition of UBE2T/FANCL-Mediated Ubiquitylation in the Fanconi Anemia Pathway. *ACS Chemical Biology* **2019**, *14*, 2148-2154, 10.1021/acschembio.9b00570.
51. Marily Theodoropoulou; Luis Perez-Rivas; Adriana Albani; Gunter Stalla; Michael Buchfelder; Joerg Flitsch; Juergen Honegger; Walter Rachinger; Martin Reincke; Efficacy of pharmacological USP8 inhibition in human Cushing's disease tumours in vitro. *Endocrine Abstracts* **2018**, *null*, , 10.1530/endoabs.56.gp197.
52. Olivier Humbert; Luther Davis; Nancy Maizels; Targeted gene therapies: tools, applications, optimization.. *Critical Reviews in Biochemistry and Molecular Biology* **2012**, *47*, 264-81, 10.3109/10409238.2012.658112.

53. Helen L. Scott; Nicola Buckner; Francesc Fernandez-Albert; Elisa Pedone; Lorena Postiglione; Gongyu Shi; Nicholas Allen; Liang-Fong Wong; Lorenzo Magini; Lucia Marucci; Gregory A. O'sullivan; Sarah Cole; Justin Powell; Peter Maycox; James B. Uney; A dual druggable genome-wide siRNA and compound library screening approach identifies modulators of parkin recruitment to mitochondria.. *Journal of Biological Chemistry* **2020**, *295*, 3285-3300, 10.1074/jbc.RA119.009699.

54. Silke Miller; Miratul M.K. Muqit; Therapeutic approaches to enhance PINK1/Parkin mediated mitophagy for the treatment of Parkinson's disease.. *Neuroscience Letters* **2019**, *705*, 7-13, 10.1016/j.neulet.2019.04.029.